

# The behaviour of EDDHA isomers in soils as influenced by soil properties

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Received: 7 June 2006 / Accepted: 28 September 2006 / Published online: 16 December 2006  
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**Abstract** FeEDDHA products are applied to correct iron chlorosis in plants and consist of a mixture of EDDHA isomers chelated to iron. In this study such mixtures have been divided into four (groups of) isomers: racemic *o,o*-EDDHA, meso *o,o*-EDDHA, *o,p*-EDDHA and rest-EDDHA. The physical and chemical properties of these isomers differ and hence does their ability to deliver Fe to plants. To come to a soil-specific iron fertilization recommendation, the behaviour of the EDDHA isomers in the soil needs to be understood. This behaviour has been examined in a soil interaction experiment as a function of time, and it has been related to soil properties. The isomer fractions remaining in solution can be ranked racemic *o,o*-FeEDDHA > meso *o,o*-FeEDDHA > rest-FeEDDHA > *o,p*-FeEDDHA, regardless of soil properties. The *o,o*-EDDHA isomers largely

determine the Fe concentration in solution. Although rest-EDDHA also consists of compounds that chelate Fe more strongly than meso *o,o*-EDDHA, the latter is on average better capable of keeping Fe in solution upon interaction with soil. The principal adsorption surface differs per EDDHA isomer. For racemic *o,o*-FeEDDHA it is organic matter, for meso *o,o*-FeEDDHA it is iron (hydr)oxide and for *o,p*-FeEDDHA clay minerals. Cu and Al are important competing cations. Cu forms soluble complexes with *o,p*-EDDHA, and Al with meso *o,o*-EDDHA not chelated to Fe. Al is likely to affect the effectiveness of a potential shuttle effect. The tendency of *o,p*-FeEDDHA and rest-FeEDDHA to be removed from solution, makes these isomers less effective as iron fertilizer in soil application, in particular on clay soils.

**Electronic Supplementary Material** Supplementary material is available for this article at <http://dx.doi.org/10.1007/s11104-006-9135-y> and is accessible for authorized users.

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**Keywords** Adsorption · Cation competition · EDDHA isomers · FeEDDHA · Iron chelates · Iron chlorosis

## Abbreviations

*o,o*-FeEDDHA iron (3+) ethylene diamine-*N,N'*-bis(2-hydroxy phenyl acetic acid) complex  
*o,p*-FeEDDHA iron (3+) ethylene diamine-*N*-(2-hydroxy phenyl acetic acid)-*N'*-(4-hydroxy phenyl acetic acid) complex

DOC	Dissolved organic carbon
DTPA	Diethylene triamine penta acetic acid
HFO	Hydrous ferric oxide
ICP MS/AES	Inductively coupled plasma mass spectroscopy/atomic emission spectroscopy
SOC	Soil Organic Carbon
CEC	Cation Exchange Capacity

## Introduction

Iron is an essential micronutrient for its role in the formation of chlorophyll and in various enzymatic processes (Marschner 1995). Although generally present in the soil in sufficient quantities, iron is not always sufficiently available to plants. A low bioavailability of iron may lead to iron chlorosis in crops. Iron chlorosis is a nutritional disorder characterized by a significant decrease of chlorophyll in the leaves. It reduces crop quality and depresses crop yields and hence causes economic losses. In particular in alkaline and calcareous soils, the bioavailability of iron can be severely limited (Chaney 1984; Mortvedt 1991). This mainly results from the low solubility of iron (hydr)oxides at high pH (Lindsay 1979), and from the elevated bicarbonate concentration in the soil solution (Boxma 1972; Mengel et al. 1984; Shi et al. 1993). Bicarbonate either impairs Fe uptake mechanisms of the plant (Marschner 1995; Venkatraju and Marschner 1981) or inactivates Fe in the leaf apoplast (Mengel 1994). Most alkaline and calcareous soils are found in areas of the reference soil groups calcisols and solonchaks which cover nearly 10 million km<sup>2</sup> of the earth's surface (FAO/AGL 2000).

The most common practice to overcome iron deficiency in plants is the application of synthetic iron chelates (Chen and Barak 1982). FeEDDHA (iron ethylene diamine-*N,N'*-bis(hydroxy phenylacetic acid)) is among the most effective iron fertilizers on neutral and alkaline soils (Lucena et al. 1992b; Papastylianou 1990; Reed et al. 1988). Its agronomical performance has been intensively studied since the 1950s (Hill-Cottingham and Lloyd-Jones 1958; Kroll 1957; Wallace et al. 1955). Several studies have been dedicated

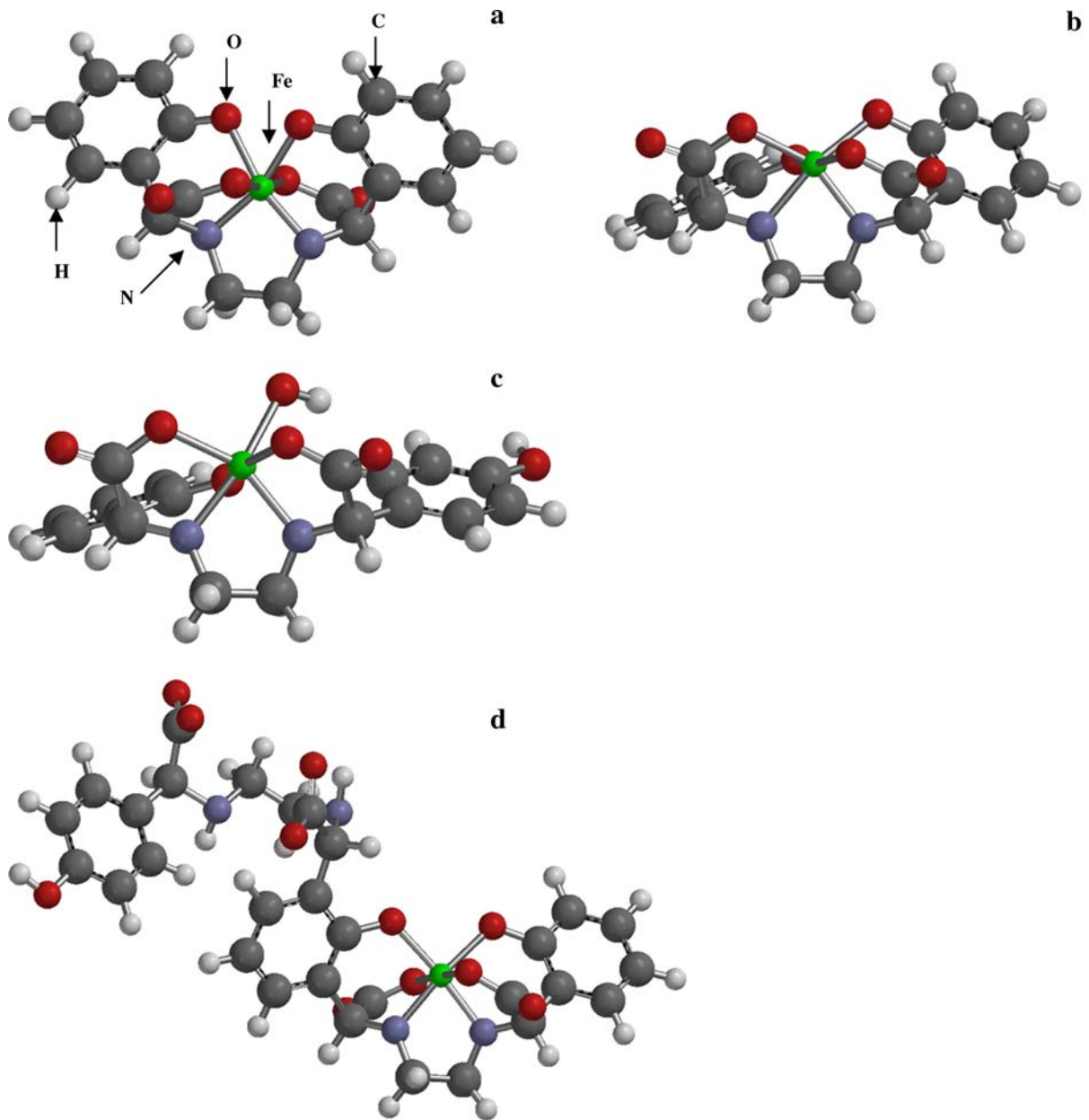
to the determination and the quantification of FeEDDHA (Barak and Chen 1987; Hernandez-Apaolaza et al. 1997; Lucena et al. 1996).

The synthesis pathway applied for manufacturing commercial FeEDDHA formulations is a Mannich-like reaction between phenol, ethylene diamine and glyoxylic acid (Julien and Aymard 1989; Petree et al. 1978). The reaction produces a mixture of (1) positional isomers, (2) enantiomers and (3) polycondensates. This is because: (1) the reaction pathway allows for aromatic substitution in both ortho (*o*) and para (*p*) position, (2) two chiral centres are introduced into the molecule, leading to (*R,R*), (*R,S*), (*S,R*) and (*S,S*) enantiomers and (3) undesired addition reactions take place between reactants and half products (Cremonini et al. 2001).

Throughout this paper, the EDDHA-synthesis products are divided into four categories (see Fig. 1), labelled as follows: (1) racemic *o,o*-EDDHA (referring to the (*R,R*) and (*S,S*) *o,o*-EDDHA enantiomers; the enantiomers are mirror images, differing in the direction they deviate polarized light but identical in binding strength), (2) meso *o,o*-EDDHA (referring to the (*R,S*) = (*S,R*) enantiomer; due to the internal mirror plane of the molecule, the (*R,S*) and (*S,R*) configurations are identical (Bailey et al. 1981; Hill-Cottingham 1962; Ryskievich and Boka 1962)), (3) *o,p*-EDDHA (referring to the four *o,p*-EDDHA enantiomers) and (4) rest-EDDHA (referring to the 3 *p,p*-EDDHA enantiomers and a variety of polycondensates and half-products (Cremonini et al. 2001; Hernandez-Apaolaza et al. 2006)). In general these four groups are referred to as the different EDDHA isomers.

Because the physical and chemical properties of these EDDHA isomers differ, so will their ability to bind iron and deliver it to the plant. Binding strength parameters such as protonation constants and complexation constants for iron and several other metals have been determined for the most important EDDHA isomers and enantiomers (Ahrland et al. 1990; Bannochie and Martell 1989; Frost et al. 1958; Yunta et al. 2003a, b).

The isomeric compositions of commercially available FeEDDHA formulations differ, and therefore the need for a quality parameter arose. Several parameters have been proposed



**Fig. 1** Spatial structures of the isomers (a) racemic *o,o*-FeEDDHA; (b) meso *o,o*-FeEDDHA; (c) *o,p*-FeEDDHA with  $\text{OH}^-$  on the coordination complex; (d) rest-FeEDDHA (one possible polycondensate)

**Table 1** log  
Complexation constants  
of EDDHA and Fe  
(Yunta et al. 2003a, b)  
( $I = 0.1$  (NaCl))

	Racemic <i>o,o</i> -EDDHA	meso <i>o,o</i> -EDDHA	<i>o,p</i> -EDDHA
$[\text{Fe}^{3+}][\text{L}^{4-}] \leftrightarrow [\text{FeL}^-]$	35.86	34.15	28.72
$[\text{Fe}^{3+}][\text{L}^{4-}][\text{H}^+] \leftrightarrow [\text{FeLH}]$	35.08	36.56	35.02
$[\text{Fe}^{3+}][\text{L}^{4-}][\text{H}^+]^2 \leftrightarrow [\text{FeLH}_2^+]$			37.35

(Hernandez-Apaolaza et al. 1995; Lucena et al. 1992a, b). At present, the quality aspect is assured in the European fertilizer law (Regulation (EC) No. 2003/2003) through two parameters: (1) soluble Fe-content of the product and (2) percentage of the Fe chelated by the *o,o*-EDDHA isomers. The latter (2) is considered a suitable parameter, because the *o,o*-EDDHA isomers were found to form the most stable complexes with iron (Hernandez-Apaolaza et al. 1997; Yunta et al. 2003b, c) (Table 1).

Because of the requirement to have a certain percentage of the iron chelated by *o,o*-EDDHA and the limitation of the synthesis pathway in producing *o,o*-EDDHA, there is no incentive to maximize the amount of iron in the FeEDDHA product. As a result commercial EDDHA formulations tend to contain significant amounts of EDDHA isomers other than *o,o*, that are not chelated to iron.

The characteristics of an iron chelate that determine its effectiveness in agronomic practice are: (1) its ability to remain in solution, (2) its susceptibility to competition from other metal ions, (3) its ability to deliver iron to the plant, and (4) its selectivity to pick up iron from the soil, either after having delivered an iron ion to the plant (shuttle effect) or upon initial contact with the soil (Lucena 2003). In the case of soil application of iron chelates, at least three out of four of these features are co-determined by the characteristics of the soil.

To improve the understanding on the interaction of FeEDDHA isomers with soil and soil constituents, several studies have been done (Alvarez-Fernandez et al. 1997, 2002; Cantera et al. 2002; Garcia-Marco et al. 2006; Garcia-Mina et al. 2003; Hernandez-Apaolaza et al. 2006; Hernandez-Apaolaza and Lucena 2001; Siebner-Freibach et al. 2004). Soil organic matter (acid peat) and iron (hydr)oxides (ferrihydrite) have been identified as the most reactive, and calcium carbonate and clay (Ca-montmorillonite) as less reactive soil constituents with respect to FeEDDHA sorption (Alvarez-Fernandez et al. 1997, 2002). The meso *o,o*-FeEDDHA was found to be more susceptible to sorption than the racemic *o,o*-FeEDDHA isomer (Alvarez-Fernandez et al. 2002; Hernandez-Apaolaza and Lucena 2001). The speciation of

certain metals other than Fe is also affected from soil application of dissolved FeEDDHA products: Cu and Mn have been reported to go into solution. (Alvarez-Fernandez et al. 1997, 2002; de Krij 1998; Gil-Ortiz and Bautista-Carrascosa 2004). Whether this is the result of Fe displacement or of complexation by chelating compounds initially not chelating Fe has not been cleared up. *O,p*-EDDHA not chelated to Fe has been reported to dissolve Cu from soils (Garcia-Marco et al. 2006).

Up until now, interaction studies with EDDHA isomers have mainly focused on isolated and synthesized soil constituents, while actual soils have been largely approached as black boxes. In this study a novel approach was followed, in which the influence of reactive soil constituents and competing cations on EDDHA isomer behaviour was examined within the soil system itself. An understanding of this issue is crucial to come to an adequate, soil-specific iron fertilization recommendation.

The aim of this research was (1) to examine EDDHA isomer behaviour upon interaction with soils as a function of time, and (2) to relate this behaviour to soil properties. More specifically, an attempt was made to pinpoint per isomer which reactive surfaces and competing cations are dominant in determining their behaviour upon soil interaction. Because *o,p*-EDDHA and rest-EDDHA are present in commercial formulations both chelated to and non-chelated to iron, this distinction was also included in this study.

An experiment was done in which a number of soils were allowed to interact with a number of EDDHA solutions. The EDDHA solutions differed in (1) isomeric composition, primarily the *o,o*-EDDHA content, and (2) degree to which the chelating capacity of the EDDHA solutions was saturated with Fe. The aqueous phase was examined as a function of reaction time.

## Materials and methods

### Soils

Soils were collected from seven sites, located in Italy (Bologna), Spain (Xeraco and Santomera),

Saudi Arabia (Nadec and Hofuf) and the Netherlands (Droevendaal and Herveld). The soils are named after the location of collection. The sites were selected so that there were ranges in soil properties and constituents reported to interact with FeEDDHA isomers (Alvarez-Fernandez et al. 1997). Four clay soils and four sandy soils were included. At all sites the top layer (0–20 cm) was sampled. From one site (Xeraco, Spain), soil material from both the top layer and the layer directly underneath (20–40 cm) were sampled separately. The top layer is relatively rich in organic material. In crops grown at the sites in Spain, Italy and Saudi Arabia, iron chlorosis was manifest. The two Dutch sites were included to extend the ranges of potentially relevant soil characteristics. Pre-treatment consisted of drying (40°C) and sieving (2 mm). The chemical and textural properties of the soils were analysed, the prime results of which are presented in Table 2.

### Experimental solutions

Seven FeEDDHA solutions and a blank were used in the interaction experiment. The FeEDDHA solutions were prepared from three sodium-EDDHA stock solutions and solid *o,o*-H<sub>4</sub>EDDHA<sup>1</sup> (99% pure). The stock solutions were synthesized through the aforementioned Mannich-like reaction (Petree et al. 1978) and differed in *o,o*-EDDHA-content: approximately 20%, 40% and 60% on an ethylene diamine input basis. Out of each stock solution, two experimental solutions were prepared through the addition of different amounts of FeCl<sub>3</sub>·6H<sub>2</sub>O. To the first solution an amount of Fe was added, equal to the molar equivalent of *o,o*-EDDHA. This solution was given the P-suffix for “Partly chelating Fe”. To the second solution an amount of Fe was added, 5% in excess based on a 1:1 stoichiometry between Fe and ethylene diamine. This solution was given the F-suffix for “Fully chelating Fe”. The pH was raised to 7 (±0.5) and the solutions were left standing over-night in the dark in order for excess iron to precipitate as (hydr)oxides. The experimental solution from solid *o,o*-H<sub>4</sub>EDDHA was prepared as described

by Alvarez-Fernandez et al. (2002). The following day, the solutions were filtered through a 0.45-µm nitrocellulose micropore filter (Schleicher & Schuell, ref-no: 10401114). After filtration the solutions were further diluted. The final experimental solutions had total chelating capacities in between an equivalent of 12 and 15 mg Fe l<sup>-1</sup> (see Fig. 2b). They were named after their *o,o*-EDDHA content (*o,o*20%; *o,o*40%, *o,o*60% and *o,o*100%) and their degree of chelation (P or F).

In order to impose ionic strength CaCl<sub>2</sub> was added, so that the final solutions including the blank had a 0.01 M CaCl<sub>2</sub> concentration. The composition of the experimental solutions was analysed through ICP-AES, ICP-MS and HPLC analysis at time *t* = 0 and at the different sampling moments as described under sampling and measurement. Prior to analysis the samples were filtered through a 0.45-µm cellulose acetate micropore filter (Schleicher & Schuell, ref no: 10462650). To avoid contamination, the preparation of the experimental solutions and dilution of samples for measurement were done with analytical grade chemicals and ultra pure water.

### Soil–FeEDDHA interaction studies

The selected soils were allowed to interact with the different experimental solutions in a soil-solution ratio of 1:1 (w/v) for respectively 1, 2, 4 and 6 weeks in 50 ml polypropylene test tubes (Greiner bio-one, Cat No 210296). The tubes were placed in an end-over-end shaker, rotating at 18 rpm in absence of light. Room temperature was kept at 20 (±1)°C. To avoid drastic changes in redox conditions throughout the experiment, the tubes were taken out of the shaker, opened and left standing for 30 min every three to four days.

The experiment was carried out in triplicates. Control treatments with the different experimental solutions without soil were included for *t* = 0 and the four reaction times.

### Sampling and measurement

After interaction, the samples were centrifuged for 15 min at 3,000 rpm. The pH and EC of the supernatant were measured. Subsequently the

<sup>1</sup> These chemicals were kindly provided by AKZO-Nobel.

**Table 2** Soil characteristics

Origin/name	Country	Soil classification	0.01 M CaCl <sub>2</sub>			Oxalate <sup>a</sup>			DTPA <sup>h</sup>							
			pH- CaCl <sub>2</sub>	SOC <sup>b</sup> (g kg <sup>-1</sup> )	Clay content <sup>c</sup> (%)	CaCO <sub>3</sub> <sup>d</sup> (%)	CEC <sup>e</sup> (cmol eq kg <sup>-1</sup> )	DOC <sup>e</sup> (mg kg <sup>-1</sup> )	Reactive Fe (g kg <sup>-1</sup> )	Reactive Al (g kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Co (mg kg <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )
Bologna	Italy	Entisol	7.9	8.7	23	14	17.2	53	1.64	0.50	18.6	8.63	2.83	0.60	0.03	0.73
Xeraco top	Spain	Entisol	7.8	43.7	10	42	33.1	234	1.57	0.79	82.2	3.77	1.13	7.13	0.00	0.50
Xeraco lower	Spain	Entisol	7.8	13.7	36	15	30.3	78	0.86	1.62	10.5	5.33	3.03	6.70	0.00	0.20
Santomera	Spain	Entisol	8.0	5.4	26	52	10.3	30	0.05	0.19	3.5	4.57	4.13	0.90	0.00	0.20
Nadec	Saudi Arabia	Aridisol	8.1	8.7	7	14	5.5	93	0.22	0.29	2.1	5.67	0.13	0.47	0.00	0.13
Hofuf	Saudi Arabia	Aridisol	7.9	7.1	4	6	3.5	53	0.26	0.13	6.7	3.80	2.30	5.03	0.00	0.10
Droevendaal	The Netherlands	Spodosol	6.5	15.2	4	0	3.3	54	1.76	1.50	60.0	1.50	1.07	1.77	0.00	0.13
Herveld	The Netherlands	Spodosol	7.2	15.2	26	3	24.0	139	2.29	0.75	30.5	21.53	5.30	10.77	0.00	0.80

<sup>a</sup> Schwertmann (1964)<sup>b</sup> Walinga et al. (1992)<sup>c</sup> Houba et al. (1997)<sup>d</sup> ISO 10693, Soil Quality—Determination of carbonate content, volumetric method<sup>e</sup> Houba and Novozamsky (1998)<sup>f</sup> ISO/DIS 10390 Soil Quality—Determination of pH<sup>g</sup> ISO/DIS 11260 Soil Quality—Determination of cation exchange capacity and base saturation—method using barium chloride solution<sup>h</sup> Lindsay and Norvell (1978) and Quevauvillier et al. (1996)

supernatant was filtered through a 0.45- $\mu\text{m}$  cellulose acetate micro pore filter (Schleicher & Schuell, ref no: 10462650). The filtrate was further analysed.

Fe, Ca and Mg concentrations were measured by ICP-AES (Varian, Vista Pro); Cu, Al, Mn, Zn, Ni and Co concentrations were measured by ICP-MS (Perkin Elmer, ELAN 6000). The samples were acidified with nitric acid before measurement.

FeEDDHA isomers were separated through high-performance liquid chromatography (HPLC). HPLC was performed on a Waters HPLC system consisting of a Waters 600E Multisolute Delivery System, an Alltech online degasser, a Waters 717plus Autosampler, a Waters 2487 Dual Wavelength Absorbance Detector and Millennium32 software. A Waters Spherisorb ODS2 cartridge,  $d_p = 5 \mu\text{m}$ ,  $250 \times 4.6 \text{ mm}^2$  with guard column was used. The injection volume was  $20 \mu\text{l}$  and the flow rate was  $1 \text{ ml min}^{-1}$ . Detection was done at  $277 \text{ nm}$ . The mobile phase was prepared by mixing 915 ml of a

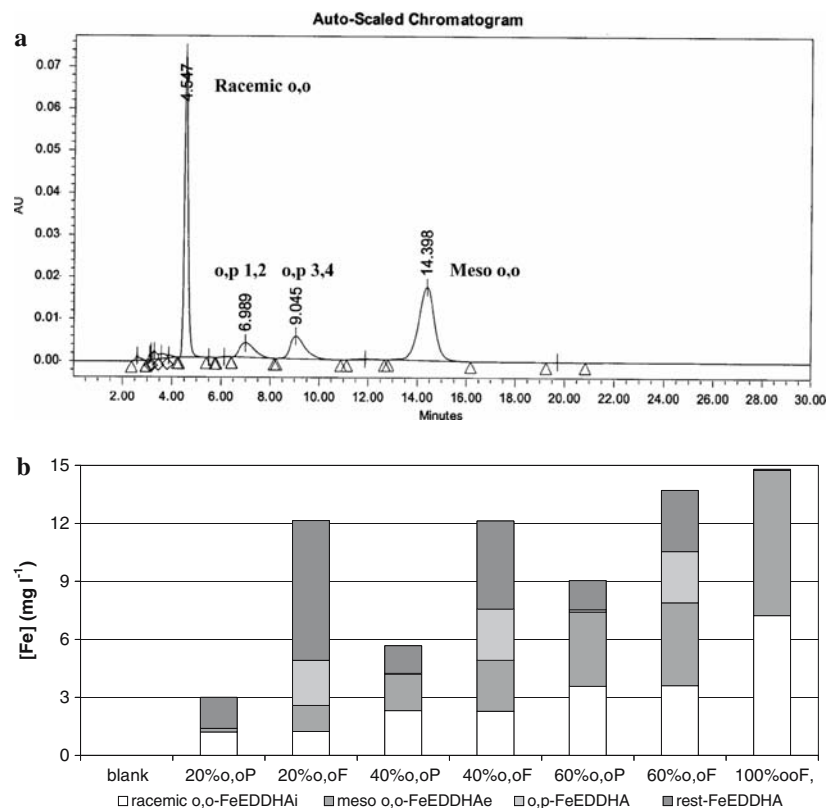
filtered formate buffer (0.015 M sodium formate adjusted to  $\text{pH} = 3.0$  with HCl) with 85 ml of acetonitrile. The concentrations of Fe chelated by racemic *o,o*-EDDHA, meso *o,o*-EDDHA and *o,p*-EDDHA were determined using an external calibration method. The Fe concentration chelated by rest-EDDHA was calculated by subtracting the Fe concentrations chelated by the other three isomers and the Fe concentration in the blank from the total Fe concentration as measured by ICP-AES.

## Results and discussion

### Experimental solutions

Figure 2a depicts the HPLC chromatogram of the 60%*o,o*F experimental solution. It illustrates that the peaks were clearly separated. The two *o, p*-EDDHA peaks were not calibrated separately, but combined. In chromatograms of soil interaction samples a drift in elution time

**Fig. 2** (a) The HPLC chromatogram for the 60%*o,o*F experimental solution. *o,p*-FeEDDHA elutes in two sets of two enantiomers *o,p*1,2 and *o,p*3,4. (b) The isomeric composition of the experimental solutions before interaction with soil. The concentrations of the isomers are expressed in terms of Fe concentration chelated by a particular isomer. All solutions had a 0.01 M  $\text{CaCl}_2$  background, pH in between 6 and 7



was observed but the peaks remained readily identifiable. Peaks resulting from DOC did not interfere disturbingly for integrating the FeEDDHA isomer peaks. From this and similar chromatograms of the different experimental solutions their isomeric composition was determined.

In Fig. 2b the Fe concentrations of the experimental solutions are presented, specified per FeEDDHA isomer. All experimental solutions with the F-suffix were expected to have Fe concentrations of  $15 \text{ mg l}^{-1} \text{ Fe}$ . A deviation was observed, increasing with decreasing *o,o*-EDDHA content. This observation might be explained from the following three factors: First, the Fe addition was based on a 1:1 stoichiometry between Fe and ethylene diamine. However, polycondensates may contain more than one ethylene diamine group, but may not be able to bind equally more Fe. Solutions containing more polycondensates will therefore have an over-all stoichiometric ratio of ethylene diamine to Fe, further from 1. Secondly, the relatively large Fe chelating polycondensates might be more susceptible to sorption to iron (hydr)oxides formed from excess Fe. The iron (hydr)oxides and adsorbed complexes are removed from the experimental solutions through filtration. Thirdly, the addition of  $\text{CaCl}_2$  might lead to precipitation and competition effects. Where *o,o*- and *o,p*-FeEDDHA concentrations are hardly affected by Ca, rest-FeEDDHA concentrations might be.

In the experimental solutions with the P-suffix, all *o,o*-EDDHA was expected to chelate Fe, due to its high complexation constants (Table 1). The compositions in Fig. 2b however show, that not all meso *o,o*-EDDHA is chelating Fe and a significant amount of Fe is being chelated by rest-EDDHA. This is most evident in the 20%*o,o*P solution. Since *p,p*-EDDHA binds Fe much less strongly than meso *o,o*-EDDHA, polycondensates must be responsible for chelating Fe. This demonstrates that there are polycondensates in commercial EDDHA formulations that form iron complexes of higher chemical stability than meso *o,o*-FeEDDHA. Hernandez-Apaolaza et al. (2006) recently found that rest-FeEDDHA complexes remain in solution over a smaller pH range than *o,o*-FeEDDHA and

concluded from that rest-FeEDDHA complexes are less stable. The findings from this study contradict this conclusion. Possibly, these polycondensates have racemic *o,o*-EDDHA resembling segments enabling them to bind Fe this strong. The higher chemical stability of such complexes compared to meso *o,o*-FeEDDHA does not necessarily imply a higher stability in solution in the presence of reactive surfaces. The stability of the various EDDHA isomers in solution upon interaction with soil will be discussed further on.

### Soil–EDDHA interaction

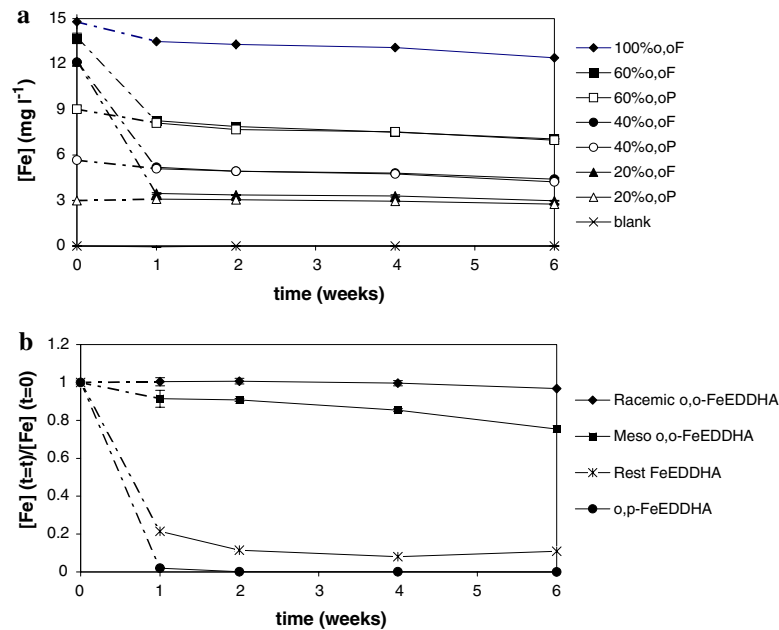
The results from the interaction experiment are presented and discussed in the following four sections: (1) Fe and FeEDDHA isomer concentrations over time, (2) Reactive surfaces, dealing with the relations between FeEDDHA isomer concentrations and the contents of reactive surface compounds in the soil, (3) Degree of chelation, dealing with the differences in Fe and FeEDDHA isomer concentrations between F- and P-treatments, and (4) Competing cations, dealing with the effect of EDDHA treatments on the concentrations of competing cations.

#### *Fe and FeEDDHA isomer concentrations over time*

*Fe.* The observed changes in Fe concentration over time for the different treatments were similar for all soils. The effects of treatment are illustrated for the Bologna soil in Fig. 3a. (Results for the Santomera soil, the Nadec soil and the Hofuf soil are presented as Supporting Information.) The measured Fe concentrations can be fully attributed to the addition of EDDHA, because the Fe concentration in the blank-treatment was below the determination limit of the ICP-AES ( $11 \text{ } \mu\text{g l}^{-1} \text{ Fe}$ ).

The Fe concentrations in the F-treatments decreased strongly within the first week and were relatively constant afterwards, whereas the Fe concentrations in the P-treatments were relatively constant from the beginning onward. After one week, the differences in Fe concentration between the P- and F-treatment of the same *o,o*-EDDHA content had become small relative





**Fig. 3** (a) The Fe concentrations after interaction with the Bologna soil over time for the different treatments. Concentrations at  $t = 0$  are prior to soil interaction. The lines in between the initial concentration and the concentration after 1 week are dashed, because the data do not

to the differences between the treatments with different *o,o*-EDDHA contents. Hence, the *o,o*-EDDHA content of the experimental solution largely determines how much Fe remains in solution during interaction.

The initial decrease in Fe concentration in the F-treatments is caused by EDDHA isomers, which form Fe complexes in plain solutions, but either adsorb, precipitate or have Fe displaced by a competing cation when interacting with soil. Cantera et al. (2002) and Garcia-Mina et al. (2003) found a similar strong decrease in Fe concentration as observed in the F-treatments for a number of FeEDDHA products, already within the first day.

Except for in two sandy soils (Hofuf and Droevendaal), the EDDHA isomers that do not initially chelate Fe in the P-treatments, do not establish a net increase in Fe concentration upon interaction with soils. To determine whether Fe is in fact chelated by the same EDDHA isomers before and after soil interaction requires HPLC analyses.

**FeEDDHA isomers.** The overall Fe concentration is the resultant of the contributions of the

show how quickly the decrease in concentration took place within the first week. Error bars indicate the standard deviation. (b) Fractions of the FeEDDHA isomers remaining in solution over time for the 60%*o,o*F treatment with the Bologna soil. Error bars indicate the standard deviation

different FeEDDHA isomers. In Fig. 3b the remaining fractions of these different FeEDDHA isomers in solution ( $[\text{FeEDDHA}]_{t=t}/[\text{FeEDDHA}]_{t=0}$ ) are plotted over time for the 60%*o,o*F treatment upon interaction with the Bologna soil (Results for the Santomera soil, the Nadec soil and the Hofuf soil are presented as Supporting Information.) After one week, *o,p*-FeEDDHA had disappeared from solution almost completely and the rest-FeEDDHA concentration had strongly decreased (a remaining fraction of 0.21). Both *o,o*-FeEDDHA isomers remained in solution to a much larger extent. In accordance with other adsorption and soil interaction studies, the racemic *o,o*-FeEDDHA concentration is most constant, hardly displaying any decrease (Alvarez-Fernandez et al. 2002; Cantera et al. 2002; Hernandez-Apaolaza and Lucena 2001). The meso *o,o*-FeEDDHA concentration showed some initial decrease in concentration. From one to six weeks of soil interaction, the isomer concentrations remained largely constant, except for meso *o,o*-FeEDDHA. Its remaining fraction declined from 0.91 to 0.75. Similar observations were found for the other soils.

After 6 weeks of interaction with the different soils in the 60%*o,o*F treatment, the remaining fractions of the FeEDDHA isomers ranged from 0.85 to 1.04 for racemic *o,o*-FeEDDHA, from 0.58 to 0.94 for meso *o,o*-FeEDDHA, from 0.11 to 0.42 for rest-FeEDDHA and from 0 to 0.16 for *o,p* FeEDDHA.

For all F-treatments, similar trends in remaining fractions were found. The remaining fractions can be ranked as follows for all soils: racemic *o,o*-FeEDDHA > meso *o,o*-FeEDDHA > rest-FeEDDHA > *o,p*-FeEDDHA. It should be added that rest-EDDHA consists of a variety of compounds, some of which are able to maintain Fe in solution better than *o,p*-EDDHA, while others are not. The composition of the rest-EDDHA fraction differs between treatments with different *o,o*-content. The average chain length of the polycondensates increases with increasing rest-EDDHA fraction in the experimental solutions. This complicates the comparison of the rest-FeEDDHA concentration between treatments with different *o,o*-contents.

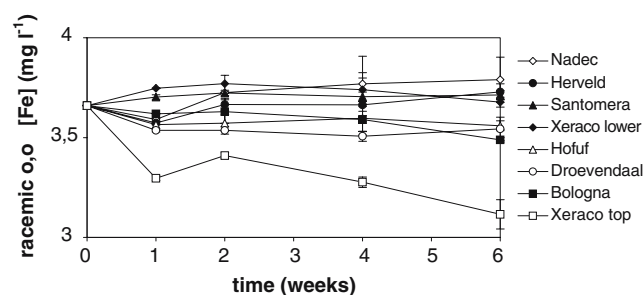
In the P-treatments an increase in meso *o,o*-FeEDDHA concentration and a decrease in rest-FeEDDHA concentration were found, compared to the experimental solutions. The increase in meso *o,o*-FeEDDHA was largest in the 20%*o,o*P-treatment, amounting 0.45–1.00 mg l<sup>-1</sup> Fe for the different soils. Apparently the stability of the meso *o,o*-FeEDDHA complex is lower, but its ability to keep Fe in solution during interaction with soil is larger. Whether meso *o,o*-EDDHA chelated native Fe or Fe initially chelated by rest-FeEDDHA remains unclear.

### Reactive surfaces

The chemical properties and the sorption behaviour of the FeEDDHA isomers differ. Therefore it was expected that different soil constituents might play a dominant role in their sorption behaviour. Relations were studied between the contents of reactive soil constituents and FeEDDHA isomer concentrations.

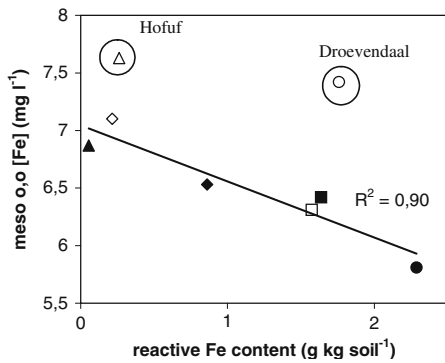
**Racemic *o,o* FeEDDHA.** In Fig. 4 the racemic *o,o*-FeEDDHA concentrations are shown as a function of time for all soils. The concentrations hardly decrease over time. This complicates the interpretation of which soil characteristics affect the racemic *o,o*-FeEDDHA concentration. The soil that stands out, in that relatively much of the racemic *o,o*-FeEDDHA isomer is removed from solution is the Xeraco top soil (Fig. 4). The distinctive feature of this soil is its relatively high organic matter content (Table 2). Alvarez-Fernandez et al. (1997, 2002) and Hernandez-Apaolaza and Lucena (2001) pointed out that organic matter might be an important reactive soil constituent with respect to *o,o*-FeEDDHA sorption. However, the overall sorption of the racemic *o,o*-FeEDDHA isomer remains small; approximately 15% after 6 weeks at an organic matter content of 9%.

At pH 7–8 organic matter is substantially negatively charged. As a consequence there is an electrostatic repulsion between the deprotonated carboxylate groups of soil organic matter and the negatively charged racemic *o,o*-FeEDDHA complexes. Adsorption to organic matter might be established through bridging mechanisms with



**Fig. 4** Fe concentrations chelated by racemic *o,o*-EDDHA, for the different soils over time in the 60%*o,o*F treatment. Concentrations at  $t = 0$  are prior to soil

interaction. Error bars indicate the standard deviation. Open symbols represent sandy soils, closed symbols represent clay soils



**Fig. 5** Fe concentration chelated by meso *o,o*-EDDHA after 4 weeks in all soils for the 100%*o,o*F treatment versus reactive iron in the soils. Standard deviations fall within the data points. Open symbols represent sandy soils, closed symbols represent clay soils. The two data points indicated with a circle were not included for calculating the linear relation

di- or trivalent cations. Such mechanisms have been reported for linking functional groups within and between DOC molecules. Van der Waals interaction between the uncharged areas of organic matter and the aromatic rings of the complex might also play a role (Stevenson 1994).

*Meso o,o-FeEDDHA.* The extent to which meso *o,o*-FeEDDHA is removed from solution appears to be related to the amount of reactive Fe (oxalate extractable Fe) present in the

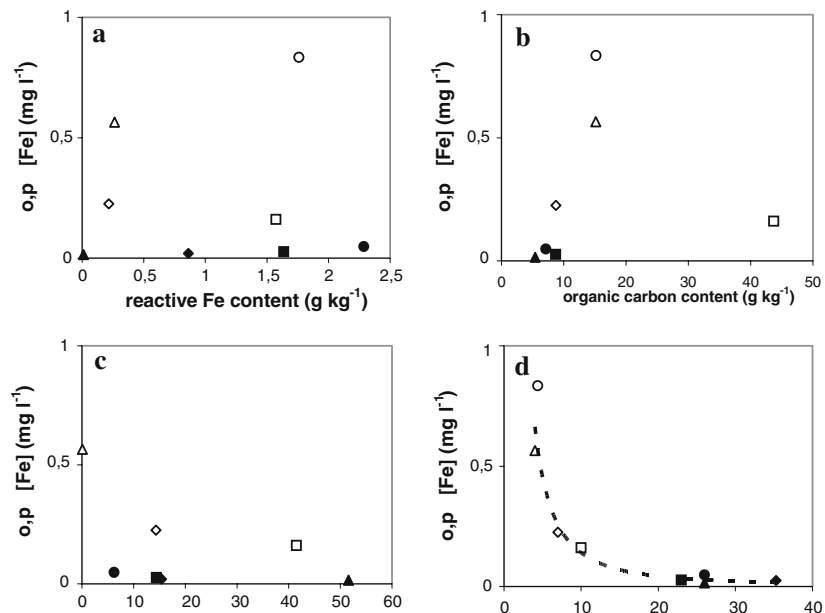
soil (Fig. 5). This suggests adsorption of meso *o,o*-FeEDDHA to reactive iron (hydr)oxide. The data points for the Hofuf and the Droevendaal soil do not match the trend line. An explanation for this might be sought in the soil texture, since these two deviating soils have the lowest clay content.

The potential relevance of iron (hydr)oxide with respect to FeEDDHA sorption in soils has already been outlined by Alvarez-Fernandez et al. (1997, 2002) and Hernandez-Apaolaza and Lucena (2001). Iron (hydr)oxides have positively charged surface groups and a net positively charged surface at pH values below the pristine point of zero charge (7.9–8.2 for HFO (Dzombak and Morel 1990); 9.2–9.3 for goethite (Filius et al. 1997)). The electrostatic attraction between the negatively charged meso *o,o*-FeEDDHA complex and a positively charged iron (hydr)oxide surface will enhance sorption.

Why adsorption of meso *o,o*-FeEDDHA to iron (hydr)oxides is stronger than of racemic *o,o* FeEDDHA is not clear. Both complexes have the same charge. More adsorption of the isomer with the lower stability constant could indicate the breaking of chemical bonds to establish sorption (Hernandez-Apaolaza and Lucena 2001).

*o,p-FeEDDHA.* In Fig. 6, the *o,p*-FeEDDHA concentrations after one week of interaction are plotted against the contents of different reactive

**Fig. 6** Fe concentration chelated by *o,p*-EDDHA as a function of the reactive surface contents of the different soils (a) reactive iron; (b) organic carbon; (c) calcium carbonate; (d) clay. Open symbols represent sandy soils, closed symbols represent clay soils



constituents of the respective soils. No relations were found with reactive Fe (Fig. 6a), soil organic carbon (Fig. 6b) and  $\text{CaCO}_3$  (Fig. 6c). However, a relation between *o,p*-FeEDDHA concentration and clay content is evident: an increase in clay content leads to a decrease in *o,p*-FeEDDHA concentration in solution (Fig. 6d). An effect of soil texture on Fe concentration upon interaction of commercial FeEDDHA formulations with soil has been reported by Gil-Ortiz and Bautista-Carrascosa (2004). *O,p*-FeEDDHA is always present in commercial formulations (Garcia-Marco et al. 2006) and its tendency to adsorb to clay minerals can explain Gil-Ortiz and Bautista-Carrascosa's observation.

The adsorption of *o,p*-FeEDDHA to clay minerals is counter-intuitive because both have an overall negative charge and the two repel each other. A bridging mechanism with Ca has been suggested to bind FeEDDHA complexes to the clay surface (Wallace and Wallace 1992). A similar mechanism has been demonstrated for the adsorption of DOC to clay (Muneer and Oades 1989). The sixth position on the coordination complex of Fe chelated by *o,p*-EDDHA is not occupied by a phenolic hydroxyl group of the chelating agent, but, depending on pH, by either a separate hydroxide anion or a water molecule (Yunta et al. 2003a) (Fig. 1c). This hydroxide anion or water molecule may play a central role in the adsorption behaviour, because it is displaced relatively easily and may hence facilitate binding to a negatively charged surface group on a clay-edge, or it may act as a bridge between complex and surface.

Another explanation for the enhanced removal of *o,p*-FeEDDHA in clay soils could be that these soils generally contain more competing cations like Cu that might replace Fe from the *o,p*-FeEDDHA complex. Competition effects will be further discussed in a following section.

In this study, the soil with the highest organic matter content, Xeraco top soil, sorbed most racemic *o,o*-FeEDDHA, but did preserve a remaining *o,p*-FeEDDHA fraction of 0.05 over 6 weeks, while in clay soils all *o,p*-FeEDDHA was removed from solution. There are several possible explanations for this. First, organic matter may be less specific in its affinity for the

different FeEDDHA isomers, because sorption may be partly based on the interaction between the non-polar sections of both soil organic matter molecules and the EDDHA complexes. Secondly, organic matter is a source of DOC. DOC might act as a competitor for adsorption sites on clay minerals, which are shown to provide the key adsorption surface for *o,p*-FeEDDHA.

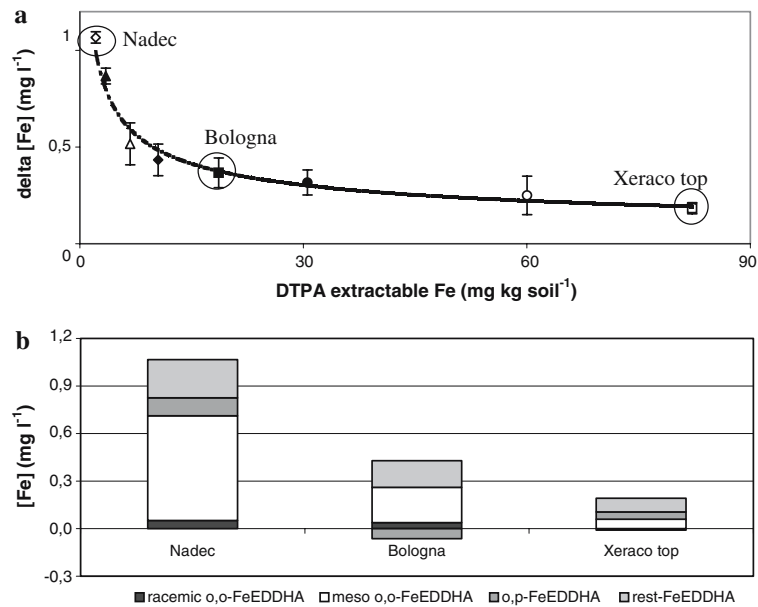
In literature the role of organic matter with respect to sorption of FeEDDHA seems to be overestimated. Alvarez-Fernandez et al. (1997, 2002), and Hernandez-Apaolaza and Lucena (2001) have shown substantial degrees of sorption for peat over a wide pH range; this however is not representative for the soils where FeEDDHA products are actually applied, which have a much lower organic matter content.

*Rest-FeEDDHA.* Due to the varying composition of rest-FeEDDHA fraction between the experimental solutions, no unambiguous comments on the adsorption behaviour of rest-FeEDDHA are possible. For all treatments containing rest-FeEDDHA, the largest fraction remained in solution for the soils with least clay: Droveendaal and Hofuf (see Supporting Information), again supporting Gil-Ortiz's and Bautista-Carrascosa's observations with respect to soil texture (2004).

#### *Degree of chelation*

Figure 3a shows that differences in Fe concentration were found between the P- and F-treatments of the same isomeric composition. This was most evident for the 20%*o,o* treatments. For all isomeric compositions, the Fe concentrations in the P-treatments were lower. The differences in Fe concentration varied per soil and after one week they ranged from 0.18 to 1.07 mg l<sup>-1</sup> Fe for Xeraco top soil and Nadece respectively. For Nadece the difference of 1.07 mg l<sup>-1</sup> Fe amounted to little over a third of the Fe concentration of the P-treatment after interaction (2.98 mg l<sup>-1</sup> Fe). The most obvious explanation for the difference in Fe concentration is the following: in the P-treatments EDDHA molecules are not able to pick up Fe, either from the soil or from adsorbed FeEDDHA complexes, to such an extent that the same Fe concentration is reached as in the

**Fig. 7** (a) The difference in Fe concentration between the fully (F) and partly (P) chelating 20%*o,o* treatments after 1 week, versus the DTPA-extractable Fe in the soils. Open symbols represent sandy soils, closed symbols represent clay soils. Error bars indicate the standard deviation. (b) Difference in Fe concentration between the fully (F) and partly (P) chelating 20%*o,o* treatments after 1 week specified per isomer



corresponding F-treatments. The difference in Fe concentration between the P- and F-treatments should then be a function of Fe availability. Figure 7a, in which the difference in Fe concentration is given as a function of the Fe availability parameter DTPA-extractable Fe (DTPA-Fe) confirms this relation: with increasing DTPA-Fe, the difference in measured Fe concentration between the P- and F-treatment becomes smaller. At low DTPA-Fe, the relative decrease is largest and the slope becomes gradually less steep with increasing DTPA-Fe.

The differences in Fe concentration have been examined in terms of isomers. For Nadec, Bologna and Xeraco top soil (indicated in Fig. 7a), these differences are specified in Fig. 7b. Generally, the largest contribution is from meso *o,o*-FeEDDHA, followed by rest-FeEDDHA. This large contribution of meso *o,o*-FeEDDHA is remarkable in view of its high stability constant (Table 1). The amount of DTPA-Fe suffices in all soils to have all meso *o,o*-EDDHA in the P-treatment chelate Fe. Apparently a lasting kinetic effect is limiting the ability of meso *o,o*-EDDHA to chelate Fe from the soil. With commercial FeEDDHA formulations this is not a direct concern, because all *o,o*-EDDHA tends to be chelated to Fe. When, however, Fe has been delivered to the plant and EDDHA returns into

solution to chelate a new Fe-ion (the shuttle effect), it becomes of relevance.

#### Competing cations

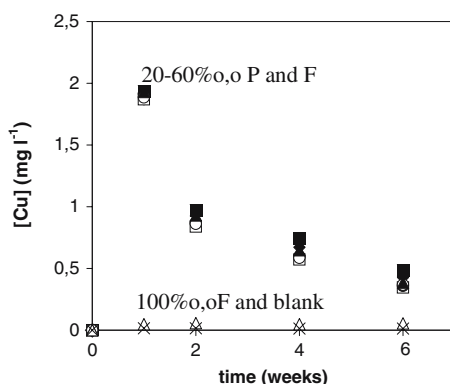
The effects of EDDHA-treatments on Cu and Al concentrations are discussed here in more detail. Findings on the other cations are briefly commented on afterwards.

*Cu.* In several studies, elevated Cu concentrations were found due to the addition of commercial FeEDDHA formulations. Addition of an *o,o*-FeEDDHA standard solution however, hardly led to an increase in Cu concentration (Alvarez-Fernandez et al. 1997, 2002). Therefore one of the other isomers must be responsible for the increase in Cu concentration. In Fig. 8 the Cu concentrations over time are shown for the different treatments of the Santomera soil. Hardly any increase in Cu concentration for the 100%*o,o*F treatment compared to the blank-treatment (up to 0.06 mg l<sup>-1</sup> Cu), and increases up to 1.90 mg l<sup>-1</sup> Cu after 1 week for the other treatments. The increases are approximately equally large for the treatments other than 100%*o,o*F and the blank. The data shown in Fig. 8 have three important implications.

First, it is irrelevant if the EDDHA isomer responsible for Cu solubilization initially chelates

Fe or not; there is hardly any difference in Cu concentration between P- and F-treatments after interaction with the soil. Because Cu concentrations are about equal for P- and F-treatments, there is no lag time and the displacement of Fe from the complex can not be the time-determining step in Cu solubilization.

Secondly, *o,p*-EDDHA determines the extent of Cu solubilization. The ability of *o,p*-EDDHA to dissolve Cu from the soil has been reported by Garcia-Marco et al. (2006). For the six treatments in this study containing *o,p*-EDDHA (20%*o,o*; 40%*o,o* and 60%*o,o*; both F and P), *o,p*-EDDHA was the only isomer of which the initial concentrations were approximately the same (Fig. 2b). The Cu concentrations after interaction with soil were also approximately the same for these treatments, for all four interaction times individually (Fig. 8). A similar effect of treatment was found for all eight soils. The Cu concentrations varied between the soils ranging from 0.06 to 1.93 mg l<sup>-1</sup> Cu after one week, amongst others due to differences in Cu availability. The treatments containing *o,p*-EDDHA gave equal Cu concentrations, regardless if DTPA-extractable Cu exceeded the amount of *o,p*-EDDHA added (in the case of Santomera and Herveld; Cu-DTPA amounted 4.13 and 5.30 mg kg<sup>-1</sup> Cu respectively), or not (in the case of the remaining soils). This advocates that Cu is not just preferentially, but exclusively dissolved through complexation with *o,p*-EDDHA.

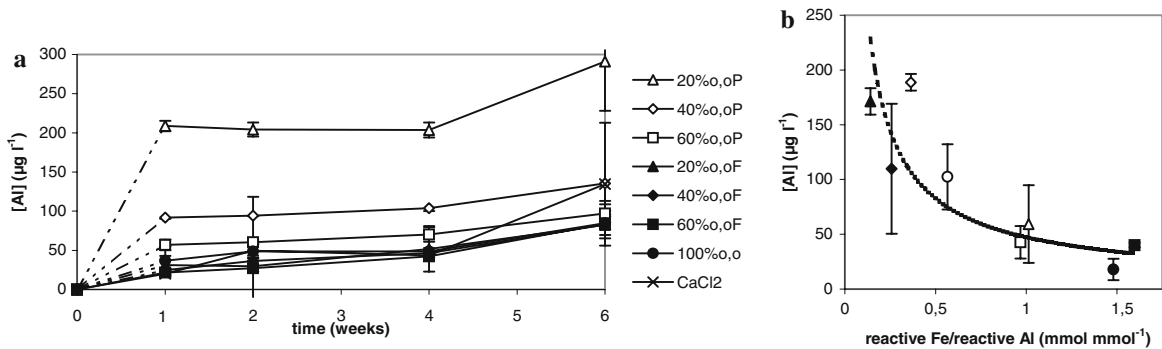


**Fig. 8** The Cu concentration after interaction with the Santomera soil over time for the different FeEDDHA treatments. Concentrations at  $t = 0$  are prior to soil interaction

Thirdly, the Cu concentrations decrease over time for all treatments containing *o,p*-EDDHA, in all soils except for Hofuf. Adsorption of *o,p*-CuEDDHA seems the most likely cause, because no other trace metal concentrations increase and competition with Ca and Mg is unlikely. The fact that Hofuf has low reactive surface contents, and in particular the lowest clay content, supports the adsorption hypothesis, but degradation can not be excluded. However, ion adsorption equilibria are generally reached within a few days in the soil and in between week 4 and 6 Cu concentrations still decrease with 25–70% for the soils other than Hofuf and Droevendaal. Parallel occurring processes like Cu complexation/solubilization, and slow adsorption kinetics may extend the time required to reach equilibrium. Further research is needed to resolve the adsorption and exchange mechanism of metal-EDDHA complexes.

Al. Al has not yet been reported as a competing cation, potentially affecting the performance of FeEDDHA products. The data in Fig. 9a show the Al concentrations over time for all treatments with the Nadec soil. The Al concentrations of the F-treatments do not differ from those in the blank treatment. The Al concentrations in the P-treatments are however higher, increasing with increasing concentration of EDDHA isomers not chelating Fe. Contrary to other competing cations, the Al concentrations are constant over time, at least up until and including 4 weeks of interaction time. In week 6 an increase in Al-concentration was observed. Similar results were observed for the other soils. These results imply that Al does not displace Fe from FeEDDHA complexes. However, when EDDHA, not chelated to Fe, is brought into contact with soil, it can form AlEDDHA complexes. In turn, Fe does not displace Al from AlEDDHA complexes.

The observed trend in Al concentrations for the different treatments excludes racemic *o,o*-EDDHA and *o,p*-EDDHA as Al complexing isomers. Further examination of the data shows that meso *o,o*-EDDHA is the dominant EDDHA isomer chelating Al, for two reasons. First, when considering the 20%*o,o* P- and F-treatments for all soils, the differences in Al concentration correlate better with the differences in meso *o,o*-FeEDDHA concentration (–0.95) than with



**Fig. 9** (a) The Al concentration after interaction with the Nadec soil over time for the different FeEDDHA treatments. Concentrations at  $t = 0$  are prior to soil interaction. The lines in between the concentration at  $t = 0$  and the concentration after 1 week are dashed, because the data do not show how quickly the increase in Al concentration took place within the first week. Error

bars indicate the standard deviation. (b) Al concentration in the 20%*o,o*P treatment corrected for the Al concentration in the blank treatment for all soils, as a function of the ratio of reactive Fe and reactive Al. Open symbols represent sandy soils, closed symbols represent clay soils. Error bars indicate the standard deviation

the differences in rest FeEDDHA concentration ( $-0.57$ ). Secondly, the differences in rest-FeEDDHA concentration between the 20%*o,o* P- and F-treatment are not for all soils large enough to account for the increase in Al concentration, whereas the differences in meso *o,o*-FeEDDHA are. Complexation of Al is to a large extent able to explain the differences in Fe concentration observed in the *degree of chelation* section.

In this study it was found that meso *o,o*-EDDHA is able to dissolve both Fe and Al from the soil. As a consequence Fe and Al compete for meso *o,o*-EDDHA. Because neither drives off the other from the complex within the time span examined, a relation between relative availabilities and concentrations is expected. Relative availability can be expressed as a ratio of two availability parameters. The oxalate extractable fractions of Al and Fe were used as availability parameters. The expected relation was found and is displayed in Fig. 9b: an increased relative availability of Al results in higher Al concentrations in solution. Hence, in soil application Al complexation may cause the alleged shuttle effect to become partly impeded. The impeding effect will be stronger with increasing relative availability of Al.

*Other cations.* The remaining trace metals measured (Zn, Mn, Ni and Co) were generally present in solution in lower concentrations than

Al and Cu. When elevated concentrations were found as a result of EDDHA-treatments, this effect diminished over time, like in the case of Cu, except in the Hofuf soil for Ni and Co. Elevated Zn concentrations were only found in the Hofuf soil (up to  $160 \mu\text{g l}^{-1}$  Zn after 1 week) and elevated Mn concentration were found in four soils (up to  $80 \mu\text{g l}^{-1}$  Mn after 1 week). Elevated Co and Ni concentrations were found in all soils (up to  $52 \mu\text{g l}^{-1}$  Co and  $150 \mu\text{g l}^{-1}$  Ni, respectively). Co and Ni were generally present in higher concentrations than Zn and Mn as a result of the EDDHA treatments. Even if only to a small extent ( $5.3 \mu\text{g l}^{-1}$  Co), unlike Al, Ni, Zn and Mn, Co was able to displace Fe from *o,o*-FeEDDHA in the 100%*o,o*F treatment.

## Conclusion

The results from this study have shown that regardless of soil properties, the *o,o*-fraction of the FeEDDHA treatment largely determines how much Fe remains in solution upon interaction with soil. The fractions of the EDDHA isomers have been relatively constant from week 1–6 of the interaction experiment. Although rest-EDDHA also consists of compounds that chelate Fe more strongly than meso *o,o*-EDDHA, the latter is on average better capable of keeping Fe in solution upon interaction with soil. The principal adsorption

surface in the soil differs per FeEDDHA isomer. For racemic *o,o*-FeEDDHA it is organic matter, for meso *o,o*-FeEDDHA it is iron (hydr)oxide and for *o,p*-FeEDDHA clay minerals. For rest-FeEDDHA no single surface has been identified, but the clay fraction seems to be of relevance.

The differences in Fe concentration after interaction with soil, between corresponding Partly and Fully chelating treatments are dependent on the iron availability of the soil. These differences can be largely attributed to differences in meso-FeEDDHA and to a lesser extent rest-FeEDDHA concentrations. When meso *o,o*-EDDHA that is not chelated to Fe is brought in contact with soil, it will not exclusively form complexes with Fe but also with Al. The fact that meso *o,o*-EDDHA can chelate Al from the soil, while Fe does not displace Al from the complex, has implications for the effectiveness of a possible shuttle effect. Concentrations of other chelated trace elements (Cu, Zn, Mn, Ni and Co) generally decrease during the experiment. In terms of concentration, Cu is by far the most important competing cation. Cu is practically exclusively chelated by *o,p*-EDDHA, regardless if the *o,p*-EDDHA was initially chelating Fe or not.

The tendency of *o,p*-FeEDDHA and rest-FeEDDHA to be removed from solution and the tendency of *o,p*-FeEDDHA to exchange Fe for Cu, make these isomers less effective as iron fertilizer in soil application, in particular on clay soils. The agronomic value of the different FeEDDHA isomers, however, depends on more factors than their ability to preserve Fe in solution. Additional studies involving plants are needed to comment on this.

**Acknowledgements** The authors wish to express their sincere appreciation and gratitude to the following people: AKZO Nobel for financing this project which was initiated by P. Weijters and M. Bugter, P. Nobels for his help with the ICP-measurements, T. Scheperman for the synthesis of the EDDHA stock solutions and G. Vink for practical support.

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