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Influence of Chelating Ligands on Bioavailability and Mobility of Iron in Plant Growth Media and Their Effect on Radish Growth

H. Hasegawa*; M. Azizur Rahman; K. Saitou; M. Kobayashi; C. Okumura

Graduate School of Natural Science and Technology, Kanazawa University,
Kakuma, Kanazawa 920-1192, Japan

*Corresponding author

E-mail: hhiroshi@t.kanazawa-u.ac.jp

Tel/Fax: 81-76-234-4792

Abstract:

In this study, the effects of chelating ligands on iron movement in growth Medium, iron bioavailability, and growth of radish sprouts (*Raphanus sativus*) were investigated. Iron is an important nutrient for plant growth, yet the insoluble state of iron hydroxides in alkaline conditions decreases its bioavailability. Iron chelates increase iron uptake and have been used in agriculture to correct iron chlorosis. While previous studies have reported the effects of chelating ligands on iron solubility and bioavailability, the present study elucidates the pattern of iron movement by chelating ligands in plant growth Medium. The apparent mobility of iron in growth Medium was calculated using a '4-box' model. Ethylenediaminedisuccinic acid (EDDS) and hydroxy-iminodisuccinic acid (HIDS) produced the highest apparent mobility of iron from the bottom layer of the medium (initially 10^{-4} M Fe(III)) to the upper layer (no iron), followed by glutamicdiacetic acid (GLDA), ethylenediaminetetraacetic acid (EDTA), methylglycinediacetic acid (MGDA), and iminodisuccinic acid (IDS). Iron movement in the growth Medium was influenced by the chelating ligand species, pH, and ligand exposure time. The iron uptake and growth of radish sprouts were related to the iron mobility produced by the chelating ligands. These results suggest that, in alkaline media, chelating ligands dissolve the hardly soluble iron hydroxide species, thus increasing iron mobility, iron uptake, and plant growth. HIDS, which is biodegradable, was one of the most effective ligands studied; therefore, this compound would be a good alternative to other environmentally persistent chelating ligands.

Keywords: Chelating ligands, HIDS, Iron, Radish sprouts (*Raphanus sativus*), Bioavailability.

60 Introduction

61 Iron is an essential micronutrient for plants (Boyer et al., 1988; Zancan et al., 2008) and
62 plays an important role in respiration, photosynthesis, DNA synthesis, nitrogen fixation,
63 hormone production, and many other cellular functions (Vert et al., 2002). Although abundant
64 in nature, Fe exists in alkaline soil as hardly soluble hydrated oxide states, including
65 $(\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O})$, Fe^{3+} , $\text{Fe}(\text{OH})_3$, and $\text{Fe}(\text{OH})^{2+}$ (Aston and Chester, 1973; Barry et al., 1994).
66 These Fe species are poorly absorbed by plant roots (Cohen et al., 1998; Guerinot and Yi,
67 1994) and cause defective growth of the plant (Robin et al., 2008; Yousfi et al., 2007).
68 Insoluble ferric hydroxide complexes are also known as Fe plaques. Formation of Fe plaques
69 in the rhizosphere results in a deficiency of Fe and other nutrients (including P, Cu, Mn, Zn, Pb,
70 and Cd) in the plants (Batty et al., 2000; Christensen and Sand-Jensen, 1998; Otte et al., 1989;
71 Ye et al., 1998; Ye et al., 2001; Zhang et al., 1998). Under such conditions, plants have two
72 distinct natural strategies to assimilate Fe from the environment. Grasses release
73 phytosiderophores, which are low-molecular-weight, high-affinity Fe(III)-chelate compounds
74 that solubilize ferric Fe in the rhizosphere and are recognized by specific membrane
75 transporters (Bienfait, 1988; Chaney, 1987; Romheld, 1987; Romheld and Marschner, 1986a,
76 b). Fe uptake in dicots and non-grass monocots is mediated by a plasma-membrane-bound
77 ferric reductase that transfers electrons from intracellular NADH (Buckhout et al., 1989) to
78 Fe(III)-chelates in the rhizosphere (Chaney et al., 1972). The ferrous ions released from the
79 chelates by this process are subsequently transported into the cytoplasm via a separate
80 transport protein (Fox et al., 1996; Kochian, 1991). In addition, some rhizospheric microbes
81 exude siderophores at the root-plaque interface. These siderophores solubilize ferric iron in the
82 rhizosphere and are recognized for uptake by specific membrane receptors, thus rendering the
83 iron bioavailable (Bienfait, 1988; Chaney, 1987; Romheld and Marschner, 1986a).

84 Research on the interaction between plants and chelating ligands started in the 1950s
85 with the goal of reducing deficiencies of the essential nutrients Fe, Mn, Cu, and Zn (Wenger et

al., 2005). Chelators increase the mobility of iron in alkaline media by dissolving the hardly soluble iron hydroxide species (Lucena, 2006; Lucena, 2003; Lucena et al., 1996; Lucena and Chaney, 2006; Tagliavini and Rombolà, 2001; Villen et al., 2007; Yona et al., 1982). Among all soil-applied Fe fertilizers, synthetic Fe(III) chelates are the most effective and commonly used. These compounds originate mainly from polyaminecarboxylic acids with phenolic groups such as ethylenediamine di(*o*-hydroxyphenylacetic) acid (EDDHA) and ethylenediamine di(2-hydroxy-4-methylphenylacetic) acid (EDDHMA) (Alvarez-Fernandez et al., 2005). Ethylenediaminetetraacetic acid (EDTA) has been a popular choice to achieve this purpose (Claudia and Rodríguez, 2003; Nowack and Sigg, 1997; Urrestarazu et al., 2008), but it does not dissolve easily in water or soil, it persists in the environment (Bucheli-Witschel and Thomas Egli, 2001; Nortemann, 1999; Villen et al., 2007), and it affects the material cycle of various elements. This, in combination with its high affinity for heavy metal complexation, results in an increased risk of leaching. EDTA also severely impairs plant growth, even at very low concentrations (Bucheli-Witschel and Thomas Egli, 2001). Therefore, EDTA use is prohibited in some European countries.

Biodegradable chelating ligands, such as ethylenediaminedisuccinic acid (EDDS) and hydroxyl-iminodisuccinic acid (HIDS), would be good alternatives to EDTA. In this study, we investigated the biodegradable chelating ligand hydroxyl-iminodisuccinate (HIDS). The physicochemical properties of EDDS, EDTA, and IDS have already been established by a number of researchers (Evangelou et al., 2007; Helena et al., 2003; Jaworska et al., 1999). However, HIDS is a new chelating ligand introduced by Nippon Shokubai Co. Ltd. It is classified as one of the safest and most biodegradable chelating ligands, with a biodegradation rate of about 22.4% within 48 h. HIDS traps and inactivates various metal ions, particularly Fe^{3+} and Cu^{2+} as well as Ca^{2+} and Mg^{2+} , over a wide range of pH values. In addition, HIDS is highly stable in harsh conditions and high temperatures (80°C) and highly soluble in aqueous alkaline solutions (Sokubai, 2009). HIDS forms water-soluble complexes with various metal

ions over a wide pH range. In particular, it shows superior performance in chelating Fe^{3+} ions in alkaline solutions (Sokubai, 2009). Because of its high degradation rate and high stability constant with Fe^{3+} , we investigated the effectiveness of HIDS on Fe bioavailability and mobility patterns in growth Medium. EDTA, EDDS, and IDS were also studied for comparison. The effects of both biodegradable and non-biodegradable chelating ligands on the mobility and bioavailability of iron in plant growth medium are discussed using a '4-box' model. This is the first report on Fe mobility due to chelating ligands in plant growth Medium.

Materials and Methods

Culture of radish sprouts

Murashige and Skoog (MS) culture medium (Murashige and Skoog, 1962) was used for radish sprout growth. The concentration of chelating ligands in the medium was 10^{-3} M. After adjusting to pH 10 using 0.1 M NaOH, the medium was sterilized by high-pressure sterilization in an autoclave (120°C, 30 min) and UV irradiation. Before the agar hardened, 4 mL of the medium (25 mm depth) was dispensed into a 14-mL sterilized polystyrene tube.

Radish seeds were collected from a local market and stored at 4°C until use in the experiment. The seeds were sterilized in a solution of 0.25% NaClO and 25 μM Tween20 for 2 minutes, and then rinsed 5 times with 5 mL of deionized water (EPW) using an E-pure system (Barnstead). Germinating seeds were planted in the agar medium and cultured for a week in a 20°C growth chamber with 180 μM photon $\text{m}^{-2} \text{s}^{-1}$ light intensity from cool white fluorescent lights on a 14:10 h light/dark schedule.

Extraction of extracellular iron fractions and chemical analysis

Intra- and extracellular iron fractions in the radish sprouts were determined by radiochemical measurements of ^{55}Fe . To determine intracellular iron concentrations, samples were successively rinsed with 5 mL of EPW, 5 mL of 0.047 M Ti(III)-citrate-EDTA solution,

and again with 5 mL of EPW. Samples used to determine total iron (corresponding to intra- and extracellular iron) were rinsed with 5 mL of EPW. Both types of samples, in which $^{55}\text{Fe(III)}$ was retained as a tracer, were directly added to 5 mL of liquid scintillation solution (3.0 g of 2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole per 500 mL toluene) in 20 mL vials. The radiochemical activity of $^{55}\text{Fe(III)}$ was measured using a liquid scintillation counter (LSC-6101, Aloka, Japan) in tritium mode. The concentration of Fe(III) was calculated from the Fe(III)/ $^{55}\text{Fe(III)}$ ratio in solutions.

Determination of Fe mobility

A 2-layered modified MS medium was used to measure Fe mobility. The bottom layer contained 10^{-4} M FeCl_3 with 370 MBq/l of ^{55}Fe , and the upper layer contained no FeCl_3 (Fig. 1). The MS agar medium was collected after 48, 96, and 144 h during the experiment to measure iron concentrations. The tubes were divided into 5 mm sections, and the agar was removed from each section and dried for 24 h in an electric oven. The iron content was measured by a 370 MBq/l radioactive tracer ^{55}Fe using a liquid scintillation counter.

Fe mobility in the nutrient medium was calculated from the transfer coefficient of iron movement using a 4-box model. The details of the model are described in the Results and Discussion.

Chemicals

A stock solution of Fe(III) was prepared by dissolving $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Nacalai Tesque, Kyoto) in 1 M HCl (TAMAPURE-AA-100, Tama Chemicals, Tokyo) and standardized using inductively coupled plasma atomic emission spectrometry (Optima 3300XL, Perkin-Elmer, USA). A stock solution of $^{55}\text{Fe(III)}$ was prepared by dissolving $^{55}\text{FeCl}_3$ (PerkinElmer Life & Analytical Sciences, specific activity; 370 MBq/l) in 1 M HCl (TAMAPURE-AA-100). The solutions were diluted to the desired concentration ratios of Fe(III)/ $^{55}\text{Fe(III)}$. Stock solutions of

EDTA, HIDS, IDS, MGDA, GLDA and EDDS were prepared by dissolving ethylenediamine-N,N,N',N'-tetraacetic acid (Dojindo Molecular Technologies, Japan), tetrasodium 3-hydroxy-2,2'-iminodisuccinate (Nippon Syokubai), tetrasodium iminodisuccinate (Bayer), methylglycine-N,N-diacetic acid (BASF), L-glutamate-N,N-diacetic acid, and ethylenediamine-N,N'-disuccinic acid (Chelest), respectively, in 0.1 M sodium hydroxide. The reagents were of analytical grade and used without further purification. All solutions were prepared with purified water (EPW) using an E-pure system (Barnstead).

Results

Iron movement in the growth medium

Radish sprouts were grown in 2-layered culture medium to investigate the effect of chelating ligands on Fe movement in the medium. The layers of the growth medium were distinguished by the initial concentration of Fe(III), which was 10^{-4} M in the bottom layer while the upper layer initially contained no Fe(III) (Fig. 1). A solution of 0.1 mM chelating ligand was added to the bottom layer of semisolid MS-agar culture medium. The medium in the test tubes was divided into 5 mm sections, and samples from each section were collected and analyzed for Fe after 48, 96, and 144 h. The presence of chelating ligands increased Fe movement from the Fe-rich bottom layer to the Fe-free upper layer of the Medium (Fig. 3).

To investigate the pattern of Fe movement, a Fe gradient was created across two layers of semisolid MS-agar growth medium in the presence of chelating ligand. Each of the two layers was further divided into two layers, and a '4-box' model was established (Fig. 1) to estimate the amount and pattern of Fe movement in the medium. The highest concentration of Fe was measured in box 3 (B_3), although the initial concentrations of Fe in B_3 and box 4 (B_4) were the same. The Fe adsorbed on the bottom surface of the test tubes, which was not desorbed by the addition of the chelating ligand, could explain this phenomenon. The Fe concentration in B_3 differed greatly from box 2 (B_2), where the initial Fe concentration was

zero.

Four-box model for the determination of Fe mobility

Fe mobility was calculated from the transfer coefficient of iron movement using a '4-box' model of the 2-layered growth medium. The transfer rate of total Fe between layers is related proportionally to the differences in dissolved Fe and inversely to the volume of growth medium in the corresponding layer. The '4-box' model is shown in [Figure 1](#). Using this system, the transfer coefficient of total Fe was calculated from the following equations:

$$Q_{t1} = \frac{1}{C_{d2} - C_{d1}} V_1 \frac{\Delta C_{t1}}{\Delta T_1} \dots\dots\dots(1a)$$

$$Q_{t2} = \frac{1}{C_{d3} - C_{d2}} V_2 \frac{\Delta C_{t2}}{\Delta T_2} \dots\dots\dots(1b)$$

$$Q_{t3} = \frac{1}{C_{d4} - C_{d3}} V_3 \frac{\Delta C_{t3}}{\Delta T_3} \dots\dots\dots(1c)$$

Where Q_t is the transfer coefficient of total Fe; C_d and C_t are the concentrations of dissolved and total Fe, respectively; V is the volume of the medium; and T is transfer time. The four boxes are defined as B_1 , B_2 , B_3 , and B_4 , and the volumes of medium in each box are labeled as V_1 , V_2 , V_3 , and V_4 , respectively, where $V_1=V_4 = 1.5 \text{ cm}^3$, and $V_2=V_3 = 1.0 \text{ cm}^3$ ([Fig. 1](#)).

Iron in growth media can exist as either dissolved ($[\text{Fe}]_{\text{dis}}$) or undissolved fractions ($[\text{Fe}]_{\text{undis}}$). Therefore, total iron ($[\text{Fe}]_t$) in the medium can be calculated as:

$$[\text{Fe}]_t = [\text{Fe}]_{\text{undis}} + [\text{Fe}]_{\text{dis}} \dots\dots\dots(2)$$

The dissolved and undissolved fractions of iron contain both inorganic iron species ($[\text{Fe(III)}']$), such as Fe^{3+} , Fe(OH)^{2+} , Fe(OH)_2^+ , and so forth, as well as organic iron, as in the FeL complex. Since agar was used in the preparation of the growth medium, some fractions of the iron might have adsorbed onto agar particles and become undissolved.

$$[Fe]_t = \left\{ [Fe(III)']_{undis} + [FeL]_{undis} \right\} + \left\{ [Fe(III)']_{dis} + [FeL]_{dis} \right\} \dots\dots\dots (3)$$

After the addition of chelating ligands, most of the FeL was expected to be in the dissolved form, and the existence of Fe in the insoluble form ($[FeL]_{undis}$) was negligible. Thus,

$$[Fe]_t = [Fe(III)']_{undis} + [Fe(III)']_{dis} + [FeL]_{dis} \dots\dots\dots (4)$$

The concentrations of Fe^{3+} and undissolved fractions of $[Fe(III)']$ in the medium were proportional to the concentration of dissolved fractions:

$$[Fe(III)']_{undis} = f(\alpha) [Fe(III)']_{dis} \dots\dots\dots (5)$$

$$[Fe^{3+}] = f(\beta) [Fe(III)']_{dis} \dots\dots\dots (6)$$

The dissolution of Fe in the medium depended on the conditional stability constant of the chelating ligands with Fe^{3+} . The stability constant of chelating ligands (K_{FeL}) can be defined as:

$$K_{FeL} = \frac{[FeL]_{dis}}{[Fe^{3+}][L]} \dots\dots\dots (7)$$

Subsequently, the total Fe concentration in the medium can be calculated by the following equation derived from equations (4), (5), (6), and (7):

$$[Fe]_t = \{ f(\alpha) + 1 + f(\beta)[L]K_{FeL} \} [Fe(III)']_{dis} \dots\dots\dots (8)$$

Thus, total Fe concentration in B_1 and B_2 can be calculated as

$$[Fe(III)']_{dis1} = \frac{[Fe]_{t1}}{F'} \dots\dots\dots (9a) , \text{ and}$$

$$[Fe(III)']_{dis2} = \frac{[Fe]_{t2}}{F'} \dots\dots\dots (9b), \text{ where } F' = f(\alpha) + 1 + f(\beta)[L]K_{FeL} .$$

Furthermore, the transfer coefficient of dissolved Fe from B_1 to B_2 can be calculated from the following equation derived from equation (1a):

$$\begin{aligned}
230 \quad Q_{t1} &= \frac{1}{\left\{ \left[\text{Fe(III)}' \right]_{\text{dis2}} + [\text{FeL}]_{\text{dis2}} \right\} - \left\{ \left[\text{Fe(III)}' \right]_{\text{dis1}} + [\text{FeL}]_{\text{dis1}} \right\}} V_1 \frac{\Delta C_{t1}}{\Delta T_1} \\
231 \quad &= \frac{1}{\left\{ \{1 + f(\beta)[L]K_{\text{FeL}}\} \frac{[\text{Fe}]_{t2}}{F'} \right\} - \left\{ \{1 + f(\beta)[L]K_{\text{FeL}}\} \frac{[\text{Fe}]_{t1}}{F'} \right\}} V_1 \frac{\Delta C_{t1}}{\Delta T_1} \\
232 \quad &= \frac{1}{\frac{1}{F} \cdot \{[\text{Fe}]_{t2} - [\text{Fe}]_{t1}\}} V_1 \frac{\Delta C_{t1}}{\Delta T_1} \dots \dots \dots (10)
\end{aligned}$$

$$233 \quad \text{Where, } F = \frac{f(\alpha) + 1 + f(\beta)[L]K_{\text{FeL}}}{1 + f(\beta)[L]K_{\text{FeL}}}$$

234 In addition, the coefficient (Q/F) of Fe movement from B₁ to B₂ in the medium can be defined

$$235 \quad \text{as - } \frac{Q_{t1}}{F} = \frac{1}{[\text{Fe}]_{t2} - [\text{Fe}]_{t1}} V_1 \frac{\Delta C_{t1}}{\Delta T_1} \dots \dots \dots (11a), \text{ and the Q/F from B}_2 \text{ to B}_3 \text{ and from B}_3$$

236 to B₄ would be –

$$\frac{Q_{t2}}{F} = \frac{1}{[\text{Fe}]_{t3} - [\text{Fe}]_{t2}} V_2 \frac{\Delta C_{t2}}{\Delta T_2} \dots \dots \dots (11b)$$

$$237 \quad \frac{Q_{t3}}{F} = \frac{1}{[\text{Fe}]_{t4} - [\text{Fe}]_{t3}} V_3 \frac{\Delta C_{t3}}{\Delta T_3} \dots \dots \dots (11c)$$

238

239 Iron movement coefficient by chelating ligands

240 A ‘4-box’ model was established to calculate the apparent coefficient of Fe movement
 241 due to chelating ligands in the growth medium. Using this model, the apparent coefficient
 242 (Q/F) of Fe movement in the medium was calculated from equations (11a), (11b), and (11c),
 243 and the results are presented in [Table 1](#) and [Fig. 4](#).

244 Iron concentrations in B₄ for all ligands and the control treatment were lower than those
 245 in B₃ ([Fig. 3](#)). This might be attributable to the adsorption of additional Fe on the bottom wall
 246 of the test tubes. All sections of the test tubes had a common surrounding wall, while B₄ had a

bottom wall in addition to the surrounding wall. Therefore, some of the Fe in B₄ could have adsorbed on this additional surface, resulting in the inconsistent apparent movement of Fe from B₄ to B₃ (Q_{t1}/F) compared to the movement from B₃ to B₂ (Q_{t2}/F) and B₂ to B₁ (Q_{t3}/F) (Table 1). In contrast, the Q_{t2}/F and Q_{t3}/F showed a unique and consistent pattern. While the Q_{t3}/F was higher than the Q_{t2}/F in growth medium that lacked chelating ligand, this outcome was reversed in the ligand-treated samples (Fig. 4): the Q_{t2}/F was significantly higher than the Q_{t3}/F in samples treated with chelating ligands. These results suggest that the Q/F of Fe is favored by chelating ligands, and the Fe movement is high across concentration gradients in growth media.

The highest Q_{t2}/F values, representing apparent movement of Fe from B₃ to B₂, were 0.0103 ± 0.0012 and 0.0116 ± 0.0026 in growth Medium treated with HIDS or EDDS, respectively, followed by GLDA, MGDA, EDTA, and IDS. The same pattern of Q_3/F for Fe was observed with few exceptions (Fig. 4). The coefficients of Fe movement by chelating ligands in the growth Medium would relate to the conditional stability constant of each ligand ($\text{Log}K_{\text{FeL}}$). Therefore, the conditional stability constant of the chelating ligand could be an important indicator of Fe bioavailability and movement in growth Medium.

Fe uptake and radish growth

The growth of radish sprouts was correlated with the Fe concentration in the plant tissues. The heights of the radish sprouts increased with higher tissue Fe concentrations (Fig. 5). The Fe concentration in the tissues of the radish sprouts was dependant on the chelating ligands, since the Fe was not readily bioavailable under experimental conditions (at pH 10) before the addition of ligands. Compared to the control, the Fe concentration in the sprouts increased with the addition of chelating ligands (Fig. 5). The Fe uptake in radish sprouts was increased by 79% with the addition of HIDS to the growth medium. Other chelating ligands also significantly increased Fe uptake, as follows: 0.4% with IDS, 28% with MGDA, 37% with EDTA, 56% with GLDA, and 58% with EDDS. This increase in Fe uptake by the chelating

ligands correlated with radish growth. Compared to the control, the height of the radish sprouts was increased by 34%, 30%, 22%, and 19% with the addition of HIDS, GLDA, EDDS, and EDTA, respectively.

Discussions:

Effect of chelating ligands on Fe uptake in and growth of radish

Although abundant in nature, iron is often unavailable to plants, especially at neutral or alkaline pH, because of the formation of insoluble ferric hydroxide under oxic conditions (Guerinot and Yi, 1994; Robinson et al., 2006). Precipitation of Fe in the rhizosphere may result in an Fe deficiency in the plants and reduce growth. Chelating ligands have been used in agriculture as an additive in micronutrient fertilizers in order to increase Fe bioavailability (Alvarez-Fernandez et al., 2005), and the growth of all organisms is dependent on the acquisition of the proper quantities of trace elements. Iron is an important micronutrient for plants and plays vital roles in respiration, photosynthesis, and many other cellular functions including DNA synthesis, nitrogen fixation, and hormone production (Vert et al., 2002). Ferric ions and their complexes have low solubility in aquatic systems, but they are extensively buffered by chelation (Morel and Hering, 1993), which increases their dissolved concentration. The dissolved concentration of Fe determines its rate of uptake by organisms. Anderson and Morel (1982) observed that the Fe uptake rate in laboratory cultures of the marine diatom *Thalassosira weissflogii* was a unique function of the free ferric ion (Fe^{3+}) concentration and the presence of various chelating ligands. Although the influence of EDTA and EDDS on Fe uptake and plant growth is not new, HIDS is a new biodegradable chelating ligand that shows improved performance in Fe acquisition and plant growth. When researchers, industries or users are looking for environmentally safe and biodegradable chelating ligands that perform well, HIDS would be a good alternative to the environmentally persistent and widely used EDTA.

299

300 Influence of chelating ligands Iron movement in the growth medium

301 Chelating ligands form a soluble Fe-ligand complex (FeL) in the rhizosphere and
302 increase Fe bioavailability and uptake in plants. Therefore, chelating ligands such as EDTA and
303 EDDS have been widely used in agriculture, to increase Fe levels in crops ([Alvarez-Fernandez](#)
304 [et al., 2005](#); [Gil-Ortiz and Bautista-Carrascosa, 2004](#); [Hernandezapaolaza et al., 1995](#); [Ignatova](#)
305 [et al., 2000](#); [Lucena, 2006](#); [Marques et al., 2008](#)); however, the pattern and efficiency of Fe
306 movement by chelating ligands is poorly understood. The present study elucidates the
307 enhancement of Fe mobility and bioavailability in growth Medium due to the presence of
308 chelating ligands. A unique pattern of Fe movement in the growth Medium was observed after
309 the addition of chelating ligands. This movement of Fe increased Fe concentration in the
310 rhizosphere soils and assisted the uptake of Fe in plants.

311 The movement of Fe in the growth medium is was dependent upon the type of
312 chelating ligands as well as the pH of the medium. Fe movement was several times higher at
313 pH 6 than at pH 10 ([Fig. 2](#)). The stability constant of the Fe-complexing chelating ligands was
314 another important factor that affected Fe movement in the growth medium. Chelating ligands
315 produce soluble FeL complexes ([Alvarez-Fernandez et al., 2005](#); [Bell et al., 2005](#)) and
316 consequently increase bioavailability of Fe. This study hypothesizes that the Fe moves from
317 the deeper rhizosphere to the shallow rhizosphere as a result of its increased bioavailability.

318 Results indicate an apparent movement of Fe from B₃ to B₂ due to the addition of
319 chelating ligands. Some of the Fe also moved from B₂ to box 1 (B₁), the topmost layer of the
320 medium, which initially had no Fe. These results demonstrate that the increase in Fe
321 bioavailability and uptake by chelating ligands is useful not only for desorption and/or
322 solubilization of Fe oxides ([Lucena, 2003](#); [Schwertmann, 1991](#)) but also for movement of Fe
323 from a higher concentration area to a lower concentration area within growth Medium.

324 Fe movement in the growth medium was influenced by the chelating ligand species.

Compared to the control, the highest amount of total Fe moved from the bottom layers (B₄ and B₃) to the upper layers (B₂ and B₁) was achieved using EDDS and HIDS, followed by GLDA, EDTA, MGDA, and IDS (Fig. 3). Both EDDS and HIDS are more biodegradable than EDTA (Table 1). Specifically, the biodegradation rate of HIDS is about 22.4% within 72 h. Iron movement from the bottom layer to the upper layer also increased with an increase in ligand exposure time.

Conclusions

Iron deficiency in plants is a common phenomenon in areas of calcareous and/or alkaline soils and produces chlorotic symptoms. Many physiological and biochemical aspects of this nutritional disorder have been studied in order to resolve this problem. Synthetic Fe(III)-chelates, such as EDTA and EDDS, are the most common and effective ligands used to increase Fe bioavailability. An important concern, however, is that most of the commercially used chelating ligands are poorly biodegradable and therefore rather persistent in the environment. EDTA, for example, occurs at higher concentrations in European surface waters than any other anthropogenic organic compounds identified. As a result, the development of more effective and easily biodegradable chelating ligands is essential.

HIDS is a new chelating ligand with high biodegradability and a high stability constant with Fe³⁺. The present study revealed that the performance of HIDS with respect to Fe movement in growth Medium and radish growth is higher than that of other chelating ligands tested. Thus, HIDS would be a good alternative to EDTA and other poorly biodegradable chelating ligands.

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Table 1: Apparent mobility of iron in the growth medium affected by Fe-complexing chelating ligands

Chelating ligands	$\frac{Q_1}{F}$	$\frac{Q_2}{F}$	$\frac{Q_3}{F}$
Control	0.0716±0.0052	0.0015±0.0002	0.0038±0.0009
EDTA	-0.1432±0.0017	0.0066±0.0004	0.0026±0.0019
HIDS	0.0214±0.0089	0.0103±0.0012	0.0057±0.0001
IDS	0.0169±0.0156	0.0075±0.0018	0.0027±0.0005
MGDA	0.0006±0.0007	0.0058±0.0014	0.0032±0.0009
EDDS	0.0105±0.0081	0.0116±0.0026	0.0034±0.0017
GLDA	-0.0373±0.0845	0.0105±0.0006	0.0052±0.0005

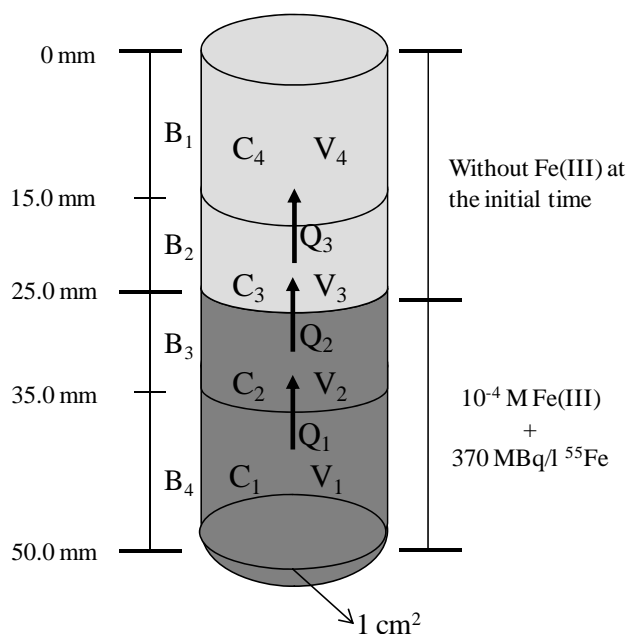


Fig. 1: Experimental set up of two-layered culture medium. Initially, the lower layer of the medium contained Fe(III) (10^{-4} M) while the upper layer had no Fe. The two-layered medium was divided into four sections and apparent Fe mobility was measured in each section.

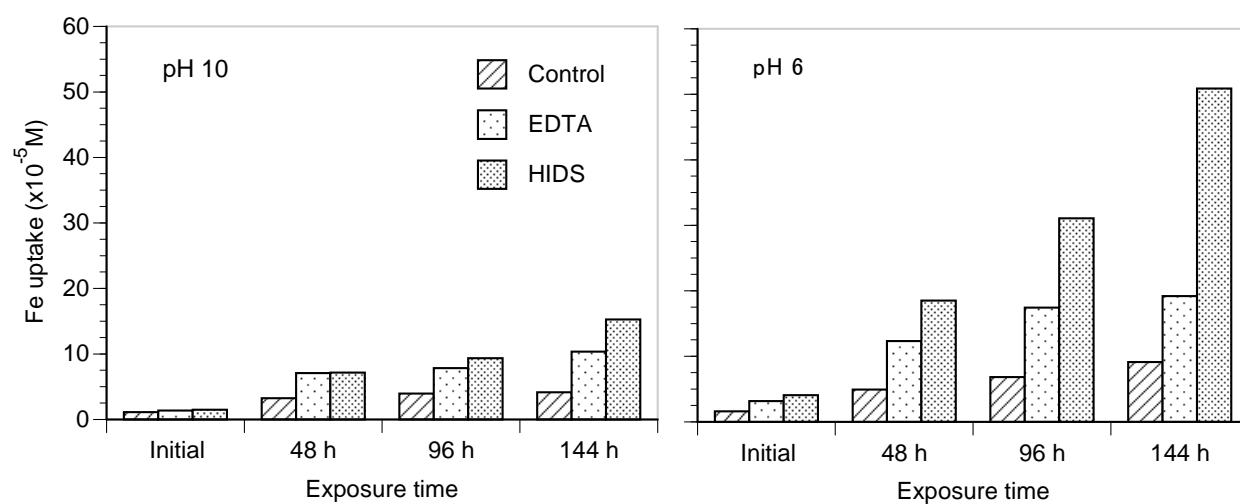


Fig. 2: Effect of pH on Fe mobility in the growth Medium.

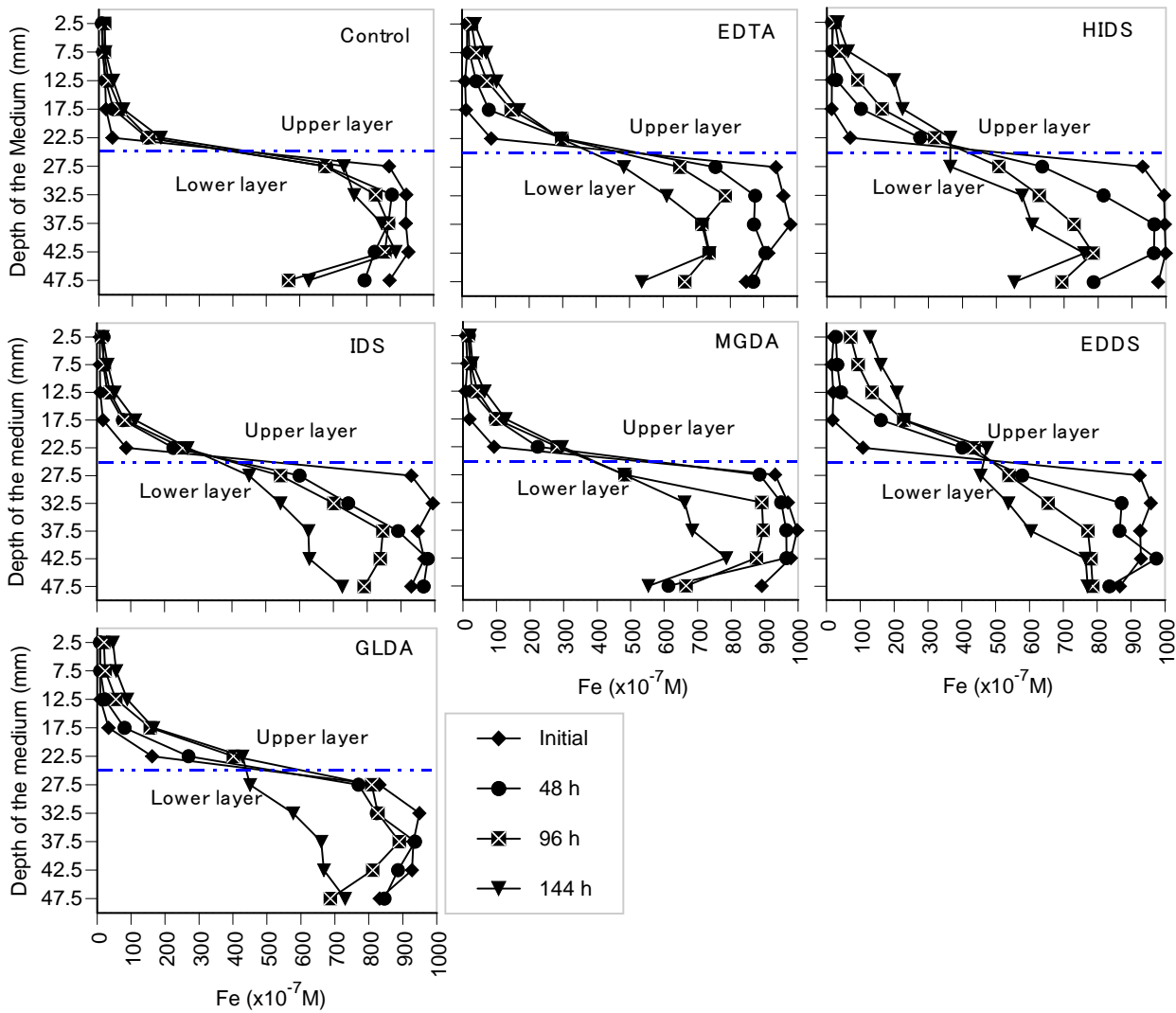


Fig. 3: Effect of chelating ligands on Fe movement from lower to upper layers of the culture medium (pH 10).

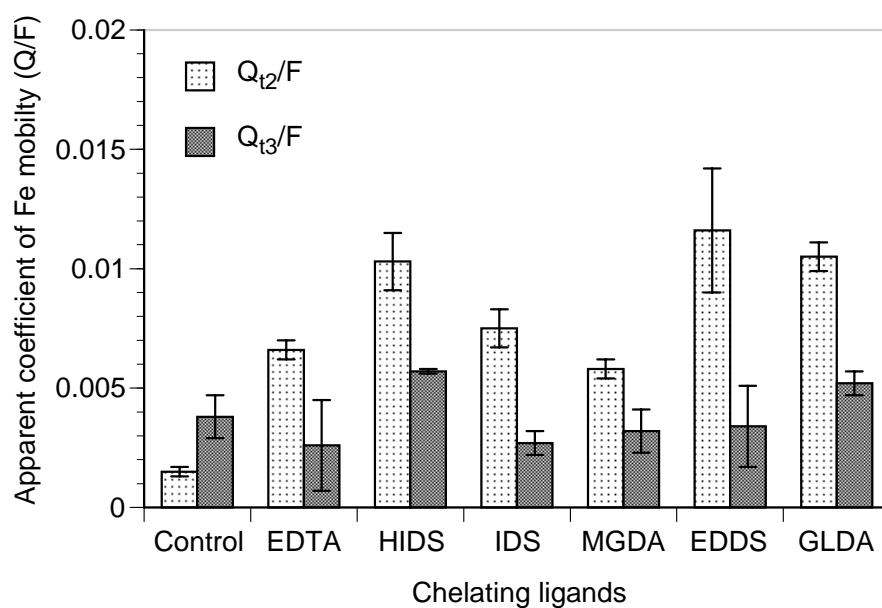
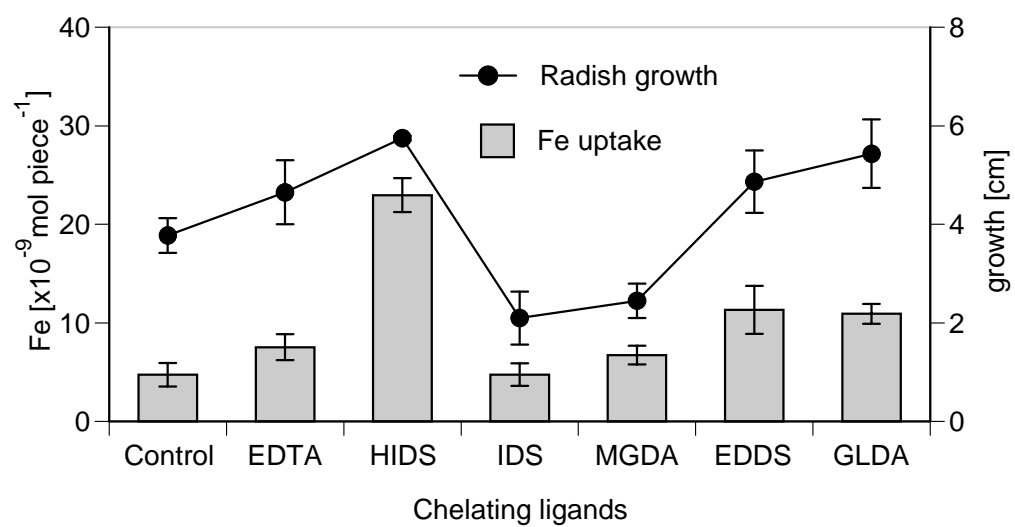


Fig. 4: The apparent Fe movement in the growth medium explained by a '4-box' model.



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573 Fig. 5: Iron uptake and growth of radish sprouts in Medium with Fe-complexing chelators.

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