Significance of the concentration of chelating ligands on Fe

Significance of the Concentration of Chelating Ligands on Fe^{3+}-Solubility, Bioavailability, and Uptake in Rice Plant

Hiroshi Hasegawa[1,*]; M. Mamunur Rahman[2, 3]; Kouta Kadohashi[2]; M. Azizur Rahman[2, 4]; Yui Takasugi[2]; Yousuke Tate[5]; Teruya Maki[1]

[1]Institute of Science and Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

[2]Graduate School of Natural Science & Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

[3]Bangladesh Rice Research Institute (BRRI), Sonagazi, Feni 3930, Bangladesh

[4]Centre for Environmental Sustainability, School of the Environment, Faculty of Science, University of Technology Sydney, P.O. Box 123, Broadway, NSW 2007, Australia

[5]Department of Chemistry and Chemical Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

Corresponding author

E-mail: hhiroshi@t.kanazawa-u.ac.jp (H. Hasegawa)

Tel/Fax: +81 76 234 4792

Abstract

Present study investigated the significance of the concentration of chelating ligand on Fe^{3+}-solubility in growth medium and its influence on Fe bioavailability and uptake in rice plant. Rice seedlings were grown in modified Murashige and Skoog (MS) hydroponic growth medium with moderate (250 μM) and high (500 μM) concentrations of ethylenediaminetetraacetate (EDTA) and hydroxyiminodisuccinate (HIDS) under sterile and non-sterile conditions. Concentrations of soluble Fe in the growth medium increased with increasing ligand concentrations, and the growth of rice seedlings was higher at moderate ligand concentration than at control (without chelant) and high ligand concentration. This explains the relationship between Fe solubility and bioavailability in the growth medium, and its effect on Fe uptake in rice plant. Fe exists in the growth medium predominantly as particulate (insoluble) forms at low ligand concentration, and as soluble [$Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, Fe-L complex] and apparently soluble (colloidal) forms at moderate ligand concentration. At high ligand concentration, most of the Fe^{3+} in the growth medium forms soluble Fe-L complex, however, the bioavailability of Fe from Fe-L complex decreased due to lopsided complex formation equilibrium reaction (CFER) between Fe and the ligands. Also, Fe is solubilized forming stable and soluble Fe-L complex, which is then detached as less stable, but soluble and bioavailable substance(s) after (time-dependent) biodegradation. Therefore- i) ligand concentration and stability constant of Fe-L complex ($K_{Fe\text{-}L}$) influence Fe bioavailability and uptake in rice plant, and ii) the biodegradable ligands (e.g., HIDS) would be more effective Fe fertilizer than the environmentally persistent and less biodegradable ligands (e.g., EDTA).

Keywords: Iron, Bioavailability, Solubility, Chelating ligand, HIDS, EDTA, Rice (*Oryza sativa* L.)

1. Introduction

Iron (Fe) is the sixth most abundant element in the earth crust, and is an essential nutrient for plants. Despite its abundance in the soil, Fe is only slightly soluble under aerobic conditions, especially in high-pH and calcareous soils [1]. Fe deficiency is one of the most common constraints to the growth of rice plant in acid soils, and together with Zn deficiency, it is the most commonly observed micronutrient disorder in wetland rice [2]. Higher plants have two distinct strategies to acquire insoluble/slightly soluble Fe from the rhizosphere; i) the reduction strategy of nongraminaceous (strategy I) plants, and ii) the chelation strategy of graminaceous (strategy II) plants [3-5]. In a recent review, Kobayashi & Nishizawa [3] discussed the roles of phytosiderophores in Fe uptake, translocation, and regulation in nongraminaceous and graminaceous plants. In addition to the Fe^{3+}-solubilising phytosiderophores, rhizospheric microbes also exude siderophores to solubilize ferric Fe in the rhizosphere, and thus render Fe soluble and bioavailable, resulting in uptake by graminaceous plants via specific membrane receptors [4, 5]. Synthetic chelating ligands belonging predominantly to the two groups: aminopolycarboxylates and polyphosphonates, are also effective and have widely been used for increasing Fe bioavailability and uptake [6, 7], and for correcting Fe-chlorosis in plants [8].

The synthetic chelating ligands form Fe-ligand (Fe-L) complexes in the rhizosphere that increase Fe solubility and subsequent bioavailability and uptake in plants. Aminopolycarboxylates such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetate (NTA), and diethylenetriaminepentaacetate (DTPA) are popular and widely used chelating agent to achieve this purpose. However, these aminopolycarboxylates are quite persistent in the environment because of its low biodegradability [9]. This, in addition to their high affinity for heavy metal complexation, results in the increased risk of leaching [10]. Therefore, these aminopolycarboxylates are being replaced by new generation biodegradable complexing agents [9, 11]. The biodegradability of hydroxyiminodisuccinate (HIDS; Fig. 1A) is higher

than EDTA (Fig. 1B) [12], and therefore, HIDS was proposed to be a better choice and substitute to less biodegradable EDTA [13]. It traps various metal ions, particularly Fe^{3+}, over a wide range of pH, shows high stability in harsh conditions and high temperature, is highly soluble in aqueous alkaline solution [14].

In the previous study, we found that the concentrations of chelating ligands (EDTA, EDDS, HIDS, IDS) significantly influence Fe uptake in rice seedlings and subsequent growth of the plant [13]. Fe uptake and growth of rice seedlings were found to be better at 0.25 mM ligand concentration compared to that at lower (0.1 mM) and higher (0.5, 1.0, and 2.5 mM) concentrations of the ligands. From the results of those studies, it was hypothesized that the concentration and stability constant of Fe-L complexes ($K_{Fe\text{-}L}$) are important factors for Fe solubility in the growth medium that influence the bioavailability and uptake of Fe in rice plants. In the present study, we investigated the influence of ligand-induced Fe^{3+}-solubility in the growth medium on Fe bioavailability and uptake in rice plant to justify the effect of ligand concentration and $K_{Fe\text{-}L}$ by growing rice seedlings in modified Murashige and Skoog (MS) hydroponic growth medium with moderate (250 µM) and high (500 µM) concentrations of EDTA and HIDS under sterile and non-sterile conditions. Chelating ligands (EDTA and HIDS) and their concentrations (250 and 500 µM) were selected on the basis of the results of previous studies [13] for a scientific contribution, but these concentrations might not be suitable for practical implications since ligands are not used alone for Fe nutrition. Fe concentrations in root surfaces, growth medium, and inside roots of rice were measured at different ligand concentrations using ^{55}Fe radioisotope tracer to investigate the influence of ligand concentrations on Fe bioavailability and uptake in rice plants.

2. Materials and Methods

2.1. Seed sterilization and germination

The rice seeds of BRRI Dhan29 were collected from Bangladesh Rice Research

Institute (BRRI), Bangladesh. The seeds were surface-sterilized before using them in the experiment. For sterilization, 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate (Sumitomo Chemical Co. Ltd., Japan) solution for 10 min. After that, the seeds were washed by deionized (DI) water (E-pure® water system, Models D4631, Barnstead, USA)) and kept in DI water at 20 ºC for 24 h. The seeds were then washed and transferred to DI water of 45 and 52 ºC for 2 and 10 min, respectively.

2.2. Chemicals

Stock solutions of EDTA and HIDS were prepared by dissolving ethylenediamine-N,N,N',N'-tetraacetic acid (Kanto chemical, Japan) in 0.1 M sodium hydroxide and tetrasodium 3-hydroxy-2,2'-iminodisuccinate (Nippon Sokubai, Japan) in DI water, respectively. All chemical reagents used in the experiment were of analytical grade. Glassware and dishes were washed with detergent and 5M HCl solution, and rinsed with DI water for eight times before use.

2.3. Nutrient solution

Modified Murashige and Skoog (MS) hydroponic solution [15] was used as growth medium for rice seedlings (Table 1). The nutrient salts of MS medium were dissolved in DI water and the pH of the growth medium was adjusted to 5.7 using 0.1 M KOH and HCl. In preparing MS culture solution, $FeCl_3$ was used instead of $FeSO_4 \cdot 7H_2O$ as Fe source. Sodium salt of EDTA ($Na_2EDTA \cdot 2H_2O$) was not used in preparing the growth medium. The chemicals used for the preparation of MS growth medium were of analytical grade and were purchased from Kanto chemical, Japan.

2.4. Experimental setup

Sterilized rice seeds were germinated and allowed to grow for 5 days with DI water on pre-sterilized bloating paper. Rice seedlings were then transferred to the modified MS growth medium (Table 1). The medium of control treatment did not contain chelating ligand, while the other treatment contained 250 and 500 µM of chelating ligands (EDTA or HIDS). To observe if there was any influence of microbial contamination on ligand degradation and Fe bioavailability, the experiment was conducted in sterile (medium with antibiotic) and non-sterile (medium without antibiotic) medium. Medium was sterilized with 50 mg L^{-1} of oxytetracycline hydrochloride (Wako, Japan). The pH of the solution was adjusted to 5.7 using 0.1 M KOH every day though out the experiment. A 250 mL of the solution was taken into 250 mL polycarbonate bottles with five replications, and three uniform seedlings were planted in each bottle. Rice plants were grown in a plant growth chamber with conditions of 14/10 h light/dark schedule, 100-125 $\mu Em^{-2}s^{-1}$ light intensity, and 20 (±2) ºC. Rice seedlings were grown in the growth medium for 2 weeks, and 5 mL sample was collected everyday throughout the experiment and filtered through 0.2 µm membrane filters (mixed cellulose ester, Advantec, Japan). The volume of the medium was corrected by adding fresh medium from the stock having respective amount of chelating ligands. Fe and chelating ligand concentrations were measured in filtered samples.

Radioisotope ^{55}Fe tracer was used to investigate the changes of Fe concentration in growth medium, and the uptake of Fe in root surface and roots of rice seedlings. In this experiment, radioisotope ^{55}Fe tracer was added to the modified MS growth medium contained 0, 250 and 500 µM HIDS or EDTA at the 1^{st} day before growing rice seedling in the medium. Rice seedlings were grown in the growth medium at 1^{st} and 11^{th} day of the addition of ^{55}Fe tracer for 24 h, and ^{55}Fe concentrations were measured in rice root surface and inside the roots. Fe from rice root surfaces was washed by titanium(III)-citrate-EDTA (Ti(III)-citrate-EDTA) solution [16]. On the other hand, ^{55}Fe concentrations were measured in the growth medium at 1^{st}, 6^{th} and 11^{th} day of the addition of ^{55}Fe tracer.

2.5. Chemical analysis for Fe and chelating ligands

For total Fe determination, rice roots were rinsed with DI water four times, and the samples were placed on a clean absorbent paper to remove the water from the root surfaces. Then the samples were dried at 65 ºC until they reached a constant weight. Then the entire dried samples were weighed (10-30 mg) and put into 50-mL polyethylene tubes (DigiTubes, SCP Science, Canada) for digestion. A 5 mL of 65% nitric acid (HNO_3) was added to each of the sample, and the samples were heated on a heating block (DigiPREP, SCP Science, Canada) at 95 ºC for 2 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide (H_2O_2) was added to each sample and the samples were heated again to 105 ºC for 30 min. Then, the digests were diluted to 30 mL with DI water and total Fe concentration in the samples was determined by using graphite-furnace atomic absorption spectrometer (GF-AAS, Perkin Elmer, AAnalyst 800).

The concentration of ^{55}Fe was determined by liquid scintillation counting (LSC) method in a liquid scintillation counter (LSC-6101, Aloka, Japan). Polycarbonate filters (Corning, USA) of different pore-size were used to determine soluble and insoluble (particulate) fractions of Fe in the growth medium. A 0.025 μm filters were used to separate particulate Fe from its soluble fraction corresponding to 200 kDa of molecular weight, and 0.20 μm filters were used to separate colloidal fraction from particulate fraction. An inductively coupled plasma atomic emission spectrometer (ICP-AES; Optima 3300XL, PerkinElmer, USA) equipped with an ultrasonic nebulizer (U-5000AT, Cetac, USA) was used for the determination of size-fractioned Fe concentrations in the growth medium.

EDTA and HIDS were analyzed using high performance liquid chromatography (HPLC: 8020+PD8020, Tosoh, Japan; column: ODS-80TM, Tosoh, Japan; mobile phase: 5 mM of ammonium dihydrogenphosphate; flow rate: 0.5 mL/min). A 25 μL of 0.1 mol/L $CuCl_2$/1M HCl solution was added to 500 μL filtered sample in order to increase the

absorption intensity at 254 nm. In each analytical batch, at least two reagent blanks and three replicate samples (n = 3; three plants from three pots) were included.

3. Results and Discussion

3.1. Effect of ligand concentrations on growth of rice seedling

The growth of rice seedlings was influenced by the concentrations and the type of the ligands. In both sterile and non-sterile conditions, the growth of rice seedlings was better in moderate concentration (250 µM) of EDTA and HIDS than that in control and high ligand concentration (500 µM) (Fig. 2). Although the growth of rice seedlings in sterile (EDTA and HIDS treated; Figs. 2A and 2B) and non-sterile (HIDS treated; Fig. 2D) medium in all concentrations of the ligands were gradually increased for first few days (5-6 days) and remain constant for the rest of the days, a different growth pattern was observed in EDTA treated non-sterile medium (Fig. 2C). Rice seedlings growth almost stopped throughout the experiment in 500 µM EDTA treatment in non-sterile medium, while the growth increased steadily in control and 250 µM EDTA treatments. This might be attributable to the effect of ligand concentration on solubility and bioavailability of Fe in the growth medium.

The concentration of soluble Fe in the growth medium was highest in 500 µM EDTA and HIDS treatments in both the sterile and non-sterile medium followed by 250 µM of the ligand treatments (Fig. 3). The soluble fraction of Fe in the medium was extremely low in control treatment. However, high seedlings growth at moderate ligand concentration (250 µM EDTA and HIDS) in both sterile and non-sterile medium (Fig. 2) indicate that increased solubility of Fe, mediated by the formation of Fe-L complex at high concentrations (500 µM) of the ligands, does not favor rice growth. Rahman et al. [13] also found that the growth of rice seedlings decreased with increasing ligand concentrations in hydroponic medium. Thus, being a good Fe fertilizer for rice plants [8], the concentration of chelating ligands in the growth medium would be an important factor for increasing Fe bioavailability and uptake in

rice. However, ferric ions and their complexes, which have low solubility in water, are extensively buffered by chelation [17] and increase their dissolved concentration. In this study, the growth of rice seedlings increased for increasing Fe uptake at moderate ligand concentration. The rice plant may take up Fe as ferric chelants; however, most of the Fe in the medium may complex with the ligands at high ligand concentrations to form soluble Fe-L complexes which may not be bioavailable for the rice plants.

3.2. Fe solubility and bioavailability in the growth medium

The Fe-L complexes solubilize the ferric Fe in the growth medium. Therefore, the influence of ligand concentration on the formation of Fe-L complex and Fe solubility are important in understanding ligand-induced Fe bioavailability and plant uptake. In the present study it was observed that the concentration of soluble Fe, which is considered to be predominantly Fe-L complex, was increased with increasing concentration of the chelating ligands (Fig. 3). On the other hand, ligand concentrations decreased with increasing growing time in both sterile and non-sterile medium (Fig. 3). In control treatments, the soluble Fe concentration in the growth medium was 5 μM or less that remained unchanged throughout the experiment (Fig. 3), and most of the Fe was in insoluble (particulate) ferric hydroxides (e.g., Fe_2O_3, $Fe(OH)_3$, FeOOH) forms. This was because the source of Fe nutrient in the MS growth medium was $FeCl_3$ instead of sodium salt of EDTA ($FeSO_4 \cdot 7H_2O$).

Fe solubility in both the sterile and the non-sterile growth medium varied significantly for EDTA and HIDS treatments. In sterile medium of 250 and 500 μM EDTA treatments, initial soluble Fe concentration was about 50 μM which decreased to about 35 μM after 14 d (Fig. 3A). However, soluble Fe concentration remained constant (about 50 μM) in non-sterile medium of 250 and 500 μM EDTA treatments throughout the experiment (Fig. 3C). In contrast, the pattern of soluble Fe concentration in 250 and 500 μM HIDS treatments differed from that of EDTA treatments. In sterile medium, soluble Fe concentration in medium of 500

μM HIDS treatment was about 10 μM lower than the medium of 250 μM of the ligand treatment, and this difference was observed to be invariable throughout the experiment (Fig. 3B). The soluble Fe concentrations did not differ significantly between 250 and 500 μM HIDS treatments in non-sterile medium (Fig. 3D). This may be because Fe in the growth medium exists mostly as Fe-L complexes at high ligand concentration (500 μM EDTA and HIDS in this case) due to lopsided complex formation equilibrium reaction (CFER) of the ligands with Fe^{3+}. A generalized model of CFER of ligands with Fe^{3+} in the ligand-treated growth medium is presented in Figure 4 that describes the facts of ligand-induced Fe solubility and bioavailability in the growth medium.

Chelating ligands increase the concentration of soluble Fe in the growth medium to make it bioavailable [8, 18-20]. When the concentration of the ligand was high (500 μM of EDTA and HIDS in this case), the bioavailability of Fe decreased due to the lopsided CFER between Fe^{3+} and chelating ligands that results in the decrease of Fe uptake and growth of rice seedlings (Fig. 1). At moderate concentration of the HIDS, Fe exists in the growth medium predominantly as inorganic [Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, etc.] and colloidal (apparently soluble) forms, while it exists mainly as 'Fe-L' form at the same concentration of EDTA (Fig. 4). This was related to the stability constant of Fe-L complex (K_{FeL}) since the stability constant of Fe-HIDS complex is lower than that of Fe-EDTA complex [12], and since Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$ and colloidal (apparently soluble) forms of Fe are more bioavailable than Fe-L complex. Therefore, moderate concentration of HIDS is more efficient than that of EDTA for Fe bioavailability and uptake in the rice plants.

3.3. Fe uptake in rice plant influenced by chelating ligand concentration

The influence of concentrations and type of the chelating ligands on Fe uptake in plant was studied using radioactive ^{55}Fe tracer. ^{55}Fe concentrations were higher in root surfaces than in roots and shoots of control and ligand-treated rice seedlings (Figs. 5A, 5B). In control

treatment, ^{55}Fe concentration in rice root surfaces was about 86.12±11.25 nmol g^{-1} wet weight (w. wt.) at 1st day that decreased significantly to 55.26±2.56 nmol g^{-1} w. wt. at 11th d (Fig. 5A). The concentration of ^{55}Fe in rice shoots of 11th day was also significantly lower than that in shoots of 1st d in control treatment (Fig. 5B). This was due to the decrease of Fe solubility and bioavailability resulted from the precipitation of ferric oxides in the bottom of the growth medium [21].

At moderate HIDS concentration (250 µM), ^{55}Fe concentration in rice root surfaces was 147.88 nmol g^{-1} w. wt. at 1st d which decreased by 31% at 11th d. In contrast, ^{55}Fe concentration in rice roots was 52.20 nmol g^{-1} w. wt. at 1st d which increased by 45% at 11th d (Fig. 5A). The concentrations of ^{55}Fe in root surfaces of rice seedlings at 1st and 11th d did not differ significantly for high concentration (500 µM) of HIDS (Fig. 5A). The results reveal that most of the Fe is stored in rice roots and the increase of Fe uptake by the ligand occurs in roots, but not in root surfaces. Previous studies also showed that chelating ligands increased Fe uptake in rice roots [13]. The present study showed that Fe uptake in roots of rice seedlings increased significantly at moderate concentration of HIDS compared to that at high concentration of the ligand (500 µM).

HIDS concentration in the growth medium did not affect ^{55}Fe uptake in shoots of rice seedlings at 1st and 11th d (Fig. 5B). The results reveal that Fe uptake in aerial parts of rice plant is not a factor of its concentration in the growth medium. The concentrations of ^{55}Fe in root surfaces, roots and shoots of rice seedlings were very low for EDTA treatments (Figs. 5A, 5B) indicating that HIDS is more effective than EDTA for Fe bioavailability and uptake in rice plant.

The changes of ^{55}Fe concentrations in growth medium were measured at 1st, 6th and 11th d. Results showed that ^{55}Fe concentrations in the medium of control treatment (without chelating ligand) decreased by 54% and 68% at 6th and 11th day, respectively, while its concentrations remain stable when HIDS and EDTA were added to the medium (Fig. 5C).

This trend of ^{55}Fe concentration changes in the growth medium explains the solubility of Fe at different concentrations of chelating ligands as shown in Figure 4. The decrease of ^{55}Fe concentration in growth medium of control treatment might be due to the precipitation of the oxides of ferric Fe , which eventually influenced its concentrations in root surface and inside the roots.

3.4. Biodegradation of EDTA and HIDS in growth medium

A unique pattern of ligand concentrations in sterile and non-sterile growth medium was observed for EDTA and HIDS treatments. The concentrations of EDTA and HIDS in sterile medium decreased gradually (Figs. 6A, 6B), while a different pattern in their concentration changes was observed in non-sterile medium (Figs. 6C, 6D). Irrespective of the added concentration, HIDS concentration in non-sterile medium decreased dramatically by 40% on the second day, and then gradually decreased to be disappeared after two weeks (Fig. 6D). At moderate (250 μM) EDTA treatment, EDTA concentration in non-sterile medium was observed to maintain its initial value up to 4th day, and then decreased gradually. In contrast, EDTA concentration decreased gradually at high (500 μM) ligand treatment in non-sterile medium, and a 50% decrease of the ligand concentration was recorded after two weeks of incubation (Fig. 6C). The result indicates the microbial degradation of EDTA and HIDS in the growth medium. HIDS is more biodegradable than EDTA, which in consistent with the result of Hyvonen and Aksela [12].

4. Conclusion

In general, solubility of Fe in the growth medium is important factor affecting its bioavailability and uptake in rice plant. The composition and stability (and subsequently bioavailability) of the Fe-L complex is determined by the ligand concentration in the growth medium. The stability of Fe-L complex increases at high ligand concentration that lowers the

bioavailability and uptake of Fe in rice plant. The higher ligand concentration, the stable Fe-L complex, and the bioavailability of Fe-L complex is low due to the lopsided CFER between Fe and the ligands. A medium concentration of a chelant, at which Fe exists predominantly as soluble [$Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, Fe-L complexes] and apparently soluble (colloidal) forms, would be the optimum concentration for increasing Fe bioavailability and uptake in hydroponic rice.

in addition to the ligand concentration, stability constant of Fe-L complex ($K_{Fe\text{-}L}$) is also important factor that influence Fe bioavailability and uptake in rice plant significantly. The other factor that may influence Fe bioavailability and uptake in hydroponic rice plant is the biodegradability of ligands. Fe is solubilized by forming stable and soluble Fe-L complex, which is then detached as less stable, but soluble and bioavailable substance(s) after (time-dependent) biodegradation (the higher chelant content, the longer biodegradation). A number of aminopolycarboxylates such as EDTA, NTA, and DTPA have been widely used as Fe fertilizers; however, these are not biodegradable and hence, persist in the environment for longer time. Therefore, non-biodegradable chelating ligands are losing their acceptance in the environmentally sustainable agriculture. The present study showed that the biodegradable ligands (e.g., HIDS) would be a better Fe-fertilizer than the environmentally persistent non-biodegradable ligands (e.g., EDTA).

Acknowledgement

This research was supported partly by Grants-in-Aid for Scientific Research (20·08343 and 21651101) from the Japan Society for the Promotion of Science (JSPS). The contribution of the University of Technology, Sydney (UTS), Australia, to this work is also acknowledged. The authors also wish to thank the anonymous reviewers for their constructive comments and suggestions that have improved the quality of the manuscript greatly.

References

[1] H. Marschner, Mineral Nutrition of Higher Plants 2nd ed., Elsevier, London, 1995.

[2] H.U. Neue, C. Quijano, D. Senadhira, T. Setter, Strategies for dealing with micronutrient disorders and salinity in lowland rice systems, Field Crops Res., 56 (1998) 139-155.

[3] T. Kobayashi, N.K. Nishizawa, Iron uptake, translocation, and regulation in higher plants, Annu. Rev. Plant Biol., 63 (2012) 131-152.

[4] V. Romheld, Different strategies for iron acquisition in higher plants, Physiol. Plant, 70 (1987) 231-234.

[5] V. Romheld, H. Marschner, Evidence for a specific uptake system for iron phytosiderophores in roots of grasses, Plant Physiol., 80 (1986) 175-180.

[6] R. Gil-Ortiz, I. Bautista-Carrascosa, Effects of Fe-EDDHA chelate application on evolution of soil extractable iron, copper, manganese, and zinc, Commun. Soil Sci. Plant Anal., 35 (2004) 559-570.

[7] M.A. Rahman, H. Hasegawa, K. Kadohashi, T. Maki, K. Ueda, Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction, Chemosphere, 77 (2009) 207-213.

[8] A. Alvarez-Fernandez, S. Garcia-Marco, J.J. Lucena, Evaluation of synthetic iron(III)-chelates (EDDHA/Fe^{3+}, EDDHMA/Fe^{3+} and the novel EDDHSA/Fe^{3+}) to correct iron chlorosis, Eur. J. Agron., 22 (2005) 119-130.

[9] B. Kos, D. Lestan, Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching, Plant. Soil., 253 (2003) 403-411.

[10] M. Bucheli-Witschel, T. Egli, Environmental fate and microbial degradation of aminopolycarboxylic acids, FEMS Microbiol. Rev., 25 (2001) 69-106.

[11] A. Cao, A. Carucci, T. Lai, P. La Colla, E. Tamburini, Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria, Eur. J. Soil Biol., 43 (2007) 200-206.

[12] H. Hyvonen, R. Aksela, Complexation of 3-hydroxy-2, 2′ -iminodisuccinic acid (HIDS) with Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} ions in aqueous solution, J. Coord. Chem., 63 (2010) 2013-2025.

[13] M.A. Rahman, H. Hasegawa, K. Kadohashi, T. Maki, K. Ueda, Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction, Chemosphere, 77 (2009) 207-213.

[14] N. Sokubai, Biodegradable Chelating Agent: HIDS, in, Nippon Sokubai Co., Ltd., Tokyo, Japan. http://www.shokubai.co.jp/eng/products/hids.html, 2011.

[15] T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plant, 15 (1962) 473-497.

[16] R.J.M. Hudson, F.M.M. Morel, Distinguishing between extracellular and intracellular iron in marine-phytoplankton, Limnol. Oceanogr., 34 (1989) 1113-1120.

[17] F.M.M. Morel, J.G. Hering, Principles and applications of aquatic chemistry: Complexation, in, John Wiley, New York, USA, 1993, pp. 319-420.

[18] S. García-Marco, N. Martínez, F. Yunta, L. Hernández-Apaolaza, J. Lucena, Effectiveness of ethylenediamine-N(o-hydroxyphenylacetic)-N′ (p-hydroxyphenylacetic) acid (o,p-EDDHA) to supply iron to plants, Plant Soil, 279 (2006) 31-40.

[19] L. Hernandez-Apaolaza, A. Garate, J.J. Lucena, Efficacy of commercial Fe(III)-EDDHA and Fe(III)-EDDHMA chelates to supply iron to sunflower and corn seedlings, J. Plant Nutr., 18 (1995) 1209-1223.

[20] R.A. Jeffreys, V.Q. Hale, A. Wallace, Uptake and translocation in plants of labeled iron and labeled chelating agents, Soil Sci., 92 (1961) 268-273.

[21] H. Hasegawa, M.A. Rahman, K. Saitou, M. Kobayashi, C. Okumura, Influence of chelating ligands on bioavailability and mobility of iron in plant growth media and their effect on radish growth, Environ. Exp. Bot., 71 (2011) 345-351.

Table 1: Composition of modified Murashige and Skoog (MS) growth medium used for growing rice seedlings hydroponically. Sodium salt of EDTA ($Na_2EDTA \cdot 2H_2O$) was not included in preparing the growth medium. $FeCl_3$ was used instead as iron source.

Nutrients	**Concentration (mg L^{-1})**
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
K_2HPO_4	170
$MnSO_4 \cdot 5H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeCl_3$	8.11
pH	5.7

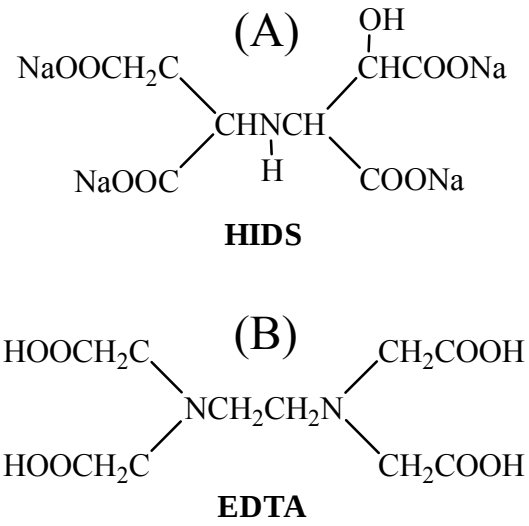

Fig. 1: The chemical structures of ethylenediaminetetraacetate (EDTA) and hydroxyiminodisuccinate (HIDS).

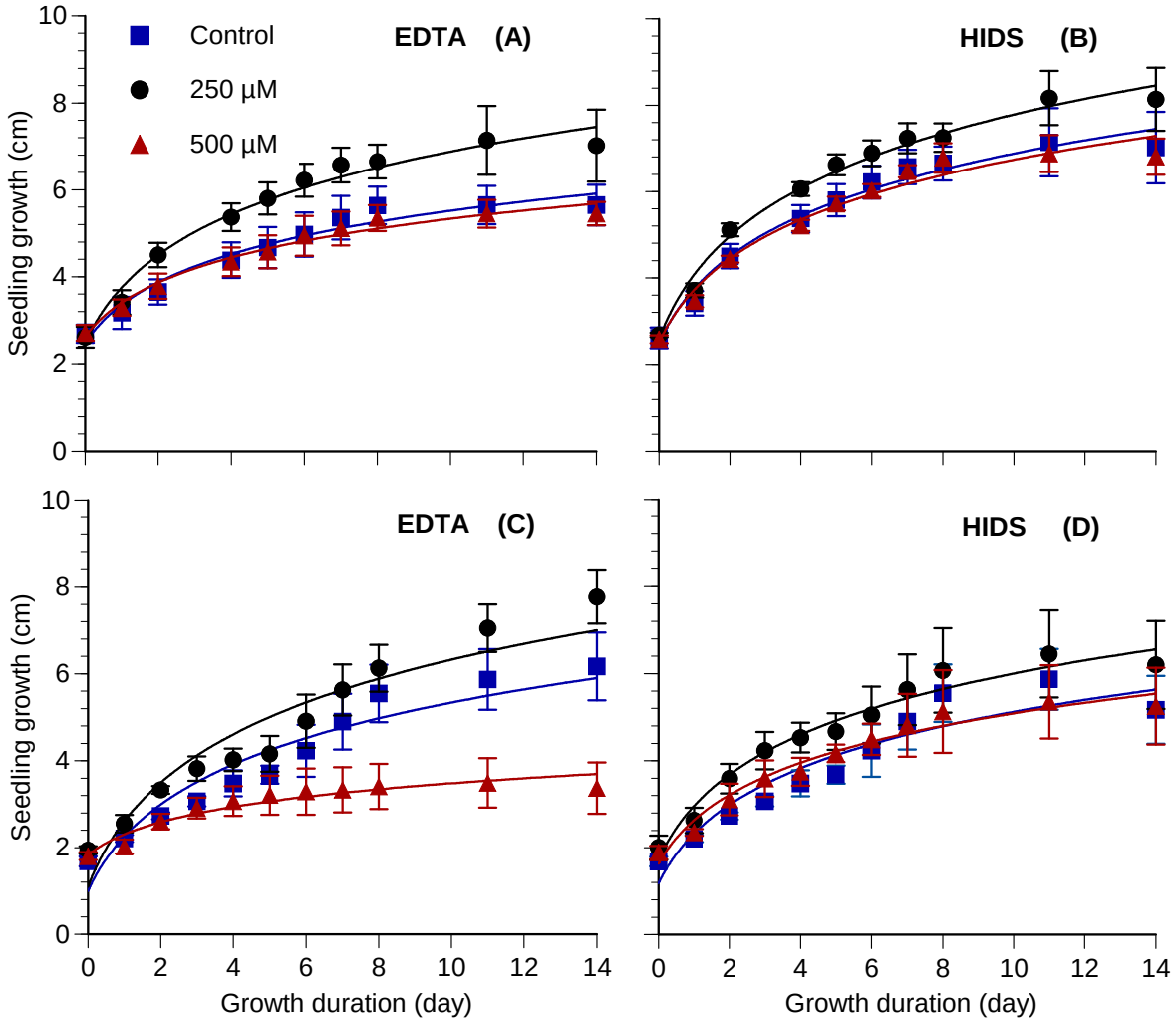

Fig. 2: Rice growth under different concentrations of EDTA and HIDS in sterile (A, B) and non-sterile (C, D) hydroponic medium. The medium was sterilized by oxytetracycline hydrochloride (50 µg mL^{-1}). Values are mean ± SD (n = 3 plants from 3 pots).

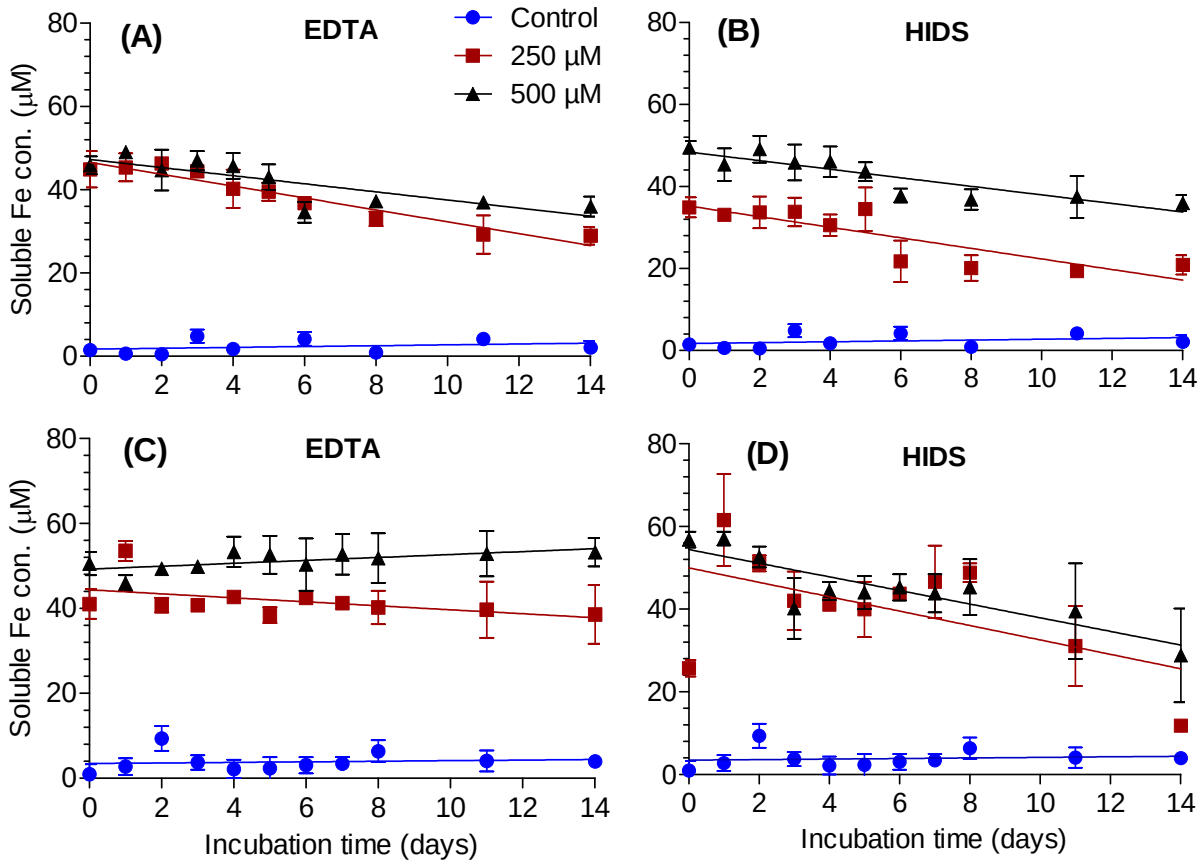

Fig. 3: Time-dependent changes of soluble Fe concentrations affected by EDTA and HIDS in sterile (A, B) and non-sterile (C, D) hydroponic medium. The medium was sterilized by oxytetracycline hydrochloride (50 µg mL^{-1}). Values are mean ± SD (n = 3 plants from 3 pots).

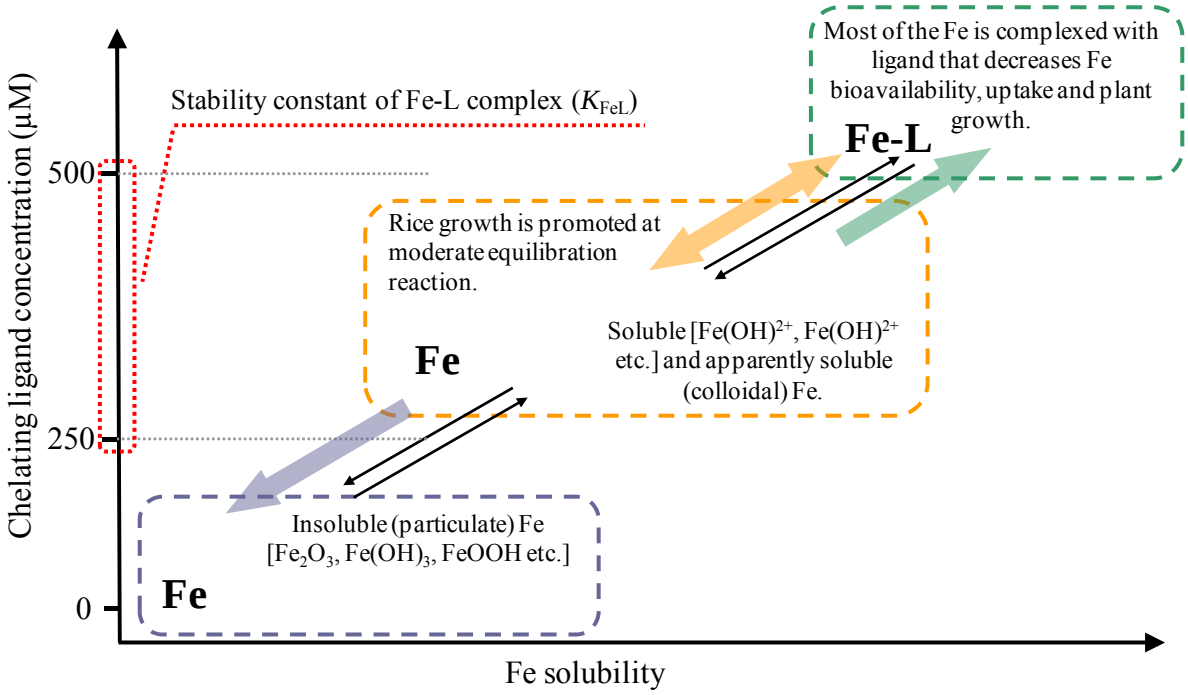

Fig. 4: Complex formation equilibrium reaction (CFER) between chelating ligand and Fe^{3+} in the ligand-treated growth medium influence Fe bioavailability and uptake in rice plant. At moderate ligand concentration, Fe exists in the growth medium predominantly as inorganic [$Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, etc.], Fe-L complexes, and colloidal (apparently soluble) forms, while it exists mainly as Fe-L complexes at high ligand concentration. Fe solubility and bioavailability is also related to the conditional stability constant of Fe-L complex (K_{FeL}) and loop-sided equilibrium reaction between chelating ligand and Fe^{3+}. ⬅ and ➡ indicate the lopsided CFER of Fe-L complex.

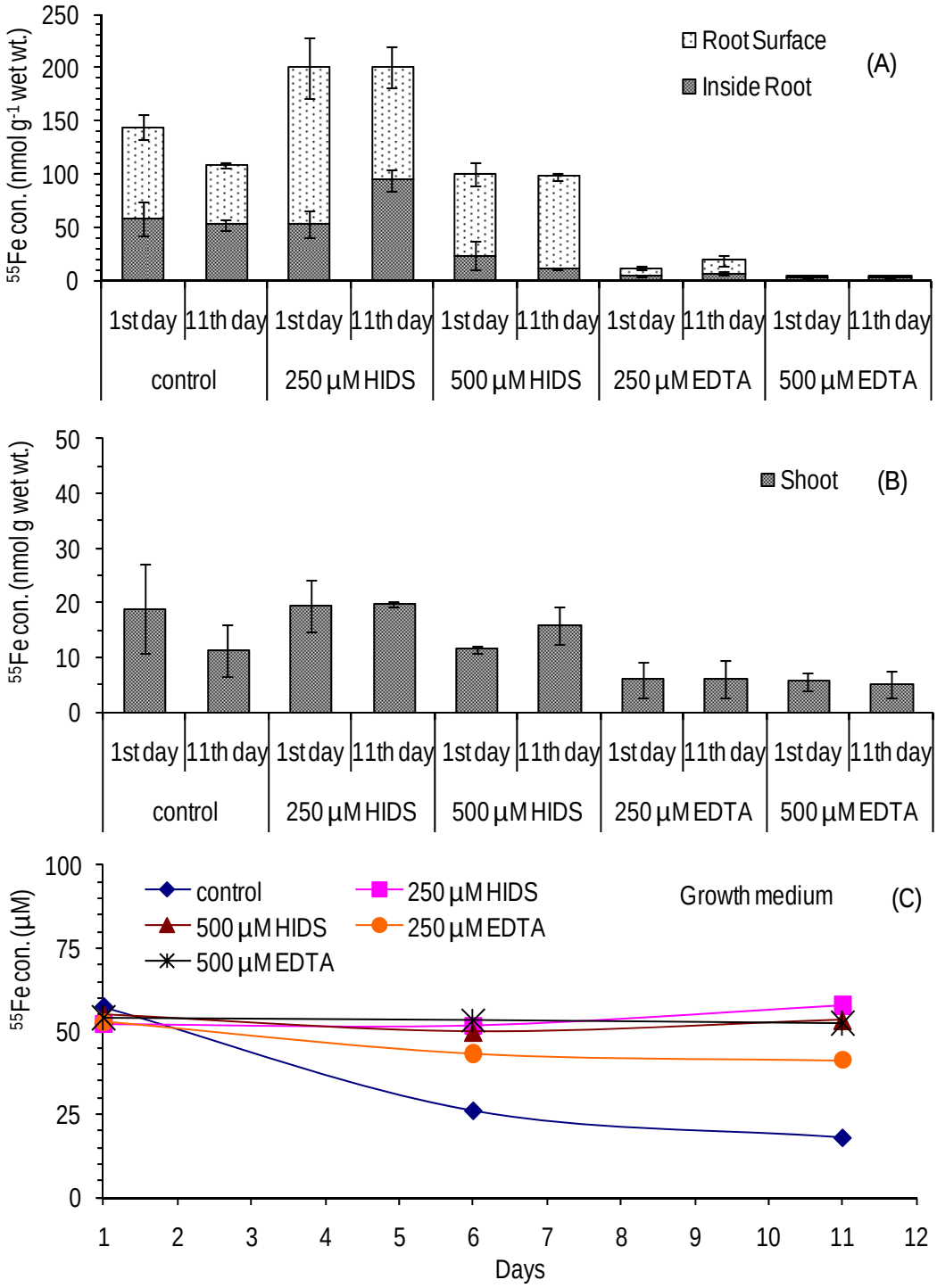

Fig. 5: Concentrations of ^{55}Fe in rice root surfaces, roots, shoots and growth medium. Rice seedlings were incubated in EDTA or HIDS treated growth medium for 24 hours on 1st and 11th day, and the concentrations of radioactive ^{55}Fe were measured in root surfaces and in roots **(A)** and shoots **(B)**. The concentrations of radioactive ^{55}Fe in growth medium were measured at 1st, 6th, and 11th day (C). Values are mean ± SD (*n* = 3 plants from 3 pots).

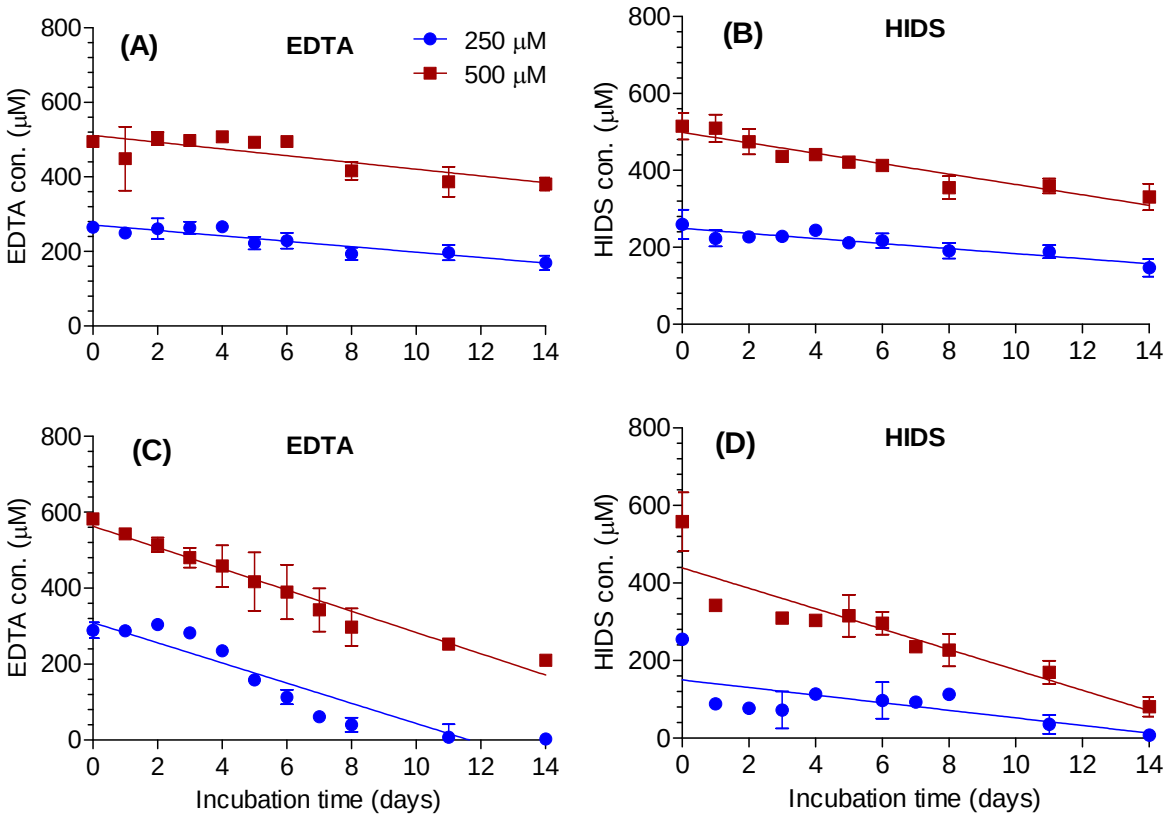

Fig. 6: Time-dependent changes of EDTA and HIDS concentrations in hydroponic MS growth medium in sterile (A, B) and non-sterile (C, D) conditions. The medium was sterilized by oxytetracycline hydrochloride (50 µg mL^{-1}). Values are mean ± SD (*n* = 3 plants from 3 pots).