Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction

1 **Hydroxyiminodisuccinic acid (HIDS): A Novel Biodegradable**

2 **Chelating Ligand for the Increase of Iron Bioavailability and**

3 **Arsenic Phytoextraction**

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24 **Abstract**

25 The influence of biodegradable chelating ligands on arsenic and iron uptake by hydroponically

26 grown rice seedlings (*Oryza sativa* L.) was investigated. Even though the growth solution

27 contained sufficient Fe, the growth of rice seedlings gradually decreased up to 76% with the

28 increase of pH of the solution from 7 to 11. Iron forms insoluble ferric hydroxide complexes at

29 neutral or alkaline pH in oxic condition. Chelating ligands produce soluble 'Fe-ligand complex'

30 which assist Fe uptake in plants. The biodegradable chelating ligand hydroxyiminodisuccinic

31 acid (HIDS) was more efficient then those of ethylenediaminetetraacetic acid (EDTA),

32 ethylenediaminedisuccinic acid (EDDS), and iminodisuccinic acid (IDS) in the increase of Fe

33 uptake and growth of rice seedling. A total of 79±20, 87±6, 116±15, and 63±18 mg dry biomass

34 of rice seedlings were produced with the addition of 0.5 mM of EDDS, EDTA, HIDS, and IDS

35 in the nutrient solution, respectively. The Fe concentrations in rice tissues were 117±15, 82±8,

36 167±25, and 118±22 μmol g^{-1} dry weights when 0.25 mM of EDDS, EDTA, HIDS, and IDS

37 were added to the nutrient solution, respectively. Most of the Fe accumulated in rice tissues was

38 stored in roots after the addition of chelating ligands in the solution. The results indicate that the

39 HIDS would be a potential alternative to environmentally persistent EDTA for the increase of Fe

40 uptake and plant growth. The HIDS also increased As uptake in rice root though its translocation

41 from root to shoot was not augmented. This study reports HIDS for the first time as a promising

42 chelating ligand for the enhancement of Fe bioavailability and As phytoextraction.

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1. Introduction

Iron is an essential micronutrient for plants, which plays important roles in respiration, photosynthesis, and many other cellular functions such as DNA synthesis, nitrogen fixation, and hormone production (Vert et al., 2002). Although abundant in nature it forms insoluble ferric hydroxide complexes (also known as Fe-plaque) at neutral or alkaline pH in oxic condition (Guerinot and Yi, 1994). The Fe-plaque formation in the rhizosphere soils, however, results in the Fe deficiency to plants. In nature, rhizospheric microbes exude siderophores to the root-plaque interface. These siderophores solubilize ferric iron in the rhizosphere, render its bioavailability, and plants uptake the Fe by specific membrane receptors (Romheld, 1987).

Elevated levels of As in soil from natural and anthropogenic sources is a threat to plants' health (Rahman et al., 2008). Remediation of contaminated soil is important to prevent As deposition in food crops and its subsequent transfer into the human body through the food chains (Rahman et al., 2008). Phytoremediation becomes a promising alternative and environmentally safe technology for the remediation of environmental pollutants (Raskin et al., 1997; Tu et al., 2002). An essential prerequisite for phytoremediation of contaminated soil is solubility and bioavailability of As (Fitz and Wenzel, 2002). But the solubility and bioavailability of As becomes reduced by adsorption to variable charged minerals (Fe and Al) at alkaline pH (Xu et al., 2008). In the past decade, chelant-enhanced phytoremediation has received much attention (Pastor et al., 2007). This technique aims to cleanse polluted soils by solubilizing the toxic metals, allowing it to be accumulated in plants that would subsequently remove toxic metal from the site. Publications on chelant-enhanced phytoremediation have increased steadily to about 15-20 per year in the last few years, indicating that this is a growing and active research field (Nowack et al., 2006).

71   Research on the interaction of plants with chelating ligands started in the 1950s with a

72   view to reduce the deficiencies of the essential nutrients such as Fe, Mn, Cu, and Zn (Wenger et

73   al., 2005). Among all soil-applied Fe fertilizers, synthetic Fe(III)-chelates, mainly Fe(III)-

74   chelates of polyaminecarboxylic acids with phenolic groups, such as ethylendiamine di(*o*-

75   hydroxyphenylacetic) acid (EDDHA), and ethylendiamine di(2-hydroxy-4-methylphenylacetic)

76   acid, are the most effective and commonly used (Alvarez-Fernandez et al., 2005). On the other

77   hand reports on As phytoextraction by chelating ligands is limited though a number of

78   investigations have been conducted on chelant-enhanced phytoextraction of Pb, Zn, Hg, Cu and

79   some other heavy metals (Luo et al., 2005). Ethylenediaminetetraacetic acid (EDTA) has been

80   very popular to achieve this purpose, but it is quite persistent in the environment because of its

81   low biodegradability. This, in combination with its high affinity for heavy metal complexation,

82   results in an increased risk of leaching. EDTA also impairs plant growth severely, even at low

83   concentrations (Bucheli-Witschel and Egli, 2001).

84   Biodegradable chelating ligands, such as ethylenediaminedisuccinic acid (EDDS),

85   Hydroxyiminodisuccinic acid (HIDS), and iminodisuccinic acid (IDS) would be good choice and

86   alternative to less biodegradable EDTA. The physicochemical properties of EDDS, EDTA, and

87   IDS have already been discussed and tested for the phytoextraction of heavy metals by a number

88   of researchers (Helena et al., 2003; Evangelou et al., 2007). HIDS is a new chelating ligand

89   introduced by Nippon Shokubai Co. Ltd. It is one of the highly biodegradable (biodegradation

90   rate is about 22.4% within 48 h) and safe chelating ligands. It traps and inactivates various kinds

91   of metals ions over a wide rage of pH, particularly Fe^{3+} and Cu^{2+}, as well as Ca^{2+} and Mg^{2+};

92   shows high stability in harsh conditions and high temperature (80 ºC); is highly soluble in

93   aqueous alkaline solution (Sokubai, 2009). Because of high degradation rate and high stability

94   constant with Fe^{3+} ($pKaFe^{3+}$ is 12.5) of HIDS, we become interested to investigate the

95   effectiveness of the chelating ligand for the increase of Fe bioavailability and phytoremediation

96   of As. The EDTA, EDDS, and IDS were also used in the present study to compare the results of

97   HIDS. Our research approach was to find a biodegradable and eco-friendly chelating ligand that

98   is more desirable then EDTA or EDDS for Fe bioavailability and As phytoextraction.

99

100   **2. Materials and Methods**

101   *2.1. Seed sterilization*

102   Rice seeds of BRRI dhan 29 were collected from Bangladesh Rice Research Institute.

103   The seeds were surface-sterilized before using them in the experiment. For sterilization, about

104   100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate

105   solution for 10 min. After that, the seeds were washed by deionized (DI) water (using an E-pure

106   system (Barnstead)) and kept in DI water at 20 ºC for 24 h. The seeds were then washed and

107   transferred to DI water of 45 ºC for 2 min, and of 52 ºC for 10 min.

108

109   *2.2. Chemicals*

110   Stock solutions of EDTA, EDDS, HIDS, and IDS were prepared by dissolving

111   ethylenediamine-N,N,N',N'-tetraacetic acid (Dojindo Molecular Technologies, Japan),

112   ethylenediamine-N, N'-disuccinic acid (Chelest), tetrasodium 3-hydroxy-2,2'-iminodisuccinate

113   (Nippon Syokubai, Japan), and tetrasodium iminodisuccinate (Bayer) in 0.1 M sodium hydroxide,

114   respectively. Other reagents were of analytical grade or better. All solutions were prepared with

115   DI water.

116

117   *2.3. Nutrient solution*

118   Sterilized rice seeds were germinated on pre-sterilized bloating paper (seed bed) with

119   standard murashige and skoog (MS)(Murashige and Skoog, 1962). Iron concentration in the

120 experimental solution was 0.36 mM while its concentration was 27.8 mg L^{-1} in pre-experimental

121 solution (used for growing rice seedling prior to the experiment). The pH of the pre-experimental

122 solution was adjusted to 6.5 while the pH of experimental solution was 9.0. Rice seedlings were

123 grown on the seed bed for 1 wk. In preparing MS culture solution, $FeSO_4 \cdot 7H_2O$ was used as Fe

124 source instead of NaFe(III)-EDTA.

125

126 *2.4. Experimental setup*

127   Rice seedlings were transferred to the experimental solution after one week of growth in

128 pre-experimental solution. In the experimental solution, rice seedlings were grown in two steps.

129 In the first step, rice seedlings were grown with different concentrations of chelating ligands (up

130 to 2.50 mM) to observe the effect of chelating ligands on Fe uptake. In the second step, 6.0 μM

131 of As ($Na_2HAsO_4 \cdot 7H_2O$) was added to the nutrient solutions containing 1.0 mM of chelating

132 ligands to see the effect of chelating ligands on Fe and As uptake. Iron concentration in the

133 experimental solution was 0.36 mM, and the pH of the solution was adjusted to 9 using 0.1 M

134 KOH. About 100 mL of the solution was taken into 250-mL polystylene bottles with three

135 replications, and three uniform seedlings were cultivated in each bottle. The experiment was

136 performed following randomized design. Rice plants were grown in a plant growth chamber and

137 the conditions in the chamber were set as 14:10 h light/dark schedule, 100-125 μ E $m^{-2} s^{-1}$ light

138 intensity, 22(±2) ℃ temperatures. Rice seedlings were grown in experimental solution for 5 d.

139

140 *2.5. CBE-extraction of Fe-plaques*

141   At harvest, the shoots were cut from 1 cm above the roots and separated. The Fe-plaques

142 from root surfaces were extracted using citrate-bicarbonate-ethylenediaminetetraacetate (CBE)-

143 technique, a modified method of dithionite-citrate-bicarbonate extraction by Taylor and Crowder

144 (1983) to determine the real amount of Fe and As contents in rice tissues. The CBE solution was

145 prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate, and EDTA,

146 respectively. Roots were treated with 30 mL of CBE solution for 60 min at room temperature.

147 The roots were then rinsed with deionized water for 3 times, and the rinsed water was added to

148 the CBE-extracts to make a total of 30 mL.

149

150 *2.6. Sample preparation*

151 After rinsing with deionized water for four times, the root samples were kept on clean

152 absorbent paper to remove the water from the root surfaces. Both the root and the shoot samples

153 were dried at 65 ºC until they reached in a constant weight. Then the dried samples were

154 weighted and taken into 50-mL polyethylene tubes for digestion. Five mL of 65% HNO_3 were

155 added to the sample and kept for 12 h. The samples were heated on a heating block at 95 ºC for 2

156 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide were added, and the

157 samples were heated again at 105 ºC for 20 min. Then, the digests were diluted to 30 mL with DI

158 and analyzed for As and Fe.

159

160 *2.7. Chemical analysis*

161 Arsenic and Fe were analyzed using graphite-furnace atomic absorption spectrometer (Z-

162 8100, Hitachi, Japan). Certified standard reference material 1573a (tomato leaf from NIST,

163 USA) was used to check the accuracy of analysis. Arsenic concentration in certified standard

164 reference materials was 0.112 ± 0.004 µg g^{-1} dry weight (all the reported data in this article are

165 expressed as dry weight) while the measured concentration was 0.114 ± 0.002 µg g^{-1}. The

166 concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01

167 µM in water sample).

168 All chemical reagents used in this experiment were of analytical grade. Glassware and

169 dishes were washed with detergent and 1 N HCL solution, and rinsed with DI water for eight

170   times before use. In each analytical batch, at least two reagent blanks and three replicate samples

171   were included.

172

173   **3. Results and Discussions**

174   *3.1. Effect of pH on rice growth*

175   Rice seedlings were grown in nutrient solution adjusted to different pH ranging between

176   6 and 11. Results show that the biomass production of rice seedlings was affected by the pH

177   significantly. The highest biomass of rice seedling (83±7 mg) was observed at pH 7, which was

178   about 16, 19, 43, and 76% higher than those at pH 8, 9, 10, and 11, respectively (Fig. 1). The rice

179   growth remain unchanged, and even died at pH 10 and 11. Rice plants have a tendency of higher

180   Fe uptake than that of other plants (Becker and Asch, 2005). But the pH of the growth medium

181   plays an important role in Fe bioavailability and uptake. Even though the Fe is sufficient in

182   growth medium, it forms insoluble ferric hydroxide complexes at alkaline pH in oxic condition

183   (Cohen et al., 1998). Therefore, Fe bioavailability and uptake decreases drastically. In the present

184   study, it was observed that the Fe concentrations in tissues of rice seedlings were highest at pH 7

185   compared to those at other pHs (Fig. 2). This trend of Fe uptake in rice tissues is correlated to

186   that of biomass production of rice seedlings (Fig. 1). The result implies that the influence of pH

187   on rice growth is the ultimate effect of reduced Fe bioavailability and uptake. Moreover, Fe

188   concentrations on root surfaces of rice seedlings were lowest at pH 7 and 8 compared to those at

189   other pHs (Fig. 2). High level of Fe on root surfaces of rice seedling at pH 11 reveals the

190   formation of Fe-hydroxides (Fe-plaque) on root surfaces, which decreased the Fe uptake in rice

191   tissues. Formation of Fe-plaques on the roots of wetland plants (Hansel et al., 2001) and

192   hydroponically grown rice seedling (Hu et al., 2005) have also been reported. The precipitation

193   of ferric (oxyhydro)-oxides (FeO_x) and its association with phytoplankton surfaces, both in

194 natural conditions and laboratory cultures, has been reported by Tang and Morel (2006).

195 Robinson et al. (2006) also found the occurrence of Fe-plaque on aquatic macrophytes collected

196 from the Taupo Volcanic zone, New Zealand.

197   The Fe deficiency results in Fe-chlorosis in green leaves, which retards plant growth, and

198 leads to the reduction of crop yields (Guerinot and Yi, 1994). The results of the present study

199 also reveal that the growth of rice seedling decreased drastically at higher pH, which is the

200 consequence of Fe-chlorosis.

201

202 *3.2. Influence of chelating ligands on Fe uptake-translocation*

203   Influence of EDDS, EDTA, HIDS, and IDS on Fe uptake and translocation in rice

204 seedlings were investigated at different concentrations of the ligands ranging between 0.1 and

205 2.5 mM. Results showed that Fe uptake in rice seedling differed significantly with the type and

206 concentrations of the chelating ligands. Iron uptake was highest at 0.25 mM of the chelating

207 ligands compared to the control treatment. Iron uptake decreased gradually with the increase of

208 chelating ligand concentrations above 0.25 mM (Fig. 3). The effectiveness of HIDS and EDDS

209 in the increase of Fe uptake in rice tissues was higher than that of EDTA and IDS. Iron

210 concentrations in roots of rice seedling were 35 ± 3 and 44 ± 2 $\mu mol\ g^{-1}$ when the HIDS

211 concentrations in the nutrient solution were 0.10 and 0.25 mM, respectively. These

212 concentrations were significantly higher than those of other chelating ligands.

213   Iron concentrations in shoots of rice seedlings were significantly lower than those in roots,

214 and were about identical up to 0.25 mM of chelating ligand treatment. Iron content in shoots

215 decreased with the gradual increase of chelating ligands from 0.25 to 2.50 mM (Fig. 3). The

216 results indicate that the translocation of Fe from roots to shoots was not affected by lower dose

217 of the chelating ligands. The translocation of Fe was inhibited by the chelating ligands at higher

218 doses (> 0.25 mM).

219   Although abundant in nature, Fe is often unavailable to plants, especially at neutral or

220   alkaline pH, because of the formation of insoluble ferric hydroxide complexes in oxic condition

221   (Robinson et al., 2006). Precipitation of Fe in the rhizosphere, however, may result in the Fe

222   deficiency to the plants. Chelating ligands are used in agriculture as additives in micronutrient

223   fertilizers for the increase of Fe bioavailability. Although some chelating ligands have been

224   reported to increase Fe uptake/translocation in plant, inhibition of Fe uptake/translocation by

225   ligands   has   also   been   reported.   Chaney   et   al.   (1972)   reported   that

226   bathophenanthrolinedisulfonate   (BPDS)   was   the   most   effective   inhibitor   of   Fe

227   uptake/translocation, followed by EDTA > DTPA (diethylenetriaminepentaacetic acid) > CDTA

228   (diaminocyclohexanetetraacetic acid) >> EDDHA. The BPDS inhibited ^{59}Fe movement to the

229   exudate by 99.7% even at the lowest level of competitor. The BPDS inhibits Fe translocation by

230   10-100 times compared to those of EDTA, DTPA, or CDTA. Chaney et al. (1972) also observed

231   that EDDHA, the chelator with the highest Fe^{3+} stability constant, only slightly inhibited or

232   actually promoted Fe uptake/translocation, whereas the BPDS with the highest Fe^{2+} stability

233   constant was a severe inhibitor. Thus, stability constant of Fe-ligand ($logK_{FeL}$) would be one of

234   the important determinants for the promotion or inhibition of Fe uptake/translocation.

235   *3.3. Effect of chelating ligands on rice growth*

236   Rice seedlings were grown in alkaline nutrient solution (pH 9) containing 0.10, 0.25, 0.50,

237   1.00, and 2.50 mM of chelating ligands and 0.36 mM of Fe. Results show that the growth of rice

238   seedlings was increased with the increase of HIDS and EDTA concentrations up to 1.0 mM, and

239   the growth was decreased at 2.5 mM of chelating ligand concentrations (Fig. 4). The highest

240   biomass production (141±21 mg) of rice was observed when 1.0 mM of EDTA was added to the

241   nutrient solution followed by 127±8, 82±19, and 75±4 mg for HIDS, EDDS, and IDS,

242   respectively.

243   Chelating ligands have been used to enhance Fe bioavailability (Alvarez-Fernandez et al.,

244   2005). The concentration of chelating ligands in the nutrient medium is important for the

245   solubilization of precipitated Fe and the increase of its bioavailability. In the present study, it was

246   observed that the rice seedling produce highest biomass at 1.0 mM chelating ligand

247   concentrations, and the growth remain unchanged, and even died at higher concentration (>1.0

248   mM).

249   Although the growth of all organisms is dependent on the acquisition of the proper

250   quantities of trace elements, excess amount of some metals such as Fe, zinc, manganese, and

251   copper produce toxic effects (Morel and Hering, 1993). However, ferric ions and their complexes,

252   which have low solubility in aquatic system, are extensively buffered by chelation (Morel and

253   Hering, 1993), and increase their dissolved concentration. The dissolved concentration of Fe

254   determines its rate of uptake by the organisms. Anderson and Morel (1982) reported that the Fe

255   uptake rate in laboratory cultures of the marine diatom *Thalassosira weissflogii* is a unique

256   function of the free ferric ion concentration at the presence of 10^{-5} M of various chelating ligands

257   (1.4×10^{7} cells L^{-1}). Hudson and Morel (1990) reported that in Fe-limited culture of marine

258   diatom *Thalassosira weissflogii* (10^{7} cells L^{-1}) containing 10^{-8} M Fe and 10^{-5} M EDTA and with

259   white-light illumination, both the thermal dissociation of FeEDTA and its photoreduction and

260   reoxidation contribute to the formation of the dissolved inorganic Fe(III) pool responsible for the

261   Fe uptake. In this case, growth of rice seedlings was inhibited by the free ferric ion that was

262   increased by the addition of higher level of chelating ligands.

263   Toxicity of chelating ligands on plants has not been studied extensively. So, it is difficult

264   to interpret the direct toxicity of chelating ligands on plants. Since most of the chelating ligands

265   are synthetic compounds, no nutrient carriers in the plasma membrane are thought to exist

266   (Berne and Levy, 1998). Also, synthetic chelates cannot slip through the plasma membrane as

267   they are too large and polar to move through the plasma lemma lipid bilayer (Berne and Levy,

268 1998). Tanton and Crowdy (1972) observed that most solutes moved into some endodermal

269 passage cells adjacent to the casparian strip intracellularly to the other side of the strip, and then

270 extracellularly to the xylem. The passage cells may include the aquaporins and there may be

271 selectivity toward molecules. Paul et al. (2003) reported that Swiss chard uptakes a considerable

272 amount of EDTA from chelator-buffered hydroponic solution through transpirational flow that

273 occurs via apoplastically.

274

275 *3.4. Influence of chelating ligands and As on rice growth*

276 Chelating ligand treated rice seedlings were grown with and without As to investigate the

277 effect of As and chelating ligands on rice growth. Results show that As does not have a

278 consistent effect on rice growth as chelating ligand has. Rice growth was not affected by As

279 when chelating ligand was not treated. The highest growth of rice seedling was observed in

280 HIDS treated medium. The inconsistent effect of chelating ligand and As on rice growth suggest

281 that in the presence of chelating ligands lower level of As in the growth medium does not affect

282 rice growth significantly. It has been reported that rice growth is not affected by low level of As

283 though the growth decrease drastically with the increase of As in the soil. Abedin and Meharg

284 (2002) also reported that low level of As in water (about 2.0 mg L^{-1}) does not show toxicity to

285 both rice germination and rice growth, but the rice germination and growth were aversely

286 affected by higher As level.

287

288 *3.5. Influence of chelating ligands and As on Fe uptake/translocation*

289 Iron uptake in rice seedling was affected by chelating ligands and As significantly. Iron

290 concentration was measured both in root surfaces and plant tissues. Results show that the Fe

291 concentration was higher in rice root surfaces of control treatment (without chelating ligands)

292 while its concentration was higher in plant tissues of ligand treated nutrient solution (Fig. 5). The

293   highest Fe contents were found in tissues of rice seedlings treated with EDTA or HIDS and As.

294   Increasing Fe uptake by chelating ligands, especially EDTA and HIDS, can be explained by the

295   adsorption of As(III)-EDTA/-HIDS complex on the Fe-plaques of rice root surfaces and

296   dissociation of the complex to release of Fe(III)-EDTA/-HIDS into solution. The release of

297   Fe(III)-EDTA/-HIDS into the culture solution results in the increase of Fe uptake. Adsorption of

298   metal-EDTA to the surface of Fe oxides and dissociation of the complex and release of Fe(III)-

299   EDTA has been reported by Nowack and Sigg (1997).

300   Strong ligands, such as EDTA, complex with metals in natural systems. Adsorption of

301   uncomplexed EDTA on metal oxides (Fe-oxides, Al-oxides) has been studied previously

302   (Bowers and Huang, 1985; Blesa et al., 2000). The EDTA has been reported to exist as complex

303   species of metals (mainly CaEDTA, ZnEDTA, and Fe(III)EDTA) in natural waters (Xue et al.,

304   1995). Dissolution reactions of Fe-oxides in the presence of metal-EDTA complexes have also

305   been reported by Nowack and Sigg (1997).

306

307   *3.6. Arsenic uptake/translocation affected by chelating ligands*

308   Arsenic contents in roots, shoots, and root surfaces of rice seedling were determined to

309   asses the effect of chelating ligands on As uptake. Results show that As was stored mostly in

310   roots followed by shoots and root surfaces (Fig. 6). Previous studies with rice also reported

311   higher content of As in rice roots (Abedin et al., 2002). The higher storage of As in roots and

312   lower translocation to shoots can be explained by the reduction of arsenate to arsenite in roots,

313   complexation with thiols, and sequestration in the root vacuoles (Zhao et al., 2009).

314   Formation of Fe-plaque on rice root surfaces and its effect on As uptake in rice have been

315   well explained in literature (Liu et al., 2006). Although Fe-plaque inhibits the As uptake (Zhang

316   et al., 1998), increase of the uptake of toxic and nutrient elements in plants and organisms by Fe-

317   plaque has also been reported (Ye et al., 2001). The effects of Fe-plaque on the uptake of nutrient

318 and/or toxic elements depend on the amount of Fe-plaque on root surfaces (Zhang et al., 1998).

319 Otte et al. (1989) reported higher concentration of Zn in roots of *Aster tripolium* L. coated with

320 500-2000 nmol Fe cm^{-2} compared to those coated with less than 500 or more than 2000 nmol Fe

321 cm^{-2}. Even though the increasing amount of Fe-plaque elevates As accumulation on the root

322 surfaces, it does not affect As uptake in rice shoots. The Fe-plaque acts as "buffer" to prevent the

323 translocation of As from roots to shoots Liu et al. (2004).

324 Present study also report that the As contents in roots and shoots were higher in rice

325 seedlings grown with chelating ligands compared to those grown without chelating ligands (Fig.

326 6). Arsenic content in roots was highest when the rice seedlings were grown with HIDS while

327 the content was identical when grown with EDTA, EDDS, or IDS. The results suggest that

328 chelating ligands increased As uptake in rice root significantly, though its translocation form root

329 to shoot was not increased. The use of chelating ligands, especially the EDTA, EDDS, IDS, etc.

330 for the increase of heavy metals have been studied extensively (Jean et al., 2008; Marques et al.,

331 2008). Present study reports a better/comparable performance of HIDS to that of others studied

332 previously for the first time.

333 Arsenate has a high adsorptive affinity to Fe oxides (Zhao et al., 2009). Chelating ligands

334 solubilization/desorption As from the Fe-plaque of rice roots, and rice plant readily uptakes

335 desorbed/soluble As from the nutrient solution. The results of the present study reveal that the

336 HIDS is stronger then EDTA, EDDS, or IDS for dissolution/desorption of precipitated As. Since

337 the EDTA is not readily biodegradable, and is persistent in the environment, the biodegradable

338 HIDS would be a good alternative to EDTA in the phytoextraction/phytoremediation of As.

339

340 **4. Conclusions**

341 The use of chelating ligands in the phytoextraction of toxic metals and in the increase of

342 essential nutrient elements is not new at all. Especially, the EDTA and EDDS have been widely

343 used in agriculture for long time to serve the above purposes. The use of EDTA, however, has

344 the disadvantage that it is quite persistent in the environment due to its low biodegradability.

345 Therefore, looking for biodegradable chelating ligands is an important concern to the researchers.

346 In this study the effectiveness of HIDS for the increase of Fe bioavailability and As

347 phytoextraction was investigated, and the results were compared with those of EDTA, EDDS,

348 and IDS. The Fe limiting condition was induced by increasing the pH of the growth solution.

349 Results show that the performance of HIDS was more effective then that of other chelating

350 ligands. HIDS is a newly introduced, biodegradable and environmentally harmonious chelating

351 ligands with high chelating capability. Therefore, it would be a good alternative to the EDTA.

352

353 **Acknowledgement**

356

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492   Figure Captions:

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494   Fig. 1: Growth of rice (*Oryza sativa* L.) affected by the pH of nutrient solution. Results are
495   presented as mean and the error bars express ± SD (*n* = 3).

496

497   Fig. 2: Fe concentration in roots, root surfaces, and shoots of rice seedling (*Oryza sativa* L.) as
498   affected by the pH of nutrient solution. Results are presented as mean and the error bars
499   express ± SD (*n* = 3).

500

501   Fig. 3: Fe uptake and translocation in rice seedling (*Oryza sativa* L.) as affected by chelating
502   ligand concentrations in the nutrient solution. Results are presented as mean and the error
503   bars express ± SD (*n* = 3).

504

505   Fig. 4: Growth of rice seedling (*Oryza sativa* L.) affected by chelating ligand concentrations in
506   the nutrient solution. Results are presented as mean and the error bars express ± SD (*n* = 3).

507

508   Fig. 5: Fe concentration in root surfaces and plant tissues (roots and shoots) of rice seedling
509   (*Oryza sativa* L.) as affected by chelating ligands and arsenic in the nutrient solution.
510   As(+) and As(-) indicate with and without arsenic, respectively.
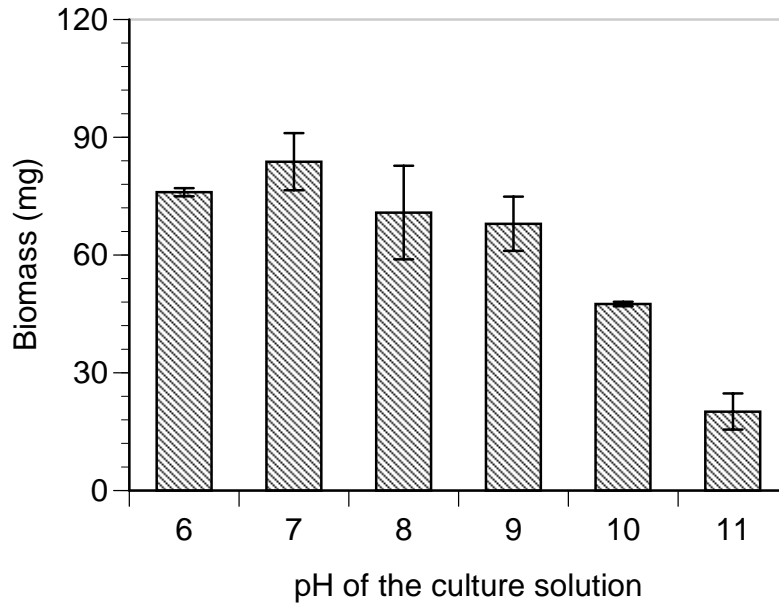
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512   Fig. 6: As concentration in roots, shoots, and root surfaces of rice seedling (*Oryza sativa* L.) as
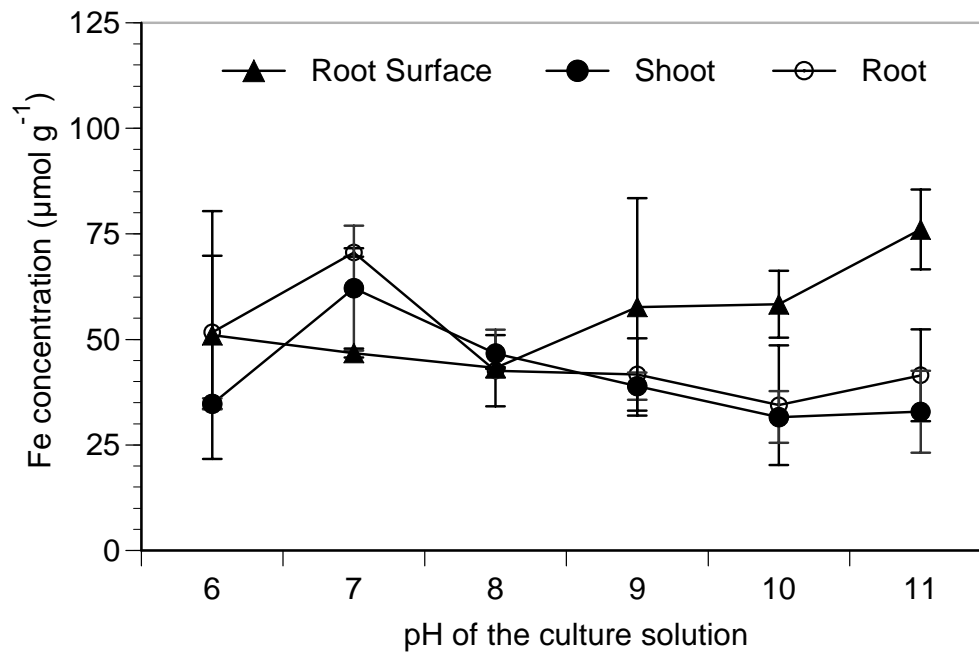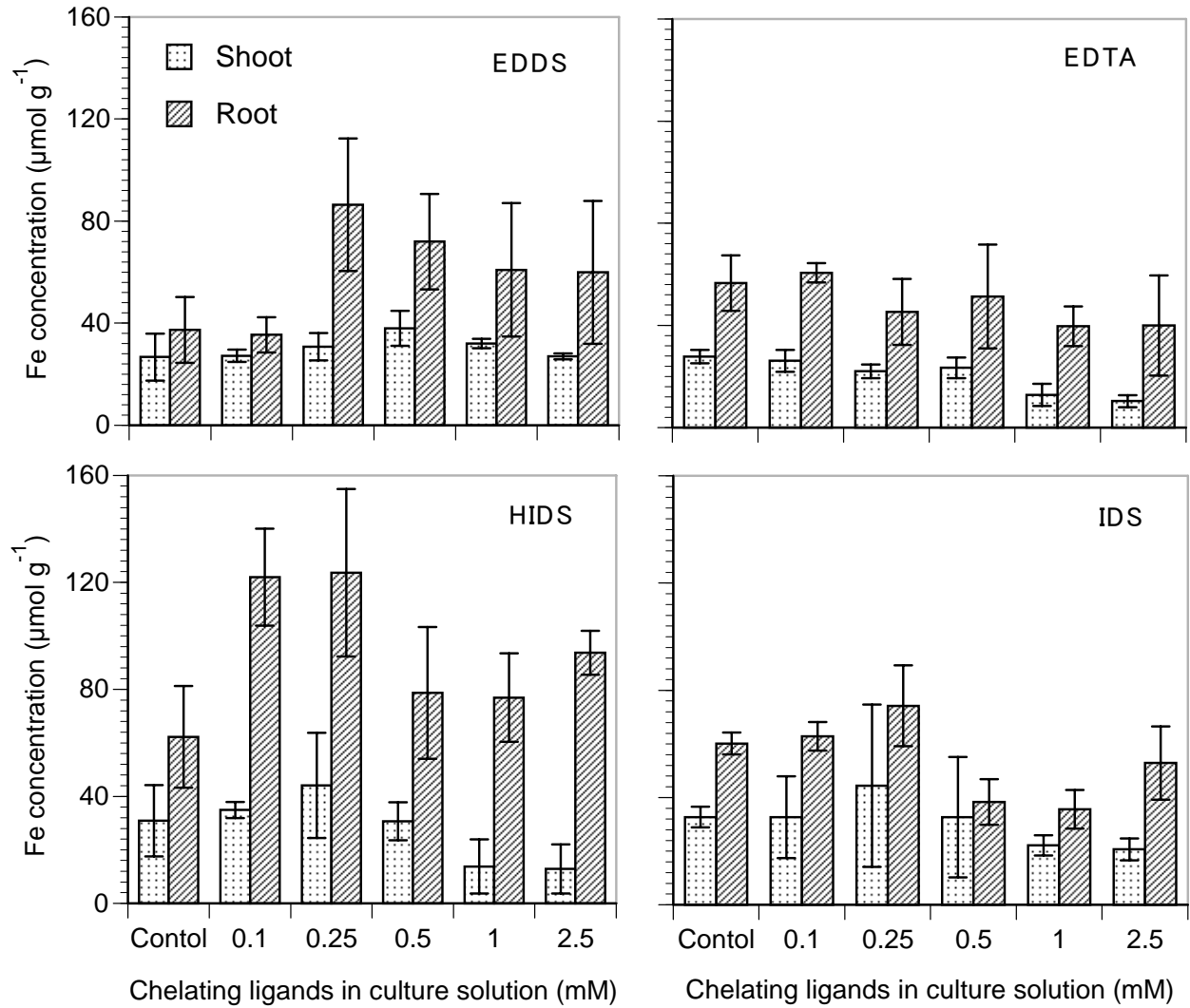513   affected by chelating ligands in the nutrient solution.

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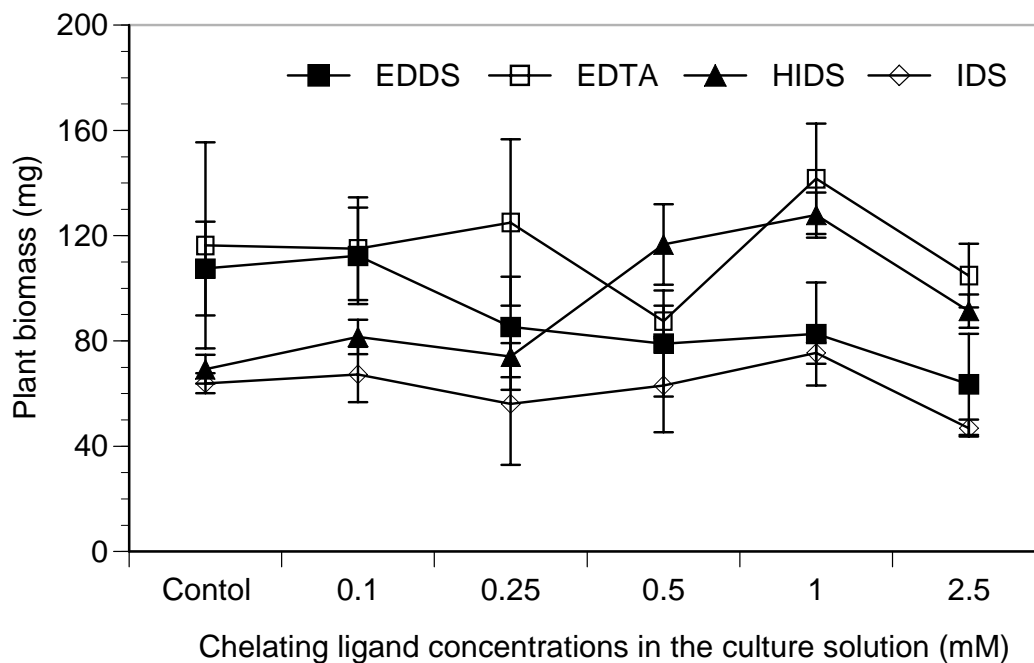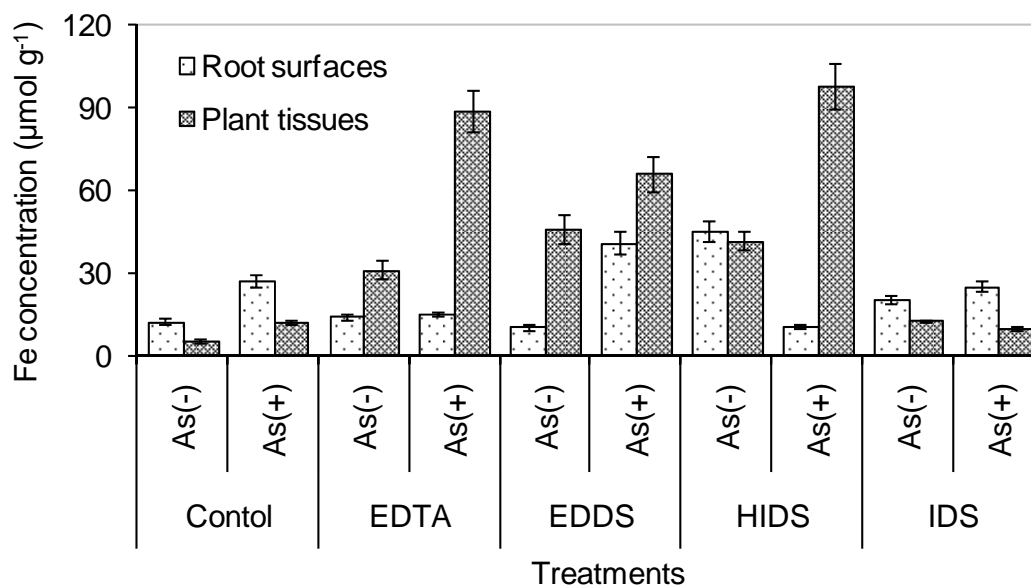
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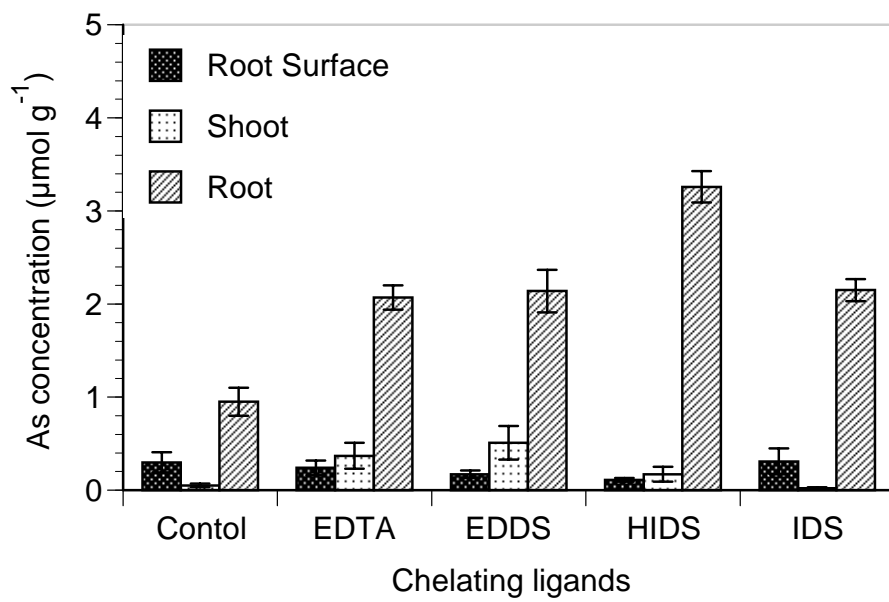540

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