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Iron-chelate stability and speciation in a nitrogen fertilizer matrix: implications for micronutrient fertilizer formulation

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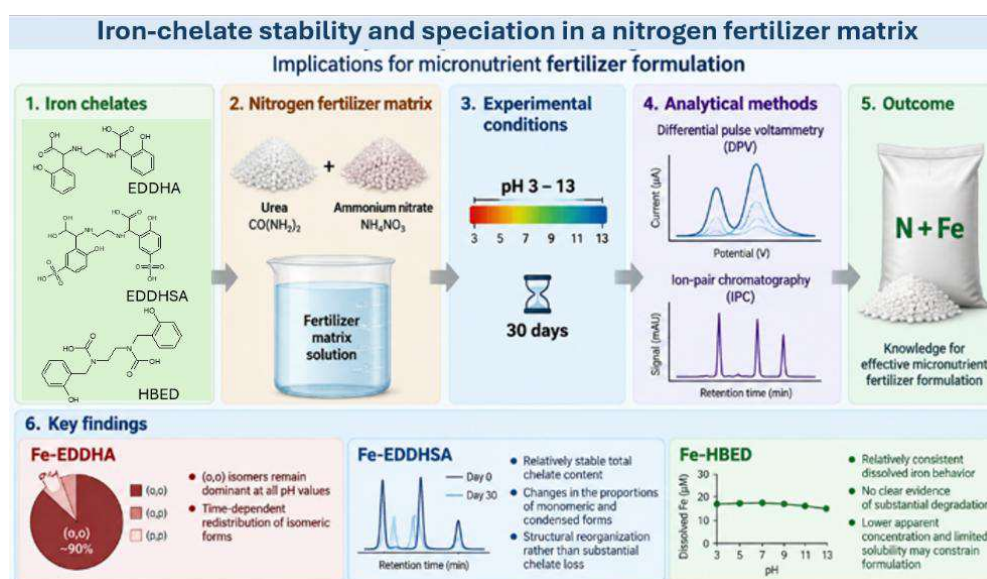
Abstract:

Background Maintaining iron in chemically stable and soluble forms is important for the effective incorporation of micronutrients into nitrogen-based fertilizer formulations. This study evaluated the stability and speciation of Fe-EDDHA, Fe-EDDHSA, and Fe-HBED in a model nitrogen fertilizer matrix based on urea and ammonium nitrate.

Results Iron speciation was investigated by differential pulse voltammetry over a pH range of 3–13, while chelate composition and structural changes were monitored by ion-pair chromatography over 30 days. All three chelates showed substantial iron complexation under the investigated fertilizer-matrix conditions, although their behaviour depended on pH and ligand structure. Fe-EDDHA remained dominated by the more stable (o,o) isomers, with gradual redistribution among isomeric forms during storage. Fe-EDDHSA maintained relatively stable total chelate content, despite changes in the relative proportions of monomeric and condensed forms, suggesting structural reorganization rather than substantial chelate loss. Fe-HBED exhibited relatively consistent dissolved iron behaviour and no clear evidence of substantial degradation; however, its lower apparent concentration and limited solubility may constrain its use in fertilizer formulations. Compared with the reference electrolyte, the nitrogen fertilizer matrix altered the concentration of dissolved uncomplexed Fe(III), indicating that fertilizer composition affects iron speciation under these conditions.

Conclusion The results demonstrate that both ligand structure and fertilizer matrix composition influence the stability and speciation of chelated iron. The observed differences among Fe-EDDHA, Fe-EDDHSA, and Fe-HBED highlight the need to consider matrix effects when evaluating chelated micronutrients for fertilizer formulations. These findings provide a chemical basis for further studies of storage stability, nutrient release, solubility, and agronomic performance of chelated iron in nitrogen-based fertilizers.

Graphical abstract:



Keywords: chelates, micronutrients, stability, Fe-EDDHA, Fe-EDDHSA, Fe-HBED

1. Introduction

The transition toward more sustainable agricultural systems requires the development of fertilizers that maximize nutrient use efficiency while minimizing environmental impact. Conventional fertilization strategies often treat macronutrients and micronutrients separately, leading to suboptimal nutrient delivery and increased risk of environmental losses. Integrating micronutrients into nitrogen-based fertilizers represents a promising approach to improve nutrient management and reduce application rates.

Iron deficiency remains a major limitation in crop production, particularly in calcareous and alkaline soils, where low solubility of iron compounds restricts plant availability despite sufficient total iron content in soil systems [1–3]. In alkaline and calcareous soils, iron supplied in soluble forms is readily affected by precipitation and soil interactions, which limits its persistence in solution [4, 5]. As a result, synthetic chelating agents are widely used to maintain iron in soluble forms and improve its availability over a broad pH range [6–8].

Chelating agents, particularly aminopolycarboxylates, form strong metal–ligand complexes characterized by high thermodynamic stability and water solubility. These properties underpin their effectiveness in agricultural applications but also raise environmental concerns. Persistent chelators such as EDTA can reach aquatic environments and may enhance metal mobilization [4, 9–11]. Differences in chelate structure significantly influence their chemical behaviour and

interaction with metals and soils, highlighting the need for careful selection of ligands in sustainable fertilizer design [12–14].

Among available ligands, phenolic chelators such as EDDHA and EDDHSA are effective in maintaining iron in solution under alkaline conditions [1, 14, 15], while HBED has also demonstrated high Fe(III) stability and effectiveness under agronomic conditions [16, 17]. Their chemical stability is related to ligand structure and the resulting metal–ligand equilibria [12, 18]. These chelates are therefore used to correct iron chlorosis in crops grown on calcareous soils [14, 18].

EDDHA-based chelates are structurally complex systems consisting of multiple isomers formed during synthesis via the Mannich reaction. The relative distribution of (o,o), (o,p), and (p,p) isomers significantly influences chelation efficiency and agronomic performance [19–22]. The (o,o) isomers exhibit the highest stability, while (o,p) forms contribute to faster plant uptake and broader micronutrient complexation capacity [21–25]. In contrast, the (p,p) isomer shows negligible chelating ability and is typically considered an inactive byproduct [26].

EDDHSA represents a structurally distinct class of chelators that exist as both monomeric species and condensation products. Despite this heterogeneity, these forms display comparable complexation properties and have been shown to provide effective iron delivery at lower application rates [1, 15]. Their higher solubility and reduced soil interaction suggest a potentially lower environmental impact compared to more persistent chelators [18, 27].

HBED, in contrast, is a structurally well-defined chelator without the isomeric complexity characteristic of EDDHA. It exhibits very high Fe(III) stability and has demonstrated effectiveness in maintaining iron in solution and supplying iron under agronomic conditions [16, 17, 28]. However, its behaviour in complex fertilizer systems remains less explored.

Although the stability of iron chelates in aqueous systems and soils has been extensively studied [14, 22, 29], their performance in multicomponent fertilizer matrices is not yet fully understood. Unlike our previous study, which examined chelate stability under aqueous conditions [30], the present work evaluates Fe-EDDHA, Fe-EDDHSA and Fe-HBED in a defined urea–ammonium nitrate matrix, allowing the effect of fertilizer components on iron speciation and time-dependent chelate transformations to be assessed. Because the fertilizer matrix contains multiple nitrogen-containing components, its combined chemical environment may affect metal speciation and ligand behaviour. These interactions are particularly relevant in the context of multifunctional fertilizers, where compatibility between components is essential to prevent precipitation and maintain nutrient availability.

In this context, the present study investigates the stability of Fe-EDDHA, Fe-EDDHSA, and Fe-HBED in a model nitrogen fertilizer environment.

Materials and Methods

Materials

All reagents used for synthesis and analytical measurements were of analytical grade and were comparable to those previously reported by Klem-Marciniak et al. [30]. The HBED chelating agent was obtained as a commercial product (ADOB, Poland).

A simulated nitrogen fertilizer matrix was prepared using urea (p.a., POCH, Gliwice, Poland) and ammonium nitrate (p.a., POCH, Gliwice, Poland) mixed in a 1:1 molar ratio. The resulting solution contained 3.5 wt% total nitrogen, comprising 1.75 wt% amide nitrogen and equal contributions of nitrate and ammonium nitrogen.

Synthesis and Experimental Design

Chelator synthesis was performed under laboratory conditions following procedures analogous to those described in the literature [30]. The chemical stability of Fe-EDDHA, Fe-EDDHSA, and Fe-HBED chelates in the simulated nitrogen environment was evaluated using differential pulse voltammetry (DPV) and ion-pair chromatography.

Voltammetric measurements enabled the quantification of non-complexed iron ions present in the fertilizer matrix as a function of pH. Experiments were conducted at pH values of 3, 5, 7, 9, 11, and 13. Chromatographic analyses were applied to determine the total chelate content (EDDHA, EDDHSA, HBED), as well as the distribution of individual EDDHA isomers and EDDHSA forms at pH 3, 5, and 7. The restricted pH range for chromatographic measurements resulted from the operational limits of the analytical column. Both analytical approaches were applied over a 30-day observation period.

Differential Pulse Voltammetry

The degree of iron complexation was determined by differential pulse voltammetry using an AUTOLAB PGSTAT 12 potentiostat controlled by GPES software. Measurements were carried out with a static mercury drop electrode (SMDE, 663 VA Stand) as the working electrode. A silver/silver chloride electrode was employed as the reference electrode, and a glassy carbon electrode served as the auxiliary electrode.

Because ligand complexation behavior is strongly pH-dependent, measurements were performed at pH 3, 5, 7, 9, 11, and 13. The pH was adjusted with 0.1 M HCl or NaOH solutions. Analyses were conducted both in a reference electrolyte and in the simulated fertilizer matrix. The reference electrolyte consisted of 0.1 M potassium perchlorate (KClO₄).

Each analyzed sample contained defined amounts of the micronutrient cation, chelating agent, gelatin, and either the supporting electrolyte or fertilizer solution. After pH adjustment, the final solution volume (25 cm³) was transferred to the electrochemical cell. Prior to measurement, dissolved oxygen was removed by purging with nitrogen gas (Messner). All sample preparation and measurements were performed at room temperature and atmospheric pressure under constant background electrolyte concentration. Each measurement was performed in triplicate, and the reported values represent the arithmetic mean of three replicate determinations

Ion-pair Chromatography

The qualitative and quantitative analysis of EDDHA and HBED isomers was performed in accordance with EN 13368 [31], which enables the determination of EDDHA, EDDHMA, and HBED, as well as the separation of *d,l-rac* Fe-(*o,o*)EDDHA, *meso* Fe-(*o,o*)EDDHA, Fe-(*o,p*)EDDHA, and Fe-(*p,p*)EDDHA isomers.

Chromatographic measurements were carried out using a Dionex ICS 5000+ system equipped with a Eurospher II 100 Vertex Plus C18A column (4.0 × 150 mm, 5 μm) and a guard column. Isocratic elution was applied at a flow rate of 1.0 cm³ min⁻¹ with an injection volume of 20 μL. Detection was performed using a UV–Vis detector at 280 nm. The total analysis time was 15 min. Demineralized water served as the solvent. Eluents were degassed in an Emmi 30 HC ultrasonic bath (EMAG AG), and all samples were filtered through nylon syringe filters (0.45 μm, 25 mm).

The distribution of individual Fe-EDDHSA forms, including condensation products formed during synthesis, was determined following EN 15451 [32]. Analyses were conducted using the same chromatographic system and column configuration. Gradient elution was applied using two eluents differing in acetonitrile content. The flow rate was maintained at 1.0 cm³ min⁻¹, with an injection volume of 20 μL. UV–Vis detection was performed at 480 nm, and the total run time was 20 min. Chromatographic data were processed using Chromeleon software. The chromatographic determinations were performed according to EN 13368-2 and EN 15451 [31, 32].

2. Results and discussion

The objective of this study was to evaluate the temporal stability of Fe-EDDHA, Fe-EDDHSA, and Fe-HBED chelates under conditions representative of nitrogen-based fertilizer formulations. Changes in the concentration of uncomplexed Fe(III) ions as well as variations in chelate composition expressed as isomer distributions for EDDHA and structural forms for

EDDHSA were monitored over a 30-day period. Voltammetric measurements were performed in a simulated nitrogen fertilizer matrix at pH values ranging from 3 to 13, while chromatographic analyses were conducted at pH 3, 5, and 7, in accordance with the operational constraints of the analytical column (pH 1–8).

The combined electrochemical and chromatographic data provide a comprehensive picture of chelate behavior in the presence of nitrogen containing compounds and allow assessment of their suitability for incorporation into multinutrient fertilizer systems.

Effect of nitrogen fertilizer matrix on iron speciation

For experiments involving Fe-EDDHA, the chelate synthesized via the Mannich reaction under laboratory conditions was used. The initial concentration of voltammetrically detectable dissolved Fe(III) ions in the simulated nitrogen environment ($t = 0$) is summarized in Table 1.

Table 1. Content of voltammetrically detectable dissolved iron(III) ions at $t = 0$ in a simulated nitrogenous fertilizer environment at different pH values for Fe-EDDHA

pH	Iron(III) ion content [%]
3	0.02
5	2.14
7	0.03
9	5.13
11	1.41
13	2.59

At neutral pH, the concentration of dissolved uncomplexed iron was close to zero, indicating rapid and efficient complexation. Elevated levels of unbound Fe(III) were detected

at alkaline pH values, particularly at pH 9, suggesting partial destabilization of the complex under these conditions.

The chromatographic profile of the Fe-EDDHA chelate used in stability studies is shown in Figure 1.

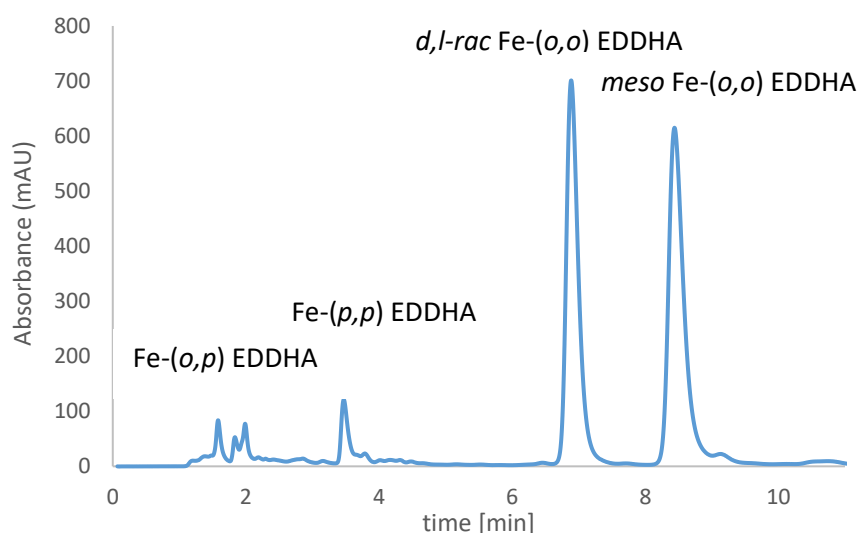


Figure 1. Chromatogram of Fe-EDDHA used to study changes in chelate and iron(III) ion content over time

Table 2 presents the initial relative distribution of Fe-EDDHA isomers at pH 3, 5, and 7. The (*o,o*) isomers were dominant at all investigated pH values, whereas the relative proportions of the (*o,p*) and (*p,p*) forms varied with pH.

Table 2. Initial content of Fe-EDDHA isomers in a simulated nitrogenous fertilizer environment at pH 3, 5, and 7 ($t = 0$)

pH	Fe-(<i>o,p</i>) EDDHA [%]	Fe-(<i>p,p</i>) EDDHA [%]	<i>d,l-rac</i> Fe-(<i>o,o</i>) EDDHA [%]	<i>meso</i> Fe-(<i>o,o</i>) EDDHA [%]
3	2.67	4.53	45.20	47.60
5	3.58	6.82	43.63	45.97
7	2.61	6.30	44.32	46.77

The (*o,o*) isomers remained dominant at all investigated pH values, while the proportions of (*o,p*) and (*p,p*) isomers varied with pH. These observations confirm the sensitivity of EDDHA speciation to environmental conditions and highlight the importance of pH control in fertilizer formulations.

For Fe-EDDHSA, the initial concentration of unbound iron ions in the nitrogen matrix is presented in Table 3.

Table 3. Iron(III) ion content at $t = 0$ at different pH values in a simulated nitrogenous fertilizer environment for Fe-EDDHSA

pH	Iron(III) ion content [%]
3	3.80
5	5.31
7	3.38
9	4.51
11	3.09
13	0.49

Reliable determination of the degree of complexation was only possible at pH 7. At lower pH values, overlap between the Fe(III) signal and the hydronium ion peak limited quantification, whereas at higher pH iron hydroxide precipitation interfered with voltammetric measurements. The chromatogram used to monitor Fe-EDDHSA stability is shown in Figure 2.

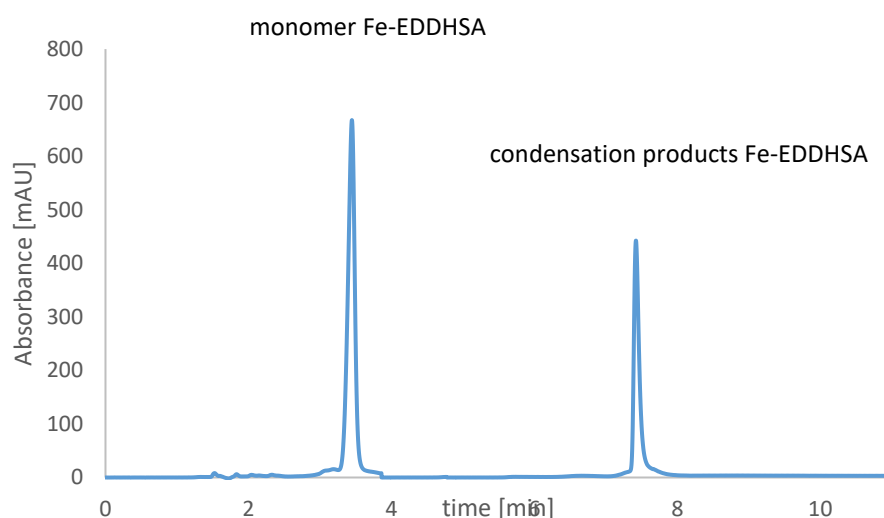


Figure 2. Chromatogram of Fe-EDDHSA used to study changes in chelate and iron(III) ion content over time

The initial proportions of Fe-EDDHSA monomer and condensation products are summarized in Table 4.

Table 4. Initial content of Fe-EDDHSA monomer and condensation products in a simulated nitrogenous fertilizer environment at pH 3, 5, and 7 ($t = 0$)

pH	Monomer [%]	Condensation products [%]
3	48.74	51.26
5	57.20	42.80
7	49.97	50.03

Compared with the previously studied aqueous system [30], the measured total Fe-EDDHA content was not reduced in the fertilizer matrix at $t = 0$. The relative distribution between monomeric and condensed forms depended on pH.

Commercial Fe-HBED was used to investigate iron complexation under identical conditions. The initial concentration of unbound Fe(III) ions is shown in Table 5.

Table 5. Iron(III) ion content at $t = 0$ at different pH values in a simulated nitrogenous fertilizer environment for Fe-HBED

pH	Iron(III) ion content [%]
3	3.08
5	3.22
7	3.31
9	2.53
11	1.41
13	0.65

Compared to EDDHA and EDDHSA, Fe-HBED exhibited a more uniform distribution of voltammetrically detectable dissolved iron across the investigated pH range, with the lowest values observed at highly alkaline pH due to iron hydroxide precipitation.

The chromatographic profile used for subsequent stability analysis is presented in Figure 3.

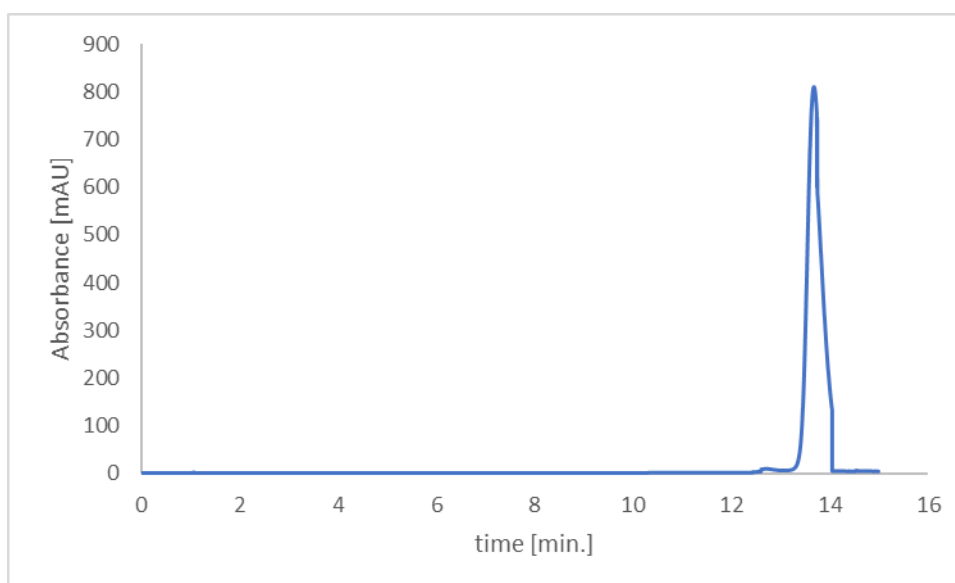


Figure 3. Chromatogram of Fe-HBED used to study changes in chelate and iron(III) ion content over time

Table 6 summarizes the initial Fe-HBED content at pH 3, 5, and 7.

Table 6. Fe-HBED content in a simulated nitrogenous fertilizer environment at pH 3, 5, and 7 (t = 0)

pH	Fe-HBED [%]
3	35.21
5	26.11
7	23.87

Among the tested chelates, Fe-HBED exhibited the lowest initial concentration in the nitrogen environment.

The presence of nitrogen-containing components significantly influenced iron speciation in all investigated systems. In comparison with reference electrolyte conditions, the fertilizer matrix consistently reduced the concentration of uncomplexed Fe(III) ions across a broad pH range. This observation suggests additional interactions between Fe(III) species and matrix components; however, the specific mechanisms cannot be conclusively determined based on the available data.

Such matrix-induced effects are particularly relevant from a formulation perspective, as they may affect the distribution of dissolved and precipitated iron species. At the same time,

these findings indicate that iron speciation in fertilizers cannot be directly extrapolated from simplified aqueous systems.

pH-dependent complexation behavior

All studied chelates exhibited strong pH dependence, although the extent and nature of this dependence varied significantly between ligands. Changes in the concentration of unbound Fe(III) ions in Fe-EDDHA over 30 days are shown in Figure 4.

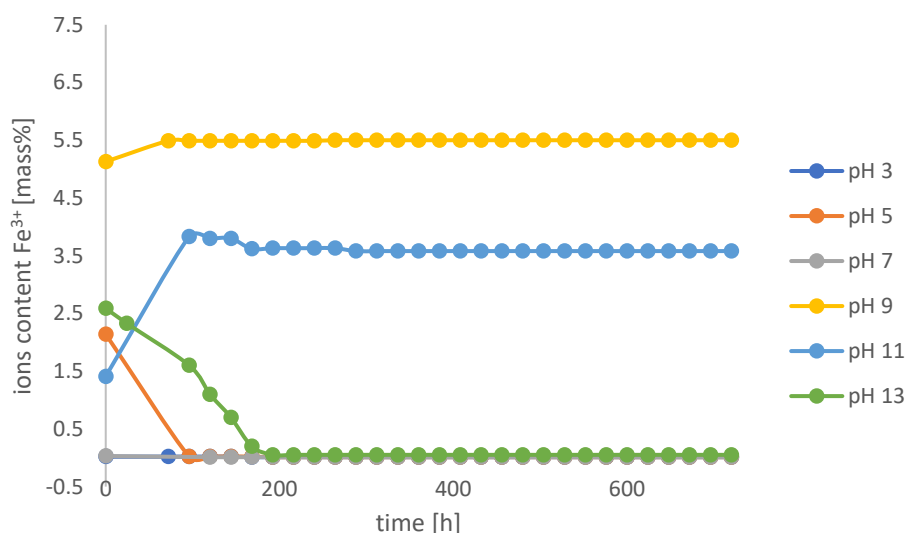


Figure 4. Change in Fe³⁺ ion content over time at different pH values in a simulated nitrogenous fertilizer environment for Fe-EDDHA

In comparison with measurements performed in a reference electrolyte [30], the nitrogen environment significantly reduced the concentration of voltammetrically detectable dissolved iron, indicating interactions between Fe(III) ions and nitrogen containing species. For Fe-EDDHA, near-complete complexation was observed at neutral pH, whereas increased levels of unbound Fe(III) were detected under alkaline conditions, particularly around pH 9. This behavior indicates partial destabilization of the complex, likely associated with competing hydrolysis reactions and changes in ligand protonation state. Despite this, the system maintained relatively high stability across the investigated pH range.

For Fe-EDDHSA, the evolution of voltammetrically detectable dissolved Fe(III) ions over time is presented in Figure 5.

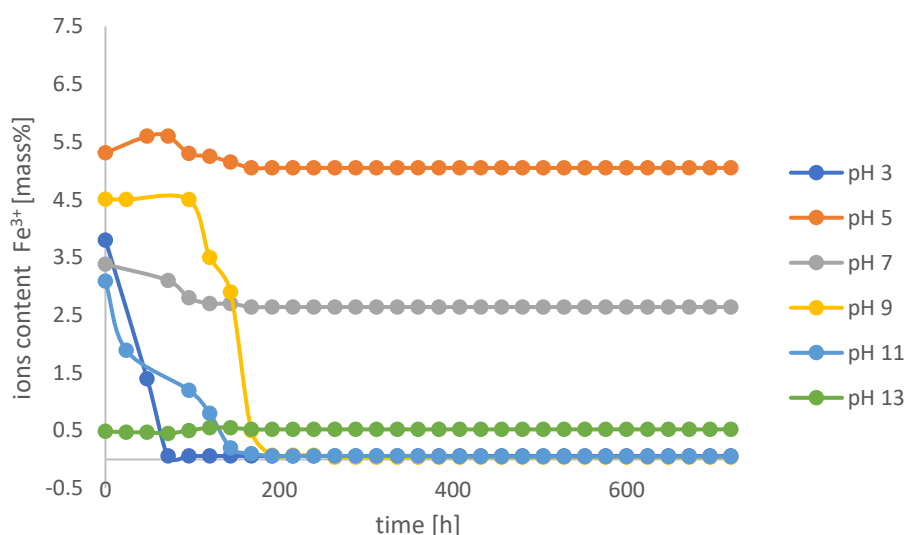


Figure 5. Change in the content of Fe^{3+} ions over time at different pH values in a simulated nitrogen fertilizer environment in the Fe-EDDHA solution.

Fe-EDDHA displayed more uniform behavior, with high levels of iron complexation maintained over time. Although quantitative determination at extreme pH values was limited by analytical constraints, the available results suggest that nitrogen-containing species do not significantly destabilize the complex. At neutral pH, the degree of complexation remained close to quantitative, indicating high stability under fertilizer-relevant conditions.

In the case of Fe-HBED (Figure 6), variations in voltammetrically detectable dissolved Fe(III) concentration were smaller than those observed for the other chelates.

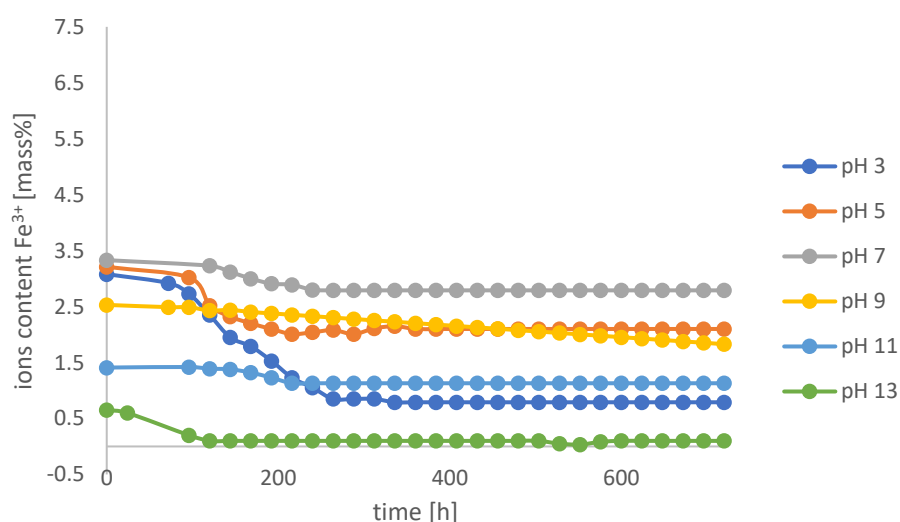


Figure 6. Changes in the content of Fe^{3+} ions over time at different pH values in a simulated nitrogenous fertilizer environment in the Fe-HBED solution.

Fe-HBED exhibited the most consistent complexation profile, with relatively small variations in voltammetrically detectable dissolved iron concentration across the pH range. The observed decrease in Fe(III) concentration at high pH is likely associated with precipitation of iron hydroxides rather than enhanced complex stability. This distinction is critical, as voltammetric measurements reflect only dissolved iron species and do not differentiate between complexed and precipitated forms. Therefore, lower Fe(III) signals at alkaline pH should not be interpreted solely as improved chelation efficiency.

Stability of chelate structures in the nitrogen environment

Chromatographic analysis of Fe-EDDHA over time revealed gradual changes in the relative abundance of individual isomers (Figures 7–9).

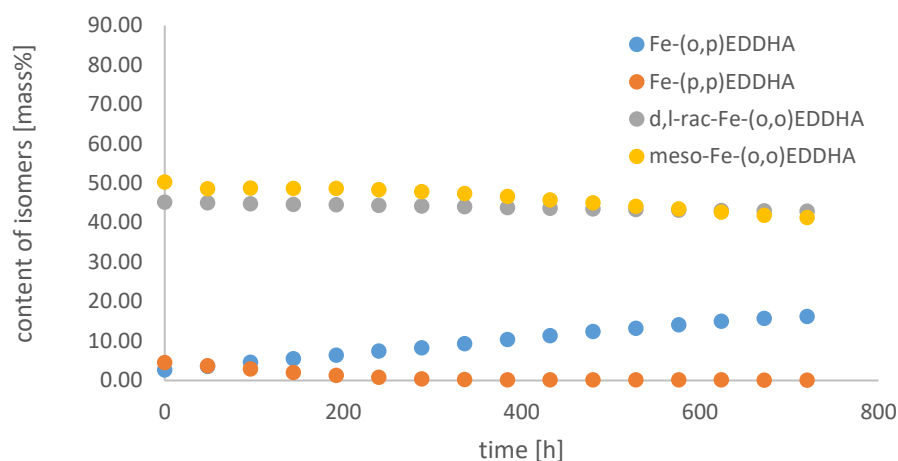


Figure 7. Changes in Fe-EDDHA isomer content over 30 days in a simulated nitrogenous fertilizer environment at pH 3

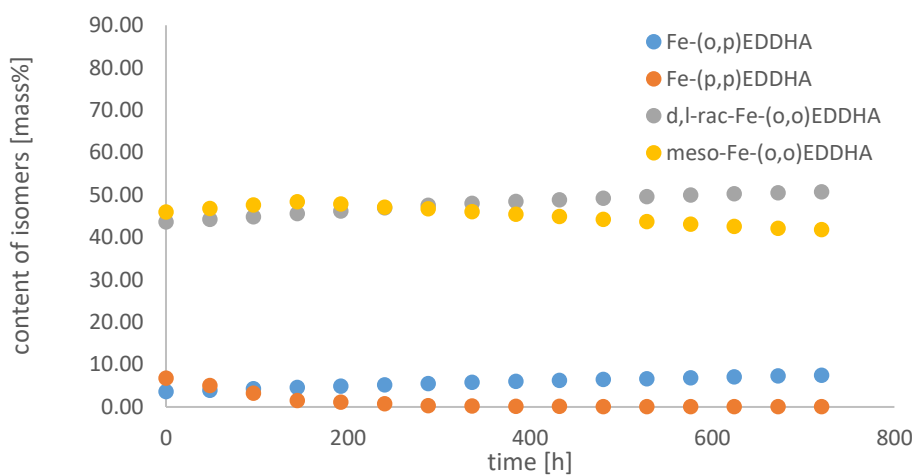


Figure 8. Changes in Fe-EDDHA isomer content over 30 days in a simulated nitrogenous fertilizer environment at pH 5

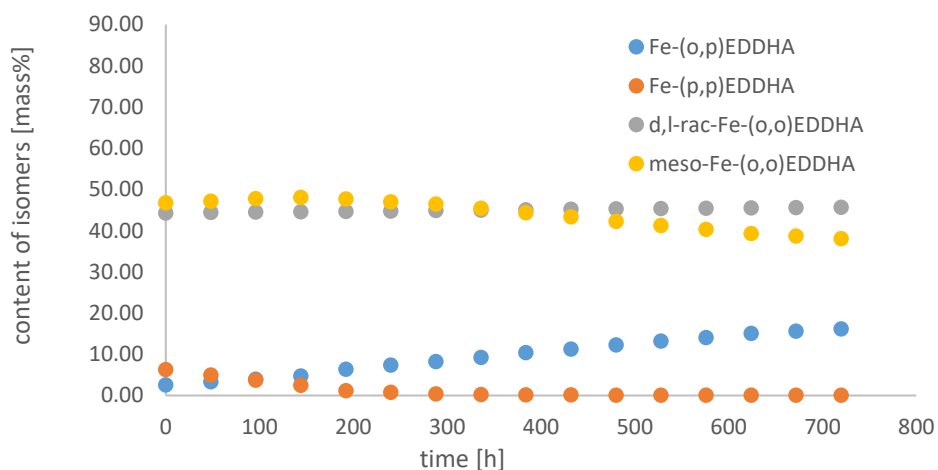


Figure 9. Changes in Fe-EDDHA isomer content over 30 days in a simulated nitrogenous fertilizer environment at pH 7

At pH 3, a slight increase in total chelate concentration was observed, most likely reflecting slow dissolution of the solid phase under acidic conditions. At pH 5 and 7, a minor decrease in total Fe-EDDHA concentration occurred over the 30-day period.

Chromatographic analysis revealed that Fe-EDDHA undergoes gradual redistribution of isomeric forms over time. The *d,l-rac* Fe-(*o,o*) EDDHA isomer showed the greatest persistence in the present experiment, particularly at pH 5 and 7, consistent with previous reports on the behaviour of EDDHA isomers in soil systems [20]. The *meso* Fe-(*o,o*) isomer decreased at all pH values, in agreement with literature data linking its instability to competitive interactions and environmental factors [22, 29, 33]. Changes in isomer distribution in the nitrogen environment were less pronounced than those previously observed in aqueous systems [30], which is consistent with a possible stabilizing effect of the fertilizer matrix; however, this interpretation requires further confirmation.

In contrast, the (*p,p*) isomer showed a systematic decrease, consistent with its low stability and limited chelating ability. The relative increase in the (*o,p*) isomer suggests either enhanced stability under the investigated conditions or improved solubility within the fertilizer matrix [33].

For Fe-EDDHSA, chromatographic data (Figures 10–12) showed systematic changes in the relative content of monomeric and condensed forms.

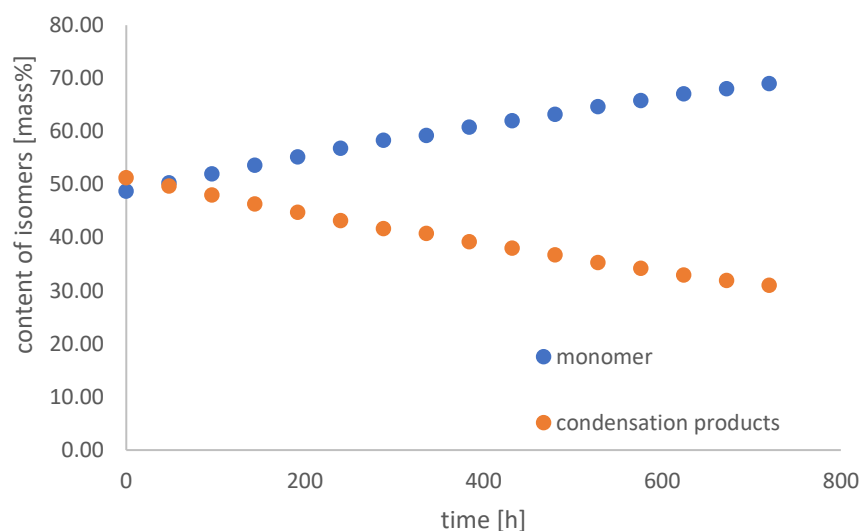


Figure 10. Changes in the content of monomer and condensation products of Fe-EDDHSA over time in a simulated nitrogenous fertilizer environment at pH 3

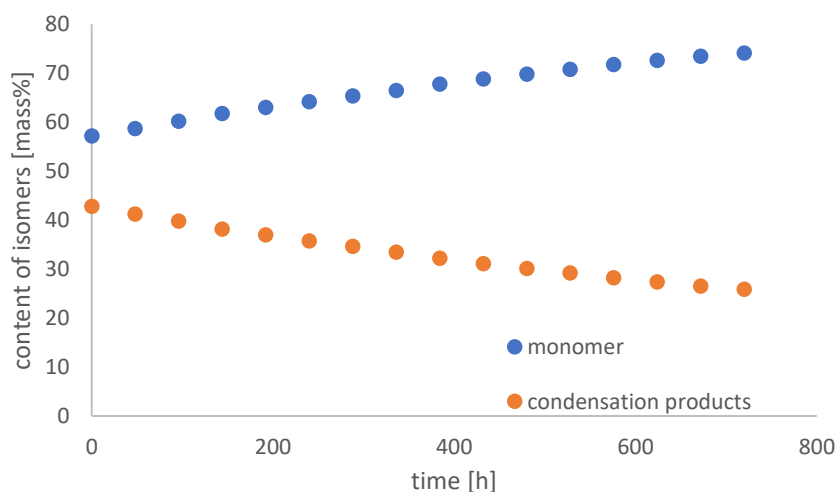


Figure 11. Changes in the content of monomer and condensation products of Fe-EDDHSA over time in a simulated nitrogenous fertilizer environment at pH 5

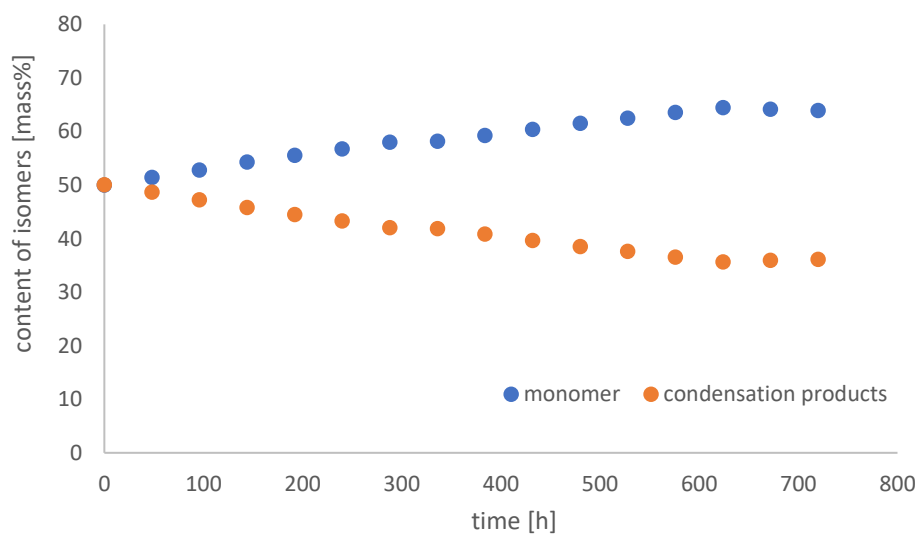


Figure 12. Changes in the content of monomer and condensation products of Fe-EDDHSA over time in a simulated nitrogenous fertilizer environment at pH 7

Unlike EDDHA, Fe-EDDHSA did not exhibit significant loss of total chelate content. Instead, its behavior was characterized by internal structural reorganization, reflected in the gradual increase of monomeric forms at the expense of condensation products.

This transformation was most pronounced under acidic conditions and least significant at neutral pH, where the system remained nearly unchanged over time. The preservation of total chelate concentration suggests that the observed structural changes were not accompanied by substantial loss of total chelate content. This behavior indicates that chelate stability in the fertilizer matrix may involve structural reorganization rather than simple degradation.

The results for Fe-HBED in simulated nitrogenous fertilizer environment are presented in the figures 13–15.

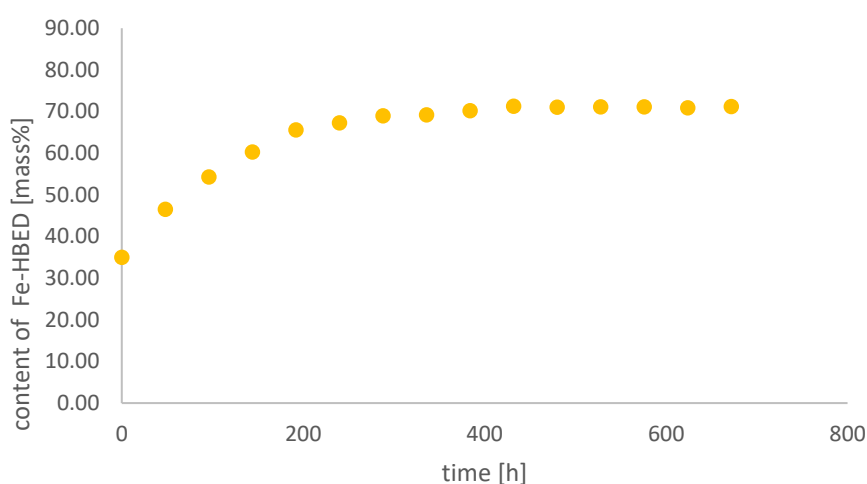


Figure 13. Changes in Fe-HBED content over time in the simulated nitrogenous fertilizer environment at pH 3

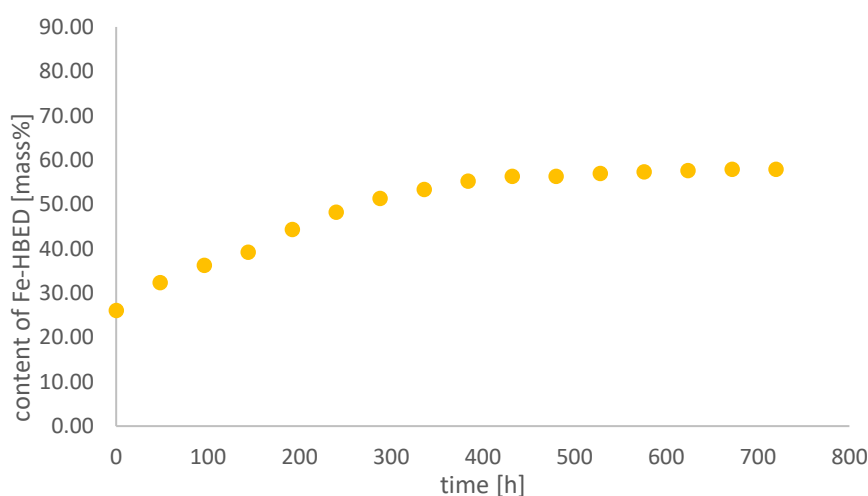


Figure 14. Changes in Fe-HBED content over time in the simulated nitrogenous fertilizer environment at pH 5

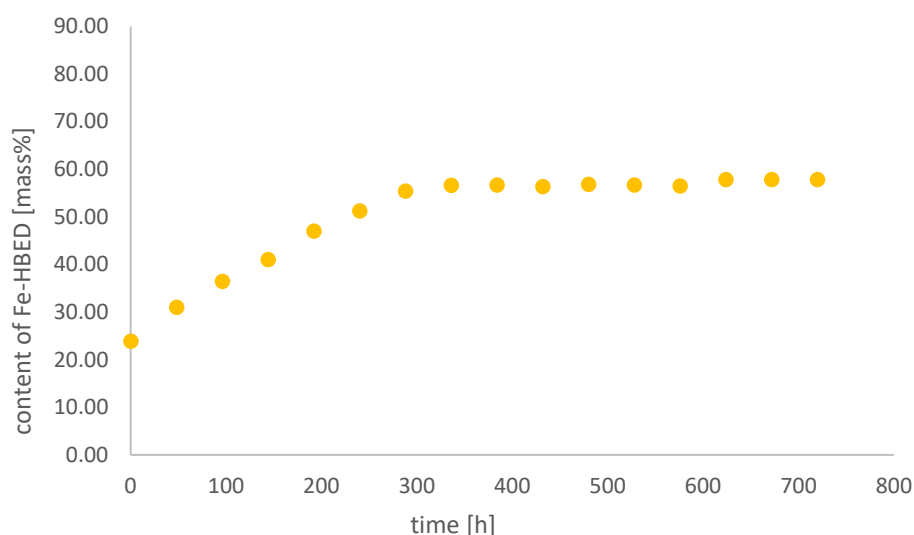


Figure 15. Changes in Fe-HBED content over time in the simulated nitrogenous fertilizer environment at pH 7

Chromatographic data indicated lower apparent concentrations compared to aqueous systems, accompanied by shorter retention times. HBED combined high apparent chemical stability with a formulation-related solubility limitation.

Implications of fertilizer matrix effects for micronutrient formulation

The observed decrease in dissolved uncomplexed Fe(III) concentration in the presence of nitrogen fertilizer components suggests that the matrix influences iron speciation beyond simple dilution effects. This behavior may be attributed to competitive coordination equilibria, changes in ionic strength, and possible interactions between nitrogen species and iron–chelate complexes, which together can shift the equilibrium toward more stable forms.

Differences between the investigated chelates further indicate that ligand structure governs not only intrinsic stability but also responsiveness to matrix-induced perturbations. The pH-dependent isomer redistribution observed for Fe-EDDHA suggests a distinct time-dependent redistribution pattern, whereas the structural resilience of Fe-EDDHSA points to a different structural response. In contrast, the apparent limitation observed for Fe-HBED is likely associated with solubility constraints rather than complex instability, highlighting the role of phase behavior in determining effective performance.

These findings indicate that fertilizer matrices can alter the observed speciation and time-dependent behavior of iron chelates. From a formulation perspective, such interactions are

relevant for understanding how formulation composition influences nutrient behavior in complex, multicomponent systems.

3. Conclusions

The results demonstrate that the chemical environment of a nitrogen fertilizer matrix substantially influences the stability and speciation of iron chelates. Fe-EDDHA, Fe-EDDHSA, and Fe-HBED exhibited distinct responses to pH and storage time, reflecting differences in ligand structure and formulation behavior.

Fe-EDDHA remained dominated by the more stable (*o,o*) isomers, while gradual redistribution among isomeric forms occurred during storage. Fe-EDDHSA showed comparatively stable total chelate content accompanied by changes in the relative proportions of monomeric and condensed forms, indicating that structural reorganization may occur without substantial loss of total chelate. Fe-HBED exhibited relatively consistent dissolved iron behavior and no clear evidence of substantial degradation, although its lower apparent concentration and solubility limitations may restrict its use in concentrated fertilizer formulations.

The nitrogen fertilizer matrix consistently altered the concentration of dissolved uncomplexed Fe(III) compared with the reference electrolyte, demonstrating that iron speciation cannot be directly inferred from simplified aqueous systems. However, the mechanisms responsible for these matrix effects cannot be established conclusively from the present data and may involve changes in ionic strength, phase behavior, and metal–ligand equilibria.

Overall, the findings highlight the importance of considering fertilizer matrix composition when evaluating iron-chelate stability and selecting chelating agents for micronutrient fertilizer formulations. The observed differences among Fe-EDDHA, Fe-EDDHSA, and Fe-HBED provide a basis for further formulation studies focused on solubility, storage stability, nutrient release, and agronomic performance under practical fertilizer application conditions.

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4. References

- [1] Álvarez-Fernández, A., García-Marco, S., & Lucena, J. J. (2005). Evaluation of synthetic iron(III)-chelates (EDDHA/Fe³⁺, EDDHMA/Fe³⁺ and the novel EDDHSA/Fe³⁺) to correct iron chlorosis. *European Journal of Agronomy*, 22, 119–130. <https://doi.org/10.1016/j.eja.2004.05.003>

- [2] Briat, F., Dubos, C., & Gaymard, F. (2015). Iron nutrition, biomass production, and plant product quality. *Trends in Plant Science*, 20(1), 33–40. <https://doi.org/10.1016/j.tplants.2014.07.005>
- [3] Lucena, J. J. (2006). Synthetic iron chelates to correct iron deficiency in plants. In R. Hell & J. Mendel (Eds.), *Iron nutrition in plants and rhizospheric microorganisms* (pp. 103–128). Springer. https://doi.org/10.1007/1-4020-4743-6_5
- [4] Álvarez-Fernández, A., Sierra, M. A., & Lucena, J. J. (2002). Reactivity of synthetic Fe chelates with soils and soil components. *Plant and Soil*, 241, 129–137. <https://doi.org/10.1023/A:1015875509055>
- [5] Cantera, R. G., Zamarreño, A. M., & García-Mina, J. M. (2002). Characterization of commercial iron chelates and their behavior in alkaline and calcareous soil. *Journal of Agricultural and Food Chemistry*, 50(26), 7609–7615. <https://doi.org/10.1021/jf025745y>
- [6] Cao, X., Jia, X., Liu, L., Cao, H., Qin, W., Li, M., & Li, J. (2021). Iron deficiency leads to chlorosis through impacting chlorophyll synthesis and nitrogen metabolism in *Areca catechu* L. *Frontiers in Plant Science*, 12, 710093. <https://doi.org/10.3389/fpls.2021.710093>
- [7] Madhupriyaa, D., Baskar, M., Sherene Jenita Rajammal, T., Kuppusamy, S., Rathika, S., Umamaheswari, T., & Mohanapragash, A. G. (2024). Efficacy of chelated micronutrients in plant nutrition. *Communications in Soil Science and Plant Analysis*, 55(22), 3609–3637. <https://doi.org/10.1080/00103624.2024.2397019>
- [8] European Parliament and Council. (2019). Regulation (EU) 2019/1009 on the making available on the market of EU fertilising products. *Official Journal of the European Union*, L 170, 1–114.
- [9] Wu, H., Luo, Y. M., Xing, X. R., & Christie, P. (2004). EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agriculture, Ecosystems & Environment*, 102(3), 307–318. <https://doi.org/10.1016/j.agee.2003.09.002>
- [10] Sinigani, A. A. S., Tahmasbian, I., & Sinigani, M. S. (2015). Chelating agents and heavy metal phytoextraction. In I. Sherameti & A. Varma (Eds.), *Heavy metal contamination of soils: Monitoring and remediation* (pp. 367–393). Springer. https://doi.org/10.1007/978-3-319-14526-6_20
- [11] Bucheli-Witschel, M., & Egli, T. (2001). Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiology Reviews*, 25(1), 69–106. [https://doi.org/10.1016/S0168-6445\(00\)00055-3](https://doi.org/10.1016/S0168-6445(00)00055-3)
- [12] Yunta, F., García-Marco, S., Lucena, J. J., Gómez-Gallego, M., Alcázar, R., & Sierra, M. A. (2003). Chelating agents related to ethylenediamine bis(2-hydroxyphenyl)acetic acid (EDDHA): Synthesis, characterization, and equilibrium studies of the free ligands and their Mg^{2+} , Ca^{2+} , Cu^{2+} , and Fe^{3+} chelates. *Inorganic Chemistry*, 42(17), 5412–5421. <https://doi.org/10.1021/ic034333j>
- [13] Zuluaga, M. Y. A., Cardarelli, M., Roupheal, Y., Cesco, S., Pii, Y., & Colla, G. (2023). Iron nutrition in agriculture: From synthetic chelates to biochelates. *Scientia Horticulturae*, 312, 111833. <https://doi.org/10.1016/j.scienta.2023.111833>
- [14] López-Rayó, S., Nadal, P., & Lucena, J. J. (2015). Reactivity and effectiveness of traditional and novel ligands for multi-micronutrient fertilization in a calcareous soil. *Frontiers in Plant Science*, 6, 752. <https://doi.org/10.3389/fpls.2015.00752>
- [15] Hernández-Apaolaza, L., García-Marco, S., Nadal, P., Lucena, J. J., Sierra, M. A., Gómez-Gallego, M., Ramírez-Lopez, P., & Escudero, R. (2006). Structure and fertilizer properties of byproducts formed in the synthesis of EDDHA. *Journal of Agricultural and Food Chemistry*, 54, 4355–4363. <https://doi.org/10.1021/jf0603897>
- [16] Nadal, P., García-Delgado, C., Hernández, D., López-Rayó, S., & Lucena, J. J. (2012). Evaluation of Fe-N,N'-Bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetate (HBED/Fe³⁺) as Fe carrier for soybean (*Glycine max*) plants grown in calcareous soil. *Plant and Soil*, 360, 349–362. <https://doi.org/10.1007/s11104-012-1153-7>
- [17] López-Rayó, S., Hernández, D., & Lucena, J. J. (2009). Chemical evaluation of HBED/Fe³⁺ and the novel HJB/Fe³⁺ chelates as fertilizers to alleviate iron chlorosis. *Journal of Agricultural and Food Chemistry*, 57, 8504–8513. <https://doi.org/10.1021/jf9019147>

- [18] Sierra, M. A., Gómez-Gallego, M., Alcázar, R., Lucena, J. J., Yunta, F., & García-Marco, S. (2004). Effect of the tether on the Mg^{2+} , Ca^{2+} , Cu^{2+} and Fe^{3+} stability constants and pM values of chelating agents related to EDDHA. *Dalton Transactions*, 3741–3747. <https://doi.org/10.1039/B408730E>
- [19] López-Rayó, S., Correas, C., & Lucena, J. J. (2012). Novel chelating agents as manganese and zinc fertilisers: Characterisation, theoretical speciation and stability in solution. *Chemical Speciation & Bioavailability*, 24(3), 147–158. <https://doi.org/10.3184/095422912X13409631969915>
- [20] Rojas, C. L., Romera, F. J., Alcántara, E., Pérez-Vicente, R., Sario, C., García-Alonso, I., et al. (2008). Efficacy of Fe(o,o-EDDHA) and Fe(o,p-EDDHA) isomers in supplying Fe to strategy I plants differs in nutrient solution and calcareous soil. *Journal of Agricultural and Food Chemistry*, 56, 10774–10778. <https://doi.org/10.1021/jf8022589>
- [21] García-Marco, S., Martínez, N., Yunta, F., Hernández-Apaolaza, L., & Lucena, J. J. (2006). Effectiveness of ethylenediamine-N(o-hydroxyphenylacetic)-N'(p-hydroxy-phenylacetic) acid (o,p-EDDHA) to supply iron to plants. *Plant and Soil*, 279, 31–40. <https://doi.org/10.1007/s11104-005-2030-7>
- [22] Rodríguez-Lucena, P., Tomasi, N., Pinton, R., Hernández-Apaolaza, L., Lucena, J. J., & Cesco, S. (2009). Fe chelates and plant nutrition in calcareous soils. *Plant and Soil*, 325, 53–63. <https://doi.org/10.1007/s11104-009-0052-3>
- [23] Yunta, F., García-Marco, S., & Lucena, J. J. (2003). Theoretical speciation of ethylenediamine-N(o-hydroxyphenylacetic)-N'(p-hydroxyphenylacetic) acid (o,p-EDDHA) in agronomic conditions. *Journal of Agricultural and Food Chemistry*, 51, 5391–5399. <https://doi.org/10.1021/jf034304r>
- [24] López-Rayó, S., Sanchis-Pérez, I., Ferreira, C. M. H., & Lucena, J. J. (2019). [S, S]-EDDS/Fe: A new chelate for the environmentally sustainable correction of iron chlorosis in calcareous soil. *Science of The Total Environment*, 647, 1508–1517. <https://doi.org/10.1016/j.scitotenv.2018.08.209>
- [25] Álvarez-Fernández, A. (2000). Calidad y eficacia de quelatos férricos (FeEDDHA, FeEDDHMA, FeEDDHSA y FeEDDCHA) como fertilizantes [Doctoral dissertation, Universidad Autónoma de Madrid].
- [26] Chaney, R. L. (1988). Plants can utilize iron form Fe-N,N'-di-(2-hydroxybenzoyl)-ethylenediamine-N,N'-diacetic acid, a ferric chelate with 10^6 greater formation constant than Fe-EDDHA. *Journal of Plant Nutrition*, 11, 1033–1050. <https://doi.org/10.1080/01904168809363591>
- [27] Albano, J. P., & Merhaut, D. J. (2012). Influence of FeEDDS, FeEDTA, FeDTPA, FeEDDHA, and $FeSO_4$ on Marigold growth and nutrition, and substrate and runoff chemistry. *HortScience*, 47(1), 93–97. <https://doi.org/10.21273/HORTSCI.47.1.93>
- [28] López-Rayó, S., Nadal, P., & Lucena, J. J. (2016). Novel chelating agents for iron, manganese, zinc, and copper mixed fertilisation in high pH soil-less cultures. *Journal of the Science of Food and Agriculture*, 96, 1111–1120. <https://doi.org/10.1002/jsfa.7183>
- [29] Schenkeveld, W. D. C., Reichwein, A. M., Temminghoff, E. J. M., & Van Riemsdijk, W. H. (2007). The behaviour of EDDHA isomers in soils as influenced by soil properties. *Plant and Soil*, 290, 85–102. <https://doi.org/10.1007/s11104-006-9135-y>
- [30] Klem-Marciniak, E., Huculak-Mączka, M., Marecka, K., Hoffmann, K., & Hoffmann, J. (2021). Chemical stability of the fertilizer chelates Fe-EDDHA and Fe-EDDHSA over time. *Molecules*, 26(7), 1933. <https://doi.org/10.3390/molecules26071933>
- [31] EN 13368-2. (2017). Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 2: Determination of Fe chelated by [o, o] EDDHA, [o, o] EDDHMA and HBED, or the amount of chelating agents, by ion pair chromatography.
- [32] EN 15451. (2008). Fertilizers. Determination of chelating agents. Determination of iron chelated by EDDHSA by ion pair chromatography.
- [33] Schenkeveld, W. D. C., Temminghoff, E. J. M., Reichwein, A. M., & Van Riemsdijk, W. H. (2010). FeEDDHA-facilitated Fe uptake in relation to the behaviour of FeEDDHA components in the soil-plant system as a function of time and dosage. *Plant and Soil*, 332, 69–85. <https://doi.org/10.1007/s11104-009-0274-9>