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CHELATION IN NUTRITION

Review of Chelation in Plant Nutrition

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The use of synthetic chelating agents as a means of supplying Fe and to a lesser extent Zn and Mn to plants is increasing. The manner in which chelating agents function is not as yet fully understood. Chelating agents and their metal components can be absorbed by plant roots and transported to leaves, but there is evidence that chelating agents do not penetrate plant cells. Especially under low pH conditions the chelating agent either remains outside the plant root or is excreted by the plant root with only the metal component being accumulated. At high pH, there is a greater tendency for accumulation of both components. Some chelating agents increase yields independently of their effect on supplying deficient micronutrients. This effect continues to be investigated and may be of practical importance.

ALTHOUGH a biological phenomenon of great importance, chelation of metals was not taken advantage of as a practical means of supplying micronutrients to agricultural crops until about 9 years ago. As yet, economics does limit the use of synthetic chelating agents in fertilizers. They are used, however, as a means of supplying iron and, to a lesser extent, Zn and Mn to high value crops. Chelated Fe is exceptionally valuable for a wide variety of ornamental and fruit trees, vines, and shrubs.

Many woody plants are very susceptible to iron deficiency, and for these species and especially for certain ornamental species the fertilizers should routinely contain chelated iron. Specialty fertilizers for use with plants such

as roses, azaleas, rhododendrons, gardenias, camellias, hydrangeas, and other acid-loving plants should contain chelated iron.

Chelated iron is especially valuable for growing plants in soil-less culture (hydroponics) both experimentally and commercially. The synthetic agent, ethylenediamindi(o - hydroxyphenylacetate) (EDDHA) has proved most adaptable for this use.

The method of application most widely used in agriculture is on the soil. For this reason, the chelated metals can be mixed with complete fertilizers with which they are very compatible. Since very low application rates of chelated metals are required, their use in mixtures ensures

proper distribution. This is particularly so for band placement of fertilizers.

Foliage application of chelated metals to fruit trees would be more economical than soil application. The results, however, from foliar application with the polyaminopolycarboxylic acid chelating agents have not been as successful as those from soil application. Some other chelating agents prepared from waste forestry and agricultural products as foilage sprays have given better results but in general not good enough.

The use of chelating agents in practical agriculture is increasing slowly. The amount is measured in the hundreds of thousand pounds. As the use increases, it is increasingly important to understand the total picture of what

the chelating agents do chemically in the soil, to plants, and in plants. Studies in this laboratory have been directed towards each of these problems.

Absorption Phenomena

Certain synthetic chelating agents have the ability to keep Fe and other micronutrients soluble in the soil and to facilitate the absorption by plant roots of the chelated metals. Just how the chelating agents function in these processes is not adequately known.

The first studies from this laboratory on the ratio of Fe *vs.* chelating agents absorbed by plants indicated close to a 1:1 ratio (*44*). Later studies indicated that the two may not be equal (*46*). More recent studies indicated a very pronounced effect of pH of external solution on the relative amounts of Fe and EDDHA absorbed from a solution of FeEDDHA (*46*). On a whole plant basis, the Fe:EDDHA for pH 4.0, 5.5, 7.0, and 8.5 was 4.2, 2.9, 0.9, and 0.7. Low pH actually favored the accumulation of Fe, and pH had little effect on the absorption of EDDHA. The differences were much more pronounced in roots than in leaves. The possibility exists that some FeEDDHA is absorbed and transported to leaves, and other FeEDDHA is dissociated at the root surface with the chelating agent remaining in the external solution especially at low pH. These observations may help explain some of the results obtained in this laboratory that appeared to be in conflict with results obtained in other laboratories.

Brown *et al.* have reported that chelating agents are not absorbed by plants to the same extent as the metals but that, in general, metals are separated from the chelating agents at the root surface with a competing action between the chelating agents and the roots (*5–7, 28–31*). They have indicated the absorption of both components and translocation of both components to leaves although Fe-deficient plants accumulated and transported much more Fe than chelating agent (*29*).

The ability of EDDHA to decrease Fe accumulation in (or on) roots has been known for some time (*26, 45, 46*). Recent studies verify this for both the Hawkeye and PI 54619-5-1 soybeans (*35*). Fe^{59} in roots was decreased as the level of EDDHA was increased from $10^{-5}M$ to $10^{-3}M$ when the Fe level in the external solution was $10^{-5}M$ (tagged with Fe^{59}). Simultaneously, the level of Fe^{59} in leaves was increased in both varieties as the level of chelating agents was increased. The excess of the chelating agent may have limited the absorption of Fe^{59}, but facilitated translocation of it to the leaves under the conditions.

When equimolar amounts of K and chelating agents were present in the nutrient solution, the accumulation of K was increased in both tops and roots supposedly because Ca when chelated with EDTA did not compete with K (*36*). When the concentration of the chelating agent exceeded that of either Sr or K, the accumulation of each was inhibited, and the inhibition was much more pronounced in leaves than in roots. There was an insufficient amount of the chelating agents accumulated and translocated to account for these results. Chelated Sr was very unavailable to plants.

The amounts of the EDDHA in the leaves and roots of soybean plants receiving 10^{-5}, 10^{-4}, and $10^{-3}M$ levels of C^{14}-EDDHA was essentially directly proportional to that in the external solution (*35*). A 10-fold increase in the level in the external solution resulted in very close to a 10-fold increase in the amount in both leaves and roots. This possibly is indicative of a passive uptake mechanism.

Brown *et al.* (*4, 5, 28, 29, 31*) based their conclusion that chelating agents were not accumulated with the metals to a great extent by plants in part on the observation that Fe but not chelating agents was in the exudate from detopped soybean plants. In studies in this laboratory, high concentrations of Fe-EDDHA resulted in the presence of its reddish color in leaves within minutes. With similarly high concentrations, the chelating agent was present in the stem exudates. With low concentrations of chelating agents, none were found in the exudates. In Table I are data representing the behavior of chelating agents in the stem exudate. Instead of solving the problem of whether or not the chelating agent was accumulated by plants, the absence of the chelating agent in the exudate raises the question of the meaning of the presence or absence of solutes in the stem exudate. C^{14}-chelating agents are transported to eaves but not in the exuded sap of detopped plants. Some other solutes have been studied. In 5-hour studies, two or three times as much C^{14}-mannitol and C^{14}-biuret was present in leaves as in roots, but neither was transported in the stem exudate. C^{14} from urea was transported to leaves but also was absent in the exudate. Fe^{59}, K^{42}, Sr^{85}, and Br^{85} were present in the stem exudate when supplied to the solution in which the roots were bathed. These differential observations open a fruitful field for additional studies.

Even within a variety of a plant, variability is encountered in the relative amounts of metal and chelating agent that are accumulated and transported to leaves. From a $10^{-3}M$ solution of FeEDDHA some soybeans had leaves that were colored with the red Fe-EDDHA and some plants did not have red-colored leaves even when growing

Table I. Fe and EDDHA in a 24-Hour Exudate from Detopped Bush Beans

In External Solution, $M \times 10^4$		In Exudate, $M \times 10^4$	
Fe	EDDHA	Fe	EDDHA
1.0	0	1.08	0.00
1.0	1.0	2.90	0.00
1.0	10.0	0.40	1.10

in the same container (*35*). There was 2.6 times as much EDDHA in the red leaves as in the green leaves. There was also 1.4 times as much Fe^{44} in the red leaves as in the green leaves.

Plant species differ in their ability to absorb Fe from chelating agents. Some actually fail when chlorotic to respond to applications of Fe chelates (*4–6*). This differential behavior has not been adequately explained and is of utmost importance in the use of metal chelates.

As mentioned earlier, there has been some question as to whether or not chelating agents facilitated the translocation of Fe to the leaves of plants (*4–7, 28–31*). This question is intimately related to the question of absorption and translocation of the chelating agent as discussed above. In studies with Chippewa soybeans with a factorial experiment of varying Fe and EDDHA levels, increasing Fe greatly increased both the absorption and translocation to leaves of the chelating agent (*35*). As usual, a high level of the EDDHA relative to a low level decreased Fe^{59} in the roots. It resulted, however, in an increase of Fe^{59} in leaves at 10^{-3} and $10^{-4}M$ EDDHA but not at $10^{-5}M$.

The results just described indicate that metals and chelating agents may be separated at or near the root surface and that the absorption or accumulation of each may follow separate mechanisms. A limited number of inhibitor studies indicated that cyanide and azide decreased the uptake of Fe but not chelating agents (*20*). Further exploration of these effects is necessary. An interesting aspect of some of the inhibitor studies was that Fe accumulation into roots was inhibited but not that into leaves.

The observation that P can result in a precipitation of Fe^{59} in roots from Fe^{59}EDDHA (*13*) is further evidence that Fe can be separated from EDDHA either at the root surface or in the root. The further observation that HCO_3^- and $H_2PO_4^-$ appeared to compete competitively with Fe^{59} when supplied as Fe^{59}EDDHA which is an anion suggests strongly that Fe^{59} was taken up as an anion (*13*). If taken up as the anion FeEDDHA$^-$, the separation of Fe from the chelating agent would of necessity occur inside the root.

The Hawkeye soybean is very re-

sistant to lime-induced chlorosis, and the PI 54619-5-1 soybean is very susceptible to lime-induced chlorosis (3–5). These two soybean varieties have proved very useful in the study of the chlorosis and of chelating agents. Brown, Holmes, and Tiffin (3) have postulated that the reason for the difference between the two varieties is that the Hawkeye soybean has a greater reducing capacity at the root surface than does the Pi 54619-5-1 soybean. Results obtained in this laboratory may result in another hypothesis (37). A small quantity of calcareous soil that had been equilibrated with Fe^{59} was added to a nutrient solution (10 grams per liter). This served as the source of Fe and other micronutrients. The two soybean varieties were grown separately in this mixture and also in combination. About 40 times as much Fe^{59} was transported to leaves of the Hawkeye soybean than to those of the PI 54619-5-1. About three times as much was in the roots of Hawkeye. When the two varieties were grown together, the Hawkeye had no effect on Fe^{59} content of the PI 54619-5-1, but the latter decreased by 50 to 75% and by 25 to 50% the amount of Fe^{59} in leaves and roots, respectively, of the Hawkeye. The PI 54619-5-1 evidently did something that resulted in inhibition of Fe accumulation not only by itself but also by the Hawkeye. One of the possibilities is that the PI 54619-5-1 excreted a substance that inhibited Fe accumulation.

The two varieties of soybeans were grown in the same type of soil-solution mixtures with substances that are known to be excreted by plant roots. Glycine, citrate, and flavin inhibited Fe^{59} accumulation, but the addition of the sodium salt of EDDHA greatly increased it. More research is needed to ascertain the differences between the two soybean varieties.

Even though the FeEDDHA is more stable at pH 8 than 4, more Fe was accumulated by the soybeans at pH 4 than at pH 8 (1). This may support the contentions of Brown and Tiffin (6, 7) that Fe is separated from the chelating agent at the root surface before accumulation of the Fe. Whether or not this occurs, both components are accumulated by the plants, at least to varying degrees (20, 29, 35, 44, 46).

The PI 54619-5-1 soybeans accumulated Fe from a nutrient solution of pH 4 and also from the soil-solution mixture containing acid soil as effectively as did the Hawkeye soybean (1, 37). It is at alkaline pH values that PI 54619-5-1 fails to accumulate Fe.

Since chelating agents, at least to a certain extent, are absorbed by roots and translocated to leaves of plants and since the question of metabolism of chelating agents is still an unknown

subject, information concerning the retranslocation of chelating agents is of importance. Even though there is no apparent health hazard whatsoever from the levels of synthetic chelating agents found in plants, questions may be asked concerning levels in the edible parts of plants. To assess retranslocation, bean plants were grown in solutions of C^{14}-chelating agents for 1 week, removed to solutions free of chelating agents, and then allowed to grow to maturity (12). Seed pods which grew in the latter part of the test contained essentially no radioactivity. The same obtained for the new growth of both shoots and roots. There was a gradient of C^{14} from the oldest to the newest parts.

The studies just described provide an indirect method for measuring the possible metabolism of chelating agents in plants. The C^{14}-label of EDDHA, DTPA, and EDTA was in each case on the carboxyl group which could be lost as $C^{14}O_2$ in any decomposition. During the period of the test with beans, some 50% of the C^{14}-label was lost (12). When the C^{14}-chelating agents were injected into tree branches (25), about 75% of the C^{14} could not be accounted for after a period of several weeks. These results indicate metabolism but a more direct approach is necessary. Hill-Cottingham has also reported data of this indirect type, but with ethylene groups labeled with C^{14}, that indicate metabolism of EDTA (18).

Translocation of C^{14}-chelating agents in woody plants was studied by injecting the agents into the xylem of orange and avocado trees and measuring the C^{14} in the various plant parts and in fruit that had been set both before and after the time of injection (12). The injection techniques have been described (25). Comparisons were made with soil applications to small trees grown in pots. Some of these were ringed (girdled) by removing a section of the bark to ascertain the tissues involved. The results all indicated that the chelating agents were readily translocated in the xylem to leaves and to fruits if fruits were already on the trees. Very little of the C^{14} was retranslocated to fruits that were set after the time of application of the C^{14}-chelating agents.

Absorption of chelating agents (FeEDDHA) into potato tubers which had been allowed to develop roots and shoots was studied. The red color of the chelating agent could be traced into the tuber and into the shoots. The conducting elements of the tuber were outlined by the chelating agent. The eyes (buds) of the tubers are all connected in a continuous manner. The chelating agent moved very little in a lateral direction from the conducting elements into the tuber. When a cut tuber was exposed to the Fe chelating

agent, the red color penetrated about 1 cm. into the main part of the tuber but completely filled the conducting tissues around the sides of the tuber. Prolonged washing in water resulted in complete removal of the color (FeEDDHA) from the tuber. More precise studies with C^{14}-EDDHA indicated a trace of C^{14} that could not be removed by washing.

Soil applications of Fe chelates often result in a decreased uptake of Mn by plants, and sometimes Mn deficiency results (22, 32, 33). Mn toxicity has been overcome by the use of chelating agents. The mechanism by which an Fe chelate inhibits Mn uptake appears to be competitive chelation (32). FeEDDA has a pronounced effect on inhibiting Mn uptake, but MnEDDHA is very available to plants (33). Fe chelates sometimes enhance Zn uptake by plants. Zn chelates are reasonably available to plants and are proving to be a better source of Zn for plants than some had suspected (40, 48).

DeKock (9) has found inhibitory effects of chelating agents on the uptake of divalent heavy metals by plants. Studies were conducted in this laboratory to determine if a similar condition would result for some of the radioactive fission products of nuclear bomb fallout. Many chelating agents have been tested with contaminated soil, and the greatest inhibitory effect on Sr^{85} uptake was with EDDHA and amounted to about 25%. This amount of inhibition is considered to be too little for any possible use in decontamination. The results in nutrient solutions were of greater interest but are of academic importance only.

The use of microbially stable chelating agents by soil application in practical agriculture can result in the solubilization of some ionic species that may not otherwise be available to plants. Some of the fission products are of concern. For example, with Sorrento loam soil, very little Y^{91} that had been added to the soil was available to bean plants in a 58-day growing period (10). When 100 p.p.m. of the Na salts of chelating agents was added to 1600 grams of soil, EDDHA had no effect of Y^{91} in bean plants, but EDTA resulted in a 50-fold increase in leaves relative to the control, and DTPA resulted in a 1500-fold increase in leaves and was in leaves as YDTPA. The chelating agents had much less effect on Sr^{90} and Zn^{65} with the same soil.

Effect of Chelating Agents on Enzyme Reactions

In vitro studies have been made of the influence of chelating agents on two systems that fix carbon dioxide in plants (2, 11, 19, 34, 41, 42, 47). The substrates involved are, besides CO_2,

ribose-5-phosphate (R5P) and phosphoenolpyruvic acid (PEP). Most chelating agents that were studied stimulated the two reactions, but others inhibited them. Weissbach et al. (50) had observed earlier that EDTA or glutathione was necessary for maximum CO_2 fixation in leaf preparations with R5P or its phosphorylated product, ribulose 1-5 diphosphate. This reaction involves the step for CO_2 fixation in photosynthesis. An important consideration is whether or not chelating agents when applied to intact plants can have the same effect. This would necessitate the uptake of the chelating agents through the roots and their transport to leaves and then into the cells of leaves.

Effort has been directed toward elucidating the nature of the chelating agent effect on CO_2 fixation. There is no doubt that chelating agents can overcome heavy-metal toxicity for the two enzymatic reactions that were investigated (19, 34). Heavy metals catalyze decomposition of some of the products of CO_2 fixation (19, 34, 47). Some of the effect of chelating agent is in decreasing the decomposition of the products.

The question remains as to whether or not the chelating agents have an effect in addition to decreasing heavy-metal toxicity. Cyanide and azide both inactivate heavy metals and both resulted in increased CO_2 fixation with PEP as a substrate (47). Cyanide additionally results in a greater CO_2 fixation because it combines with and stabilizes oxaloacetate, the product to the reaction. Azide resulted in an increased CO_2 fixation with R5P as a substrate. Cyanide fails with R5P because it combines with ribulose-5-phosphate and stops the reaction. When EDTA was included in the reaction mixtures, the percentage increase for EDTA was just as great where cyanide and azide were incluned as when they were omitted. The effects of cyanide, azide, and EDTA then were additive. This implies that the effect of the chelating agent was in addition to preventing heavy-metal inhibition.

This possibility was further tested by the use of dialysis and sephadex (Pharmacia, Uppsala, Sweden—this noncharged, resinous product differentially retains low molecular weight substances and allows high molecular weight substances such as proteins to pass through first) to decrease the endogenous levels of contaminating heavy metals. Leaf preparations were ground in 10^{-2} and $10^{-1}M$ EDTA and EDDHA to dissolve heavy metals and to remove bound metals from the proteins. The metal chelates were removed from the systems as described. Chelating agents added to reaction mixtures had just as much effect on these systems as on systems from which heavy metals had not been removed

(47). The main effect of the chelating agents remain unknown.

Chelating agents sometimes can be toxic to plants. Ordinarily EDDHA is not toxic, and plants can tolerate high levels of it (38, 43). Levels high enough to color the foliage seem to have no ill effect. Of academic interest is the observation that when Fe is deficient in a nutrient solution, EDDHA without Fe can be very toxic (38, 46). The same is not usually encountered in soil because EDDHA can mobilize enough insoluble Fe from the soil to improve Fe-deficient plants.

Hydroxyethylethylenediaminetriacetic acid is the most toxic of the few chelating agents used to supply Fe in agriculture when excessive applications were used. This chemical also was the most toxic of the chelating agents in the in vitro CO_2-fixing reactions (11). The technique of double reciprocal plots indicated a noncompetitive type of inhibition. This implies that additional substrates did not overcome the inhibition and that the inhibitor combined with or inactivated the enzyme at a site other than that at which the substrate is combined.

Yield Increases beyond Correcting Micronutrient Deficiencies

Chelating agents have been observed to result in yield increases supposedly beyond their ability to supply deficient micronutrients to plants (15, 16, 49). There are several possible reasons for this, and more than one may be involved. These include a better micronutrient balance, calcium inactivation in the cytoplasm, growth regulator effects, and stimulatory effects on some enzyme reactions.

Some of these effects have been referred to elsewhere in this report. Iron chelating agents have been reported to decrease a Mn toxicity (22). A high level of EDDHA overcame toxic effects of high Cu, Zn, and Mn (46). Conversely a high level of Fe in a nutrient solution prevented toxicity from a high level of chelating agent (26).

The effects of chelating agents on enzymatic CO_2 fixation has been described in this report. Chelating agents may have inhibitory or stimulating effects on many other systems. A critical analysis of effects of chelating agents on growth is warranted.

Studies have been made elsewhere of the growth stimulating effects of chelating agents that resemble growth regulators (15, 16, 27, 49). Thimann and Takahashi (27) report that the effect is not like that of a growth regulator.

Hanson (14) is exploring the possibility of chelating agents protecting mitochondria from Ca^{+2} ions as an explanation of growth stimulation. The theory demands the accumulation of

chelating agents into the cytoplasm. In an exploratory test here, $10^{-4}M$ EDDHA inhibited bush bean growth 10% when the Ca was omitted from the external nutrient solution (seedlings were 7 days old at transplanting and contained some Ca). At 10^{-3} and $10^{-2}M$ Ca, the EDDHA increased the yield 90% and 50%, respectively. Hanson's theory deserves more evaluation.

A possible commercial use of chelating agents may result from the work of Miller et al. (23, 24) where chelating agents were included in foliage sprays of 2,4-D that were used to increase bean yields. The EDDHA not only increased the yield but also greatly widened the range of 2,4-D that can be tolerated by beans. The EDDHA resulted in the 2,4-D sprays being much safer.

Continued exploration may make it possible to take practical advantage of the 20 to 30% yield increases that are often associated with the use of chelating agents independently of correcting micronutrient deficiencies.

Behavior of Chelating Agents in Soil

Earlier studies in this laboratory indicated that some chelating agents became fixed in soil with the result that the chelated metal was at least temporarily unavailable to plants (21, 39). Fixation of Fe chelates on clay was postulated, but this was questioned by Hemwall (17). An important observation was that FeEDTA and FeDTPA were fixed in the soil, but FeEDDHA was not (39). Recent studies indicate that Fe chelates have amphoteric properties and that they may be subject to cation exchange.

It is generally believed that synthetic polyamino polycarboxylic acid chelating agents are very resistant to microbial decomposition. In a study in which C^{14}-EDDHA was added to an avocado tree potted in soil, only $1/6$ of the C^{14} could be accounted for in the tree, soil, or leachate (13). C^{14}-EDDHA and C^{14}-DTPA were incubated with soil to which undecomposed organic matter had been added as a microbial substrate. Fifty per cent of the C^{14} from EDDHA was lost in about 1 week.

Practical Use of Chelating Agents

Several new chelating agents have been screened for possible soil and spray use in agriculture. No compound appears as yet to be better than EDDHA for supplying Fe to plants in calcareous soil. This chemical is overtaking diethylenetriaminepentaacetic acid (DTPA) for use on such soils. DTPA is the chemical that enhanced the Y^{91} uptake by plants, but the use of this chemical will decrease as that of EDDHA increases.

Table II. Fresh Weights of Bean Plants Grown with FeEDDHA and Fe(CN)$_6$$^{-3}$

Treatment, M Fe	Fresh Weight, Grams/ Four Plants	
	Tops	Roots
0	23.1	8.9
10^{-5} K$_3$Fe(CN)$_6$	45.0	18.3
10^{-4} K$_3$Fe(CN)$_6$	49.3	15.8
10^{-3} K$_3$Fe(CN)$_6$	23.2	6.3
10^{-5} FeEDDHA	55.3	19.4
10^{-4} FeEDDHA	62.1	19.9
10^{-3} FeEDDHA	53.2	19.0

Glucylglycine proved to be a good chelating agent for Fe when used as a foliage spray for macadamia. It was not as effective on some other plant species, however. Reasons for the ineffectiveness of spray applications need study.

Buc (8) has reported that ferrocyanide and ferricyanide are good sources of iron for control of lime-induced chlorosis. In Table II are some top and root weights of bush beans grown for 4 weeks in complete nutrient solutions with iron either as Fe(CN)$_6$$^{-3}$ or as FeEDDHA. The iron from Fe(CN)$_6$$^{-3}$ was available to the plants but was toxic at 10^{-3}M. This level is in excess of that necessary for iron. Although the EDDHA treatments out-yielded the Fe(CN)$_6$$^{-3}$ treatments, this is not indicative of the iron supply. EDDHA increases yields independently of the Fe supply.

In the 10 years that have elapsed since the beginning of the use of synthetic chelating agents as a means of correcting micronutrient deficiencies in plants, considerable information concerning the nature of and behavior of the agents in soils and plants has been accumulated. Considerable uncertainty still exists, however, in regard to their influences and mode of action. The concept of chelation is relatively new in plant nutrition as a means of explaining metal behavior, and much progress can be expected in improving fertilizer usage through its application.

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