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Chacko J. KaUulruzhy

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RECYCLE EFFICIENCY OF SELECTED CHELATING
AGENTS AFTER PLANT NUTRIENT UPTAKE

THESIS

BY

CHACKO J. KALLUKUZHY

1996

RECYCLE EFFICIENCY OF

SELECTED CHELATING AGENTS

AFTER PLANT NUTRIENT UPTAKE

THESIS

Presented in Partial Fulfillment of the Requirements for
the Degree Master of Science in the Graduate School
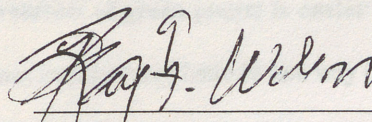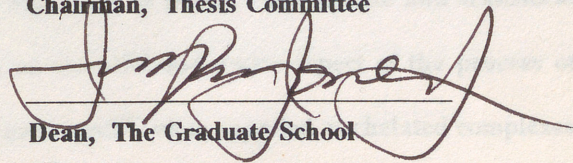**of Texas Southern University**

By

Chacko J. Kallukuzhy, B.S

Texas Southern University

1996

Approved By

(signature)

Chairman, Thesis Committee

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Dean, The Graduate School

RECYCLE EFFICIENCY OF SELECTED CHELATING AGENTS
AFTER PLANT NUTRIENT UPTAKE

By

Chacko J. Kallukuzhy, B. S

Texas Southern University, 1996

Professor Ray F. Wilson, Advisor

From the knowledge of Chelation Chemistry of plants we can predict the composition and function of metals in green plants. Hydroponic plant growth technologies are developed to accommodate the need for food production during long duration space missions. Controlled-environment agricultural systems such as the hydroponic system provide out of season, top quality produce. The continued delivery of ions in hydroponic systems ensures that the leaves are supplied with the mineral nutrient essential to the growth and development of plants. The metals required for the growth of plants exists in cells and tissues as complexes and especially as chelates. Iron is an essential element for growth, metabolism and survival of plants. Low affinity chelators such as EDTA help keep iron soluble and available for cell growth in plants. Chelators added to the growth medium promote iron uptake. Severe iron deficiency can occur if pH is out of the 5 .8 to 6.5 range.

The study of inorganic nutrient requirements of green plants is easier than that of other kinds of organisms. The reason is that green plants require very little in the way of organic growth factors so that growth media may be prepared in high state of purity. The uptake and translocation of inorganic solutes within the whole plant is an essential and major aspect of the process of plant nutrition. Iron is taken up and transported more readily when supplied as chelated complexes, such

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chlorophyll synthesis, and many other enzymatic activities. In EDTA Fe chelate complex, the six donor atoms bond to the central atom. A ligand capable of binding Fe^{3+} and Fe^{2+} will bind the Fe^{3+} more tightly than it will bind with Fe^{2+}. The Stock solution I was Prepared by dissolving 5.05g KNO_3, 0.012 g $MnSO_4.H_2O$, 0.002g $CuSO_4.5H_2O$, 0.005g $ZnSO_4.7H_2O$, 1.36g KH_2PO_4, 4.92g $MgSO_4.7H_2O$ and 0.58 g NaCl in DI water and the volume was made up to 100ml. Stock solution II was prepared by dissolving 0.44g Na_2EDTA and 11.8g of $Ca(NO_3)_2.4H_2O$ in 50ml Deionized water. Excess $FeCl_3$ (100 ppm) solution was added to stock solution II. The volume was adjusted to 100 ml. Working nutrient solution was prepared by adding 20 ml each of stock solutions I and II to 3000ml DI water and diluting to 4 liters.

The pH was adjusted to 6.0. At 15 days the plants looked healthy. They were 5" tall. Halogen lamps were used as light source for photosynthesis. pH was maintained between 5.8 and 6.3. The ionic strength was measured regularly. Stock refill nutrient solution was prepared by dissolving 12.1g KNO_3, 2.04g KH_2PO_4, 4.80g $MgSO_4.7H_2O$, 0.58g NaCl, 0.01g $MnSO_4.H_2O$, 0.002g $CuSO_4.5H_2O$, and 0.005g $ZnSO_4.7H_2O$ in DI water and the volume was adjusted to 100ml. The volume of nutrient solution was maintained at two liters. An air pump was used to circulate air through the medium. The plant roots were protected from light after observing algae growing in the medium. Forty five days after germination, the plants were 12" tall. At sixty days, flowering buds were observed. Small beans were seen on the plants on October 20, 1995. On November 6 1995, the beans were ready to be picked. The matured beans were picked. The beans weighed 10.5 grams. On November 13, beans were picked again and the beans weighed 8.5 grams.

All the reagents and chemicals used were of analytical grade. A stock standard solution was prepared by dissolving one gram Na_2EDTA in one liter deionized water. From this solution, calibration standards ranging 10 to 80 $\mu mol/L$ EDTA were prepared. One milliliter $FeCl_3$ solution (100ppm) was added before the last dilution. The chelated EDTA in the standards were analyzed by HPLC using the BAS 200A analyzer and the calibration graph was obtained. The original concentration of EDTA in the sample was 60 μmol. Four samples were collected from the nutrient

concentration of EDTA in the sample was 60 μmol. Four samples were collected from the nutrient medium at two weeks interval. They were filtered using 0.02 μm filter paper. To one ml of each sample, one ml $FeCl_3$ solution (100 ppm) and three ml mobile phase solution were added. Twenty four hours after mixing the samples with $FeCl_3$ solution, they were analyzed using the BAS 200A HPLC analyzer. The instrument was allowed to reach equilibrium prior to analysis. The injection volume was 20μl and the detection wave length was 254 nm. The electro chemical cell temperature was 30° C. The temperature of mobile phase in bottle A was set at 50°C and the temperature of mobile phase in bottle B and C were 35°C. The pressure was monitored during analysis and was between 2310 and 2350. The recovery of EDTA from the sample ranged from 84.05% to 91.87%. From these result it can be concluded that EDTA concentration does not decrease significantly during and after plant nutrient uptake. EDTA can be considered as an efficient chelating agent. The advantage of this method for analysis are the low cost, lower limit of detection and the good reproducibility.

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ACKNOWLEDGEMENTS

My research has been devoted to a study of the recycle efficiency of ethylenediamine tetraacetic acid in plant nutrient solution after nutrient uptake. Many people have contributed in one way or another to the realization of this study. In conserving space, only a few of them are mentioned below. The fact that other staff members from the Chemistry Department who have helped to write this thesis by their suggestions are not mentioned does not make my gratitude to them less sincere.

I would like to express my sincere thanks to Dr. Ray F. Wilson, Professor of Chemistry, Texas Southern University, who made helpful suggestions in the planning at various stages of the research. His guidance in writing this thesis made the task much easier. Finally, I should acknowledge the support of my wife, Ruby, and my children, Noble, Nibin and Nason. This thesis could not have been written without their understanding.

CHAPTER 1

INTRODUCTION

Chelation is a well developed branch of chemistry. From the knowledge of chelation chemistry of plants in hydroponic solutions, we can predict the composition and function of metals in green plants and the recycle efficiency of chelating agents. As the United States advances in space exploration and interplanetary transportation, the establishment of out posts on other planets can become realities in the near future. Success in these explorations depend on many different technologies. Particularly crucial are the technologies that support human life. Longer-duration missions and larger crew sizes will require increased degree of self sufficiency of the life support system. Normal life support consumables[1] required for a human being are 219 kg/yr food (dry mass), 329 kg/yr oxygen, 657 kg/yr drinking water, 840 kg/yr sanitary water, and 6132 kg/yr domestic water. Available technology can be utilized for food production and processing. Currently, spacecraft life support systems rely on nonrecycling technologies. These are simple and sufficiently reliable for human space-flight missions of relatively short duration, small crew size, and limited power availability. But longer duration missions, larger crew size, and changes in crew compliment during the mission will require maximizing crew safety by increasing the degree

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[1]Steven H. Schwartzkopf, <u>Design of a Controlled Ecological Life Support System</u> (Bio Science Vol. 42 No. 7, 1992), 527.

of self sufficiency of the life support system, and minimizing the economic costs associated with resupply. The five basic functions required of any regenerative life support system are atmosphere regeneration, water purification, waste processing, food production and food processing. Hydroponic plant growth technologies are developed to accommodate the need for food production. These techniques are best applied when chelating agents are recycled after the plant uptake of nutrients. Self-sufficiency of food is one of the most important aspects for the success of space exploration.
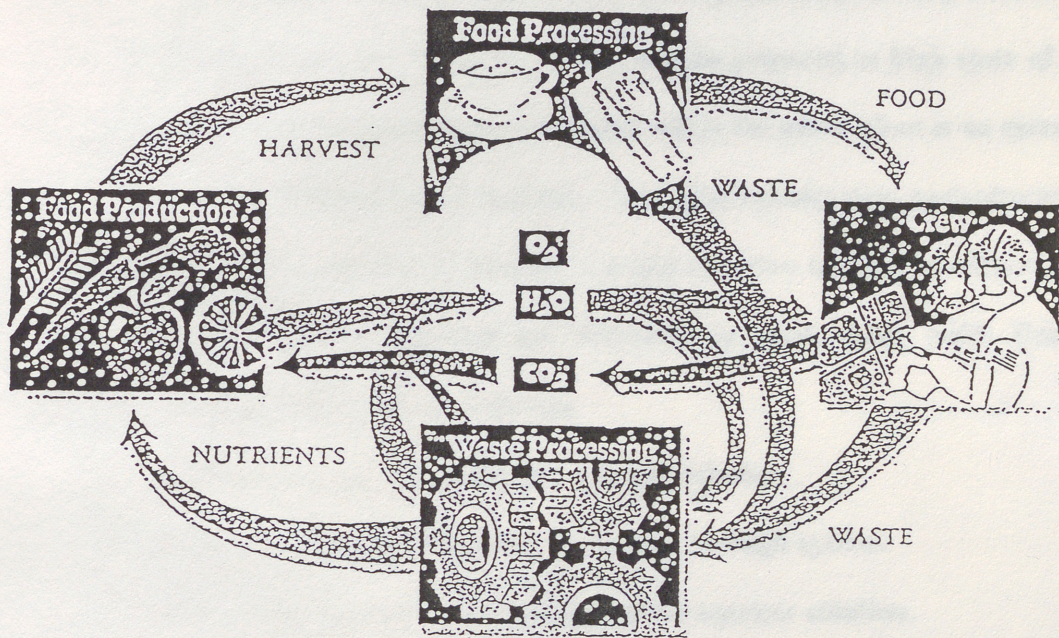
Figure 1. Generic diagram of CELSS, Illustrating Primary Mass Flows Among Subsystems
Source: Design of a Controlled Ecological Life Support System, Steven H. Schwartzkopf. Bio Science Vol. 42 No. 7 (1992)

Nutrient availability and composition are subject to accurate control in a hydroponic system. Water requirements never go low enough to be stressful to the plant. The pH can be accurately controlled. The precise control of nutrient composition provides constant ionic strength and continuous nutrient supply to the plants. The pH of the solution should

be regulated between 5.8 and 6.3. With the nitrogen supplied as NO_3^-, the pH of the solution rises due to the removal of nitrate ions. As the completely dissociated anion (NO_3^-) is replaced by bicarbonate ion, which forms the undissociated carbonate molecule, hydrogen ions are removed from the system and pH rises requiring acid addition to keep the pH in the recommended range. Plant growth can be affected when the solution is below pH 5.0 or above pH 7.0.

The study of the inorganic nutrient requirements of green plants is easier than that of other kinds of organisms. The reason is that green plants require very little in the way of organic growth factors so that growth media may be prepared in high state of purity. The uptake and translocation of inorganic solutes within the whole plant is an essential and major aspect of the process of plant nutrition. Controlled-envoirnment agricultural systems provide out of season, top quality produce. Careful attention to light conditions, nutrient solutions, harvesting and handling are necessary to attain high yield. Commercial hydroponic system consists of the following.

1. A nutrient reservoir that holds the nutrient solution.

2. A pump that circulates the nutrient solution through system.

3. A delivery line that allows the passage of the nutrient solution.

4. Lay flat plastic tubes which contain plant roots and nutrient solution.

5. Drain line that catches solution from the tubing for delivery back in to the reservoir.

Advantages of nutrient film technique

1. Plants give high yield.

2. The medium will be weed free.

3. The nutrient film technique maintains optimum moisture at the root surface.

4. The precise control of composition of the nutrient solution provides a continuous nutrient supply to the plants.

5. Root temperature is maintained at optimum by heating or cooling the nutrient solution.

6. The space requirement is minimum.

The processes involved in the uptake and translocation of solutes are the initial acquisition of cations by the plant root, their transport from root to shoot through the xylem, the subsequent accumulation by the tissues of the shoot, and the redistribution of these cations within the plant through the phloem pathway. Plants will absorb to some extent any element presented to them in the nutrient media but the ionic content of plants will vary, reflecting selectivity and species variation, and the stage of development of the plant. The continued delivery of metal ions such as iron, copper, zinc, manganese, nickel, vanadium, calcium and potassium ensures that the leaf is supplied with the mineral nutrients essential to its growth and development. Ions cross the plasma membrane of the root cell and this movement across the membrane permit uptake to occur.

CHAPTER 2

LITERARY REVIEW

There are fascinating examples of the deductive use of chemistry in tracing metal ions in plant nutrients during metabolism. A deficiency of the element makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle. Such deficiency is specific to the element in question. It can be prevented or corrected only by supplying that element. The quantities of nutrients required by plants are of enormous theoretical and practical importance. It is clearly not enough to know how much of a nutrient is necessary for a plant to survive, but how much is necessary for optimum growth. A plant responds to changes in the concentration of nutrients by changes in the rate of plant growth.

Table 1

Typical Concentrations of Mineral Elements in Normal Plants

Element	p.p.m in dry matter	mM in cell sap	Nutrient solution (mM)
N	15,000-35,000	150-350	15
P	1500-3000	7-14	1
S	1000-3000	7.5-140	1.5
Ca	10,000-50,000	35-175	5
Mg	2500-10,000	15-60	1.5
K	15,000-50,000	55-180	5
Na	200-2,000	1-12	1
Fe	50-300	0.15-0.75	0.1
Mn	25-250	0.06-0.6	0.01
Cu	5-15	0.01-0.03	0.001
Zn	15-75	0.03-0.15	0.002
Co	0.2-29	0.005-0.05	0.0002
Mo	0.5-5	0.004-0.075	0.0005
Cl	100-1000	0.4-4	0.1

Source: Uptake of Cations and Their Transport. Baker, D.A (1976)

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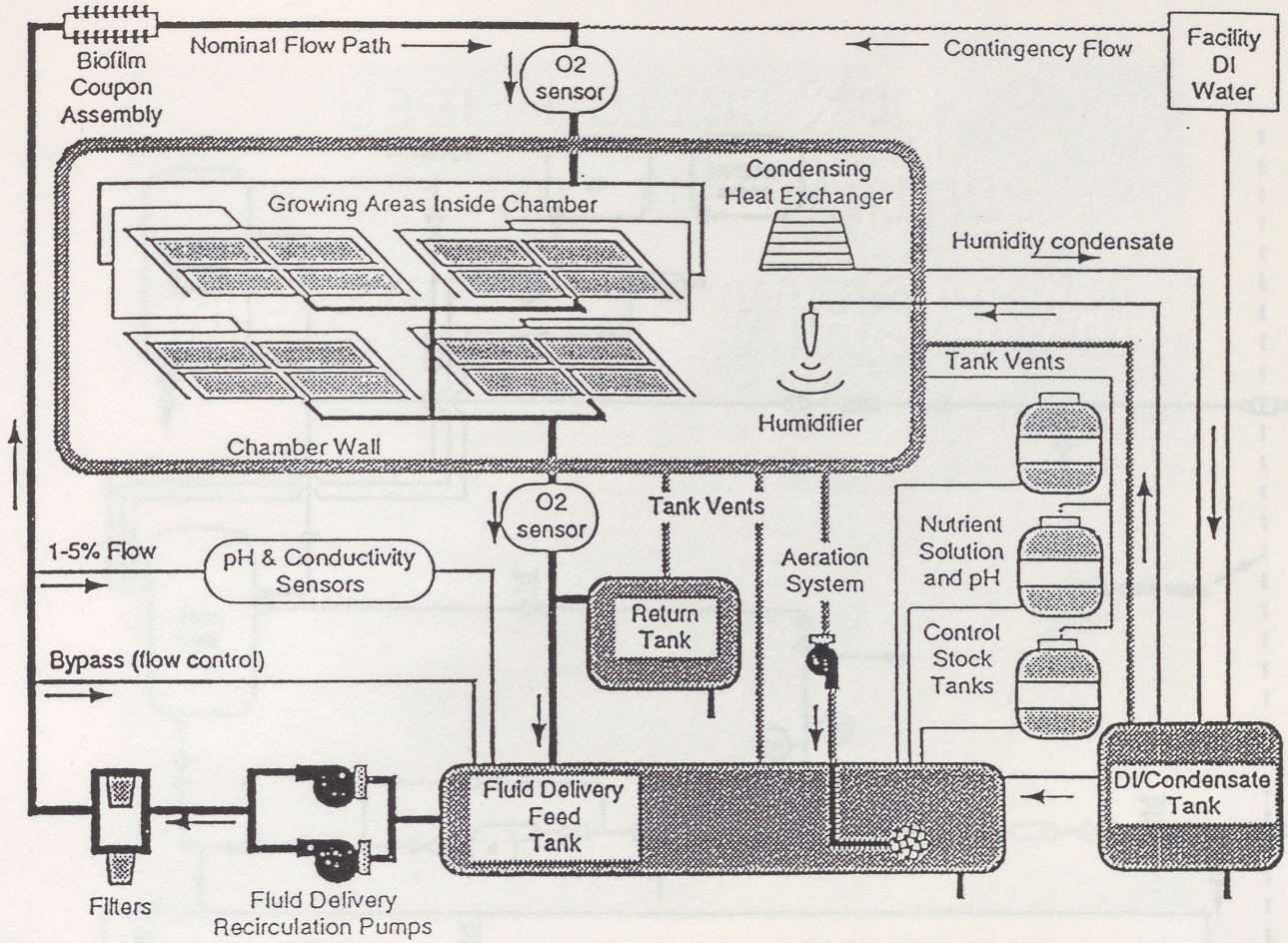
Figure 2. Schematic Diagram of the Operational Features of Hydroponic Systems in Space Shuttles
Source: Design of a Controlled Ecological Life Support System. Steven H. Schwartzkopf. Bio Science Vol 42 No.7 (1992)

Figure 3. Schematic Diagram of Hydroponic/Solid Substrate Irrigation Subsystem in Space Shuttles
Source: Design of a Controlled Ecological Life Support System. Steven H. Schwartzkopf. Bio Science Vol 42 No.7 (1992)
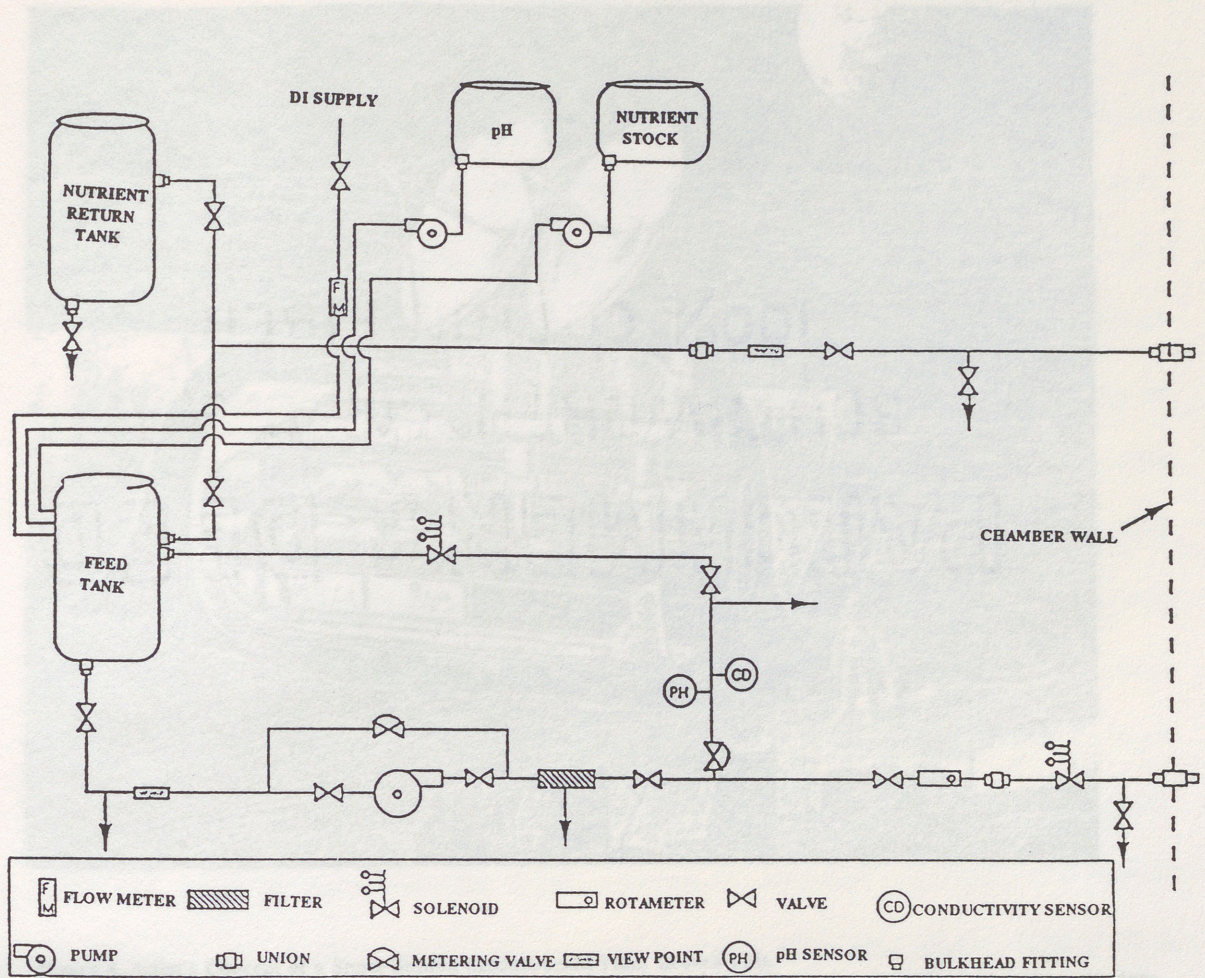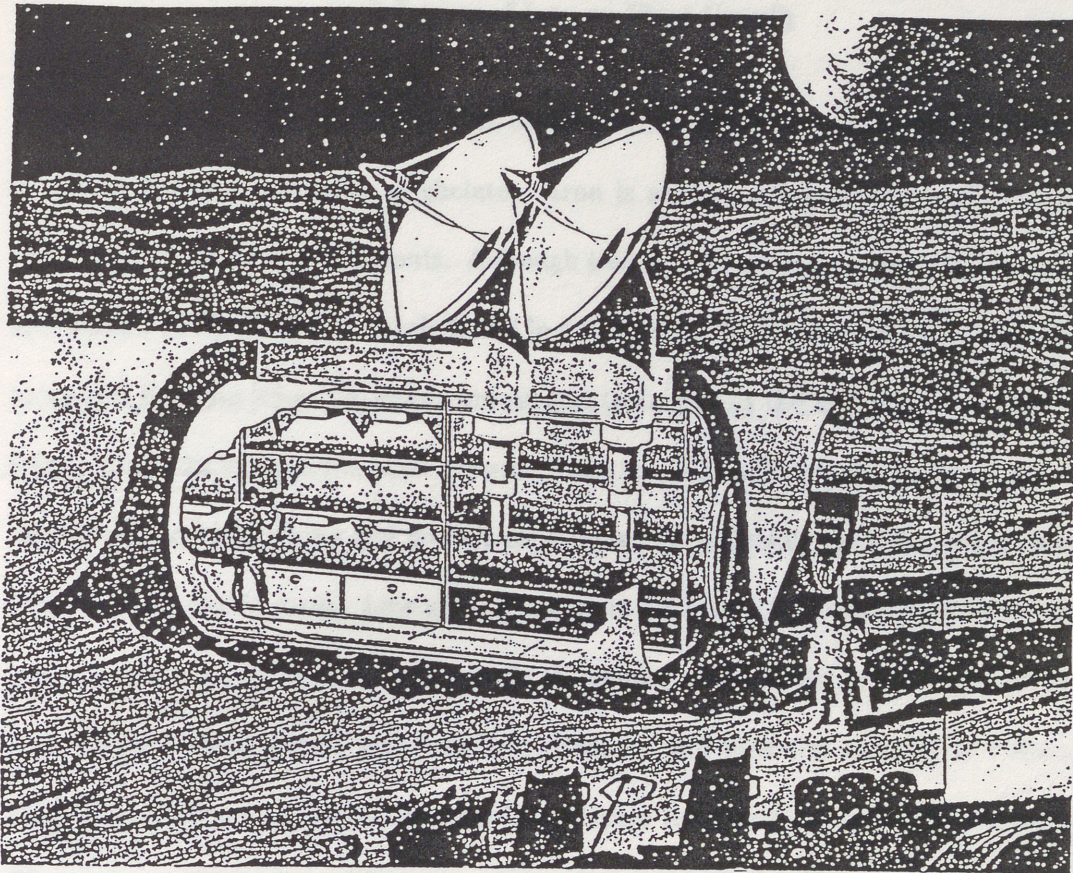
Figure 4. Artist's Concept of a Space Station Module-based Plant Growth Unit.
Source: Design of a Controlled Ecological Life Support System. Steven H. Schwartzkopf. Bio Science Vol 42 No.7 (1992)

Influence of Iron on Plant Growth

The metals required for the growth of plants exists for the most part in cells and tissues as complexes and especially as chelates. Iron is an element that is essential for growth, metabolism, and survival of plants. Although iron is a common element found in soil, water and tissues of all types, it is rarely found in a free form. Some plants employ iron chelators to secure the iron from the envoirnment and to supply to the cell in a form that can be transported. In hydroponic plant growth, iron should be added to the media in a form that will remain soluble under the growth conditions. In an acidic medium simple iron salts remain in a soluble form. Low affinity chelators such as EDTA and citric acid help keep iron soluble and available for cell growth. Chelators added to the growth medium promote iron uptake. Most plants maintain iron efficiency and have the capacity to reduce ferric chelators. Mineral deficiencies caused by disease are common in plants (Huber, 1978). They may occur by immobilization of the minerals by microbial pathogens and iron in particular is not readily mobile even in healthy plants (Chenand Barak, 1982). It is reasonable to suspect that microbial pathogens may cause local iron deficiency. Disease through iron limitation has been observed during infection of beans by the microbial pathogen Fusarium Solani (Guerra and Anderson, 1985). In their experiment, plants were starved for iron by being grown in iron deficient hydroponic medium. Chelated iron is added to produce iron sufficiency. Addition of iron to healthy plants did not show any significant effect on plant height or leaf area. But in infected plants, four times higher chlorophyll content was detected. Low iron availability to bean plants induced an increase of 183% in size of lesions caused by Fusarium Solani. The peroxidase are iron containing

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enzymes that catalyze oxidation-reduction reactions, and are involved in the formation of suberin and lignin which act as significant structural resistance barriers against pathogens. Iron deficiency generally increases disease severity in plants (Barash, 1990). Severe iron deficiency of plant can occur if pH is not controlled in 6.0 to 6.5 range on a daily basis. If the pH is below 5.0, the plant roots are damaged by the hydrogen ions in the solution and if it rises above pH 7.0, the availability of iron is affected. In excess of nitrogen, ethylenediamine tetraacetic acid will not decompose above pH 6.5.

Iron plays an essential role in respiration, DNA and chlorophyll biosynthesis, and many other enzymatic activities. The enzyme ferrochelatase catalyzes the insertion of Fe^{2+} ions in to porphyrins to make haeme-protein complexes in animals and plants. Haeme synthesis is essential for the survival of nearly all species. The plastids are structures characteristic of plant cells, present in green tissue as chloroplasts which are the site of all chlorophyll and photosynthetic reactions. Ferrochelatase is present in plastids and mitochondria (Porra and Lascelles, 1968). Complexed iron has a lower oxidation-reduction mid-point potential than free iron and the oxidized state is favored in complexes.

Recycle Efficiency of EDTA

Iron is taken up and transported more readily when supplied as a chelated complex, such as iron(111) ethylenediamine tetraacetic acid. In EDTA Fe chelate complex, the six donor atoms bond to the central atom. A complex is a combination of a metal ion and an electron donor group called a ligand. Bonds between the metal and the ligands are called coordination bonds. These bonds may be either high ligand-field or low ligand-field. A chelating ligand is one containing two or more functional groups with each able to donate electrons to a single metal ion. The fact that several functional groups of a chelating ligand are more or less fixed in space greatly increase the probability that the metal will form

complexes with all these groups simultaneously. This greatly increases the stability of chelating ligands. Ethylenediamine tetraacetate ion is a hexadentate ligand.

$$^-O_2CCH_2 \quad\quad\quad CH_2CO_2^-$$
$$\rangle NCH_2CH_2N \langle$$
$$^-O_2CCH_2 \quad\quad\quad CH_2CO_2^-$$

When the number of chelate ring is increased, the stability of the chelate increases.

In the equilibrium between the metal ions and ligands,

$M^{+m} + L^n \rightleftharpoons ML^{m-n}$, the formation constant K_f of the complex is

$$K_f = \frac{[ML^{m-n}]}{[M^{+m}][L^n]}$$

TABLE 2

Some of the Functional Groups Typically Found in Ligands are the Following

---

-O-	Enolate
$-NH_2$	Amino
-N=N-	Azo
N	Ring N

$$
\begin{array}{l}
\quad\; O \\
\quad\; \| \\
-C \\
\quad\; \backslash \\
\quad\; O
\end{array}
\qquad \text{Carboxylate}
$$

$$
\begin{array}{c}
\;\;|\quad\quad| \\
-C-O-C- \qquad \text{Ether} \\
\;\;|\quad\quad|
\end{array}
$$

$$
\begin{array}{l}
\backslash \\
C=O \qquad \text{Carbonyl} \\
/
\end{array}
$$

-OH	Alcohol
-SH	Sulfhydril
$-SO_2O^-$	Sulfonate
$-PO_3^-$	Phosphate

The number of functional groups with which a metal will complex is said to be the coordination number. These numbers are obtained by comparing the formation constants for complexes of a metal with increasing numbers of ligands found. Coordination numbers of a metal typically equal the number of additional electrons required to fill the metals noble gas shell. This number may differ for different oxidation states.
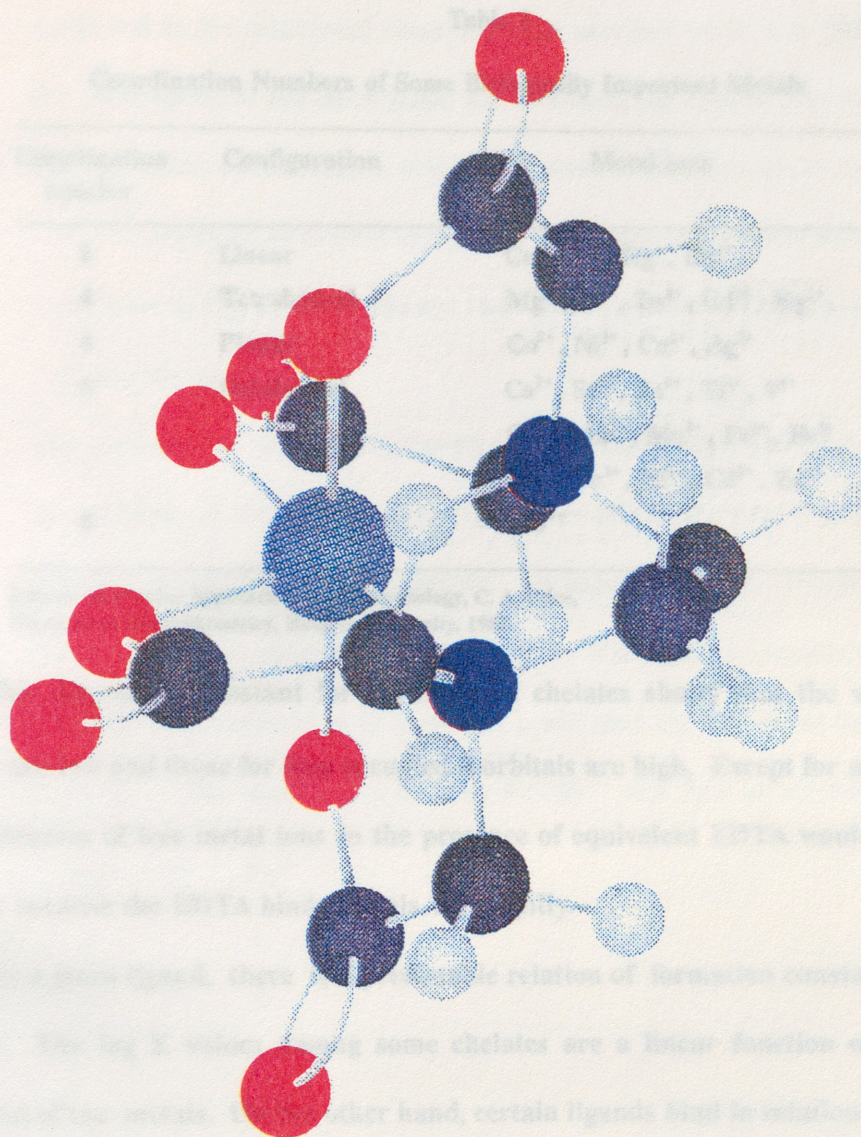
Figure 5. Ball-and-Stick Model of Metal Ion Complexed with the Hexadentate Ligand EDTA
Source: General Chemistry - J. B. Umland, University of Houston, 1993

Table 3

Coordination Numbers of Some Biologically Important Metals

Coordination number	Configuration	Metal ions
2	Linear	Cu^+, Ag^+, Hg^+, Hg^{2+},
4	Tetrahedral	Mg^{2+}, B^{3+}, Zn^{2+}, Cd^{2+}, Hg^{2+},
4	Planar	Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^{2+}
6	Octahedral	Ca^{2+}, Sr^{2+}, Ba^{2+}, Ti^{4+}, V^{4+}
		Cr^{3+}, Mn^{2+}, Mn^{3+}, Fe^{2+}, Fe^{3+},
		Co^{2+}, Co^{3+}, Ni^{2+}, Cd^{2+}, Zn^{2+}
8		Mo^{4+}

The formation constant for EDTA-metal chelates shows that the values for alkali metals are low and those for ions occupied d-orbitals are high. Except for alkali metals, the concentration of free metal ions in the presence of equivalent EDTA would be very small. This is because the EDTA binds metals very tightly.

For a given ligand, there is a predictable relation of formation constants for different metals. The log K values among some chelates are a linear function of the ionization potential of the metals. On the other hand, certain ligands bind in relation to the solubility products of the corresponding metal sulfides.

The structure and composition of the chelating ligand is obviously crucial to the stability of the chelate. The following factors contribute to their stability.

(1) Number of chelate rings. The notion that entropy decreases when ligands are tied together led Schwarzenbach and Adamson (cf. Charberk and Matell, 1959) to predict an increment of approximately 2 log K units for each chelate ring formed

compared with the corresponding monodentate complex. The additional stability conferred by the additional rings has been ascribed by R. J. P Williams (1959) to the overcoming of repulsive polar forces among the complexing ligands.

(2) The size of the chelate ring is critical. It must be long enough to accommodate the directed angles of electronic orbitals of the metal, but it must be short enough to contribute to the negative entropy and heat of reaction. Five membered rings are typically the most stable.

(3) The composition of chelating ligand can have highly specific effects. For example, the addition of alcoholic hydroxyl group usually adds little or strongly decrease stability, except for the Fe^{3+} chelate. An important factor affecting oxidation potentials is the presence chelating agents. If a ligand L capable of binding Fe^{3+}, and Fe^{2+} is introduced in to a system, L will bind the Fe^{3+} more tightly than it will bind Fe^{2+}. The equilibrium between Fe^{2+}/Fe^{3+} will then be altered:
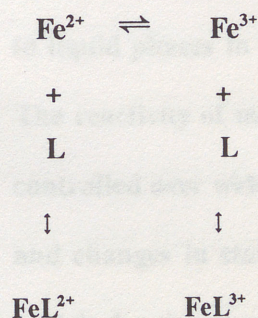
$$Fe^{2+} \quad \rightleftharpoons \quad Fe^{3+}$$
$$+ \qquad\qquad +$$
$$L \qquad\qquad L$$
$$\updownarrow \qquad\qquad \updownarrow$$
$$FeL^{2+} \qquad FeL^{3+}$$

Since the oxidized form (Fe^{3+}) is stabilized, the presence of L will clearly raise the oxidation potential. In the range of pH 3.5 to 6.5 Fe^{2+} and Fe^{3+} react with EDTA as follows.

$$Fe^{2+} + EDTA^{4-} \quad \rightleftharpoons \quad Fe(EDTA)^{2-} \quad \log \ K_f = 14.3$$

$$Fe^{3+} + EDTA^{4-} \quad \rightleftharpoons \quad Fe(EDTA)^{-} \quad \log \ K_f = 25.1$$
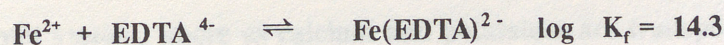
In the presence of 100 mM EDTA^{4-}, the concentration of metal ion will be

$$[Fe^{2+}] = \frac{[Fe(EDTA)^{2-}]}{[EDTA^{4-}] \times 10^{14.3}} \quad and$$

$$[Fe^{3+}] = \frac{[Fe(EDTA)^{-}]}{[EDTA^{4-}] \times 10^{25.1}}$$

The catalytic activity of a metal in oxidation reduction reactions can be enhanced by chelation. For example, ascorbic acid oxidation by cupric ion is 100 times greater in ascorbic acid oxidase than in free ion (Meikljohn and Stewart, 1955). The absorption spectra of metal chelates have been the principle means of assessing their electronic structure. Complexation, in general, and chelation, in particular, are important to the plant in the following ways:

(1) Chelates may stabilize structural elements.

(2) The electrical charge on a metal may be neutralized so that it can be brought in to liquid phases in concentrations otherwise unattainable.

(3) The reactivity of metal ions, and especially their oxidation potentials, may be controlled over wide ranges through changes in ligands. The stability of chelates, and changes in stability through changes in molecules bearing ligands, serve as vehicle for the accumulation of nutrients.

The distribution of cations within the plant may be heterogenous. Elements such as iron, lead, nickel, copper, zinc, manganese and chromium being preferentially retained by the root system, where as calcium and potassium are transported in major part to the shoot system. The pattern of distribution of these elements may be considerably affected by their concentration in the external medium. Plants live in a dilute ionic environment and they accumulate ions several times over the concentrations in the environment.

CHAPTER 3

EXPERIMENTAL SECTION

Purpose

The purpose of this experiment is to determine the recycle efficiency of EDTA when used as a chelating agent in plant nutrient solutions in hydroponic systems.

Reagents and Chemicals

All chemicals used in these studies were reagent grade level. EDTA, KH_2PO_4, $MgSO_4.7H_2O$, $CuSO_4.5H_2O$, H_3BO_3, $Na_2MoO_4.2H_2O$, $MnSO_4.H_2O$, NaCl and $FeCl_3$ were obtained from Fisher Scientific. KNO_3, $Ca(NO_3)_2.4H_2O$ and $ZnSO_4.7H_2O$ were obtained from J. T. Baker Chemical Company.

Instruments

The pH of the solutions were measured using the Digital Ionalyzer/501 (Orion Research). The ionic strength was measured using the Dist Conductivity meter (Hanna Instruments). A BAS 200A (Bio Analytical System Inc., West Lafayette, Indiana) high performance liquid chromatography (HPLC) was the instrument used for the determination of EDTA as FeEDTA in excess iron in the nutrient solutions.

Procedure

The stock nutrient solution I (200 ml) was prepared by completely dissolving 10.1000g KNO_3, 2.7200g KH_2PO_4, 9.8600g $MgSO_4.7H_2O$, 0.0240g $MnSO_4.H_2O$, 0.0040g $CuSO_4.5H_2O$, 0.0100g $ZnSO_4.7H_2O$, 0.0470g H_3BO_3, 0.0001g $Na_2MoO_4.2H_2O$ and 1.1600g NaCl in 150 ml deionized water and bringing up to the final volume (200 ml). The stock

solution II was prepared by dissolving 0.8800g Na_2EDTA and 23.6000g $Ca(NO_3)_2.4H_2O$ in 200 ml deionized water. One ml $FeCl_3$ solution (100 ppm) was added to stock solution II prior to adjusting the volume. The working solution was prepared (4000 ml) by diluting 20 ml stock solution I and 20 ml stock solution II to the final volume. The pH of the working solution was adjusted to 6.0 prior to supplying the nutrients to germinated bean plants. The pH of the nutrient solution was measured regularly and adjusted manually. When the solution was above pH 6.3, nitric acid (HNO_3) was added to the nutrient solution to provide hydrogen ions and when the pH was below 5.8, potassium hydroxide (KOH) was added to provide the hydroxyl ions to adjust the pH to the desired value. The stock refill nutrient solution was prepared by dissolving 12.1000g KNO_3, 2.0400g KH_2PO_4, 4.8000g $MgSO_4.7H_2O$, 0.5800g NaCl, 0.0100g $MnSO_4 .H_2O$, 0.0020g $CuSO_4.5H_2O$, 0.0430g H_3BO_3, 0.00008g $Na_2MoO_4.2H_2O$ and 0.0050g $ZnSO_4.7H_2O$ in 50 ml deionized water and the volume was adjusted to 100 ml. Five milliliters stock refill solution was diluted to 1000 ml. The nutrients were supplied as needed to maintain the ionic strength at desired range. The ionic strength of the nutrient medium was measured using the Dist conductivity meter.

Lima bean seeds (Phaseolus) were germinated on August 14, 1995. The plant normally produce crops in 45 days. The hydroponic systems were set up in triplicate. After one week the plants started growing and they appeared healthy. The temperature was monitored and was 23˚C. Halogen lamps were used as light source for photosynthesis. Air was circulated through the nutrient medium using an air pump. The pH and the ionic strength of the solutions were measured regularly and maintained at desired values. The root system of the plants were protected from light. Fifteen days after germination, the plants were 5" tall. The nutrient solutions were added and the volume of solutions in the dishes were adjusted to two liters. The pH of the nutrient solution in tray # 1 was

maintained at 5.4 - 5.8. The pH of nutrient solution in tray # 2 was between 6.0 - 6.5 and tray # 3 had pH in the range of 7.0 - 7.5. In the early stage of growth, plants in all trays appeared healthy. After three weeks, the plant leaves in tray # 3 started drying up. The leaves were fallen off after a few days. The new leaves were smaller in size than the plant leaves in tray # 2. Eventually the plant lost all leaves and stopped growing. Five weeks after germination, it was noticed that the root system of plants in tray# 1 were decaying. The leaves turned yellow and had fallen off. The plants appeared weaker than those in tray # 2. After forty-five days it became evident that plants in tray # 1 and tray # 3 will not survive. The plants in tray # 3 died in two days and they were discarded. The plants in tray # 1 died a few days later.

TABLE 4

pH and Ionic Strength of Nutrient Solutions and Condition of Plants

Date	pH			Ionic strength*			Condition of plants		
	Tray #1	Tray #2	Tray #3	Tray #1	Tray #2	Tray #3	Tray #1	Tray #2	Tray #3
8 - 14 - 95	5.60	6.00	7.30	42	42	42			
8 - 16 - 95	5.62	6.04	7.30	42	42	41			
8 - 18 - 95	5.63	6.10	7.33	41	41	41			
8 - 21 - 95	5.63	6.16	7.38	42	42	42			
8 - 23 - 95	5.64	6.01	7.36	42	43	42	healthy	healthy	healthy
8 - 25 - 95	5.61	6.06	7.33	41	41	40			
8 - 28 - 95	5.59	6.10	7.34	40	42	41			
8 - 31 - 95	5.60	6.09	7.32	41	42	40	5" tall	5" tall	5" tall
9 - 06 - 95	5.59	6.08	7.34	42	41	41			
9 - 08 - 95	5.63	6.10	7.38	40	42	42	weak	healthy	lost leaves
9 - 11 - 95	5.58	6.02	7.40	41	43	43			
9 - 13 - 95	5.61	6.07	7.37	40	43	42			
9 - 15 - 95	5.66	6.10	7.41	42	42	42			
9 - 18 - 95	5.70	6.13	7.39	41	42	40			
9 - 20 - 95	5.65	6.15	7.40	40	42	41	roots dead	healthy	stops growth
9 - 22 - 95	5.66	6.14	7.36	42	41	41			
9 - 25 - 95	5.64	6.10	7.33	42	41	42			
9 - 27 - 95	5.66	6.12	7.38	42	40	41			
9 - 29 - 95	5.60	6.10	7.40	41	42	41	dead	12" tall	dead
10 - 02 - 95		6.14			42				
10 - 05 - 95		6.17			41				
10 - 09 - 95		6.18			41				
10 - 12 - 95		6.21			42				
10 - 16 - 95		6.22			42				
10 - 18 - 95		6.21			41				
10 - 20 - 95		6.27			41				
10 - 26 - 95		6.24			40				

*The displayed value from the conductivity meter was multiplied by factor 10 to get the reading in ppm (manufacturer's instruction)

On September 30, 1995, blooms were appeared on plants in tray # 2. A few flowers were fallen off from the plants. New flowers were seen on the plants at sixty days from the date of germination. Small beans were noticed on the plants on October 20, 1995.

By November 6, 1995, the beans were ready to be picked. The matured beans were picked and weighed. The beans weighed 10.5 grams. On November 13, beans were picked again and the beans weighed 8.5 grams.

Figure 9. Approximately Grown Pinto Bean Plant with Beans

Figure 6. Hydroponically Grown Lima Bean Plants with Beans.
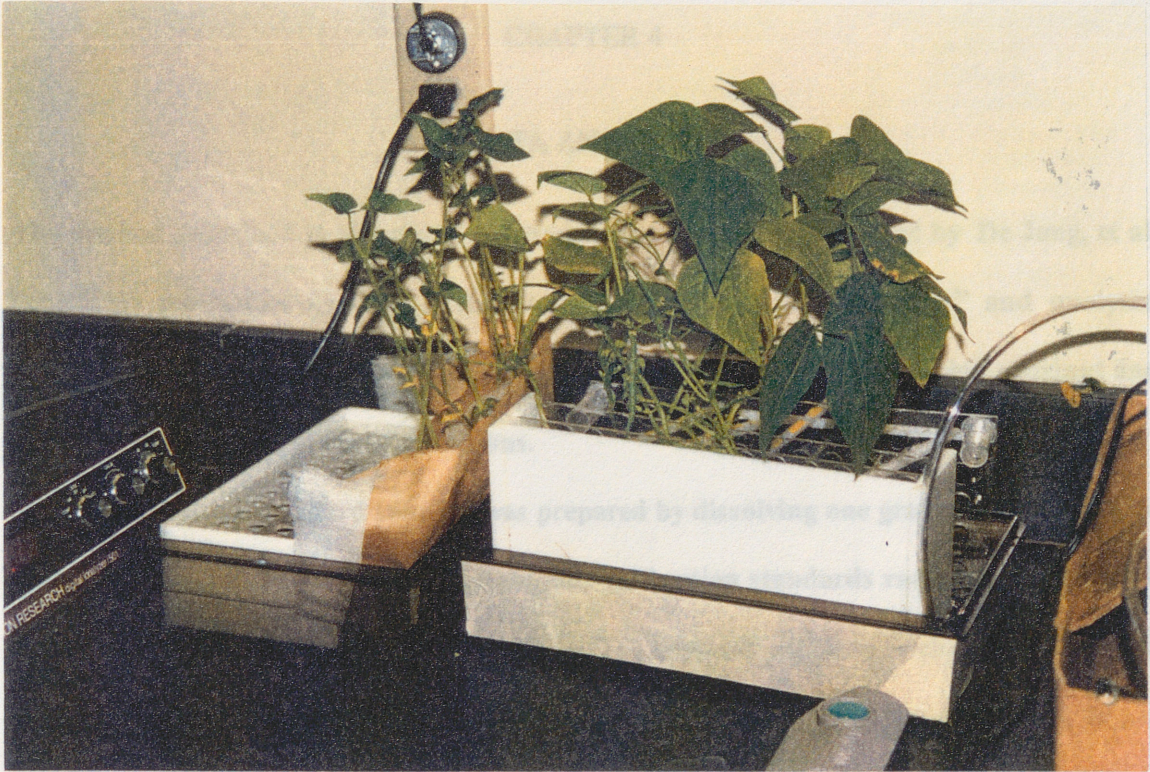
Figure 7. Another View of Hydroponically Grown Plants in the Laboratory.

CHAPTER 4

DATA ANALYSIS

The method described in this thesis is based on the procedure described by De Jong, et al. (1991). In the studies reported in this thesis EDTA was chelated with Fe^{3+} and analyzed using the BAS 200A analyzer. The Fe^{3+}/EDTA complex has a large formation constant and minimal interference of other metal ions.

EDTA stock standard solution was prepared by dissolving one gram of Na_2EDTA in one liter deionized water. From this solution, calibration standards ranging from 10 to 80 μmol/L EDTA were prepared. The EDTA in the standards which were chelated with $FeCl_3$ solution and were analyzed using the BAS 200A analyzer. The injection volume was 20 μL. The electrochemical cell temperature was 30°C. The mobile phase in bottle A was set to 50°C. The mobile phase in bottles B and C were set to 35°C. The detection wave length was 254 nm. The calibration graph was obtained by plotting the values of standards with EDTA concentrations ranging from 10 to 80 μmol/L.

Table 5

Calibration Data

Standards	Concentrations (μmol/L)	Area
Level 1	10	418678
Level 2	20	809440
Level 3	40	1550634
Level 4	60	2351164
Level 5	80	3176025
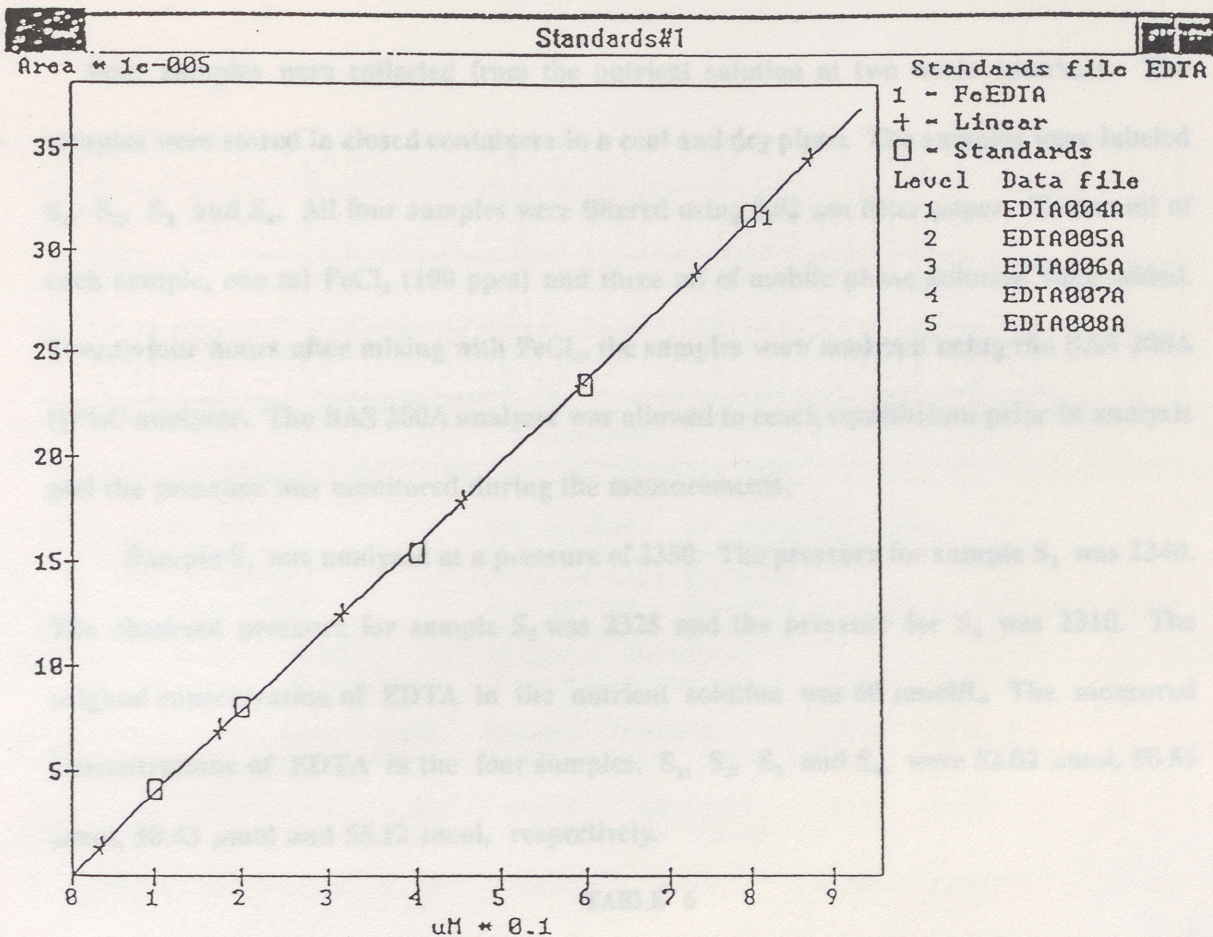
Figure 8. Graphical Report of Calibration Standards with EDTA Concentrations Ranging from 10 to 80 μmol/L

Four samples were collected from the nutrient solution at two weeks intervals. The samples were stored in closed containers in a cool and dry place. The samples were labeled S_1, S_2, S_3 and S_4. All four samples were filtered using 0.02 μm filter paper. To one ml of each sample, one ml $FeCl_3$ (100 ppm) and three ml of mobile phase solution were added. Twenty-four hours after mixing with $FeCl_3$, the samples were analyzed using the BAS 200A HPLC analyzer. The BAS 200A analyzer was allowed to reach equilibrium prior to analysis and the pressure was monitored during the measurements.

Sample S_1 was analyzed at a pressure of 2350. The pressure for sample S_2 was 2340. The observed pressure for sample S_3 was 2325 and the pressure for S_4 was 2310. The original concentration of EDTA in the nutrient solution was 60 μmol/L. The measured concentrations of EDTA in the four samples, S_1, S_2, S_3 and S_4, were 52.02 μmol, 50.56 μmol, 50.43 μmol and 55.12 μmol, respectively.

TABLE 6

Dates Samples Collected and Their Concentrations

Sample #	Date collected	Concentration (μ mol/L)
S1	8 - 31 - 95	52.02
S2	9 - 20 - 95	50.56
S3	10 - 05 - 95	50.43
S4	11 - 06 - 95	55.12
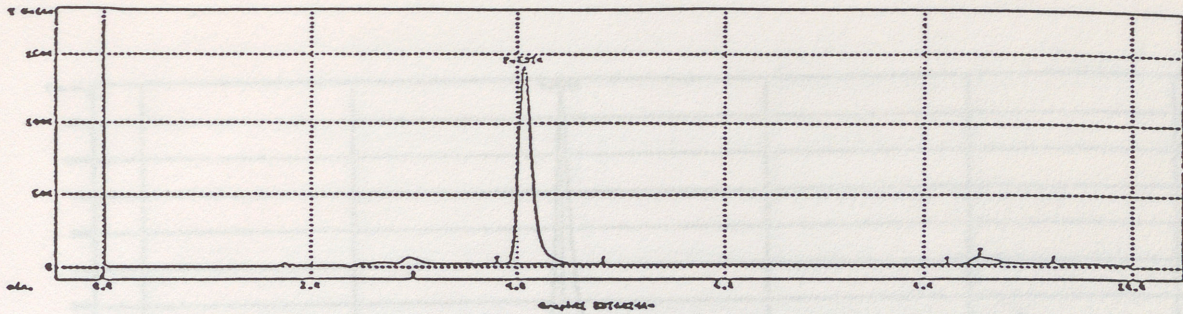
```
------------------------------------------------------------------
      ChromGraph REPORT 1.2      Tue Nov 21 19:07:47 1995
------------------------------------------------------------------

Data filename: EDTA              Run number:  24  Detector: A=UV
Vial number:  0                  Data Set 1 name: EDTA024A
Time of run:   13:29             Date of run: 11/21/95
Method filename: TEMP            Run notes:   Analysis of EDTA in Nutrient Solut
Operator: CHACKO                 Conditions:  1 ml/min
Standards ( Area ): TEMP         Dilution multiple: 5
Time zero offset:  0             Time scale factor: 1

Peak Minutes Type  Skew  Width    Height  %Height       Area   %Area *
  Peak-Name        Grp   400 um   +/-Error Std  dTime DP

   1   4.07  BB   1.24   0.14     45492    96.73      421358  93.09
 FeEDTA           0     10.403     0.685    1    0.03  1

   2   8.56  BB   1.39   0.28      1538     3.27       31299   6.91


                                 47029   100.00      452657 100.00 0
 Totals                 10.403

                                                      421358 100.00
 Total for Group   0    10.403   100.00%

                                                      421358 100.00
 Total Identified       10.403   100.00%
```

Figure 9. Chromatograph Report for Sample # S_1
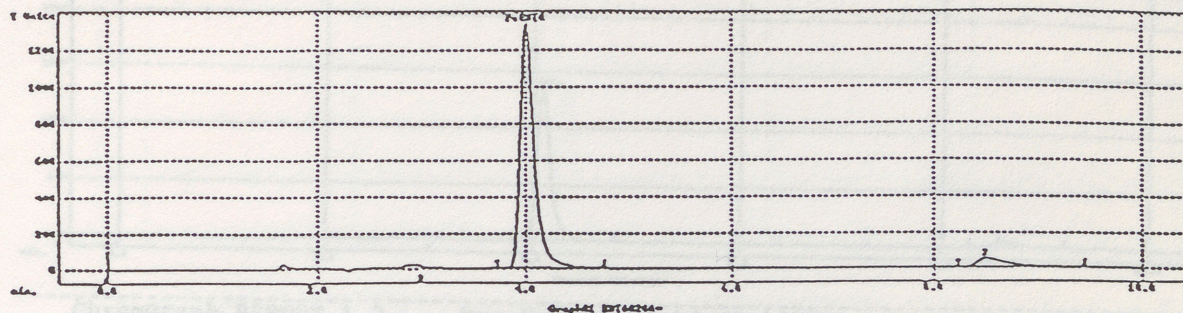
ChromGraph REPORT 1.2   Tue Nov 21 19:12:16 1995

Data filename: EDTA	Run number: 28 Detector: A=UV
Vial number: 0	Data Set 1 name: EDTA028A
Time of run: 14:33	Date of run: 11/21/95
Method filename: TEMP	Run notes: Analysis of EDTA in Nutrient Solut
Operator: CHACKO	Conditions: 1 ml/min
Standards (Area): TEMP	Dilution multiple: 5
Time zero offset: 0	Time scale factor: 1

Peak Peak-Name	Minutes	Type	Skew Grp	Width 400 um	Height +/-Error	%Height Std	dTime DP	Area	%Area *
1	4.01	BB	1.13	0.14	43839	96.66		409906	92.59
FeEDTA			0	10.111	0.685	1	-0.03 1		
2	8.51	BB	1.42	0.30	1514	3.34		32792	7.41
Totals				10.111	45354	100.00		442698	100.00 0
Total for Group 0				10.111		100.00%		409906	100.00
Total Identified				10.111		100.00%		409906	100.00

Figure 10. Chromatograph Report for Sample # S_2
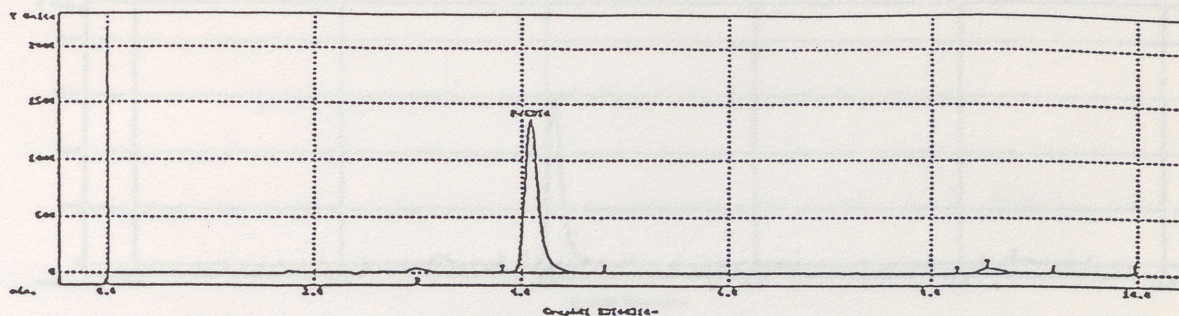
```
- - - - - - - - - ChromGraph REPORT 1.2      Tue Nov 21 19:16:41 1995 - - - - - - - - -

Data filename: EDTA              Run number:  31  Detector: A=UV
Vial number:   0                 Data Set 1 name: EDTA031A
Time of run:   15:10             Date of run: 11/21/95
Method filename: TEMP            Run notes:   Analysis of EDTA in Nutrient Solut
Operator: CHACKO                 Conditions:  1 ml/min
Standards ( Area ): TEMP         Dilution multiple: 5
Time zero offset:   0            Time scale factor: 1

Peak Minutes Type  Skew  Width    Height  %Height     Area   %Area *
  Peak-Name        Grp   400 um   +/-Error Std  dTime DP

  1    4.09   BB   1.12  0.14     44193   97.04      408904  93.91
FeEDTA             0     10.086    0.685   1    0.05  1

  2    8.56   BB   1.24  0.29      1349    2.96       26528   6.09

                                  45542  100.00      435432 100.00 0
Totals                   10.086

                                                     408904 100.00
Total for Group  0       10.086  100.00%

                                                     408904 100.00
Total Identified         10.086  100.00%
```

Figure 11. Chromatograph Report for Sample # S₃
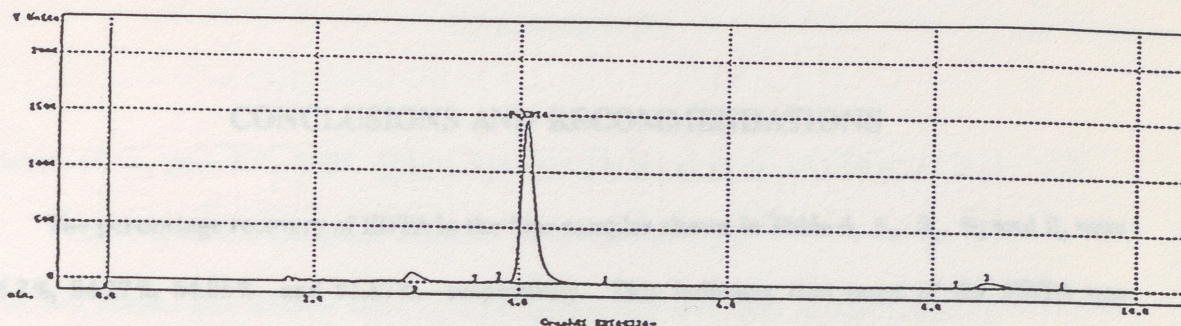
```
--------------------------------------------------------------------------------
        ChromGraph REPORT 1.2          Tue Nov 21 19:19:52 1995
--------------------------------------------------------------------------------

Data filename: EDTA            Run number:  32  Detector: A=UV
Vial number:   0               Data Set 1 name: EDTA032A
Time of run:   15:27           Date of run: 11/21/95
Method filename: TEMP          Run notes:    Analysis of EDTA in Nutrient Solut
Operator: CHACKO               Conditions:  1 ml/min
Standards ( Area ): TEMP       Dilution multiple: 5
Time zero offset: 0            Time scale factor: 1

Peak Minutes Type  Skew   Width    Height  %Height        Area   %Area *
  Peak-Name        Grp    400 um   +/-Error Std  dTime DP

  1   3.81   BV    0.11   0.11     433      0.87           3023    0.63

  2   4.08   VB    1.12   0.14     47556    95.89          445687  92.40
 FeEDTA            0       11.023   0.685   1    0.04   1

  3   8.54   BB    1.31   0.29     1608     3.24           33632   6.97

                                   49597    100.00         482343 100.00 0
  Totals                  11.023

                                                           445687 100.00
  Total for Group  0      11.023   100.00%

                                                           445687 100.00
  Total Identified        11.023   100.00%
```

Figure 12. Chromatograph Report for Sample # S_4

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The percentage recovery of EDTA in the four samples shown in Table 6, S_1, S_2, S_3 and S_4 were 86.7%, 84.27%, 84.05% and 91.87%, respectively. This indicates that most of the EDTA was recovered from the nutrient solution. From these results it can be concluded that EDTA concentration does not decrease significantly during and after plant nutrient uptake.

Degradation of EDTA can happen due to microbial contamination of nutrient solutions. Prolonged photolysis of EDTA also may cause degradation. The excess nutrient solution that was not supplied to the plants was analyzed and a slight decrease in the EDTA concentration was observed. The toxicity of the chemical, if any, when used as a chelating agent in plant nutrient solutions need to be studied. Based on the present knowledge of EDTA, it can be considered as an efficient chelating agent which can be used in hydroponic solutions. Any degradation of EDTA in the plant nutrient solution appeared minimal.

There are several advantages of using high performance liquid chromatography for analysis of EDTA in plant nutrient solutions. The most important among them are the low cost, lower limit of detection and the good reproducibility.

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