1937

A study of the assimilation of some trace elements by plants

Robert A. Caughey
University of Massachusetts Amherst

Caughey, Robert A., "A study of the assimilation of some trace elements by plants" (1937). *Masters Theses 1911 - February 2014*. 1384.
Retrieved from https://scholarworks.umass.edu/theses/1384

A STUDY OF THE ASSIMILATION OF SOME TRACE ELEMENTS BY PLANTS

CAUGHEY · 1937

A STUDY OF THE ASSIMILATION
OF SOME TRACE ELEMENTS BY PLANTS

Robert A. Caughey

Thesis submitted for
the degree of
Master of Science
Massachusetts State College
June, 1937

TABLE OF CONTENTS

INTRODUCTION

The presence of the so-called trace elements in plant and animal tissues was early demonstrated, and considerable attention has been directed toward the role of these elements in plant and animal nutrition. The demonstration of the existence of iron in blood, and the therapeutic use of iron compounds in the correction of anemic conditions, naturally led to an investigation of the iron content of foodstuffs. The apparent catalytic effect of copper in the production of hemoglobin, and possibly chlorophyll, tended to stimulate an interest in the copper content of plants and the soils in which they are grown.

Many experiments have been performed to determine the effect of various elements on both the growth of the plant and the correction or prevention of diseases. The toxic effect produced by excessive amounts of some of these elements have also been studied. That the presence of at least a trace of the elements iron, copper, manganese, boron, and possibly zinc, is essential to normal growth has often been shown.

From the standpoint of the possible therapeutic value of foodstuffs having an unusually high content of one or more of these minor elements, it would be of value to determine whether such a high content could readily be obtained.

REVIEW OF LITERATURE

Obviously, a reasonable method of studying the effect which the addition of an element to the soil will have on the rate of assimilation of that element by a plant grown in that soil, would be to add the element, or a compound of the element to the soil and then, after growing a plant in it, determine the amount of the element present in the tissues of the plant. Comparison of this amount with the amount found in the tissues of a similar plant grown in normal soil would then demonstrate whether there was a definite effect as a result of such a fertilization.

The elements studied in this experiment, namely iron, copper, manganese, and iodine have long been known to exist in plant tissues, but in very small amounts. Brenchley (16) has described numerous water culture experiments in which the action of minute amounts of these elements in the nutrient solutions has been observed. All of them seem to be necessary for growth, but if present in a concentration substantially greater than that necessary for good growth, a stimulating effect may be replaced by a toxic one. Accordingly, although toxicity does not appear at such low concentrations when applied to the soil as in water solution, care must be exercised in the application of these elements. The type of soil, its acidity, moisture content and texture all contribute to the relative availability of the element to the plant (17, 18, 19 and 20).

The determination of the actual amounts of trace elements in plant and animal tissues has been the subject of much investigation, and has been limited considerably by the inadequacy of the available methods.

Iron, because it is ordinarily present in larger amounts than are copper, manganese, zinc, lead, etc., and because of its nutritional functions, has received more attention. Elvehjem and Peterson (21) determined the percentage of iron in about 150 samples of fruits and vegetables, and found it to range from 0.00015 per cent in lemon juice to 0.0192 per cent in parsley (dry basis). The iron content varied, in descending order, as follows: dried legumes, green leafy vegetables, dried fruits, nuts, cereals, green legumes, roots and tubers, non-leafy vegetables, fish and fresh fruits.

Copper and manganese are also of interest, since it has been shown (18, 19) that total absence of either may result in chlorosis or other malnutritional effects in a plant, and also because of their function in the animal body. Less complete data on the copper and manganese content are available than in the case of iron.

Elvehjem and Lindow (10) determined the copper content of several types of material and found the following amounts of copper present (dry basis): codfish, .00298 per cent; dates, 0.00053 per cent, lettuce, 0.00116 per cent; oats, 0.00101 per cent; plums, 0.00097 per cent. Lindlow, Elvehjem, and Peterson (22), using the Biazzo method (10) have analyzed 158 common food materials, and give values ranging from 1.9 ppm in strawberries to 28.7 ppm in radishes.

The manganese content of feeding stuffs has been investigated by Skinner and Peterson (23). Their results range from 5.4 ppm in corn to 712.5 ppm in sugar beet tops. Lindlow and Peterson (24) using the periodate method of Willard and Greathouse (8), examined some 84 samples of common foodstuffs and found manganese in amounts ranging from none in fish to 0.02162 in northern grown lettuce (dry basis).

However, in the determination made by the workers mentioned above, only a very limited number of samples of each type of material was analyzed, and the results cannot be said to represent the average content.

PURPOSE OF INVESTIGATION

The purpose of this investigation was to determine, first, the average normal amount of some of the trace elements contained in various plant materials; and second, if the amount of these elements might be appreciably increased by the application of compounds of the elements to the soil in which the plants are grown.

To accomplish this purpose, it was proposed to develop analytical methods which are sufficiently rapid and accurate to permit the routine analysis of a considerable number of samples; it was also proposed to design and carry out a lysimeter experiment by means of which the effect in question might be studied.

PART I. METHODS OF ANALYSIS

It was desired to examine the ash of plant materials for the iron, copper, manganese and also phosphorus which they contain. Most of the methods used are modifications of existing methods, applied to the particular case in hand.

A. Preparation of the Ash Solution

In view of the large number of determinations to be made, it was, of course, advantageous to prepare an ash solution from which a number of determinations might be made directly. The wet combustion method, using concentrated nitric and sulfuric acids was found to be very satisfactory.

Method: Ten grams of the air-dry material, ground to pass a 1 mm. screen, are transferred to a 500 ml. Kjeldahl flask, and 8 or 10 glass beads are added; after adding 25 ml. of concentrated sulfuric acid and mixing thoroughly, the flask is heated over a small flame; 50 to 75 ml. of concentrated nitric acid are added, drop by drop, from a dropping funnel until the solution becomes clear. It is then cooled, 25 ml. of water added, and boiled until all the nitric acid is expelled. After again cooling, 100 ml. of water are added and the solution is allowed to stand overnight. It is then transferred to a 500 ml. volumetric flask, made to volume at 25°C. and filtered into glass stoppered bottles. Aliquots of this solution are taken for analysis.

In cases where uncontrollable frothing begins upon application of heat, it may be necessary to add the nitric acid first, using about 25 ml. and gradually introduce the sulfuric acid later, after which the remainder of the nitric acid is added in the usual manner.

If after boiling off the excess nitric acid with 25 ml. of water a yellow color remains, the water must be boiled off, and small portions (5 ml.) of nitric acid added, drop by drop, to the boiling solution until the color disappears, when it may be treated as before.

Since these solutions were to be used for the determination of very small amounts of material, only the best grade of acids was used; all water used was redistilled from glass.

B. Determination of Iron

Kennedy (1) determined the iron in plant tissues colorimetrically by first ashing the material with sulfuric and perchloric acids, diluting with water, adding nitric acid, and developing the ferric thiocyanate color with sodium thiocyanate. The colored compound was extracted with amyl alcohol and compared with a standard.

Elvehjem and Hart (2) ashed the plant material in a muffle, and removed the interfering phosphorus by precipitation with ammonium molybdate from the nitric acid solution of the ash. Potassium permanganate was added to convert all the iron to the ferric state, and potassium thiocyanate was added to produce color.

Colorimetric determination of iron by means of the ferric thiocyanate was found to be quite satisfactory in this experiment.

Method:

Reagents Required:

Standard Iron Solution:

Iron wire (for standardizing)	0.2000 gm.
Sulfuric acid, 1-1	5.0 ml.
Nitric acid, concentrated	1.0 ml.
Iron free water at 25°C. to make	2000.ml.

The wire is thoroughly cleaned to remove rust, and dried. 0.2000 grams are dissolved by boiling with about .200 ml. water, 5 ml. sulfuric acid, and 1 ml. nitric acid. When solution is complete, and oxides of nitrogen have been expelled, the volume is made to 2 liters at 25° in a volumetric flask. 1 ml. contains .0001 gm. of iron.

Dilute iron solution:

200 ml. of the standard iron solution are placed in a 2-liter volumetric flask with 200 ml. of 1-1 sulfuric acid and made to volume at 25°C. 1 ml. contains .00001 gm. iron.

Potassium thiocyanate	400 gm. per 2000 ml.
Nitric acid, concentrated	
Iron-free water, redistilled from glass.	

Procedure: An aliquot (25 ml.) of the original ash solution containing about .0001 gm. of iron is transferred to a 50 ml. volumetric flask. 1 ml. of concentrated nitric acid and 10 ml.

of the potassium thiocyanate solution are added, and the solution made to volume and mixed. The solution is compared in a colorimeter using a blue filter, with 10 ml. of dilute iron solution treated in exactly the same manner. Results are calculated by the equation:

$$\text{Per cent Fe} = 0.02\ R/R'$$

where R = mm. standard

R' = mm. unknown

The limit of error between parallel solutions should not exceed two per cent.

Best results are obtained when the color development in the standard and in the unknown are brought about simultaneously. Comparison should be made within a half hour after the development of the color, and sooner, if possible.

C. Determination of Phosphorus

Fiske and Subbarow (4) applied the method of Bell and Doisy (5) and Briggs (6) to the determination of phosphorus in blood and urine. In the method used by Bell and Doisy phosphomolybdic acid is reduced to molybdenum blue with hydroquinone. Briggs found that the fading of the blue color could be largely eliminated by using an acid rather than an alkaline solution. Fiske and Subbarow used 1-amino-2-naphthol-4-sulphonic acid instead of hydroquinone as a reducer.

Method:

Reagents Required:

Standard Phosphate Solution:

Monopotassium phosphate (Sorenson's)	0.7022 gm.
10 N Sulfuric Acid	20. ml.
Distilled water at 25°C. to make	2000 ml.

1 ml. contains .00008 gm. phosphorus.

Ammonium Molybdate solution: 2.5 per cent

Ammonium molybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	50 gm.
10 N Sulfuric acid	1000 ml.
Distilled water at 25°C. to make	2000 ml.

Dissolve the molybdate in 400 ml. water and pour slowly into the acid, and dilute to 2000 ml.

Amino-naphthol-sulfonic acid

1-amino-2-naphthol-4-sulfonic acid, recrystallized	2.5 gm.
Sodium bisulfite solution (150 gm/liter)	975 ml.
Sodium sulfite solution (20 gm/100 ml.)	25 ml.

Dissolve the aminonaphtholsulfonic acid in the combined sulfite and bisulfite solutions and filter into an amber bottle. It may be necessary to add a little more of the sulfite to insure complete solution.

Procedure: An aliquot (10 ml.) of the original ash solution containing about 0.0004 gm. of phosphorus is pipetted into a 100 ml. volumetric flask and diluted to 70-75 ml.; 10 ml. of molybdate and 5 ml of amino-naphthol-sulfonic acid solution are added. Aftermaking to volume and mixing thoroughly, the solution is allowed to stand for 7 to 10 minutes to develop maximum color. It is then compared with 5 ml. of the standard phosphate solution treated simultaneously in exactly the same manner. The use of an orange filter in the colorimeter aids in the comparison. The per cent of phosphorus is calculated by the equation:

$$\text{Per Cent P} = 0.2\ R/R'$$

The limit of error between parallel solutions should not exceed one per cent.

D. Determination of Manganese

The official methods of the A.O.A.C. (7) for the colorimetric methods for the determination of manganese employ ammonium persulfate, sodium bismuthate, or potassium periodate to oxidize the manganese to permanganate.

Willard and Greathouse (8) employed sodium periodate as the oxidizing agent in place of the then used persulfate or bismuthate. The difficulty with persulfate is that the results obtained by its use are apt to be erratic; also extraneous colors may frequently be developed. Since a catalyst is necessary and silver nitrate is used for this purpose, the absence of the chloride ion is essential. Sodium bismuthate was early suggested and used as an oxidizer, and was found to be more reliable than the persulfate; however, the excess reagent must be removed by filtration. A further difficulty arises here in that the color of the solution may be somewhat bleached in passing through the filter.

By the use of periodate only a small excess of reagent is required, but a sufficient concentration of acid is necessary.

Willard and Greathouse applied the periodate method to the determination of manganese in steels.

Richards (9) used the periodate method in the determination of manganese in plant ash. It was here found that the percentage acidity in the solution should be between 5 to 20 per cent, to prevent fading. The percentage of nitric acid should not exceed 2 per cent. Since chlorides are acted upon by permanganate, the chloride ion should be removed; however, a slight amount of chloride may be taken care of by a little excess periodate. The principal interfering element in the ash solution is iron, but this interference may be eliminated by the addition of orthophosphoric acid.

In these experiments it was found that the persulfate method gave unsatisfactory results. Oxidation with bismuthate and subsequent titration against arsenite was also found to give too low results. The colorimetric method as outlined below was used with good success.

Method:

Reagents Required:

Standard sodium oxalate solution:

Sodium oxalate (Sorenson's)	0.3049 gm.
Distilled water at 25° to make	1000 ml.

A fresh solution must be used for each standardization.

Potassium permanganate solution:

Potassium permanganate	0.2877 gm.
Distilled water at 25° to make	2000 ml.

Each ml. contains 0.00005 gm. Manganese.

Potassium periodate, solid
Nitric acid, concentrated
Phosphoric acid, sp. gr. 1.7
2N sulfuric acid
Sulfuric acid, concentrated

Standardization of permanganate solution: The potassium permanganate solution is titrated against 10 ml. of the oxalate solution to which has been added 5 ml. of 2N sulfuric acid. The titration is carried out in a 150 ml. Erlenmeyer flask at 60 to 80°C. to a faint pink that persists for several minutes.

Procedure: An aliquot (100 ml.) of the original ash solution containing about 0.00025 gm. manganese is placed in a 150 ml. beaker and evaporated on the steam bath with 10 ml. concentrated nitric acid. The sides of the beaker are washed down with 10 to 15 ml. of water, and 5 ml. concentrated nitric acid, 5 ml. syrupy phosphoric acid, and about 0.1 gm. of potassium periodate added; the beaker is placed on the steam bath for about a half-hour, at the end of which time the solution should show some development of the permanganate color. After removing the beaker to an electric hot plate, a little more periodate is added, and the solution brought to boil, when the full permanganate color develops very rapidly. The hot solution is filtered by suction through a sugar tube with asbestos filter into a 50 ml. volumetric flask, in the bottom of which have been placed a few crystals of periodate. The solution is made approximately to volume, mixed, and placed on the steam bath for an hour or so to insure complete development of color. It is then cooled to room temperature, made to volume, mixed, and compared with

5 ml. of standard permanganate solution which has been treated in exactly the same manner after the addition of 3 ml. concentrated sulfuric acid. A green color filter aids in the comparison. The limit of error between parallel solutions should not exceed five per cent.

Notes and precautions: Filtration of the solution after heating on the hot plate may not always be necessary, but generally is, since a precipitate of calcium sulfate usually forms. Too much periodate may tend to produce a turbidity when the solution becomes cool, and therefore should be avoided. When the color has been completely developed in the presence of an excess of periodate, it will remain permanent for a considerable length of time, provided the flasks are kept stoppered.

Results obtained by this method were compared with those obtained by the use of persulfate or bismuthate, and found to be more reliable. Results obtained by the periodate method were invariably higher than when the manganese was oxidized with bismuthate, and the resulting permanganate titrated against arsenite. Results are compared in the following table.

Sample	Periodate, Mn. %	Arsenite, Mn. %
19 Manamar	.00122	.0004
20 Linseed Meal	.00351	.0011
22 White Hominy Meal	.00127	.0010
27 English Hay	.00474	.0008
617 Manganese Dioxide	61.47	59.59

E. Determination of Copper

The determination of copper in such minute amounts as are present in most plant materials has been difficult and generally unsatisfactory. The amounts are so small as to render gravimetric or volumetric methods either impractical or impossible; consequently attention has been turned to the more sensitive colorimetric methods.

Elvehjem and Lindow (10) have used the Biazzo (11) method fairly successfully. This method depends on the formation of a green precipitate of copper dipyridine thiocyanate $[Cu(C_5H_5N)_2(CNS)_2]$ in neutral solution, which may be extracted with chloroform, the intensity of the color in the chloroform layer being proportional to the concentration of the copper present. Ferric iron is said to be the only other ion which interferes appreciably.

Callan and Henderson (12) used sodium diethyldithiocarbamate as a colorimetric reagent for copper.

McFarlane (13) found that in the absence of iron the carbamate reagent gave more accurate and reliable results than did the Biazzo method. The golden-brown copper salt could be extracted with amyl alcohol. The ferric salt is of a browner color, although its intensity is only approximately one-tenth that of the copper salt. Extraction is most easily accomplished in alkaline solution. It is necessary to remove precipitates (R_2O_3) by filtration or by centrifuging. Iron interference may be eliminated by precipitation with pyrophosphate.

Sylvester and Lampitt (14) extracted copper from a hydrochloric acid solution of milk ash, using chloroform and diphenylthiocarbonazone. Calcium sulfate interferes. The carbamate reagent was applied to ammoniacal solution.

Winter (15) used citric acid to remove the ferric ion, and substituted carbon tetrachloride in place of amyl alcohol in the carbamate extraction.

Of the available methods mentioned above the application of sodium diethyldithiocarbamate seemed the most promising; Winter's method, with various modifications, was used as described below.

<u>Method:</u>

Reagents Required:

Standard Copper Solution:

Electrolytic copper foil (standard)	0.2000 gm.
Sulfuric acid, 1-1	5.0 ml.
Nitric acid, concentrated	1. ml.
Copper-free water at 25° to make	2000 ml.

The foil is cleaned in dilute nitric acid to remove the oxide, rinsed thoroughly with distilled water and dried. 0.2000 gm. are dissolved in 200 ml. water in which has been added 5 ml. 1-1 sulfuric acid and 1 ml. concentrated nitric acid, boiling until the solution is complete and the oxides of nitrogen are expelled. 1 ml. contains 0.0001 gm. copper. The copper content may be checked by electrodeposition from 200 ml. of the solution.

Dilute Copper solution:

Standard copper solution	200 ml.
Copper free water at 25°C to make	2000 ml.

Each ml. contains 0.00001 gm. copper

Sodium diethyldithiocarbamate - 1 gm. per 500 ml.

Shake vigorously to dissolve as much as possible, allow to stand overnight, filter.

Carbon tetrachloride, reagent.

Citric acid, reagent, 10 per cent solution

Sulfuric acid, 1-1

Ammonium hydroxide, concentrated

Copper free water, redistilled from glass

Procedure: An aliquot (100 ml.) of the original ash solution containing about 0.00005 gm. (0.02 - 0.10 mg.) of copper is evaporated with 10 ml. of concentrated nitric acid on the steam bath. The sides of the beaker are washed down with about 15 cc. of copper free water, a small piece of litmus paper added, and the solution neutralized with strong ammonia. After cooling, 10 ml. of citric acid are added, and the solution neutralized to litmus with ammonia. (The development of a yellow color during the first neutralization with ammonia necessitates a second evaporation with 10 ml. more nitric acid). The solution is transferred to a 100 ml. glass stoppered graduate, the volume made to 85 ml. and exactly 10 ml. of carbon tetrachloride and 5 ml. of the carbamate reagent are added. The cylinders are then stoppered and shaken vigorously for about two minutes, after which the combined solutions are transferred to a separatory funnel. A few drops of the carbon tetrachloride layer are let out to rinse the stem, and the remainder of the same layer run into a glass stoppered weighing bottle. The color is compared in a colorimeter, using micro cups and a blue color filter, against 5 ml. of dilute copper standard treated simultaneously in exactly the same manner, after first adding 1 ml. of 1-1 sulfuric acid. The percentage of copper is calculated from the equation:

$$Cu = 0.0025\ R/R'$$

The limit of error between parallel solutions should not exceed five per cent.

The color comparison should be made within a very few minutes after the extraction, since it may change somewhat after about half an hour.

In some cases, where the iron content of the sample is very high, it may be necessary before adding the citric acid, to remove the precipitated hydroxides (R_2O_3) by centrifuging. It is advantageous to re-dissolve the precipitate with a little dilute sulfuric acid, re-precipitate with ammonia, and after centrifuging, combine the supernatant liquid with that obtained before, and proceed as usual from this point.

PART II. LYSIMETER EXPERIMENT

The lysimeters used in this experiment were a set of fifty concrete tanks, each a meter cube, constructed in a single row lying in a north-south direction. The soil in all the tanks was from the same source, and of the type known as Merrimac sandy loam.

In 1934, the year before the present experiment was begun, the following applications had been made.

Tanks	Material	Rate of Application
1-5	$CaSO_4 \cdot 2H_2O$	1785 lb./acre
6-10	"	3569 "
11-15	"	5354 "
16-20	KI	7.1 "
21-25	"	14.3 "
26-30	"	21.4 "
31-35	---	---
36-40	$Ca(H_2PO_4)_2 \cdot H_2O$	1307 "
41-45	"	2614 "
46-50	"	3921 "

In addition a basic fertilizer furnishing 10 grams nitrogen, 5 grams P_2O_5, and 10 grams K_2O per tank (89.7 lbs. nitrogen, 44.8 lbs. P_2O_5, and 89.7 lbs. K_2O per acre respectively) was applied to all tanks except 46-50, which received 10 grams Nitrogen, 10 grams K_2O, and P_2O_5 as indicated above.

In the spring of 1935 a basic fertilizer was applied, each tank receiving the same amount. This fertilizer was applied at the rate of 2500 lb. per acre, and had a formula 4-6.4-5.6, the nitrogen, phosphorus, and potash being furnished by cottonseed meal, calcium nitrate, urea, calcium acid phosphate, and sulfate of potash and magnesia, in the proportions indicated in the following table:

Cottonseed meal (1/2)	Total N	7.10%	21.197 lb.
Calcium nitrate (1/4)	"	14.84	5.054 lb.
Urea (1/4)		45.75	1.639 lb.
Acid phosphate - avail. P_2O_5		16.91	28.386 lb.
Sulfate of K_2O and MgO			
	Water sol. K_2O	24.34	17.256 lb.
Sand			
(0.6178 lb. or 280.23 gm. per tank)			1.538 lb.
			75.000 lb.

The copper, iron, manganese, and iodine were applied in the form of basic copper sulfate, Princess metallic (ferric oxide), manganese dioxide, and potassium iodide, respectively, the first three being applied by mixing the required amount for each tank with one pound of sifted soil, the mixture then being spread on the tank and worked in. The iodide was applied in water solution. The analyses of these materials, and the rate of application are given in the following table.

Basic copper sulfate	- 53.70% Cu	at rate of 100 lb/A (11.21 gm./tank)
Princess metallic	- 51.56% Fe	" " of 200 lb/A (22.42 gm./tank)
Manganese dioxide	- 61.47% Mn	" " of 100 lb/A (11.21 gm/tank)
Potassium iodide	- 76,? % I	" " of 10 lb/A (1.12 gm./tank)

These materials were all applied in the above indicated amounts just before the planting of crops in 1935, and again in 1936, to the plots as listed in table 1. In the spring of 1936 sulfur was applied in addition to the iron on tank 38.

Five different crops were grown simultaneously, arrangement being such that for each crop there were two tanks with each type of application, and also two control tanks.

The crops grown in 1935 were beets, spinach, lettuce, carrots, and onions, planted in the order indicated in table 1. The indicated arrangement was deemed advisable in order to equalize as much as possible the effects of the previous applications to the tanks.

TABLE I.

Arrangement of Lysimeter Crops

Crops	Application	Tanks
Beets	Iron	1 and 36
"	Copper	6 and 41
"	Manganese	11 and 46
"	Iodine	21 and 26
"	Control	16 and 31
Spinach	Iron	2 and 37
"	Copper	7 and 42
"	Manganese	12 and 47
"	Iodine	22 and 27
"	Control	17 and 32
Carrots	Iron	3 and 38
"	Copper	8 and 43
"	Manganese	13 and 48
"	Iodine	23 and 28
"	Control	18 and 33
Lettuce	Iron	4 and 39
"	Copper	9 and 44
"	Manganese	14 and 49
"	Iodine	24 and 29
"	Control	19 and 34
Onion Sets	Iron	5 and 40
"	Copper	10 and 45
"	Manganese	15 and 50
"	Iodine	25 and 30
"	Control	20 and 35

In 1936 the same crops were planted, and in the same arrangement used in 1935. Due to adverse weather conditions in the early part of the 1936 season, it was necessary to replant most of the crops, and as a result of the late start, growth was none too good. After harvesting the lettuce crop, swiss chard was planted in the vacated tanks. Endive was planted after spinach was removed, but came along so late and was so poor in quality that analysis was not considered worthwhile, and the crop was discarded.

It was endeavored to harvest each crop when development was such as would correspond to that of the ordinary marketable product. The two samples of each crop with the same fertilization were combined. All samples were washed as free from soil as possible without injury to the sample, and dried in a hot air drier at 50°C. The root crops were cut into small pieces in order to shorten the time required for drying. When dry, the samples were ground in a Wiley mill to pass a 1 mm. screen, and preserved in glass stoppered bottles or Mason jars. All analyses were made on portions of the samples thus prepared. Moisture determinations were made by drying 2 grams of the above material to constant weight in a vacuum oven at 50°C. Results of this determination were used to convert the results of the other determinations to the dry matter basis.

In addition to the analyses made for the trace elements, proximate analyses (crude protein, crude fat, crude fiber, ash and nitrogen free extract) were made by the usual methods of the A.O.A.C. The results of both the proximate analyses and the determinations of trace elements are given in tables III and IV.

TABLE III.

Analysis of Plant Materials.

1935 Crop

No.	Crop	Fertilization	Drude Protein %	Crude Fat %	N. Free Extract	Crude Fiber %	Acid insoluble ash %	Acid sol. ash %	Iron ppm	Copper ppm	Manganese ppm	Phosphorus ppm
316	Spinach	Fe	31.68	3.33	36.10	8.29	2.03	18.57	1110	105	94	2890
317	"	Cu	31.12	3.64	36.36	8.50	1.80	18.58	1080	238	90	2980
318	"	Mn	32.28	2.96	33.94	8.26	2.77	19.59	1260	139	127	3020
319	"	I	32.93	2.87	34.09	9.00	1.98	19.13	1170	146	122	2900
320	"		31.27	2.92	35.69	8.56	2.00	19.56	1140	143	133	2670
	Average		31.86	3.14	35.24	8.52	2.16	19.09	1160	133	110	2890
	Increase								-50	+105	+17	
322	Lettuce	Fe	18.80	3.73	58.24	9.65	0.57	9.01	289	78	50	1920
323	"	Cu	17.86	3.54	60.33	9.75	0.46	8.06	243	150	46	2090
324	"	Mn	17.64	2.24	61.10	10.72	0.35	7.95	220	114	57	2390
325	"	I	18.28	2.73	60.31	9.91	0.40	8.37	238	106	52	2030
326	"		18.21	3.12	59.21	10.63	0.47	8.36	239	55	47	1980
	Average		18.16	3.09	59.84	10.13	0.45	8.35	235	88	49	2080
	Increase								+54	+68	+8	
347	Onions	Fe	11.06	0.75	79.18	5.01	0.05	3.95	51	5.1	18	1480
348	"	Cu	11.07	1.07	78.28	5.52	0.04	4.02	38	6.2	17	1460
349	"	Mn	10.70	1.00	79.29	5.15	0.03	3.33	45	4.8	16	1410
350	"	I	10.70	0.80	79.47	5.17	0.00	3.86	36	7.3	14	1630
351	"		11.36	0.74	78.42	5.38	0.01	4.09	43	9.6	16	1670
	Average		10.98	0.87	78.93	5.25	0.03	3.95	41	6.7	16	1530
	Increase								+10	+0.5	0	

TABLE III.
(Continued)

No.	Crop	Ferti-lization	Crude Protein %	Crude Fat %	N.Free Extract	Crude Fiber %	Acid Insol. Ash %	Acid Sol. Ash %	Iron ppm	Copper ppm	Manganese ppm	Phosphorus ppm
352	Beets	Fe	7.74	0.50	81.86	5.19	0.02	4.69	70	27.5	49	1600
353	"	Cu	8.65	0.49	80.92	5.20	0.01	4.73	79	9.2	57	1960
354	"	Mn	7.55	0.38	82.27	5.05	0.01	4.74	71	10.0	57	2120
355	"	I	8.00	0.30	81.79	5.07	0.03	4.81	69	14.0	56	1760
356	"		7.75	0.39	81.98	5.22	0.04	4.62	70	10.5	56	1840
	Average		7.94	0.41	81.67	5.15	0.02	4.72	72	17.8	55	1860
	Increase								-2	-8.6	+2	
257	Beet Tops	Fe	13.79	3.66	49.04	11.35	0.80	21.36	713	23.4	342	3710
358	"	Cu	14.91	3.49	48.93	11.96	0.67	20.04	517	49.8	271	3790
359	"	Mn	14.02	3.75	47.91	11.05	1.25	22.02	895	30.4	425	4890
360	"	I	14.91	3.51	48.08	12.00	1.61	19.79	891	26.9	358	2650
361			14.49	3.57	48.70	11.88	1.23	20.13	741	27.8	336	2690
	Average		14.42	3.60	48.53	11.67	1.11	20.67	761	27.1	327	3550
	Increase								-48	+22.7	+98	
362	Carrots	Fe	7.84	1.08	79.17	6.17	0.05	5.69	59	12.0	25	1480
363	"	Cu	7.05	0.96	80.81	5.75	0.02	5.41	52	11.9	26	1420
364	"	Mn	7.81	1.05	79.84	5.83	0.02	5.46	49	8.4	27	1490
365	"	I	8.52	0.96	78.49	6.05	0.03	5.95	52	10.8	28	1470
366			8.12	0.75	79.02	6.07	0.07	5.97	65	12.3	29	1480
	Average		7.87	0.96	79.46	5.97	0.04	5.70	55	10.9	27	1480
	Increase								+4	+ 1.0	0	

TABLE IV.

Analysis of Lysimeter Crops for 1936

No.	Crop	Fertilization	Crude Protein %	Crude Fat %	N. Free Extract	Crude Fiber %	Acid Insol. Ash %	Acid Sol. Ash %	Iron ppm	Copper ppm	Manganese ppm	Phosphorus ppm
468	Lettuce	Fe	21.58	4.41	51.82	9.77	0.47	11.95	476	7.8	75	1460
469	"	Cu	20.19	4.50	53.36	9.77	0.44	11.74	366	11.6	64	1550
470	"	Mn	24.08	4.64	48.82	9.28	0.45	12.73	517	7.8	103	1750
471	"	I	26.74	4.60	43.91	9.16	0.52	15.08	526	4.0	125	1340
472	"		26.29	3.89	45.10	9.39	0.46	14.66	506	3.7	95	1260
	Average		23.78	4.41	48.60	9.47	0.47	13.23	479	5.8	90	1470
	Increase								-3	+5.8	+13	
473	Spinach	Fe	18.41	2.42	42.62	16.14	4.84	15.57	2430	33.2	117	2000
474	"	Cu	18.44	2.37	41.14	16.55	5.54	15.96	3000	42.9	124	2250
475	"	Mn	19.08	2.41	40.86	16.07	4.79	16.79	2570	10.5	133	2480
476	"	I	20.58	2.47	40.12	15.51	4.80	16.54	2280	14.9	120	2400
477	"		20.91	2.51	41.90	14.37	4.38	15.93	1870	20.6	118	4730
	Average		19.48	2.44	41.33	15.73	4.87	16.16	2860	15.8	120	2283
	Increase								-430	+27.1	+13	
478	Onions	Fe	14.99	0.72	76.00	3.66	0.04	4.59	85	4.9	13	3470
479	"	Cu	15.88	0.73	74.96	3.67	0.03	4.73	70	9.3	13	3680
480	"	Mn	15.19	0.76	75.61	3.61	0.03	4.80	53	4.2	13	3430
481	"	I	15.07	0.71	75.95	3.55	0.02	4.71	60	4.7	12	3520
482	"		15.90	0.74	74.92	3.56	0.01	4.87	45	5.1	12	3610
	Average		15.41	0.73	75.49	3.61	0.03	4.74	57	4.7	13	3540
	Increase								+28	+4.6	0	

TABLE IV.
(Continued).

No.	Crop	Ferti-lization	Crude Protein %	Crude Fat %	N.Free Extract	Crude Fiber %	Acid Insol. Ash %	Acid Sol. Ash %	Iron ppm	Copper ppm	Manganese ppm	Phosphorus ppm
483	Beets	Fe	5.55	0.44	88.30	5.99	0.38	4.34	290	10.1	119	2470
484	"	Cu	5.92	0.41	83.50	5.67	0.27	4.23	182	21.5	134	2000
485	"	Mn	5.98	0.45	83.35	5.71	0.25	4.26	212	11.5	142	2470
486	"	I	5.70	0.42	83.81	5.77	0.22	4.08	210	8.6	156	2000
487	"		5.68	0.45	83.68	5.64	0.30	4.25	243	9.5	126	2370
	Average		5.79	0.43	83.53	5.56	0.28	4.23	212	9.9	134	2260
	Increase								+78	+11.6	+8	
488	Beet Tops	Fe	15.38	3.16	48.57	10.50	1.77	20.62	1260	14.6	482	5690
489	" "	Cu	15.90	3.15	48.40	10.33	1.71	20.53	1096	41.5	425	6750
490	" "	Mn	15.83	3.26	48.20	10.26	1.81	20.64	1256	14.4	488	7370
491	" "	I	16.16	3.31	47.42	10.36	1.86	20.89	1497	13.4	653	4290
492	" "		17.13	3.33	46.93	10.23	1.91	20.47	1341	17.0	508	4380
	Average		16.08	3.24	47.90	10.34	1.81	20.63	1298	14.9	517	5710
	Increase								-38	26.6	-29	

TABLE IV.
(Continued).

No.	Crop	Ferti-lization	Crude Protein %	Crude Fat %	N. Free Extract	Crude Fiber %	Acid Insol. Ash %	Acid Sol. Ash %	Iron ppm	Copper ppm	Manganese ppm	Phosphorus ppm
493	Carrots	Fe	6.11	0.96	79.26	7.83	0.05	5.79	217	12.0	22	2180
494	"	Fe & S	5.99	0.85	79.62	7.55	0.09	5.90	171	13.8	56	2260
495	"	Cu	6.38	0.98	78.86	7.39	0.08	6.01	144	10.1	20	2250
496	"	Mn	6.28	0.98	79.27	7.62	0.06	5.79	127	10.7	16	2340
497	"	I	6.54	0.90	78.20	7.92	0.08	6.36	141	6.6	26	2160
498	"		6.45	0.90	78.56	7.80	0.08	6.21	125	6.9	34	1950
	Average		6.29	0.93	78.96	7.74	0.07	6.01	134	10.0	26	2190
	Increase								+60	+0.1	-10	
499	Carrot T.	Fe	13.70	2.32	49.75	15.65	2.91	15.67	1876	9.2	155	1590
500	" "	Fe&S	13.27	2.16	50.67	14.38	4.78	14.74	2918	9.9	533	1630
501	" "	Cu	13.70	2.28	50.21	14.64	4.26	14.97	2701	27.2	195	1600
502	" "	Mn	12.80	2.25	49.66	15.00	5.57	14.72	3384	12.6	246	1730
503	" "	I	13.56	2.25	47.88	14.75	6.81	15.25	3852	18.2	247	1550
504	" "		14.28	2.27			6.83	14.80	4096	16.6	241	1550
	Average		13.55	2.25			5.12	15.03	3508	14.2	205	1620
	Increase								-1111	+13.0	+41	
516	Swiss C.	Fe	13.03	1.64	51.07	9.01	11.32	13.73	5770	12.6	166	5370
517	" "	Cu	14.58	1.97	50.48	9.07	9.66	14.24	3220	37.2	147	5500
518	" "	Mn	16.95	1.88	47.04	8.45	10.31	15.37	3370	13.8	202	5290
519	" "	I	13.43	1.63			9.68	14.55	3370	13.7	182	5140
590	" "		15.14	1.86	51.29	8.83	8.24	14.64	2880	10.4	167	5110
	Average		14.65	1.84			9.84	14.63	3210	12.6	166	5280
	Increase								+2560	+24.6	+36	

TABLE V.

Weather Conditions During the Growing Seasons of 1935 and 1936

Month	Mean Temperature Obs.	Mean Temperature Normal	Hours of Sunshine Obs.	Hours of Sunshine Normal	Mean Relative Humidity Obs.	Mean Relative Humidity Normal	Precipitation Obs.	Precipitation Normal
1935								
May	54.4	56.8	224	250.1	60	70.7	2.17	3.63
June	65.9	65.4	179	257.4	72	73.9	5.50	3.54
July	73.2	70.6	307	267.0	72.7	67.9	3.10	4.33
August	69.4	68.4	254	237.0	69.7	69.9	0.82	4.24
September	59.1	61.6	179	202.5	73.0	73.2	4.67	3.79
October	50.4	50.4	203	171.0	64.9	69.0	0.88	3.35
	62.0	62.2	1346.0	1385.0	68.7	70.7	17.14	22.88
1936								
May	59.4	56.8	308	250.1	60	70.7	1.76	3.63
June	67.0	65.4	252	257.4	65.6	73.9	3.28	3.54
July	70.8	70.6	323	267.0	63.7	67.9	1.45	4.33
August	69.7	68.4	266	237.0	67.9	69.9	4.85	4.24
September	62.3	61.6	185	202.5	76.2	73.2	3.80	3.79
October	50.6	50.4	174	171.0	74.2	69.0	4.90	3.35
	63.3	62.2	1508	1385.0	67.9	70.7	20.04	22.88

A summary of the weather conditions during the course of the experiment is given in table V, showing that the months of May and June in 1936 were hotter and drier than normal, and may account for the poor start which some of the earlier crops made.

Soil

In order to gain some idea as to the relative amount of iron, copper, and manganese already present in the soil, analyses were made on samples taken from each tank. The first set of samples was taken in the spring of 1935, before the application of any of the elements in question. Another set of samples was taken in the fall of 1936, after all the crops had been removed.

In making the analysis for trace elements in the soil, 2 grams of soil were digested with concentrated nitric and sulfuric acids, as in the wet combustion method described previously in this paper. Constituents were determined in aliquots of this ash solution.

This method, by showing the total concentration of the element present, gave an indication as to whether there was an appreciable increase due to the application. It seemed reasonable that, since the amount of the application was so small, the availability of the applied material would approach that already present, provided the conditions of pH and other factors remained the same. Results of the analyses are given in table VI.

TABLE VI.

Soil from Lysimeters, Before Application, 1935

Samples	Plot Nos.	Application	Total Ash	Iron %	Copper ppm	Manganese ppm	Phosphorus ppm
642	1-5	None	95.30	2.531	39.9	481	1650
643	6-10	"	95.41	2.542	36.2	474	1560
644	11-15	"	95.38	2.591	27.2	418	1650
645	16-20	"	95.50	2.493	25.1	447	1650
646	21-25	"	95.34	2.564	19.2	431	1660
647	26-30	"	95.43	2.521	21.4	472	1590
648	31-35	"	95.58	2.606	20.5	482	1580
649	36-40	"	95.60	2.600	23.9	466	1690
650	41-45	"	95.61	2.655	21.6	457	1870
651	46-50	"	95.70	2.537	19.7	432	1940
	Average		95.49	2.564	25.5	456	1680

TABLE VI.

Soils from Lysimeters, After Harvesting Crops, 1936.

Samples	Plot Nos.	Application	Total Ash	Iron %	Copper ppm	Manganese ppm	Phosphorus ppm
592	1-5	Iron	95.05	2.390	33.0	479	1710
593	6-10	Copper	95.17	2.477	85.4	535	1790
594	11-15	Manganese	95.10	2.512	31.5	596	1810
595	21-25	Iodine	95.12	2.573	31.8	581	1760
596	16-20		95.32	2.577	31.7	583	1790
597	36,37,39,40	Iron	95.41	2.444	31.0	490	1790
598	38	Iron & Sulfur	95.63	2.891	22.8	530	1840
599	41-45	Copper	95.37	2.536	81.5	516	2050
600	46-50	Manganese	95.39	2.578	20.2	597	2160
601	26-30	Iodine	95.51	2.622	21.4	411	1720
602	31-35		95.45	2.579	21.3	477	1580
	Averages		95.32	2.560	27.2	522	1820
	Average Increase			None	56.3	75	

In determining the average value of the iron, copper, and manganese in the soils, the values for those plots to which an application of an element was made were not included in the calculation for that element.

A pH determination was made on the soil samples taken in the fall of 1936; results of the determination are given in table VII.

TABLE VII.

Results of pH Determinations

Sample	Plots	Application	1936 pH 22°	1935 pH 22°
592	1-5	Iron	5.32	5.55
593	6-10	Copper	5.50	5.52
594	11-15	Manganese	5.32	5.36
595	21-25	Iodine	5.12	5.69
596	16-20		5.32	5.59
597	36,37,39,40	Iron	5.57	5.81
598	38	Iron & Sulfur	5.25	
599	41-45	Copper	5.50	5.60
600	46-50	Manganese	5.59	5.91
601	26-30	Iodine	5.45	5.79
602	31-35		5.51	5.79
Average			5.40	5.66

Interpretation of Results

Soils - The data given in table VI shows that while the iron content of the soil was not changed by two years of application, the copper content showed an increase of about 270 per cent over the amount present in plots to which no copper had been applied; the increase in the manganese content was about 14 per cent.

The ash, and consequently the organic matter content of the soil remained very constant. Table VII shows that the pH also was practically the same in all plots. In tank No. 38, to which sulfur had been added, there was no indicated decrease in the pH.

Iron - From the fact that the amount of iron normally present in the soil is so large as compared to the very small amount and unavailable form of the application, only slight, if any effect would be expected. Inspection of results seems to indicate that in general, after application there is a decrease in the iron content in the leafy parts of the plants, and an increase in the iron content in the roots.

Copper - The effect of the copper application is quite marked. The copper content generally increased, the greatest increase being in the leafy parts. In the roots (beets, carrots, onions) the increase was only slight, or more often negative. There was in every case a positive increase with the leaf crops, amounting to 171 per cent in spinach and 178 per cent in beet tops.

A further effect noted with copper application was that when the copper content increased, the iron content was quite often found to decrease.

Manganese - The increase in the manganese content was quite comparable with that of copper although in a considerably less degree, as might be expected from the fact that there was a proportionately smaller increase in the manganese content of the soil. The greatest increase was shown in beet tops, and amounted to 30 per cent.

Iodine - Having no satisfactory method available for the determination of the iodine content in plant materials, it was possible only to observe the effect on assimilation of other elements. This effect is best illustrated by the following table:

Crop	Iron	Copper	Manganese	Phosphorus
Spinach	+	+	+	0
Lettuce	0	+	+	0
Onions	-	+	-	+
Beets	-	-	+	-
Beet Tops	+	-	+	-
Carrots	-	0	+	0
Lettuce	+	-	+	-
Spinach	-	-	0	+
Onions	+	0	-	0
Beets	0	-	+	-
Beet tops	+	-	+	-
Carrots	+	-	0	0
Carrot Tops	+	+	+	-
Swiss Chard	+	+	+	-

The above table shows that iodine application does not produce a definite effect with any appreciable degree of regularity. It does seem to have a depressing effect on the assimilation of copper, both in the roots and in the leaves. The effect on the iron and manganese content was about equal, in general tending toward an increase, the effect being more marked in the leafy parts. With phosphorus the effect was only slight, being more often negative than positive.

Phosphorus - In nearly every case, the results of the phosphorus determinations show that even after two years, there is still a residual effect from the $Ca(H_2PO_4)_2 \cdot H_2O$ which was applied in 1934. The phosphorus content of the plant varied directly with the concentration of phosphate in the soil.

Sulfur - Plants (carrots) grown on tank 38, to which sulfur has been applied, showed an increase in iron, copper, manganese, and phosphorus in the roots; in the tops a decrease in copper, and increase in iron and manganese, the latter increase being about 250 per cent.

The various effects of the iron, copper and manganese applications are summarized in table II.

TABLE II.

Effects of Iron, Copper, and Manganese Applications

Crop	Iron (Added)	Copper (Added)	Manganese (Added)	Phosphorus (mn. App.)
Spinach	-4	79	15	5
Lettuce	23	77	16	15
Onions	24	-8	0	-8
Beets	-3	-48	4	14
Beet Tops	-6	84	30	38
Carrots	7	9	0	1
Lettuce	-1	100	14	19
Spinach	-15	171	11	9
Onions	49	98	0	0
Beets	37	117	6	9
Beet Tops	-3	178	-6	23
Carrots	-45	1	-38	3
Carrot Tops	-32	92	20	-1
Swiss Chard	80	195	22	0

Values given are the per cent change in the iron, copper, and manganese content, with respect to the average normal content, in those cases where the iron, copper, and manganese, respectively, have been applied. The per cent change in the phosphorus content was calculated for the sample receiving manganese fertilization.

CONCLUSIONS

Analytical methods which are reasonably accurate and rapid have been applied to the determination of iron, copper, manganese, and phosphorus in plant materials.

An increase in the copper, manganese, or phosphorus content of a soil will result in an increase in the copper, manganese, or phosphorus content of the plant, the effect being more marked in the leafy portions than in the roots.

Manganese may tend to enhance the assimilation of phosphorus.

Copper may decrease the assimilation of iron.

Iodine may have a slight influence on iron, manganese, or copper, increasing the assimilation of the first two, and decreasing the assimilation of the copper.

There is no recognizable change in the crude protein, crude fat, crude fiber, or nitrogen free extract as a result of making small applications of ferric oxide, basic copper sulfate, manganese dioxide, or potassium iodide to the soil.

BIBLIOGRAPHY

(1) Kennedy, R. P.
The Quantitative Determination Iron in Tissues
J. Biol. Chem. 74, 385 (1927)

(2) Elvehjem, C. A. and Hart, E. B.
II. Quantitative Methods for the Determination of Iron in Biological Materials.
J. Biol. Chem. 67, 43 (1926)

(3) McFarlane, W. D.
Application of the Sodium Diethyldithiocarbamate Reaction to the Micro-colorimetric Determination of Copper in Organic Substances.
Biochem. J. 26, pt. 1, 1022 (1932)

(4) Fiske, C. H. and Subbarow, Y.
The Colorimetric Determination of Phosphorus
J. Biol. Chem. 66, 375 (1925)

(5) Bell, R. D. and Doisy, E.A.
Rapid Colorimetric Methods for the Determination of Phosphorus in Urine and Blood.
J. Biol. Chem. 44, 55 (1920&

(6) Briggs, A. P.
A.Modification of the Bell-Doisy Phosphate Method
J. Biol. Chem. 53, 13 (1923)
Some Applications of the Colorimetric Phosphate Method
J. Biol. Chem. 59, 255 (1924)

(7) Methods of Analysis;
A.O.A.C., Ed. IV. (1935)

(8) Willard, H.H. and Greathouse, L.H.
Colorimetric Determination of Manganese by Oxidation with Periodate.
J. Am. Chem. Soc. 39, 2366 (1917)

(9) Richards, M. B.
The Colorimetric Determination of Manganese in Biological Materials.
Analyst 55, 554 (1929)

(10) Elvehjem, C. A. and Lindow, C.W.
The Determination of Copper in Biological Materials
J. Biol. Chem. 81, 435 (1929)

(11) Redfield, A. C., Coolidge, T., and Schotts, M.A.
The Copper Content and the Minimal Molecular Weight of the Hemocyanin of Limulus Polyphemus.
J. Biol. Chem. 76, 185 (1928)

(12) Callan, T., and Henderson, J.A.R.
A New Reagent for the Colorimetric Determination of Minute Amounts of Copper.
Analyst 54, 650 (1929)

(13) McFarlane, W. D.
The Determination of Iron in Organic Substances.
Biochem. J. 26, I. 1034 (1932)

(14) Sylvester, N. D. and Lampitt, L.H.
The Determination of Copper in Foods with Special Reference to Milk.
Analyst 60, (1935)

(15) Winter, O.B.
Correspondence with Dr. W. S. Ritchie

(16) Brenchley, W. E.
Inorganic Plant Poisons and Stimulants
Cambridge University Press (1927)

(17) Willis, L. G. and Piland, J. R.
The Influence of Copper Sulphate on Iron Absorption by Corn Plants.
Soil Sci. 37, 79 (1934)

(18) Sideris, C. P. and Kraus, B. H.
The Effect of Sulfur and Phosphorus on the Availability of Iron to Pineapple and Maize Plants.
Soil Sci. 37, 85 (1934)

(19) Lundegardh, Henrik
The Influence of the Soil Upon the Growth of the Plant.
Soil Sci. 40, 89 (1935)

(20) Olsen, C.
Uber die Manganaufnahme der Pflanzen
Biochem. Z. 269, 329 (1934)

(21) Peterson, W. H., and Elvehjem, C.A.
The Iron Content of Plant and Animal Foods.
J. Biol. Chem. 78, 215 (1928)

(22) Elvehjem, C.A., Lindow, C. W., and Peterson, W. H.
The Copper Content of Plant and Animal Foods.
J. Biol. Chem. 82, 465 (1929)

(23) Skinner, I. T. and Peterson, W. H.
The Iron and Manganese Content of Feeding Stuffs.
J. Biol. Chem. 79, 679 (1928)

(24) Lindow, C. W., and Peterson, W. H.
The Manganese Content of Plant and Animal Materials.
J. Biol. Chem. 75, 169 (1927)

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation for the assistance and advice of Dr. E. B. Holland, under whom the project was carried out, and To Dr. W. S. Ritchie and Dr. W. S. Eisenmenger for many helpful suggestions.

Thanks are also due Dr. J. E. Goldthwait for financial assistance which made the project possible, and to the Agronomy Department of Massachusetts State College for the use of the lysimeters.

Approved by:

Edward B. Holland

Walter S. Eisenmenger

____

Date:

____