THE EFFECTS OF COPPER APPLIED TO ORGANIC SOIL, ALONE AND IN ASSOCIATION WITH MANGANESE AND ZINC, ON COMPOSITION OF CROPS AND REACTIONS IN THE SOIL

By

Robert Elmer Lucas

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

1947

ACKNOWLEDGEMENT

The writer wishes to express his appreciation to Dr. Paul M. Harmer for his effective guidance throughout this investigation, and for use of plant and soil samples from the Michigan State College Muck Experimental Farm. He also wishes to express appreciation to Dr. C. E. Millar, Dr. Nathan Hall, and other members of the Soil Science Department for their cooperation and advice; and to Dr. E. J. Benne and other members of the Agricultural Chemistry Department for their helpful cooperation and suggestions.

CONTENTS

ILLUSTRATIONS

TABLES

Table		Page

THE EFFECTS OF COPPER APPLIED TO ORGANIC SOIL, ALONE AND IN ASSOCIATION WITH MANGANESE AND ZINC, ON COMPOSITION OF CROPS AND REACTIONS IN THE SOIL

GENERAL INTRODUCTION

It has long been recognized that calcium, potassium, magnesium, and phosphorus are essential mineral elements for the normal development of plants. These elements compose a large proportion of the mineral content of plants. In addition to these elements, many investigators have shown that manganese, iron, and boron are required in minute amounts for plant growth. Two other elements, copper and zinc, have been proven essential, but because they present nutritional problems in restricted areas, they have not been studied as extensively as other elements. In the United States, the areas where copper and zinc deficiencies have been reported are the Gulf States, California, and muck soils of the Northern States.

Certain muck soils of Michigan have been shown to need copper and other minor elements for normal crop production (23). At times the lack of sufficient available copper in the soil has caused a complete crop failure. From these observations it has become the general policy to include copper in the fertilizer (4) for responsive crops grown on muck soils. Even though crops may not always benefit from copper fertilization, nevertheless, its use is considered a good insurance practice. Results of experiments from surrounding states on the need of copper for muck soils are not in agreement. For example, Comin (14) found that the use of copper sulfate for crops growing on a muck of high fertility in Ohio gave little or no conclusive results. Likewise, Ellis (17), in minor element experiments at the Purdue Muck Experimental Farm was not able to obtain any response in plant growth from the use of copper sulfate.

With the objective of determining some of the factors affecting the behavior and reactions of copper in plants and in the soil, the approach to the problem was broad rather than specific. No attempt was made to determine the specific physiological role of copper in plants. In general, three distinct phases of research were followed which will be described as individual sections in this report. Part I will show some of the effects of the addition of copper sulfate, and other minor elements to organic soils on the crop yields and absorption of these elements. Part II deals with the effect of these nutrients on the quality of plant products as measured by the protein, ascorbic acid, and carotene contents of plants. Part III attempts to show some of the chemical reactions of copper in the soil and interpret the results in terms of the availability of copper.

PART I. The Effect of the Addition of the Sulfates of Copper, Zinc, and Manganese on the Absorption of These Compounds by Plants Grown on Organic Soils.*

Introduction --

Results obtained from field experiments are a practical means for determining a copper deficiency. Field experiments, however, fail to uncover the fundamental role of the element in plant nutrition, or its behavior in the soil. From a scientific viewpoint, one is concerned with the problems that cause the deficiency and what takes place when it is corrected. For this reason it is important to have a knowledge of the role of copper in plant nutrition and the factors that affect absorption of copper by the plant. Once copper is used, the question is often asked, "Does the use of one minor element require the addition of other minor elements to the soil?" It is general knowledge that the quantity of a minor element applied to the soil is considerably greater than the amount absorbed by plants. Again the question is asked, "What has happened to the rest of the nutrients?" In the case of manganese, it has been proven that in slightly acid to alkaline mucks, there is an oxidation of the readily available manganous form to the rather unavailable manganic form (50). In highly alkaline soils, manganese is almost always a yearly problem. Is the problem for copper similar

*A portion of the results of this work was presented at the 1945 meeting of the Soil Science Society of America (37).

to manganese?

In this section some of the questions of nutrient absorption and interrelationship are discussed.

Historical --

The exacting precautions and methods of Sommer (54) with water cultures proved the essential need of copper in plant-nutrition. The investigations of Elvehjem and Hart (19) were among the early reports on the need of copper in animal nutrition. They showed that suckling pigs, which were not able to eat soil, developed anemia due to a deficiency of copper and iron in the diet. The same workers reported that the addition of copper sulfate to the soils of Wisconsin showed no increases in plant growth, nevertheless, within certain limits the copper content of a crop could be increased by the use of copper salts in the fertilizer. Workers in Australia (8,59) report wide-spread deficiencies of copper both in plants and in animals. The deficiencies were prevented by top dressing the pastures with copper compounds.

The investigations of Felix (20) were among the first to indicate the need of copper on muck soils in New York. He reported that 100 to 200 pounds per acre of pulverized copper sulfate resulted in normal production of lettuce and onions. Painting the leaves of affected lettuce with a weak solution of copper sulfate also caused the plants to outgrow abnormalities. In more detailed experiments, Allison, et al. (3) showed marked benefit from the use of copper on the raw peat soils of Florida. In Europe many workers have reported the need of copper compounds for combating "Heather-Moor Disease".

The use of copper for crops grown on mineral soils is essential in large areas of Australia and in parts of New Zealand. In this country it has benefited some crops grown in the Gulf States and in limited areas of California. In 1913, Floyd (21) noticed that the use of bordeaux successfully cured a disease long observed in citrus groves called "Die-Back" or "Exanthema". Shortly thereafter, Grossenbacher (22) reported successful results from soil applications of copper sulfate.

Investigations reported by Rademacher (46) showed evidence that the amount of copper deficiency in a plant is an innate varietal characteristic connected with the ability of the plant to absorb copper from the soil. Even strains within a variety showed marked differences. For example, the copper content of oat straw varied between 3.1 to 7.3 p.p.m., while that of the grain varied between 4.2 to 12.2 p.p.m. of copper in the dry material. A fairly resistant strain of oats contained 5.7 to 9.5 p.p.m. of copper in the straw and 8.8 to 12.3 p.p.m. in the grain. The higher copper content of the resistant strains was due to their greater ability to absorb copper from copper deficient soil. On normal soil the different varieties showed little difference in copper content.

In a comparison of feed, Beck (8) reported that samples of pasturage from areas where copper deficiency symptoms occurred contained 5 p.p.m. or more. Svanberg and Nydahl (58) reported that the copper content of hay from organic soils varied from very low (0.5 p.p.m.) to high (13 p.p.m.) according to the locality. Neal (42) reported symptoms of copper deficiency in cattle when the forage dry matter contained less than 1.5 to 3.0 p.p.m. of copper.

Chapman, et al. (13) found that copper in nutrient solutions would sometimes cause iron chlorosis. They suggested that the action of copper may be through reactions affecting the oxidation of iron.

Liebig, et al. (33) noticed that the addition of 0.1 p.p.m. of aluminum to purified nutrient solutions containing a trace of copper and other micro-nutrients slightly depressed plant growth. They believed it caused a copper deficiency. According to Willis and Piland (63) the addition of copper to a soil may be beneficial or not, depending on the state of oxidation of the iron and manganese. The interrelationship of minor elements to copper may account for some of the results that are to be discussed.

Considerable data has been collected on the copper and other minor element content of feeds and foods. A good compilation was published by Beeson (9). The copper content of many foods is reported by Sherman (51). For more detailed reviews on the role of copper in plants and animals, papers by Sommer (55) and Elvehjem (18) are rather complete.

Experimental Procedure --

Greenhouse and field studies were made with several crops on a somewhat fibrous, deep virgin muck (pH 6.0 to 6.3) from the Michigan State College Muck Experimental Farm. Studies were also made on a very acid peat (pH 3.65) on which crops were known to be responsive to the addition of copper sulfate. A third soil was obtained from unproductive muck in Indiana. This soil was naturally very acid but had been recently limed with about 5 tons per acre of limestone.

Plant samples from the crops grown in the field were collected from experimental plots under investigation by Harmer (25) since 1942. The plants were cut at ground level at the normal time of harvest, dried at 102°C., and then ground to pass a 1 m.m. mesh screen.

The soil for greenhouse study was passed through an asphalt coated $\frac{1}{4}$-inch square-mesh wire screen. Crops were grown in equal quantities of the thoroughly mixed soil placed in two gallon glazed jars. At intervals the soil was brought to an optimum moisture content with distilled water. Chemically pure nutrients were added to the soil of each jar at the equivalent rate of 2000 pounds per acre of a 3-9-18 fertilizer. Where limestone was added, it was mixed thoroughly in the soil before the addition of fertilizer. The minor elements were mixed in the fertilizer before being applied to the soil. Rates of nutrient application, expressed in pounds per acre, were based on average surface area of the soil. Copper was added as pulverized $CuSO_4 \cdot 5H_2O$, zinc as

$Zn \cdot SO_4 \cdot H_2O$, and manganese as $MnSO_4 \cdot H_2O$, with rates of application expressed on the basis of the hydrated weights. Each treatment was replicated three times. Wherever possible the chemical composition of a crop reported herein was an average of single determinations from each of three replications of a treatment. In some treatments it was necessary to conbine replicates because there was not a sufficient amount of dry material for all the desired analyses.

The amounts of the minor elements in plants were evaluated colorimetrically with the aid of a photo-electric colorimeter. Copper was determined by the diethyldithiocarbamate method (5), zinc by the dithizone method (15), and manganese by the periodate method (62). The use of these new methods for determining minor elements proved very satisfactory. Although some investigators go so far as to report the amounts of these elements in plants to the closest 0.1 p.p.m., the accuracy in this research did not warrant significant figures closer than 1 p.p.m. (0.001 mgm./gm.).

The procedure for determining the amount of copper in muck was as follows: The soil was ashed at dull red heat, digested in 25 mls. of aqua regia, and the solution evaporated to dryness. The residue was then taken up with dilute hydrochloric acid, boiled for several minutes, and filtered into a volumetric flask. A suitable aliquot was then taken for the copper determination (5).

Results and Discussion --

Three crops, onions, spinach, and Sudan grass, were grown on muck soil in a series of plots which had the same experimental design. Data in Tables 1, 2, and 3 show the yields and chemical analyses. In the case of spinach and Sudan grass, zinc analyses were not determined.

The data in Table 1 show that the response of onions to copper was not very great, but, nevertheless, the difference was statistically significant. In the acid soil the response amounted to 16 per cent, and on the limed muck it was 19 per cent increase in weight. The copper content increased from 7 p.p.m. to 15 p.p.m. with an application equivalent to 100 pounds per acre of copper sulfate. (One p.p.m. is equivalent to .001 mgm./gm.). Soils which received both copper and zinc contained 10 p.p.m. of copper. Onions grown on the limed soil doubled in copper content when copper sulfate was added to the soil.

From data shown in Tables 2 and 3, it is quite evident that spinach and Sudan grass showed a decided response to copper when grown on the muck soil used in this investigation. A comparison of the 10 pound and 100 pound application for all the three crops indicates little or no important differences in yield. The addition of 10 pounds of copper sulfate to the acre did not change the percentage content of copper in the spinach, but because of the increased growth the absorption was more than doubled. The 100 pound application caused a considerable increase in both percentage

TABLE 1. Yield and Composition of Onion Bulbs (dry weight) Grown on Muck Soil in the Greenhouse.

Treatment, mgms per jar*	Yield, grams per jar	Copper		Manganese		Zinc	
		Mgms. per gram	Total mgms.	Mgms. per gram	Total mgms.	Mgms. per gram	Total mgms.
		Acid Muck, pH 6.1					
Control	49	0.007	0.34	0.038	1.86	0.040	1.96
Cu 9.9	56	0.009	0.50	0.034	1.90	0.036	2.02
Cu 99	57	0.015	0.85	0.032	1.82	0.032	1.82
Cu 99, Zn 70	66	0.010	0.66	0.032	2.18	0.041	2.70
Cu 99, Zn 280	70	0.010	0.70	0.032	2.24	0.049	3.44
Zn 70	31	0.006	0.19	0.030	0.93	0.046	1.43
Zn 280	28	0.006	0.17	0.030	0.84	0.059	1.65
		Limed Muck, pH 7.5					
Mn 1375	53	0.005	0.26	0.030	1.58	0.030	1.58
Mn 1375, Cu 99	63	0.011	0.69	0.028	1.76	0.030	1.89
Mn 1375, Cu 99, Zn 280	64	0.010	0.64	0.026	1.66	0.040	2.56
Cu 99, Zn 280	8	0.013	0.11	0.009	0.07	0.063	0.50

* Amounts are equivalent to 10 and 100 lbs/A. of copper sulfate, 50 and 200 lbs/A. of zinc sulfate, and 600 lbs/A of manganese sulfate.

TABLE 2. Yield and composition of spinach on dry-weight basis grown on muck soil in the greenhouse.

Treatment Mgms. per jar*	Yield Grams per jar	Copper		Manganese	
		Mgms. per gram	Mgms. per jar	Mgms. per gram	Mgms. per jar
		Acid Muck, pH 6.1			
Control	4.4	0.009	0.040	0.145	0.64
Cu, 9.9	9.5	0.009	0.086	0.135	1.28
Cu, 99	11.4	0.015	0.171	0.132	1.50
Cu, 99; Zn, 70	11.8	0.012	0.142	0.118	1.39
Cu, 99; Zn, 280	11.7	0.010	0.117	0.095	1.11
Zn, 70	4.0	0.008	0.032	0.115	0.46
Zn, 280	3.8	0.006	0.023	-----	----
		Limed Muck, pH 7.5			
Mn, 1375	2.5	0.008	0.020	0.091	0.23
Mn, 1375; Cu, 99	11.5	0.012	0.138	0.076	0.87
Mn, 1375; Cu, 99; Zn, 280	12.0	0.010	0.120	0.059	0.71
Cu, 99; Zn, 280	7.0	0.010	0.070	0.014	0.01

*Amounts are equivalent to 10 and 100 lbs./A. of copper sulfate, 50 and 200 lbs./A. of zinc sulfate, and 600 lbs./A. of manganese sulfate.

TABLE 3. Yield and composition of Sudan grass on dry-weight basis grown on muck soil in the greenhouse.

Treatment Mgms. per jar*	Yield grams per jar	Copper		Manganese	
		Mgms. per gram	Mgms. per jar	Mgms. per gram	Mgms. per jar
	Acid Muck, pH 6.1				
Control	13.8	0.008	0.110	0.138	1.90
Cu, 9.9	29.7	0.010	0.297	0.058	1.72
Cu, 99	30.1	0.011	0.331	0.040	1.20
Cu, 99; Zn, 70	34.9	0.010	0.349	0.038	1.33
Cu, 99; Zn, 280	39.3	0.011	0.432	0.028	1.10
Zn, 70	12.8	0.008	0.102	0.118	1.51
Zn, 280	10.4	0.008	0.083	0.100	1.04
	Limed Muck, pH 7.5				
Mn, 1375	1.5	0.008	0.012	0.044	0.07
Mn, 1375; Cu, 99	22.8	0.012	0.274	0.020	0.46
Mn, 1375; Cu, 99; Zn, 280	19.5	0.010	0.195	0.012	0.23
Cu, 99; Zn, 280	1.6	0.014	0.022	0.012	0.002

*Amounts are equivalent to 10, and 100 lbs./A. of copper sulfate, 50 and 200 lbs./A. of zinc sulfate, and 600 lbs./A. of manganese sulfate.

Fig. 1. Injurious effect of zinc on onions. 518 = control, 522 = zinc sulfate, 524 = copper and zinc sulfate, 520 = copper sulfate.

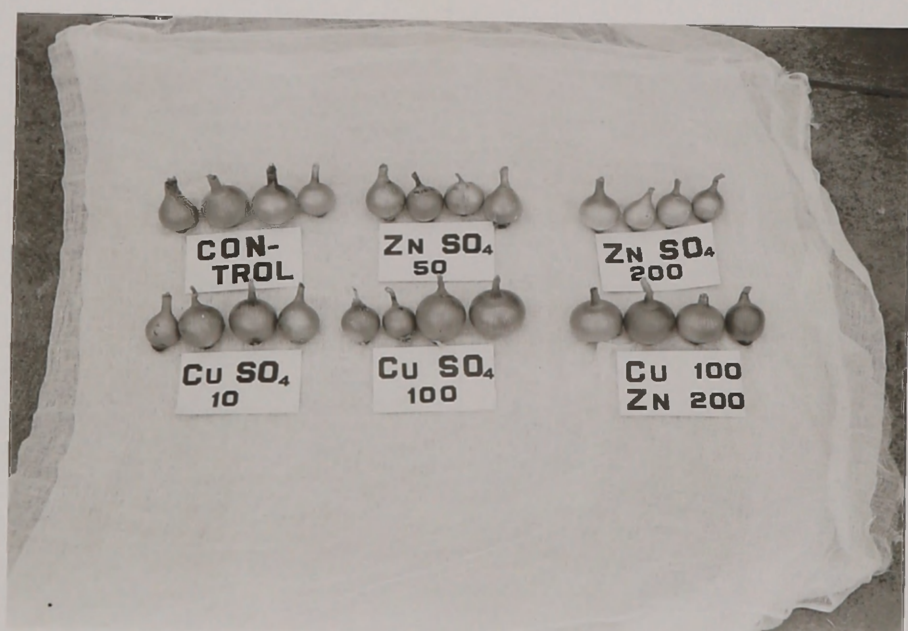

Fig. 2. Effect of zinc on onion bulb size and skin color. Zinc without copper causes lemon colored skin.

content and absorption.

The addition of zinc without copper decreased the yield; this was especially evident in the growth of onions. The lack of copper apparently weakened the plants and aided the development of neck rot disease. The onions shown in Figure 1 illustrate the break-down.

The plants grown in jar 522 received an equivalent of 200 pounds per acre of zinc sulfate. Plants in jar 524 received in addition to the zinc, 100 pounds per acre of copper sulfate. When the onion bulbs were mature the effect of zinc without copper was very marked in that it caused a light lemon colored onion skin. Onions shown in Figure 2 illustrate the color effect.

Knott (31) has shown that a lemon colored onion is a symptom of copper deficiency. It appears that zinc applications on copper deficient soils accentuated the deficiency in the plant.

In the presence of copper, in the unlimed soil, zinc increased the yields both of Sudan grass and onions, while spinach showed no response. The use of zinc had a marked effect on the copper content. In spinach the copper content decreased from 9 p.p.m. in the control down to 6 p.p.m. in the soil which received 200 pounds to the acre of zinc sulfate. A similar trend was obtained when 100 pounds of copper sulfate was applied with varying amounts of zinc sulfate. In view of these results it is believed that the application of zinc to a copper deficient soil accentuates a copper deficiency in plants. In the presence of copper, the addition of zinc to the soil was not toxic, in fact a beneficial response is suggested.

The addition of copper slightly reduced the zinc and manganese absorption by onions. The data in Table 3 show the effect of copper on the manganese content of Sudan grass. These differences were so marked that one could pick out the plant ash. The high manganese content caused a green ash, which when dissolved in a little hydrochloric acid turned pink. The use of zinc also depressed the manganese content from 138 p.p.m. down to 100 p.p.m. In the limed soil the manganese content of Sudan grass was the same as the content of the plants which showed extreme manganese deficiency. From the results of the chemical analyses, it would appear that the low manganese content was retarding the plant growth on limed soils which were treated with copper and zinc.

Since the content of manganese in the Sudan grass was affected considerably by the addition of zinc and copper, another greenhouse experiment was set up to include additional comparison of these elements. Manganese sulfate equivalent to 50 pounds per acre was added to each treatment. The muck used for this experiment proved to be only slightly responsive to copper. Two crops of Sudan grass were harvested and analyzed chemically. The analyses of the two cuttings were very similar in trend and composition for each treatment. The results given in Table 4 show the analyses of the first cutting. In these experiments the addition of copper sulfate

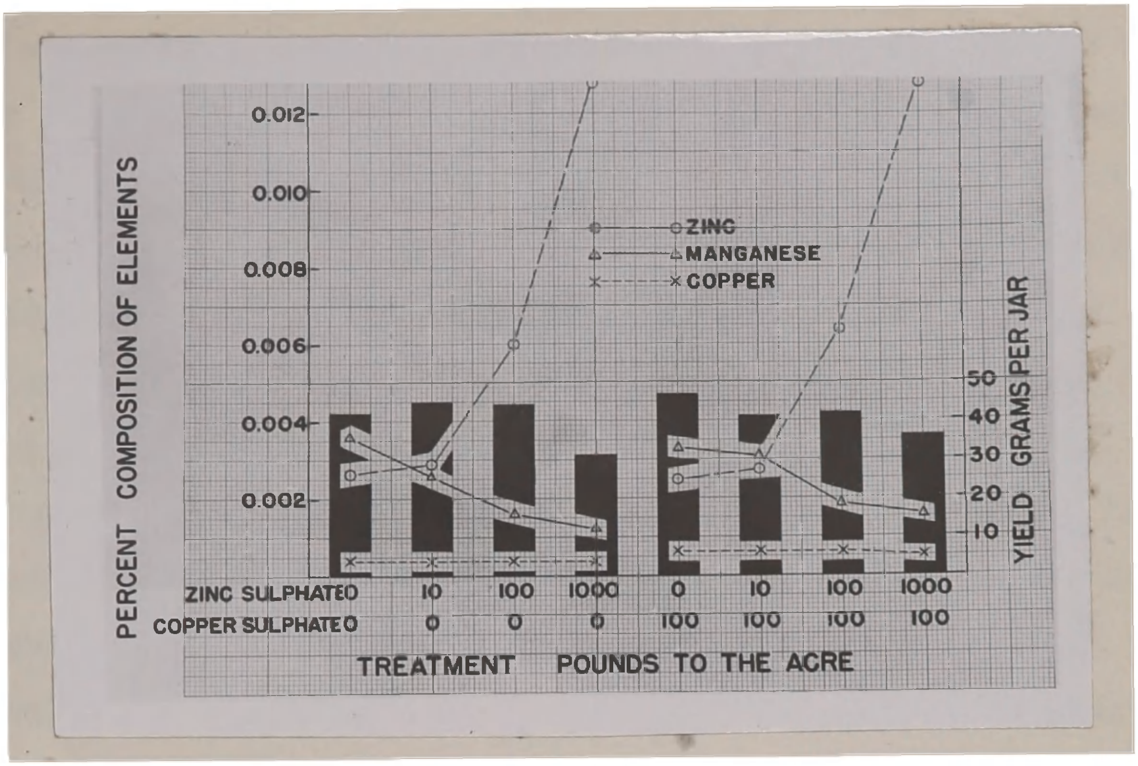

Fig. 3. The effect of the addition of copper sulfate and zinc sulfate upon yield and composition of Sudan grass.

TABLE 4. Composition and yield of Sudan grass as affected by the addition of copper sulfate.

Copper Sulfate Treatment lbs./acre	Yield* Dry Material gms./jar	Copper p.p.m.	Manganese p.p.m.	Zinc p.p.m.
Control	42	4	36	26
1	44	4	34	25
10	45	5	34	25
100	47	6	33	25
1000	48	16	36	27
2000	42	16	35	27
4000	41	15	35	27

*Difference in yield not significant.

up to an equivalent of 4000 pounds per acre had little or no effect on the zinc and manganese contents. It would appear that the high manganese content of plants shown in Table 3 was caused by plants deficient in copper. The physiological unbalance of the elements and the depressed yields caused the high manganese content. The use of copper sulfate up to 1000 pounds per acre showed no injurious effect on growth. The copper content of the plants increased from 4 p.p.m. in the control treatment to 17 p.p.m. in the 1000 pound per acre application.

Figure 3 shows two sets of comparisons for increasing amounts of zinc, one without any copper applied to the soil, and the other with 100 pounds per acre of copper sulfate. The yield and composition of Sudan grass are nearly the same for both comparisons except that the addition of copper sulfate increased the copper content approximately 60 per cent. Zinc had no effect on the copper content, but it depressed the manganese absorption considerably. Yields were noticeably reduced when the equivalent of 1000 pounds per acre of zinc sulfate was applied to the soil. The content of zinc in the plant increased markedly with increasing rates of zinc application.

Data in Table 5 show the response of tomatoes to copper. Near the end of the harvest, the number of fruits per plant was nearly the same for the four treatments, but the weight per tomato averaged more for the copper treated soil.

Figures 4 and 5 show the effect of copper on early plant growth and on the development of the fruit. The picture shows that the use of copper in this soil has developed plants with large petioles. The marked response on growth early in the growing season accounted for the ripening of fruit nearly 10 days before any fruit matured on plants grown in soil without copper. The mature fruit on these plants was more fleshy, which accounted for the greater weight as compared to the fruit grown on soil without copper fertilization.

TABLE 5. The total number and fresh weight (gms.) of ripe tomatoes per plant as affected by the use of copper and zinc (100 pounds per acre). Data show total fruit harvest up to date of record. (Average of duplicate determinations.)

Treatment	12/15/45		1/1/46		1/25/46		Copper in Dried Fruit p.p.m.
	No.	Wt.	No.	Wt.	No.	Wt.	
Control	0	0	7	605	17	1446	4
Copper	2.5	298	9	1050	18	2043	8
Copper, Zinc	2.5	407	8.5	1100	21	2330	8
Zinc	0	0	8	710	18	1525	4

Fig. 4. Effect of copper on the early growth of tomatoes. Plants showed no response to zinc.

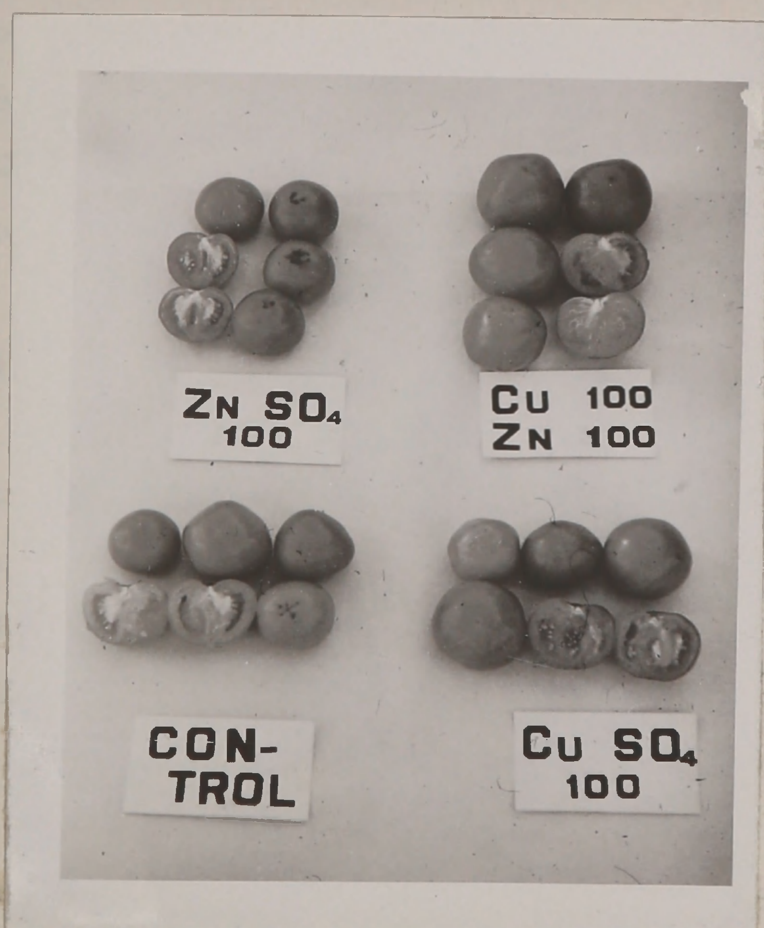

Fig. 5. Effect of copper and zinc on tomato fruit.

Composition of Crops Grown in the Field

Through experiments set up at the Michigan State College Muck Experimental Farm in 1942, it was possible to obtain plant samples in 1945 for the determination of the minor element content of some field crops. All plots received 1000 pounds to the acre of 0-20-20 fertilizer. The minor elements were applied broadcast and disked into the soil. Many crops grown on this soil showed response to copper fertilization, but not to manganese. Zinc increased the yield of onions. For the control treatment, plants were collected from plots 1, 5, and 11, which did not receive any minor elements; plots 3 and 13, which received 50 pounds of copper sulfate in 1942 and none thereafter; and plots 4 and 14, which received 50 pounds of copper sulfate annually from 1942 through 1945. In order to compare the effect of the addition of zinc sulfate on composition, it was necessary to harvest and analyze crops grown on plots 32 and 39 which received 50 pounds to the acre annually of copper and manganese sulfates, and plots 33 and 40 which received 50 pounds to the acre of zinc sulfate in addition to the copper and manganese. These plots were a considerable distance from plots 1 to 14.

Copper fertilization of this soil increased the copper content of the plants as much as 200 per cent. It slightly decreased the manganese content of all crops except head lettuce. The greater the copper fertilization, the smaller was the zinc content of the plant. The application of manganese still further decreased the zinc content. The plants growing on the control plots were always high in zinc. On the basis of three field crops, an application of 50 pounds to the acre of zinc sulfate did not appear to affect either the copper or manganese absorption. The addition of manganese did not increase the manganese content in lettuce and peppermint but did increase it in onions.

Sufficient analyses were made to compare the copper content of several crops grown on soils which did or did not receive copper sulfate. The data in Table 7 show these comparisons. In general most of the plants showing copper deficiency contained around 5 p.p.m., while the addition of 100 pounds of copper sulfate per acre approximately doubled the copper content. Exceptions to this general observation are the copper content in oats and barley. These two crops were grown on very acid soils (pH 4.3) while analysis of other crops were grown on soil with pH 6.1. It is believed that an adequate level of copper in any crop is not a specific figure, but is related to the composition of other elements and organic compounds in the plant. Such a statement would not be without foundation since it is known that plant needs for boron are related to calcium (28), manganese to iron (56), and potassium to the level of calcium and magnesium (38). From data shown in Figure 14, which is discussed in Part III, it is possible that either the level in the plant of iron or aluminum, or both, affect the copper requirement of plants. In acid soils these elements are

TABLE 6. Copper, manganese, and zinc contents of crops grown on muck. Calculated on dry-weight basis, (1945 harvest).

Treatment	Copper p.p.m.	Manganese p.p.m.	Zinc p.p.m.
	Head Lettuce		
Control	3	15	54
Cu (1942 only)*	7	16	50
Cu†	9	17	45
Cu, Mn†	9	17	42
Cu, Mn, Zn†	9	18	58
	Peppermint		
Control	8	32	26
Cu (1942 only)	9	26	25
Cu	12	28	18
Cu, Mn	11	26	14
Cu, Mn, Zn	11	26	24
	Onion Bulbs		
Control	2	16	42
Cu (1942 only)	3	15	36
Cu	5	15	35
Cu, Mn	6	19	21
Cu, Mn, Zn	5	20	33
	Onion Tops		
Control	5	24	9
Cu(1942 only)	7	20	9
Cu	10	20	9
Cu, Mn	8	30	8
Cu, Mn, Zn	8	28	10
	Wheat, Prior to Heading		
Control	8	26	40
Cu (1942 only)	12	27	42
Cu	13	23	36
	Dill Seed, 1944 Harvest		
Control	6	40	84
Cu (1942 only)	11	33	78
Cu	12	29	76

*Received 50 pounds to the acre of copper sulfate.

†Received annually from 1942 to 1945, inclusive, 50 pounds to the acre of the sulfate for each minor element indicated.

TABLE 7. Comparison between the copper contents of plants grown on control plots and those receiving copper fertilization (dry-weight basis).

Crops	Place Grown	Degree of Response	Copper in Plants p.p.m. Control	Copper Sulfate
Alfalfa	Field	Fair	5	10
Barley (preheading)	Greenhouse	Good	10	14
Carrot Tops	"	Good	4	6
Carrot Roots	"	Good	3	5
Dill Seed	Field	Good	6	12
Head Lettuce	"	Good	3	9
Ladino Clover	"	None	7	14
Oats (preheading)	Greenhouse	Good	11	15
Onion Bulbs	Field	Fair	2	5
Onion Tops (mature)	"	Fair	5	10
Peppermint	"	None	8	12
Red Clover	"	None	7	15
Sudan Grass	Greenhouse	Good	5	10
Sugar Beets (tops)	"	Good	6	7
Spinach	"	Good	8	12
Tomato Fruit	"	Fair	4	8
Wheat Grain	Field	Good	6	8
Wheat (preheading)	"	Good	8	12

known to be very soluble and are quite available to plants. For this reason, though the copper content of the oats and barley appears to be high, the plants showed extreme deficiency because of an unbalance in the plant of other unknown elements. Further investigations on this subject are needed.

Though some crops did not respond to copper fertilization, nevertheless, the copper content in the plant increased. This increased absorption by both responsive and nonresponsive crops, is a very small fraction of the amount added to the soil. On a basis of 10 p.p.m., two tons of alfalfa would contain 0.04 pounds (18 grams) of copper. Likewise, the content in 30 bushels of wheat would amount to about 0.013 pounds. The efficiency of recovery by one crop would be less than 0.2 per cent if 100 pounds of copper sulfate per acre were applied to the soil. It is easily understandable why an application of 10 pounds per acre would be sufficient copper sulfate, providing soil fixation was not too high.

Response of plants to the addition of copper sulfate grown on an unproductive muck obtained in Indiana is shown in the series of Figures 6, 7, 8, and 9. This soil was taken from a field of corn which resulted in a complete crop failure (1946), even though large applications of phosphate and potash were used. Occasionally a corn plant matured in the field, but most of them remained dwarfed or died during mid season. The nutritional symptoms were a general light chlorosis of the plant, especially in the newly developed leaves. These leaves also showed longitudinal light streaks.

A greenhouse experiment was set up in November using corn as the indicator crop. Treatments consisted of soil with and without an application of six tons per acre of calcic limestone. The pHs of the treated and untreated soil were 4.3 and 5.2, respectively. In both the limed and unlimed soils copper, manganese, zinc, boron, and magnesium were added, either singly or in combination. Because of the unfavorable light conditions in the greenhouse, the use of corn as an indicator crop was discontinued. Sugar beets were then planted in the same jars.

The sugar beets shown in Figure 6 illustrate the response to copper on this soil. All treatments which failed to receive copper grew plants very similar to the control treatment. Likewise, all combinations of minor elements with copper showed no greater benefit over the treatment containing copper along.

When the results of the sugar beets became so apparent, an experiment was set up to demonstrate the response of several crops to copper in the soil which received sufficient nitrogen, phosphorus and potassium. The crops used in this experiment were barley, oats, spinach, alfalfa, and wheat. All crops responded markedly to copper fertilization. The pictures are attestations to this fact. It could be logically assumed that the malnutrition of corn observed growing in the field was also a copper deficiency.

Copper deficiency symptoms are variable for different

Fig. 6. Response of sugar beets to copper fertilization.

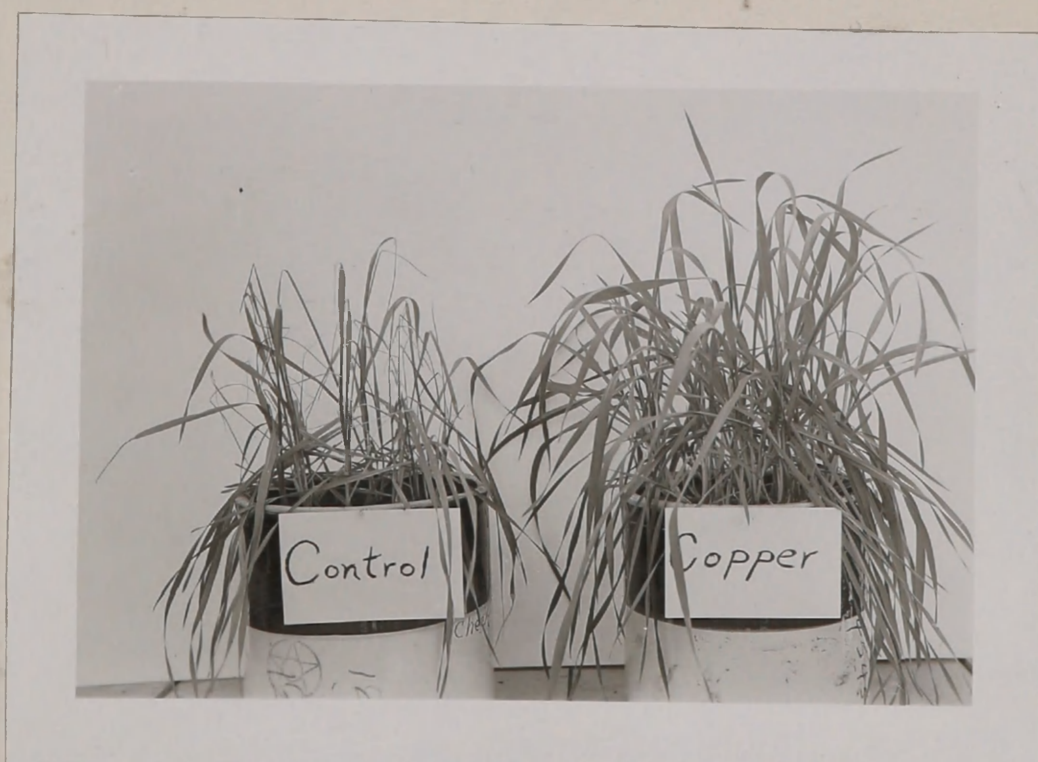

Fig. 7. Effect of the addition of copper sulfate to muck on growth of wheat.

Fig. 8. Barley with and without copper fertilization.

Fig. 9. Beneficial effect of copper on oats.

crops. In Figure 10 is shown a normal oat leaf as compared to three leaves showing different types of deficiencies. The mature leaf on the left of the picture showed yellowing and death of the tip. The young deficient leaf tended to remain rolled. The deficiency symptoms in wheat are similar to oats. In barley the lack of copper produced a very characteristic chlorosis. Sugar beets and spinach showed no early symptom. As the spinach plant aged, it showed a wilting and crinkling appearance near the tip of the leaf followed by a yellow to yellow-brown coloration around the leaf blade. Aged sugar beet leaves developed white necrotic areas on the lower leaves. Similar symptoms were observed in alfalfa.

Fig. 10. Comparison between three copper deficient oat leaves and a normal leaf.

PART II. The Effect of Copper on Quality as Measured by the Carotene, Ascorbic Acid and the Protein Contents of Plants.

Introduction --

The supply of available nutrients within a soil affects the nutritional value as well as the yield of crops produced on the soil. The quality or food value of plants cannot be adequately measured by determining any one constituent but rather by the measure of several constituents. In general a food of high nutritional value is one which is high in proteins, carbohydrates, and vitamins. Perhaps food values can best be measured by actual feeding tests. Such tests do not show, however, which constituents have actually been affected by the fertilizers applied to the crops. In this section is reported the effect of copper deficiency upon the carotene (Pro-Vitamin A), ascorbic acid (Vitamin C), and the protein contents of plants.

Historical --

Recently a few papers have been published on the use of minor elements for improving nutritional quality. The results have not been in agreement. Bernstein, Hamner, and Parke (11) have contributed considerably to this phase of research. In an experiment with turnip greens they found that fertilizers, either in pot cultures or on field plots did not affect the content of ascorbic acid. These results were obtained in spite of the fact that fertilizer did affect the growth and appearance of the plants. Likewise, there was no appreciable effect of fertilizer on carotene content, except in the case of visibly chlorotic plants growing in sand cultures.

Lyon, Beeson, and Ellis (39) reported that fresh tomatoes grown in a complete nutrient solution contained 20.0 $\pm$ 0.46 mgms./100 of ascorbic acid, and 0.4 $\pm$ 0.02 mgms./100 of Pro-Vitamin A. Fruit harvested from copper deficient plants contained 20.5 $\pm$ 1.05 and 0.5 $\pm$ 0.04 mgms./100, respectively, of these vitamins. The differences between the two treatments were not considered significant.

The investigations of Sherman (50) showed that plants grown on a soil deficient in available manganese contained less reduced and total ascorbic acid than did plants grown on soils containing sufficient manganese. Hester (26) found that fruit from manganese deficient tomato plants had less ascorbic acid than fruit harvested from plants which were not deficient in manganese. Barnes (7) obtained a 16 per cent increase in yield of carrots, and a 9 per cent increase in carotene content, as a result of an application of 100 pounds per acre of copper sulfate.

Scripture and McHargue (49) noted that alfalfa plants showing boron deficiency contained 0.78 per cent reducing sugars and an additional 0.51 per cent sugar after hydrolysis, while normal plants contained 0.42 and 0.35 per cent, respectively, of the two forms of sugar. They concluded that the abnormal accumulation of sugars, and also soluble nitrogen compounds, in boron deficient plants suggests that protein metabolism may not be proceeding normally. Harmer (25) reported that the sugar content of carrots and table beets was appreciably reduced in copper deficient plants. This fact indicates that the role of copper in plants is entirely different from that of boron.

Loustalot, et al. (33) found a highly significant reduction in the rate of carbon dioxide assimilation in both slightly or markedly copper deficient leaves of tung trees. They believe that the apparent decrease in photosynthesis was not primarily associated with the appearance of chlorosis or necrosis of the leaf tissue. The average rate of carbon dioxide assimilation for leaves on normal trees was 11.58 milligrams per 100 sq. cm. per hour as compared with 8.15 and 5.20 milligrams, respectively, for normal appearing leaves growing on zinc and copper deficient trees. The average rates for abnormal leaves on zinc and on copper deficient trees were 4.45 and 2.19 milligrams, respectively. It seems that the results of these workers are quite in agreement with the conclusion drawn by Harmer (25) that copper aids in carbohydrate formation.

Orth, et al. (43) found that a copper deficiency in oranges affected the chlorophyll content of the leaves. Trees treated with copper contained 4.6 times as much chlorophyll in their leaves as did those from untreated trees.

Meiklejohn and Stewart (42) reported that cucumber juice contains a copper protein complex, which was described as ascorbic acid oxidase. This enzyme was much more active catalytically than the same amount of copper in the ionic form. The complete removal of copper by dialysis inactivated the juice. The dialyzed enzyme did not contain a polyphenol oxidase. They believed that their preparation was nearly identical to an enzyme found in potatoes previously described by Kubowitz (32). This enzyme oxidized polyphenols and in the pure state contained about 0.2 per cent copper. Lovett-Janison and Nelson (35) isolated from crook-neck squash a blue to bluish-green enzyme which contained 0.15 per cent copper and was free from peroxidase activity. It catalyzed the oxidation of ascorbic acid. Other copper enzymes purified and described are Laccase (30), and Polyphenol oxidase (29).

Mapson (40) reported that the complete removal of copper from a solution renders ascorbic acid stable to oxygen (O_2). The copper concentration has to be reduced to 1.5×10^{-9} before the oxidation of the acid is virtually suppressed.

In a study of the effect of minor elements on the enzyme activity of tomato plants, Bailey and McHargue (6) found an interesting relationship between copper concentration in the nutrient solution and oxidase activity in the leaves. Assuming that the plants grown without copper had a relative oxidase activity value of 100, they showed that plants growing in nutrient solutions containing 0.01, 0.05, and 0.1 p.p.m. of copper had a relative value of 110, 122, and 140, respectively. The enzyme invertase was increased markedly in the tomato fruit by increasing the amounts of copper in the nutrient solution. Again using the control value as 100 the values for the copper treatments 0.01, 0.05, and 0.10 p.p.m. were 123, 170, and 190, respectively. The amount of peroxidase and catalase activity diminished progressively with increasing copper.

The role of copper in animal nutrition has been associated with the formation of normal blood. Schultze (47,48) has shown that copper fed to anemic and copper deficient rats produced accelerated cytochrome oxidase of the bone marrow, and a gradual increase in the erythrocyte count and of the blood hemoglobin content. Rats fed milk containing iron, copper, and manganese had a Q oxidase value of 33.4, milk containing copper and manganese had a value of 31.9, while milk containing iron and manganese without copper had a value of only 5.6. From these results it was concluded that copper and not iron was essential for oxidase formation. It was also shown that the Q oxidase value of rats changed from 10.4 to 29.0 when fed 0.1 mg. of copper 24 hours before the measurement.

Experimental Procedure --

In the study of the effect of copper on quality, analyses were made for the carotene, ascorbic acid, and protein contents of plants grown in the greenhouse and in the field. The cultural methods were the same as described in Part I. Ascorbic acid was measured by the 2,6-dichlorophenolindophenol method, as modified by Lucas (36), in which 20 to 30 grams of plant material were dispersed with a Waring Blendor in 180 to 280 mls. of metaphosphoric acid solution. Carotene was measured by the procedure described by Benne, et al. (10). At times it was difficult to get representative samples from 3 to 5 grams of plant material. In such cases, sufficient material was dispersed in acetone with the Waring Blendor. After refluxing and filtering a sufficient aliquot was taken for the separation and measurement of the carotene. Protein was measured by determining total nitrogen in the dried sample (5) and multiplying by use of the factor 6.25.

The samples of field plants to be used for carotene and ascorbic acid measurements were collected in the early morning and analyzed the same day. Moisture was determined on a portion of the fresh material. Where differences were significant, the chemical analyses were corrected to the average moisture content of the whole lot. The same general procedure was followed for the carotene analyses of plants grown either in the field or in the greenhouse. All ascorbic acid determinations of plants grown in the greenhouse were made in the greenhouse laboratory. No effort was made to determine the moisture content of these plants, for they were harvested, weighed, and a representative sample taken within a period of five minutes for ascorbic acid analysis.

Results and Discussion --

Data shown in Table 8 are values for the ascorbic acid and carotene contents of onions determined at three stages of maturity. The differences in carotene are not great in the young plants. The application of copper sulfate caused an increase of 0.38 mgm. of carotene per 100 gms. of green plant material. On the limed soil, the addition of copper in the presence of manganese did not affect the carotene content of onions but the addition of manganese in the presence of copper and zinc, increased the carotene content by 0.42 mgm. per 100 gms. of green material. The application of minor elements did not affect the ascorbic acid content of young plants. The differences in both ascorbic acid and carotene as affected by the acidity of the muck were quite noticeable. This was probably caused by the differences in the size of plants, as those growing on the limed soil were smaller. Mature bulbs which were harvested from zinc treated acid muck and from limed muck deficient in manganese were higher in ascorbic acid than were those from all other plots. Bulbs harvested from plots which received these treatments were very stunted (see Table 1).

In Table 9 are shown the results of the analyses of fall and spring grown spinach. Though the increase in yield caused by copper fertilization was much greater in the spring than in the fall, the increase in ascorbic acid content resulting from the copper treatment was much greater in the fall. It was observed that there were no copper deficiency symptoms on the spring grown plants. The plants grown in the fall without copper developed a mottling and were not as dark green as plants which were fertilized with copper.

The chemical analyses shown in Table 10A are the results obtained from wheat grown in the field. They indicate that copper had little effect on the ascorbic acid content of wheat at two stages of maturity. The only significant difference obtained as a result of copper treatment was an increase in the carotene content at the preheading stage.

As shown by the data reported in Table 10B, the carotene and ascorbic acid contents of field grown spinach and onions were not markedly affected by copper sulfate treatments. The only apparent affect of the copper was to increase the carotene content of the spinach.

In Tables 11 and 12 are shown the results obtained from growing barley and carrots on soil from the College Muck Farm. Both crops responded to copper fertilization. The barley plants growing on soil which did not receive copper were of a yellow to yellowish-green color. The application of 10 pounds of copper sulfate did not entirely eliminate the deficiency symptoms but was equal to the 100 pound application so far as yield was concerned. The carotene and ascorbic acid contents of the barley were significantly increased by the 10 pound per acre application of copper sulfate. A larger application of copper caused a further increase in carotene content but did not further increase the content of ascorbic acid. The data presented in Table 12 show that the yield and carotene content of carrots was greatly increased by an application of 100 pounds per acre of copper sulfate. The data show that the difference in carotene content, as effected by copper, diminished as maturity advanced. The plants shown in Figure 11 show the effect of copper on carrot tops and roots.

Tomato fruit harvested from plants receiving minor elements did not show any significant differences at the 5 per cent level in ascorbic acid as a result of treatment. (See Table 5 for response). The values varied between 18.8 and 21.2 mgms. per 100 gms. of fresh fruit.

The data reported in Table 13 show that yields, ascorbic acid and carotene contents of oats were greatly increased by applications of copper sulfate, irrespective of the degree of acidity of the muck. The greatest response, as far as yield was concerned, occurred on the unlimed muck where the pH was 3.6. As the pH was raised by liming to pH values of 4.6, 5.9, and 7.2, response to the copper seemed to become less and then to increase again at the highest pH. The same trend seemed to occur in the effect of the copper on the carotene content of the oats, the greatest increase in carotene occurring at the low soil pH and the least increases occurring at the intermediate pH levels. That particular trend did not occur in the case of ascorbic acid. With that constituent there seemed to be greater increases, as affected by the application of copper sulfate, at the

TABLE 8. Effect of copper, manganese, and zinc on the ascorbic acid and carotene contents of onions grown in greenhouse.

Treatment Mgms./jar	Moist weight basis (mgms./100 gms.)				
	Carotene	Ascorbic Acid			
	Young Plants	Young Plants	Bulbing Stage Tops	Bulbing Stage Bulbs	Mature* Bulbs
Acid Muck pH 6.1					
Control	2.92	37.2	23.5	15.0	7.5
Cu 9.9	3.12	36.5	24.2	14.8	7.7
Cu 99	3.30	36.5	22.0	14.7	7.7
Zn 70	3.18	38.2	25.5	18.0	10.0
Zn 280	3.08	38.5	26.5	19.0	10.0
Cu 99, Zn 50	3.28	36.5	23.5	15.0	7.0
Limed Muck pH 7.5					
Mn 1375	3.65	44.5	----	----	7.4
Mn 1375, Cu 99	3.56	44.0	----	----	7.6
Mn 1375, Cu 99, Zn 280	3.68	44.1	----	----	8.1
Cu 99, Zn 280	3.26	46.4	----	----	10.3

*See Table 1 for weight differences.

TABLE 9. Influence of minor elements upon the ascorbic acid contents of spinach grown in the greenhouse.

Treatment	Fall Grown		Spring Grown	
	Wt. of Fresh Material	Ascorbic Acid* Mgms./100 gms.	Wt. of Fresh Material	Ascorbic Acid† Mgms./100 gms.
Control	97	57	49	95
Manganese	109	57	--	--
Copper	123	72	127	102
Zinc	92	61	42	96
Cu, Mn	114	73	--	--
Cu, Mn, Zn	118	71	130	100

*Differences significant at 5% level.

†Differences not significant at 5% level.

TABLE 10A. Ascorbic acid and carotene contents of wheat grown on Minor Element Series. (Mgms./100 gms.)

Treatment	Wheat Harvested 6/19/45		Wheat, preheading* Harvested 6/30/45	
	Ascorbic Acid	Carotene	Ascorbic Acid	Carotene
Control	63	4.5	25	1.9
300 $CuSO_4$ in 1942	56	4.6	26	2.8
50 $CuSO_4$ in 1942	58	4.5	27	2.5
50 $CuSO_4$ in 1942, '43, '44, '45	56	4.7	25.	2.6

*Corrected to 90% moisture.

TABLE 10B. Ascorbic acid and carotene contents of spinach and onions grown on Minor Element Series. (Mgms./100 gms.)

Treatment	Spinach Petiole*		Fresh Onions** Early Bulbing	
	Ascorbic Acid	Carotene	Ascorbic Acid in Tops	Ascorbic Acid in Bulbs
Control	71	5.9	26.0	16.6
50 $CuSO_4$ in 1942	74	6.4	24.4	15.1
50 $CuSO_4$ annually	70	6.3	24.8	15.3

*Corrected to 90% moisture.

**Corrected to 91% moisture.

TABLE 11. The effect of copper sulfate applied to muck soil in pot cultures on the carotene and ascorbic acid contents of barley.

Treatment lbs./acre	Green Weight gms./jar	Ascorbic Acid mgms./100 gms.	Carotene mgms./100 gms.
Control	63	29.2	4.00
10 $CuSO_4$	122*	45.1*	5.40*
100 $CuSO_4$	122	46.5	6.60*

*Differences significantly greater than the results from the control treatment.

TABLE 12. The effect of copper sulfate applied to muck soil in pot cultures on the carotene content of carrot roots harvested at various intervals. Expressed in mgms./100 gms. of fresh material. Yield based on weight of 12 representative roots.

Treatment	12/15/45		1/2/46		1/21/46	
	Yield	Carotene	Yield	Carotene	Yield	Carotene
Control	24	2.16	104	4.30	123	5.06
100 $CuSO_4$	110	3.50	432	6.08	590	5.96

Crop planted 10/15/45.

intermediate pH levels.

With the assumption that plants growing on subsoil and topsoil would vary in their response to copper, five widely separated virgin soils were collected from the College Muck Farm. The results of the average fresh weights shown in Table 14 indicate that plants growing on the topsoil were more responsive to copper than plants growing on subsoil samples. The increase in the ascorbic acid content of the leaves amounted to about 15 per cent for the subsoil, and 33 per cent for the topsoil.

The conclusion drawn from the chemical analyses of plants deficient in copper is that the carotene and ascorbic acid are lower, but varying with different crops. In view of the results of this research, it may be stated that a copper deficiency can disturb some organic constituents in the plant. For this reason, feeds and food from copper deficient plants may be lower in nutritional value as well as in copper. One of the physiological functions of copper is to form a protein enzyme which is specific for the oxidation of ascorbic acid (29,35). The increased absorption of copper by plants grown on soil fertilized with copper does not decrease the ascorbic acid content. Apparently the plant cell has inhibiting enzymes which under certain circumstances counteract the action of the copper enzyme.

One of the symptoms of copper deficiency in wheat, oats, and barley is the general chlorosis of the plant. The grain from wheat having copper deficiency is pale brown in color

TABLE 13. Carotene and ascorbic acid contents of oats grown on muck soil at various pH levels with and without the addition of copper sulfate.

pH	Fresh Weight gms./jar		Ascorbic Acid mgms./100 gms.		Carotene mgms./100 gms.	
	No Copper	Copper Added	No Copper	Copper Added	No Copper	Copper Added
3.6	14	102	22.5	29.5	6.50	8.83
4.6	126	146	23.0	36.0	7.25	8.10
5.9	143	177	26.8	35.0	8.10	8.52
7.2	136	192	27.2	32.5	7.15	8.92
Average			24.9	33.2	7.25	8.59

Data are averages of duplicate determinations.

TABLE 14. Effect of copper on ascorbic acid content of fresh oat leaves (preheading stage), grown on five different pairs of topsoil and subsoil.

Soil Sample	pH	Total Yield gms./jar		Ascorbic Acid Content mgms./100 gms.	
		No Copper	Copper	No Copper	Copper
1 - Top	6.7	226	208	51	67
1 - Sub	6.7	199	212	75	79
2 - Top	7.3	199	212	62	68
2 - Sub	6.6	305	312	56	77
3 - Top	6.5	183	232	48	66
3 - Sub	6.5	200	205	66	77
4 - Top	6.3	195	207	59	69
4 - Sub	6.5	185	181	64	74
5 - Top	6.5	168	234	48	68
5 - Sub	6.8	183	184	65	68
Av. Topsoil		194	219	51	68
Av. Subsoil		214	223	65	75

Data are averages of duplicate determinations.

Fig. 11. Effect of copper on carrots. For carotene content see Table 12.

while grain from normal plants is dark reddish-brown. These symptoms appear similar to nitrogen deficiency. Actually this malnutritional condition is not due to a nitrogen deficiency, for, as shown in Table 15, the protein content in copper deficient plants was found to be exceptionally high. Quick tests by the diphenolamine method for nitrates in the stem or petioles indicated that both normal and copper deficient plants were adequately supplied with nitrate-nitrogen. Evidently a copper deficiency does not interfere in the conversion of carbohydrates to proteins. Since the reports of Loustalot (34) and Harmer (25) show that copper aids in photosynthesis and the formation of simple sugars, the role of copper is probably involved in the early stages of food synthesis.

TABLE 15. Protein content in copper deficient and normal plants. (Oven dried samples)

Crop	Per Cent Protein		Per cent Decrease
	Copper Deficient Plants	Copper Fertilized Plants	
Alfalfa	32.6	30.2	7.4
Barley (preheading)	38.9	27.2	30.1
Oats (preheading)	33.2	29.4	11.4
Wheat (preheading)	34.2	32.9	3.8
Wheat Grain	21.5	19.4	9.8
Sugar Beets	24.4	19.9	18.4
Tomato Fruit	15.9	13.9	12.6
Carrot Roots	19.6	13.2	33.6
		Average =	16.0

PART III. A Study of the Chemical Reactions and Physical Effects of Copper in Organic Soils.

Introduction --

No practical fertilizer recommendation can be made for a plant-nutrient if a knowledge of the physical and chemical effects of the element in the soil is not known. In the case of major nutrients, such as phosphorus and potash, the amount applied to soils of low fertility is governed to a considerable extent by the amount used by the crop. For minor elements, as pointed out in Part I, the amount utilized by the crop is only a very small fraction of that normally applied to the soil for correcting the plant deficiency. For this reason an understanding of the behavior of minor elements in soils is especially important. In this section an attempt is made to answer some of the questions about the chemical properties of copper in muck soil.

Historical --

Very little study has been made of the effects which occur in the soil itself. The workers in Florida have probably contributed the most. Jamison (27) reported that the particle size of copper sulfate affected its solubility in Norfolk sand. Leaching the soil with 29 inches of water carried downward a distance of one foot, 91 mgms. of copper out of a total application of 379 mgm., when copper sulfate was applied in the form of pea sized lumps. A similar comparison with very fine crystals showed that the leachings at the one foot depth contained only 0.6 mgms. of copper. It was concluded from these results that the use of coarse copper sulfate was more practical than very fine copper sulfate.

Allison (2) reported that a considerable portion of the copper cation applied to organic soils was held rather tenaciously in the top few inches of the soil. Skaptason (53) accounted for an increase of 180 pounds per acre of copper in the top six inches of Long Island Soils, when approximately 20 pounds of copper per year for 32 years had been applied in the form of bordeaux sprays.

Most workers report that copper availability decreases with increased alkalinity of the soil. Piper (45) found that an increase in acidity for any given level of copper increased the availability somewhat when measured either chemically or by total copper absorbed by plants. Peech (44) reported similar data. Teakle, et al. (60) reported plants were acutely deficient in copper when grown on marly soils of Western Australia. Teakle (59) also reported that applications of 2 to 10 pounds per acre of copper sulfate to these soils were sufficient to correct a copper deficiency. The residual values of such small applications of copper were so substantial that the succeeding crops showed little or no response to additional applications of copper.

The areas where copper deficiency in plants have been reported are not necessarily found to be alkaline soil. Harmer (25) generally observed that the more acid the organic soil, the greater was the relative response to copper, and the greater the number of crops which were likely to respond to the copper application. Teakle and Stewart (61) were able to show spectacular benefits from the application of copper to a very acid muck (pH 4.2). In view of these findings on the response of plants to copper on both alkaline and very acid soils, one can conclude either that the relationship between availability of copper and pH is not important if the copper content in the soil is low, or that the need of copper is more dependent upon the balances of nutrients within the plant.

Bower and Truog (12) found that the base exchange capacity of a soil, as shown by the use of copper acetate, was nearly twice as high as values obtained by monovalent cations. For example, two soils with exchange capacity values of 117 and 60, as determined by standard methods, had values of 230 and 115, respectively, for the two soils when exchange capacity was determined by the use of copper acetate. They believed that the exchange adsorption was $(HO\text{-}Cu)_{2x}$-Clay instead of Cu_x-Clay.

Probably the best work so far reported on the cation exchange reactions of zinc is that of Elgabaly and Jenny (16). Since the chemical reactions of zinc are similar to copper, probably many of their findings would apply to reactions of copper in the soil. These investigators found that the addition of zinc chloride to either a sodium or calcium bentonite (clay) released less calcium or sodium than the total milliequivalents of zinc adsorbed. In other words, the reaction is not stoichiometric. It was also noted that the adsorption of zinc involved an adsorption of chloride anions. Additions of zinc chloride to a calcium bentonite produced a complex colloidal system of adsorbed cations containing Ca^{++}, Zn^{++}, $(ZnCl)^{+}$, and $(ZnOH)^{+}$. The use of zinc acetate instead of zinc chloride showed a similar complex system. The force of zinc adsorption by the clay was found to be similar to hydrogen adsorption. At 4 S. (symmetry) concentration of hydrochloric acid, the release of adsorbed zinc amounted to about 70 per cent.

Wood (64) believed that the copper extracted by boiling normal nitric acid represents the copper which, through weathering and other processes, may become available for plant use as the exchangeable supply is lowered. The amount of copper leached out by ammonium acetate from a 5 gram soil sample was less than 1 p.p.m.

Experimental Procedure --

Most of the laboratory studies on muck were determined on samples obtained from the Muck Experimental Farm. Ten to twelve borings with an auger type sampler were collected from each plot (3 replications per treatment) on the Minor Element Series. Borings from each treatment were combined, thoroughly mixed, and bottled in a moist condition. Chemical properties of the soil were determined on moist samples for which the moisture content was corrected from soil samples dried at 90°C.

Procedures as described in Part I were used. Most of the copper determinations were measured by means of an Evelyn photoelectric colorimeter. The carbamate method was used for micro-concentration of copper (5). In the study of the copper adsorption at various symmetry values the copper in solution was measured as the cupric-ammonia complex by adding 20 mls. of dilute ammonia to 5 mls. of copper solution (52). For the latter determination a 6200 Angstrom filter was used.

In the study of the adsorption of copper at various pH levels, equal samples of moist soil were weighed. This amounted to 7.1 grams of dry soil. All samples of soil were leached with 0.2N HCl in a Buckner funnel until the pH of the soil was around 3.0. The soil was then leached with water, and the pH of the soil adjusted to the desired value with lime water. The soil was then dispersed in 100 mls. of water containing 2.0 mgms. of copper sulfate. After intermittent stirring for several hours, the solution was again filtered and copper not adsorbed was measured. A similar procedure was followed in which the soil sample received 100 mls. of 0.5N copper sulfate. The large amounts of copper sulfate produced considerable acidity. The soil suspension was adjusted back to the original pH with concentrated sodium hydroxide. For these soils the copper sorbed was measured rather than copper not sorbed. This was done by leaching out excess copper from the soil with

300 mls. of carbon dioxide free distilled water, and then leaching with 500 mls. of 0.2N hydrochloric acid to remove adsorbed copper. Copper was measured colorimetrically as the cupra-ammonia complex.

In the preparation of a Cu-humus, the soil was first leached with dilute hydrochloric acid to remove all the bases. This was followed by leaching with water, and then finally with 200 mls. of 0.5N copper solution. Excess copper was leached out with 300 to 400 mls. of cold water. Total adsorbed copper was measured as described previously.

In the study of the chemical reaction of copper humus with sodium hydroxide or hydrochloric acid, a Fisher Tritrimeter was used to measure the electrical resistance (ohms). Readings were made one minute after the addition of the base or acid. The reciprocal of ohms. (conductivity) was used in plotting the curve.

In the preparation of 0.5N copper acetate solution about two mls. of concentrated acetic acid were added per liter to dissolve all traces of copper hydroxides. The pH of the solution was around 5.3 instead of 5.6.

Results and Discussion --

The first consideration of most plant nutrients in the soil is to determine the relationship between total and available nutrients. In Table 16 is shown the data for the total copper content of soil sampled from the Minor Element Plots at the Muck Experimental Farm. Plot 1 received no copper fertilization; Plot 3 received 50 pounds of $CuSO_4$ per acre in 1942; Plot 2, received 300 pounds per acre in 1942; and Plot 4 received annually 50 pounds per acre of copper sulfate from 1942 to 1946, inclusive, or a total of 250 pounds. It is evident that the soil analysis of the 0 - 8" depth correlates with the treatment. The content of copper in soil sampled from Plot 3 was nearly treble the content of copper in soil from the control treatment. Analysis of the 24" - 30" soil depth indicates only slight accumulation of copper in the lower horizons. Since practically all the copper applied to the soil was in the 0 - 8" depth, it can be concluded that leaching was not a great factor in muck soils. The figures in the right column illustrate this fact. Assuming that the 0 - 8" depth weighed 500,000 pounds of oven dry soil--a figure which is nearly correct by actual measurements--the copper sulfate equivalent in Plot 3 was 76 pounds. The difference between the amount in Plot 1 and Plot 3 is 48 pounds, which practically accounts for all the original application even though it had been applied five years before soil sampling. Similar comparison of Plot 2 showed that a total of 232 pounds of the original 300 pound application was still in the topsoil. Plot 4 showed that approximately 212 pounds remained from a total application of 250 pounds.

Attempts were made to differentiate between available and total copper from soil sampled from the Minor Element Plots. The data in Table 17 show some of these results.

Wood (64) suggested the use of boiling 1N nitric acid for extracting from the soil the copper considered available for plants. This procedure was not suitable for organic soils because of the extreme frothing. As shown in the Table, leaching the soil with 400 mls. of cold nitric acid extracted out a major portion of the total copper. The copper utilized yearly by crops grown on the control treatment generally amounts to less than 0.03 lbs. per acre. The copper extracted by nitric acid from Plot 1 was equivalent to 4 lbs. per acre. This was nearly 100 times as great as the amount of copper consumed annually by plants, which shows that the method does not measure readily available copper. When hydrochloric acid was used instead of nitric acid about the same amount was extracted. The use of these strong acids for extracting copper may serve to indicate the total copper in muck.

Leaching the soil with 400 mls. of neutral ammonium acetate indicated no copper removal as the copper impurities in the blank solution were greater than the leachings from the soil. Using the same ammonium acetate with the pH adjusted to 4.5 by concentrated acetic acid likewise failed to extract any appreciable amounts of copper. Electrodialysis of the 20 grams of moist soil produced results somewhat in agreement with plant utilization, but not the treatment. Because of the variable and inconsistent results, the method was not satisfactory. The values which were obtained suggest that it measured soluble copper and not exchangeable

TABLE 16. Fixation of copper in muck soil (pH 6.1). Samples collected in the Fall of 1946 from Minor Element Series.

Treatment	Total Copper Content p.p.m.		$CuSO_4$ Equivalent in Topsoil* lbs./acre
	Top 0-8"	Subsoil 24"-30"	
Plot 1 Control	14	8	28
Plot 3, 50 lbs./A. $CuSO_4$ in 1942 only	38	9	76
Plot 2, 300 lbs./A. $CuSO_4$ in 1942 only	130	13	260
Plot 4, 50 lbs./A. $CuSO_4$ annually 1942-46	120	10	240

*Calculated on basis of 500,000 lbs./acre of dried soil.

TABLE 17. Solubility of copper (p.p.m. of soil).

Treatment	Total	Cold 1N HNO_3	1N NH_4AcO	Electrodialysis*
Plot 1	14	8	None	2
Plot 3	38	29	"	5
Plot 2	130	104	"	9
Plot 4	120	92	"	7

*Results of duplicate determinations were not reproducible.

copper.

It can be concluded from the results shown in Table 17 that copper in slightly acid soil does not exist in the exchange complex. When copper as copper sulfate is added to the soil under field conditions it probably changes to the oxide and hydroxide form. When combined as an oxide, copper is easily dissolved by strong acids.

To determine the reactions of copper sulfate under laboratory conditions, twenty grams of moist soil (7.1 gms. dried) were suspended in 100 mls. of water containing copper sulfate. The data in Table 18 show the amounts of copper in solution three hours after equilibria had been established with the soil. When the copper was added at levels equivalent to 10, 100, and 1000 p.p.m., by dry soil weight, the amounts of copper not sorbed were 2.9, 0.9, and 0.4 per cent, respectively. After removal of the copper not sorbed, the soil was leached with 400 mls. of neutral normal ammonium acetate solution. The results show that increasing the amount of copper sulfate increased the water-soluble and exchangeable copper, but decreased the per cent recovery.

When it became evident that pH affects the reaction of copper in the soil, two experiments were set up. One consisted of soil samples which were given only a small amount of copper. In the second experiment the soil was treated with excess copper sulfate (about 5 times the exchange capacity). The methods used in this experiment were des-

TABLE 18.- Sorption of copper by muck

Mgms. of Copper Applied	Copper Not Sorbed		Cu Extracted by 400 mls. of Neutral NH_4AcO	
	Total Mgms.	Per cent Recovery	Total Mgms.	Per cent Recovery
Control*	None	---	None	---
0.07 (10 p.p.m.)	0.002	2.9	0.003	4.3
0.70 (100 p.p.m.)	0.006	0.9	0.022	3.1
7.00 (1000 p.p.m.)	0.030	0.4	0.206	2.9

*Soil contained about 0.1 mgm. of copper.

TABLE 19. Sorption of copper by 20 gms. of moist soil at various pH levels. Ex. capacity about 8.9 M.E. in sample.

pH	Mgms. of Copper Not Sorbed on Addition of 2.0 mgms. of $CuSO_4$	M.E. of Copper Sorbed from 100 mls. of 0.5N $CuSO_4$
3.0	0.012	5.3
3.3	0.011	6.3
3.7	0.011	6.6
4.0	0.011	7.3
4.3	0.009	7.5
4.7	0.009	10.6
5.0	0.008	$Cu(OH)_2$ ppt.
5.3	0.008	"
5.8	0.006	"
6.1	0.006	"
6.4	0.007	"
7.2	0.006	"

cribed in the procedure. The data are shown in Table 19. The amount of copper not adsorbed (water soluble) doubled from neutrality to the very acid soil. The results are in agreement with the statement that copper solubility increases with greater acidity.

The soil which received excess copper sulfate showed low adsorption at extremely acid levels. Attempts to adjust the pH of the suspension above 4.7 obviously showed that all the copper hydroxide precipitated before the adjustment point could be reached.

The high amount of copper sorption at pH 4.7 indicates that there possibly was some copper hydroxide precipitated at this point. It can be concluded that copper goes into the exchange reaction in soils more acid than pH 4.7. In soils less acid than pH 4.7, the reaction of copper sulfate most probably would be a precipitation of copper as the hydroxide as well as some exchange reactions.

In order to study the relative ease of adsorption of copper to hydrogen, two systems of humus were prepared. One soil was saturated with hydrogen ions by leaching a muck with dilute hydrochloric acid until all the bases were removed, and then leaching out the excess hydrochloric acid. The other soil system was a Cu-humus derived from an H-humus and prepared either from copper sulfate or copper acetate. The Cu-humus was prepared by slowly leaching the soil with 300-400 mls. of <u>0.5N</u> copper solution and then leaching out the excess copper with CO_2^- free distilled water. The use of alcohol was not found to be satisfactory because of the precipitation of some of the copper in solution. To investigate equilibrium reactions a given weight of humus was suspended in water and brought up to a volume of 200 mls., after the addition of either the acid or the copper salt. The suspension, after standing for several hours with occasional stirring, was filtered. The adsorbed copper was measured by leaching the soil with excess 0.2N hydrochloric acid.

In Table 20 are given values for displaced copper after equilibrium had been established between the humus and a definite amount of hydrochloric acid. Since the exchange capacity of the soil is about 8.9 M.E., the equilibrium on addition of 10.0 M.E. of hydrogen is approximately the reaction at 1.1 symmetry concentration. The per cent replacement of copper by hydrogen was similar for copper-humus derived either from copper sulfate or copper acetate, even though the total amount replaced was considerably different. There appeared to be little difference for either salt at the different concentration levels. Since HCl equal to 1.1 times the symmetry value released approximately two-thirds of the total sorbed copper, the ratio of copper to hydrogen in the solution was 2 to 1. The following equations illustrate possible reactions at a state of equilibrium which would account for the values obtained:

(1) $Cu_3\text{-humus} + 6H^+ = 2Cu^{++} + (H_4Cu)\text{-humus} + 2H^+$

(2) $3Cu(x)\text{-humus} + 3H^+ = 2Cu(x)^+ + (Cu(x)H_2)\text{-humus} + H^+$

Nearly 90 per cent of the copper was replaced from the colloid on addition of 40 M.E. of hydrochloric acid which suggests that hydrogen can effectively replace adsorbed copper on organic soils. An interesting difference in the two copper salts is shown in Table 20. The total copper adsorbed by soil from copper sulfate amounted to about 6.0 M.E., while the value for copper acetate averaged 13.5 M.E. Since 6.0 M.E. is less than the exchange capacity of 8.9 M.E., it is possible that most of the copper sorbed was as the divalent copper. The difference between the two values is mostly the amount of exchangeable hydrogen. The large amount of exchangeable hydrogen was caused by the strongly acidic properties of copper sulfate. The chemical reaction of copper acetate is quite different from copper sulfate. First fact noticed is that the total sorbed is greater than the exchange capacity of the soil; however, it has not doubled in value, which Bower and Truog (12) were able to show. One also can see that the reactions may not be stoichiometric for the addition of 2.5 and 5.0 M.E. of H^+ released 2.66 and 5.04 M.E., respectively, of copper which is an efficiency greater than 100. Obviously each adsorbed copper cation is not neutralizing two charges on the colloid, but is acting as a monovalent cation with an acetate or hydroxyl anion neutralizing one of the two copper valences.

In Table 21 are shown the data for the amounts of copper sorbed by H-humus on addition of copper sulfate or copper acetate to the suspension. The values for copper sulfate are much in agreement with those obtained in Table 20. For example, 31 per cent of the copper was sorbed by the soil on addition of 10.0 M.E. of copper, or 32 per cent remained sorbed on the addition of 10.0 M.E. of hydrogen ions to the Cu-humus. In the case of the addition of copper acetate to the acid humus, entirely different values are obtained. At approximately one symmetry concentration (10 M.E.) 90 per cent of the copper was sorbed. Additions higher than one symmetry concentration naturally had low percentage sorption values because the soil was nearly saturated with copper. This behavior of copper acetate made possible its use for measuring the exchange capacity of soils (52). The formation of acetic acid which has a low dissociation value permitted the copper to replace a greater proportion of hydrogen on the colloid than was possible with copper sulfate, which formed some sulfuric acid by hydrolysis.

The same soil used for data shown in Tables 19, 20, and 21 was used to further study the reactions of copper as determined by conductivity methods. It was believed that this method could help one differentiate the various proportions and types of adsorbed copper on the colloid. Determination of adsorbed ammonium by the standard ammonium acetate method (5) showed that the exchange capacity of the soil sample was approximately 8.9 M.E. The exchange capacity as determined by the Titrimetric method indicated that the value was around 10.3 to 10.6 M.E. when a H-humus was titrated with 0.48N NaOH. The difference in values for the two methods made it difficult to decide which method was preferable for evaluating exchange capacity. The value of 8.9 M.E. was the most suitable for interpretating Figures 12 and 13.

The principle upon which a titrimetric measurement is based is that, as each colloidal system becomes saturated with a different cation, one would detect a change in the electrical conductivity of the suspension. In a simple colloidal system, as for example a Ca-humus titrated with HCl, there are only two breaks in a titrimetric curve. The first represents the replacement of calcium by hydrogen on the colloid, and the second measures the effect of increasing concentrations of HCl on conductivity. In the case of a Cu-humus, the system is much more complex. The curves shown in figure 12 well illustrate this fact. They are the results of titrating with NaOH, a Cu-humus derived from a H-humus either by leaching with an excess of <u>0.5N</u> copper sulfate or with copper acetate. The dots on the figure are actual measurements. The straight lines are lines considered to best fit the curve. At each intersection of the lines the milliequivalent value is shown.

Any interpretation of Figure 13 must coincide with other known facts. For example in Table 20 the data showed that the total M.E. of copper sorbed by the soil when derived from $CuSO_4$ and CuAcO was 6.0 and 13.5 M.E., respectively. The exchange capacity of the soil was about 8.9 M.E. In addition to these facts, the milliequivalent values shown

TABLE 20. Replacement of copper from a Cu-humus at different concentrations of hydrochloric acid in a soil-water suspension. Ex. Cap. = 8.9 M.E. in samples.

M.E. HCl^+ Added	Soil Saturated with $CuSO_4$ pH 3.2			Soil Saturated with CuAcO pH 5.3		
	M.E. Cu^{++} Replaced	Total M.E. Sorbed	Per Cent Replaced	M.E. Cu^{++} Replaced	Total M.E. Sorbed	Per Cent Replaced
2.5	1.80	5.74	31	2.66	13.0	20
5.0	2.61	5.65	46	5.04	13.7	37
7.5	3.76	6.08	62	7.30	14.2	51
10.0	4.33	6.40	68	8.84	13.2	67
20.0	4.71	5.78	81	11.20	14.1	79
40.0	5.42	6.22	87	11.20	12.6	89
	Av.=	6.0		Av.=	13.5	

TABLE 21. Replacement of hydrogen from an H-humus by addition of varying amounts of cupric ions.

M.E. Cu^{++} Added	Added as $CuSO_4$			Added as Copper Acetate		
	pH at Equilibrium	M.E. Copper Sorbed	Per Cent Sorbed	pH at Equilibrium	M.E. Copper Sorbed	Per Cent Sorbed
2.5	2.6	1.87	75	3.1	2.48	99
5.0	2.5	2.44	49	3.35	4.75	95
7.5	2.45	2.67	36	3.6	6.82	91
10.0	2.4	3.08	31	3.85	9.04	90
20.0	2.35	3.64	18	4.2	10.6	53
40.0	2.3	4.59	11	4.5	11.1	28

in Figure 12 must likewise coincide with the values shown in Figure 13. The curve for copper acetate shows that the final equivalent point is approximately 13.3, which is similar to the total milliequivalents of adsorbed copper. If the copper is adsorbed as $Cu(OH)^{+}$, which was suggested by Bower and Truog (12), no reasonable explanation could account for all these breaks in the curve nor could the total milliequivalents be greater than the exchange capacity of the soil, providing one assumes that copper hydroxide is the end product. For example, if a soil system had 4 M.E. of Cu^{++}, and 5 M.E. of $Cu(OH)^{+}$, the reactions would be:

(1) $(2Cu \cdot 5CuOH)(humus + 2NaOH) = (2Na \cdot 7CuOH)\text{-}humus.$

(2) $(2Na \cdot 7Cu \cdot OH)\ humus + 7NaOH = (9Na)\text{-}humus + 7Cu(OH)_2.$

In such a reaction it would take 9 M.E. of Na^{+} to replace 14 M.E. of Cu^{++} on the colloid. One then could expect equivalent points at 2.0 and 9.0 M.E. The latter would be identical with the soil exchange capacity.

The points shown in Figure 13 substantiate the belief that copper does not exist as $Cu(OH)^{+}$. There was only one obvious equivalent point which is 9.0 M.E., a point nearly identical to the exchange capacity of the soil. In order to account for the 13.5 M.E. of copper known to be adsorbed, it would have to be combined with an acidic compound such as $(CuAcO)^{+}$ instead of the hydroxyl group.

The curve for the NaOH titration of Cu-humus appears to show that a double reaction is taking place since the point 4.1 is approximately mid-distant from 8.3. The third reaction

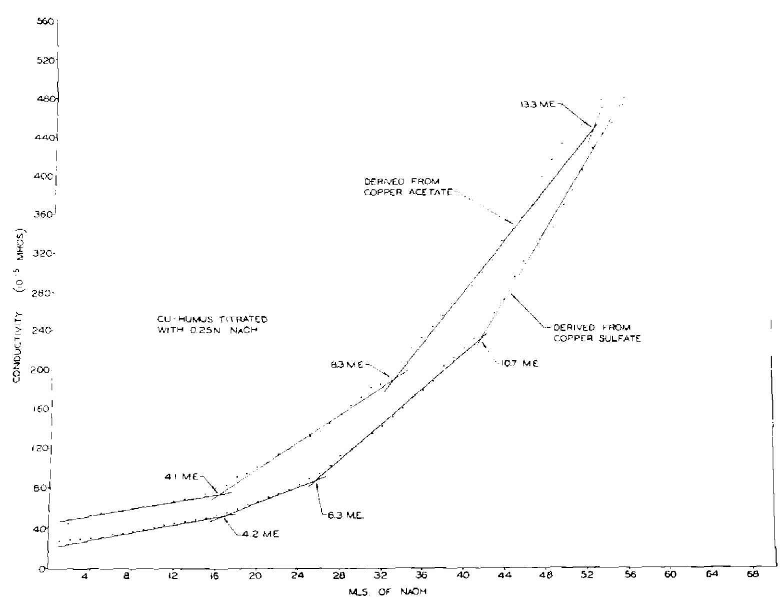

Fig. 12. Conductivity measurements of Cu-humus when titrated with NaOH.

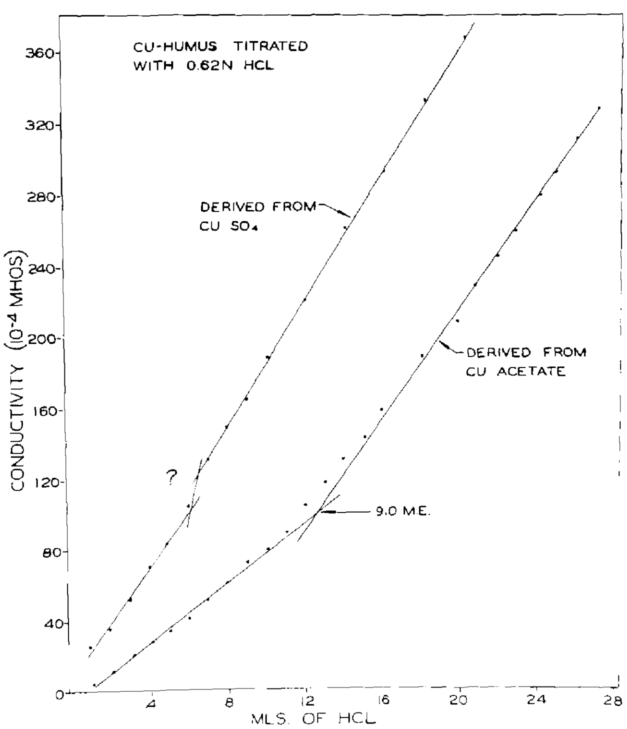

Fig. 13. Conductivity measurements of Cu-humus when titrated with HCl.

requires a replacement of 5.0, which is the difference between 13.3 and 8.3. The following reactions are proposed to fit these conditions:

(1) $(2.5Cu \cdot 4.15CuAcO)$-humus + $4.15NaOH$ = $(2.5Cu \cdot 4.15Na)$-humus + $4.15Cu(AcO \cdot OH)$.

(2) $4.15Cu(AcO \cdot OH)$ + $4.15NaOH$ = $\underline{4.15Cu(OH)}_2$ + $4.15Na^+$ + $4.15AcO^-$.

(3) $(2.5Cu \cdot 4.15Na)$-humus + $5NaOH$ = $(9.15Na)$-humus + $\underline{2.5Cu(OH)}_2$

It can be observed that it required 13.3 M.E. of Na^+ to replace and precipitate the adsorbed copper. The exchangeable copper was, likewise, 13.3 M.E. The actual amount of Na^+ on the colloid amounted to 9.15 M.E. It was first thought that some exchangeable hydrogen was still on the humus-complex. Apparently there is very little hydrogen, for it could not be accounted for on any portion of the curve. The ease of replacement of exchangeable hydrogen by copper acetate, as shown in Table 21, would tend to confirm this fact. Furthermore, the hydrogen ion concentration of water at pH 5.3 amounts to less than 0.01 M.E. per liter.

Using the Cu-humus complex proposed in the sodium hydroxide reaction, there would then have to be two reactions when the complex is titrated with hydrochloric acid. The following equations illustrate these reactions:

(1) $(2.5Cu \cdot 4.15CuAcO)$-humus + $4.15HCl$ = $(2.5Cu \cdot 4.15H)$-humus + $4.15Cu^{++}$ + $4.15AcO^-$ + $4.15Cl^-$.

(2) $(2.5Cu \cdot 4.15H)$-humus + $5HCl$ = $(9.15H)$-humus + $2.5Cu^{++}$ + $5Cl^-$.

The primary difference in the reactions with H^+ and Na^+ is that copper remains ionized in acid solutions, and is precipitated in alkaline solutions. One also notices that the addition of 4.15 M.E. of H^+ releases 8.3 M.E. of Cu^{++}. The total of 9.15 M.E. of H^+ required for the two reactions is considered to be in good agreement with the 9.0 point shown in Figure 13. If there is a break in the curve between the first and second reaction in the acid titration, it could not be detected. This is not surprising since the conductivity of Cu(AcO•Cl) would be very similar to $CuCl_2$. Both compounds would be highly dissociated.

The reactions for Cu-humus derived from copper sulfate would be different than the colloid from copper acetate in that a considerable amount of hydrogen is adsorbed. The first reaction of NaOH would be to replace any hydrogen. This required 4.2 M.E. The difference between 4.2 and 10.7 represents the exchange and precipitation of Cu^{++} and $(CuSO_4)^+$. The titration of the colloid with hydrochloric acid would only show the reactions with the adsorbed copper. In Figure 13 is shown the curve for this reaction. Because of the peculiarity of the titration curve, no definite intersection could be prescribed for the Cu-humus derived from copper sulfate.

If further study of copper reactions on the soil were attempted, pH should be determined along with the conductivity measurements. To prove the existence of $(CuSO_4)^+$, the amount of $SO_4^=$ should be determined. A titrimetric study of the salt also should be run. The determination of these reactions would help to interpret the data shown in Figures 12 and 13.

In order to study the effect of soil reaction on the benefit of copper, several greenhouse experiments were conducted. Two of the experiments were with alkaline mucks obtained from different areas. The alkalinity in each case had resulted from burning the surface muck in the past. No naturally alkaline soil was obtained.

Spinach and Sudan grass were grown on the two alkaline mucks to test for response to copper. Copper did not increase the growth of Sudan grass, but increased the yield of spinach 20 per cent on one of the soils. Data in Tables 22 and 23 show the effect of the addition of minor elements on these two soils on the growth of spinach. These results are in agreement with the observations of Harmer (25).

In order to study the effect of pH on copper availability, oats were grown on a very acid virgin muck. The copper content of the dried soil was 8 p.p.m. The pH was varied by mixing different amounts of limestone in the soil. After the plants were up, manganese sulfate was applied in solution at the rate of 100 pounds per acre to the soils with pH values higher than 5.2.

In Figure 14 are shown the yields for oats when grown on muck with the following treatments: (a) without any copper treatment, (b) copper sprayed on the plants, (c) copper sulfate applied to the soil. In the spray trial, copper sulfate in a 1-4000 dilution was applied with a small hand sprayer.

TABLE 22. Spinach grown on alkaline muck from Muck Expt. Farm expressed as gms. of fresh material per jar.

Treatment lbs./acre	Replication A	B	C	Average
Control	101	82	108	97
Manganese (300)	106	107	115	109
Zinc (100)	89	92	96	92
Copper (100)	109	132	127	123
Cu + Mn	122	104	116	114
Cu + Mn + Zn	112	109	132	118

TABLE 23. Spinach grown on alkaline muck obtained from Old Experimental Plots. Expressed as gms. of fresh material per jar.

Treatment lbs./acre	Replication A	B	C	Average
Control	86	77	82	82
Manganese (300)	98	79	84	87
Zinc (100)	79	94	91	86
Copper (100)	91	92	95	93
Cu + Mn	92	85	80	86
Cu + Mn + Zn	87	94	82	88

To guard against copper falling on the soil, the jars were placed horizontally.

Irrespective of the pH, the copper benefited the crop; however, the response to this nutrient increased sharply as the soil became more acid than pH 5.2. As the acidity decreased from pH 5.2, the difference in yield increased between the plants which received copper and those which did not receive copper. The sprayed plants would not compare with plants grown on copper treated soil because of the delay in applying the spray.

There is no definite explanation of the curve for the growth of oats on the soil which did not receive copper. Piper (46) reported that copper was more available as the soil became more acid. In a study of minor elements on mineral soils at various pH values, Peech (45) showed that the availability of copper increased as the soil became more acid. In the light of these investigations, it is believed that the portion of the curve at pH values above 5.2 represents the relationship between soil acidity and the availability of copper. These results are in agreement with data formerly discussed and presented in Table 2. They showed that liming an acid soil (pH 6.1) did not decrease the response of spinach to copper. Actually there was an increase in response since the yield of spinach grown on soil which received manganese and lime was lower than the yield of plants grown on the control treatment. Similar values for Sudan grass (Table 3) showed a greater response to copper fertilization when grown on alkaline muck due to liming. With Sudan grass copper fertilization increased the yield from 13.8 to 30.1 grams on the acid soil, and from 1.5 to 22.8 grams on the limed soil.

The reason that the response to copper did not continue to decrease as the acidity increased is probably due to factors other than availability. It was observed that there was poor root development of oats on all treatments on the very acid soil. It was first believed that the poor root development restricted the feeding capacity of the plant for obtaining the limited supply of available copper. It is now believed that this is not the only factor. It is suspected that an unbalance exists in the plants between copper and some unknown element. In Part I it was shown that oats and barley grown on a very acid soil had a moderate content of copper even though extreme copper deficiency existed. If the problem of copper is one of balance, the question then is, what element or elements are readily being absorbed by plants in very acid soils? It has been shown that zinc (57) and manganese (24,50) are absorbed in greater amounts as acidity increases. It is also a well known fact that soluble iron and aluminum increase quite appreciably if the pH of the soil falls below 5.0. Abbot, Conner, and Smalley (1) have reported that an unproductive acid sandy loam high in organic matter was caused by a high content of soluble aluminum. In nutrient solutions, Liebig, et al. (23) have reported a relationship existing between aluminum and

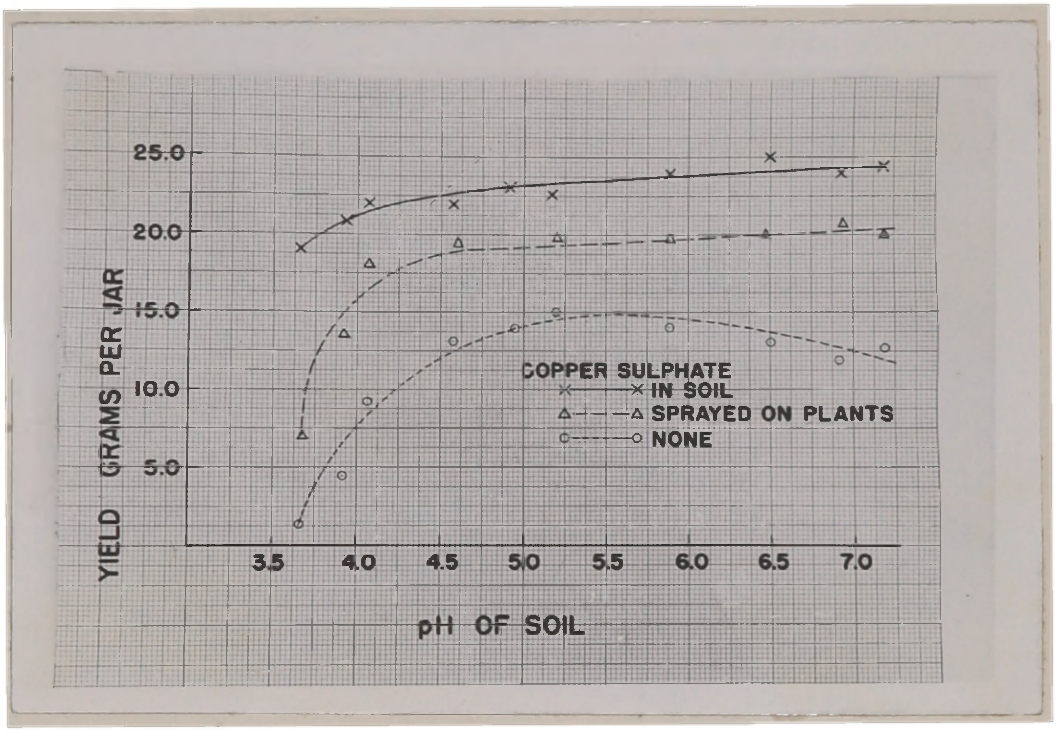

Fig. 14. Yield of oats at different pH values as affected by copper. Grown on an acid muck which received various amounts of limestone.

copper availability. These facts indicate that in very acid soils plants absorb certain other elements relatively more rapidly than they do copper. For this reason the role of copper in plants is to balance oxidation-reduction processes, then this would also imply there would need to be greater amounts of copper. The data in Table 24 show the copper content of the soils studied, their pH values and the response of plants to copper fertilization. In the soil collected from the Brown Farm, little difference was observed in pH; yet, the copper content varied considerably, depending on the distance from the edge of the muck area that the sample was collected. From the data on plant response to copper it appears that on slightly acid soils a copper content of 40 to 50 p.p.m. would be sufficient for most crops. Once the copper content has been brought up to this level, additional applications of this element as a nutrient would not show any growth response, since it has been shown that copper does not leach out very readily. This fact probably accounts for the failure of Comin (14) and Ellis (17) to obtain any response to the use of copper sulfate as a plant-nutrient in their experiments.

The need for copper and manganese as minor elements on organic soils is based upon an entirely different chemical problem. Manganese deficiency can be corrected by proper adjustment of the soil acidity. If the acidity is not corrected, manganese may be an annual problem in spite of large accumulations in the soil. The availability of manganese (24,50) is

TABLE 24. Copper content of some mucks. Oven-dried basis.

Location	pH	Plant Response to Copper Addition	Total Copper Content p.p.m.
Minor Element Series*	6.1		
Plot 1		Good	14
Plot 3		None	38
Plot 4		None	120
Plot 2		None	130
Muck Near Lansing Airport	3.65	Good	9
Muck-North Judson, Indiana	4.3	Good	10
Alkaline Muck (Old Plots)	7.5	None	33
Burned Muck (N. End of Expt. Farm)	6.8	Slight	26
Edge of Brown Farm, 27% ash	6.7	N.D.	25
200 yds. out-Brown Farm, 19% ash	6.8	N.D.	19
400 yds. out-Brown Farm, 18% ash	6.9	N.D.	12

*Crop response from unpublished data by Harmer (25).

related to the amount of reduced manganese in the soil. The chemistry of copper is definitely related to pH, but not to the same extent as manganese. The problem is not one of chemical form, but mostly one of quantity in the soil. Nearly all virgin mucks are low or deficient in available copper. There would be little need for annual applications of copper to a muck soil after one large application has been applied.

Harmer (23) reported that the copper oxide content for 10 samples of virgin muck varied between 11 and 21 p.p.m. There appeared to be little correlation between pH and copper content. Analyses of muck collected from different areas at the Muck Experiment Station, likewise, showed no correlation. A high copper content of 26 p.p.m. was found on a burned muck. Samples from areas not known to be burned contained around 15 p.p.m. The burning of the topsoil presumably had increased the copper content. An analysis of some ash clods collected in the same area confirmed this supposition, for they contained 55 p.p.m. of copper.

The beneficial effects of burning of a very acid muck might not all be attributed to a decrease in acidity. Where copper deficiency existed and was not corrected, the burning could increase the copper content. For example, a soil contained only 4 pounds of copper per acre in each six inch depth of muck. If the soil were burned so as to lower the level three feet, the copper content they would be approximately 24 pounds per acre in the topsoil.

SUMMARY

A study of the physical and chemical reactions of copper in organic soils and on the effect of this cation, alone and with manganese and zinc, on the absorption of these elements by crops, and the subsequent effect of these elements on the production of carotene, ascorbic acid, and protein in plants has resulted in the following conclusions:

1. The application of copper considerably increased the yield of most of the crops studied, increased the copper content of different crops from 50 to 200 per cent, and sometimes decreased the manganese and zinc content of the crops. The amount of increase in copper content varied with different crops, but an increase was not always attended by more growth. A low of 6 p.p.m. in the 15 crops investigated generally was indicative of copper deficiency, but the grain crops and spinach showed a somewhat higher content in the deficient plants.

2. The application of zinc when copper was applied increased the yields of onions and Sudan grass, but did not affect those of spinach and tomatoes. The copper and manganese contents were sometimes slightly decreased while the zinc content was generally increased. On copper deficient soils, zinc slightly depressed the yields of onions. spinach, and Sudan grass. With these crops, zinc appeared to accentuate a copper deficiency.

3. The marked response to copper on the acid mucks

studied was in some cases not attended by any response to other minor elements, including manganese, zinc, boron or magnesium, applied either alone or in combination.

4. In greenhouse trials the application of copper to deficient mucks increased the ascorbic acid content of barley, oats and fall-grown spinach but did not affect the ascorbic acid content of onions, spring-grown spinach, and tomato fruit. In field-grown crops, onions, spinach and wheat showed no little differences in ascorbic acid content.

5. In greenhouse trials, the application of copper increased the carotene content of wheat, spinach, barley, carrots, and oats.

6. The protein content of plants deficient in copper was found to be abnormally high. This would indicate that copper does not aid in the formation of proteins.

7. Applications of copper sulfate to organic soils were found to be held rather tenaciously in the zone of placement. Soils collected five years after a 50 pound per acre application showed that approximately an equivalent of 48 pounds of copper sulfate remained in the upper eight inches of soil. From an original application of 300 pounds per acre of copper sulfate, 232 pounds were found to be in the top soil.

8. A large portion of the copper in organic soils could be extracted with normal nitric or hydrochloric acid, but very little with ammonium acetate.

9. Copper sulfate was precipitated probably as the hydroxide, when the pH of the soil-water suspension was

greater than 4.7.

10. Studies of equilibrium reactions of copper and hydrogen in muck soils showed that the ratio of adsorbed cations at symmetry concentration was approximately two equivalents of hydrogen to one equivalent of copper.

11. Saturating an H-humus with copper by leaching with copper acetate showed that there was no simple stoichiometric reaction. Titrimetric studies indicate that the copper was adsorbed as the divalent cation Cu^{++}, and the monovalent cation complex $(CuAcO)^{+}$.

12. The need for copper by plants is related to the copper content and pH of the soil, and possibly to nutrient balances within the plant. It is believed that the marked response of plants to copper on very acid soils is due to restricted plant-root development and possibly to an unbalance of nutrients within the plant. The decrease in plant growth on soils made alkaline by liming is believed due to a decrease in available copper in the soil. Burning of muck soils increases the copper content of the topsoil.

LITERATURE CITED

1. Abbott, J. B., Conner, A. D., and Smalley, H. R. The reclamation of an unproductive soil of the Kankakee Marsh region. Purdue Un. Agr. Exp. Sta. Bul. 170, 27:329-374, 1913.

2. Allison, R. V. Studies upon the role of special elements in plant development grown on peat and muck soil of the Everglades. Fla. Agr. Sta. Ann. Rpt. pp. 159-160, 1931.

3. ______________, Bryan, O. C., and Hunter, J. H. The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulfate and other chemicals. Fla. Agr. Exp. Sta. Bul. 190:33-80, 1927.

4. Anonymous Fertilizer recommendations. Soil Science Staff. Mich. Sta. Col. Ext. Bul. 159 (Revised), 1946.

5. Ass. of Official Agr. Chem. Official and tentative methods of analysis. (Ed. 5 pp. 14), (Ed. 4 pp. 25, (Ed. 5 pp. 122). Banta Press, Menasha, Wis. 1935, 1940, 1945.

6. Bailey, L. F., and McHargue, J. S. Effects of boron, copper, manganese, and zinc, on the enzyme activity of the tomato, and alfalfa plants grown in the greenhouse. Plant Physiol. 19:105-116, 1944.

7. Barnes, W. C. Effect of some environmental factors on growth and color of carrots. Cornell Un. Agr. Sta. Memoir 186, 1936.

8. Beck, A. B. A survey of copper content of Western Australian pastures. J. Dept. Agr., W. Australia 18:285-300, 1941.

9. Beeson, K. C. The mineral composition of crops with particular reference to the soil in which they were grown. A review and compilation. U.S.D.A. Misc. Pub. 369, 1941.

10. Benne, E. J., Wolman, W., Hibbard, R. P., and Miller, E. J. A comparison of the Petering-Wolman-Hibbard procedure for determining carotene and two modifications thereof with the Peterson-Hughes-Freeman Technic. Jour. Ass. of Official Agr. Chem. 23:709-716, 1940.

11. Bernstein, L., Hamner, K. C., and Parke, R. O. The influence of mineral nutrition, soil fertility, and climate on carotene and ascorbic acid content of turnip greens. Plant Physiol. 20:540-571, 1945.

12. Bower, C. A., and Truog, E. Base exchange capacity determinations as influenced by nature of cation employed and formation of basic exchange salts. Soil Sci. Amer. Proc. 5:86-89, 1940.

13. Chapman, H. D., Leibig, G. F., and Vanselow, A. P. Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus. Soil Sci. Soc. Amer. Proc. 4:196-200, 1939.

14. Comin, D. The response of some muck crops to the application of minor elements. Ohio Sta. Bimo. Bul. 228, pp. 144-147, 1944.

15. Cowling, H., and Miller, E. J. Determination of small amounts of zinc in plant material. Ind. and Eng. Chem. Anal. Ed. 13:145-149, 1941.

16. Elgabaly, M. M., and Jenny H. Cation and anion interchange with zinc montmorillonite clays. Jour. Phys. Chem. 47:399-408, 1943.

17. Ellis, K. Unpublished data from Purdue Muck Expt. Farm.

18. Elvehjem, C. A. The biological significance of copper and its relation to iron metabolism. Phys. Review 15:471-507, 1935.

19. ________________, Hart, E. B. The necessity of copper as a supplement to iron for hemoglobin formation in the pig. Jour. Biol. Chem. 95:363-370, 1932.

20. Felix, E. A.
Correction of unproductive muck by the addition of copper. Phytopath 17:44, 1927.

21. Floyd, B. F.
Die-back or "Exanthema" of citrus trees. Fla. Agr. Exp. Sta. Bul. 140, 1-31, 1917.

22. Grossenbacher, J. G.
Some bark diseases of citrus trees in Florida. Phytopath 6:29-50, 1916.

23. Harmer, P. M.
The muck soils of Michigan, their management and uses. Mich. Agr. Exp. Sta. Spec. Bul. 314, 1941.

24. ________________
The occurrence and correction of unproductive alkaline organic soils. Soil Sci. Soc. Amer. Proc. 7:378-386, 1943.

25. ________________
Studies on the yield of copper sulfate applied to organic soil on the yield and quality of several crops. Soil Sci. Soc Amer. Proc. 10: 284-294, 1945.

26. Hester, J. B.
Influence of soil fertility upon the quality of tomatoes. Soil Sci. Soc. Amer. Proc. 6:243-245, 1942.

27. Jamison, V. C.
The effect of particle size of copper and zinc source material and of excessive phosphates upon the solubility of copper and zinc in a Norfolk sand. Soil Sci. Soc. Amer. Proc. 8: 323-326, 1943.

28. Jones, H. E., and Scarseth, G. D.
The calcium-boron balance in plants as related to boron needs. Soil Sci. 57:15-24, 1944.

29. Keilin, D. and Mann, T.
Polyphenol oxidase. Purification, nature, and properties. Proc. Roy. Soc. Lond. B 124:187-203, 1938.

30. ________________
Laccase, a blue copper protein oxidase from the latex of Rhus Sucedanea. Nature 143:23-24, 1939.

31. Knott, J. E.
The effect of certain mineral elements on the color and thickness of onion scales. Cornell Un. Agr. Exp. Sta. Bul. 552, 1933.

32. Kubowitz, F.
Über die chemische Zusammensetzung der Kartoffelozydase. Biochem. Ztschr. 292:221-229, 1937.

33. Liebig, G. F., Vanselow, A. P., and Chapman, H. D.
Effects of aluminum on copper toxicity as revealed by solution culture and spectrographic studies of citrus. Soil Sci. 53: 341-351, 1942.

34. Loustalot, A. J., Burrows, F. W., Gilbert, S. G., and Nason, A.
Effect of copper and zinc deficiency on the photosynthatic activity of the foliage of young tung trees. Plant Physiol. 20:283-288, 1945.

35. Lovett-Janison, P. L., and Nelson, J. M.
Ascorbic acid oxidase from summer crookneck squash. Jour. Amer. Chem. Soc. 62: 1409-1412, 1940.

36. Lucas, E. H.
Determining ascorbic acid in large numbers of plant samples. Ind. Eng. Chem. 16:649-652, 1944.

37. Lucas, R. E.
The effect of the addition of sulfates of copper, zinc, and manganese on the absorption of these elements by plants grown on organic soils. Soil Sci. Soc. Amer. Proc. 10:269-274, 1945.

38. ____________, Scarseth, G. D., and Sieling, D. H.
Soil fertility level as it influences plant nutrient composition and consumption. Purdue Agr. Exp. Sta. Bul. 468, 1942.

39. Lyon, C. B., Beeson, K. C., and Ellis, G. H.
Effects of micronutrient deficiencies on growth and vitamin content of tomato. Bot. Gaz. 104: 495-514, 1943.

40. Mapson, L. W.
The influence of halides on the oxidation of ascorbic acid. Biochem. Jour. 35:1322-1333, 1941.

41. Meiklejohn, G. T., and Stewart, C. P.
Ascorbic acid oxidase from cucumber. Biochem. Jour. 35:755-760, 1941.

42. Neal, W. M.
Some effects of copper intake on cattle. Amer. Chem. Soc. Mtg. 103, 1942.

43. Orth, O. L., Wickwire, G. C., and Burge, W. E.
Copper in relation to chlorophyll and hemoglobin formation. Science 79:34, 1934.

44. Peech, M.
Availability of ions in light sandy soils as affected by soil reaction. Soil Sci. 51:473-486, 1941.

45. Piper, S. C.
Investigations of copper deficiency in plants. Jour. Agr. Sci. 32:143-178, 1942.

46. Rademacher, B.
Variation in content, course of assimilation, and uptake of copper by oat plants. Bodenkunde u. Pflanzenernähr. 19:80-108, 1940. Cited from Chem. Abst. 35, 3293.

47. Schultze, M. O.
Relation of copper to cytochrome oxidase and hematopoietric activity of the bone marrow of rats. Jour. Biol. Chem. 138:219-224, 1941.

48. ________________, and Simmons, S. J.
Use of radioactive copper in studies on nutritional anemia of rats. Jour. Biol. Chem. 142:97-106, 1942.

49. Scripture, P. N., and McHargue, J. S.
Effects of boron deficiency on the soluble nitrogen and carbohydrate content of alfalfa. Jour. Amer. Soc. Agron. 35:988-992, 1943.

50. Sherman, G. D.
Activation of iron in plants by manganese and other chemicals in a lime-induced chlorosis. Ph.D. Thesis, Mich. State College, 1940.

51. Sherman, H. C.
Chemistry of Food and Nutrition. 6th Ed. Macmillan Pub. Co., 1941.

52. Sieling, D. H.
Base exchange capacity determination of soils by means of a rapid colorimetric copper method. Jour. Amer. Soc. Agron. 33:24-36, 1941.

53. Skaptason, J. B.
Copper content of Long Island soils in relation to tuber rot of potatoes. Amer. Potato Jour. 17:88-92, 1940.

54. Sommer, Anna L.
Copper as an essential for plant growth. Plant Physiol. 6:339-345, 1931.

55. ________________
Copper and plant growth. Soil Sci. 60:71-79, 1945.

56. Sommer, I. I., and Shive, J. W.
The iron-manganese relation in plant metabolism. Plant Physiol. 17:582-602, 1942.

57. Staker, E. V., and Cummings, R. W.
The influence of zinc on the productivity of certain New York peat soils. Soil Sci. Soc. Amer. Proc. 6:206-214, 1943.

58. Svanberg, O., and Nydahl, F.
Kyl Lantbr. Akad. Tedshr. 80:457-480, 1941
Cited in Bibl. Minor Elements pp. 58, 1944.

59. Teakle, L. H.
Copper deficient soils in Western Australia. Jour. Aust. Inst. Agr. Sci. 8:70-72, 1942.

60. ______________, and Burvill, G. H.
Experiments with microelements for the growth of crops in Western Australia. Jour. Dept. Agr. W. Aust. 18:125-132, 1941.

61. ______________, and Stewart, A. M.
Response to copper as a fertilizer on certain soils in Western Australia. Jour. Aust. Inst. Agr. Sci. 5:50-53, 1939.

62. Willard, H. H., and Greathouse, L. H.
Colorimetric determination of manganese using periodic acid as the oxidizing agent. Jour. Amer. Chem. Soc. 39:2366-2377, 1917.

63. Willis, L. G., and Piland, J. H.
The function of copper in soils and its relation to the availability of iron and manganese. Jour. Agr. Res. 52:467-476, 1936.

64. Wood, L. K.
Copper studies with Oregon soils. Jour. Amer. Soc. Agron. 37:282-291, 1945.