SPECTROGRAPHIC DETERMINATION OF COPPER
IN WHEAT AND SOIL

by

WILLIAM BOONE BURGER

B. S., Washburn College, 1940

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1941

TABLE OF CONTENTS

INTRODUCTION

Within recent years the growing knowledge in the fields of
biology and chemistry has brought about a gradual recognition of
the importance of trace elements in plant and animal life. Ele-
ments such as copper, manganese, zinc, and boron appear to be
essential to plant life, and are generally referred to as trace
elements because of their extremely low concentration in the
plant tissue. Although copper was discovered in plants in 1899
by Frankforter (4), the exact role played by this element in
plant metabolism is not yet fully understood. Orth, Wickwire,
and Burge (11) thought that copper is necessary in the formation
of chlorophyll molecules.

A deficiency of copper in plants is one of the causes of the
phenomenon of "chlorosis", which is a yellowing of plant leaves
due to chlorophyll deficiency. Too great a concentration of
copper, however, has a poisoning effect. In certain localities
it has been found advantageous to add copper salts to the soil.
The Florida Everglades have recently been made agriculturally
productive in this manner. In other localities the problem is
to render ineffective the trace elements which are present in too
large quantities. If fields are to produce crops at a maximum
efficiency, the soil content of the minor elements must be con-
trolled by first analyzing the soil qualitatively and quanti-
tatively for minor elements and then adding the elements in which

it has been found deficient. Such analyses have been carried out by chemical methods, but these are both inaccurate and time consuming because of the minute amounts of the minor elements contained in a large amount of sample.

The spectrograph now offers great possibilities in making analyses of soil and plant material because it requires a minimum of sample, it gives good precision in the low concentration range, and when the method is once developed for a particular sample, it is extremely rapid. These advantages have been made possible mainly by two developments, the improvement of the reproducibility of discharge conditions and improvements in methods of measuring line intensities.

It is the purpose of this investigation to study the applicability of the spectrograph to the problem of analyzing plants and soils for copper. Since wheat is one of the most important crops in the state of Kansas from an economic point of view, it was chosen as the specimen for analysis. In addition to the wheat plants, soil samples were taken from the plots on which the wheat was grown, and the fertilizers which had been used on the soil were analyzed for copper.

Several types of discharges which are possible means of exciting atoms of the sample are the uncondensed spark, the direct current condensed spark, the high frequency spark, the high voltage condensed spark, and the direct current carbon arc. This investigation has been limited to the direct current carbon arc because this method of excitation seemed most feasible for use with plant and soil samples. The main difference between the arc

and the spark is that ions in the former are produced by therm-
ionic emission whereas in the latter, they are formed by the
strong electrostatic field. Since arc sources are more sensitive
than spark sources, they are desirable when a large amount of
sample is available for analysis of traces of material.

SURVEY OF THE LITERATURE

The application of the spectrograph to the analysis of ma-
terials for trace elements is a comparatively new process, and
few investigators have used this method to analyze soils and
plants. Milbourn (10) reported qualitative and semi-quantitative
results in analyzing peas for boron and manganese. He also ana-
lyzed soil and fertilizers for these elements. Milbourn's method
was to place 0.01 gm. of the material, dried and ground, on the
negative electrode of a graphite arc. The length of this arc was
maintained at 1 mm. and the current at 7.5 amperes. The photo-
graphic plate was exposed for two minutes from the time of
striking the arc. This arc was placed 22 cm. from the slit of a
Hilger E315 spectrograph without a condensing lens between the
arc and slit.

Ewing, Wilson, and Hibbard (3) used a more elaborate pre-
liminary treatment of the sample. The sample was first ashed,
and 50 mg. of this ash was treated with hydrochloric acid and di-
luted to 10 ml. with a sodium chloride-ammonium chloride buffer
solution. One-tenth ml. of this solution was excited on the
positive electrode of a 15 ampere direct current arc, and a
microphotometric comparison of the blackenings of the lines of

the test elements in the sample with those in standard solutions
was made. The use of a greater current in the arc seems ad-
visable from the standpoint of maintaining a standard arc.
Duffendack, Wolfe, and Smith (2) found that the ratio of the in-
tensities of selected lines may vary with fluctuations of
current, but that the magnitude of this variation is negligible
at higher current values.

Although there is a gain in sensitivity when the sample is
arcked on the negative electrode, the arc is more uniform and
reproducible when the anode contains the sample. The high
temperature of the electrodes melts the sample into a bead in the
anode crater, and if the upper electrode is pointed, the arc
strikes directly into this bead. Pierce, Torres, and Marshall
(13) have found that the cathode arc vaporizes more carbon and
hence causes more background formation on the plate by wandering
about the rim of the crater. From these considerations the anode
arc was chosen for the discharge method in this work.

The internal standard method of Gerlach has been used by
Rogers (14) in determining copper in orange leaves. Rogers sug-
gested the use of either tin or cadmium as an internal standard
because the ionization potentials of these elements as well as
their boiling points are near that of copper. Cadmium and tin
also emit lines in the vicinity of the copper line of wave length
3273.97 Angstrom units. Rogers's data for these constants are
shown in Table 1. Cholak and Story (1) analyzed various biologi-
cal materials for copper using cobalt as an internal standard.

Table 1. Rogers's data for ionization potentials
and boiling points.

Element	Ionization potential in electron volts	Boiling point in degrees C.	Wave length in Angstrom units
Cu	7.68	2310	3273.97
Sn	7.30	2270	3262.33
Cd	8.96	767	3261.05
Co[1]		2900	3283.45

Jaycox and Ruehle (8) criticized the internal standard
method because of the principles which it assumes. The first of
these is that any variability in the arc will affect both the
test element and the standard element to the same degree. The
second principle states that the difference in photographic
blackening of two lines on the plate is proportional to the
logarithm of their concentrations in the source. Although the
first of these assumptions has been justified under certain con-
ditions by experimental evidence, the second holds only for the
straight line portion of the characteristic curve for the plate.
Thus, unless the method of homologous pairs is employed, a cali-
bration of each plate is necessary.

Rogers (14) stated that any preliminary treatment of the
sample was disadvantageous because of the risk involved of intro-
ducing small traces of copper into the sample during the manipu-
lation. He ashed the samples, weighed them onto the electrodes,

---

1. Data for cobalt taken from Handbook of Chemistry and Physics,
Hodgman (7).

and added the internal standard. Hess, Owens, and Reinhardt (5) however, have used a method of wet ashing for the destruction of organic materials after which the sample was analyzed spectrographically for metallic impurities. Miltner (6) in determining metals in organic materials first digested his sample with sulfuric and nitric acids after which the solution was cooled and filtered.

Other types of discharge besides the direct current arc have been used, the type employed depending on the nature of the sample being analyzed. A discussion of the different sources used in trace analysis was published by Owens (12) in which he recommended the direct current arc for the analysis of plant material. A comparison of absolute sensitivities of different discharges is given in Table 2.

Table 2. Owens's data for sensitivity.

Type of discharge	: Mg. determinable
Direct current arc	$10^{-5} - 10^{-4}$
Cathode layer of direct current carbon arc	$10^{-6} - 10^{-5}$
High voltage, alternating current arc	$10^{-6} - 10^{-5}$
Direct current condensed spark	$10^{-6} - 10^{-4}$

Pierce, Torres, and Marshall (13) attributed the sensitivity of spectrographic detection to a great number of factors, three of which are background intensity, interfering lines, and conditions of vaporization. These factors assume an even greater degree of importance in spectrographic determinations and hence

should be carefully considered. A low background, i. e., one approaching an optical density of zero, is preferable, but is attainable only with great difficulty in the region of the copper line (3273.97 A) because of the nearness of Cn bands (3400-5000 A) and bands due to silicon in the sample. Elements which emit lines of nearly the same wave length as the line under consideration may cause considerable difficulty. Usually a qualitative identification is positive only when a series of lines of an element can be identified, while quantitative determinations by the line density method require the line to be isolated. When a sample contains several elements, some of which are more volatile than others, the more volatile ones are excited in the arc first. Slavin (15) pointed out the necessity of using sufficiently long exposure to consume the element being determined under these conditions.

The spectroscopic buffer has come into being since the advent of photoelectric densitometers. These instruments measure the opacity of a line with a much greater degree of fineness than is possible with the human eye. Hence, small variations previously not observable now have become quite prominent. Among these variations is the effect of the presence of one element upon the spectral intensity of another element. This phenomenon is due to the transport mechanism of ions across the arc. Transport phenomena in arc sources may be the result of an equilibrium between thermal diffusion of material from the region of the electrode and a migration of ions of the sample due to electrical forces. Langstroth and McRae (9) have investigated the

transport phenomenon and have enumerated the following results:

1. Lines of different elements have, in general, different intensity distributions along their lengths.

2. The relative distributions depend on ionization potentials and masses of the elements, and on the ionization potentials of other atoms present in the discharge.

3. When easily ionized atoms are present in the discharge, the distribution of an element relative to that of a more easily ionized element of comparable mass falls off more sharply toward the unloaded electrode than it does when easily ionized atoms are present.

The purpose of the spectroscopic buffer is to minimize variations in the transport mechanism from spectrum to spectrum. The buffer should be of such a nature that it does not emit a complicated spectrum of its own, and that it has a low ionization potential.

EXPERIMENTAL PROCEDURE

Description of Apparatus

The spectrograph used in this investigation was a Bausch and Lomb large Littrow with a quartz optical system. This instrument had a wave length range of 2100-8000 Angstrom units and a linear dispersion of 7.1 Angstrom units per mm. at a wave length of 3556 Angstroms. The quartz prism was 57 mm. high with a refraction face 95 mm. long, the reflecting coating being of the evaporated aluminum type. The lens in front of this prism had a focal length of 1827 mm. and was provided with two stops for reducing the aperture.

The illuminating system consisted of the electrode holders, a spherical condensing lens, a rotary sector disc, and a cy-

lindrical lens.

Line densities were read by means of a Bausch and Lomb density comparator, consisting of a projection system, a photoelectric cell for reception, and a galvanometer mounted on a Julius suspension. No voltage regulator was used since the source was operated on direct current from a bank of cells which remained at constant potential.

All qualitative work was done on a spectrum viewing box, and line separations were determined with a spectrum measuring magnifier which allowed the operator to read distances of 0.1 mm. and to estimate .01 mm.

Collection and Treatment of Samples

Samples for this investigation were obtained from the Department of Agronomy of Kansas State College. Wheat samples were collected in June, 1940 from plots of series II and III which were 16 year rotations of corn, alfalfa, and wheat. Fertilizers used on the different plots in these series are shown in Table 3.

Table 3. Fertilizer data on series II and III.

Plot :	Fertilizer
1	Superphosphate
2	Check I
3	Rock phosphate, green manure
4	Superphosphate, potassium sulfate
5	Check II
6	Superphosphate, potassium sulfate, sodium nitrate
7	Manure, superphosphate
8	Check III
9	Manure
10	Manure, lime
11	Check IV
12	Manure, brome grass

The samples were taken from an area of four square feet from the middle of each plot.

After the samples were collected, as much earth as possible was removed from the roots of the plants by mechanical means, and the samples were divided into roots, stems, leaves, and heads. Root samples were then washed with distilled water, and all samples were dried for 24 hours at $98^{\circ}C$. Following this drying period the samples were stored in paper bags at room temperature.

Soil samples were taken from the same area on the plots from which the wheat samples were taken. A-horizon samples were from the first 12 inches of top soil, B-horizon from the layer 12 to 36 inches deep, and C-horizon from the layer below 36 inches. After collection, these samples were stored in cardboard butter cartons.

Calibration of the Rotating Sector

Exposure may be defined as the product of the intensity of illumination and the time of exposure. It was found necessary to decrease the exposure without decreasing the over-all exposure time, so the intensity of illumination was reduced by means of a rotating sector.

The intermittent effect is the variability of exposure with exposure time depending on whether exposure time is one continuous interval or several short intervals. In order to avoid the intermittent effect, a total of more than fifty exposure time intervals must be made. It was therefore desirable to calibrate the rheostat which controlled the speed of the rotating sector.

This calibration was done with a strobatac from the Department
of Physics. At the several speeds of the rotating sector the
strobatac was adjusted to give one image. Then the number of
flashes per second was read and multiplied by two, and when the
strobatac was set at this doubled value, a double image was
observed. The reason for this double reading was to avoid errors
due to setting the strobatac on harmonics instead of on funda-
mentals. Results of this calibration are shown in Table 4.

Table 4. Calibration of rotating sector against strobatac.

Scale	:	Revolutions per minute
1		700
2		1020
3		1570
4		2120
5		2950
6		3650
7		4450

Calibration Curve for Spectrograph

The two persistent lines of copper having wave lengths of
3247.55 and 3273.97 Angstrom units can be photographed con-
veniently with the Bausch and Lomb large Littrow spectrograph
at position 3 which covers the wave length range of 2960 to 4350
Angstrom units. For this position the focus was set at 59.0
and the camera tilted at 230. The two copper lines then fell at
59.8 and 64.5 respectively on the scale. Wave lengths corre-
sponding to the remainder of the scale were determined by
plotting known wave lengths against scale divisions. Elements

whose emission lines could be found in tables (7) were arcked on carbon electrodes and the scale positions of the lines on the photographic plate were measured. The results of this calibration are shown in Table 5 and Fig. 1.

Table 5. The calibration of the Littrow spectrograph.

Scale reading	Wave length (15)	Element emitting line
1.1	2961.2	Cu
3.8	2973.3	Fe
4.7	2973.0	Fe
7.0	2989.0	Bi
8.0	2993.3	Bi
9.4	2997.4	Cu
12.5	3010.9	Cu
13.1	3009.1	Sn
18.0	3036.1	Cu
18.3	3032.8	Sn
23.9	3063.4	Cu
24.2	3067.7	Bi
50.0	3194.1	Cu
52.5	3208.2	Cu
59.8	3247.6	Cu
64.5	3274.0	Cu
65.5	3279.8	Cu
84.2	3397.2	Bi
101.2	3510.9	Bi
111.2	3581.0	Fe
111.3	3581.0	Fe
119.9	3647.9	Fe
128.5	3719.9	Fe
130.2	3737.1	Fe
131.9	3748.3	Fe
137.5	3801.0	Fe
139.6	3820.4	Fe
197.9	4526.0	Fe
198.0	4528.0	Fe

Structure of Copper and Tin Lines
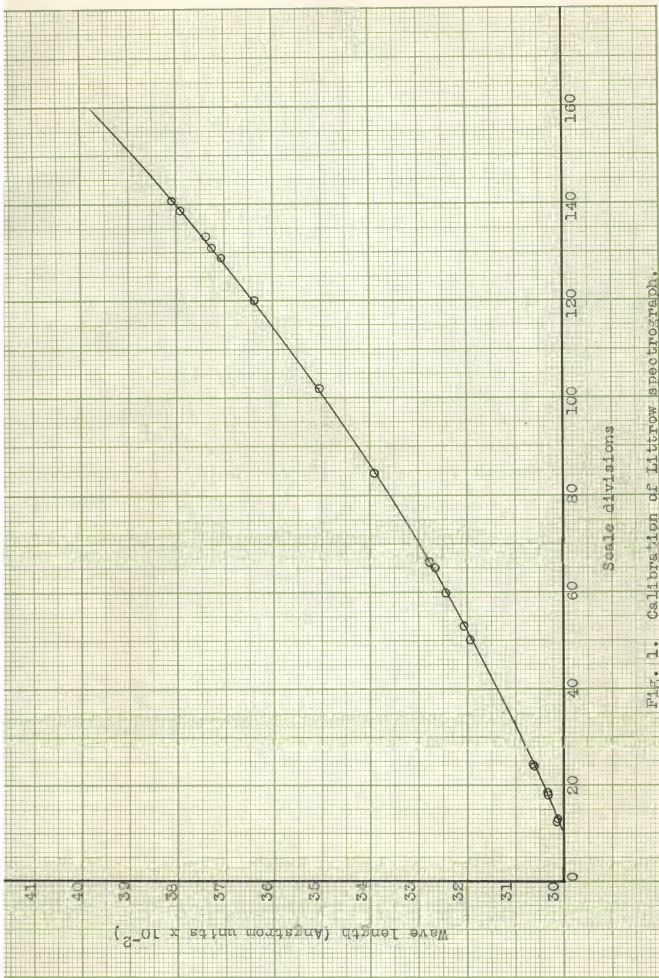
The structures of the copper line at 3273.97 and the tin

Fig. 1. Calibration of Littrow spectrograph.

EUGENE DIETZGEN CO. NO. 345

PRINTED IN U.S.A.

line at 3262.33 Angstrom units were studied by means of the
microdensitometer in the Department of Physics. This instru-
ment is of the recording type and plots the relative intensity
of a line against the relative distance across the width of the
line. Fig. 2 is a copy of the graph made by the densitometer
moving across the copper and tin lines. The lines both appear
to have a doublet structure because the curves have a definite
break in symmetry on their left-hand sides. This lesser in-
tensity was ignored in making measurements for analyses, all
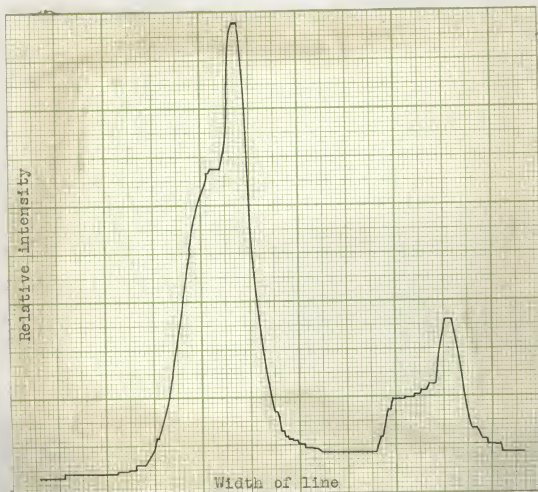measurements being made from the peaks of the curves.

Fig. 2. Microdensitometer graph.

Preparation of Electrodes

Electrodes were prepared from quarter-inch graphite rods which were manufactured by the National Carbon Company as spectroscopic carbons. The rods were cut into one-inch lengths and a layer from the outer surface was removed by filing in a lathe. Cathodes which were designed to contain the sample were drilled with a 3/16" bit encased in a sharpened outer metal shell which served to remove a layer from the outside of the crater simultaneously with the drilling. The result was a thin-walled crater 3/16" in diameter and 3/16" deep. The anode was placed in the lathe and filed to a point, this design being used to help prevent wandering of the arc.

A qualitative spectrographic analysis of these electrodes indicated that copper, tin, and cobalt were absent. No preliminary purification treatment was therefore necessary for these carbons.

Photographic Plates and Their Development

Eastman process plates were chosen for this work because they give good sensitivity in the region of the spectrum in which measurements were made, and because they give a low background density. Other plates which were tried and discarded because they seemed less desirable were Eastman "50" and Eastman D C Ortho plates.

Two developers were used, Eastman D-11 and Eastman x-ray developer. The D-11 developer was originally used, development

being carried out in glass trays and the developer being stored
at a temperature of $12^{\circ}C$. when not in use. Eastman's x-ray
developer, however, seemed to give the same quality of develop-
ment on Eastman process plates and was substituted for the D-11
developer because its keeping qualities allowed it to be stored
at higher temperatures making tank development possible.

Working Curve for Copper in Soil Using Tin as an Internal Standard

The working curve is a plot of the ratio of the optical
densities of copper to tin lines against the logarithm of the
concentration of the copper on the electrode. To obtain data for
this curve, a series of standard copper solutions were first pre-
pared. Crystals of copper nitrate which were spectrographically
free of tin were used as the reagent for the stock solution of
copper. Approximately 45 gms. of the hydrate, $Cu(NO_3)_2 \cdot 3H_2O$,
were dissolved and diluted to one liter.

An aliquot portion of this approximated solution was then
analyzed for copper by the electrolytic method. Ten ml. of the
solution was diluted to 50 ml. with distilled water in a 150 ml.
beaker. One and one-half ml. of concentrated sulfuric acid and
10 drops of concentrated nitric acid were then added, and the
solution was heated to $80^{\circ}C$. after being diluted with water to
80 ml.

Platinum electrodes were then immersed in the solution and a
potential difference great enough to maintain a current of about
1.5 amperes was applied to them. Immediate deposition of copper

on the cathode was evidenced by its sudden change in color from a white to reddish yellow. To avoid loss of sample by spattering, a split cover glass was placed over the beaker, and this was rinsed down into the solution from time to time during the electrolysis. Current was allowed to flow through the cell for forty-five minutes before the first test was made. After this time a drop of solution was removed and analyzed qualitatively for copper by adding to it a drop of potassium ferrocyanide solution. Failure to produce a brown color indicated that copper

$$K_4Fe(CN)_6 + 2 Cu \rightarrow Cu_2Fe(CN)_6 + 4 K$$
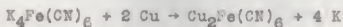
was completely deposited from the electrolyte. Two gms. of urea was added to the electrolyte and the current was allowed to flow for another 15 minutes. The purpose of the urea was to guard against the loss of copper, when the circuit was broken, by destroying the oxides of nitrogen. At the end of this time the electrodes were lifted out of the solution while the current was still on, being rinsed down with water during this removal. This technique prevented any of the deposited copper from dissolving off the electrode into the acid electrolyte.

The cathode was next weighed after being washed with water and ethanol and dried. The deposited copper was then dissolved off with nitric acid and the cathode again dried and weighed. The difference in weight between these two weighings gave accurately the amount of copper deposited. This method of analysis based on Faraday's law, one of the most exact laws of physical

chemistry, is the most precise method known for determining copper in solutions whose concentration is of the same order of magnitude as this one. The reaction occurring at the cathode may be illustrated by the following equation:
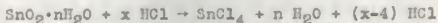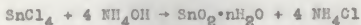
$$Cu^{++} + 2\ e \rightarrow Cu^{o}$$
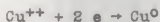
Results of this analysis indicated that the quantity of solution analyzed contained 0.1390 gm. of copper. The volume of copper solution added was 10.03 cc. as determined by previously calibrating a 10 cc. pipette at room temperature (32.7° C.). Since duplicate samples were run, a total of 20.06 cc. of the liter of solution prepared was used for the analysis. The remaining 979.94 cc. portion was diluted to 1357 cc. to make the stock solution, data for which are shown in Table 6.

Table 6. Data for preparing standard copper solutions.

Solution :	Copper content :	:	Preparation from stock solution					
3-O	0.001 mg./ml.		Dilute	0.1 ml. stock to 1000 ml.				
3-N	0.005 "		"	0.5	"	"	"	"	"
3-C	0.01 "		"	1.0	"	"	"	"	"
3-G	0.05 "		"	5.0	" .	"	"	"	"
3-B	0.1 "		"	10.0	"	"	"	"	"
3-P	0.5 "		"	50.0	"	"	"	"	"
3-A	1.0 "		"	100.0	"	"	"	"	"

The second prerequisite to making a working curve was to obtain a solution of tin free from copper for the internal standard. The chemically pure stannic chloride available was arced on the electrodes of the spectrograph and was found to

contain copper. A solution of the salt was then prepared, and the tin was precipitated, centrifuged, and redissolved thirteen successive times. Precipitation was brought about by making the solution basic with ammonium hydroxide, and solution by acidifying with hydrochloric acid. A second spectrographic analysis

$$SnCl_4 + 4 \ NH_4OH \rightarrow SnO_2 \cdot nH_2O + 4 \ NH_4Cl$$

$$SnO_2 \cdot nH_2O + x \ HCl \rightarrow SnCl_4 + n \ H_2O + (x-4) \ HCl$$

showed that all copper had been removed by this process.

The concentrated acid solution of stannic chloride was analyzed for tin by diluting an aliquot portion to 300 ml. and adding a few drops of methyl orange indicator solution. Ammonium hydroxide was then added until the indicator turned from red to yellow. Ten ml. of 3 N ammonium nitrate solution was added to the neutralized solution, and it was heated and allowed to boil for 2 minutes. The solution was then filtered and the precipitate washed with an aqueous solution of ammonium nitrate. The sample was ashed in a muffle and weighed as SnO_2. The tin solution was found to contain 14.644 mgs. of tin per ml. From this the standard tin solution was prepared by diluting 25 ml. of stock solution to one liter with water and sufficient hydrochloric acid to prevent precipitation.

The third step in preparing a working curve was to make a photographic plate, exposed so as to work in the correct gamma region for that plate which contains spectra of different amounts of copper photographed simultaneously with a constant amount of tin. Each electrode was therefore filled with two

solutions, the copper solution of desired concentration and the tin solution whose quantity remained constant throughout the series of pictures.

Since the small sample used in the arc was not always the same weight, the logarithm of the number of milligrams of copper present was plotted instead of the conventional concentration.

A separate photographic plate was used to determine the exposure time necessary to accomplish this result. An electrode was loaded with the most concentrated copper solution used plus the internal standard plus the spectroscopic binder which in this case was silicic acid. The arc was allowed to burn continuously for $4\frac{1}{2}$ minutes, the plate being moved to a new position every half minute after the first two minutes. This method was preferred to closing the shutter and turning off the arc while the plate was being moved, because the temperature of the arc was maintained just as it would be in the picture for the working curve. The result of this exposure test photograph is shown in Table 7. The solutions were measured onto the electrode with a

Table 7. Exposure test data.

Spectra	Solution	Exposure interval (min.)	Cu line (3273.97)	Sn line (3262.33)
1	0.1 ml. 3-A	0-2	Present	Present
2		2-2.5	"	"
3		2.5-3	Absent	Absent
4		3-3.5	"	"
5		3.5-4	"	"
6		4-4.5	"	"

microburette and allowed to dry uniformly. One-tenth ml. of the tin solution was added after the copper solution had dried, and an excess of dry silicic acid was added upon complete drying of the tin solution. The data in Table 7 show that all the copper and tin were consumed during the first two minutes of arcking.

Two minute exposures were then used in taking pictures for the working curve. In order to keep down the background due to silicon and cyanogen bands, a rotating sector set at one-fourth and turning at a speed of 1020 revolutions per minute (fast e-nough to avoid the intermittency effect) was placed in the path of the beam between the arc and the slit.

The photograph was developed in the usual manner, the density comparator was used to measure the blackness of lines, and optical densities were calculated from the data which is shown in Table 8. In this table G_{Cu} and G_{Sn} are galvanometer deflections for the copper and tin lines, and D refers to their respective

Table 8. Data for working curve.

Spectra	Mgs. Cu	St. $SnCl_4$	G_{Cu}	G_{Sn}	$\dfrac{G_{Sn}}{G_{Cu}}$	$\dfrac{D_{Cu}}{G_{Sn}}$	Log mgs. Cu
1	.001	.1	15.2	5.8	.381	-.419	-3.000
2	.001	.1	15.6	6.1	.301	-.408	-3.000
3	.005	.1	5.0	3.7	.740	-.131	-2.301
4	.005	.1	7.9	8.8	1.11	.045	-2.301
5	.005	.1	6.5	7.7	1.18	.072	-2.301
6	.01	.1	5.3	7.9	1.49	.173	-2.000
7	.01	.1	5.1	7.8	1.53	.185	-2.000
8	.05	.1	2.2	5.2	2.36	.373	-1.301
9	.05	.1	2.8	6.9	2.46	.391	-1.301
10	.1	.1	2.6	9.3	3.59	.554	-1.000
11	.1	.1	3.5	11.2	3.20	.505	-1.000

optical densities. The development of the optical density ratios are shown below. G_0 is the galvanometer reading for a clear portion of the plate. Zero readings were ignored since they remained constant and nearly zero on the scale. Data in Table 8

$$D_{Sn} = \log G_0/G_{Sn}$$
$$D_{Cu} = \log G_0/G_{Cu}$$
$$D_{Cu}/D_{Sn} = \log G_0/G_{Cu} - \log G_0/G_{Sn}$$
$$D_{Cu}/D_{Sn} = \log G_0/G_{Cu} \times G_{Sn}/G_0 = \log G_{Sn}/G_{Cu}$$

are plotted in Fig. 3. The increase of line density with concentration is shown in Fig. 4 of Plate I.

Previous to preparing the working curve from data on copper nitrate solutions, many attempts were made to obtain the data by using copper sulfate solutions, but without success.

Analysis of Soil

In analyzing a soil sample for copper, about 0.05 gm. of the dried sample was weighed onto an electrode which had been drilled to a depth of 3/16". A volume of 0.1 ml. of the standard tin solution was added on top of the soil sample and was dried uniformly at room temperature. When the sample was thoroughly dry, it was arced on the spectrograph just as the copper samples had been arced. After the plate was developed, the intensities of copper and tin lines were determined and the ratio of their optical densities were calculated. This ratio was referred to the working curve to determine the milligrams of copper present. The per cent copper in the sample was then
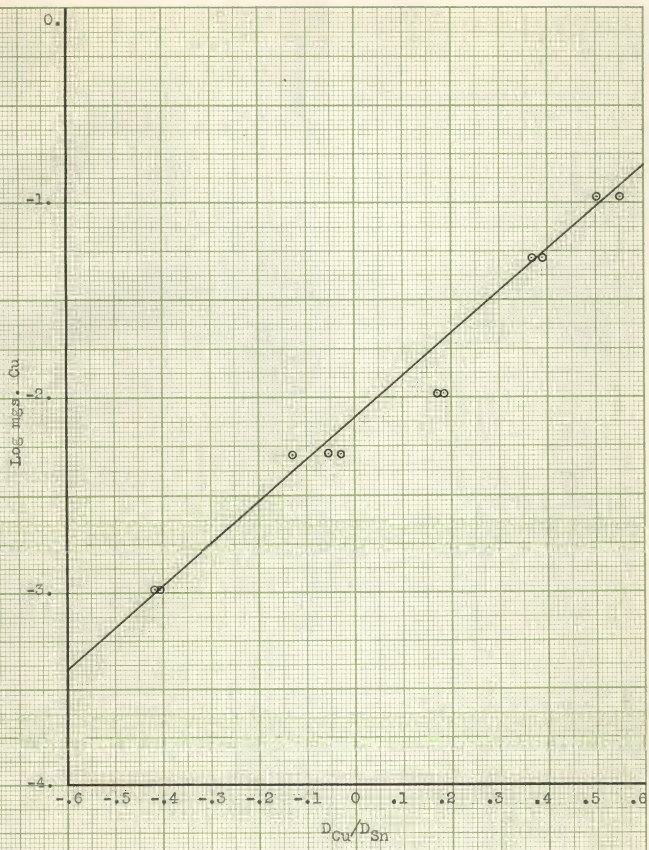
Fig. 3. The working curve for copper in soil.

EXPLANATION OF PLATE I

Figure 4 of Plate I is designed to show the variation
in intensity of a line with the variations in concentration
of the element emitting that line. This photograph is one
of the spectrum of copper whose concentration is varied and
of the internal standard, tin, whose concentration is the
same for the whole series of pictures. Two copper lines,
whose wavelengths are 3247.55 and 3273.97 Angstrom units,
are indicated by the black arrows at the bottom of the figure.
The black arrow at the top of the figure points out the
tin line whose wavelength is 3262.33 Angstroms. It is noted
that the brightness of this latter line is about the same in
each picture while the other two lines are dim in the top
pictures and gradually increase in brightness towards the
bottom of the figure. The top spectrum represents a copper
concentration of 0.00032 mg. while the concentration in the
bottom picture was 0.01 mg. The sample arcked also contained
silicic acid and sodium nitrate, and the bright line, marked
x, is the sodium line at 3302.34, 3302.94 (doublet structure);
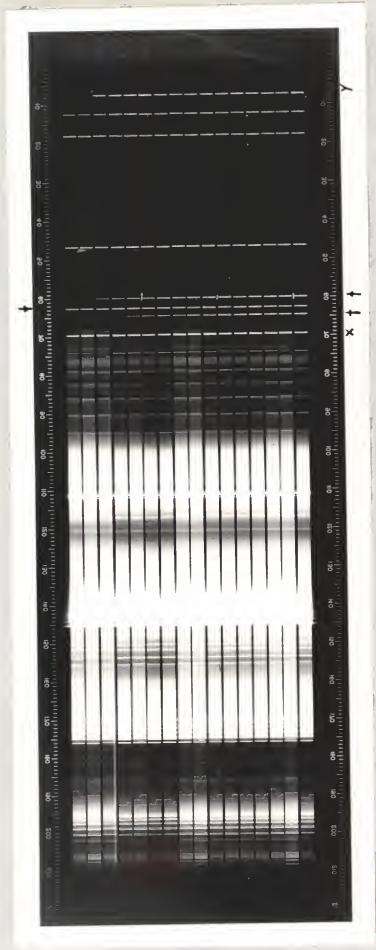the line marked y is the silicon line at 2881.59 Angstrom
units.

PLATE I

Fig. 4.

EXPLANATION OF PLATE II

Figure 5 of Plate II is a picture of a series of soil spectra showing the copper and tin lines (indicated by the white dots) which appear in Figure 4. Other lines present in this figure are due to such extraneous elements as iron, manganese, magnesium, silicon, and others. Beginning with the ninth spectrogram in Figure 5, spectra of the fertilizers sodium nitrate, potassium sulfate, rock phosphate, and superphosphate are shown. The last six spectrograms are solutions of copper and tin.
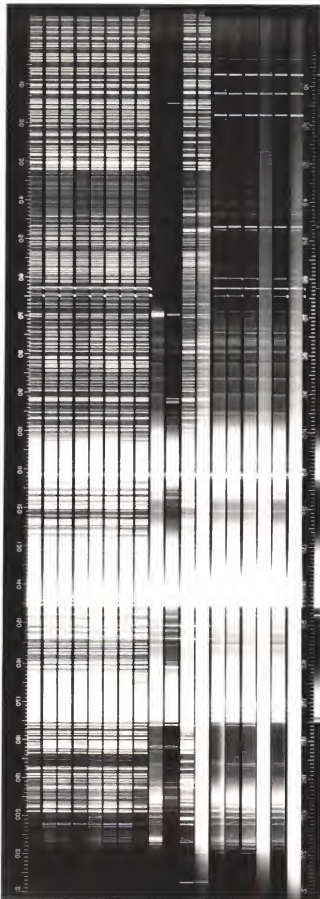
PLATE II

Fig. 5.

calculated from the value obtained and the initial weight of the sample.

Method of Soil Analysis Using Spectroscopic Buffer

The application of the principles enunciated by Lang-stroth and McRae (9) involved several modifications of the method already described. Sodium nitrate, because it was easily obtainable in a form so pure as to warrant no further treatment and because it has a low ionization potential and emits no complicated spectrum of its own, was chosen as a spectroscopic buffer.

The first difficulty encountered was that of keeping the solution of sodium nitrate from diffusing through the thin wall of the crater in the electrode. Different materials such as kerosene and collodion failed to prevent this action, but it was discovered that palmitic acid applied to the electrode by first dissolving it in the volatile solvent, benzene, would hold the solution in the crater.

In order to complete the analysis in a reasonable amount of time, it was necessary to dry the solutions in the electrodes at temperatures higher than room temperature. This created a second problem, that of preventing the internal standard, stannic chloride, from vaporizing. It was found that 25 ml. of the standard tin solution when treated with 25 ml. of nitric acid remained on the electrode. The sodium nitrate so enhanced the tin line that it was necessary to dilute this solution with three volumes of water to keep the line within a reasonable range of optical densities.

Data for the working curve obtained by using these modifications are shown in Table 9 and plotted in Fig. 6.

Table 9. Data for working curve with buffer.

Spectra	Mgs. Cu	St. Sn Soln. (ml.)	G_{Cu}	G_{Sn}	$\dfrac{G_{Cu}}{G_{Sn}}$	$\dfrac{D_{Sn}}{D_{Cu}}$	Log mgs. Cu
1	.0005	.1	16.8	3.6	4.67	.669	-2.301
2	.0005	.1	17.9	4.1	4.37	.641	-2.301
3	.0001	.1	28.9	4.5	6.26	.796	-3.000
4	.0001	.1	24.6	4.8	5.12	.710	-3.000
5	.001	.1	8.9	4.6	1.94	.236	-2.000
6	.001	.1	9.2	4.6	2.00	.301	-2.000

Working Curve for Wheat Samples

A working curve for wheat samples was established in the same manner as the one for soil samples. Sodium nitrate was added to each electrode to act as a spectroscopic buffer, and silicic acid was added as a binding material. Data for this working curve are shown in Table 10 and are plotted in Fig. 7.

Table 10. Data for working curve of wheat.

Spectra	Mgs. Cu	St. Sn Soln. (ml.)	G_{Cu}	G_{Sn}	$\dfrac{G_{Cu}}{G_{Sn}}$	$\dfrac{D_{Sn}}{D_{Cu}}$	Log mgs. (Cu x 10^4)
1	.00032	.1	28.0	4.2	6.76	.83	.505
2	.00032	.1	28.1	4.5	6.25	.80	.505
3	.0005	.1	21.3	4.7	4.58	.66	.699
4	.0005	.1	20.8	2.9	7.05	.85	.699
5	.0007	.1	14.7	3.2	4.60	.66	.845
6	.0007	.1	12.2	3.1	4.02	.60	.845
7	.0010	.1	8.9	2.7	3.30	.52	1.000
8	.0010	.1	9.4	2.9	3.19	.50	1.000
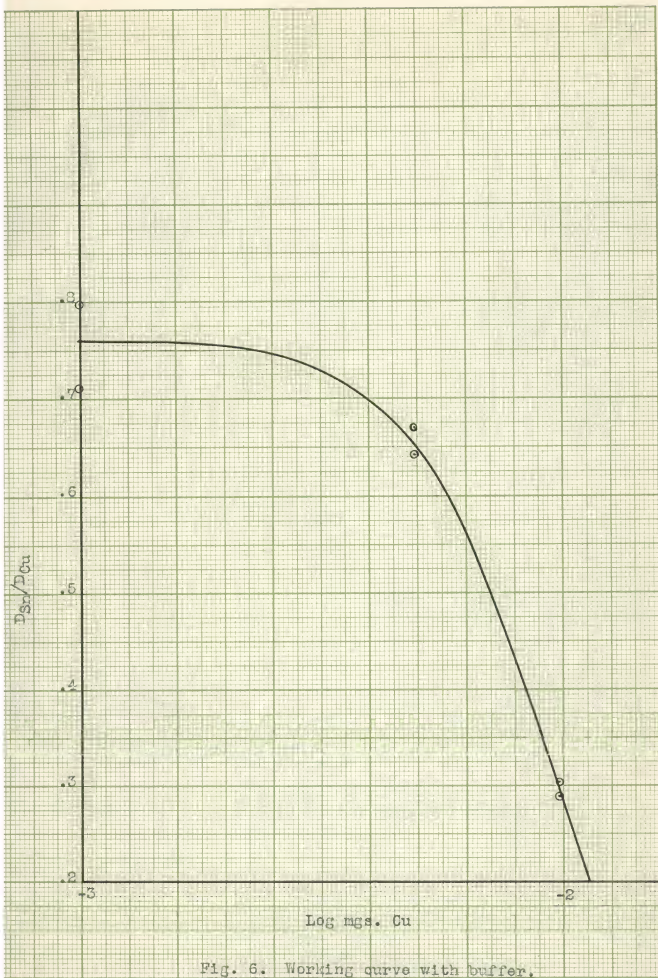
Fig. 6. Working curve with buffer.

Table 10 (concl.).

Spectra	Mgs. Cu	St. Sn. Soln. (ml.)	C_{Cu}	C_{Sn}	$\frac{C_{Cu}}{C_{Sn}}$	$\frac{D_{Sn}}{D_{Cu}}$	Log mgs. (Cu x 10^4)
9	.0032	.1	5.1	3.7	1.40	.15	1.505
10	.0032	.1	4.5	3.0	1.50	.18	1.505
11	.0070	.1	2.0	2.7	.74	-.13	1.845
12	.0070	.1	3.0	3.6	.85	-.07	1.845
13	.010	.1	2.5	3.7	.68	-.17	2.000
14	.010	.1	2.3	3.0	.76	-.12	2.000

The preliminary treatment for wheat differed from that of the soil samples in that the dry wheat was ground in a Wiley mill equipped with a screen which was plated with silver to avoid contact of the sample with brass. After the grinding process, the sample was weighed in a platinum dish and ashed in a preheated muffle at 560° C. for 2 hours. The ash was then weighed onto the electrodes for arcking.

RESULTS

Analysis of Soil Using Stannic Chloride
as an Internal Standard

Two plates were taken of a set of soil samples from series II and III, the results of the duplicate analyses being shown in Table 11. The sample number is designated by three terms, a Roman numeral indicating the series, the Arabic numeral indicating the plot, and a capital letter indicating the horizon (see Table 3). Other samples photographed on these same plates did not yield results close enough to warrant consideration.
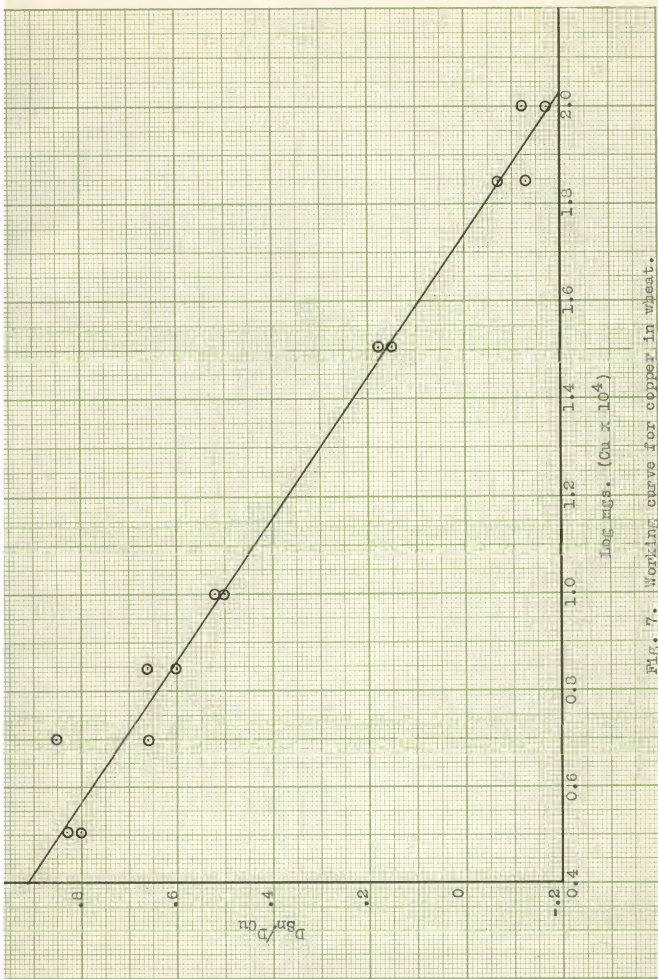
Fig. 7. Working curve for copper in wheat.

log mgs. (Cu x 10⁴)

$log\ mgs.\ (Cu \times 10^4)$

$\overline{Dsn}/Dcu$

Table 11. Data for soil analysis.

Sample	Per cent copper	
	Trial 1	Trial 2
III- 4-A	.0026	.0021
II-10-A	.0035	.0038
III- 5-A	.0041	.0041
II- 7-A	.0044	.0039
III- 9-A	.0047	.0050
III- 7-A	.0039	.0056
III- 6-A	.0033	
III- 8-A		.0037
III-10-A		.0024
II- 2-A		.0043

Analysis of Soil Using Buffer

A set of soil samples from series II, including plots 1 to 6 were analyzed by the method which employs spectroscopic buffers. Results of this analysis are shown in Table 12.

Table 12. Data for soil analysis with buffer.

Sample	Per cent copper	
	Trial 1	Trial 2
II-1-A	.0016	.0014
II-2-A	.0018	.0013
II-3-A	.0015	.0015
II-4-A	.0017	.0013
II-5-A	.0050	.0021
II-6-A	.0019	.0017

Analysis of Wheat Using Tin
as an Internal Standard

Plant samples which were ground, ashed, and analyzed for copper yielded the results shown in Table 13. Spectra of wheat

Table 13. Data for wheat analysis.

Sample	Per cent copper	
	Trial 1	Trial 2
Series II, leaves		
Plot 1	.00063	.00091
Plot 2	.00069	.00080
Plot 3	.00054	.00046
Plot 4	.00047	.00052
Series II, stems		
Plot 1	.00031	.00048
Plot 2	.00040	.00073
Plot 3	.00024	.00041
Plot 4	.00028	
Series II, roots		
Plot 1	.00151	.00109
Plot 2	.00153	.00157
Plot 3	.00174	.00086
Plot 4	.00120	.00078
Series II, grain		
Plot 1	.00061	.00045
Plot 2	.00074	.00053
Plot 3	.00049	.00034
Plot 4	.00056	.00068

samples are shown in Fig. 8, Plate III.

EXPLANATION OF PLATE III

Figure 8 of Plate III is a series of spectrograms of wheat samples containing the internal standard, tin. The first eight spectrograms are root samples, and the remaining ones are grain samples. The increased number of lines in the root spectrograms is due to soil which adhered strongly to the plant roots.
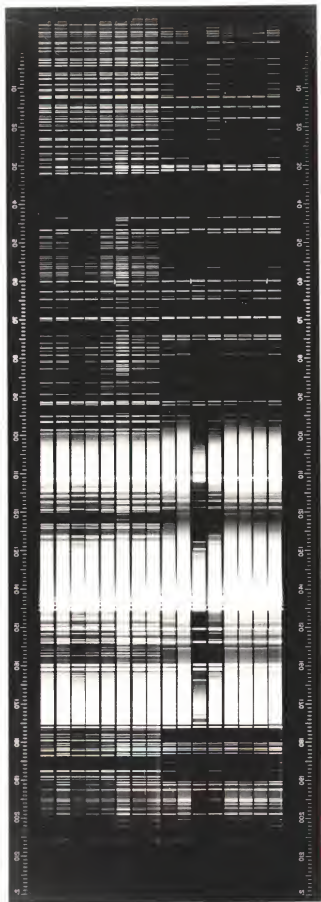
PLATE III

Fig. 8.

SUMMARY

Several soil and wheat samples from plots on which differ-
ent fertilizers had been used were analyzed for copper by means
of the spectrograph. Samples of wheat were divided into roots,
stems, leaves, and grain and were dried before storing.

The spectrograph was calibrated with known samples, and
wave lengths were correlated with scale divisions. The nearly
straight line graph obtained indicated an excellent degree of
dispersion of the instrument throughout the spectral region
which it covered.

The method using a sodium nitrate spectroscopic buffer
gave most consistent results in the analysis of soil samples.

Plant samples were ashed and their ash was analyzed for
copper by placing the dry ash on the electrodes and adding a
buffer and an internal standard.

About four times as much copper was found in the soil as
in the plant material. However, different soils did not vary
appreciably in copper content.

Of the several parts of the wheat plants analyzed, roots
were found to contain more copper than leaves, and leaves con-
tained more copper than either stems or grain.

CONCLUSIONS

It appears that soil analyses may be carried out with smaller error by means of the buffer method than without the buffer. Although buffer materials are probably in soils normally, they are not present in sufficient quantities to maintain a constant arc over a two minute exposure.

The copper content of the soil samples ranged from .0013 per cent to .0050 per cent. The fertilizers used seemed to have no effect on the concentration of copper in the soil. This is to be expected because copper was found to be present in the fertilizers in only trace concentrations.

The range of copper concentration in the wheat samples was from .00020 per cent to .00174 per cent. Such a difference in the concentrations of copper between wheat and soil indicate that none of the soils tested was deficient in copper. These values were lower than those reported by Webster and Jansma (16) who analyzed wheat for copper by chemical methods and found from .0025 to .0050 per cent.

Higher concentrations of copper in roots was probably due to adhering soil particles which were not completely removable by the methods employed. In general, the leaves seemed to contain more copper than either stems or grain. This bears out the chlorophyll formation theory of Orth, Wickwire, and Burge (11), because the major part of the photochemical reaction in

the plant takes place in the leaf whose area is exposed to the action of light quanta.

The spectrograph offers a successful method of quickly and accurately determining trace elements in plants and soil. However, some variable factors which are still unrecognized must be solved and controlled before the method reaches its perfection.

ACKNOWLEDGMENTS

LITERATURE CITED

(1) Cholak, Jacob, and Story, Robert V.
    Spectrographic analysis of biological material. Indus.
    and Engin. Chem., Analyt. Ed., 10: 619-622. Nov., 1938.

(2) Duffendack, O. S., Wolfe, R. A., and Smith, R. W.
    Quantitative analysis by spectroscopic methods. Indus.
    and Engin. Chem., Analyt. Ed., 5: 226-229. July, 1933.

(3) Ewing, D. T., Wilson, Mabel F., and Hibbard, R. P.
    Spectroscopic determination of metals in small samples.
    Indus. and Engin. Chem., Analyt. Ed., 9: 410-414. Sept.,
    1937.

(4) Frankforter, G. B.
    The occurrence of copper in the plant world. Chem.
    News. 79: 44-45. Jan., 1899.

(5) Ness, T. M., Owens, J. S., and Reinhardt, L. G.
    Analysis of organic material for traces of metallic
    impurities. Proceedings of the seventh summer confer-
    ence on spectroscopy and its applications. New York.
    John Wiley and Sons. 154 p. 1940.

(6) Hiltner, Werner.
    Bestimmung kleinster Mengen Kupfer and Mangan in
    Geweben. Z. Analitische Chemie. 110: 241-251. July,
    1937.

(7) Hodgman, Charles D.
    Handbook of chemistry and physics, ed. 16. Cleveland,
    Ohio. Chemical Rubber Publishing Co. 2067 p. 1931.

(8) Jaycox, E. K., and Ruehle, A. E.
    Quantitative spectrochemical analysis by measurement
    of relative intensities. Indus. and Engin. Chem.,
    Analyt. Ed., 12: 195-196. Apr., 1940.

(9) Langstroth, G. O., and McRae, D. R.
    Transport of material in sources for spectroscopic
    analysis. Canad. Jour. Res., 16A: 61-74. March, 1938.

(10) Milbourn, M.
    The spectrographic analysis of soils and plant materials
    for minor elements. Soc. Chem. Indus. Jour., Trans. 56:
    205-209. June, 1937.

(11) Orth, O. S., Wickwire, G. C., and Burge, W. E.
Copper in relation to chlorophyll and haemoglobin formation. Science. 79: 33-34. Jan., 1934.

(12) Owens, J. S.
Spectrographic methods of trace analysis. Indus. and Engin. Chem., Analyt. Ed., 11: 59-63. Feb., 1939.

(13) Pierce, W. C., Torres, O. Ramirez, and Marshall, W. W.
Qualitative spectrographic analysis in the arc, with graphite electrodes. Indus. and Engin. Chem., Analyt. Ed., 12: 41-45. Jan., 1940.

(14) Rogers, Lewis H.
Spectrographic microdetermination of copper. Indus. and Engin. Chem., Analyt. Ed., 11: 47-48. Jan., 1939.

(15) Slavin, Morris.
Quantitative analysis based on spectral energy. Indus. and Engin. Chem., Analyt. Ed., 10: 407-411. Aug., 1938.

(16) Webster, James E., and Jansma, Frank.
A comparison of copper content of Oklahoma wheat with those of other states. Science. 70: 174-175. July, 1929.

Date Due		