A STUDY OF COBALT IN
MICHIGAN SOILS

Thesis for the Degree of Ph. D.
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Costas George Apostolakis
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This is to certify that the

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Costas Apostolakis

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Ph.D. degree in Soil Science

R. L. Cook
Major professor

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A STUDY OF COBALT IN MICHIGAN SOILS

By

Costas George Apostolakis

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and
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of the requirements for
the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

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Approved R. L. Cook

In view of the interest in the cobalt problem from the standpoint of the nutrition of animals in the State of Michigan a study was originated (1) to study spectrographic procedures for the estimation of cobalt in plant and soil material, (2) to evaluate the cobalt content of some surface soils, and (3) to determine its availability to plants under different soil treatments.

A spectrographic procedure involving the preconcentration of cobalt using 8-hydroxyquinoline was developed and found to give very satisfactory results in determining trace amounts of cobalt in plants, soils and their fractions.

The yield of red clover grown in the greenhouse on two soils was not influenced by applications of cobalt sulphate. However, an increase in the cobalt content of clover resulted from increased applications of this cobalt salt to the soil.

Various applications of lime material caused a decrease in the cobalt content of red clover. The highest application of lime resulted in the lowest cobalt content in the plant tissue, especially in the Coloma sand soil. The results obtained with plants grown on the Hillsdale sandy loam were erratic.

Laboratory studies consisted of the determination of the available cobalt of the soils, the total cobalt content of alfalfa grown thereon and the total cobalt content of the soils and their fractions.

The available cobalt content of the soils studied varied from 0.08 to 0.68 parts per million, while the cobalt content of alfalfa grown on them ranged from a minimum of 0.03 to a maximum of 0.24 parts per million. The total cobalt content of the soils ranged from 0.47 to 2.17 parts per million.

No significant correlation was obtained between the cobalt content of the plant and the total or available cobalt content of the soils although in some cases, a direct trend was evident.

As a rule the cobalt content of the various fractions increased as particle size decreased. No significant correlation was obtained when the total cobalt content of the soil was compared to that of the individual sand, silt or clay fractions.

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A THESIS

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1955

TO MY MOTHER

Whose encouragement and understanding
have greatly helped throughout my
academic work, these results
are dedicated.

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VITA

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I. INTRODUCTION

Early interest in the cobalt content of soils and plants developed when the importance of this element in ruminant nutrition was recognized. The recent discovery that cobalt is required for the synthesis of vitamin B_{12} has broadened this interest to include the whole field of animal and human nutrition. With ruminants, a lack of cobalt in the feed creates certain physiological disorders, which though known under various names, present similar symptoms often resulting in the death of the animal.

The above mentioned disorders have been reported in several countries and as early as 1909 the coloquially known "Grand Traverse" or "Lake Shore Disease" of the dairy cattle was observed and reported in the state of Michigan. This nutritional anemia of the cattle came to be recognized as a cobalt deficiency in the herbage used for feeding the animals.

In view of the existing interest in this state in relation to the cobalt problem, a study dealing with the first two phases of the soil-plant-animal relationship was originated.

Greenhouse and laboratory studies on the status of cobalt in Michigan soils were undertaken in an effort to obtain information pertaining to the amount and forms of cobalt in the soil, some of the factors affecting its availability to the plants, the distribution of the element in the soils of

Michigan, and the related methodology for solving the above points of interest.

II. REVIEW OF LITERATURE

The greatest portion of the total cobalt content of the soil has its origin in minerals of the soil parent material. Goldschmidt (1937), in his study on the distribution of cobalt in rocks, concluded that the greatest portion of the original amount of cobalt in the earth was concentrated in the iron core with very small quantities left in the silicate rocks. This distribution is the result of a close similarity in the chemical properties of iron and cobalt. According to a later report (1945) by the same author, cobalt is closely associated with lithium, magnesium, nickel, iron, zinc, lead, tin, and it can replace any of these elements in the ferromagnesian minerals present in basic igneous rocks. Important host minerals for the above group of elements are olivines, pyroxenes, amphiboles and biotite micas.

Wager and Mitchell (1943), determining cobalt in a series of rocks originating from the same magma in the Skaergaard Intrusion of East Greenland and ranging from ultra-basic to acidic type, found a higher cobalt content (100 parts per million) in the ultra-basic rocks with the amount decreasing as the fractional crystallization of the rocks progressed towards the acidic type. Based on the above results they concluded that deficiencies in cobalt would not likely be found in areas where soils are derived from ultra-basic rocks.

An examination of American igneous rocks by Sandell and Goldich (1943) suggested a pattern of cobalt distribution similar to the one observed by Wager and Mitchell. Their results indicate 0.5 to 5.0 parts per million of cobalt were present in the granites as compared with 26 to 45 parts per million in the diabases. They also estimated the average cobalt content of the earth's crust as being 23 parts per million.

From Australia it was reported by Carroll (1945) that acid gneisses, the parent material of the soils of the Denmark area which are low in ferromagnesian minerals, had a low to medium cobalt content, while a fresh unmetamorphosed intrusion of dolerite containing augite and feldspar had a rather high cobalt content.

Limestone parent material was found to be low in its cobalt content. From the work of Dixon and Kidson (1940), McNaught and Paull (1940), Maunsell (1945), Rigg (1940-b) and other investigators, it has been shown that the range of cobalt in limestone is 0.2 to 12.5 parts per million of cobalt with most values falling between 1.0 and 5.0 parts per million.

Beeson, Gray and Hamner (1944) as well as Lyford, Percival, Keener and Morrow (1945) report that most of the cobalt deficiency cases in New Hampshire occur on soils developed from granites.

Mitchell (1944) believes that if a soil can be related directly to an igneous rock the cobalt content of the soil can be approximated, but the problem becomes complicated when the soil is derived from glacial drift composed of various rocks and rock materials.

Beeson (1948) concluded that the age of the soil, in relation to time available for the operation of the soil-forming processes is the most important factor resulting in the low cobalt content of certain soils. He illustrated his point by the fact that the cobalt deficient areas of the Northeast region of the U. S. are those in which the soils are young and immature.

Studies of the quantity of cobalt in soils were started by Bertrand and Mokragnatz (1922) who reported 2.8 parts per million of cobalt in a soil near Belgrade, Yugoslavia and 3.7 parts per million of cobalt in a garden soil at the Pasteur Institute in France. The same investigators reporting on later work (1924), listed the cobalt content of several soils from various countries as ranging from 0.26 to 11.7 parts per million. In this country, McHargue (1925) reported 1.5 parts per million of cobalt found in a virgin soil in Kentucky.

As it became evident that a specific nutritional deficiency in cattle and sheep was due to a lack of cobalt in the herbage, a rather thorough investigation of cobalt in soils started especially in the countries mostly affected by this "disease", namely Australia and New Zealand.

In New Zealand, Askew and Maunsell (1937) reported the cobalt content of three pasture soils as 41, 18, and 17 parts per million. Rigg (1939, 1940_b) noted that soils of the Nelson district derived from granite, contained approximately one part per million of cobalt as compared with only 0.2 parts per million in the parent material. It was observed by Kidson (1938) that in the Dartmoor area deficient soils contained three to four parts per million of cobalt as compared to 11 to 30 parts per million for soils of healthy sections. Grimmett (1937) reported that certain North Auckland soils associated with cobalt-deficient pastures contained ten parts per million of cobalt. Rigg (1940_a) found 3.8 to 18.8 parts per million of cobalt for soils of the Marlborough district. He also reported (1937) that Glenhope, Kaharoa and Taupo soils contained less than two parts per million of cobalt, while Taranaki soils averaged 12 parts per million and Morton Mains soils contained four to five parts per million.

McNaught and Paull (1940) observed that certain highly calcareous soils of New Zealand contained only 0.030 to 0.045 parts per million of cobalt.

From work done in Australia by Harvey (1937) it was found that surface soils of Denmark, Western Australia where the nutritional disorder was prevalent among the livestock, usually contained less than three parts per million of cobalt while soils of healthy localities ranged from 4.2 to 40 parts per million. Approximately similar results were obtained by

Underwood and Harvey (1938) who reported that the cobalt content of 22 samples of soil, on which "enzootic marasmus" in grazing animals occurs, ranged from 0.1 to 1.5 parts per million, while 27 soils from healthy locations contained 0.2 to 32.0 parts per million of cobalt.

In England, Patterson (1937) observed a sheep disease on Dartmoor moorland soils having a cobalt content of 3.9 parts per million while healthy soils had 16.7 parts per million. Stewart, Mitchell and Stewart (1941) reported that the cobalt content of all soils examined spectrographically at the Macaulay Institute in Scotland ranged between one and three hundred with a mode of about ten parts per million. Most of the deficient soils in Scotland contained less than five parts per million of cobalt.

The cobalt content of Italian soils examined by Cambi (1949) ranged from 4.59 to 20.00 parts per million.

Malyuga (1944) reported that various soil types in Russia do not vary much from 10.0 parts per million of cobalt. Chernozem soils rich in humus contained slightly higher than the average amount of cobalt, while podzol soils were below the average.

Wright and Lawton (1954) investigated 14 soils from Nova Scotia, Canada, and concluded that the cobalt content of the light-textured soils ranged from 3.6 to 10.8 parts per million, whereas that of the medium and heavy textured soils ranged from 9.0 to 21 parts per million.

In the United States interest in studies of soil cobalt has been increasing. Most of the early work in this regard is in relation to the nutritional disorders in livestock. Beeson (1945) has mapped the areas of the United States in which nutrition troubles in the dairy animals due to a cobalt deficiency in the herbage have been observed. The states of New Hampshire, Massachusetts, North Carolina, Florida, Michigan, and Wisconsin have reported nutritional disorders in the cattle due to lack of cobalt.

The analyses of several virgin soils in Florida reported by Gammon, Henderson et al. (1953) indicated a cobalt content ranging from one to five parts per million with a mode of one part per million.

In a recent work, Hill, Toth and Bear (1953) found that New Jersey soils contained between 0.2 and 30.8 parts per million of cobalt.

The distribution of cobalt in the soil profile was studied by Bertrand and Mokragnatz (1924) who reported an accumulation of cobalt in the surface horizons of various soils. Data by Slater, Holmes and Byers (1937) also indicate that the upper horizons of several soil profiles were higher in cobalt than the lower horizons. However, studies by Hill, Toth and Bear (1953) indicated that although in several soils of the Appalachian Province cobalt content was higher in the A horizon than in the B, the situation was reversed in the soils of the coastal plain province. They also reported that the cobalt content of the different particle-sized fractions from two soils, increased with a decrease in the size of the particles.

The quantity of cobalt in plants, mainly pasture herbage, has been reported by several investigators. Askew and Maunsell (1937) and Stanton and Kidson (1939) reporting on the cobalt content of pasture herbage from the Nelson district, New Zealand found 0.06 to 1.26 parts per million and 0.03 to 0.05 parts per million. Rigg (1940_a) found that pastures of the Marlborough district of New Zealand, contained 0.04 to 0.16 parts per million of cobalt. Analyses by Underwood and Harvey (1938) of pasture plants in the Denmark region of Australia indicated that healthy pastures had an average value of 0.04 parts per million.

Beeson, Gray and Adams (1947) determined the cobalt content of several common grasses grown on a fine sandy loam soil and the range of cobalt was found to be from a low of 0.05 parts per million in Natal grass to a high of 0.14 parts per million for the Kentucky bluegrass.

The cobalt content of various plant species grown in New Jersey under uniform soil conditions were recently reported by Hill, Toth and Bear (1953). These workers found that legumes ranged from 0.12 to 0.27 parts per million for the mature soybean plant and second cutting alsike clover respectively. It was noted that grasses contained from 0.05 parts per million for the sudan grass to 0.20 parts per million for Kentucky bluegrass. The cobalt content of cereals varied from 0.04

parts per million for the corn plant to 0.70 parts per million for rye, while for vegetable crops it was reported that the onion bulb contained as little as 0.02 parts per million and the pepper plant was high with a value of 0.31 parts per million. In the weeds examined, the cobalt content varied from 0.08 to 0.32 parts per million. Wright and Lawton (1954) reported the cobalt content of oat plants grown on various soils as ranging from 0.04 to 0.38 parts per million and that of timothy plants as varying from 0.05 to 0.20 parts per million.

Along with an increasing amount of research on the cobalt content of soils and plants, attempts were made to enrich the existing data with information pertaining to general factors influencing the availability of cobalt and its uptake by various plants. Askew and Dixon (1937) observed that in a pot experiment with dogstail ryegrass and white clover, a soil treatment of 200 pounds of cobalt chloride per acre increased the cobalt content of the dry plant tissue from an average of 0.2 parts per million to as high as 122.0 parts per million. However, such large applications of cobalt chloride were toxic to many pasture plants, especially white clover.

Riceman and Donald (1938) carried out plot tests and observed that as much as five pounds of cobalt chloride per acre depressed the growth of oats, wheat and barley.

Rigg (1940_b) found that a southland ground limestone containing five parts per million of cobalt when applied at the rate of three tons per acre increased the cobalt content of the pasture to the same extent as four ounces of cobalt sulphate per acre. A somewhat similar study by Askew (1943), using a southland limestone which contains five parts per million of cobalt, indicated the cobalt content of herbage was increased slightly, although this effect was of short duration. When a Nelson limestone having a very low cobalt content was used, a depression was observed in the cobalt content of the grass. He also reported that on a poor pasture an application of 200 pounds of cobaltized superphosphate per acre, carrying the equivalent of 16 ounces of cobalt sulphate, resulted in a marked improvement in the cobalt status of the forage.

Maunsell and Simpson (1944) demonstrated that the cobalt content of pasture grasses could be satisfactorily increased by adding the cobalt sulphate mixed with various carriers such as superphosphate, superphosphate and lime, lime, beach sand and other media. It was also observed by Askew (1946) that the cobalt content of a pasture increased from 0.03 to 0.22 parts per million three weeks after an application of four ounces of cobalt sulphate per acre. However, the amount of cobalt in the pasture dropped rapidly to 0.09 parts per million after 16 months. Application of any one of several cobalt compounds, including the sulphate, hydroxide, carbonate and phosphate was found by Askew and Watson (1946) to effectively increase the cobalt content of pasture herbage over a period of 16 months.

Stewart (1946) observed that top-dressing with two pounds of cobalt sulphate per acre on first-year grass was sufficient to ensure adequate cobalt in the third-year grass. It was noted by Rossiter, Curnow and Underwood (1948) that the use of cobalt had no significant effect during its growth stage and on the yield of subterranean clover. They also indicated that plant uptake of applied cobalt was low. At the rates of application used, four ounces and eight ounces of cobalt sulphate per acre, about one percent of the applied cobalt was recovered in the clover plants over a two-year period. These investigators noted that uptake of cobalt by plants was approximately related to the amount supplied.

One of the problems which has been studied rather intensively is that of the effect of liming on the cobalt uptake by plants. Askew and Dixon (1937) reported that the use of ground limestone in conjuction with cobalt resulted in a reduction of the cobalt content by plants.

In an anonymous (1943) publication from New Zealand data similar to the above observations were reported. It was also concluded that the addition of magnesium carbonate or sulphate to the fertilizer did not affect the cobalt content of the pasture forage.

According to Mitchell (1946) an application of five tons of ground limestone per acre decreased the uptake of cobalt by red clover from 0.22 to 0.18 parts per million. In cases where cobalt chloride was applied at the rate of two pounds

per acre, the limestone decreased the cobalt content of the plants from 0.89 to 0.53 parts per million. On liming, a somewhat similar reduction in the absorption of cobalt by the plant where cobalt was applied was observed by Beeson, Gray and Hamner (1948). However no significant effect on the absorption of cobalt was noted in cases where no cobalt addition to the soil was administered.

The work of Hill, Toth and Bear (1953) showed that a reduction of the available cobalt occurred as the pH of the soil increased.

Wright and Lawton (1954) pointed out that dolomite caused a decrease in the uptake of native soil cobalt by oat plants. Plant uptake was reduced considerably by the application of dolomitic limestone, even with soil treatments of added cobalt. In contrast to the above reports, Watson (1943) noted that the cobalt content of pasture herbage was not significantly changed by additions of low and high cobalt content limestone to the soil.

Less data have been published concerning the relation between the total cobalt content of soils and the amounts available to and taken up by the plant. Askew and Maunsell (1937) were unable to find a relationship between the total cobalt content of the soil and the amount determined in the pasture herbage growing on it. This conclusion was also confirmed by the work of Stanton and Kidson (1939).

Mitchell (1945) pointed out that the bulk of the cobalt of soils is bound up in the crystal lattice of minerals and is not readily available to the plant. From his data he concluded that the cobalt extracted with 2.5 percent acetic acid cannot be considered truly exchangeable as are potassium and magnesium.

The solubility relationships of cobalt to various cations and anions were studied by Young (1949). According to his observations, the presence of cations like Na, K, Ca, Mg, and NH_4 had only a slight effect on the solubility of cobalt. Although this element was found to be soluble in acid solutions, it precipitated rapidly when certain pH values were reached, depending upon the anion used. In the presence of chloride, nitrate, sulphate and acetate ions precipitation of cobalt was almost completed at pH 8.5 while with the phosphate ion such precipitation occurred at pH 6.1.

As the use of radioactive isotopes in soil chemical studies has expanded, information has been obtained by several investigators on chemical problems of the nature and exchange of plant and soil cobalt by using tracer techniques with radio cobalt 60.

Thus, Banerjee, Bray and Melsted (1953) reported on two forms of cobalt in soils; that of an easily exchangeable form extracted with neutral normal ammonium acetate like the other exchangeable bases and a strongly adsorbed form which was extracted almost completely by leaching with 0.1 normal hydrochloric acid. They also observed that cobalt in the easily exchangeable form was held by the soil colloids more strongly than the other common cations with the exception of hydrogen.

The results of studies by Spencer and Gieseking (1954) with ion exchangers and radio cobalt agree with those obtained above in relation to the exchangeability of cobalt. They concluded that the ease of exchange of cobalt in field soils decreased as the amount of adsorbed cobalt became less. Their studies also showed that cobalt is adsorbed on the exchangers in two ionic states as (1) the monovalent hydroxy-cobaltous ion and (2) the divalent cobaltous ion.

III. EXPERIMENTAL AND RESULTS

A. Analytical Procedures

Because the heterogeneity of the samples involved necessitated various methods in their preparation for a common spectrographic procedure, it was thought justifiable to present the analytical methods in two separate parts, the chemical and the spectrographic.

1. Chemical

The chemical portion of the analytical procedures for the plant and soil samples is discussed up to the point preceding the preconcentration of cobalt, which was applied to all the samples regardless of their varying nature.

a. Preparation of plant samples

Ten grams of well ground, oven dry plant material were weighed in porcelain crucibles and placed in the muffle furnace. The samples were ashed overnight at 500° C.

After ashing, the residue was taken up in two milliliters of concentrated nitric acid and the mixture was evaporated to dryness under an infrared lamp. The crucibles were then placed back in the muffle furnace for a second ashing at 400° C for approximately four hours.

Upon completion of the second ashing, the residue was treated with ten milliliters of 1:1 hydrochloric acid solution and the silica was removed by filtering through a Whatman #42 filter paper.

The silica residue was washed several times with distilled water and the combined filtrate and washings were received in 400 milliliter beakers and made up to an approximate volume of 150 milliliters with distilled water.

b. Preparation of soil samples

For the determination of the available cobalt content of the soils under study, a modification of the Mitchell (1948) method has been employed.

Twenty grams of soil, previously screened through a two millimeter sieve, were weighed and shaken in an end over end shaker with 500 milliliters of 2.5 percent acetic acid for an overnight period.

The extract was filtered through an 18 centimeter Whatman #30 filter paper. The filtrate was evaporated to near dryness and the residue was treated with nitric acid in order to oxidize the organic matter.

The residue was treated with ten milliliters of 1:1 hydrochloric acid and the silica filtered off.

The filtrate was diluted to 150 milliliters with distilled water.

For the determination of total cobalt in the soils and their fractions of sand and silt, the method presented by Holmes (1945) has been used to prepare the samples for preconcentration and the subsequent spectrographic determination.

A well mixed air-dried sample of soil, sand or silt was ground in an agate mortar and passed through a 100-mesh bolting cloth sieve.

A five gram portion of the sample was weighed and digested with five milliliters of concentrated nitric acid for two hours on a hot plate. Twenty-five milliliters of sixty percent perchloric acid was added and the digestion continued for one hour at boiling temperature. The temperature then was raised and the cover glass adjusted so the acid fumes could escape until the sample was almost dry. High temperature and reduced volume accomplish the dehydration of silica without its retention of any cobalt.

The residue was quantitatively transferred to a flask and after the addition of fifty milliliters of 6N hydrochloric acid, it was boiled under refluxing conditions for twenty to thirty minutes. The solution was cooled and quantitatively transferred through a #42 Whatman filter paper into a 400 milliliter beaker. The residue on the filter paper was washed a few times with hot 6N hydrochloric acid.

For soils, sands and silts, a diethyl ether extraction of the iron present was found to be necessary as a high amount of iron present interferes with the spectrographic determination of cobalt. The ether extraction procedure used was that described by Hillebrand et al. (1953). After the ether extraction the solutions were brought up to 150 milliliters with distilled water.

2. Spectrographic

Spectrochemical methods have been introduced and employed by several investigators in determining trace constituents of plant materials. Scott and Mitchell (1943) introduced a spectrographic method for determining cobalt along with several other trace elements in soils and plant material. This method requires a chemical preconcentration employing organic precipitation agents such as 8-hydroxyquinoline alone or a mixed reagent composed of 8-hydroxyquinoline, tannic acid and thionalide.

The technique adopted was that of the precipitation of the trace constituents by a carrier precipitate of Al and Fe quinolinates at pH 5.0 from a solution buffered with ammonium acetate.

A thorough examination of this technique was undertaken with the purpose of investigating the method of Scott and Mitchell (1943). This procedure was adapted with certain modifications, some of which were based on recent work by Borrell and Paris (1950) on the chemistry of the metal quinolinates, to the facilities of the spectrographic laboratory of the Michigan Agricultural Experiment Station and the investigator's research work.

a. Apparatus, Reagents, Standard solutions

The special apparatus used in the spectrographic analysis of the samples were as follows:

Excitation source:	National Spectrographic Laboratory source unit.
Spectrograph:	Hilger littrow quartz spectrograph.
Recording equipment:	Kodak spectrum analysis No. 1 spectrographic plate.
Microphotometer:	Jarrell Ash Company recording microphotometer.
Developing equipment:	National Spectrographic Laboratory developing and drying equipment.

The special reagents used were:

1. 8-hydroxyquinoline (oxine, C_9H_7ON), five percent solution in 2N acetic acid.

To 114 milliliters of glacial acetic acid, 50 grams of oxine powder were added and the solution was made to one liter with distilled water. If difficulty was experienced in dissolving the oxine, the solution was warmed on a steam bath.

2. Ammonium acetate, 2N neutral solution.

To 144 milliliters of glacial acetic acid, 150 milliliters of distilled water were added. Gradually and with agitation, 135 milliliters of concentrated ammonium hydroxide were added and the pH of the solution was adjusted to 6.9 with glacial acetic acid or ammonium hydroxide added as seen necessary. The solution is diluted to one liter with distilled water.

3. Standard solutions.

   a. Aluminum standard solution, 1 milliliter = 5 milligrams of aluminum in solution.

The solution was prepared by dissolving 69.5475 grams of aluminum nitrate ($Al(NO_3)_3 \cdot 9H_2O$) in distilled water and diluting to one liter.

b. Cobalt standard solution.

A stock solution containing 100 milligrams of cobalt per liter of solution or 100 parts per million of cobalt was made by dissolving 0.4040 grams of a pure grade salt of cobalt ($CoCl_2.6H_2O$) in distilled water and diluting to one liter. From the above solution, solutions containing the desired cobalt concentrations were obtained by dilution.

c. Standard synthetic mixture.

A standard synthetic mixture was prepared to approximate the composition of the plant ash or soil extracts in the elements which are complexed by 8-hydroxyquinoline.

In Table 1 data are presented relative to the kind and amount of salts used in preparing the stock solutions. The amounts of the stock solutions used to prepare the synthetic standard were based on the approximate chemical composition of the plant or soil material and the quantity of the material taken. To this solution the amounts of cobalt standard solution necessary for establishing the desired range of cobalt concentration were added and the solutions diluted to 150 milliliters with distilled water.

b. Preconcentration

To the standard and the sample solutions which were diluted to 150 milliliters volume with distilled water, three milliliters of the stock aluminum solution (1 ml = 5 mg) and ten milliliters of five percent 8-hydroxyquinoline solution were added.

TABLE 1.

CONCENTRATIONS OF STOCK SOLUTIONS OF VARIOUS ELEMENTS USED IN PREPARING THE SYNTHETIC STANDARD MIXTURE

Element	Compound	Grams of compound in 200 ml of solution*	Concentration
Fe	Fe powder	0.2000	1 ml. = 1 mg Fe
Mg	MgO	1.6579	1 ml. = 5 mg Mg
Cu	$CuSO_4.5H_2O$	3.9281	1 ml. = 5 mg Cu
Zn	ZnO	0.6224	1 ml. = 2.5 mg Zn
Mo	$H_2MoO_4.H_2O$	0.9379	1 ml. = 2.5 mg Mo
Mn	$MnCl_2.4H_2O$	0.7205	1 ml. = 1 mg Mn

* All compounds were dissolved in distilled water with the exception of the iron powder and magnesium oxide which were dissolved in 1:1 hydrochloric acid solution and zinc oxide which was dissolved in dilute acetic acid.

Concentrated ammonium hydroxide, added dropwise, was used to raise the pH of the solution to the desired pH of 1.9. The reaction was controlled with a pH meter. At the proper pH the solution has an emerald green color, while at a pH of 2.5 or above, a precipitate starts to form. The precipitate may be dissolved by lowering the pH with the dropwise addition of 1:1 hydrochloric acid solution.

The solution was buffered with fifty milliliters of 2N neutral ammonium acetate solution to a pH of about 5.0. Rapid stirring is advisable for the formation of a flaky precipitate which is allowed to stand for about twelve hours with occasional stirring and then filtered through a 12.5 cm #40 Whatman filter paper and washed several times with distilled water.

Filter paper and precipitate were transferred to porcelain crucibles and dried under the infrared lamp. The crucibles were then placed in the muffle furnace and the temperature was slowly raised to 700° C for the final ashing which lasted approximately five hours.

c. Preparation of arcing mixture and electrodes

After ashing, the residue, composed of metallic oxides, was weighed and made up to 80 milligrams for the plant samples and to 100 milligrams for the soil samples with purified carbon powder. The mixture was then pulverized in an agate mortar and stored in a dry container ready to be used in the preparation of the electrodes.

The amount of arcing mixture used on the electrodes depends upon the amount of cobalt present in the material to be analyzed. The various amounts used are presented along with the standard curves.

Standard spectroscopic grade carbon rods, twelve inches in length and three-sixteenths of an inch in diameter, were employed. Craters approximately four millimeters in diameter were drilled in the lower as well as the upper electrode to a depth of 3.5 millimeters with a wall thickness of 0.5 of a millimeter.

The arcing mixture was placed in the lower crater with the help of a special plastic funnel which fitted tightly on the electrode, thus eliminating any possible loss while transferring the mixture.

After packing with a glass rod and immediately preceding the arcing, the mixture in the lower electrode was wetted throughout with a very small amount of 1:1 hydrochloric acid solution.

d. Excitation conditions

The electrodes were positioned and spaced three millimeters apart on the arc stand unit of the spectrograph.

The arcing period varied with the amount of the mixture on the electrode and was selected on the basis of complete burning of the sample. The voltage on the line was 220 volts and the amount of direct current required for arcing was nine amperes.

A slit width of 25 microns was used and an aperture setting was established for a spectrum three millimeters wide. The range of the spectrograms was from 2,650 to 4,200 angstrom units wavelength.

A condensing lens for even illumination of the slit was used, and a ten percent filter incorporated into the optical path which serves to divide each spectrum into two portions of varying intensity (10% and 100% transmission). This setting was selected in order to provide a greater range for comparison.

e. Calibration of photographic emulsion

Since the internal standard method involves the determination of intensity ratios, the first step was to convert the data obtained from the microphotometer to relative intensities. This was accomplished by utilizing a pair of lines having similar excitation characteristics and the intensity ratio of which remains constant under ordinary operation conditions. This method is the so called two-line method of emulsion calibration.

The carbon lines, 2,836 and 2,837 angstrom units, the intensity ratio of which is 1.5, were selected for this caliration. The illumination of the slit was varied in small steps by varying the distance from the source to the slit from exposure to exposure.

From the percent transmission data, a graph was prepared with logarithmic coordinates by plotting each deflection recorded for one of the members of the calibration pair against the deflection for the other member in the same spectrum. This so called preliminary curve provides a means of determining for any particular deflection produced by an intensity I the deflection that would be produced by an intensity equal to 1.5 I.

The final emulsion calibration curve was prepared by plotting a series of points taken from the preliminary curve against the intensity ratio of the line pair. The intensity ratio of the internal standard line and the analysis line may be determined by referring the deflection obtained on the microphotometer to the emulsion calibration curve and dividing the numerical values of relative intensity obtained. This procedure is simplified when the emulsion calibration curve is projected onto a metal strip and used in conjunction with a sliding logarithmic scale.

f. Plate processing

The plate was developed for three minutes at 70° F in D-19 developer, rinsed in a five percent acetic acid stop bath for about fifteen seconds and fixed with Kodak acid fixer for ten minutes.

The plate was washed under a continuous stream of water for ten to twenty minutes and then was spray washed with distilled water, sponged and dried over a warm current of air.

g. Photometry

The internal standard method was employed to provide some degree of correction for variations in excitation, exposure, and response of the photographic emulsion. A weak line of the major constituent aluminum (3064.30 angstrom units) was selected as the internal standard, due to its stability and proximity to cobalt line.

Unfortunately, the small amount of cobalt in plant material limited the choice of cobalt line to the "raies ultimes" located at 3453.51 angstrom units. Since this region of the spectrum exhibits considerable background due to carbon (CN band spectra), a background correction was applied when necessary by adjusting the microphotometer deflection to 100 for the background near the cobalt and aluminum lines. By adjusting the background deflection to 100 and the deflection of an image of infinite density (closed slit) to zero, the microphotometer readings were considered percent transmission.

The vertical system of photometric measurement was used in which the operator measures a spectrum line of a given wavelength in all the spectra on the plate before proceeding to the next line.

To facilitate calculation of intensity ratios the emulsion calibration curve was projected onto a scale which was used slide rule fashion with a logarithmic abscissa scale (Churchill, 1944).

h. Standard curves

A series of standard curves was established. This was found to be necessary because of the varying nature of the three groups of samples analyzed (plant, soil and soil separates) and the initial weight of the sample used for the determination of cobalt. In addition the method of preparation of the sample and the range of the cobalt content of each group of samples affected the constants on which the standard curves were based.

The values were plotted on 2 x 2 cycle logarithmic paper with the logarithm of the relative intensity of the cobalt to the aluminum line plotted on the abscissa while the concentration of cobalt expressed in parts per million was plotted on the ordinate.

In drawing the curves, the average value of several determinations of the logarithm of relative intensity for each standard amount of cobalt was used in establishing the path of the curve.

The constants entering the construction of each standard curve are presented in Tables 2 to 6 inclusive and Figures I to V inclusive.

TABLE 2.

CONCENTRATIONS OF COBALT IN THE STANDARD SOLUTIONS AND LOGARITHM OF RI VALUES USED FOR ESTABLISHING A STANDARD CURVE FOR THE EVALUATION OF COBALT IN PLANT MATERIAL (FIGURE I)

Cobalt	Logarithm of Relative Intensity (RI)							
ppm	1	2	3	4	5	6	7	Average
0.04	0.154	0.159	0.168	0.158	0.149	0.126	0.137	0.150
0.08	0.186	0.175	0.184	0.235	0.213			0.198
0.12	0.171	0.182	0.261	0.204	0.239			0.211
0.16	0.280	0.248	0.245	0.275	0.285			0.266
0.20	0.280	0.330	0.320	0.32				0.310
0.25	0.370	0.350	0.370					0.360
0.30	0.350	0.410	0.340					0.370
0.40	0.490	0.460	0.430					0.450

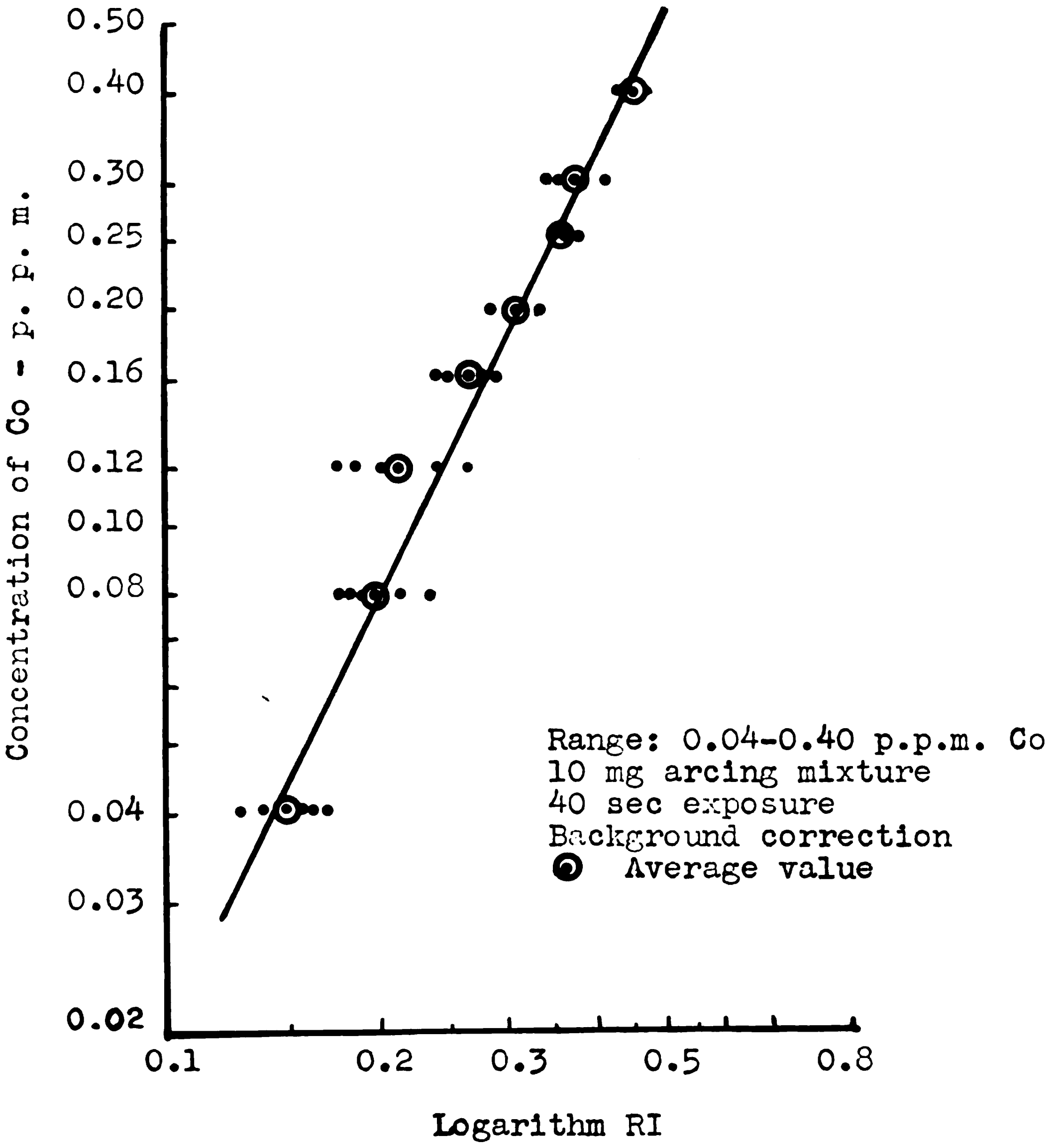

Figure I. Standard curve for the evaluation of cobalt in plant material.

TABLE 3.

CONCENTRATIONS OF COBALT IN THE STANDARD SOLUTIONS AND LOGARITHM OF RI VALUES USED FOR ESTABLISHING A STANDARD CURVE FOR THE EVALUATION OF COBALT IN PLANT MATERIAL (FIGURE II)

Cobalt	Logarithm of Relative Intensity (RI)							
ppm	1	2	3	4	5	6	7	Average
0.40	0.53	0.41						0.47
0.50	0.60	0.77						0.68
0.75	0.72	0.82	0.76	0.76	0.89	0.71		0.78
1.00	0.94	1.05	1.18	0.92	0.82	1.10		1.00
1.50	1.42	1.72	1.68	1.94	1.42	1.49	1.89	1.65
2.00	2.24	1.91	2.40	2.16	1.96	2.36	2.43	2.21
3.00	3.18	4.28	3.14	3.54	3.08	4.08		3.55
5.00	7.15	4.10	6.80	4.90	6.90			5.97
7.50	7.30	9.20	8.60	8.70				8.45

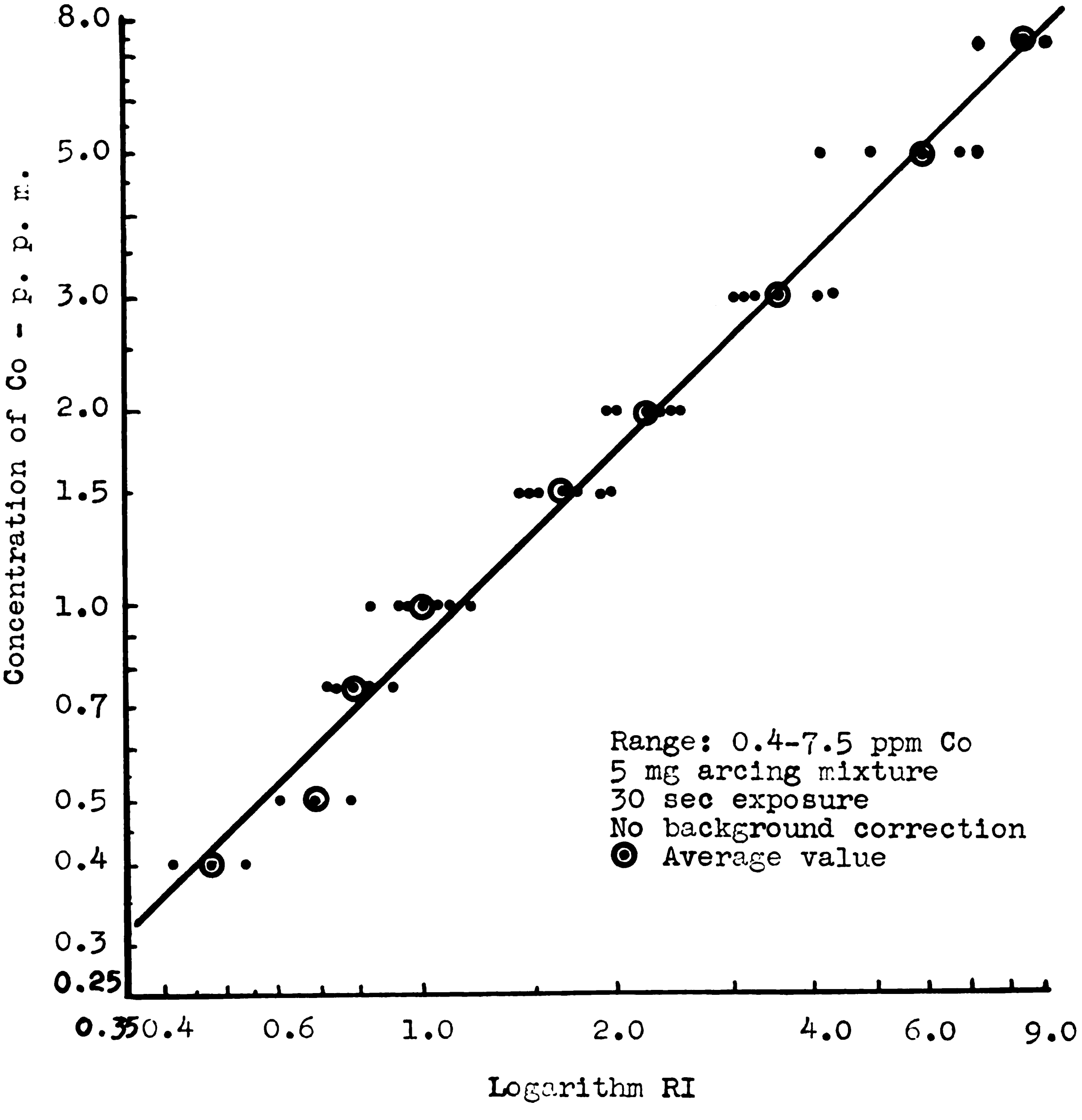

Figure II. Standard curve for the evaluation of cobalt in plant material.

TABLE 4.

CONCENTRATIONS OF COBALT IN THE STANDARD SOLUTIONS AND LOGARITHM OF RI VALUES USED FOR ESTABLISHING A STANDARD CURVE FOR THE EVALUATION OF COBALT IN PLANT MATERIAL (FIGURE III)

Cobalt ppm	Logarithm of Relative Intensity (RI) 1	2	3	4	Average
1.0	1.53	1.08	1.28		1.30
1.5	1.53	1.57	1.88	1.72	1.67
2.0	2.04	1.92	2.49	1.84	2.07
3.0	4.44	2.53	3.12		3.36
5.0	6.55	6.15	6.30	6.94	6.48
7.5	6.52	8.79	8.97	7.60	7.97
10.0	9.22	13.15	11.20	11.10	11.17
20.0	18.42	19.68	15.57		17.89

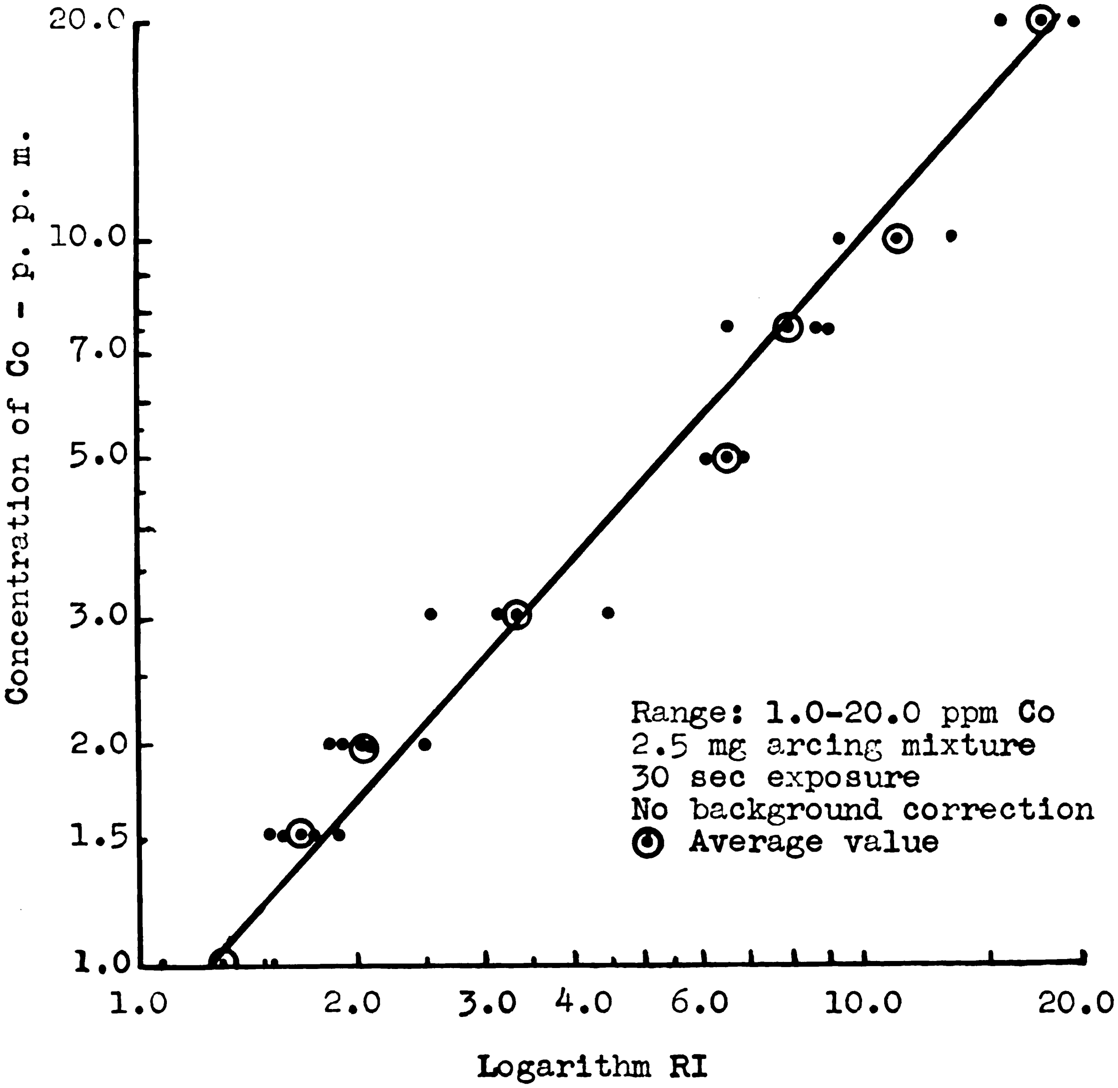

Figure III. Standard curve for the evaluation of cobalt in the plant material.

TABLE 5.

CONCENTRATIONS OF COBALT IN THE STANDARD SOLUTIONS AND LOGARITHM OF RI VALUES USED FOR ESTABLISHING A STANDARD CURVE FOR THE EVALUATION OF COBALT IN ACETIC ACID EXTRACTS OF SOILS (FIGURE IV)

Cobalt	Logarithm of Relative Intensity (RI)					
ppm	1	2	3	4	5	Average
0.10	0.990	0.945	0.975	0.963	0.977	0.970
0.20	1.140	1.030	1.380	1.180	1.110	1.170
0.40	1.720	2.720	2.080	1.520	1.770	1.960
0.70	2.290	1.690	1.940	1.640	1.660	1.840
1.00	2.480	3.580	2.190	1.840	2.010	2.420

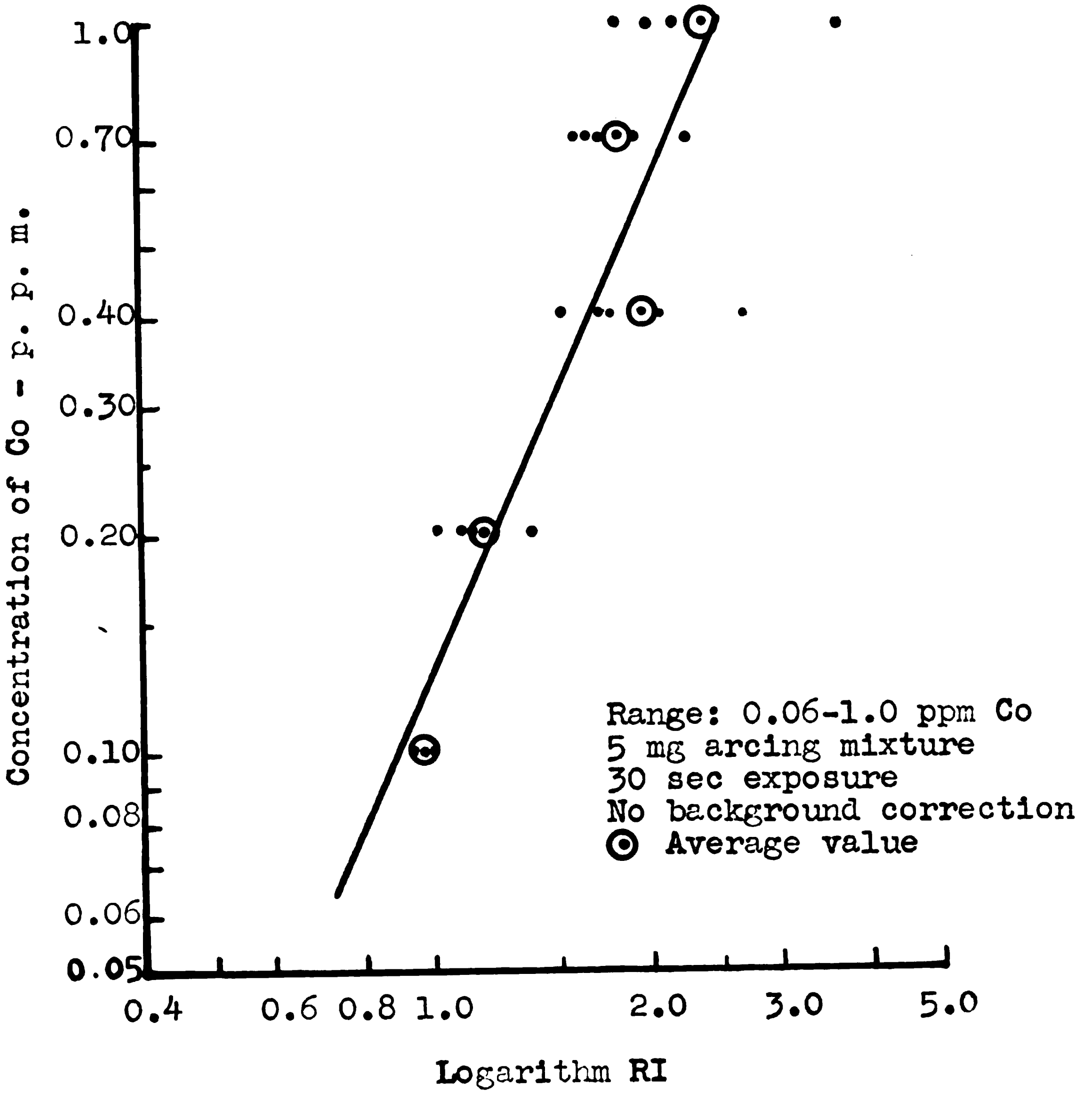

Figure IV. Standard curve for evaluating available cobalt in soils.

TABLE 6.

CONCENTRATIONS OF COBALT IN THE STANDARD SOLUTIONS AND LOGARITHM OF RI VALUES USED FOR ESTABLISHING A STANDARD CURVE FOR THE EVALUATION OF TOTAL COBALT IN SOIL SAND AND SILT SAMPLES (FIGURE V)

Cobalt	Logarithm of Relative Intensity (RI)					
ppm	1	2	3	4	5	Average
0.50	0.610	0.800	0.645	0.610		0.666
1.00	0.895	1.010	0.975	0.825		0.926
2.00	1.205	1.410	1.275	1.495		1.350
3.00	1.450	2.250	1.395			1.690
4.00	1.910	1.880	1.840	1.730	2.110	1.890
5.00	1.840	1.670	2.240	2.380		2.030
7.00	2.490	3.700	3.090			3.090

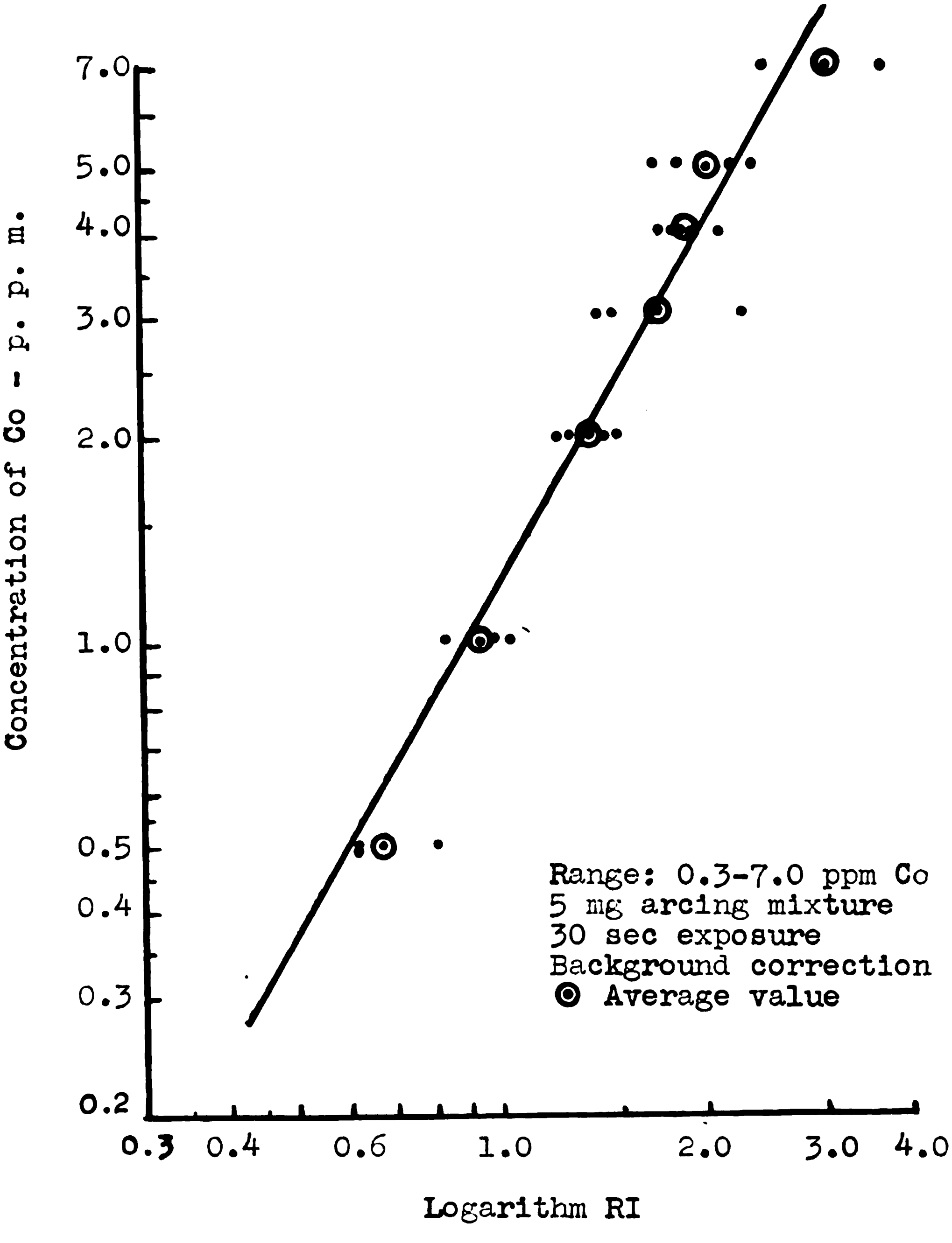

Figure V. Standard curve for evaluating the total cobalt content of soils, sand and silt.

i. Comparative tests

Comparison of results from the spectrographic procedure and from the chemical method involving use of nitroso-R-salt were made with plant samples received upon request from Dr. E. J. Benne of the Agricultural Chemistry Department of Michigan State University and from Dr. K. C. Beeson of the United States Plant, Soil and Nutrition Laboratory, Ithaca, New York.

The results of these tests are presented in Table 7.

The data presented in Table 7 indicate that most of the results of the spectrographic method agree very well with those obtained by the chemical method. The usual deviation between values obtained by the two methods is 0.01 of a part per million which may be considered very satisfactory. It is also observed that the spectrographic method tends to give lower results.

j. Discussion

During the course of the investigation of the method, several problems pertaining to the accuracy of the procedure and the reproduceability of the results have been solved.

The control of pH values at the stages of complexing and precipitation, the preparation of the arcing mixture, the selection of the proper shape for the carbon electrodes, the elimination of background difficulties, the proper amounts of

TABLE 7.

COMPARATIVE STUDY BETWEEN THE SPECTROGRAPHIC AND COLORIMETRIC PROCEDURES IN DETERMINING COBALT IN PLANT MATERIAL

	Parts per million of cobalt in plant material							
	Spectrographically					Colorimetrically		
Sample	1	2	3	4	Ave.	1	2	Ave.
Hay 1	0.08	0.07	0.04	0.02	0.05	0.063	0.063	0.06 *
Hay 2	0.18	0.11	0.26	0.12	0.17	0.171	0.171	0.17
Hay 3	0.08	0.06	0.03	0.03	0.05	0.063	0.046	0.05
Hay 4	0.20	0.06	0.12	0.13	0.12	0.074	0.069	0.07
Hay 5	0.05	0.05			0.05			0.06
Hay 6	0.08	0.08			0.08			0.08
Hay 7	0.04	0.05			0.05			0.08
Hay 8	0.05	0.06			0.06			0.09
Hay 9	0.07	0.06			0.07			0.08
Hay 10	0.04	0.05			0.05			0.06
Hay 11	0.08	0.07			0.08			0.09
Alfalfa Hay	0.12	0.13			0.13			0.14**
Timothy Hay	0.06	0.16			0.06			0.05**
Buckwheat Flour	0.06	0.07			0.07			0.05**

* Determinations of cobalt in hays 1-11 inclusive, by Mrs. Doris Jerrim, Agricultural Chemistry Department, Michigan State University, East Lansing, Michigan.

** Average of values obtained by several investigators, Dr. K. C. Beeson, associate referee, A. O. A. C., Ithaca, New York.

the 8-hydroxyquinoline and aluminum internal standard solutions used during the procedure, and certain other minor points have been investigated.

It is important that pH of 1.9 be closely controlled, a fact enabling the correct buffering of the solution to pH 5.0 at which precipitation takes place. Consequently the use of a pH meter is recommended for establishing the pH value of 1.9 in order to avoid variances that may occur if other means are employed. The pH of the buffered solution was also checked immediately after the addition of 50 milliliters of 2N ammonium acetate solution and it was found to be 5.0 to 5.1, which is within the desirable range for the precipitation of aluminum and cobalt (Willard and Diehl, 1943).

An attempt was made to simplify the quantitative determination of cobalt by eliminating the chemical complexing and precipitation from the procedure. The ash of two different plant materials containing 0.27 and 0.09 parts per million of cobalt, as determined colorimetrically, was taken up in three milliliters of 2:1 hydrochloric acid. From these solutions, measured amounts were placed on flat carbon electrodes and were dried under an infrared lamp. This procedure was repeated as many as five times on different electrodes in an effort to increase the amount of cobalt in the arc. From the sample containing 0.09 parts per million of cobalt, the spectrograms revealed no measurable cobalt lines, even with the electrodes receiving five additions of the solution. In the case of the plant material containing 0.27 parts per million of cobalt, measureable lines appeared, the density of which varied proportionately with the amount placed on the electrodes. As the scope of this investigation is concerned with much smaller concentration of cobalt than 0.09 parts per million, elimination of chemical preconcentration was not considered advisable.

With the sudden release of energy accompanying the formation of the arc, the arcing mixture shows a tendency to spatter or spray out of the crater. In order to eliminate this sample loss in the semi-final stage of the procedure, various shapes of upper electrode were investigated. The shapes tried were flat, pointed and cratered to the same dimensions as the lower electrode. From observations during arcing, as well as from studying the spectrograms on the plate, it was decided that cratered, upper electrodes were by far more satisfactory than the other types.

Thorough wetting of the arcing mixture with 1:1 hydrochloric acid solution was employed and gave satisfactory results by eliminating the spattering of the sample to a very minimum. The above practices have been found to be very satisfactory for this procedure.

In an effort to eliminate photometric difficulties due to high background on the spectra, the amperage of the arc, and the exposure time were examined. Several methods for the preparation of the arcing mixture were studied.

A plate was prepared in which the amount of the arcing mixture in the electrode was kept constant (10 milligrams) while the amperage and the exposure time were varied. It was concluded that the mixture which was arced with nine amperes for 60 seconds exposure time gave the most satisfactory results.

A comparison carried out between high purity carbon electrodes and regular spectroscopic grade carbon electrodes indicated that in the case of the former an extremely high background on the spectrograms prevented the accurate measurement of the density of the cobalt lines.

Because of the appreciable amount of sample lost during excitation with the d.c. arc, a trial plate was prepared employing the a.c. spark as the means of excitation. Although it is known from the tables by Harrison (1939) and the report of Heggen and Strock (1953), that the density of the cobalt line produced by means of a.c. spark excitation is far less than that produced by the higher energy d.c. arc, it nevertheless warranted a trial in view of the definite advantages offered by this means of excitation. For instance, spark excitation brings about a minimum of spattering of the sample resulting in more uniform results and less background on the spectrogram. Comparing the spectrograms obtained from these studies, it was observed that although the above advantages are apparent, this approach was not advisable due to the very low density of the cobalt line resulting from the incomplete burning of the sample. This situation, resulting from the a.c. spark excitation, could probably be remedied by extremely long exposure times.

The amount of the aluminum internal standard solution as well as that of the 8-hydroxyquinoline solution has been investigated. To the filtrate, after complexing and precipitation of the metals, ten milliliters of five percent 8-hydroxyquinoline solution were added and after the necessary pH adjustments it was left to stand overnight. Formation of new precipitate did not take place, indicating thus that all metals present in the solution had been precipitated following the addition of the first ten milliliters of 8-hydroxyquinoline. The proper amount of the aluminum standard solution to use was determined by comparative tests.

Varying the amount of aluminum from 15 to 500 milligrams and maintaining the amount of cobalt the same in a series of five standards, it was concluded from studying the spectrograms, that the relative intensity of the 3,064.30 angstrom aluminum line as compared to the 3453.51 cobalt line remained virtually unchanged. It was decided that three milliliters of the aluminum solution or 15 milligrams of aluminum was a satisfactory amount for the complete complexing and precipitation of the cobalt present in the solution.

The temperature of the final ignition stage was raised to 700° C for four hours. This temperature of ignition of the metallic quinolinates, was found to be necessary as it became apparent from comparative tests, that at 450° C the density of the aluminum lines was erratic. Such variance is most probably due to partial volatilization of the aluminum quinolinate in this stage of decomposition of the compound. At 700° C the completely decomposed compound reached a definite composition, that of alumina Al_2O_3 (Borrell and Paris, 1950).

B. Greenhouse Studies

A greenhouse experiment was established in which various amounts of lime material and cobalt were applied to two soils. The test plant was red clover (Trifolium pratense) and the purpose of the experiment was to determine the effect of lime on the absorption of cobalt by plants.

1. Materials and Methods

A pot experiment was set up in August of 1951 using two soils, a Hillsdale sandy loam with an original pH of 5.1 and a Coloma sand with an original pH of 5.7. Each soil was air dried, screened and mixed thoroughly. The amount of lime in the form of precipitated calcium carbonate to bring these soils to desired pH levels was determined experimentally in moist incubation trials.

The soils were placed in glazed one-gallon clay pots, the necessary amounts of calcium carbonate were added and mixed thoroughly with the soil and the whole mass was thoroughly wetted with distilled water.

A three weeks moist incubation period was considered long enough to allow the lime to react with the soil. After incubation the pH of the soils was determined in a 1:1 soil to water mixture using a Beckman pH meter.

The amounts of lime applied and the resulting pH values are presented in Table 8.

At the end of the incubation period, various chemical compounds were applied to the soil in water solutions at the rates shown in Table 9 to insure an adequate supply of nutrients for plant growth.

Compounds of all the major elements were added together in one solution and those of the minor elements in another, with the exception of boron which was added separately.

Cobalt, as cobalt sulphate heptahydrate ($CoSO_4.7H_2O$) in water solution, was applied to both soils at the rate of five, ten and twenty pounds of cobalt per acre.

The experiment was thus established with twelve different treatments for each soil. Each treatment was replicated three times. A summary of the various treatments for the two soils are presented in Tables 10 and 11.

On September 10, 1951, approximately twenty red clover seeds were planted in a circle about one-quarter of an inch below the soil surface and approximately one inch from the wall of the pot. The pots were covered with paper in order to maintain optimum soil moisture for the germination and emergence of seedlings. Ten days after seeding, the plants were thinned to ten plants per pot.

TABLE 8.

AMOUNTS OF $CaCO_3$ ADDED AND RESULTING pH VALUES OF THE SOILS

Soil type	Pounds of $CaCO_3$ per acre	Soil pH
Coloma sand	None	5.70
	250	6.20
	500	7.10
	1,000	7.50
Hillsdale sandy loam	None	5.10
	1,000	5.85
	3,000	6.50
	5,000	7.20

TABLE 9.

AMOUNTS AND FORMS OF NUTRIENT ELEMENTS APPLIED TO SOILS

Element	Form*	Amount of element Pounds per acre
Nitrogen	$(NH_4)_2SO_4$	250
Phosphorus	$Ca(H_2PO_4)_2 \cdot H_2O$	100 as P_2O_5
Potassium	KCl	100 as K_2O
Boron	$Na_2B_4O_7$	25
Manganese	$MnCl_2$	75
Copper	$CuSO_4 \cdot 5H_2O$	10
Zinc	$ZnSO_4 \cdot 7H_2O$	5
Molybdenum	$(NH_4)_2Mo_7O_{24} \cdot 4H_2O$	2.5

* All the compounds were dissolved in distilled water.

TABLE 10.

SOIL TREATMENTS* USED FOR THE COLOMA SAND

Treatment number	Pounds of $CaCO_3$ per acre	Pounds of Co per acre
1	None	5
2	250	5
3	500	5
4	1,000	5
5	None	10
6	250	10
7	500	10
8	1,000	10
9	None	20
10	250	20
11	500	20
12	1,000	20

* Each treatment was replicated three times.

TABLE 11.

SOIL TREATMENTS* USED FOR THE HILLSDALE SANDY LOAM

Treatment number	Pounds of $CaCO_3$ per acre	Pounds of Co per acre
1	None	5
2	1,000	5
3	3,000	5
4	5,000	5
5	None	10
6	1,000	10
7	3,000	10
8	5,000	10
9	None	20
10	1,000	20
11	3,000	20
12	5,000	20

* Each treatment was replicated three times.

Throughout the growing period, the maintenance of a definite moisture content in the soil was not attempted but an adequate level of moisture was maintained by surface applications of distilled water whenever needed.

The first sampling was made on February 10, 1952. The plants were cut off approximately one inch above the crown. The plant material was dried in an oven at 60° C for 48 hours and the oven dry weights recorded. A few days after the first sampling, a blanket application of nitrogen, phosphorus, and potassium was made to all pots at the rates shown in Table 9.

The second and final cutting took place on May 9, 1952 following the same procedure as before. During both cuttings extreme care was taken in order to avoid any contamination of the plant samples, especially from the soil.

The average yield values of the three replications for the first and second sampling are presented in Table 12.

After drying, the samples were ground in a small Wiley mill and the replicates of each treatment were thoroughly mixed together. This was necessary in view of the rather large analytical portions (ten grams) required for the spectrographic determination of cobalt.

The results obtained are presented and discussed in the following section.

2. Results and Discussion

a. Effect of various treatments on the yield of red clover

The average yields of the two cuttings of the red clover plants are presented in Table 12. In both cuttings the average yields of red clover were higher on the Hillsdale sandy loam than on the Coloma sand soil. Even though an adequate supply of plant nutrients were added the variation between soils was probably due to a higher inherent fertility level of the Hillsdale soil.

The analysis of variance for the yield of red clover from the two soils at two sampling dates is presented in Tables 13 to 16 inclusive. The data indicate a highly significant effect of lime on the yield of red clover. The lime treatment was not significant in affecting the second cutting of plants grown on the Coloma sand, but in this case the effect of the lime-cobalt interaction was significant. This interaction was highly significant in the yield averages of the first cutting red clover grown on the Hillsdale sandy loam soil.

In agreement with the results of other workers on the effect of cobalt on higher plants, the application of cobalt alone did not significantly affect the yields of red clover grown on soils treated with cobalt.

TABLE 12.

AVERAGE YIELD OF RED CLOVER PLANTS

Treatment	Coloma sand	Hillsdale sandy loam	Coloma sand	Hillsdale sandy loam
	First sampling		Second sampling	
	Oven dry weight per pot in grams			
1	6.56*	10.68	4.17	8.88
2	8.01	10.85	3.60	8.32
3	7.58	12.21	4.46	8.72
4	8.28	9.89	4.90	11.12
5	5.66	9.33	3.40	6.82
6	7.51	10.08	4.90	9.73
7	8.36	12.16	4.36	10.29
8	9.09	11.73	4.77	10.40
9	6.88	7.59	4.75	7.60
10	7.28	14.12	4.21	7.83
11	9.32	11.83	4.08	8.96
12	8.97	16.89	3.54	11.01

* All values represent the mean oven dry weight of three replicates.

TABLE 13.

ANALYSIS OF VARIANCE OF THE YIELD OF FIRST CUTTING RED CLOVER PLANTS GROWN ON A COLOMA SAND SOIL

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Standard deviation	F value
Total	35	70.42			
Replications	2	7.13			
Treatments	11	39.33			
Cobalt	2	1.75	0.88		0.807
Lime	3	30.72	10.24		9.394**
Cobalt x Lime	6	6.86	1.14		1.046
Error	22	23.96	1.09	1.046	

L. S. D. (P. 05) 1.046 x 0.847 = 0.886

TABLE 14.

ANALYSIS OF VARIANCE OF THE YIELD OF FIRST CUTTING RED CLOVER PLANTS GROWN ON A HILLSDALE SANDY LOAM SOIL

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Standard deviation	F value
Total	35	257.00			
Replications	2	20.05			
Treatments	11	167.39			
Cobalt	2	3.90	1.95		0.617
Lime	3	47.15	15.72		4.975**
Cobalt x Lime	6	116.34	19.39		6.136**
Error	22	69.56	3.16	1.778	

L. S. D. (P. 05) 1.778 x 0.847 = 1.506

TABLE 15.

ANALYSIS OF VARIANCE OF THE YIELD OF SECOND CUTTING RED CLOVER PLANTS GROWN ON A COLOMA SAND SOIL

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Standard deviation	F value
Total	35	20.18			
Replications	2	1.66			
Treatments	11	9.38			
Cobalt	2	.26	0.13		0.310
Lime	3	.40	0.13		0.310
Cobalt x Lime	6	8.72	1.45		3.452*
Error	22	9.14	0.42	0.648	

L. S. D. (P. 05) 0.648 x 0.847 = 0.549

TABLE 16.

ANALYSIS OF VARIANCE OF THE YIELD OF SECOND CUTTING RED CLOVER PLANTS GROWN ON A HILLSDALE SANDY LOAM

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Standard deviation	F value
Total	35	95.51			
Replications	2	2.22			
Treatments	11	63.78			
Cobalt	2	1.55	0.78		0.582
Lime	3	46.14	15.38		11.478**
Cobalt x Lime	6	16.09	2.68		2.000
Error	22	29.51	1.34	1.158	

L. S. D. (P. 05) 1.158 x 0.847 = 0.981

b. Effect of lime application on the cobalt content of red clover.

The results of lime and cobalt treatments on the cobalt content of red clover are reported in Tables 17 and 18 and are presented graphically in Figures VI and VII.

In the Coloma sand soil the uptake of cobalt by clover was influenced by the rate of application of lime. Generally, with an increase in the applied amount of calcium carbonate a decrease in the amount of cobalt absorbed by the plant occurred. With one exception, red clover invariably absorbed a two to four times greater amount of cobalt from the check pot than the one treated with the highest application of lime. Intermediate amounts of cobalt were absorbed from the treatments in which intermediate amounts of lime were applied.

The amount of cobalt absorbed during the growing period of the plant prior to the second cutting was considerably less in comparison to the amount absorbed prior to the first cutting.

The variation in the cobalt content of first cutting red clover grown on the Hillsdale sandy loam soil was very small, as affected by different rates of lime. This was particularly true when the lowest rate of cobalt was applied to the soil. When higher amounts of cobalt were added to this soil cobalt absorption by red clover decreased with an increase in the amount of lime applied. Similar results were also found for the red clover plants of the second cutting.

TABLE 17.

EFFECT OF LIME ON THE COBALT CONTENT OF FIRST AND SECOND CUTTING RED CLOVER PLANTS GROWN ON A COLOMA SAND SOIL

Treatment number	Pounds of $CaCO_3$ per acre	Soil pH	Pounds of Co per acre	First Cutting	Second Cutting
				Cobalt content of red clover * ppm	
1	0	5.7	5	1.10	1.33
2	250	6.2	5	1.04	0.97
3	500	7.1	5	1.33	0.83
4	1,000	7.5	5	0.92	0.46
5	0	5.7	10	2.65	1.35
6	250	6.2	10	1.96	0.97
7	500	7.1	10	1.36	0.77
8	1,000	7.5	10	1.14	0.45
9	0	5.7	20	8.40	1.76
10	250	6.2	20	3.04	1.39
11	500	7.1	20	2.86	1.02
12	1,000	7.5	20	1.78	0.60

* Average of three determinations.

TABLE 18.

EFFECT OF LIME ON THE COBALT CONTENT OF FIRST AND SECOND CUTTING RED CLOVER PLANTS GROWN ON A HILLSDALE SANDY LOAM SOIL

Treatment number	Pounds of $CaCO_3$ per acre	Soil pH	Pounds of Co per acre	First Cutting	Second Cutting
				Cobalt content of red clover* ppm	
1	0	5.1	5	0.54	0.41
2	1,000	5.8	5	0.67	0.67
3	3,000	6.5	5	1.02	1.02
4	5,000	7.2	5	0.58	0.84
5	0	5.1	10	1.55	1.32
6	1,000	5.8	10	0.62	1.05
7	3,000	6.5	10	1.35	1.00
8	5,000	7.2	10	1.29	0.81
9	0	5.1	20	3.83	2.21
10	1,000	5.8	20	1.05	1.66
11	3,000	6.5	20	0.79	1.37
12	5,000	7.2	20	0.65	0.60

* Average of three determinations.

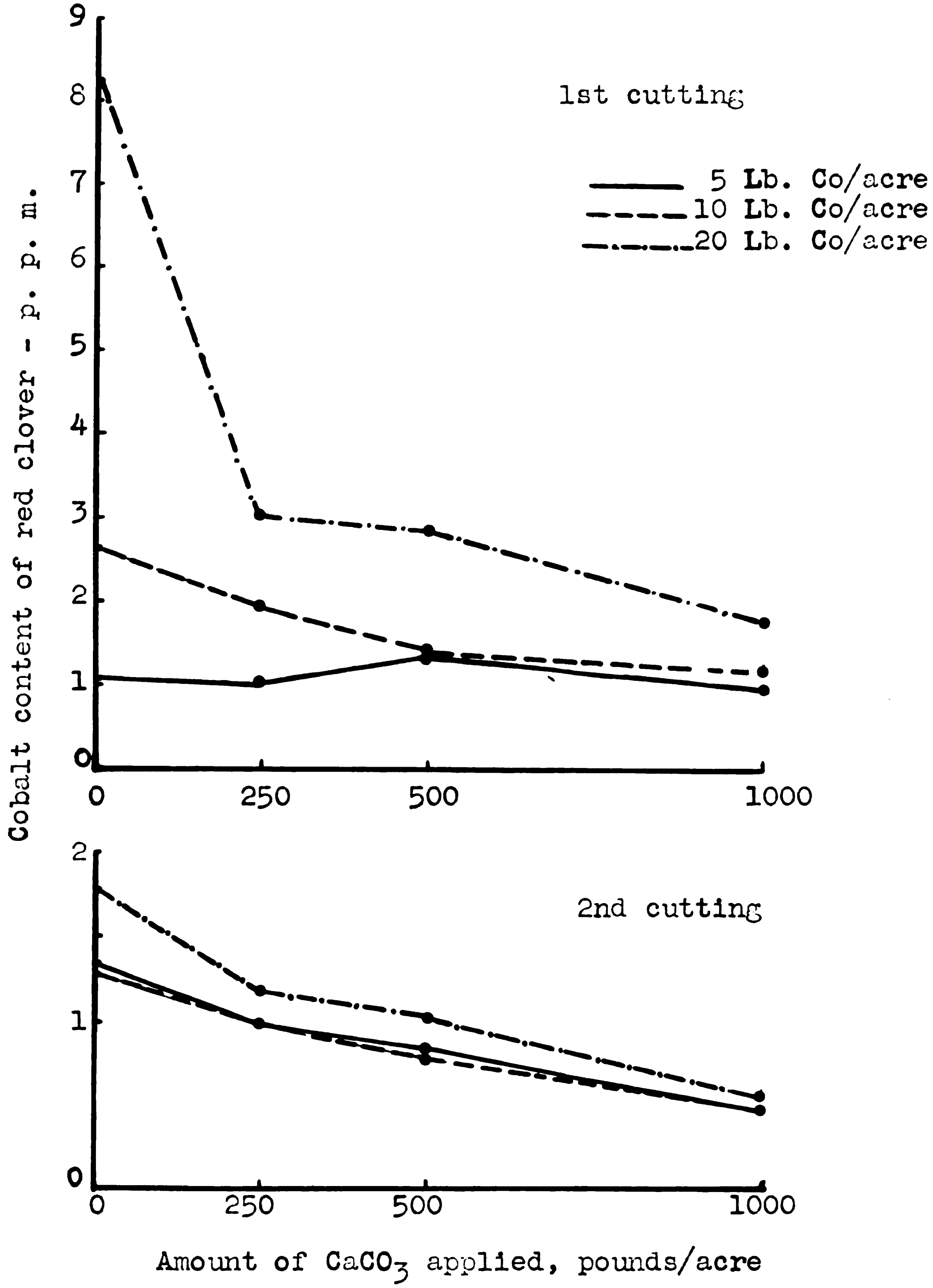

Figure VI. Effect of liming on the cobalt content of red clover grown on a Coloma soil, receiving varying amounts of cobalt.

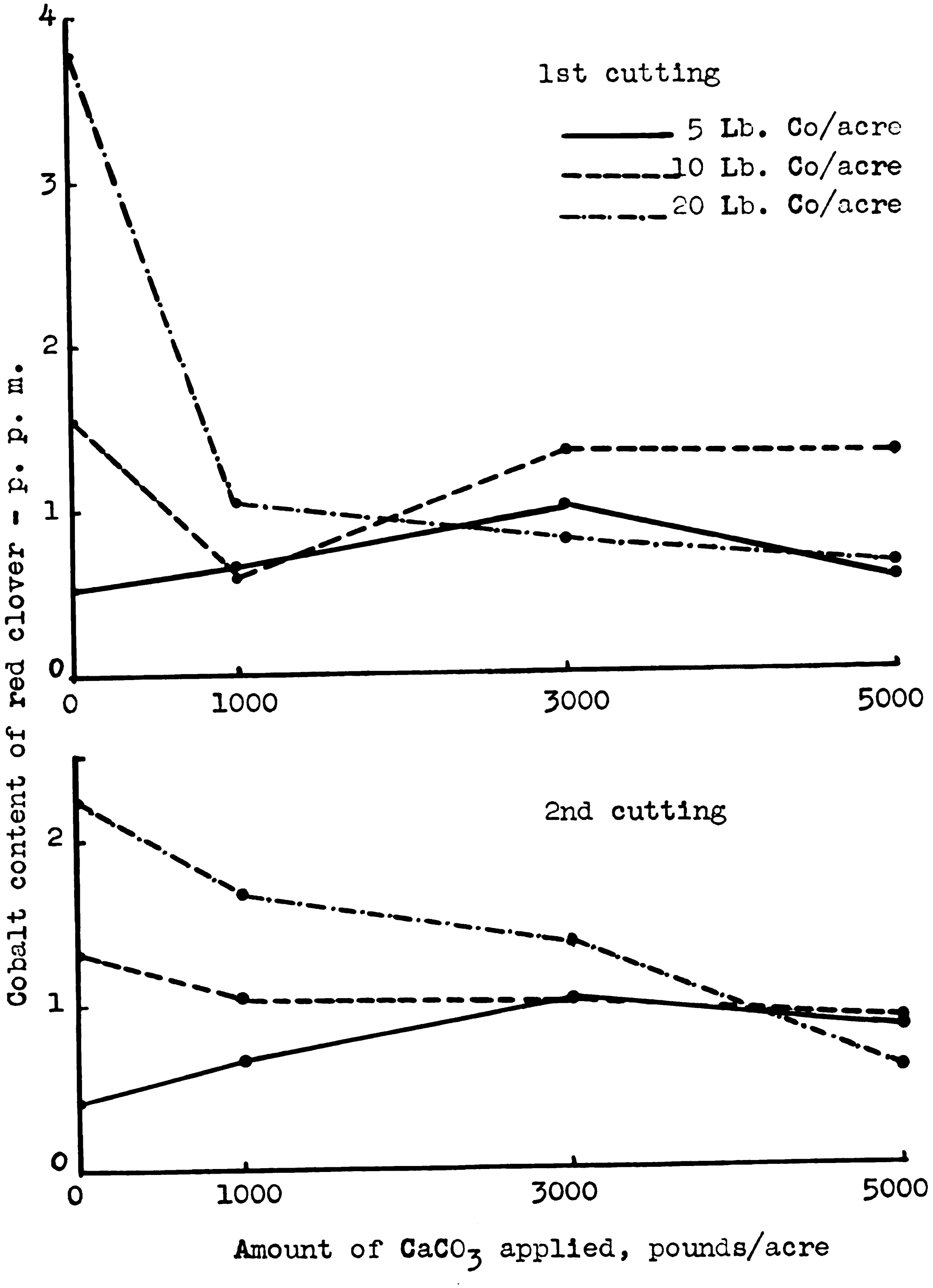

Figure VII. Effect of liming on the cobalt content of red clover grown on a Hillsdale soil, receiving varying amounts of cobalt.

c. Effect of the amount of cobalt applied to the soil on the cobalt content of red clover.

The amount of cobalt absorbed by first and second cutting red clover plants grown on the two soils to which no lime was added are presented graphically in Figure VIII.

It can be noted that with an increase in the amount of cobalt applied to the two soils the cobalt content of red clover also increased. This relationship was found for both first and second growth on the soils studied.

The amount of cobalt absorbed by the plants grown on the Coloma soil was approximately twice as great as the amount determined in the plants grown on the Hillsdale soil. The results of the second cutting did not indicate a definite relationship on the above point.

C. Laboratory Studies

The laboratory studies consisted of the spectrographic determination of cobalt in samples of alfalfa grown on various Michigan soils, the available and total cobalt content of these soils and the distribution of cobalt in the three soil fractions of sand, silt and clay.

These studies were undertaken in an attempt to detect possible relationships existing among the above mentioned factors.

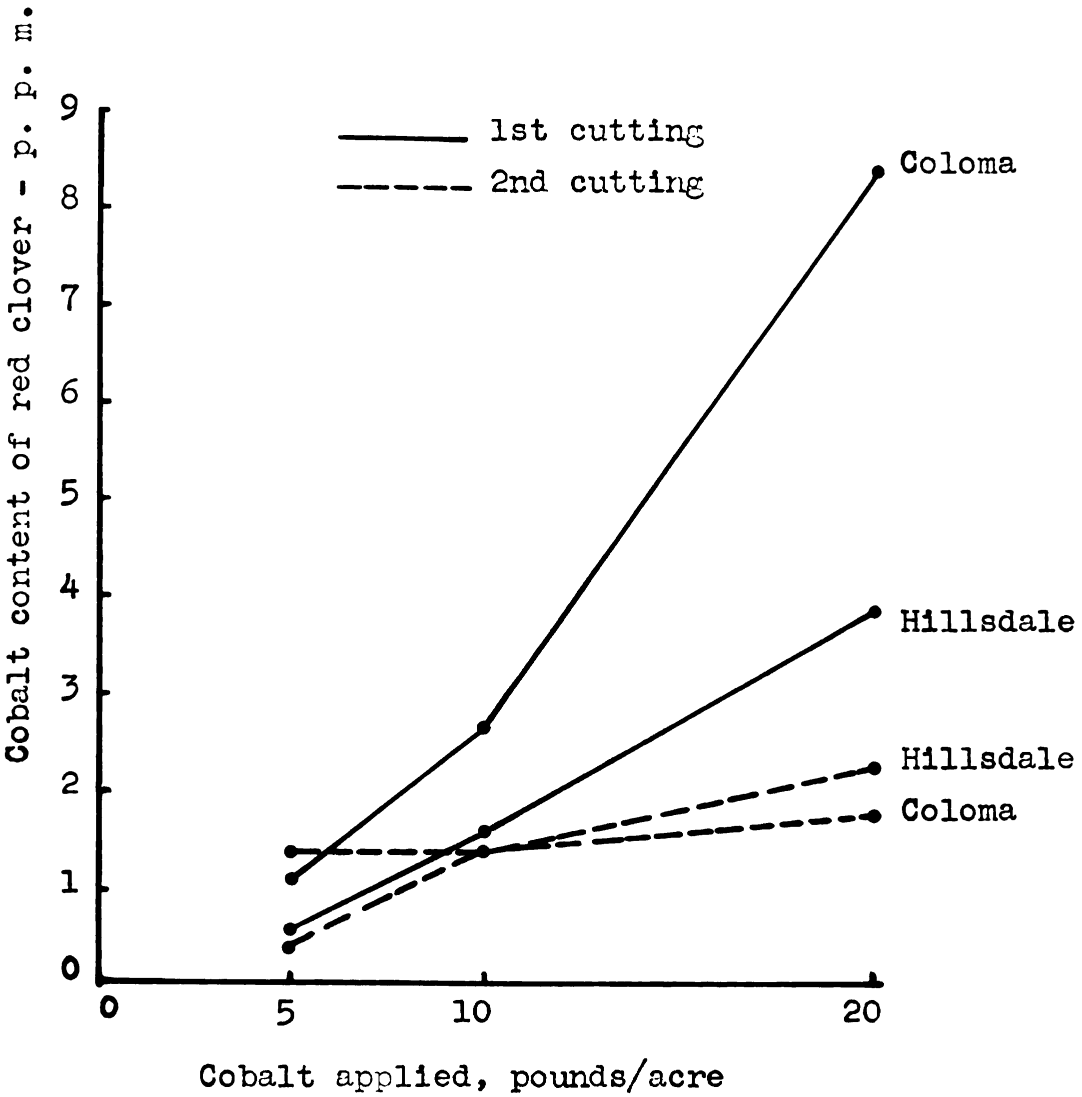

Figure VIII. Cobalt content of red clover grown on two Michigan surface soils receiving different amounts of cobalt.

1. Materials and Methods

Samples of the soils used for the laboratory studies were collected over a relatively large area of the southern half of the Lower Peninsula of the State of Michigan from fields of alfalfa in the localities shown in Table 19. After appropriate preliminary preparation, a mechanical analysis was made of each sample and its reaction was determined. The results obtained are presented in Table 20.

The general features characterizing the soil series to which the several soil types belong are presented in the following brief descriptions.

a. Description of soils

Brady series. This series includes dark-colored, very poorly drained soils in the Gray-Brown Podzolic region. The soils are developed on calcareous sands and gravel occurring as glaciofluvial outwash plains and terraces in the regions of highly calcareous glacial till.

The series is characterized by a dark-gray to brownish gray loamy surface with a relatively high content of organic matter and a neutral to slightly acid reaction.

The top soil is underlain by a mottled gray, yellow and brown loam, sandy loam or clay loam and the parent material, occurring below 40 inches, is a pale yellow and gray stratified calcareous sand and gravel.

TABLE 19.

SOIL TYPES USED IN THE LABORATORY STUDIES AND LOCALITY OF SAMPLING

Soil Type	County	Township	Section
Coloma loamy sand	Livingston	Howell	2
Brady sandy loam	Lenawee	Deerfield	11
Hillsdale sandy loam	Hillsdale	Somerset	3
Kalkaska sandy loam	Oceana	Shelby	14
Kawkawlin sandy loam	Bay	Williams	27
Nester sandy loam	Arenac	Clayton	32
Brookston loam	Monroe	Dundee	27
Conover loam	Ingham	Meridian	3
Miami loam	Clinton	Watertown	13
Selkirk loam	Iosco	Alabaster	34
Wisner loam	Bay	Merritt	15
Kent silt loam	Oceana	Claybanks	36
Brookston clay loam	Wayne	Van Buren	5

TABLE 20.

SOIL REACTION AND TEXTURAL COMPOSITION OF THE SOILS USED IN THE LABORATORY STUDIES

Soil type	Soil pH	Sand	Silt	Clay
			Percent	
Coloma loamy sand	6.2	82.6	11.9	5.5
Brady sandy loam	7.2	56.9	26.1	17.0
Hillsdale sandy loam	6.5	70.5	23.2	6.3
Kalkaska sandy loam	7.0	66.8	26.2	7.0
Kawkawlin sandy loam	7.5	77.2	16.0	6.8
Nester sandy loam	6.5	54.4	33.1	12.5
Brookston loam	7.0	33.6	40.3	26.1
Conover loam	6.6	46.0	43.0	11.0
Miami loam	6.7	48.0	40.1	11.9
Selkirk loam	7.0	46.2	34.2	19.6
Wisner loam	8.0	47.5	33.6	18.9
Kent silt loam	6.6	28.2	60.7	11.1
Brookston clay loam	7.0	20.8	47.9	31.3

The native vegetation was deciduous forests of elm, ash, soft maple, hickory and basswood, but at the present the cleared areas are used for general farming with crop yields ranging from medium to high.

Brookston series. Brookston soils are found on nearly level to depressed areas in upland till plains and glacial lake plains. They include Wiesenboden-like soils in the region of Gray-Brown Podzolic soils, developed on highly calcareous glacial till of Wisconsin age. They are very poorly drained soils and in virgin areas consist of a brown, partially decayed forest litter underlain by a dark brownish-gray loam high in organic matter and with a slightly acid to mildly alkaline reaction. This horizon is followed by a dark gray to mottled gray loam with a relatively high content of organic matter.

The parent material found below 48 inches is a mottled gray, pale yellow, highly calcareous heterogeneous mixture of all textures from clay to stones, a high percentage of which is limestone and dolomite pebbles and stones.

The native vegetation was deciduous trees but the greatest portion of it is cleared and under cultivation with corn, soybeans, wheat, oats, clover and alfalfa. These soils are considered highly productive.

Coloma series. This series includes Gray-Brown Podzolic soils developed on fine sand, sand or loamy sand glacial drift of Wisconsin age. Such soils occur in nearly level to steep slopes or moraines, till plains and outwash plains.

They are considered well to somewhat excessively drained and consist of a very dark grayish-brown, dark gray sandy to sandy loam top soil with a strong to medium acid reaction and a moderate to low organic matter content.

The parent material observed below 60 inches is a pale brown to brown loamy sand to sand with a medium to strong acid reaction.

The native vegetation was predominantly oaks with some hickory.

The soil is used for special crops, small fruits, hay and pasture.

Conover series. The soils of this series occur on nearly level or gently undulating topography. They are imperfectly drained, moderately dark-colored soils in the Gray-Brown Podzolic region, developed on highly calcareous till.

In the virgin condition they exhibit a partially decayed forest litter underlain by a dark-grayish brown to dark brownish gray loam with a relatively high organic matter content and a slightly acid to neutral reaction.

The parent material appearing below 30 inches is a gray and yellow clay loam to loam calcareous glacial till.

The native vegetation was deciduous forest, consisting of elm, ash and other deciduous species.

These soils are highly productive. Most of the land, including these soils, has been cleared and is used for the cultivation of corn, oats, wheat, beans and sugar beets.

Hillsdale series. Soils of this series are observed on undulating and rolling glacial moraines and includes Gray-Brown Podzolic soils developed from light textured siliceous and somewhat calcareous glacial till. They are well drained soils and their profile characteristics consist of a surface covered with a thin leaf litter and mold from deciduous trees under which there is a dark grayish-brown loam to sandy loam with a slightly or medium acid reaction and decreasing humus content downward to an approximate ten inches depth.

The subsoil texture varies from sand to clay loam and the parent material occurring below 50 inches depth is a brownish-yellow or pale yellow friable sandy loam calcareous glacial till.

The native vegetation was deciduous hardwood forest and at the present time the cleared areas are used for general farming with yields medium to fairly high where good management practices are applied.

Kalkaska series. The Kalkaska soils are sandy Podzols developed on glacial outwash plains. They occupy nearly level pitted outwash plains and stream terraces.

The undisturbed surface soil has a leaf litter and raw organic mat from one to three inches in thickness underlain by a humus layer below which is a pinkish-gray sand 3 to 18 inches thick.

The texture of the subsoil may range from a fine sand to a sandy loam with a medium to strongly acid reaction.

The parent material, 30 or more inches in depth, is sand of mixed composition with considerable silicate materials and some limey materials.

They are well to excessively drained soils. The native vegetation was a dense stand of hardwood forest with maple, beech, birch, and elm as the predominant trees.

Some areas are cropped to hay, oats and potatoes with moderate yields. Most of these soils support cut-over timber.

Kawkawlin series. This series occurs on nearly level to undulating areas in till plains and includes Gray-Wooded soils in the Podzol region.

They are imperfectly drained soils and the texture of the surface soils ranges from a very dark gray loam to dark grayish-brown loamy sand extending to ten inches in thickness and having a slightly acid to mildly alkaline reaction.

The subsoil from 10 to 20 inches in depth is a brown to dark brown mottled with yellowish-brown clay loam to silty clay loam with a slightly acid to neutral reaction.

The parent material, below the 20 inches depth, is a reddish-brown silty clay loam to clay loam, calcareous till.

The native vegetation was mixed hardwoods including sugar maple, red oak, ironwood, basswood and aspen. A large proportion of these soils is presently used for pasture. Cultivated areas are used for the production of small grain, beets, beans and corn.

Kent series. The Kent series includes the moderately well drained Podzol soils, occurring on undulating or rolling topography and developed on pinkish to reddish calcareous clay to silty clay glacial till.

Their profiles exhibit a surface layer covered with a thin layer of leaf litter and leaf mold or mull derived from hardwood trees. The top soil is a dark grayish-brown loam with considerable humus extending to six inches in depth and having a neutral or slightly acid reaction.

The surface layer is underlain by a reddish-brown heavy silty clay material approximately 18 inches in depth and having a medium acid reaction.

The native vegetation was hard maple, ash, beech and at the present the cleared areas are used in general farming with hay, corn, wheat and oats as the principal crops.

Miami series. The Miami series includes well drained, modal Gray-Brown Podzolic soils developed on highly calcareous till.

The color of the surface soil, extending from 6 to 12 inches in depth, is a very dark to dark grayish-brown changing to a light yellowish-brown to brown with an increase in depth. The texture varies from silt loam, loam, to sandy loam. The reaction is slightly acid.

The subsoil, extending to 28 inches in thickness, ranges in color from light yellowish-brown to dark brown and in texture from heavy silt loam to silty clay loam. It exhibits a strongly acid to slightly acid to neutral reaction.

The parent material, below 28 inches, is a heavy loam to light clay loam glacial till consisting of a heterogeneous mixture of all textures from clay to stones with a high percentage of limestone and dolomite rock fragments and stones.

The native vegetation included deciduous trees. A large proportion of the Miami soils has been cleared of timber and is used for general farming.

Nester series. This group consists of Podzols developed from moderately heavy pinkish-brown calcareous glacial till.

The soil is well drained throughout and exhibits a light gray surface horizon one to five inches in thickness and ranging in texture from loam to sandy loam.

The subsoil extends to 36 inches in depth, has a yellowish-brown to reddish color and is heavier in texture.

The parent material is a calcareous gritty and pebbly clayey till of pale reddish or pinkish cast.

These soils occur on undulating or rolling glacial till plains or subdued moraines.

Luxuriant forest, chiefly of sugar maple, beech, elm and white ash, composed the native vegetation. The greatest percentage of the forest has been cleared for general farming and dairying. Alfalfa is generally grown without liming.

Selkirk series. The Selkirk series consists of imperfectly drained soils developed over reddish or pinkish calcareous silty clay or clay till in the Podzol soil region or the transition belt between the Podzol and Gray-Brown Podzolic soils.

They occur on level or gently undulating areas and are considered imperfectly drained.

They exhibit a dark gray to light gray A horizon varying in thickness from one to six inches, in texture from loam to silt loam and in reaction from strongly to slightly acid.

The subsoil is a pale yellowish to reddish brown silt loam to silty clay loam having a slightly acid to neutral reaction.

The parent material is a pale red calcareous clay or silty clay that may include some boulders or pebbles.

Dense stand of sugar maple, beech, elm and hemlock composed the natural vegetation and at the present general farming and dairying is exercised on the cleared areas.

Wisner series. The Wisner series includes soils of the Wiesenboden group developed on calcareous medium to heavy textured lakelaid materials.

They are poorly drained soils occurring on nearly level topography.

The profile consists of a dark gray loamy A horizon extending to eight inches in thickness, containing numerous small shell fragments and exhibiting an alkaline reaction.

The subsoil, extending to 30 inches in depth, is a gray to light gray and yellowish brown clay loam to clay.

The natural vegetation is grasses, sedges, rushes and small willows and on the utilized areas general farming is practiced after artificial drainage. The yields are usually high.

b. Collection of samples for analysis

The plant samples which were analyzed for total cobalt content, were first cutting alfalfa collected from the check plots of fertilizer trials conducted with the previously described soil types in 1946.

Both chemical and spectrographic procedures were used in determining total cobalt content of the alfalfa samples and the results are presented in a following section.

For the fractionation of the soil samples, a varying quantity (100 to 500 grams) was used, the amount depending upon the textural composition of the soil and the amount of the fraction required.

The samples were treated with the amounts of ten percent and thirty percent hydrogen peroxide solutions necessary for complete oxidation of the organic matter and with 100 or more milliliters of one percent sodium hexa-metaphosphate solution. The suspensions were then dispersed by stirring for ten to fifteen minutes in a mechanical mixer.

The sand fraction was separated and collected by sieving through a 300-mesh sieve.

The fractions of silt and clay remaining in suspension were placed in sedimentation cylinders and by applying Stoke's Law of settling velocities the time and depth of sampling were calculated according to the diameters of the particles composing each fraction. After several samplings the clay fraction was separated and collected, thus leaving the silt fraction in the cylinder to be collected by decanting and discarding the supernatant liquid.

The amount of available cobalt in the soil samples was determined in a 2.5 percent acetic acid extract of the soils. The total cobalt content, as extracted with nitric and perchloric acid digestion and 6N hydrochloric acid refluxing, of the soil, sand and silt separates was determined by the procedures already presented while the total cobalt content of the clay fraction was determined by calculation as follows:

Knowing the textural composition of each soil sample and the total amounts of cobalt in the soil and in the sand and silt fractions, the amount of cobalt from the clay fraction of each sample was calculated on the five gram sample basis.

2. Results and Discussion

a. Cobalt content of alfalfa grown on various Michigan soils.

The cobalt content of alfalfa grown on various Michigan soils is presented in Table 21. The values presented were obtained spectrographically from three aliquots of each sample. The conditions required for arcing the carbon-metal oxide mixture were the same as those for the standard curve presented in Figure I, which was used for interpreting the results.

The cobalt content of alfalfa ranged from a minimum of 0.03 parts per million to a maximum of 0.24 parts per million.

TABLE 21.

COBALT CONTENT OF ALFALFA GROWN ON VARIOUS MICHIGAN SOILS

Soil type	Cobalt parts per million 1	2	3	Average
Coloma loamy sand	0.23	0.19	0.31	0.24
Brady sandy loam	0.08	0.12	0.07	0.09
Hillsdale sandy loam	0.11	0.10	0.12	0.11
Kalkaska sandy loam	0.03	0.04	0.04	0.04
Kawkawlin sandy loam	0.10	0.05	0.08	0.08
Nester sandy loam	0.11	0.09	0.08	0.09
Brookston loam	0.07	0.06	0.07	0.07
Conover loam	0.06	0.07	0.07	0.07
Miami loam	0.07	0.08	0.07	0.07
Selkirk loam	0.05	0.07	0.07	0.06
Wisner loam	0.06	0.07	0.07	0.07
Kent silt loam	0.03	0.03	0.03	0.03
Brookston clay loam	0.12	0.10	0.11	0.11
Mean				0.09

The mode was 0.07 parts per million while the arithmetic mean of all determinations was 0.09 parts per million.

The range of the cobalt content is in good agreement with the results of Hill et al. (1953) although lower than those reported for the referee sample of Beeson (1953).

The percentages of cobalt in alfalfa in reference to the pH of the soils on which the legume was grown are plotted in Figure IX. Although there was a tendency of a lower uptake of cobalt from soils of higher pH values, the data are not very conclusive. The absorption of cobalt was highest on the Coloma soil with the lowest pH of 6.2 but in the Wisner loam with a pH of 8.0 the cobalt taken up was 0.07 parts per million which is the mode of the data presented.

The coefficient of correlation between the soil pH and the cobalt content of alfalfa was -0.41, which is not significant at the five percent level.

The effect of the soil pH alone on cobalt uptake by plants is better illustrated in the greenhouse studies where varying pH values were established in each of the soils used.

It is not feasible to try to correlate the pH of the various soils with the cobalt content of the plant growing thereon. The soils probably vary considerably in their cation and anion content and their chemical and physical properties. Young (1948) has shown that in solutions the solubility of cobalt is affected not only by the pH of the solution but also by the nature of the cations and anions present.

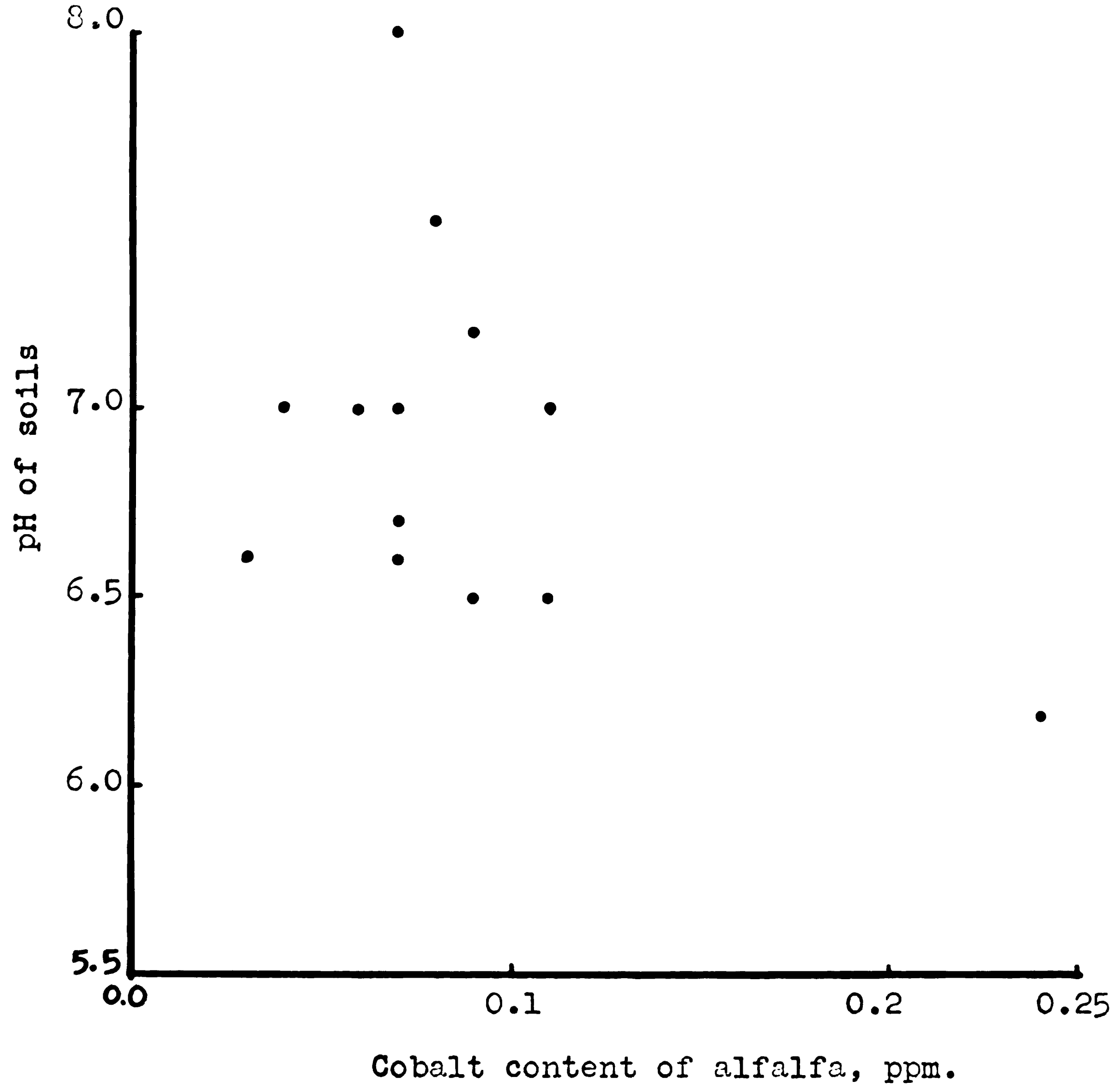

Figure IX. Cobalt content of alfalfa in relation to the pH of the soils.

b. Available cobalt in Michigan soils.

The available or 2.5 percent acetic acid extractable cobalt of selected Michigan surface soils is presented in Table 22.

For the interpretation of the logarithm of relative intensity values Figure IV was used.

The available cobalt in Michigan soils ranged from 0.08 parts per million for the Coloma soil to 0.68 for the Brookston soil with most values ranging from 0.15 to 0.30 parts per million of cobalt.

If the above data are considered in the light of Mitchell's (1945) arbitrary separation of soils into those "deficient" and "non-deficient" in cobalt, most of the soils examined would be classified as "deficient" in cobalt.

c. Total cobalt in Michigan soils.

The total cobalt content of certain Michigan surface soils is presented in Table 23. These data were obtained by using the standard curve presented in Figure V.

The total cobalt content of the soils, as extracted and determined by the procedures already presented, ranged from 0.47 parts per million for the Kalkaska sandy loam to 2.17 parts per million for the Hillsdale sandy loam. The average value for all soils was 1.09 parts per million of cobalt. As a rule, the content of cobalt was higher in the loam soils than in the sandy loams. The cobalt content of the Hillsdale

TABLE 22.

AVAILABLE COBALT CONTENT OF MICHIGAN SOILS

Soil type	Cobalt parts per million 1	2	3	Average
Coloma loamy sand	0.07	0.08	0.09	0.08
Brady sandy loam	0.24	0.20	0.29	0.24
Hillsdale sandy loam	0.21	0.13	0.22	0.19
Kalkaska sandy loam	0.10	0.08	0.09	0.09
Kawkawlin sandy loam	0.32	0.20	0.23	0.25
Nester sandy loam	0.22	0.25	0.23	0.23
Brookston loam	0.33	0.30	0.25	0.29
Conover loam	0.22	0.15	0.17	0.18
Miami loam	0.62	0.53	0.49	0.55
Selkirk loam	0.33	0.43	0.43	0.40
Wisner loam	0.35	0.30	0.43	0.36
Kent silt loam	0.15	0.14	0.17	0.15
Brookston clay loam	0.72	0.72	0.60	0.68
Mean				0.28

TABLE 23.

TOTAL COBALT CONTENT OF MICHIGAN SOILS

	Cobalt parts per million			
Soil type	1	2	3	Average
Coloma loamy sand	0.80	0.82	1.00	0.87
Brady sandy loam	0.86	0.80	0.82	0.83
Hillsdale sandy loam	2.10	2.15	2.25	2.17
Kalkaska sandy loam	0.50	0.48	0.44	0.47
Kawkawlin sandy loam	0.90	0.84	0.88	0.87
Nester sandy loam	1.07	1.05	1.03	1.05
Brookston loam	1.19	1.10	1.10	1.13
Conover loam	0.80	0.88	0.84	0.84
Miami loam	1.45	1.50	1.45	1.47
Selkirk loam	1.50	1.35	1.30	1.38
Wisner loam	1.25	1.35	1.50	1.37
Kent silt loam	0.72	0.57	0.72	0.67
Brookston clay loam	0.99	0.99	1.25	1.08
Mean				1.09

sandy loam showing the greatest deviation from the mean cannot be clearly explained on the basis of the mechanical composition of the soil. This value may be due to the presence of minerals high in cobalt. However, with no mineralogical data for this soil, this supposition cannot be substantiated.

d. Relation between plant uptake and total and available cobalt in the soil.

The values for cobalt in soils and legume hay presented in previous tables are summarized in Figure X. The cobalt content is plotted on the ordinate while the various soils given by the series name are plotted on the abscissa, the arrangement based on increasing total cobalt content of the soil. Three separate relationships are presented for the cobalt content of alfalfa, the available and the total cobalt of the soils studied.

From studying the relationships presented in Figure X, it is evident that a trend exists between total and available cobalt content of the various soils. However, the available cobalt content of the Hillsdale and Coloma soils is relatively low in relation to their total cobalt content. The apparent lack of relationship between these two pair of data are sufficient to eliminate significance of correlation for all data concerned.

The cobalt content of alfalfa from all soils was quite similar, except for that grown on Coloma sandy loam. Conse-

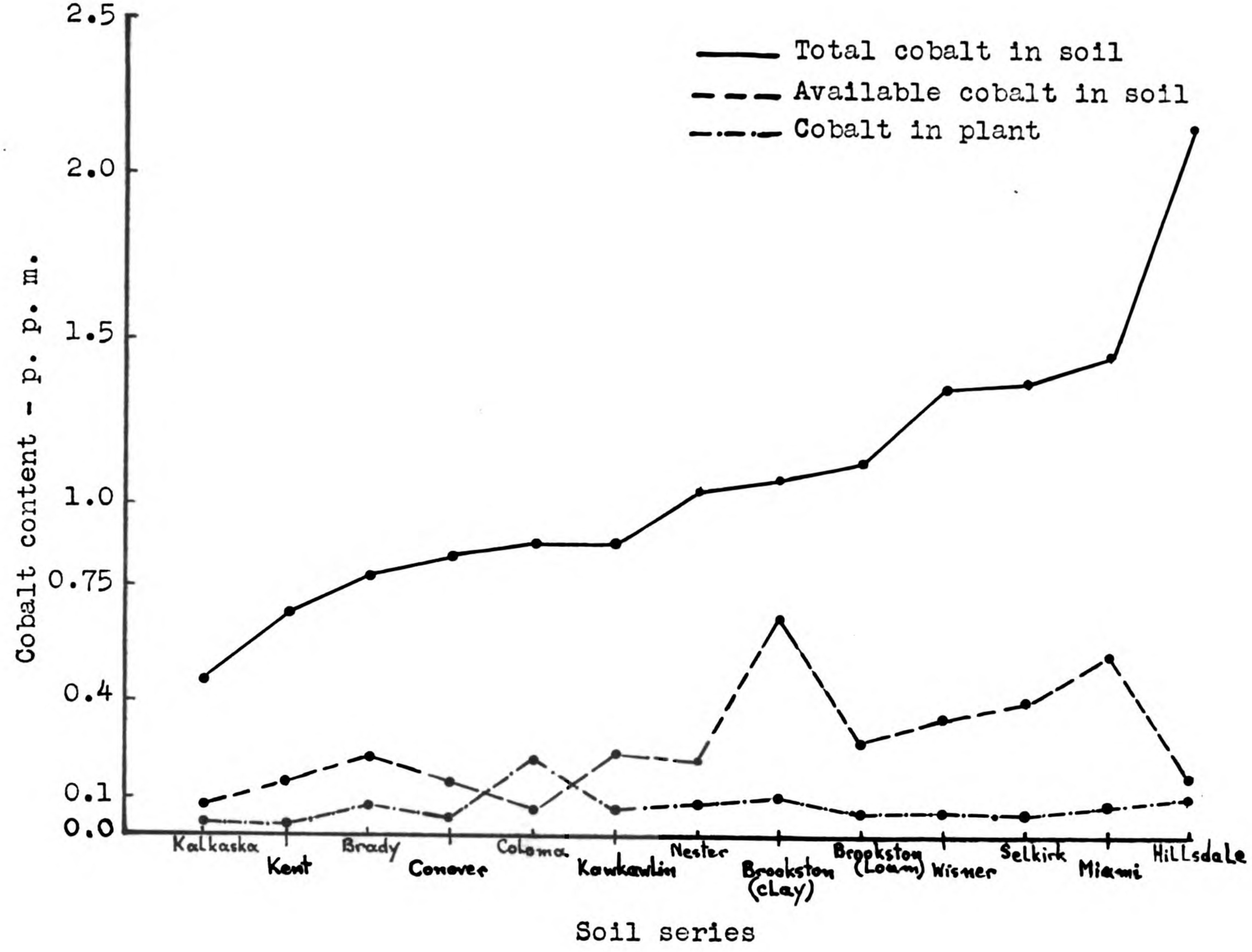

Figure X. Comparison of total and available cobalt content of various Michigan soils to the cobalt content of alfalfa grown thereon.

quently no relationship is evident between plant content of cobalt and total or available cobalt content of the soils studied.

A correlation test between total cobalt in the soils and the cobalt content of alfalfa plants grown thereon, resulted in a non-significant low correlation coefficient of 0.118. The amount of available cobalt in the 13 soils compared to the cobalt content of first cutting alfalfa yielded a low correlation coefficient of -0.132.

The relationship between total and available cobalt of the soils is also presented in a scattered diagram (Figure XI).

The correlation test between these two sets of data indicated a non-significant coefficient of 0.38. It was decided to eliminate the two pairs of values which exhibited an extreme variation; the values of 2.17 parts per million of total cobalt and its corresponding value of 0.19 parts per million of available cobalt for the Hillsdale sandy loam and that of 0.68 parts per million available cobalt and its corresponding value of 1.08 parts per million total cobalt for the Brookston clay loam. In this case a highly significant correlation coefficient of 0.89 was obtained.

Nevertheless the conclusions will have to be based on all experimental results rather than on selected ones, even if the selection may be justified in view of the general trend of the data.

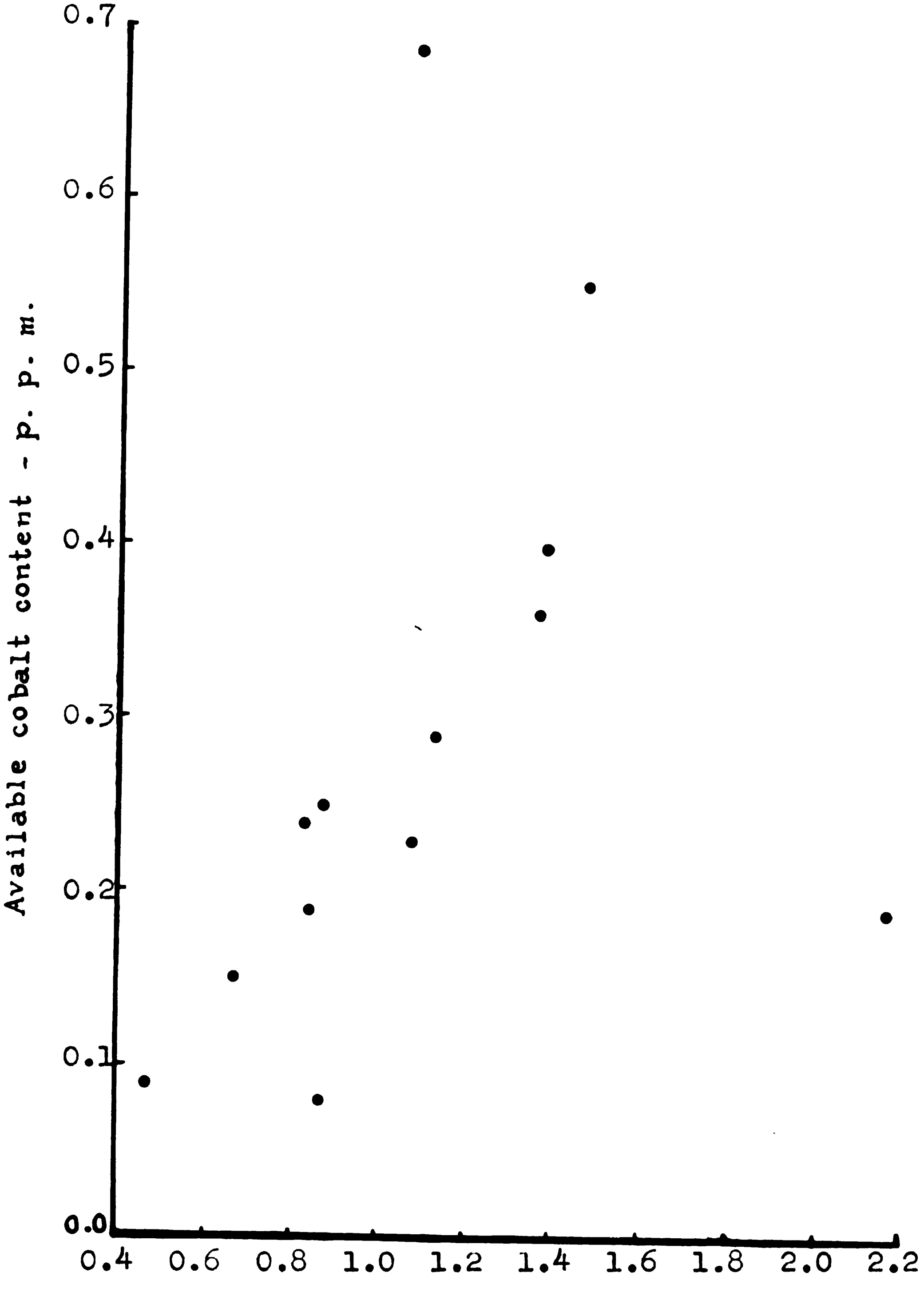

Figure XI. Relationship between total and available cobalt content of various Michigan surface soils

e. Distribution of cobalt in the sand, silt, and clay fractions of some Michigan surface soils.

The results obtained from the determination of cobalt in the sand and silt fractions of the various soils were interpreted by using the standard curve in Figure V. Data relative to the cobalt content of the sand, silt and clay fractions of 13 surface soils are given in Table 24.

The cobalt content of the various fractions increased as particle size decreased. A given weight of the clay fraction contained from two to fifty times more cobalt than the same weight of sand fraction. In every case the amount of cobalt in the silt was higher than that in the sand fraction. However, in two soils, namely Kent silt loam and Selkirk loam, the amount of cobalt in the silt was higher than that in the clay fraction.

The cobalt content of the three soil fractions as compared to the total cobalt content of the corresponding soils is also presented graphically in Figure XII. The soils are arranged in order of increasing cobalt content of the whole soil.

A trend is evident indicating some relationship between the total cobalt content of the sand fraction and the total cobalt content of the soil, although a non-signidicant correlation coefficient of 0.28 was obtained. Even lower correlation coefficients were obtained when the cobalt contents of

TABLE 24.

COBALT CONTENT OF THE SAND, SILT AND CLAY FRACTIONS OF MICHIGAN SOILS.

Soil type	Cobalt parts per million*		
	Sand	Silt	Clay
Coloma loamy sand	0.46	0.78	7.24
Brady sandy loam	0.54	0.64	2.18
Hillsdale sandy loam	0.53	0.94	25.24
Kalkaska sandy loam	0.40	0.57	0.85
Kawkawlin sandy loam	0.41	1.58	8.70
Nester sandy loam	0.64	1.45	1.75
Brookston loam	0.54	0.76	2.49
Conover loam	0.42	0.50	3.90
Miami loam	0.41	0.62	8.57
Selkirk loam	0.37	2.40	1.99
Wisner loam	0.57	1.32	3.49
Kent silt loam	0.34	0.83	0.72
Brookston clay loam	0.69	1.04	1.41

* The reported values for the sand and silt fractions are the average of three determinations. The cobalt content of clay was obtained by difference from the total cobalt content of the soil.

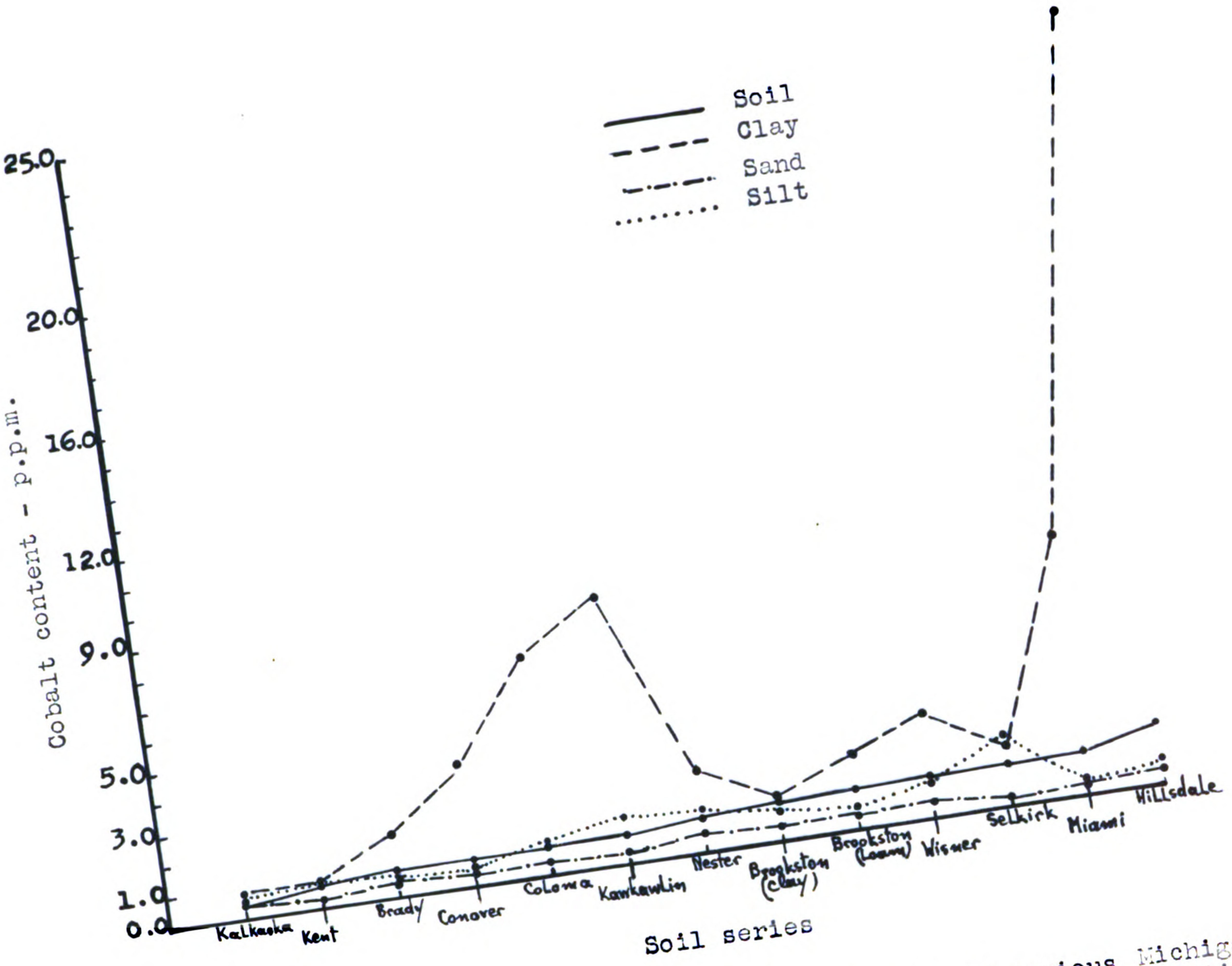

Figure XII. Relationship of the cobalt content of various Michigan soils to the cobalt content of their particle-sized fractions.

the silt and clay fraction were compared to the total cobalt content of the soils.

Due to a high content of iron in the clay fraction difficulties occurred in the spectrographic determination of the cobalt content of this separate and it was thought advisable to employ the nitroso-R-salt colorimetric method for obtaining the above data. The same method was used in determining total cobalt content of the sand fraction for comparison with the spectrographic data. These results are presented in Table 25.

Studying the results obtained by the two methods for the cobalt content of the sand fraction, very good agreement was observed. Most of the values indicated a variation from ±0 to ±20 percent. Although a higher variation exhibited by the rest of the results may seem excessive, the significance of it is questionable considering the very small amounts of cobalt with which we deal.

The agreement between the results obtained by the two methods used for determing cobalt in clay is not satisfactory. This could be expected as the values for the cobalt content of the clay obtained by difference will include all the analytical errors that may have taken place in determining cobalt content in the soils and their sand and silt fractions.

TABLE 25.

COBALT CONTENT OF THE SAND AND CLAY FRACTIONS OF MICHIGAN SOILS AS DETERMINED BY TWO METHODS

Soil type	Sand Co ppm		Clay Co ppm	
	Spectrographic	Chemical*	By difference**	Chemical
Coloma loamy sand	0.46	0.58	7.24	1.76
Brady sandy loam	0.54	0.41	2.18	1.87
Hillsdale sandy loam	0.53	0.46	25.24	-
Kalkaska sandy loam	0.40	0.40	0.85	1.82
Kawkawlin sandy loam	0.41	0.43	8.70	18.52
Nester sandy loam	0.64	0.38	1.75	2.23
Brookston loam	0.54	0.47	2.49	2.60
Conover loam	0.42	0.75	3.90	-
Miami loam	0.41	0.21	8.57	3.44
Selkirk loam	0.37	0.24	1.99	1.20
Wisner loam	0.57	0.41	3.49	2.22
Kent silt loam	0.34	0.34	0.72	-
Brookston clay loam	0.69	0.69	1.41	1.81

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* Nitroso-R-salt colorimetric determination.

** Determined by difference from total soil cobalt using results obtained spectrographically.

IV. SUMMARY

The experimental portion of this investigation has been presented in three separate parts, namely the analytical procedures, the greenhouse studies and the laboratory studies.

A thorough investigation of a spectrographic method for determining traces of cobalt in plant material, soils and their fractions was undertaken. This method necessitated a chemical preconcentration of cobalt from the various materials using 8-hydroxyquinoline. This organic agent precipitates cobalt from solution under controlled conditions of pH as cobalt quinolinate.

With this method it was possible to determine cobalt as low as 0.03 parts per million in plant material and 0.07 parts per million in soil extracts.

The reproduceability of the results was very good and comparative tests between the spectrographic method and the chemical colorimetric (nitroso-R-salt) method for determining cobalt were very satisfactory.

The greenhouse studies consisted of a pot experiment in which the uptake of cobalt by red clover was investigated. Two soils, a Coloma sand and a Hillsdale sandy loam were used after receiving various applications of calcium carbonate and cobalt sulphate. The following results were obtained.

Application of cobalt in the amount of five, ten and twenty pounds per acre did not significantly effect the yield of the plant. On the other hand, the application of lime resulted, in most cases, in a significant increase in the dry weight yield of red clover.

The amount of cobalt in the plant was influenced by the amount of lime and cobalt in the soil. Thus an increase in the amount of lime applied to the soil considerably decreased the cobalt content of red clover grown thereon. This relationship is illustrated by the results obtained with the red clover grown on the Coloma soil. The cobalt content of clover grown on the Hillsdale soil was erratic with the exception of that obtained when the crop was grown on soil receiving the highest amount of cobalt (20 pounds per acre).

The amount of cobalt applied to the soil affected the cobalt content of red clover. In all cases the higher the amount of cobalt in the soil, the greater was the cobalt content of the plant. Although there was not a proportional relationship, red clover contained up to eighttimes as much cobalt when grown on soil receiving twenty pounds of cobalt per acre as when the soil received five pounds of cobalt per acre.

For the laboratory studies samples of 13 surface soils collected from the southern half of the lower peninsula of Michigan and samples of first cutting alfalfa grown on them were used. The soils were fractionated into their sand, silt and clay fractions and after the necessary procedures, the cobalt content was determined in all samples with the exception of the clay fraction. The cobalt content of the clay fraction was obtained by calculation.

The cobalt content of the alfalfa samples ranged from a minimum of 0.03 parts per million to a maximum of 0.24 parts per million. The mode was 0.07 while the arithmetic mean of all determinations was 0.09 parts per million. No clear relationship was obtained between the cobalt content of alfalfa and the pH of the soil, although in some cases there was a tendency for cobalt to be lower in hay taken from soils with high pH values.

The available or 2.5 percent acetic acid extractable cobalt of the soils studied ranged from 0.08 to 0.68 parts per million. The total cobalt determined on soil extracts of nitric, perchloric and 1:1 hydrochloric acid solutions ranged from 0.47 to 2.17 parts per million. As a rule, the total cobalt content was higher in the loam textured soils than in the sandy loam soils.

No significant correlation was obtained between the plant content and the total or available cobalt content of the soils studied. Some apparent relationship was found between the total and available cobalt contents of the various soils, although no significant correlation was obtained when all data were considered.

The cobalt content of the various fractions increased when particle size decreased. A given weight of the clay fraction contained from two to fifty times more cobalt than the same weight of the sand fraction. No mineralogical data for the soils were available.

A non-significant coefficient of correlation was obtained when the total cobalt content of the soil was compared to that of the individual sand, silt or clay fractions.

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