START

SOME METHODS FOR DETECTING DIFFERENCES IN SOIL ORGANIC MATTER

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INTRODUCTION

That soils differ in the quantity of organic matter they contain is common knowledge. That they may differ in the kind of organic matter is not so universally recognized. In comparing two soils, differences in the kind of organic matter may be demonstrated by at least two methods, (1) by showing the presence of some definite organic compound in one soil and its absence in the other, and (2) by the use of chemical tests that are known to indicate the presence of certain groups of compounds.

The first method is necessarily tedious and limited in its application to such compounds as have been isolated from soils and for which reasonably standardized methods of isolation have been developed. In the second method it is possible to apply to soils the great number of tests and reactions known to organic chemists and biochemists as indicative of the presence or absence of certain classes or groups of organic compounds.

In applying such tests it is necessary to take into consideration the nature of the material—soil, a complex mixture of organic and inorganic material very different from that for which most of the tests were designed. Such consideration will frequently lead to im-

portan... odifications of the tests and may suggest some that are new.

In this bulletin are presented results obtained in the application of such tests that are suitable for showing differences in the kind of organic matter in soils. Some of these tests are new and bring out new facts regarding the organic matter of soils; many are new only in their application to soils.

DOES THE COLOR OF THE HUMUS EXTRACT INDICATE PHENOLIC COMPOUNDS?

Extraction of a soil with dilute alkali, sodium hydroxide, or ammonia has frequently been resorted to in studies of organic matter. Such extraction yields a so-called humus extract which, except for soils almost devoid of organic matter, ranges from light yellow to dark brown or nearly black.

This dark-colored extract suggests that obtained when phenolic compounds are treated with dilute alkali in contact with air or oxygen. Although phenolic compounds of this character, if introduced into soils, would no doubt be rapidly oxidized during the processes of decomposition and lose any property that would cause them to give a solution with alkali that would darken on exposure to the air, soils, as will be shown later, absorb oxygen when suspended in a solution of sodium hydroxide. For this reason it seemed desirable to know whether this absorption was associated with the dark color of a humus extract.

In other words, would a humus extract be lighter in color if made without contact with air? Should such be the case, the presence of phenolic compounds would seem to be indicated, and their presence or absence would afford a means of showing differences in the soil organic matter.

To determine whether the humus extract would be lighter if made without contact with air, six soils, Everglades peat from Florida, Lake Mattamuskeet peat from North Carolina, Caribou loam from Maine, Hagerstown loam from Maryland, Houston clay from Texas, and sand from the South Carolina sand-hill region, were treated as follows:

Known quantities of soil were treated with 2 per cent sodium hydroxide in the usual way. The same quantity of soil was agitated with water under reduced pressure, the usual quantity of sodium hydroxide was then added, and the suspension was agitated in contact with hydrogen or nitrogen. The colors of the resulting extracts were compared in a colorimeter. In no case was the extract prepared in contact with air darker in color. The experiment was repeated, the soil being given a preliminary treatment with 2 per cent hydrochloric acid and washed free of acid before being subjected to the action of alkali. As before, there was no increase in color in samples exposed to the air.

Since in six soils of very diverse type there was no greater depth of color in a humus extract in contact with air than in one not in contact with air, it seems fair to assume that the presence of phenolic compounds that give a dark color by absorption of oxygen in alkaline solution is not common in soils and that treatment such as that described does not show differences in respect to such compounds in soils.

MEASUREMENT OF ORGANIC MATTER BY DEPTH OF COLOR OF HUMUS EXTRACT

It has been proposed from time to time to measure the quantity of organic matter in a soil by comparing the color of the humus extract with the color of some standard, or to determine the relative quantities of organic matter in two soils by comparing the depth of color of the humus extracts.

There are several reasons why such a procedure is not generally applicable. In the first place, there is the difficulty, if not the impossibility, of preparing a color standard that represents a definite quantity of organic matter. Next, the fact that there are present in soils colorless organic compounds that do not yield colored solutions with alkali makes it impossible to gage the total quantity of organic matter by the depth of color of a humus extract. Third, the fact that the humus extract represents only a part, and usually an unknown part, of the total organic matter is sufficient to make this method inapplicable if a measure of the total is desired. Fourth, the colors of the humus extracts from different soils frequently are not comparable; that is, when the extracts are diluted to the point where they can be compared in a colorimeter they are not of the same shade. In the six soils previously mentioned, this was true in the comparison of any two, and especially true with the two peats and with Caribou loam and Hagerstown loam. When the humus extracts of two soils can not be matched in color or shade, it may be assumed that some of the organic constituents of the soils differ in character.

ABSORPTION OF OXYGEN

Though the dark color of the humus extracts is not due to the absorption of oxygen, there is an absorption of oxygen by a suspension of soil in dilute sodium hydroxide, and a carbonate, presumably sodium carbonate, is formed. When such carbonate is formed in excess of any normally present, it is evident that carbon for its formation can come only from a breaking down of some organic constituents. The realization of this fact suggests several lines of inquiry. What compounds or class of compounds in the soil are thus broken down, what products other than carbon dioxide are formed, and, finally, what is the rate or extent of this change and how is it best measured?

The first two queries can be briefly disposed of at this time by stating that so far no definite information has been obtained regarding the identity of the compound or compounds broken down or of any of the products formed other than carbon dioxide.

The extent of the breaking down of soil organic matter and the resultant formation of carbon dioxide has been studied by two methods. In the first, 20 grams of soil were placed in a flask with 200 cubic centimeters of water previously boiled, and the apparatus was so arranged that a current of air freed of carbon dioxide could be passed through a suspension of soil in water in such a way as to keep it agitated. When the current of air had been established, 10 cubic centimeters of a saturated solution of sodium hydroxide were added and the passage of air continued for some predetermined time.

At the expiration of the time set, a train for the absorption of carbon dioxide was placed between the source of suction and the flask, and enough dilute sulphuric acid to render the suspension of soil in sodium hydroxide decidedly acid was added. The passage of air was continued as long as carbon dioxide was evolved. The carbon dioxide absorbed in the train was determined and calculated to carbon percentage of soil. Two blank determinations were made, one with soil and acid without previous agitation or addition of sodium hydroxide and the other on the sodium hydroxide solution.

Two examples, in which the results were corrected for carbon dioxide from blank determinations, will suffice to show results obtained in this way. The first is Caribou loam from Maine—2.95 per cent organic carbon—20 grams, 200 cubic centimeters of water, 10 cubic centimeters of saturated sodium hydroxide solution, through which air was passed for five hours; the contents of the flask were then acidified, and air passed again for one hour. Carbon dioxide equivalent to 0.0264 gram of carbon was obtained. This is 0.132 per cent, or 4.47 per cent of the total carbon in the soil. The second is peat from the Everglades of Florida—46.8 per cent carbon—2 grams, 200 cubic centimeters of water, 10 cubic centimeters of saturated sodium hydroxide solution. Air was passed through it for 15 hours; the material was acidified; and air was again passed through it for two hours. The carbon dioxide obtained was equivalent to 0.032 gram of carbon. This is 1.6 per cent of the peat, or 3.4 per cent of the total organic carbon.

It was assumed in these experiments that the concentration of sodium hydroxide in the soil suspension was sufficient to preclude any microbiological activity and that the oxidation was purely chemical. The following data seem to support this: Caribou loam, 2.75 per cent carbon. (1) Blank—20 grams of soil; 175 cubic centimeters of water; 10 cubic centimeters of saturated solution sodium hydroxide, acidified at once, and carbon dioxide-free air passed for one hour. The carbon dioxide obtained was equal to 0.0143 gram carbon or 0.071 per cent. (2) Twenty grams of soil; 175 cubic centimeters of water; 10 cubic centimeters of saturated solution sodium hydroxide. Air was passed for six hours, acidified, and air passed for one hour. The carbon dioxide obtained was equal to 0.0488 gram carbon or 0.244 per cent which, deducting that in the blank, gives 0.173 per cent. (3) Twenty grams of soil, 175 cubic centimeters of water, 10 cubic centimeters of saturated solution sodium hydroxide were allowed to stand 22 hours without agitation, were then acidified, and air was passed for one hour. The carbon dioxide obtained was equal to 0.075 per cent, that is, was practically the same as that in the blank.

It was observed in using this apparatus in the way described that exposure to sunlight, as passed through ordinary window glass, accelerated the reaction, which was very slow (almost negligible in some cases) when carried on in the dark. Further, the rate of flow of air naturally was a factor affecting the rate of oxidation.

Difficulties in regulating the flow of air in the apparatus as set up, so that it would be the same for any duplicate periods of time, and the fact that the apparatus did not permit of the taking of samples for the purpose of determining the rate of the reaction, led to the

abandonment of this method. It showed, however, that all the soils examined contained some organic compound or compounds that broke down, giving sodium carbonate when exposed to air or to oxygen in sodium hydroxide suspension, and that soils differed in quantity of carbonate formed.

The second method resorted to for studying this reaction was as follows: A 500-cubic centimeter Kjeldahl flask, fitted with a stopper through which a glass tube with stopcock was passed, was connected by rubber tubing to a gas-measuring burette with leveling bulb. The flask was supported by a clamp midway on the neck, the clamp rod being supported in a bearing and having at its outer end a connecting rod that was attached to a small pulley in such a way that the rotation of the pulley swung the flask from side to side through an angle of about 30°. Running the pulley by a small motor at a suitable speed imparted a "slopping" motion to the liquid in the flask. The procedure followed was to place 100 grams of soil and 250 cubic centimeters of 2 per cent sodium hydroxide solution in the flask, to evacuate it with ordinary laboratory suction (about 12 millimeters) and bring it to atmospheric pressure again by allowing oxygen to run into the flask. Then, the flask being attached to the gas burette, agitation was begun with the connecting stopcock closed. From time to time the agitation was stopped, the stopcock was opened, and the volume of gas absorbed was measured.

Absorption was usually rapid at first, became gradually slower, and usually ceased in a few hours. Dilute sulphuric acid 50 per cent in excess of that required by the sodium hydroxide was then added, agitation was resumed, and the gas given off (increase in volume) was measured.

Results obtained in this way on a number of soils are shown in Table 1, volumes of oxygen and carbon dioxide being calculated to and stated in grams. The volume of liquid was the same in all cases. Correction was made for carbon dioxide obtained without previous agitation in alkali and for variation in temperature and pressure, but the figures should be regarded as relative rather than absolute.

TABLE 1.—*Oxygen absorbed and carbon dioxide formed in soils from different places*

Soil type and location	Carbon in soil	Oxygen absorbed	Equivalent CO_2	CO_2 given off	Deficiency CO_2	Excess CO_2	CO_2 given off per hundred CO_2 absorbed
	Per cent	Gram	Gram	Gram	Gram	Gram	
Marlboro sandy loam, New Bern, N. C.	0.99	0.0814	0.1119	0.0771	.0343		69
Portsmouth sandy loam, Bennettsville, S. C.	.96	.0714	.0981	.0612	.0369		62
Holdridge loam, North Platte, Nebr.	(¹)	.0789	.1081	.0573	.0508		53
Ruston fine sandy loam, Foley, Okla.	.77	.0789	.1084	.0671	.0413		62
Soil from Orlando, Fla.	(¹)	.0728	.1002	.1690		0.0688	169
Do.	(¹)	.0968	.1330	.1798		.0468	135
Washburn loam, Maine.	4.90	.1214	.1669	.2087		.0418	125
Caribou loam, Maine.	3.00	.0997	.1370	.2075		.0705	151
Clyde loam, Monticello, Ind.	(¹)	.0967	.1339	.1543		.0204	115
Haneeville fine sandy loam, Arkansas	(¹)	.0200	.0275	.1067		.0792	388
Cecil sandy loam, Atlanta, Ga.	1.32	.0414	.0569	.1166		.0597	205
Sassafras loam, Maryland.	1.20	.0614	.0844	.0256	.0588		30
Portsmouth fine sandy loam, New Bern, N. C.	.80	.0470	.0647	.0513	.0134		79

¹ Not determined.

Although the volumes of gas absorbed have been calculated· to grams, it may be noted that this volume varied from 14 to 85 cubic centimeters and the carbon dioxide given off from 13 to 106 cubic centimeters. In no case does the carbon dioxide equivalent of the oxygen absorbed correspond with the carbon dioxide given off; in some cases it exceeds it, and in others it is much smaller.

A deficiency of carbon dioxide in such a case can mean only that some of the oxygen absorbed did not contribute to the formation of carbon dioxide, and an excess can mean only that the breaking down resulting from the absorption of oxygen liberated some carbon already in combination with oxygen. Both these processes could and probably do go on at the same time, so all that the results stated indicate is that in some cases one reaction predominates and in others the other. This, of course, is in accord with the fact that the organic matter of soils differs in character in different soils.
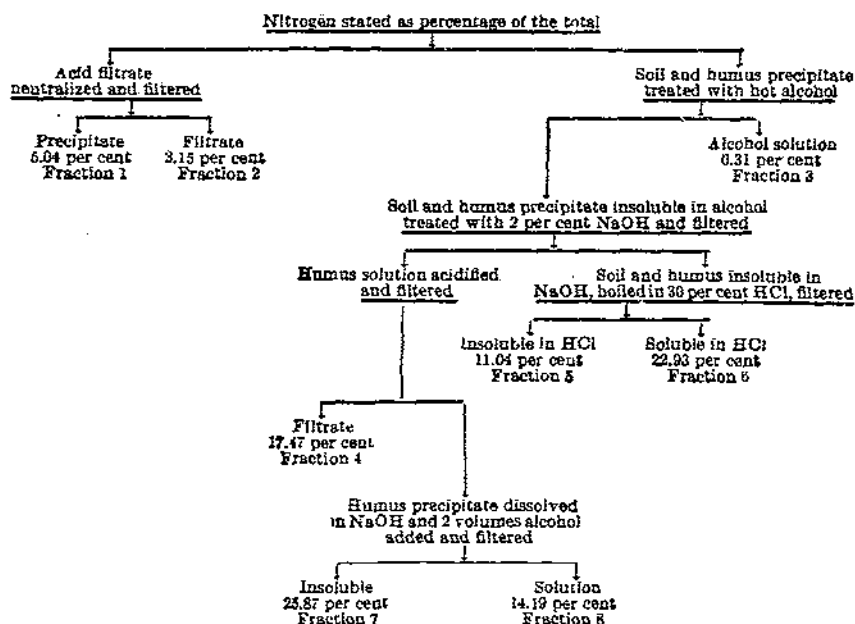
NITROGEN DISTRIBUTION

Ever since the organic material of soils has been under investigation the question whether or not nitrogen is an integral part of the material known as humus has been discussed. The fact that numerous organic compounds that do not contain nitrogen have been isolated from the humus extract of soils (12)[1] leads necessarily to the conclusion that at least some of the organic matter in such an extract is nitrogen free, but the question whether nitrogen is a component of some of the dark-colored (amorphous) colloidal organic material that often makes up a large proportion of the humus is still unanswered.

In this connection one fact stands out prominently, namely, that in fractionation of the humus material all fractions in the first steps contain nitrogen. It should be pointed out here that the numerous nitrogen-free organic compounds that have been obtained from the humus extract (hydrocarbons, carbohydrates, sterols, and acids) have been obtained in a pure form only after repeated precipitation or crystallization accompanied, of course, with large losses of material.

As an example of the nitrogen distribution obtained by fractionation of the humus extract of a soil, the results with a sample of Washburn loam from Maine, containing 0.297 per cent total nitrogen and 4.8 per cent organic carbon, are presented in Table 2. Similar results, in that all fractions contain nitrogen, have been obtained from several soils, and there is no reason to suppose that they can not be obtained from most soils by this procedure. Peats high in nitrogen content and for that reason appealing to one interested in nitrogen distribution do not lend themselves quantitatively to this treatment because of the mechanical difficulties encountered in filtration where such a large volume of colloidal material is involved.

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[1] Italic figures in parentheses refer to Literature Cited, p. 25.

TABLE 2.—*Percentage of total nitrogen in humus fractions of Washburn loam from Maine* [1]

Nitrogen stated as percentage of the total

Acid filtrate neutralized and filtered

Precipitate 6.04 per cent Fraction 1

Filtrate 3.15 per cent Fraction 2

Soil and humus precipitate treated with hot alcohol

Alcohol solution 0.31 per cent Fraction 3

Soil and humus precipitate insoluble in alcohol treated with 2 per cent NaOH and filtered

Humus solution acidified and filtered

Soil and humus insoluble in NaOH, boiled in 30 per cent HCl, filtered

Insoluble in HCl 11.04 per cent Fraction 5

Soluble in HCl 22.93 per cent Fraction 6

Filtrate 17.47 per cent Fraction 4

Humus precipitate dissolved in NaOH and 2 volumes alcohol added and filtered

Insoluble 25.87 per cent Fraction 7

Solution 14.19 per cent Fraction 8

Total N
Fractions added—0.316 per cent N
Total N in soil
    direct determination 0.297 per cent

The schematic presentation of this treatment presented in Table 2 is, to a large extent, self-explanatory and may be summarized as follows:

		Per cent		Per cent
N in soil 100 per cent	N dissolved or dispersed in NaOH solution	66.03	N in humus precipitate	45.41
			N in filtrate from humus precipitate	20.62
	N not dissolved or dispersed in NaOH solution	33.97	N dissolved by boiling HCl	22.93
			N insoluble in boiling HCl	11.04

The following comments on the character of the different fractions should be noted: Fraction No. 1 consists, for the most part, of combinations of aluminum, iron, and calcium with humus material. In this case aluminum predominates, and if the precipitate be dissolved in the smallest quantity of sulphuric acid which will effect solution and several volumes (from 6 to 10) of alcohol be added, nearly pure aluminum sulphate is precipitated. A portion of the organic matter

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[1] Treated 24 hours with 2 per cent sodium hydroxide, acidified, and filtered from soil and humus precipitate.

in the acid alcohol can be recovered by allowing the alcohol to evaporate spontaneously, adding water to maintain the volume, and filtering. There is thus obtained, in the case of this soil, a mixture of resinous and waxy material that can be further fractionated by treatment with solvents. This material is very similar to the material in fraction No. 3.

Fraction No. 2 is an acid filtrate containing a large number of compounds, some of them nitrogenous (purine bases and pyrimidine compounds, for instance). Nitrogen-free fatty acids can be obtained from this filtrate by shaking out with ether (hydroxy fatty acids) or by precipitation with metallic salts.

Fraction No. 3 is a fraction containing less nitrogen than any other, and by treatment with various solvents it can be further fractionated, most of the fractions being nitrogen free. The nitrogen may be considered contamination and not nitrogen of constitution, for while the nitrogen is 0.31 per cent of the total in the soil it is only 0.001 per cent of the fraction.

Fraction No. 4 is a fraction which might be expected to be similar in character to that obtained in the first treatment, where the sodium hydroxide solution or suspension is acidified and filtered. However, it differs from it in that no precipitate is formed on neutralizing, all the aluminum easily extracted from the soil by sodium hydroxide having been removed by the first treatment and appearing in fraction No. 1. Further, the treatment of the soil and humus precipitate with hot alcohol, whereby fraction No. 3 was obtained, has made possible the solution or dispersion of a considerable amount of additional material by the sodium hydroxide solution. This shows up in the larger percentage of the total nitrogen (17.47 per cent) appearing in this fraction.

Fractions Nos. 5 and 6 are two fractions representing the nitrogenous material not dissolved or dispersed by 2 per cent sodium hydroxide solution, the two together accounting for 34 per cent of the total nitrogen. The further fractionation of this material by treatment with hot hydrochloric acid shows that two-thirds of the nitrogen is made soluble by this treatment.

Fraction No. 6 represents the hydrochloric acid solution, which can be further fractionated by any of the treatments that are applicable to the hydrolysis products of protein. In this way fractions may be obtained that may be designated ammonia, amide, monoamino, diamino, and humic nitrogen. It is, however, doubtful whether in the application of such methods to hydrolysis products of soil there may not be included in some of the fractions compounds quite different from those formed in the hydrolysis of proteins.

Nothing definite is known regarding the compounds present in fractions Nos. 7 and 8.

POSSIBLE FORMS OF NITROGEN

Consideration of this schematic presentation of a separation of the soil nitrogen into fractions discloses the fact that despite many years' investigation and discussion of the forms of organic nitrogen in soils very little definite chemical information is available.

The definite organic nitrogenous compounds that have been identified are those that are evidently in the transitional stages between

the original organic material added to the soil and the final products that are more slowly decomposed. Such compounds are probably present at any one time in very small quantities. They are present in only two or three of the fractions just discussed.
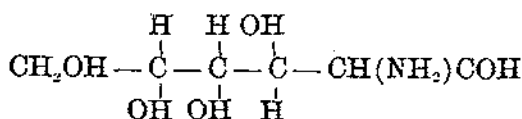
It has appeared that, in connection with investigations of the unknown soil nitrogen, it might be helpful to consider what were the possible forms of nitrogen other than those known or discussed. Such consideration is given in the following pages.

CHITIN

It is recognized that fungi are present and active in the upper layers of nearly all soils. It is further known that the cell wall of fungi is closely related chemically to chitin (*2, 3, 4, 23, 24, 25, 26, 27*), the material that forms the organic part of crab and lobster shells and the wing covers of cockroaches and other insects.

Chitin is a nitrogenous amorphous body characterized by its resistance to action by chemical reagents. Advantage of this fact is taken in preparing it from lobster shells, which are the most accessible source, the process being simply extraction with strong alkali and acid.

Were chitin present in soil, isolation by this process would be difficult, if not impossible, because of the relatively large quantity of inorganic material to be got rid of. Chitin, however, when heated with concentrated hydrochloric acid, yields glucosamine, a nitrogen-containing carbohydrate ($C_6H_{13}NO_5$) which is represented structurally by

$$\text{CH}_2\text{OH} - \underset{\underset{\text{OH}}{|}}{\overset{\overset{\text{H}}{|}}{\text{C}}} - \underset{\underset{\text{OH}}{|}}{\overset{\overset{\text{H}}{|}}{\text{C}}} - \underset{\underset{\text{H}}{|}}{\overset{\overset{\text{OH}}{|}}{\text{C}}} - \text{CH}(\text{NH}_2)\text{COH}$$

The hydrochloride obtained by this treatment is crystalline and can easily be purified and identified by its composition and properties.

The separation of glucosamine from a soil which before treatment contained no glucosamine but which was treated in such a way as to form this substance from chitin, were chitin present, would be a strong indication of the presence of the latter substance in the untreated soil.

With this in mind four soils of very diverse composition, Everglades peat, Caribou loam, Hagerstown loam, and a sandy soil from North Carolina, were examined as follows:

The soils were extracted with 10 per cent sodium hydroxide solution until the extract was colorless, or nearly so, the extracts being passed through a Pasteur-Chamberland filter. The residue was then extracted with dilute hydrochloric acid, treated with a dilute solution of potassium permanganate followed by a dilute solution of sodium bisulphite, then washed with water until no residue was left in the wash water on evaporation. The residue was then heated with concentrated hydrochloric acid and the solution so obtained treated for crystallization of glucosamine hydrochloride. In the loams and sandy soil, the solution obtained by heating with concentrated hydrochloric acid contained such a large quantity of inor-

ganic salts that crystallization and separation of any organic constituent that might have been present was not possible by any treatment usually resorted to in such cases.

In the Everglades peat, containing nearly 90 per cent organic matter, the preliminary extraction with 10 per cent sodium hydroxide solution dispersed so much of the material that extreme dilution had to be resorted to in order to accomplish filtration; and even then this process was very slow, many weeks being required to obtain sufficient residue to warrant further treatment. The residue from 1 kilo of peat was finally obtained, and after the treatment outlined above there was obtained an acid solution containing a very small quantity of inorganic salts. This solution was dextrorotary, reduced Fehling's solution. After it had been evaporated to a sirup and left standing for some days, a very few crystals were formed. All attempts to separate these for purification resulted in such losses that it was impossible to obtain sufficient crystals for confirmatory work.

However, the preliminary treatments to which the peat had been subjected would either remove or decompose any carbohydrates or any complexes yielding carbohydrates by any but the most strenuous treatment, and this, in conjunction with the fact that the final solution was optically active, reduced Fehling's solution, and that it contained crystalline material is presumptive evidence of the presence of some very resistant carbohydrate-yielding complex at least resembling chitin in nature.

INDOL COMPOUNDS

In all theoretical considerations of the possible forms of nitrogen in soils there is one class of compounds which apparently has not been studied heretofore. That one has indol,

$$C_6H_4 \underset{NH}{\overset{CH}{\diamondsuit}} C_6H_4,$$

as a nucleus and is usually spoken of as the benzpyrrol or indol group. Indigo blue or indigotin is the simplest and best known of such compounds and is represented by the structural formula

$$C_6H_4 \underset{NH}{\overset{CO}{\diamondsuit}} C = C \underset{NH}{\overset{CO}{\diamondsuit}} C_6H_4$$
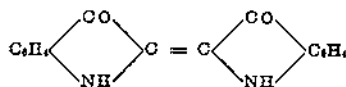
This compound is extremely insoluble and resistant to chemical reaction except that of reduction by suitable treatment (sodium hydrosulphite in alkali dispersion) by which indigotin is reduced to the leuco form, indigo white

$$C_6H_4 \underset{-NH-}{\overset{C(OH)}{\diamondsuit}} C = C \underset{-NH-}{\overset{C(OH)}{\diamondsuit}} C_6H_4$$
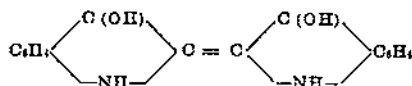
This dissolves in alkalies, the solution having a slightly yellow color. When such solution is exposed to the air oxidation to indigo blue takes place immediately.

The process of dyeing with indigo is to immerse the fabric (cotton) in a solution of the leuco form in alkali, remove the fabric, squeeze out excess of solution, and expose to the air, when indigo blue is formed in the fibers.

Other compounds of similar constitution, properties, and use are derived from indanthrene, represented by the structural formula

$$C_6H_4 \underset{CO}{\overset{CO}{\diagdown}} C_6H_2 \underset{NH}{\overset{NH}{\diagdown}} C_6H_2 \underset{CO}{\overset{CO}{\diagdown}} C_6H_4$$
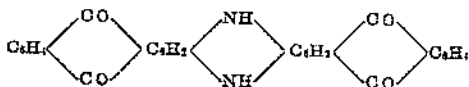
Indanthrene is very insoluble and, like indigo blue, forms on reduction a leuco compound soluble in alkali and represented as follows:

$$C_6H_4 \underset{C(OH)}{\overset{C(OH)}{\diagdown}} C_6H_2 \underset{NH}{\overset{NH}{\diagdown}} C_6H_2 \underset{C(OH)}{\overset{C(OH)}{\diagdown}} C_6H_4$$

This is again oxidized to the insoluble form on exposure to the air, which is the basis of dyeing with this class of dyes, usually called vat dyes.

The extreme insolubility and resistance to chemical reagents shown by part of the nitrogen of many soils has suggested the possibility of the presence of some compounds similar in constitution to those just discussed.

It should be mentioned in this connection that there are many compounds of similar constitution that, in the oxidized form, have some color, are very insoluble, and are reduced to a soluble, less-colored leuco form that is again readily oxidized to the insoluble form, but that are not of any value as vat dyes because the colors developed in oxidation are not of such character or brilliancy as to be of value commercially.

The orange to brown color of the humus extract of soil is not fixed on cotton in alkali dispersion. Cotton yarn or cloth treated with such an extract may be slightly stained, but the stain is almost completely removed by simple washing in water. At the most, the cotton is left a dirty white. In the case of some soils, however, if the humus extract be heated to boiling with a reducing agent such as sodium hydrosulphite, the color becomes lighter (noticeable probably only if the extract be dilute), and if cotton yarn or cloth be now treated with the hot extract, removed, the excess solution squeezed out, and the material exposed to the air for some time a color is developed in the cotton that can not be removed by washing, soap, or exposure to sunlight. This color is usually gray rather than brown, is not at all bright, and has no value in the dye industry. However, the fact that a leuco compound, which on reduction is rather readily oxidized on exposure to the air to a colored insoluble body resistant to change, is found suggests the presence in soils of compounds belonging to the indol group. It should be noted in this connection that the property of leuco formation and subsequent ready oxidation lies in the carbonyl group that joins two benzene nuclei and not in the nitrogen linkage which is in the imide form between two benzene nuclei and is not involved in the change on reduction.

All soils do not contain compounds having this property, and this fact may be made the basis of a method for showing differences in soils. Of the soils examined, Washburn loam gave the most pronounced color in cotton cloth.

ACID HYDROLYSIS

Much of our knowledge of the constitution of the proteins is based on results obtained by the hydrolysis of these compounds by boiling acid (hydrochloric or sulphuric). By this treatment proteins are split into simpler compounds, the quantity and kind varying with the kind of protein. By suitable analytical procedure these decomposition products can be separated and determined. The classes recognized in such hydrolysis usually are as follows: (1) Ammonia and amid nitrogen; (2) diamino or basic nitrogen; (3) monamino nitrogen; and (4) humin nitrogen.

In 1905 Shorey (13) applied this method of hydrolysis to soils, using the method of Hausmann as modified by Osborne and Harris, and showed that by this method part of the nitrogen was soluble in hot acid and that the nitrogen in this solution could be separated into the classes mentioned above. Later Jodidi (5, 6) applied the same method to soils, and when the method of Van Slyke (18, 19, 20) was introduced, Lathrop (7) used this modification in the examination of a number of peats and soils.

A large number of soils have been examined by some modification of the acid hydrolysis procedure as applied to proteins, and the following conclusions are warranted.

(1) Some of the nitrogen of soils and peats is found in the acid solution after such treatment; (2) the percentage of the total nitrogen so rendered soluble ranges from 30 to 90 and the percentage in peats high in nitrogen is usually lower than that in most soils; (3) the nitrogen in the acid solution can, by following the methods used in the case of proteins, be separated into similar fractions, each of which contains some nitrogen; and (4) with regard to the identity of the nitrogenous compounds in each fraction, there is, in the case of soils, some doubt, but the status of each may be summarized as follows.

AMMONIA AND AMID NITROGEN

There is little doubt that some ammonia is formed by the hydrolysis, but it is still a question whether or not some of the ammonia may not be formed from some as yet unrecognized constituent of the acid solution by the magnesium or calcium oxide used to drive off the ammonia.

DIBASIC NITROGEN

This fraction is determined by precipitation with phosphotungstic acid and in the case of protein the constituent diamines have been separated and identified. For the most part, this fraction is made up of arginine, histidine, lysine, cystin, etc. Phosphotungstic acid, however, is a somewhat general precipitant for organic bases, and in the case of soils the presence of nitrogenous bases as yet unidentified is a possibility.

MONAMINO NITROGEN

In the method of Van Slyke, which is now generally used in the study of hydrolysis products, the monamino nitrogen is measured by the volume of nitrogen liberated when the solution is subjected to diazotization under proper conditions with sodium nitrite. In the case of soils the only certainty is that there is, in this fraction, some amino compound or compounds that lose nitrogen on diazotization at room temperatures.

HUMIN NITROGEN

This is the nitrogen contained in the precipitate formed on making the acid solution alkaline with magnesium or calcium oxide. The nitrogen in this fraction may be in part a secondary product formed by polymerization or condensation of some of the primary splitting products.

In this connection it should be pointed out that pentose-yielding material has been recognized for a long time as a constant constituent of the organic matter of soils and that practically all soils, when heated with acid as in acid hydrolysis, yield furfurol. Furfurol is extremely reactive in condensing with amino compounds, and frequently in the acid hydrolysis of soils or peats under a reflux condenser a dark-colored material collects in the condenser. Where this has accumulated in sufficient quantity for analysis, it has been shown to contain nitrogen.

The proportion of the nitrogen of soils rendered soluble by acid hydrolysis is influenced to a considerable extent by the strength of the acid and the length of time of hydrolysis. The character of the hydrolysis products is to some extent influenced by the kind of acid used.

Despite the fact that part of the soil nitrogen behaves on hydrolysis as do proteins and that Walters (21) showed the presence of proteoses and peptones in an alkali extract of a sample of Norfolk sandy loam, as a rule no protein reactions can be obtained from soil or fractions of soil nitrogenous material. Although the results of hydrolysis studies of soils have not so far shed much light on the constitution of the organic nitrogen of soils, the fact that from such hydrolysis the fractions into which the product may be separated represent different percentages of the total nitrogen in different soils is conclusive proof of the varying character of the nitrogenous material itself.

ALCOHOL EXTRACTION

Extraction of a soil with alcohol to separate some of its constituents and so differentiate between soils may be applied in several ways. Extraction of the soil without any previous treatment frequently yields little material except in the case of peats containing a large proportion of resinous material. The reason for this appears to be chemical combination between some of the organic acidlike constituents and inorganic constituents such as iron, aluminum, and calcium. All such combinations are insoluble or little soluble in alcohol.

Leaching of the soil with dilute hydrochloric acid dissolves the inorganic part of the combinations, leaving the organic portion since it is little soluble in dilute acid. Treatment with alcohol, especially on boiling, after such acid treatment usually removes an appreciable quantity of organic matter which, if sufficiently large, can then be separated by the use of other solvents into fractions having different physical and chemical properties.

Failure to recognize the fact that there often are, in soils, combinations of acidlike organic materials with basic inorganic constituents, especially calcium and aluminum, and that it is necessary to break up such combinations by treatment with dilute acid before extraction with alcohol may render some proposed methods of soil examination with solvents quite misleading in results obtained.

Another method of alcoholic extraction that can be profitably used is that of extraction of the humus precipitate. When a humus solution of a soil is made with dilute sodium hydroxide many of the organic mineral combinations, especially those with aluminum, are dissolved or dispersed. When such a solution or dispersion is acidified the organic humus material is precipitated or flocculated, and on filtration the aluminum or other inorganic constituents are found in the filtrate. This humus precipitate contains most of the organic soil constituents that are acidic in nature and little soluble in water. These can be removed from the precipitate by extraction with hot alcohol, after which the extract can be treated as one made directly from the soil.

As has just been mentioned, some soils, especially peats containing a large proportion of resinous material, yield quite a large quantity of material on direct extraction with alcohol. As an example of this may be mentioned a peat from the Lake Mattamuskeet region in North Carolina. This, on prolonged extraction with hot alcohol, yielded 10 per cent of its weight in extractive material. By making a first separation of this material into that soluble and that insoluble in cold alcohol and then treating these fractions repeatedly with ether chloroform and petroleum ether, a separation into 13 fractions was accomplished. All these fractions were resinous or waxy in character. They ranged in color from white to dark brown and in melting point from 30° to 160° C.

Alcohol extraction applied in some one of the methods mentioned has, in the past, resulted in the isolation and identification of a number of organic compounds (*12, 15, 16, 22*), among which are phytosterol, hentriacontane, mono hydroxy stearic acid, lignoceric acid, and mixed glycerides, and a number, such as three resin acids, agroceric acid, agrosterol, and paraffinic acid, the identity of which is not so well established.

ALTERNATE WATER AND ALCOHOL EXTRACTION

In connection with the work on alcohol extraction it was observed that if the extraction with alcohol was continued until little color appeared in the filtrate and was then followed by an extraction with water, frequently this extract was highly colored and contained an appreciable quantity of extractive material. This procedure could be repeated several times, as is illustrated by the following figures.

One kilo of dried finely ground peat from the Florida Everglades was leached in a Buchner funnel with 2 per cent hydrochloric acid until the filtrate gave no reaction for calcium, and the acid filtrate was rejected for the purpose of this experiment. The peat was then washed with water until the washings were no longer acid, and then with hot alcohol until the filtrate was only very slightly colored. These two filtrates were evaporated to dryness and constituted water extract No. 1 and alcohol extract No. 1. Washing with water was then resorted to and continued until the filtrate was only slightly colored. Then again hot alcohol was used for washing, yielding water extract No. 2 and alcohol extract No. 2. This alternate treatment was continued until eight extracts had been obtained. The weights of the extracts are given in Table 3.

TABLE 3.—*Extracts and weights of water and alcohol*

Extract No.	Water	Alcohol
	Grams	*Grams*
1	3.30	17.85
2	1.25	6.15
3	.75	3.10
4	1.00	2.25

The peat contained 84 per cent organic matter and 2.8 per cent nitrogen. All the extracts contained nitrogen. The first water extract (washing from acid leaching) was only slightly colored, but the second water extract was highly colored. The most striking feature of the operation was the abrupt change from the last portions of the alcohol extract, which were nearly colorless, to the dark brown of the water extract immediately following it. The same changes were observed throughout but became less marked as the number of extractions increased.

The same phenomena, but of course less marked, were observed with soils containing more nearly normal quantities of organic matter. In some soils there was no increase in color in the water extract. Except for the determination of the presence of nitrogen no investigation of the composition of these extracts was made, since it was apparent that there were other less tedious methods of obtaining similar material.

The explanation of the results obtained by alternate water and alcohol extraction appears to lie in the fact that there are in many soils particles of soil material cemented together by colloidal organic matter. The leaching with dilute acid removes the more readily soluble bases, and the washing which follows removes the last of such soluble material. The alcohol then dissolves the organic acidlike compounds set free, as well as some of the cementing material. When water follows the alcohol some of the organic humus (dark-colored material) is, in the absence of soluble salts, dispersed and runs through the filter, giving a dark-colored extract which is, for the most part, a dispersion and not a solution. Concentration or the addition of a soluble salt such as sodium chloride or sodium sulphate will usually flocculate the dark-colored compounds.

The dispersion of some of the humus in water is a commonly observed phenomenon in working with such material. For instance, by acidifying an alkaline humus extract a humus precipitate is formed that can be filtered off. If washing of this precipitate be continued until all soluble salts are removed, the humus material is dispersed and runs through the filter.

A result somewhat similar to that obtained by alternate water and alcohol extraction of soil can be brought about by treating a humus precipitate in the same way.

WATER EXTRACTION

Water extraction of soils has been resorted to chiefly for the detection and determination of inorganic constituents, total soluble salts, soil reaction, nitrates, nitrites, etc., but has not been used to any extent in investigations of the organic matter. The reason for this is twofold: First, in the quantities ordinarily used in the laboratory, soils do not yield enough organic matter in solution to make possible any separation and identification of organic constituents that might be present. Some peats and some soils containing a quantity of decomposing vegetable matter are exceptions to this. The second reason lies in the scarcity of methods available for the detection of small quantities of suspected organic constituents in a water extract. This is an outstanding obstacle in this work, even when appreciable quantities of organic matter are present.

The first difficulty can be overcome by working with larger quantities of soil and extracting with hot water where that is not objectionable. Several of the organic compounds that were first isolated from an extract made with sodium hydroxide were later isolated from water extracts of the same soil (*14*). When there is much inorganic colloid material, filtration after two or more extractions with water becomes difficult because of the removal of electrolytes and the consequent dispersion of the colloid material. It is not uncommon, even with soils with little organic matter, for a hot-water extract to contain more organic than inorganic material.

The second difficulty, owing to a scarcity of methods or tests, can be overcome only by making the best use of such tests as are available and devising new ones.

FUSION WITH ALKALIES

Fusion of certain plant constituents with alkalies, usually potassium hydroxide, has frequently thrown some light on the constitution of such compounds through the identification of the products of such fusion, usually compounds of a phenolic nature. Numbers of soils and organic soil fractions have been subjected to potash fusion, and the only compound definitely identified has been pyrocatechol.

Since this phenol is formed by potash fusion from a great variety of vegetable products, its formation does not in the case of soils indicate anything very definite regarding the structure of its antecedents. However, as pyrocatechol has been obtained in this way from some soils and not from others, the fusion to that extent lends itself to some differentiation between soils.

OXIDATION WITH HYDROGEN PEROXIDE

Hydrogen peroxide has been proposed as a reagent for the oxidation of soil organic matter and as a basis for a method of determining the total organic matter of soils (11). It has also been proposed to use this reaction as a means of freeing a soil of organic matter preparatory to mechanical analysis (10).

Hydrogen peroxide, in the absence of metallic catalysts, has little or no action on pure organic compounds and the organic matter of soils when separated from mineral constituents behaves in the same way. Active catalysts of hydrogen peroxide are found in nearly all soils in sufficient quantity to bring about vigorous liberation of oxygen from hydrogen peroxide and oxidation of the organic matter present. However, in soils containing much manganese the catalysis of the peroxide is so vigorous that an enormous quantity of peroxide is rapidly decomposed without much action on the organic matter. This, according to Olmstead, Alexander, and Middleton, can be remedied by the addition of acetic acid, which changes the manganese to the manganous form (9).

As an example of what happens to the nitrogenous organic matter of soils, the following experiment is cited: A sample of Everglades peat, containing 2.57 per cent nitrogen, was treated in a dish in the steam bath with successive portions of Superoxol (30 per cent by weight hydrogen peroxide) until there was no further action. The residue, which was light gray in color, was filtered off and washed, and the nitrogen in it and in the solution was determined. The results were as follows, stated as percentage of the peat:

Per cent

$$
N \begin{cases}
\text{Insoluble} \dotfill 0.14 \\
\text{In solution 1.90 per cent} \begin{cases} \text{as } NH_3 \dotfill 1.72 \\ \text{as } NO_3 \dotfill .09 \\ \text{unknown} \dotfill .09 \end{cases} \quad 2.04 \\
\text{Unaccounted for} \dotfill .53
\end{cases}
$$

The nitrogen unaccounted for was evidently lost in gas form as free nitrogen or as oxides of nitrogen. The solution was acid in reaction, and it was evident that the ammonia was present as ammonia salts of acids that had been formed from the soil organic matter by oxidation (11).

Stated in percentage of the total nitrogen in the peat, the results of the experiment appear as follows:

Per cent

$$
N \begin{cases}
\text{Insoluble} \dotfill 5.4 \\
\text{In solution as } NH_3 \dotfill 66.9 \\
\text{In solution as } NO_3 \dotfill 3.4 \\
\text{In solution in unknown form} \dotfill 3.4 \\
\text{Lost and not accounted for} \dotfill 20.9
\end{cases}
$$

Another sample of peat containing 2.81 per cent nitrogen treated in the same way as the Everglades peat gave the following results, stated in percentage of the peat:

Per cent

$$
N \begin{cases}
\text{Insoluble} \dotfill .14 \\
\text{In solution as } NH_3 \text{ and } NO_3 \dotfill 1.53 \\
\text{Unknown form} \dotfill .55 \\
\text{Escaped and unaccounted for} \dotfill .59
\end{cases}
$$

or, stated as percentage of total nitrogen:

		Per cent
N	Insoluble	4.9
	In solution as NH_3 and NO_3	54.0
	In unknown form	20.0
	Escaped and unaccounted for	21.1

Two soils, one Chester loam from Virginia and the other Norfolk fine sandy loam from North Carolina, containing 0.18 and 0.07 per cent nitrogen, respectively, gave the figures shown in Table 4 on treatment with hydrogen peroxide:

TABLE 4.—*Hydrogen peroxide treatment of Norfolk and Chester soils*

	Norfolk fine sandy loam		Chester loam	
	Soil	Total N	Soil	Total N
	Per cent	Per cent	Per cent	Per cent
Total N	0.07		0.18	
N insoluble	.016	22.9	.042	23.3
N in solution as NH_3	.047	67.1	.113	62.7
N escaped and unaccounted for	.007	10.0	.025	14.0

Both solutions gave slight reactions for nitrites and nitrates, in quantities too small for determination. Nothing definite is known regarding the nature of the nitrogenous compound or compounds in the insoluble residue.

The transformation of a large percentage of soil nitrogen into ammonia on treatment with hydrogen peroxide occurs with all soils examined, and while there are differences in this respect, so far these differences have not yet been found to be associated with any definite organic constituents.

It is quite evident, as has been noted, that in the solution resulting from the oxidation of soils by hydrogen peroxide, some nitrogen is present as ammonium salts of organic acids produced in the oxidation. Attempts to identify such acids have so far been successful in only one case. Saccharic acid has been identified among the products of the oxidation of the peat noted above. This was identified by separation with ammoniacal lead acetate, removal of lead with hydrogen sulphide, preparation of the acid potassium salt, precipitation with cadmium sulphate, removal of cadmium with hydrogen sulphide, and purification by solution in 95 per cent alcohol (*15*).

The residue had the properties of saccharic acid and gave a silver salt containing 50.88 per cent silver (theoretical 51.02). Saccharic acid can be prepared by the oxidation of some carbohydrates with dilute nitric acid. In this case it no doubt is the result of the oxidation of some carbohydrate constituent of the peat.

It is evident from the varying figures obtained by treatment with hydrogen peroxide that when calculated in terms of nitrogen such treatment can be utilized for showing differences in the organic matter of soils.

VOLATILE CONSTITUENTS

That there are in soils organic constituents that are volatile under certain conditions is easily demonstrated.

If a current of dry air is passed over some soils, especially those of high organic content, heated to 100° to 110° C. in a closed apparatus, and is then passed into concentrated sulphuric acid, the acid is soon darkened from the decomposed organic constituents carried over.

Again, in the case of nearly all soils when subjected to steam distillation volatile compounds are carried over and may be recovered from the distillate, usually by shaking with an immiscible solvent. The material obtained in this way is usually oily and often gives aldehyde reactions. In no case, however, has sufficient material been obtained for identification of components. Free sulphur, if present in a soil, may be recovered in this way but can be much better separated directly from the soil by extraction with a suitable solvent.

A special method devised by Smith, and published by him in a dissertation (17), has thrown further light on the occurrence of volatile compounds in soils. The method is, briefly, as follows: Several kilos of a soil, preferably one high in organic content, are boiled under a reflux condenser with two or three volumes of 80 per cent alcohol, then cooled and filtered. This is repeated two or three times, and the filtrates are combined and subjected to distillation under reduced pressure. When about one-third of the volume has been distilled, this distillate is treated with a few cubic centimeters of a saturated aqueous solution of copper sulphate. A precipitate, made up of copper sulphate and a copper compound of the volatile material, is formed. The whole is allowed to stand several hours (or days) until the precipitate has settled, the supernatant liquor is decanted or siphoned off, and the precipitate is filtered off and washed with alcohol. It is then treated on the paper with a small quantity of water, which dissolves the copper sulphate, leaving the copper organic compound, which, after washing with water, is suspended in alcohol and decomposed with hydrogen sulphide. It is then filtered from copper sulphide, and the filtrate is concentrated to small volume and allowed to stand. Crystals may finally appear and if so may be separated and purified by suitable means.

The volatile compound which Smith separated in this way from a soil in Holland and which he calls "gliedene," has not been definitely identified as to structure or composition.

The method has been applied to North American soils in the soil fertility laboratory of the Bureau of Chemistry and Soils and the only case where any appreciable quantity of material has been obtained has been with a peat from the Florida Everglades. In this case the crystals had the appearance of a fatty acid and melted at about 60° C. Not enough were obtained to allow purification to a constant melting point or to make salts or other derivatives.

It is significant that the soil (Everglades peat) from which this volatile constituent was obtained is in its raw uncultivated condition infertile but improves rapidly on being cultivated so as to expose fresh portions to the air. Further, phenomenal results in increased fertility have been obtained in such soil by small applications of copper sulphate (1). Since the insoluble compound of copper with this volatile organic compound is made the basis of separation, one may be justified in speculating whether or not there is any connec-

tion between the occurrence of such volatile compounds and the results obtained by applications of copper salts to the soil.

AZOPHENINE FORMATION

In the case of some soils, if a portion be treated with aniline and heated to 100° C. and the aniline filtered off and evaporated, there is left a dark-colored residue. This is partly soluble in alcohol, and leaves a dark-red crystalline mass that can be purified by dissolving in chloroform, repeating the treatment with alcohol several times, and, finally, recrystallizing from chloroform. There are thus obtained garnet-colored crystals, usually small plates or bunches of plates, that appear as needles. These melt at 236°, are insoluble in water, alcohol, or ether, and give the characteristic reaction of azophenine (soluble in concentrated sulphuric acid, the solution being purple but suddenly changing to clear blue when heated to 300°).

Azophenine, which is represented structurally—
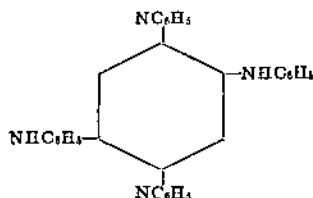
is prepared in the laboratory or industrially by several processes, nearly all of which start with aniline but none of which suggest any process by which azophenine might be formed by treating soil with aniline. There is no reason to suppose that azophenine is a soil constituent, but its formation from aniline alone through the agency of some catalyst in the soil means the condensation of 5 molecules of aniline with elimination of 11 atoms of hydrogen and 1 of nitrogen, and it is rather difficult to understand the process unless it is assumed that some soil constituent takes part in it. At the present time no indication has been obtained as to the nature of such soil constituent, but it would seem that it must be one containing a benzene nucleus.

There is formed with the azophenine a dark-purple compound soluble in alcohol and drying to an amorphous mass in which no crystalline material has been observed and regarding the constitution of which nothing is known.

It has been found that soils differ very widely in azophenine formation under the conditions described, and it may be possible to connect this property with other properties or with some characteristic of composition.

GENERAL REACTIONS

In examination of soils to determine if possible the character of the organic matter or differences in soils with respect to the character of the organic matter there are, in addition to the methods of treatment discussed above, a number of general reactions available. These, for the most part, are indicative of the presence or absence of groups of

compounds and are applicable usually to soil extracts or solutions of soil fractions. Those which have been found useful in this connection are discussed below.

PYRROL REACTION

A great many organic compounds of plant or animal origin yield pyrrol on destructive distillation. The presence of pyrrol is shown by what is known as the pyrrol or pine-splinter reaction. A splinter of pine wood that has been dipped in concentrated hydrochloric acid becomes bright red when exposed to pyrrol vapors. The color is formed by reaction of the pyrrol with aldehyde present in the wood. A strip of newsprint paper made from wood pulp can be substituted for the pine splinter, but the color developed is not so bright.

The compounds that yield pyrrol on distillation may be those containing a pyrrol nucleus, such as indol or carbazole, or nitrogen-containing bodies that on heating yield pyrrol by some rearrangement, as is the case in the diamino bases. Other nitrogenous compounds, such as leucin, alanine, and tyrosine, yield pyrrol after reduction with zinc dust, and still other compounds not containing nitrogen, such as levulinic acid, give the reaction after treatment with ammonia and zinc dust. As a means of showing that soils differ in their organic constituents the pyrrol reaction has been applied to a number of soils, (1) by dry distillation of the soil extract, (2) by distilling the soil extract with zinc dust, and (3) by distilling the soil extract with zinc dust and ammonia. The procedure was as follows:

One hundred grams of soil were treated with two hundred and fifty cubic centimeters of water, stirred for two hours, and filtered; portions of the filtrate, after evaporation to dryness, were heated in a test tube, one alone, two with zinc dust, three with ammonia added with zinc dust, and were evaporated to dryness and then distilled. In the absence of any standard depth of color for comparison the intensity of the reactions is estimated as very faint, faint, fair, etc.

There is, of course, a personal factor here, but the chief point is that soils differ in respect to the intensity of this reaction and the treatment by which it is brought about. The soils examined were such as were available in the laboratory at the time. The exact location was unknown in a few cases, so this particular has been omitted in all cases. The results obtained are shown in Table 5.

TABLE 5.—*Pyrrol reaction color developed*

Soil type	Direct	With zinc dust	With zinc dust and NH₃
Washburn loam		Strong	Strong.
Caribou loam		Very strong.	Very strong.
Florida sand	Very faint	Fair	Strong.
South Carolina sand		do	Do.
Do		Faint	Fair.
Do		do	Faint.
Carrington loam	Very faint	Fair	Strong.
Marshal loam		do	Do.
Miami sandy clay loam		Strong	Do.
Orangeburg loam		Faint	Faint.
Soil from Phoenix, Ariz		Fair	Fair.
Michigan muck	Strong	Very strong	Very strong.

It will be noted that the muck soil was the only one that gave a direct test of any intensity. Nine of the twelve soils gave a good test after reduction with zinc dust, and with one exception the color was more intense with ammonia. Several soils in this series were of undetermined type. No. 6 was the original of No. 5, the latter having had a green-manure crop plowed in for several years.

The fact that the pyrrol reaction with zinc dust and ammonia may be given by such a large number of compounds of course makes it impossible to interpret it in terms of any compound or group of compounds, but the fact that soils differ markedly in the intensity of this reaction makes it possible to utilize this test as a means of showing differences in the kind of organic matter.

IODOFORM REACTION

When a solution of iodine, in potassium iodide, is added to certain organic compounds dissolved in sodium hydroxide iodoform is formed. Some compounds, such as acetone and levulinic acid, give iodoform under these conditions immediately in the cold, others, such as ethyl alcohol, giving it only after warming the mixture. According to Lieben (8) most compounds containing the $CH_3CO.C$ and $CH_3CHOH.C$ groups give iodoform in this way.

Many alkaline extracts of soils (humus extracts) when treated with a solution of iodine in potassium iodide give iodoform, which is easily identified by its crystalline appearance, 6-sided plates or stars with lines radiating from the center. This formation of iodoform has not been connected with the presence of any known organic soil constituent, and though it has been established that some soils give it and others do not it has not thus far been shown to be characteristic of any type of soil. In carrying out this test an odor of iodoform is not conclusive. The test should not be applied to samples of soil that have previously been treated with alcohol or acetone.

CARBOHYDRATE REACTIONS

The reaction for carbohydrates most generally applicable to soil extracts or fractions prepared from soils is that known as the Molisch reaction. This is carried out by placing a few cubic centimeters of the solution to be tested in a test tube, adding a few drops of an alcoholic solution of α-naphthol, and then adding concentrated sulphuric acid in such a way that it runs down the side of the tube and forms a separate layer below. If carbohydrates are present a red or purple ring is formed at the junction of the two layers. When this color is very quickly developed it is usually worth while to carry the investigation further. The reaction is so delicate that traces of filter-paper fiber, dust, etc., are often sufficient to give it. The sulphuric acid and the solution to be tested must be free of nitrous acid, or misleading results will be obtained.

Extracts or solutions that give a positive Molisch reaction may or may not reduce Fehling's solution. In the latter case usually there is present some carbohydrate that gives a reducing sugar on hydrolysis; consequently when the Molisch test is positive and Fehling's solution negative the material should be subjected to hydrolysis

with sulphuric or hydrochloric acid. Iron should be removed from the resulting product and the solution neutralized before being tested for reducing sugars.

No definite conditions for hydrolysis can be laid down. The optimum for time of heating and strength of acid can be determined only by experiment. For instance, it has been found that some soil extracts containing pentose-yielding material give the maximum amount of reducing sugar on simply being brought to the boiling point with 10 per cent hydrochloric acid; after boiling for five minutes the reducing sugar has been completely destroyed.

Reactions of carbohydrates with phenylhydrazine and its derivatives forming hydrazones or ozazones are not applicable to soil extracts or fractions containing much other material. They are of value only when considerable of the carbohydrate has been separated in a fairly pure form.

PHENOL REACTIONS

If a blue or bluish-purple color is produced when a few drops of a dilute solution of ferric chloride are added to an aqueous extract of soil or to any aqueous fraction that has been neutralized, it indicates the presence of phenolic compounds; that is, compounds containing a benzene nucleus in which one or more hydrogens have been replaced by a hydroxol .OH. radical.

The following precautions should be observed in making this test. It should not be performed with hot solutions. Excess of ferric chloride should be avoided. The color obtained is sometimes transitory, and this should be kept in mind in making the test.

Many aromatic compounds may contain hydroxyls in the benzene ring that are not true phenols, such as hydroxy acids like salicylic or aldehydes like salicylic aldehyde. These usually give blue to purple colors with ferric chlorides. The test should, therefore, be interpreted to indicate the presence of aromatic compounds in which one or more hydrogens are replaced by hydroxyl but may also contain other substituting radicals such as carboxyl $(COOH)$.

ALDEHYDE REACTIONS

Aldehyde reactions can seldom be obtained with a water extract of a soil but often can be obtained with a distillate from steam distillation. This is especially true if such material be concentrated by extracting the distillate with ether, evaporating off the ether on the surface of a small quantity of water, and applying the tests to the water solution.

If much material is extracted from the distillate it is best to shake the ether extract with a saturated solution of sodium bisulphite by which treatment aldehydes are removed from the ether and can be recovered from the bisulphite by acidifying, driving off the sulphur dioxide, and shaking with ether again.

This treatment effects no concentration but separates the aldehydes from other material that may mask or interfere with tests applied. The quantity of aldehydes obtained on steam distillation is usually very small, but relatively large quantities can sometimes be obtained from the acid filtrate on acidifying a humus extract in sodium hydroxide. On shaking out this filtrate with ether, followed by the

bisulphite treatment outlined above, sufficient material can usually be obtained for definite establishment of its aldehyde character.

The reaction best adapted to the identification of aldehyde solutions thus prepared is that obtained with a fuchsine reagent. This is prepared by dissolving fuchsine or one of its salts in a cold saturated solution of sulphur dioxide and allowing it to stand until the pink color disappears and the colorless or yellow solution is diluted with water. Aldehydes in solution, when added to this reagent, restore the pink color in a very short time. Those difficultly soluble may require a longer time. If sufficient soil be used this aldehyde reaction can be obtained from the majority of soils. Many aromatic aldehydes contain a hydroxyl group, and these give colors from red to blue or purple with ferric chloride, as was noted in the discussion of phenol reactions.

In as much as nearly all soils give aldehyde reactions, utilization of this test in distinguishing between soils consists chiefly of observing the intensity of the reaction.

FLUORESCEIN TEST

When phthalic anhydride and resorcinol are fused together, the fused mass taken up in water, and sodium hydroxide added, a red solution is obtained, which on dilution becomes yellow, with a very strong green fluorescence. This is due to the formation of fluorescein, a compound that is the basis of several dyes (eosins). The term fluorescein is properly applied only to that formed from phthalic anhydride, derived from phthalic acid, an ortho compound

$$\text{C}_6\text{H}_4(\text{COOH})(\text{COOH}) \text{, the anhydride being written } \text{C}_6\text{H}_4(\text{CO})_2\text{O.}$$
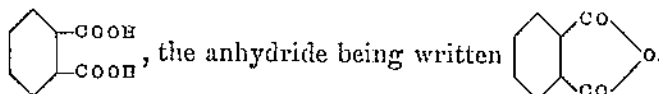
However, several anhydrides of aliphatic acids yield compounds with similar properties when fused with resorcinol, and the term fluorescein is usually applied to them.

Various soil organic fractions have been found to give the fluorescent solutions characteristic of the fluoresceins when they are fused with resorcinol, the mass taken up in water, and sodium hydroxide added.

There is, at present, no reason to suspect the presence of phthalic acid or anhydride in soils, but of the aliphatic acids (anhydrides) known to give this reaction—succinic, maleic, and citraconic—succinic has been found in soils, and a positive result may be interpreted as indicating the presence of a dicarboxylic acid or its anhydride, probably succinic. Soils differ markedly in respect to this reaction, some not giving it at all and others in different intensities.

SUMMARY

In this bulletin there are presented results obtained in the study of a number of tests and reactions used in showing differences in the organic matter of soils.

These results and the conclusions reached may be summarized as follows:

The dark color of the alkali extracts of soils (humus extract) is not due to the absorption of oxygen during the process of extraction,

and such color can not be considered an indication of the presence of phenolic compounds.

Measurement of the quantity of organic matter by comparing the depth of color of humus extracts is not possible; but the fact that the shades of color obtained differ can be offered as proof of differences in kind of organic matter.

When a soil is suspended in dilute alkali and air or oxygen is passed through it there is an absorption of oxygen and formation of an alkali carbonate. The oxygen absorbed can be measured as can the carbon dioxide liberated after acidifying the suspension. This absorption of oxygen and formation of carbonate is different in different soils.

On making an alkaline extract of a soil and fractionating this, nitrogen found in all fractions, as well as in the soil residue. The percentage of the total nitrogen found in the various fractions varies with the soil.

The presence of two classes of organic nitrogenous compounds not usually considered in discussing soil nitrogen is indicated. These are chitin, and its derivative glucosamine, and some member of the indol group.

The results of acid hydrolysis of soils from a nitrogen standpoint are discussed, and this is pointed out as a method of showing differences in soil organic matter.

The use of alcohol, water, and alternate alcohol and water extraction as a means of showing differences in the organic matter of soils is discussed.

The fate of organic nitrogen compounds in soils, when subjected to oxidation with hydrogen peroxide, is stated; and this treatment is proposed as a means of showing differences in the organic nitrogen content of soils.

The occurrence of volatile organic compounds in soils is discussed, and a new method of investigation along this line is reported.

The formation of azophenine, when some soils are heated with aniline, is described, and this formation is suggested as a means of showing the presence or absence of some as yet unknown organic soil constituents.

There is presented a short discussion of a number of general reactions that may be applied to soil extracts or fractions. Among these are the pyrrol and fluorescein reactions.

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