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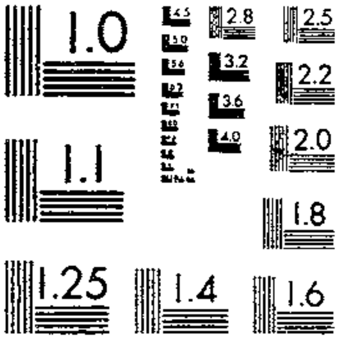

MICROCOPY RESOLUTION TEST CHART
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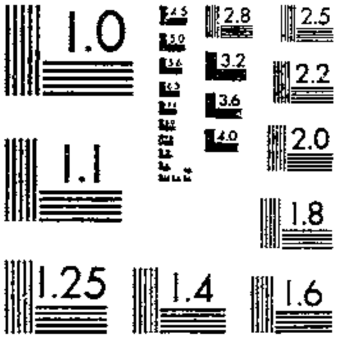

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A CRITICAL LABORATORY REVIEW OF METHODS OF DETERMINING ORGANIC MATTER AND CARBONATES IN SOIL

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INTRODUCTION

The Division of Soil Chemistry and Physics of the Bureau of Chemistry and Soils has for some years used hydrogen peroxide as a part of the procedure of the method of mechanical analysis of soils. This method is based on the international method, essentially the British official method (*1*),[1] and is modified for our own purposes as described by Olmstead, Alexander, and Middleton (*16*). The hydrogen peroxide is primarily employed to secure increased dispersion by removing the organic matter. If the treatment with hydrogen peroxide may be so carried out as also to furnish an accurate estimate of the total organic matter in the soil, the mechanical analysis would have added value.

That the hydrogen-peroxide method has certain very definite limitations was pointed out by W. O. Robinson (*22*) in the paper in which the method was first suggested. It seemed very desirable to make a new study of the method in the hope of removing these limitations.

In the course of the study the other methods which are in use for the purpose of estimating organic matter came up for comparative study, and the investigation eventually assumed the form of a critical examination of these methods. The discussion and data given herewith are the results of that study.

Essentially four methods for estimating the amount of organic matter in the soil have been suggested and used. They are as follows: The loss on ignition (with a variant), the combustion method (with

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[1] Italic numbers in parentheses refer to Literature Cited, p. 23.

many variants), the calculation of the organic matter from the quantity of nitrogen present, and the hydrogen peroxide method. These are by no means equally important or useful. The method based on an assumed constant nitrogen content of the soil organic matter has been repeatedly shown to be invalid. It appears to be true that in a given soil, and even in the soil of considerable areas, the carbon-nitrogen ratio ranges from 10 to 11.6 and therefore the organic matter-nitrogen ratio approximates 20 (*12, p. 90*). At the same time it is generally recognized that results based on these ratios are at best very rough approximations. This fact is brought out very clearly by Leighty and Shorey (*8*) who have found, in 172 samples of soil, carbon-nitrogen ratios ranging from a minimum of 3.5 to a maximum of 35.2, and by Middleton, Slater, and Byers (*15*) who have found, in the profiles of 8 soils and their colloids, an organic matter-nitrogen ratio ranging between the limits of 1.5 and 35.7. In view of these facts no further discussion of this relation will be presented.

THE HYDROGEN PEROXIDE METHOD

This method of estimation of the organic matter in the soil was proposed by W. O. Robinson (*22*) in 1927. The initial suggestion for the work came from the proposal by G. W. Robinson and Jones (*20*) in 1925 that hydrogen peroxide be used to determine the "degree of humification" of organic matter in the soil. This proposal was based on the assumption that unaltered plant tissue is not affected by hydrogen peroxide in the presence of soil mineral matter. W. O. Robinson was able to show that this assumption is not warranted in the case of soils and, by inference, that the inorganic soil material contains a catalyst (or perhaps catalysts) responsible for this effect. On this basis he developed a method which, in brief, consists in the treatment of a weighed portion of soil with a 15 per cent solution of hydrogen peroxide, heating on a water bath until effervescence ceases, and weighing the residual dried soil after filtration. The organic material of the soil is destroyed, or rendered soluble, by this process, and hence the difference between the dry weight of the soil and the dry weight of the residue represents the organic matter of the soil. Correction for dissolved inorganic matter was made by ignition of the evaporated filtrate.

The method offers a direct measure of the quantity of organic matter and is relatively so simple in procedure that if it is or could be made accurate and generally applicable its use would solve a very difficult problem. Unfortunately, as W. O. Robinson (*22*) himself pointed out, it has very serious limitations. Hydrogen peroxide does not remove all the carbon or carbon compounds from the soil. The residual carbon found after treatment with peroxide ranged from 0.04 to 1.90 per cent in the samples examined. It was believed that for the most part this residual carbon consists of adventitious charcoal, graphite, or paraffinlike bodies and is therefore not actually soil organic matter in the ordinary sense. Considerable experimental evidence is presented in justification of this view, especially in the cases of high residual carbon. This view of the matter is supported also by the previously published data of Schreiner and Brown (*26*) and by a subsequent paper by G. W. Robinson and McLean (*21*).

Further limitation of the method was shown in that the presence of any material quantity of manganese dioxide in the soil, because of its catalytic effect on the rate of decomposition of the peroxide, prevents the oxidation of the organic matter and, in the presence of considerable quantities of calcium or magnesium carbonates, the results are low and variable.

Since the publication of the method several papers which have a bearing on it have appeared. Waksman and Stevens (*32*) compared the effect of 6 per cent hydrogen and chlorine dioxide on sound chestnut wood, rotted wood, forest soil, and low-moor peat soil. They found the portions decomposed to vary from 20 to 80 per cent in the different materials, and they inferred that the hydrogen peroxide method was not suitable for such materials. In the light of Robinson's (*22*) work, it would seem that this result should have been expected and that the absence of soil mineral matter in such material is responsible for the low results. This is in harmony also with their own results which show that lignin from sound chestnut wood is even less subject to oxidation than the wood itself.

Shorey (*28*), while suggesting the use of hydrogen peroxide as a means of differentiation of soil organic matter by the use of 30 per cent hydrogen peroxide, shows that a very considerable fraction of the nitrogen of the soil remains in the insoluble residue, in peats, quantities as low as 4.9 per cent, and in soil low in organic matter, as high as 23 per cent. From his data it might be inferred that the residual nitrogen would bear a corresponding ratio to the total amount of organic matter present. Such an inference, if made, is unwarranted, since, as both he and Robinson (*22*) have shown, a very considerable fraction of the nitrogen of organic matter is converted by hydrogen peroxide to ammonia. Soil residues contain a base-exchange complex and, especially in acid soils, ammonia is strongly held by it. The high percentage of residual nitrogen is therefore no index of unconsumed organic matter.

McLean (*13, 14*) in an attempt to develop the suggestion of G. W. Robinson and Jones (*20*) concerning the use of hydrogen peroxide for determining the "degree of humification" of organic matter, reached the conclusion that 3 per cent hydrogen peroxide may be so used but that the 6 per cent reagent is not suitable for the purpose. Incident to this investigation McLean has shown that even the most drastic treatment with 6 per cent and 12 per cent hydrogen peroxide is not sufficient to remove all of either the carbon or nitrogen content of the soil. He also showed that even 3 per cent hydrogen peroxide readily removes up to 85 per cent of the total carbon except in soils containing carbonates. He made no mention of interference by manganese dioxide. In these papers McLean also failed to consider the base-exchange ammonia of his soil residues and made no mention of the content of nitrogen of the hydrogen peroxide used. In consequence, the C:N ratios of his residues have in them an element of doubt.

EXPERIMENTAL DATA

The present study was undertaken to determine means of eliminating the difficulties brought about by (1) the presence of manganese dioxide in the soil and (2) the presence of carbonates, and to ascertain how nearly complete the removal of the organic matter can be made.

MANGANESE DIOXIDE

Manganese dioxide, if present in soils, reacts catalytically to decompose the hydrogen peroxide, and this reaction has been made the basis of a semiquantitative method of its estimation by W. O. Robinson (*23*). Also, in the paper previously cited, he reported that if the soils contain but a fraction of 1 per cent of manganese dioxide, its inhibitory effect on the destruction of organic matter could be eliminated by the use of oxalic acid.

For various reasons it seemed desirable to use, if possible, an acid weaker than oxalic, and the following data were obtained, using acetic acid. The soil employed was the Wabash silt loam surface soil which contains 0.11 per cent of manganese calculated as manganese oxide. The quantity of manganese in this soil does not interfere with the removal of the organic matter.

To 10-g samples of this soil were added varying quantities of precipitated manganese dioxide and of acetic acid, and the amount of carbon dioxide developed by treatment with 20 c c of hydrogen peroxide was measured. The results are given in Table 1. Blanks were run, using acetic acid and hydrogen peroxide, and but traces of CO_2 were evolved.

TABLE 1.—*Effect of manganese dioxide and acetic acid on the liberation of carbon dioxide from 10 g of Wabash silt loam treated with hydrogen peroxide*

Manganese dioxide added	Acetic acid added	Hydrogen peroxide (6 per cent) added	Carbon dioxide evolved	Manganese dioxide added	Acetic acid added	Hydrogen peroxide (6 per cent) added	Carbon dioxide evolved
Mg	*Mg*	*Cc*	*Mg*	*Mg*	*Mg*	*Cc*	*Mg*
0	0	20	105.8	10	10	20	112.7
10	0	20	55.3	50	20	20	10.5
50	0	20	17.5	50	20	40	74.1
100	0	20	4.0	50	20	60	174.2
0	10	20	112.2				

The action of the hydrogen peroxide results in part in solution and in part in conversion of the soil organic matter to carbon dioxide. In order to determine how completely the organic matter is removed the experiments reported in Table 2 were carried out.

TABLE 2.—*Effect of manganese dioxide and acetic acid on the removal of organic matter from 10 g of Wabash silt loam*

Manganese dioxide added	Acetic acid added	Hydrogen peroxide (6 per cent) added	Organic matter removed	Manganese dioxide added	Acetic acid added	Hydrogen peroxide (6 per cent) added	Organic matter removed
Mg	*Mg*	*Cc*	*Per cent*	*Mg*	*Mg*	*Cc*	*Per cent*
100	0	40	0.81	100	100	80	1.85
100	100	40	1.38	0	0	(2)	3.10
100	0	60	1.19	10	[1] 10	(2)	2.95
100	100	60	1.73	100	[1] 100	(2)	2.85
100	0	80	1.48	200	[1] 200	(2)	2.93

[1] A slight excess over the equivalent amount of acetic acid was used.
[2] An excess of hydrogen peroxide.

It will be seen from a study of Table 2 that with relatively small quantities of acetic acid the catalytic effect of the manganese dioxide is largely eliminated, and that if repeated treatments with hydrogen peroxide are used, the organic matter may be as nearly completely removed as when manganese is not present. It should also be observed that as the quantity of manganese dioxide increases, the quantity of hydrogen peroxide used also must be increased, and in cases where the amount of manganese dioxide is in excess of 2 per cent, the cost in hydrogen peroxide and in time becomes excessive.

Although the matter was not followed to a definite decision, there are indications that traces of manganese are an aid to the oxidation process. In one case, in which the attack of the hydrogen peroxide on the soil was slow and incomplete, the addition of a milligram or two of manganese dioxide enabled the reaction to proceed rapidly to completion. It seems probable that manganese dioxide is at least one of the catalytic agents in the reaction, and since manganese is practically an omnipresent component of soils with at least some of the dioxide probably present, all soils may for this reason accentuate the attack of hydrogen peroxide on organic matter.

CARBONATES

As previously noted, soils which contain appreciable quantities of carbonates do not suffer complete, or even nearly complete, removal of their organic matter, even after repeated treatment with hydrogen peroxide. If, however, these soils are first treated with hydrochloric acid, and the acid removed, the treatment with peroxide is as effective as in ordinary soils. It is known, of course, that acid treatment of itself removes some organic matter by solution, and it has been pointed out by Shorey and Martin (*29*) that, as a consequence of the presence of compounds of the uronic acid type in soils, heating the soil with even very dilute acid may produce an evolution of carbon dioxide. It follows, therefore, that the organic matter remaining in a soil after acid treatment is not all the organic material of the soil.

It seemed desirable, therefore, to determine the magnitude of these removals of organic matter and, if possible, to compensate for them in the use of the method.

In the course of the work it became clear, both from experimental evidence and on theoretical grounds, that all soils containing exchangeable calcium and organic matter may at times, and possibly always, contain carbonates, even though the quantity is so small that the usual qualitative test of effervescence with acid does not occur. Also, it would appear that moist soils must contain carbon dioxide and that even dry soils may contain occluded or adsorbed carbon dioxide. An added incentive to this study is that, as pointed out by Shorey and Martin (*29*), some of the considerations also come into question in the use of the combustion method.

In view of these considerations the Houston black clay of Texas was selected for careful examination. It not only contains a large quantity of carbonates and organic matter but is exceptionally resistant to attack by hydrogen peroxide.

Ten-gram samples of the soil were treated with sufficient hydrochloric acid and water so that after the solution of the carbonate the resultant solution would be approximately 5 per cent of hydrochloric acid. After effervescence had ceased, the solution was filtered off by use of a Pasteur-Chamberland filter and the residual soil was washed until free from chlorides. The solution was then neutralized with sodium hydroxide. It was evaporated under diminished pressure at a temperature of 80° C. In the evaporation train there was introduced an absorption bulb containing a solution of barium hydroxide so that all gases, except the water vapor, must pass through it. No carbon dioxide evolution occurred as a result of the decomposition of organic matter.

The resulting dry residue of calcium chloride and other inorganic material was then examined for the presence of organic matter. Two methods were employed. One was the ordinary dry-combustion method. In spite of the large quantity of chlorides and the consequent difficulties, the results obtained are believed to be accurate. The second method was the wet-combustion method, using a mixture of bichromate and sulphuric acid as an oxidizing agent.

In order to prevent absorption of any material other than carbon dioxide by the alkali solution used for estimation of the carbon, the absorption train consisted of a reflux condenser, a potassium iodide trap, a water trap, and a Liebig bulb of silver sulphate. The determination of the carbon dioxide was made by titration in the usual manner.

The soil used contained 10.82 per cent of calcium carbonate and 2.27 per cent of total organic carbon. The quantities of carbon found in the evaporated residue were 0.16 per cent by the dry-combustion method and 0.18 per cent by the wet method. Further work in this direction was not deemed essential in view of the data assembled by Rather (*17*) on the quantities of organic carbon dissolved by water and by acids from a series of 12 soils, including Houston black clay, in which he showed that the quantities so dissolved vary between 0 and 0.16 per cent of the dry soil. The average quantity found was 0.06 per cent. (P. 18.)

It will be seen from the subsequent discussion that, although determination of the dissolved organic carbon in this manner is too laborious and expensive for routine work, the error introduced, if it be negelected, is of a magnitude within the limits of the accuracy of the method in other respects. The above considerations are based on the use of 5 per cent hydrochloric acid, without heating, as a solvent for the carbonates. The reason for these limitations will appear from the data presented in the following section.

ACID EFFECTS ON NONCALCAREOUS SOILS

Shorey and Martin (*29, p. 4909*) have called attention to the fact that carbon dioxide is produced by treatment of soils with boiling 12 per cent hydrochloric acid. Indeed, boiling with 1 per cent hydrochloric acid produces an evolution of carbon dioxide, but "whether this carbon dioxide is derived from inorganic carbonate, occluded carbon dioxide, or from the first states of the decarboxylation of uronic acids, is under investigation." They ascribed this evolution to the presence in the soil of organic compounds of the uronic acid type. Since the accurate determination of the carbon dioxide evolved by acid from soils is an essential step in the determination of organic matter from carbonate soils by any method, it became important to determine the magnitude of any error introduced by the action of acid on the organic matter. In the course of the work it became apparent that not all the CO_2 derived from the acid treatment came from the organic matter. There are two other possible sources—carbon dioxide "adsorbed" on the surface of the soil particles or held by soil moisture, or as acid carbonates; and carbon dioxide held as carbonates in quantities not evident by gas evolution on treatment with dilute acids. To deal with this problem a set of noncalcareous soils was selected, and the carbon-dioxide evolution studied under a variety of treatments. The apparatus used is shown in Figure 1.

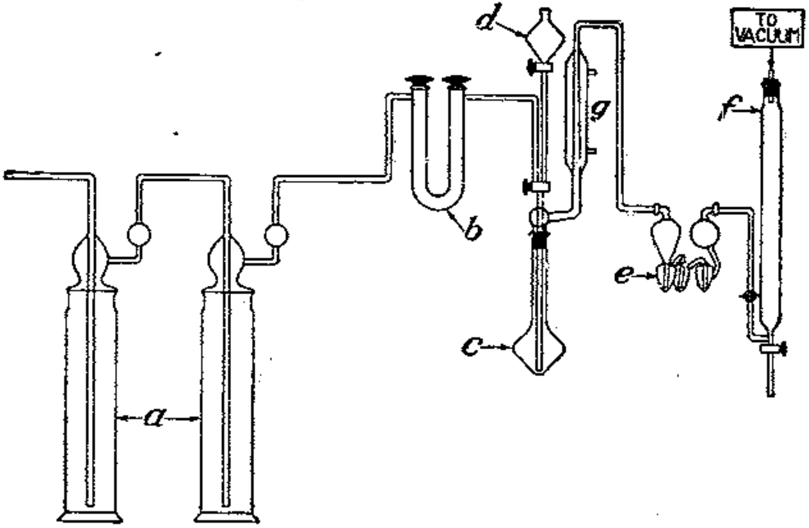

FIGURE 1.—Apparatus for determination of carbon dioxide: *a*, Gas-washing bottles containing KOH; *b*, soda-lime tube; *c*, gas-evolution flask; *d*, separatory funnel for introduction of acid; *e*, Liebig bulb containing silver sulphate and sulphuric acid; *f*, adsorption tower containing glass beads which are supported at the bottom by a platinum cone perforated by very small holes; *g*, condenser for use when contents of evolution flask are to be heated

This assemblage of apparatus presents no novelties so far as its single parts are concerned, but the purification train, evolution flask with automatic stirrer, reflux condenser, separator funnel, absorption train, and absorbing column, to the author's knowledge have not been assembled previously in any single apparatus. This device seems to be very satisfactory for accurate duplication of results of the order of magnitude encountered. When once assembled it is remarkably convenient for operation and requires relatively little manipulative skill. With it carbon-dioxide quantities as small as 0.2 mg may be duplicated accurately and, by altering the concentration of the alkali used in the absorbing tower, it may be used for even very large quantities of evolved carbon dioxide.

The samples were selected because they present a wide range of soil types, all of which are noncalcareous in the ordinary meaning of the term. Table 3 gives descriptive data on these and other soils used in this investigation.

TABLE 3.—*Soil types used in the investigation*

Soil No.	Soil type	Depth	Location
		Inches	
103	Carrington loam	0–12	Blackhawk County, Iowa.
181	Ontario loam	0–12	Rittsford, N. Y.
6797	Shelby silt loam	0–7	Bethany, Mo.
189	Wabash silt loam	0–15	Nemaha County, Nebr.
4440	Davidson loam	9–36	Guilford County, N. C.
7300	Blakely clay loam	0.3	Randolph County, Ga.
391	Hagerstown loam	0.8	Frederick, Md.
185	Cecil clay loam	0–9	Wilkes County, Ga.
190	Miami silty clay loam	0–10	Wells County, Ind.
512	Sharkey clay	0–4	Issaquena County, Miss.
5751	Houston black clay	--------	Dallas County, Tex.

The first four soil types were selected because they contain considerable quantities of organic matter that is readily removable, to a large degree, by hydrogen peroxide. The Davidson soil, by contrast, contains only a small quantity of organic matter and is not a surface soil. The Blakely soil contains 0.68 per cent of manganese calculated as manganous oxide. Hagerstown loam is a neutral soil.

The data included in Table 4, column 3, were obtained by treating 10 g of soil with 12 per cent hydrochloric acid and boiling for five hours. This is the treatment used by Shorey and Martin (*29*). It is tacitly assumed by them that all the carbon dioxide so liberated results from the decomposition of organic matter. Column 4 contains the data obtained by boiling the soils with 12 per cent hydrochloric acid for a period of 15 minutes. This series of determinations was made to ascertain the order of magnitude of the error introduced in the method of determination of carbon dioxide, as heretofore employed in the division of soil chemistry and physics (*24*). It will be observed that with the exception of the Blakely soil the quantity of CO_2 is exceedingly small, and since the laboratory method is ordinarily employed only when quantities of carbon dioxide are present sufficient to cause effervescence with HCl (1:1), the error introduced is quite negligible. This is not so unimportant relatively, however, with respect to the combustion method discussed later in this bulletin.

TABLE 4.—*Carbon dioxide evolution from certain noncalcareous soils of given pH values under diverse treatments*

Soil No.	Soil type	Percentage of carbon dioxide evolved from soil after treatment with—						
		12 per cent HCl, 5 hours boiling	12 per cent HCl, 15 minutes boiling	1 per cent HCl, 1 hour boiling	5 per cent HCl, aspirated 1 hour	H_2O only, aspirated 1 hour	5 per cent acid 1 hour after aspiration	pH
1	2	3	4	5	6	7	8	9
193	Carrington loam	0.293	0.057	0.038	0.009	0.008	0.003	5.0
181	Ontario loam	.215	.052	.078	.015	.003	.008	5.7
6797	Shelby silt loam	.203	.026	.054	.019	.008	.012	5.2
189	Wabash silt loam	.168	.035	.045	.010	.007	.003	5.6
4440	Davidson loam	.092	.052	.050	.023	--------	--------	--------
7300	Blakely clay loam	.567	.275	.402	.075 [1] .015	--------	--------	--------
391	Hagerstown loam	--------	--------	--------	--------	.003	.045	7.0

[1] In presence of ferrous chloride.

In the Blakely soil, and in any soil containing oxidizing agents sufficiently active to oxidize hydrochloric acid, chlorine may be expected to oxidize organic matter with the production of carbon dioxide, although oxidation of organic compounds by chlorine may occur without carbonic acid evolution, e. g., galactose or glucose. In such instances it is desirable to use the modification described by Schollenberger (*25*). This modification suggests the use of ferrous chloride, which is preferentially oxidized by the liberated chlorine.[2] The Schollenberger method was not employed in the data given in columns 4 and 5, but was employed in the data given in column 6 for the Blakely soil.

Column 5 gives the results obtained by boiling 10-g samples with 1 per cent hydrochloric acid one hour. It will be noted that in some instances the quantity of carbon dioxide produced was in excess of that produced by boiling with 12 per cent HCl for 15 minutes. Undoubtedly if the boiling with 1 per cent acid were sufficiently long continued even this dilution of acid would effect extensive decomposition of compounds of the uronic acid type (*9*). In this case also the effect of manganese dioxide on the quantity of carbon dioxide is very marked in the Blakely soil.

In column 6 are given the data obtained by using 10 g of soil with 5 per cent hydrochloric acid and aspirating with purified air at the room temperature for one hour. The quantities of carbon dioxide obtained are notably less than are obtained by boiling with 1 per cent acid. In the Blakely soil the second value is that obtained by addition of ferrous chloride, and the difference indicates the oxidizing effect, under these conditions, of the manganese dioxide in this soil. A point of special interest in this series is that if the aspiration is continued for successive periods of one hour each, no measurable liberation of carbon dioxide occurs. This is in marked contrast with the series with boiling acids given in column 5, in which in all cases no end point was reached though the successive increments were of diminishing value.

The obvious interpretation of these facts is that the carbon dioxide evolved by the cold acid arises from carbonates present even in these soils, most of which are distinctly acid. This conclusion seems to be in harmony with the consideration that since all fertile soils contain bases and the organic matter present is, under moist conditions, producing carbon dioxide as a result of bacterial activity, there must, therefore, at all times be an unstable condition tending toward equilibrium between the basic content of the soil and the carbonic acid content of the soil solution. When the carbonic acid content

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[2] An interesting observation was made in this connection. Cameron and Breazeale (5) comment on the observed fact that soil-containing chlorides, when treated with sulphuric acid and chromic acid, under certain conditions fail to produce chlorine evolution in sufficient quantity to affect the carbon dioxide determination by the wet-combustion method, and state that they have no explanation. The same observation was made in the treatment of the Blakely soil with hydrochloric acid, although manganese dioxide and hydrochloric acid must produce chlorine. The explanation is that the chlorine so formed reacts with water to produce hypochlorous acid which oxidizes organic matter to give carbon dioxide or decomposes to liberate free oxygen. The evidence that this occurs is found in the fact that no free chlorine can be detected in the neck of the flask below the condenser but is readily detected even by color, as well as by starch iodide paper, just above the surface of the liquid. Unless the oxidizing agent in the soil be considerable in amount, no free chlorine passes into the absorption train.

increases sufficiently the existence of bicarbonates becomes possible. On drying, these bicarbonates, so far as calcium, magnesium, and ferrous iron are concerned, will revert to carbonates and, in part, the carbon dioxide so liberated may be retained by the soil.

To determine the relative amounts of the carbon dioxide present as "adsorbed" gas, 10 of the soils were aspirated with distilled water which had been boiled and cooled in a closed vessel. The results of this series of determinations are given in column 7. It will be observed that the quantities so obtained are close to the limits of the accuracy of the method employed in the measurement. Nevertheless, the series as a whole indicates the order of magnitude of the quantities of carbon dioxide present, either as adsorbed gas or as bicarbonates. The samples were then treated with sufficient hydrochloric acid to make the acid concentration 5 per cent, and the aspiration continued for another hour. The results obtained are found in column 8. To this set was added a soil which had a normal pH of 7. The sharply increased difference between adsorbed CO_2 and that present as carbonate is striking and is considered as excellent confirmation of the general conclusion.

On the basis of the data represented by Table 4 it appears that an error of considerable magnitude is introduced into the determination of the carbonates present in calcareous soils if the boiling with hydrochloric acid (1:2) is long continued. This error is minimized when the period of heating is limited to 15 minutes. Soils, even when acid, may contain measurable quantities of carbonates; these quantities are of a negligible magnitude, except where the degree of base saturation approaches 100 per cent. Additional data on the carbon dioxide content of certain soil colloids by use of this method are given by Middleton, Slater, and Byers (*15*).

The difficulties produced by the presence of manganese dioxide and calcium carbonate in soils do not appear to be insuperable obstacles to the use of the hydrogen peroxide method. It remains to consider the question of the residual carbon content of the treated residues. For this purpose nine soils were used and the removal of the organic matter was made as complete as practicable by repeated treatment with 6 per cent hydrogen peroxide. The carbon content of the treated residues was determined by the combustion method. The results obtained are given in columns 3 and 4 of Table 5. It will be observed that the carbon content of the hydrogen peroxide residues varies between 0.15 per cent and 0.44 per cent of the whole soil and averages 0.25 per cent. This quantity corresponds to 16.4 per cent of the original carbon content of these soils. If the assumption is made that this residual carbon is from organic matter, to which can be ascribed the carbon content used as a conventional factor in the combustion method, the quantity of residual organic matter (column 5, Table 5) is considerable.[3]

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[3] That this assumption is not valid arises from the fact shown by W. O. Robinson (22) that at least a portion of the residual carbon is not organic matter (p. 2) and by the further fact, shown later in this bulletin, that even with organic matter the conventional factor is inaccurate.

TABLE 5.—*Organic matter by the hydrogen peroxide method*

Soil No.	Soil type	Organic matter by hydrogen peroxide	Carbon in residue	Organic matter in soil residue (C×1.724)	Total organic matter in soil	Total organic carbon by combustion	Organic carbon removed by H_2O_2
1	2	3	4	5	6	7	8
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
199	Miami silty clay loam	2.16	0.22	0.38	2.54	1.51	85.6
193	Carrington loam	4.62	.28	.48	5.10	2.71	89.7
512	Sharkey clay	4.29	.44	.76	5.05	2.96	86.2
185	Cecil clay loam	.39	.11	.19	.58	.42	73.8
6797	Shelby silt loam	3.01	.31	.53	3.54	1.82	83.0
189	Wabash silt loam	2.78	.25	.43	3.21	1.87	80.6
181	Ontario loam	3.34	.24	.41	3.75	2.12	88.7
391	Hagerstown loam	1.23	.24	.41	1.64	.90	73.3
5751	Houston clay	3.10	.15	.26	[1] 3.65	2.27	85.5
	Average						83.6

[1] Includes organic matter in HCl filtrate.

In order to determine the degree of removal of the organic matter by the hydrogen peroxide, it seemed best to determine the total carbon by the dry-combustion method, column 7, and from this to calculate the percentage of organic carbon removed by the peroxide treatment. These values are given in column 8, Table 5, and vary between the limits of 73.3 and 89.7 per cent. The percentage of organic matter removed is obviously of the same order of magnitude and would be identical were the residual organic matter of the same composition as that removed.

In general, the conclusions arrived at by W. O. Robinson (*22*) are confirmed. It appears certain in cases where a large quantity of carbon remains unconsumed that it may consist of organic compounds of uncertain carbon content. The evidence of this is admittedly indirect but is considered convincing by the authors. The most important indications that such is the case are as follows:

In none of the residues of the soils examined could any particle of carbon be positively identified by microscopic examination. This alone is not convincing, but when coupled with the fact shown by Robinson (*22*) that powdered charcoallike particles separated from the soil are largely consumed by hydrogen peroxide, it would seem that submicroscopic particles ought to be attacked. It has also been pointed out by Shorey and Martin (*29*) that the residue contains nitrogen, and this fact has been confirmed by the authors even when the nitrogen present as exchange ammonium was taken into account. The following data on the examination of a Chester silt loam sample are submitted as an example:

	Per cent
Organic carbon in the original soil	1.78
Organic matter removed by H_2O_2	2.41
Carbon content of residue	.37
Nitrogen content of soil	.156
Nitrogen content of residue	.032
Nitrogen as exchangeable NH_4	.016
Residual organic nitrogen	.016

It will be noted that the C:N ratio is 11.4 in the original soil and 23.1 in the residue after peroxide treatment. The important deduction to be drawn from this confirmation of the work of Shorey and Martin (*29*) is that the residual material has a carbon-nitrogen ratio not unusual in soils and therefore indicates the presence of actual organic matter in the peroxide-treated residues. More detailed data need not be given because of the data to be found in the work submitted by Leighty and Shorey (*8*) and by McLean (*13*, *14*). The former showed in a study of alteration of the carbon-nitrogen ratio in a very large number of profiles, that the ratio decreases with depth in most instances, but the reverse situation also obtained in a few cases. This result indicates an alteration in the rate of destruction of nitrogenous and nonnitrogenous material under differing soil conditions. The results of McLean on the carbon-nitrogen ratio of peroxide-treated residues also show alteration from the ratio of the original soils. It does not appear that he measured the base-exchange nitrogen, and consequently the detailed figures obtained are not significant. They do show, however, that normal soil organic matter is probably present in these residues.

That some forms of organic matter are highly resistant to hydrogen peroxide when not in contact with soil is well known. It seemed desirable to secure a few such substances, known to be present in soil organic matter or organic matter likely to find its way into soils, and determine to what extent they yield to this treatment in the presence of soil. The soil employed was a sample of Chester loam and the samples in question were mixed with this soil in the ratio of 1 to 5.

The resulting data are given in Table 6.

TABLE 6.—*Organic materials and hydrogen peroxide in the presence of soil organic matter*

Substance	Loss by H_2O_2 alone	Loss by H_2O_2 and soil	Ash	Organic matter destroyed
	Per cent	*Per cent*	*Per cent*	*Per cent*
Lemon pectin	86.7	89.5	7.9	97.2
Peat from Lake Mattamuskeet	73.9	81.5	9.7	90.2
Apple cuticle	18.5	34.8	.5	35.0
Lignin	52.3	78.9	.7	79.4
Indigotin	0	91.8		91.8
Oleanolic acid	−.9	3.4	0	3.4

The lemon pectin is a material very rich in uronic acids. The peat used is very rich in alcohol-soluble material. The apple cuticle was furnished by C. E. Sando, of the Bureau of Chemistry and Soils, and is highly resistant to oxidation. The lignin is a sample of purified material furnished by Max Phillips, of the Bureau of Chemistry and Soils. The oleanic acid is a material extracted from grape skins by C. E. Sando and known to be highly resistant to oxidation.

Certain minor, but real, sources of error in the use of the hydrogen peroxide method arise as the result of reactions between the hydrogen peroxide and the inorganic portions of the soil. The more significant of these is that hydrogen peroxide is a weak acid itself and with organic matter produces carbonic acid. Both these effect base-exchange reactions with the bases of the soil complex and whether they pass into solution or not have the effect of diminishing the apparent organic-matter loss by reason of the hydrolysis so produced. Directly or indirectly the reaction is as indicated by the following equation:

$$MX + H_2O \rightarrow MOH + HX$$

For each equivalent of base so removed there is added a molecule of water. When the soil residue is dried or the hydrogen peroxide extract ignited, the weight relation is as indicated or corresponds to half a molecule of water, depending on whether the hydroxide is converted to oxide in the process. It is clearly a task of considerable magnitude to determine in any one instance the exact effect of this type of reaction, and it does not seem worth while since each soil sample would have its own correction values. It is not difficult, however, to determine the existence of the source of error and obtain a general estimate of its magnitude. For this purpose 10-g samples of Shelby silt loam and of Carrington loam were treated with hydrogen peroxide until the organic matter was removed. At the same time duplicate samples of each were treated with the same volume of distilled water and subjected to the same procedure. The filtered solutions were evaporated to dryness and ignited. The ignited water residue amounted to 6.6 mg for the Shelby sample and 8.9 mg for the Carrington. The corresponding weights for the peroxide extracts were 33.6 and 43.3 mg.

Were the differences between the weights of these extracts due wholly to conversion of exchangeable calcium to calcium oxide, the error introduced would give too low values for the organic matter in these soils by about 0.1 per cent.

A less significant error is due to the fact that since hydrogen peroxide is an oxidizing agent, any oxidizable inorganic material in the soil will be oxidized to some extent, and to the degree that such oxidation occurs the added oxygen would make the organic matter values too low. It is not believed that this error is of moment in most soils, since almost the only oxidizable inorganic matter in soils is ferrous iron. It is probable that when such is present the oxidation primarily results in the base-exchange phenomenon discussed above. In any case the general effect of the hydrogen peroxide treatment is not greatly to intensify the color of the treated residues, even when high in iron.

In the light of all the data at present obtainable, it appears that the hydrogen peroxide method is not satisfactory as a means of determining the total organic matter of the soil. It can be a very useful means of comparing the readily oxidizable material in different soils and especially in comparing the quantities of organic material in different samples of the same soil. If it be supplemented by a combustion determination of the residue, the carbon content may be assumed to be normal organic material and multiplied by a conventional factor, or the assumption suggested by Robinson (*22*) may be used, and in either case a fairly close approximation to the real organic content be reached.

THE COMBUSTION METHOD

In the following discussion attention is not directed primarily to the means by which the quantity of carbon in a soil is determined. It may be said at once that so far as the authors can determine, both the wet and dry combustion methods, when carried out with suitable precautions, accurately estimate the quantity of carbon dioxide produced. A review of many of the available methods with critical comments was furnished by Springer (*31*) in 1928.

There seem to be three points which merit consideration in the present discussion. The first, and by far the most important, concerns the use of a factor to convert the carbon, or carbon dioxide content, into the organic matter of the soil. The second is the determination of the corrections to be applied when calcareous soils are studied, or, in the case of the wet-combustion method, when alkali soils are in question. The third concerns the residual material after dry combustion has been employed to determine the carbon content.

THE ORGANIC FACTOR

The use of the conventional factor, 1.724, to convert the carbon content of a soil to organic content, is open to serious criticism. From the literature it would seem that there is considerable uncertainty concerning the origin of this factor. There seems to be little doubt that it owes its extensive use to Van Bemmelen (*3*) who ascribes its origin to Wolff (*34*).

The earliest mention found of 58 per cent as the carbon content of humus, which carbon content is the basis of the conventional factor, is by Sprengel (*30*), who, in 1826, in a rather extensive paper, gave the composition of humus as 58 per cent carbon, 39.9 per cent oxygen, and 2.1 per cent hydrogen. He used the copper oxide combustion method but did not give any detailed data. This paper was given scant and unfavorable comment by Berzelius (*4, p. 288*) who, on other grounds, considered the work reported so inaccurate as to be untrustworthy. In the light of the data quoted by Dana (*6*) in 1858, in A Muck Manual for Farmers, as derived from Mulder, the result obtained by Sprengel for humus extracted by alkali was fairly accurate, considering the analytical methods available. Mulder's values are as follows:

Kind of soil	Percentage of carbon in humus
Fruit-orchard soil	58.49
Kitchen-garden soil	61.65
Field (mowing) soil	60.11
Oak-plantation soil	58.98

In 1864, Wolff (*34 p. 156*) made the following statement, although the analytical data on which it is based do not appear:

> Um aus dem Kohlenstoffgehalt des Bodens die Menge der Humussubstanz wenigstens annähernd genau zu berechnen, nehme ich im Humus durchschnittlich 58 Proc. Kohlenstoff an; Man braucht also den Kohlenstoff nur mit 1.724 oder die gefundene Kohlensäure mit 0.471 zu multipliciren, um auf diese Weise den Gehalt des Bodens an wasserfreiem Humus zu finden.

This statement seems to be the basis for all the later use of these factors when combustion methods are employed for determining the organic content of soils.

Detmer (*7*), in 1871, found the humus of soil to have a carbon content of 59.79 per cent. Loges (*10*), in 1883, in a study of the wet and dry methods of combustion, used the Wolff factors freely to calculate the total "humus substance" of soils. In 1890 Van Bemmelen (*3*) made the following statement concerning the humus content: "Dieser ist erhalten durch Multiplikation des Kohlenstoffgehaltes mit den factor von Wolff: 1.724." This statement may be considered as establishing the general use of the factor.

That the organic matter of the soil may be widely different in carbon content from 58 per cent and that the factors 1.724 and 0.471 are, in consequence, unreliable, has been shown repeatedly. Indeed, numerous investigations have shown that even in the extracted soil humus, the mean values of the carbon content have been found to be more nearly 50 per cent than the 58 per cent assumed in the use of the above factors. Only a few of these will be cited.

Cameron and Breazeale (*5*), in 1904, showed that the humus extracted from 19 "typical" agricultural soils by 3 per cent ammonia contained percentages of carbon varying between the limits of 33.3 and 49.22 per cent and averaged 41.77 per cent. Curiously enough, they advised the continued use of the factor 1.724. They reached this conclusion, partly at least, on the basis of the following statement (*5, p. 44*):

> Furthermore this organic matter, not extracted by the ammonia, is made up largely of cellulose, or celluloselike substances in which the percentage of carbon approaches closely the figure given by Van Bemmelen.

Since the carbon content of cellulose is 44.44 per cent, this value in reality approaches much more closely their value for the extracted material than it does the value used as the basis of the factor which they erroneously attributed to Van Bemmelen.

In 1922, Read and Ridgell (*19*), using the method of Rather (*17*), to be later discussed, on a series of 37 soils of very diverse sources, found a variation of carbon content between the limits of 30.2 per cent and 56.27 per cent, with a mean value of 49.26 per cent, in the surface soils, and a variation between 13.33 per cent and 56.55 per cent, with a mean of 39.16 per cent for subsoils of the same series of soils. The mean for the whole series of soils for both surface soil and subsoil was 43.91 per cent. These results are in general harmony with those of Cameron and Brezeale (*5*). They are distinctly out of harmony with the results obtained by W. O. Robinson (*22*) who found, for the organic matter of the material removed by hydrogen peroxide, values ranging from 37.5 to 70.1 per cent, with a mean value of 54.8 per cent. On the assumption that the residues, after treatment, consisted wholly of carbon, he calculated the total organic matter to have a carbon content which ranged between 44.5 and 78.4 per cent, with a mean value of 58.6 per cent. It should be added that in the series of soils examined, the maximum values were shown by a soil known to contain free charcoal particles. Excepting this soil, the maxima were 61.9 and 66.5 per cent.

Lunt (*11*), in 1931, in a study of the carbon-organic ratio of forest soil humus, found for freshly fallen leaves a factor of 1.89; for partly decomposed leaves, 1.85; and for well-decomposed leaves, 1.80. In this study the loss on ignition was used as a basis of estimation of total organic matter and, although this method is obviously faulty as applied to soil, it is not so seriously questionable when used on the materials studied.

A recent article by Waksman and Stevens (*32*) discusses the limitations of the hydrogen peroxide method and the use of chlorine dioxide in estimating the organic content, of certain types of material, which have a very high organic content, and finds them unsatisfactory as used. The authors state (*32, p. 114*):

> The most reliable method at present for determining quantitatively the soil organic matter is based upon the determination of organic carbon, which is multiplied by 1.724 to give a total soil organic matter.

They adduce no positive evidence from any source for this statement.

In view of the wide variance shown throughout the literature cited above, it seemed not to be essential to carry out detailed examinations for the purpose of again showing the divergence of values obtained by the different methods. However, the data given in Table 8, show that no close concordance exists between the hydrogen peroxide method and the combustion method when used on the same soils, even though "correction" be made for the residual carbon after hydrogen peroxide treatment. Incident to the use of the Rather method (see beyond) the values for the carbon content of the organic matter of these soils, which is not soluble in a mixture of hydrochloric and hydrofluoric acids, are available. (Table 8, column 8.) The range is from 46.8 per cent to 61.4 per cent.

It would appear from the accumulated data that however accurately carbon may be determined in a soil, the error introduced by the use of the conventional factor is very great. It is, therefore, futile to attempt to arrive at any factor, since existing data show that, as a matter of fact, the carbon content of soil organic matter is extremely variable. Some have endeavored to avoid the uncertainty introduced by the use of a factor by contenting themselves with the determination of the carbon and basing comparisons between soils on the quantities so found. Such procedure is analytically sound but not very satisfactory, since the quantity of the material represented by the same carbon content may be essentially different, and quantities of carbon may be quite different and yet represent like quantities of organic material. Even if reporting carbon content alone were satisfactory in general, there is one very important purpose that can not be so served. The combined water is a very important component of the soil, and its estimation depends at present on the difference between the ignition loss and the organic content. The carbon content serves in no way to obtain the water content, and, unfortunately, any error brought in by the use of the factor 1.724, or any other factor, makes a like error in the water component. This is so serious in the study of inorganic soil constitution as to make an accurate method of determination extremely desirable, entirely aside from its importance to the study of the organic matter itself.

CARBONATED SOILS

As was pointed out in the review of the hydrogen peroxide method, the accurate determination of the carbon dioxide content of a soil involves the consideration of a number of factors. Thus, if the combustion method be employed on calcareous soils, any error in the estimation of carbon dioxide resulting from acid treatment is immediately reflected in the estimation of the organic matter; this is true whether the error be caused by the decomposition of organic matter or otherwise.

The data already presented show that, although the method of determining the carbon dioxide by boiling with 12 per cent HCl for a short period is not likely to introduce significant error into the estimation of the carbonates, it is likely to produce a relatively more important error in the calculated organic content, especially since it is uncertain whether a portion of the carbon dioxide so determined has its origin in organic matter. It also seems apparent that the quantity of carbon dioxide present in noncalcareous soils is sufficiently large, especially in soils which approach or pass neutrality, to warrant a carbon dioxide determination in every case where organic matter is to be determined.

The question of the retention of carbon dioxide derived from the combustion of organic matter appears to depend somewhat on the character of the soil. In the absence of actual alkali carbonates, there seems but little likelihood of appreciable quantities of potassium or sodium carbonates being formed by reaction between the carbon dioxide and exchange bases in the soil which escape decomposition at the furnace heat. As a matter of fact this residual carbon dioxide is small but real in the nonalkaline soils under examination. The results obtained are given in Table 7.

TABLE 7.—*Carbonates in residue after combustion*

Soil No.	Soil type	Percentage of CO_2 in residue	Soil No.	Soil type	Percentage of CO_2 in residue
193	Carrington loam	0.044	181	Ontario loam	0.016
6707	Shelby silt loam	.011	512	Sharkey clay	.022
199	Miami silty clay loam	.044	5751	Houston black clay	.000
185	Cecil clay loam	.051			

So far as the authors are aware, the presence of alkali carbonates in itself offers no difficulty to the use of the dry-combustion method, but the associated so-called alkali salts may at times offer manipulative difficulties, and the wet-combustion method is to be preferred.

The combustion method seems to be a very satisfactory means of estimating the carbon content of soils, assuming proper precautions are taken to insure accuracy of results and to avoid errors incident to the presence of carbonates. When we undertake to translate the carbon content into organic-matter content, the method is useless for any purpose other than a rough comparison. It is difficult to understand why its use should have been so long continued except for the belief that no more satisfactory method existed, and it is better to use a poor tool than to do no work. Certainly the relative quantities of organic matter in soils is an important question, and a satisfactory method is greatly to be desired.

LOSS ON IGNITION

The loss on ignition has long been used as a measure of the relative organic content of soils. (*2*) That the direct application of this method is accompanied by gross error is too well known to warrant a detailed review of the proofs of its inadequacy. The hydrated minerals of the mica and zeolite types and the " water of constitution " of the colloids and of clay minerals are included in the ignition loss. It is important to determine the water content of soils and colloids as well as to determine the organic matter itself. A very valuable aid to soil study would be secured if a method were available for distinguishing between the portions of the total ignition loss due to organic matter and to the dehydration of inorganic materials.

In 1917 there appeared a bulletin of the Arkansas Agricultural Experimental Station by Rather (*17*) in which the distinction just mentioned was attempted. Rather's method consists essentially in the removal of the hydrated mineral matter of soils by repeated treatment with a mixture of dilute hydrochloric and hydrofluoric acids and subsequent washing to remove these acids and their soluble salts. The residual organic matter is then determined by ignition. It is obvious that this method involves the removal of the organic matter which is soluble in water and in acids at the dilutions employed, or which is decomposed by treatment with acids. The extent to which such removal of organic matter before ignition occurs can be approximated by determining the total carbon before and after extraction with acids. In a series of determinations on 12 soils, Rather demonstrated that the loss of carbon by leaching was small, varying between 0.01 and 0.16 per cent with an average loss of 0.06 per cent. This paper was followed by Read (*18*) who applied the method of Rather to effect a simultaneous determination of organic matter and organic carbon, and in doing so developed a wide variation in the percentage content of carbon. This was, in turn, followed by a more extended paper by Read and Ridgell (*19*). They presented the results of a study of the Rather method with reference to the question of the validity of the conventional factor to which attention has already been drawn. (P. 15.) In the 1926 edition of Wiley's Principles and Practice of Agricultural Analysis (*33*) this method of examination was somewhat briefly discussed and was dismissed with the comment that "the method gives very satisfactory results but is quite chronokleptic." In view of the importance of the matter it seemed desirable to reexamine this method, despite its time-consuming character, and apply it, or a modification of it, to the same soils which were examined by the methods previously discussed.

EXPERIMENTAL DATA

In Rather's (*17*) detailed method soils are first leached with water and then treated repeatedly on the water bath with a 1 per cent solution, consisting of 0.5 per cent hydrochloric acid and 0.5 per cent hydrofluoric acid, until a constant ignition loss is obtained. The quantity of organic matter dissolved by the water may be determined separately. In view of the observations previously discussed in this bulletin, it seemed best to modify Rather's method in certain details.

In securing the data given in Table 8, the soils were treated with a mixture of 2.5 per cent hydrochloric acid and 2.5 per cent hydrofluoric acid in platinum dishes and allowed to stand overnight at room temperature, and, after filtration, this treatment was repeated. The soil residue was washed until free from chlorides. The filtrate was not directly examined for carbon content, but this was indirectly determined as later described. The soil residue was dried at 100° to 105° C. and then ignited in a muffle furnace and the ignition loss determined. This ignition loss represents the undissolved organic matter, if the hydrated inorganic matter has actually been wholly removed, and the residue contains no inorganic material which alters in weight during ignition.

A preliminary experiment to illustrate the degree of completeness of removal of hydrated inorganic material was carried out on a sample of the fraction of the B_2 horizon of Cecil clay loam, from Rutherford County, N. C., which does not pass a 300-mesh sieve. It consisted largely of mica, together with a small quantity of colloidal aggregates. It had an ignition loss of 9.4 per cent and a carbon content of 0.12 per cent. After one treatment with the mixture of acids the ignition loss was reduced to 2.32 per cent; after a second treatment the ignition loss was 0.27 per cent; and after a third treatment was 0.15 per cent. The calculated organic content was 0.20 per cent (0.12×1.724). This material should be considerably more difficult to free from hydrated minerals than ordinary soil. The authors are confident, therefore, that no material error in the application of this method was due to loss of "combined" water.

The results obtained on 11 soils are given in Table 8. The data in column 3 are the direct ignition losses obtained after the described treatment with the hydrochloric acid-hydrofluoric acid mixture, except in the case of Houston black clay. In this case, because of the high carbonate content, the soil was given a preliminary treatment with 5 per cent hydrochloric acid.

TABLE 8.—*Organic matter by the modified Rather method*

Soil No.	Soil type	Ignition loss	Organic carbon in filtrate	Carbon in filtrate ×1.724	Total organic matter by modified Rather method	Total organic matter assuming dissolved organic matter has same carbon content as given in Column 8	Carbon in organic matter of residue
1	2	3	4	5	6	7	8
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
193	Carrington loam	3.61	0.90	1.56	5.17	5.41	50.1
181	Ontario loam	2.87	.65	1.12	3.99	4.13	51.4
6797	Shelby silt loam	2.47	.53	.92	3.39	3.48	52.3
189	Wabash silt loam	2.24	.65	1.12	3.36	3.42	55.0
7300	Blakely clay loam	6.00	1.16	2.01	8.01	8.44	47.6
391	Hagerstown loam	1.47	.14	.23	1.70	1.74	52.0
185	Cecil clay loam	.65	.12	.20	.85	.91	46.8
199	Miami silty clay loam	1.91	.41	.70	2.61	2.67	53.9
512	Sharkey clay	3.79	.63	1.08	4.87	4.82	61.4
5751	Houston black clay	3.17	.57	.98	4.15	4.24	53.4
9356	Chester loam	3.09	.24	.42	3.51	3.57	49.9
	Average						52.16

In column 4 is given the carbon content of the organic matter dissolved by the acid treatment. These values were obtained by determining the total carbon content of the original samples by the dry-combustion method and subtracting from this quantity the carbon found in the acid-treated residue. It will be observed that the quantities so found are much higher than those obtained by Rather. (P. 18.) This is not alone due to the greater concentration of the acids employed, but also to the very greatly increased period of digestion in the cold acid. The values range from 0.12 to 1.16 per cent. The high value, 1.16 per cent, was obtained in the case of Blakely clay loam. This is a soil in which 0.68 per cent of manganese is present, calculated as manganous oxide. It is present chiefly as the dioxide, and were it all so present, it would correspond to a percentage content of 0.83. Without doubt the large solution loss in this case is due in part to the oxidation of organic matter by the products of reaction between the manganese dioxide and the acids. It is certain, however, that not all the dioxide present is decomposed by this process, since in the case of the Blakely sample the ignited residue was black, whereas in all the other samples the residues were nearly white or white. With manganese dioxide present in large amounts there occurs a source of error in the application of the Rather method not encountered in the combustion method. When manganese dioxide is treated with a mixture of the acids, even of the strength employed by the authors, it is attacked to but a slight degree. When the insoluble residue is ignited it is converted to the manganous manganic form Mn_3O_4, and the loss of oxygen from this source (12.2 per cent of the weight of MnO_2), if not compensated, is counted as organic matter. In ordinary soils the error introduced in this manner is very small, and even in the case of Blakely clay loam would be at the greatest but 0.1 per cent.

In column 5 of Table 8 are given the values obtained for the organic matter present in the acid filtrates calculated on the assumption that the conventional factor, 1.724, times the carbon content, is valid. Even if this be not true, it is obvious that less error is introduced by using the factor on a part of the carbon content than when it is applied to the whole.

In column 6 of Table 8 are given the values of the total organic matter in the soils obtained by adding the values in columns 3 and 5. In column 8 is given the percentage content of carbon in the undissolved organic matter after treatment with the acid mixture. It will be observed that these values range between 46.8 and 61.4 per cent, with a mean value of 52.16 per cent. It appears that, if we were only certain that the dissolved organic matter had the same carbon content as that not dissolved, we would obtain a very accurate estimate of the organic content of the soil by multiplying the carbon content by the factor corresponding to its percentage value. It is not probable that this is permissible, since the very fact that it is soluble differentiates it from that which is not dissolved. However, since it has been repeatedly shown that the carbon content of the organic matter of soils is variable and that, in whatever fraction isolated, the mean value is less than 58 per cent (as in the soils under discussion), a lesser error is introduced when the total organic matter is calculated as indicated above than when the calculation is made as in column 6.

In column 7 are given the organic-matter contents of the soils on the assumption that the organic matter dissolved has the same carbon content as that not dissolved. It will be observed that the differences between the quantities given in columns 6 and 7 are in some cases very slight and in others quite appreciable. It is recognized that neither column 6 nor column 7 shows accurate values, but that the values in column 7 are probably somewhat closer to the real organic content of these soils.

COMPARISON OF THE METHODS

In order to facilitate comparison between the three methods under discussion the final values for each method on each soil have been brought together in Table 9.

TABLE 9.—*Comparison of organic matter determinations by the three methods studied*

Soil No.	Soil type	Organic matter by H_2O_2 method (from Table 5)	Organic matter by combustion method (C×1.724)	Organic matter by modified Rather method (C in filtrate× factor from Table 8, column 8)
1	2	3	4	5
		Per cent	*Per cent*	*Per cent*
193	Carrington loam	5.10	4.68	5.41
181	Ontario loam	3.75	3.66	4.13
6796	Shelby silt loam	3.54	3.14	3.48
189	Wabash silt loam	3.21	3.22	3.42
7300	Blakely clay loam	6.76	6.92	8.44
391	Hagerstown loam	1.64	1.55	1.74
185	Cecil clay loam	.58	.73	.91
199	Miami silty clay loam	2.54	2.47	2.67
512	Sharkey clay	5.05	5.10	4.82
5751	Houston black clay	3.62	3.91	4.24
9356	Chester loam	3.05	3.07	3.57

It will be observed that the organic matter content of these soils, as shown by the hydrogen peroxide method, is less than that shown by the modified Rather method in all the soils save Shelby silt loam and Sharkey clay. The difference in general between the values in column 3 and column 4 is small, and the quantities shown are essentially identical when the known sources of error of both methods are taken into consideration.

It appears that, if the precautions outlined in the preceding pages be used, the hydrogen peroxide method and the dry-combustion method are of equal validity, and, since both are apparently inaccurate, it is immaterial which method is employed for the purpose of estimating organic matter in soils, provided the same care is employed in each. Since the dry-combustion method is much more readily carried out and involves but one combustion, whereas the peroxide method requires in addition the careful technic of the hydrogen peroxide treatment, it follows that in general the dry-combustion method is to be preferred. It also seems to be true that where the object sought is the relative amount of organic matter in various samples of the same or similar soils, the hydrogen peroxide method may be preferred when the combustion of the residues is omitted. It may be assumed that, since the hydrogen peroxide effects the oxidation of the more readily oxidizable matter, the relative amounts so shown are more truly an index of the organic matter available for bacterial consumption than are the relative amounts of total carbon multiplied by any factor. Although this assumption may be correct, proof of its validity is lacking. The need of a method for such distinction has been pointed out by Shorey (*27*).

It may be observed that in the combustion method the results may be either above or below the actual organic content, whereas in the hydrogen peroxide methods the results should invariably be low.

When we turn to the comparison of the dry-combustion method with the modified Rather method (Table 8, columns 4 and 5) it is found that the latter, in general, gives higher results for the organic-matter content. The single exception is in the case of Sharkey clay, and this difference is chiefly due to the high carbon content of at least the organic matter not dissolved by the acid treatment. (Table 8, column 8.) Similarly, the larger values in column 5, Table 9, as compared with column 4, are to be ascribed in part to the same procedure, using the larger factor required by the carbon percentages given in column 8 of Table 8. Nevertheless, even the smaller values obtained by applying the conventional factor to the acid-soluble organic matter still leaves (Table 8, column 6) them larger than those obtained by the dry-combustion method. The widest divergence is found in the case of Blakely clay loam, where the margin is 1.52 per cent. As already mentioned (p. 20) the presence of the manganese dioxide in this soil introduces a small source of error into the modified Rather method but operates to bring about values which are too low. It follows then that if the percentage carbon content given in column 8, Table 8, actually represents the carbon content of the total organic matter of the soils in question, then the high values of column 5, Table 9, are more nearly a quantitative measure of the true organic matter of the soil than are the quantities obtained by either the hydrogen-peroxide or the dry-combustion method, using the conventional conversion factor. That the percentage carbon content of the organic matter in these soils is variable between wide limits is abundantly supported by the experimental data as well as by the data presented in the references discussed. (P. 14.) The uncertainty regarding the carbon content of the acid-soluble organic matter is the most important element of doubt in the application of the Rather principle. That it offers the most satisfactory method at present available for the determination of the true organic content is believed to be shown. That the modified Rather method, as used by the authors, is time-consuming is undeniable. It requires two combustion operations, whereas the dry-combustion method requires but one, and, in addition, requires the time delay involved in successive treatments with the mixed acids and the subsequent washing and drying of the residues. The use of hydrofluoric acid involves the use of platinum vessels in considerable numbers, if many operations are to be carried forward at the same time. It is not to be expected, therefore, that the method will be employed for routine comparative purposes. It is believed that this method, or an improvement, should be used when results of the highest possible accuracy are required.

The study of the problem of soil organic matter involved in the preparation of this bulletin lends emphasis to the contention of Waksman and Stevens (*32*) and of Shorey (*28*) that the organic matter of soils is not adequately differentiated when its quantity is determined accurately, even were that possible. That soil organic matter varying as widely in carbon content as the available data indicates should be of widely varying agricultural significance is beyond question. That the method of differentiation suggested by Waksman and the frankly qualitative method of Shorey are adequate for the purpose is more than doubtful. That better methods for the study of organic matter are urgently needed is obvious.

SUMMARY

A study of the organic-matter content of a group of soils by the hydrogen peroxide method, the dry-combustion method, and the modified Rather method is presented, together with a discussion of the defects of each method.

The conclusion is reached that none of these methods gives a satisfactorily accurate means of estimating the organic matter in soils. The attempt is made to show that the modified Rather method comes nearest to the objective sought, but that it is too slow and expensive for ordinary routine examinations. The fact that the dry-combustion method is based on a fundamentally inaccurate conversion factor does not invalidate its use for accurate determination of carbon content is pointed out.

The need for careful consideration of the carbonate content of soils as a part of the procedure in determining the carbon content of soils is emphasized and an improved procedure is suggested.

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