DETERMINATION OF CARBON IN SOILS.

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During recent years, several methods, direct as well as indirect, have been proposed for the estimation of carbon in soils. The direct methods which involve dry or wet combustion (Messinger, *Ber.*, 1888, **21**, 2910; Cameron and Breazeale, *J. Amer. Chem. Soc.*, 1904, **26**, 29; Salter, *Ind. Eng. Chem.*, 1916, **8**, 637; Dennstedt in Abderhalden's *Handbuch der Biologischen Arbeitsmethoden*, 1925, Abt. 1, Teil 3, 483; Winters and Smith, *Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 202; Friedmann and Kendall, *J. Biol. Chem.*, 1929, **19**, 727; Heck, *Soil Sci.*, 1929, **28**, 225; Methods of Analysis, *A.O.A.C.*, 1930, 4-5; Arnold and Page, *J. Agric. Sci.*, 1930, **20**, 473; Seshadri, *Madras Agric. J.*, 1933, **21**, 413; Adams, *Ind. Eng. Chem.* (*Anal.*), 1934, **6**, 277) require special equipment and are often difficult to carry out. Some of the methods are also elaborate and time-consuming. The indirect methods, which are based on the estimation of equivalent amounts of sulphur dioxide (Robinson, McLean and Williams, *J. Agric. Sci.*, 1929, **19**, 315), chromic acid (Schollenberger, *Soil Sci.*, 1927, **24**, 65; *ibid.*, 1931, **31**, 483; Degtjareff, *ibid.*, 1930, **29**, 239; Walkley and Black, *ibid.*, 1934, **37**, 29) or permanganate (Istcherecoff, cited from Degtjareff, *loc. cit.*) are very much easier to operate and yield results of comparative accuracy when a number of specimens under similar conditions are examined. The values obtained according to those methods are not, however, quite accurate, because the underlying reactions are not specific to carbon. Thus, Bhagvat, Narayanayya and Subrahmanyan (*Proc. Ind. Acad. Sci.*, 1934, **1**, 49) have shown that in the case of soils containing appreciable amounts of chloride or ferrous iron such methods give exaggerated estimates for carbon; nor are the oxygen requirements of different organic soil components proportional to their carbon contents. Furthermore, the oxidation may not always be complete, especially when the soil contains carbon compounds like acetic acid that are not readily oxidisable Such substances are known to be formed in the swamp soils (Subrahmanyan, *J. Agric. Sci.*, 1929, **19**, 627; Bhaskaran, Narasimhamurthy, Subrahmanyan and Sundara Iyengar, *Proc. Ind. Acad. Sci.*, 1934, **1**, 155), and the failure of indirect methods to yield reliable results under such conditions, as also in presence of substances like urea, has already been pointed out (Bhagvat, Narayanayya and Subrahmanyan, *loc. cit.*). The last-mentioned authors have also studied the factors influencing the oxidation of organic matter in dry as well as swamp soils and have standardised the conditions under which complete oxidation can be ensured. Their observations have shown that (*a*) soil organic matter is far less resistant to chemical oxidation than many other forms of carbon including some apparently simple substances and (*b*) the resulting carbon dioxide is more conveniently displaced by the steam issuing from the digesting mixture than by a current of air as is usually done. The apparatus and the procedure described in the present paper are based on the findings described in the previous communication.

EXPERIMENTAL.

The Apparatus.—(Fig. 1). It consists of a Kjeldahl flask (A) (800 c.c. capacity) fitted with a rubber stopper through one hole of which passes a long-stemmed dropping funnel (B), while, through the other, passes a short tube bent at right angles connecting, through a tap (C), with the absorption system which consists of a straight condenser (D) packed with glass beads. The top of the condenser is fitted with a short-stemmed dropping funnel (E) (50 c.c. capacity) and soda lime guard tube. The lower part of the condenser is fitted, through a rubber stopper, into the receiving flask which is, preferably, a wide-mouthed, flat-bottomed flask (F) of capacity about 400 c.c.

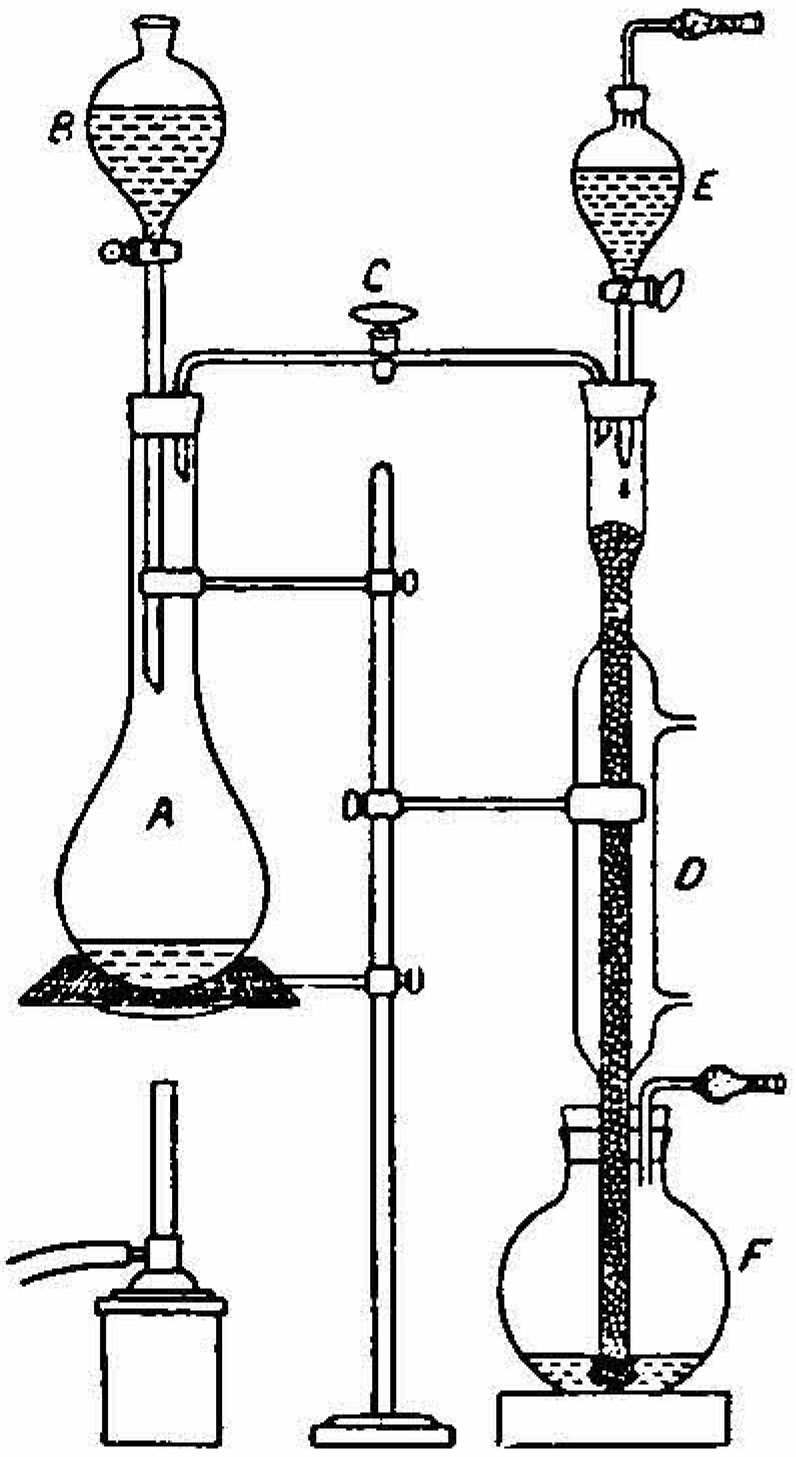

Fig. 1.

The above apparatus can be used for the estimation of carbon under a variety of conditions, only slight changes in the proportion or composition of the reagents being made to meet certain special conditions that arise in some cases. Since the previous observations (Bhagvat, Narayanayya and Subrahmanyan, *loc. cit.*) have shown that the oxidation proceeds rapidly to completion and that the vapours which pass over up to a late stage are exclusively steam and carbon dioxide, it is not considered necessary either to supplement the oxidation by additional equipment or to interpose traps to absorb acid fumes and other vapours which may interfere with the estimation of carbon dioxide.

Estimation of total carbon in dry soil.—The specimen is ground to fine powder passing the 100–mesh sieve and a known weight (5–10 g.) introduced into the Kjeldahl flask either in the dry condition or as suspension in water or alkali. It should be ordinarily possible to obtain accurate estimates upto a total of 200 mg. of carbon and, reckoning on that basis, it would be rarely necessary to take less than 5 g. of soil while, in many cases, especially when the specimens are of tropical origin, very much larger quantities, ranging from 20-25 g., can be conveniently handled. Pure, finely powdered potassium dichromate (10–15 g.) is then introduced and the sides of the flask washed down with distilled water, care being taken that the total quantity of water including that originally present does not exceed 25–30 c.c. The aqueous suspension is then shaken vigorously so as to break up any lumps that may have been formed. The flask is then stoppered and clamped in position.

The absorption system is prepared by pipetting out an excess (50–60 c.c.) of 2–4 N carbonate-free potassium hydroxide into the dropping funnel (E). About 10 c.c. of the alkali is first allowed to trickle down the condenser into the receiving flask (F). The remaining quantity is then let in drop by drop, the rate being so regulated that the alkali continues to trickle through the column of glass beads throughout the period of heating which is about 20 minutes.

A measured quantity (30–40 c.c.) of concentrated sulphuric acid is introduced into the long-stemmed dropping funnel (B). The tap is opened and the acid allowed to flow along the sides of the flask. Most soils require only 15–20 c.c. of acid for complete digestion, but there are, nevertheless, some (especially those rich in iron and alumina) which require more—hence the need for a larger quantity. It is not always possible to introduce the acid by merely opening the tap. In some cases, especially those of soils containing fairly large amounts of carbonates, there is vigorous gas production as soon as the first few c.c. of acid are added, so that, if sufficient care is not taken, a part of the carbon dioxide may escape upward, through the column of acid. A convenient procedure would, therefore, be to blow in the acid using a one-holed stopper fitted with a bent glass tube and rubber extension for the purpose. The blowing should be done in instalments and the tap closed from time to time so that there may not be too rapid a rush of gas into the absorption system. A small quantity (1–2 c.c.) of acid should be allowed to remain in the dropping funnel so as to act as seal against the carbon dioxide of the air being blown in. If there is no immediate action, the contents of the flask may be mixed together by gentle rotation and heated with a low flame in the initial stages. Bhagvat, Narayanayya and Subrahmanyan (*loc. cit.*) have shown that the most rapid evolution of carbon dioxide is between 5 and 10 minutes after commencement of heating—just before and for some time after steam begins to condense in the absorption system. If, during this period, the heating is carried out carefully, the gas passes over at a steady rate into the condenser where the large surface presented by the glass beads moistened with trickling alkali combined with the cooling serves as a very efficient absorbent system. The steam evolved from the digesting mixture displaces the last traces of carbon dioxide from the Kjeldahl flask. As the vapours get cooled in the condenser, there is fall in pressure in that section of the apparatus, so that the alkali from the receiving flask (F) rises to some extent in the condenser thus constituting an effective seal through which no carbon dioxide can escape. After steam has thus condensed for about 5 minutes, the flame can be raised and the heating continued vigorously for a further period till the oxidation is complete. The latter stage (which will ordinarily be reached in about 15 minutes after steam begins to issue) is indicated by the precipitation of the deep red chromium trioxide followed by its decomposition resulting in the evolution of oxygen. There will also be marked swelling of the digesting mass accompanied by steady change in colour (from red to green), through various stages. All the above-mentioned changes are well defined and as they continue over several minutes, serve as unmistakable indications of the stage at which the distillation ought to be stopped.

When the oxidation is complete, the central tap (C) is turned off, disconnecting the absorption system from the remaining part of the apparatus and immediately following that, the tap of the funnel (B) is fully opened to equalise pressure inside the Kjeldahl flask. The small quantity of alkali left in dropping funnel (E) is then run in rapidly and washed with about 200 c.c. of distilled water added in lots of 30–40 c.c. at a time. From time to time a few drops of phenolphthalein are run in to verify the complete removal of the last traces of any alkali that may be adhering to the glass beads. The absorption flask (F) is now disconnected after washing into it any alkali that may be adhering to the lower parts of the condenser as also to the copper gauze tied at the bottom. Barium chloride (10 c.c., 10 per cent.) is next added to the contents of F to precipitate the carbonate, and the unused alkali titrated against standard hydrochloric acid (2–3 N) to phenolphthalein end point. During titration, the flask must be continuously shaken with a rotatory motion and the acid let in slowly; otherwise, the concentration of the acid may become localised, resulting in the precipitated carbonate being acted on. It is desirable to finish the titration against 0·1–0·2 N acid so that the error of titration may be greatly reduced.

If pure alkali is used for absorption and the distillation stopped at the proper stage, the suspension left after titration should yield a clear solution on addition of a few drops of concentrated hydrochloric acid. The test would generally provide a useful indication of the accuracy of the procedure in each experiment. The persistence of even slight turbidity would ordinarily indicate the presence of sulphate and suggest that acid fumes had passed over. Correction for carbon dioxide present in the apparatus and reagents and that absorbed from the air during the titration is generally negligible, but it would, nevertheless, be desirable to carry out, from time to time, a blank distillation with only dichromate and sulphuric acid (1 : 1) and absorbing the vapours in standard alkali in the usual way.

Calculation of the carbon content is comparatively simple. 1 c.c. of normal alkali corresponds to 6 mg. of carbon and reckoning on the volume and strength of the alkali used up and the weight of soil taken, the percentage of carbon in the soil can be easily computed.

Estimation of carbon in some representative specimens of Indian soils.—Air-dry samples (10 g.) were analysed according to the procedure outlined above. The values thus obtained were compared with those secured by (*a*) calculation from loss on ignition by applying the usual factor and (*b*) dry combustion. The results have been presented in Table I.

TABLE I.

Description	PERCENTAGES				
			Total carbon by		
	Moisture	Loss on ignition	Wet combustion (new method)	Dry combustion	Calculation—$\frac{\text{Loss on ignition}}{1\cdot724}$
Paddy soil from Burma	6·1	4·67	0·52	0·55	2·71
Rain-fed soil from Punjab	2·0	2·79	0·46	0·46	1·62
Sandy soil from Ceylon	0·6	1·20	0·27	0·29	0·70
Upland soil from South Bihar	4·0	2·95	0·51	0·50	1·71
Laterite soil from Bangalore	2·2	4·54	0·72	0·71	2·64
Peaty soil from Travancore	5·6	41·56	12·40	....	24·11
Alluvial soil from Godavari Delta	8·1	5·32	0·85	....	3·09
Alkali soil from Sindh	2·4	5·85	1·42	....	3·39

.... Not determined.

It may be noted that there is close agreement between values obtained by the new method and by dry combustion while those calculated from loss on ignition are very much higher. Since the latter include combined water and volatile mineral constituents, the results obtained by that method are hardly reliable. They do not bear even an approximate relation to the values obtained by the two combustion methods.

Estimation of carbon in swamp soils.—Evidence has already been adduced to show that (*a*) incomplete oxidation of organic matter associated with the swamp soil is due partly to the resistance and partly to the volatility of some of the products associated therewith and (*b*) increasing the proportion of acid to water leads to improvement in the efficiency of oxidation while addition of different dehydrants or mineral catalysts is without any effect (Bhagvat, Narayanayya and Subrahmanyan, *loc. cit.*). The procedure for the estimation of carbon in the swamp soil would therefore be the same as the one already described except that at least 50–60 c.c. of concentrated sulphuric acid should be used for digesting 25–30 c.c. of soil suspension. The following results obtained for three specimens of soil containing known quantities of acetic acid would show that quite accurate results can be obtained according to this procedure:—(1) total carbon expected (in mg.), 174·0; found, 174·1; (2) expected, 136·0; found, 136·0; (3) expected; 130·1; found, 130·0.

Estimation of total carbon in presence of chlorides.—Most soils contain only minute quantities of chlorides, so that the correction for the alkali taken up by the chlorine or hydrochloric acid passing over into the absorption system would be generally negligible. In the case of alkali soils, however, and especially in those which are also poor in carbon, the error due to chlorides is considerable and should therefore be either eliminated altogether or the necessary correction applied to the titre values.

Cameron and Breazeale (*loc. cit.*) have drawn attention to the fact that the error due to the presence of small amounts of chloride can be eliminated by running in chromic acid cautiously and not heating the acidulated mixture until the reaction has proceeded for some time. They also suggest that if the quantity of chloride is relatively large, the soil may first be heated on the water-bath with dilute sulphuric acid until the major part of it is removed as hydrochloric acid. It is not ordinarily possible, however, to determine whether a soil is rich or poor in chlorides without conducting an analysis. In many cases, there is no visible reaction in the early stages, so it would be difficult to ascertain when the digestion should be commenced. Heating the soil with sulphuric acid, however dilute, is also undesirable, because, in addition to carbonate carbon, some organic carbon may also be thereby lost. It was considered necessary, therefore, to either completely eliminate the error due to chlorides or find an easy method of applying correction for such quantities as may pass over during the experiment. Some experiments were first carried out by fitting within the Kjeldahl flask a bulb trap containing glass beads moistened with silver sulphate so that the vapours will have to pass through it before entering the absorption system. The results obtained for parallel samples of a soil to which known quantities of chlorides were added have been presented in Table II.

TABLE II.

Chloride (as NaCl) per cent.	Total carbon as estimated (in mg.)
Nil (control)	36·0
0·2	36·0
0·5	36·1
1·0	36·5
2·0	37·3

It may be seen from the above that the trap was efficient only when the chlorides were under 0·5 per cent. It cannot therefore be relied upon for the estimation of carbon in alkali soils which often contain higher percentages of chlorides.

Attempts were next made to apply correction for the chlorine and hydrochloric acid passing into the absorption system. Free chlorine was estimated iodimetrically and total chloride by precipitation methods in both neutral and acid media. When known quantities of alkali chlorides were added to the soil, it was found that a part of the chlorine always remained behind in the Kjeldahl flask while the rest passed over as mixtures of chlorine and hydrochloric acid. Thus, in one experiment, out of 60 mg. of total chlorine (added as chloride), 38·5 mg. passed over in the elementary form and 9·5 mg. as hydrochloric acid while the rest (12·0 mg.) remained behind in the digestion flask.

It was noted that in most of the experiments, accurate estimates of carbon could not be obtained even after applying correction for the chlorine which had passed over. The values were generally discordant. Thus, in the case of a specimen of Bangalore soil, they ranged from 0·67 to 0·86 per cent. In view of these discrepancies, that procedure was given up as unsatisfactory.

Some experiments were next carried out adding excess of silver sulphate directly to the contents of the Kjeldahl flask instead of introducing it into the trap. It was then observed that there was not even a trace of chloride in the distillate, thereby suggesting that silver chloride which was presumably first formed was not acted on by sulphuric acid under the conditions of the experiment. It also appeared probable that addition of salts of other metals, especially those forming insoluble or, at any rate, sparingly soluble chlorides, may similarly arrest the formation of chlorine and hydrochloric acid. Some trials were therefore carried out adding 10 c.c. of 1 per cent. sodium chloride to 5 g. lots of a specimen of soil which was originally free from chlorides. After adding 5 g. of one of the sulphates mentioned below and 10–15 c.c. of water, the suspensions were digested with dichromate and sulphuric acid in the usual way (Table III).

TABLE III.

Treatment	Carbon (as estimated) in mg.	
	I	II
Soil alone (control) ..	36·0	36·0
„ + NaCl	38·7	37·2
„ + „ + $CuSO_4$ (anhydrous) ..	37·5	38·5
„ + „ + $PbSO_4$ ..	36·9	37·2
„ + „ + $HgSO_4$ ..	36·0	36·0
„ + „ + Ag_2SO_4 ..	36·0	36·0

The sulphates of copper and lead were not effective while those of mercury and silver were highly efficient and yielded the same results as the control.

The experiments were next repeated, varying the proportion of chloride to correspond to 0·5, 1·0, 2·0 and 5·0 per cents. respectively on the weight of the soil. The proportion of sulphuric acid to soil suspension was also increased so as to correspond to double or triple the usual quantity. In all the cases, the error due to chlorides was entirely eliminated by adding 5 g. of either silver or mercuric sulphate. These observations show that the treatment can be applied to all types of soils including those under swamp conditions.

In view of the recent observations of Krough and Keys (*Biol. Bull.*, 1934, **67**, 132) on the elimination of error due to chlorides in sea water, some trials were carried out adding thallium sulphate in sufficient quantity to precipitate all the chloride and then conducting the digestion in the usual way. The estimates thus obtained were, however, of the same order as those with copper or lead sulphate, thereby showing that the treatment is not applicable to soils.

Since silver salts are very costly and therefore not suitable for routine operations, the studies were next directed to the standardisation of conditions for the use of a mercury salt for the purpose. The trials were carried out with different concentrations of chlorides and varying quantities of metallic mercury, mercuric oxide or mercuric sulphate. The results have been presented in Table IV.

TABLE IV.

Form of mercury	Percentage of chloride in the soil (as added)	Quantity (in g.) of mercury (free or combined) added to 5 g. of soil		
		1·0	2.0	5.0
		Total carbon as estimated (in mg.)		
Mercury (metal)	0·5	42·9	43·4	44·0
	1·0	43·5	44·0	44·4
	2·0	44·2	44·6	44·4
Mercuric oxide (red)	0·5	42·5	42·5	42·5
	1·0	42·6	42·5	42·5
	2·0	42·8	42·6	42·5
Mercuric sulphate	0·5	42.5	42·5	42·5
	1·0	42·7	42·5	42·5
	2·0	42·8	42·6	42·6

Total carbon in untreated soil (control)—42·5 mg.

It was noted that in addition to yielding unsatisfactory results, addition of metallic mercury also rendered the digestion very difficult. The heavy pellet settled at the bottom and caused violent bumping. As the result of this, there was a tendency for the acid digest to shoot up in the Kjeldahl flask, so that there was also the danger of a part of the acid being mechanically carried over into the absorption system. In presence of the oxide or the sulphate, the digestion proceeded smoothly and, as will be seen from the results, the error due to chlorides (allowing for small experimental differences) was entirely eliminated when quantities equivalent to 2–5 g. of mercury were added.

The above experiment was next repeated with different soils and increasing concentrations of chloride upto 20 per cent. It was observed that although in the case of certain soils—especially those of the laterite or red gravel type which are rich in iron and alumina—addition of 10 g. of mercuric oxide was effective in completely eliminating the error, it was only partially so in other cases. It was noted, however, that any quantity upto 10 per cent. of chlorides can be easily dealt with in all the cases irrespective of the nature of the soil. Since most alkali soils of the world contain well under 5 per cent. of chlorides, it may be safely assumed therefore that by adding 5 g. of mercuric oxide to the digesting mixture, the error due to chlorides can be entirely avoided.

The mechanism of the action of mercury or silver in checking the formation of chlorine is still obscure, but the following observations may be of interest:—(*a*) whether in aqueous solution or admixed with soil suspension, alkali chlorides are readily attacked by the oxidising mixture; (*b*) when present by itself chloride of mercury or silver is not resistant, but when mixed with the soil, it does not yield even a trace of hydrochloric acid until long after the complete oxidation of carbon; (*c*) alkali chloride to which excess of mercury or silver salt is added is not resistant in presence of water alone; when combined with the soil, it withstands the action of the oxidising mixture in the same manner as silver or mercuric chloride does. These observations would show that the elimination of error is due to the combined action of silver or mercury and certain constituents of the soil.

Since the retention of chlorine was most prominent in soils which were rich in iron and aluminium oxides, it appeared probable that either or both of those mineral constituents might have contributed to such action. With a view to determining whether they could, by themselves, produce the same effect, some experiments were carried out adding 2, 5 and 10 g. respectively of either of those substances to suspensions containing 5 g. lots of quartz powder together with 10 c.c. of 1 per cent. sodium chloride. The results showed, however, that varying quantities of chlorine and hydrochloric acid had passed over in all the cases. The experiments were next repeated adding silver or mercuric sulphate (5 g.) together with iron or aluminium oxide or laterite to the solution of chloride. It was observed that appreciable quantities of chlorine and hydrochloric acid were still passing over. These observations would show that the mechanism of retention of chlorine by the soil is more complicated than it first appeared. Further work is needed to throw light on this aspect of the problem.

Total carbon in presence of nitrates.—Since most soils contain nitrates—though generally only in minute quantities—it appeared probable that treatment with the oxidising mixture would lead to the production of nitric acid and different oxides of nitrogen which might pass over and thus affect the accuracy of the estimate of carbon. With a view to obtaining an idea of the error which may thus be introduced, the following experiments were carried out:—To 5 g. lots of a specimen of black cotton soil (from Nagpur) sodium nitrate was added in quantities corresponding to 200, 400 and 600 parts per million and the suspensions (25 c.c.) oxidised in the usual way with 5 g. of dichromate and 30 c.c. of sulphuric acid. In another set of trials, the oxidations were repeated using double the quantity (60 c.c.) of sulphuric acid. In a third set, the soil suspensions were treated with 2 g. each of mercuric oxide together with dichromate and acid.

It was noted that, in all the cases, correct estimates of carbon (0·97 per cent.) were obtained. Since the nitrate contents of field soils are generally very much less than those added in the previous experiment, it may be inferred that soil nitrates do not interfere with the accuracy of the estimation of carbon by wet combustion.

The foregoing observations should not be taken to mean that there is no reaction at all between nitrates and sulphuric acid under the conditions of digestion for carbon. Traces of nitrous vapours do indeed pass over, but they do not affect the titre value. In fact, even if the entire quantity of added nitrate had been acted on, the resulting acid would not have corresponded to more than one drop of Normal alkali.

Carbonates and organic carbon: Dry soil conditions.—Several methods have been proposed for the estimation of carbonates in soils and some of them are known to give accurate values (Hall and Russell, *J.C.S.*, 1902, **81**, 81; Amos, *J. Agric. Sci.*, 1905, **1**, 322; Collins, *J. Soc. Chem. Ind.*, 1906, **25**, 518; Hutchinson, *J. Agric. Sci.*, 1914, **6**, 323; MacIntire and Willis, *Ind. Eng. Chem.*, 1915, **7**, 227). Since it is desirable, however, that the same equipment should serve for the estimation of both carbonates and organic carbon, some experiments were carried out with the apparatus described in an earlier section of the paper.

To samples (5 g.) of a Bangalore soil (which was free from carbonates), known quantities of alkali carbonate (in solution) were added and the suspensions (25 c.c.) treated with 40 c.c. each of 1:15 solution of hydrochloric, sulphuric or phosphoric acid. They were then raised to boiling and the vapours received in the absorption system in the usual way. Since there was no definite end-point as in the case of total carbon, the distillations were stopped at the end of 30 minutes, the rate of heating being so adjusted that a third of the added water was still left in the flask. The carbon contents, as determined by back titration, showed that there was complete recovery of the added carbonate in all the cases.

The foregoing observations having shown that there is no danger of acid vapours passing over under the conditions of the experiment, the trials were next extended to certain soils which were known to contain carbonates. The soil suspensions (5 g. in 25 c.c.) were treated in the same manner as in the previous experiment and the distillations stopped at the end of 10, 20 and 30 minutes respectively, the rates of distillation being so regulated that about one-third, half and two-thirds respectively of the added water had distilled over at the time of stopping the heating. The estimates of carbon thus obtained have been presented in Table V.

TABLE V.

Acid used	Time of boiling in minutes	Carbonate as mg. of carbon in soil from		
		Sindh	Mandya	Tindivanam
Hydrochloric (40 c.c., 1:15)	10 20 30	49·5 49·5 49·5	21·8 21·8 21·8	6·8 6·8 6·8
Sulphuric (40 c.c., 1 : 15)	10 20 30	49·5 49·5 49·5	21·8 21·8 21·8	6·8 6·8 6·8
Phosphoric (40 c.c., 1 : 15)	10 20 30	49·5 49·5 49·5	21·7 21·8 21·8	6·4 6·8 6·8
Boric (5 g. + 40 c.c. water)	10 20 30	13·7 13·7 13·7	6·5 6·5 6·5	3·2 3·2 3·2

It may be seen from the above that boiling for even 10 mins. was sufficient to displace all the carbon dioxide from specimens treated with hydrochloric or sulphuric acid. Those treated with phosphoric acid yielded correct estimates only after boiling for twenty mins. Specimens which were boiled with boric acid yielded consistently low values even after 30 minutes.

Although concordant values were obtained for the particular specimens of soil that were used in the above study, it was yet realised that in presence of calcium salts, sulphuric or phosphoric acid may not prove so useful as hydrochloric acid Such was indeed the case when the carbonate content of the soil mixed with a known quantity of chalk was estimated. The specimen treated with dilute hydrochloric acid yielded the correct value (96 mg.) while that treated with sulphuric acid yielded an estimate of only 81 mg. even after prolonged boiling.

The volatile character of hydrochloric acid would no doubt restrict the range of concentration within which it can usefully be employed. It is fortunate, however, that only very dilute acid (about 2·5 per cent.) is required for the decomposition of carbonates. As the result of reaction with the carbonate, as also with the mineral constituents of the soil, the strength of the acid is further reduced so that there is practically no danger of any of it passing into the distillate during the first half hour. The reaction between carbonate and acid is almost instantaneous and since the previous observations have shown that boiling for even 10 minutes is sufficient to displace all the resulting gas, it may be concluded that by stopping the distillation at a fairly early stage, the error due to acid vapours may be entirely avoided.

It should be admitted, however, that the use of hydrochloric acid for the estimation of carbonates would interfere with the determination of organic carbon in the residue. The volume and the concentration of the added acid would be so considerable that large quantities of mercury or silver salts would be required to check the acid vapours. It is doubtful if, even then, it would be effective in all soils. It may be necessary therefore to determine total carbon on a separate sample and to estimate organic carbon by difference. This duplication of procedure can be avoided if the nature of the soil can be ascertained beforehand, so that if it is not rich in lime, sulphuric or phosphoric acid may be used for the estimation of carbonates. In such cases, there will be no difficulty in digesting the residue for organic carbon.

Attention has already been drawn to the fact that the estimate of carbonate carbon is not affected even if the distillation is continued over several minutes. It was not clear, however, if the organic carbon remains unaffected by such a treatment. Thus, it appeared probable that certain non-acid, volatile, carbon compounds may pass over into the distillate and thus escape detection. With a view to determining whether such changes occur at all, the estimations of organic carbon were carried out by two methods,—(1) directly on the residue left after decomposition of carbonates, and (2) indirectly, by estimating total carbon and subtracting the value obtained for carbonate. The two sets of results thus obtained have been presented in Table VI. For reasons already stated, hydrochloric acid was not used for the direct estimation.

It may be seen from the table that both the methods yield the same values, thereby showing that none of the organic carbon is lost during boiling with dilute mineral acid. The higher estimates obtained as the result of treatment with boric acid are fictitious since they include unattacked carbonate.

TABLE VI.

Acid used for decomposing the carbonate	Organic Carbon (in mg.) by					
	Direct Method			Indirect Method		
	Soil from			Soil from		
	Sindh	Mandya	Tindivanam	Sindh	Mandya	Tindivanam
Sulphuric or Phosphoric	23·0	9·9	7·7	23·0	9·9	7·7
Hydrochloric ..	..	..	..	23·0	9·9	7·7
Boric	58·8	25·2	11·3	58·8	25·2	11·3

Carbonate and organic carbon in swamp soils.—This estimation is rendered difficult by the presence of free as well as combined fatty acids which yield acid vapours when the soil is boiled with dilute mineral acid. With a view to eliminating this source of error, some experiments were carried out treating the soil first with carbonate-free sodium hydroxide to render it alkaline and then with excess of barium chloride solution to precipitate all the carbonate. The suspension was then filtered and washed frequently with carbon dioxide-free water. The wet precipitate was transferred to the Kjeldahl flask together with the filter paper and the carbonate estimated in the usual way by treatment with excess of dilute hydrochloric acid.

It was expected that the barium salt of fatty acids being soluble, or at any rate, very much more so than the carbonate, would be washed away leaving only the carbonate and a part of the organic carbon on the filter. The estimates obtained according to the above-mentioned procedure for soil suspensions to which known quantities of carbonates and acetates had been added were compared with the quantities actually expected. The values for organic carbon were also obtained by the indirect method and compared with the expected values (Table VII).

The results show clearly that the procedure outlined above is quite reliable for the estimation of carbonate and organic carbon under swamp soil conditions.

Estimation of different forms of carbon in a soil of unknown composition: A general procedure for all soils.—It is not always possible to trace the previous history of soils under examination or to conduct preliminary analyses with a view to determining the composition and proportion of the reagents to be employed in each case. It would be desirable therefore to adopt a simple procedure that could be applied to all soils irrespective of their composition. Since the different modifications developed in the present paper are more or less additive

TABLE VII.

Composition of suspension	Carbonate as mg. of carbon		Organic carbon as mg. of carbon	
	Calculated	Found	Calculated	Found
Paddy soil (10 g.) + sodium acetate (10 c.c., 1 per cent.) + sodium carbonate (20 c.c., 1 per cent.)	22·6	22·7	52·7	52·7
Paddy soil (10 g.) + sodium acetate (10 c.c., 1 per cent.) + sodium carbonate (40 c.c., 1 per cent.)	45·2	45·0	52·7	52·6
Paddy soil (10 g.) + sodium acetate (20 c.c., 1 per cent.) + sodium carbonate (20 c.c., 1 per cent.)	22·6	22·6	82·0	82·0
Paddy soil (10 g.) + sodium acetate (20 c.c., 1 per cent.) + sodium carbonate (40 c.c., 1 per cent.)	45·2	45·2	82·0	82·0

in character and do not interfere with each other, a convenient procedure would be to assume that the soil under examination may contain some resistant organic substances like urea, volatile acid products, chlorides, nitrates and carbonates and treat it in such a manner as would yield accurate estimates in presence of all these products. A comprehensive procedure for the estimation of total carbon would be as follows.—The soil suspension (5 g. in 25 c.c.) is treated with 10 g. dichromate, 5 g. of mercuric oxide and slightly more than double the volume of concentrated sulphuric acid as that of the water in the medium. The digestion, absorption of vapours and back titration of unused alkali are then conducted in the usual way.

The estimation of carbonates may, in a like manner, be carried out on the assumption that volatile acid products would be formed on boiling the soil suspension with dilute mineral acid. The treatment for eliminating this source of error would be the same as that described for the swamp soil. Since treatment with alkali and subsequent washing may remove some of the soil organic matter together with the salts of fatty acids, estimation of organic carbon on the residue left after decomposition of carbonates would not yield accurate values. A satisfactory procedure will be to take two separate samples of soil or soil suspension and to determine total carbon on the one and carbonate on the other. The difference between the two estimates would be the value for organic carbon.

DISCUSSION.

The results of the present enquiry, as also those of the previous one (Bhagvat, Narayarayya and Subrahmanyan, *loc. cit.*) have brought into relief a number of interesting features relating to wet oxidation of organic matter in soils. Not only is the oxidation very rapid, but the type of equipment required for carrying it out is also quite simple. A considerable part of the complicated apparatus adopted by previous investigators has been eliminated, and the remaining part so adjusted that both the displacement of the vapours and the absorption of carbon dioxide have been rendered more efficient. The appendage of traps for different types of acid vapours is also done away with and the related errors eliminated by simple modifications in the composition and proportion of the digesting mixture. The procedure for estimation has been rendered so simple, and the details of manipulation so easy to follow that the determination can now be carried out as a routine operation. The whole estimation takes only about 20 minutes and if provided with three or four sets of apparatus, a single investigator can complete 15 to 20 determinations in the course of a working day. The equipment being compact, the chances of error through leakage or otherwise are also small, so that it should be possible for an experienced worker to obtain carbon estimates which would be correct to the first decimal place and very nearly agree in the second if reckoned on the usual percentage basis.

A further feature of interest about the present method is that it takes into account all types of soil conditions, including some new ones which are of considerable practical interest but have not been considered by any of the previous investigators. This would naturally extend its application not only in routine practice but also in researches having a bearing on carbon transformations in soils.

Although the present research relates primarily to standardisation of conditions for the estimation of soil carbon, the various observations would suggest that it can be easily extended to all organic substances in general. Attention has already been drawn (Bhagvat, Narayanayya and Subrahmanyan, *loc. cit.*) to the fact that the method yields accurate estimates when applied to pure organic substances. It can deal with fairly large quantities of substances so that the experimental error can be greatly reduced. The estimations can also be easily replicated, so that, by carrying out a number of parallel estimations, the error of the mean can be lowered to a minimum.

The method can also be applied to different biological products which do not easily permit of drying and those which offer considerable difficulty in handling. The extension of the technique would, in fact, open a vast line of research on various carbon transformations and economy of carbon, in general, in different types of living tissues whether of animal, plant or microbial origin. The procedure for such estimations would be similar to that described in the present paper though slight modifications with regard to details such as collection and storage of sample, quantities to be treated and such like will have to be introduced to suit the types of materials under investigation. It is hoped that the study of these and related aspects of the problem would form the subjects of later communications.

It is generally believed that the end product of sulphuric acid digestion of any nitrogenous substance (with the exception of the nitrate) is ammonia, which would be retained as the corresponding salt. It is reasonable therefore to expect that the residue after wet combustion would be suitable (or, at any rate, very nearly so) for distillation with alkali as in the Kjeldahl method. The experience of previous workers in this direction has, however, been discordant. Some state that the residue after oxidation of carbon yields correct values for nitrogen, while others point out that it is not suitable and requires further digestion prior to distillation of ammonia (Anderson, *J. Biol. Chem.*, 1924, **61**, 57; Brown, *Ind. Eng. Chem.*, 1927, **19**, 629; Adams, *loc. cit.*).

A number of experiments carried out by the present authors would show that (*a*) the residue after oxidation of carbon yields low estimates of nitrogen when distilled, as such, with excess of alkali, (*b*) further digestion conducted, as in the usual Kjeldahl method, improves the value, but a part of the nitrogen still remains unattacked, and (*c*) the incomplete digestion is traceable to the presence of large quantities of chromium salts in the medium. The following results (Table VIII) obtained from three typical soils would illustrate the above.

TABLE VIII.

Description of soil	Nitrogen per cent. as estimated by	
	Improved Kjeldahl method*	Digestion of residue after estimation of carbon
Red sandy loam—**Bangalore**	0·078	0·072
Kalar (alkali) **soil—Sindh**	0·060	0·050
Black cotton **soil—Nagpur**	0·070	0·065

* Sreenivasan and Subrahmanyan, *Indian J. Agric. Sci.*, 1933, **3**, 646.

Similar low yields were also obtained in the case of yeast. The mechanism of retention of nitrogen is still obscure, but there is evidence to show that (*a*) as the quantity of chromium is increased (either by using more dichromate for oxidation of carbon or by adding chromium salt to the digesting mixture), larger amounts of nitrogen are retained, and (*b*) the undigested nitrogen is in combination with the insoluble residue, the main constituent of which is chromium (Cr_2O_3 ?). Further work on these and allied problems is in progress and will form the subject of a later communication.

SUMMARY.

1. A simple and elegant piece of apparatus for the wet combustion of soils has been described. Its special features are (*a*) elimination of the usual equipment for aeration, (*b*) improved efficiency in the absorption of carbon dioxide, (*c*) elimination of traps for absorbing chlorine and other acid vapours, and (*d*) adaptability for all types and conditions of soils.

2. The procedure for the estimation of carbon in dry soils has been outlined. The results, which have been compared with those obtained by dry combustion, show that the method is highly reliable.

3. Complete oxidation of the organic constituents of the swamp soil can be ensured by adjusting the volume of concentrated sulphuric acid so as to correspond to at least twice that of the water in the soil suspension.

4. When the soil contains chlorides, it is not possible to obtain accurate estimates of carbon by merely applying correction for the chlorine and hydrochloric acid which pass over. The error can be entirely eliminated, however, by adding silver sulphate, mercuric sulphate or mercuric oxide to the digesting mixture. The related conditions have been standardised.

5. Even abnormally large quantities (600 parts per million) of nitrates do not interfere with the accuracy of the estimate of carbon.

6. Using the same apparatus, the conditions for the estimation of carbonates in dry as well as swamp soils have been standardised. Dilute solutions of hydrochloric, sulphuric or phosphoric acids are equally efficient in most cases, but when the soil is rich in lime, hydrochloric acid yields the most accurate results. In the case of the swamp soil, addition of baryta followed by washing the precipitate on the filter removes the volatile fatty acids which interfere with the estimation. On distillation with excess of hydrochloric acid, the residue yields the correct estimate for carbonate including dissolved carbon dioxide.

7. A comprehensive procedure for the estimation of total and organic carbon in any type of soil has been outlined.

8. The application of the method for the estimation of carbon in different types of biological products and organic substances in general has been indicated.

9. Even after prolonged digestion, the residue left after oxidation of carbon does not yield correct estimates for total nitrogen. The defect is traceable to the presence of large quantities of chromium salts in the digesting mixture. The unattacked nitrogen is present in combination with the precipitate formed during digestion.

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