A Rapid and Accurate Procedure for Estimation of Organic Carbon in Soils[1]

D. W. Nelson and L. E. Sommers[2]

Purdue University, West Lafayette, Indiana 47907

A rapid and precise method is described for routine determination of organic carbon in soils. Digestion of soil samples with $K_2Cr_2O_7$ and H_2SO_4 is carried out in Folin-Wu tubes heated for 30 minutes at 150° C in an aluminum block. Unreacted dichromate is titrated with ferrous-iron and the difference in titration values between the blank and sample is proportional to the organic carbon content of sample. Sample size was not a critical factor so long as the total amount of organic carbon in the sample did not exceed 8 to 10 mg. The precision of the proposed method was as good as commonly used techniques for carbon analysis of soils. The values for carbon content of ten reference soil samples as determined by the proposed method were the same as those determined by standard techniques known to give quantitative recovery of organic carbon in soils. The proposed procedure is more rapid than other techniques because 60 samples are digested simultaneously.

Two basic analytical approaches have been used for estimating the organic carbon content of soils. The first approach involves a wet-or-dry-combustion (oxidation) of the samples in the presence of oxygen to convert organic carbon to CO_2 followed by measurement of the CO_2 liberated by titrimetric, gravimetric, volumetric, infra-red, or thermal conductivity techniques. Wet oxidation is normally accomplished by boiling the sample with a $H_2SO_4:H_3PO_4:K_2Cr_2O_7$ mixture in a stream of CO_2-free air, whereas dry combustion is accomplished by heating the soil sample at high temperature (1,000-1,650°C) in a stream of oxygen. The second approach used is to react organic carbon in soil with a hot chromic acid solution ($K_2Cr_2O_7$ and H_2SO_4) resulting in oxidation of carbon to CO_2 and reduction of dichromate to chromium (III) according to equation 1.

(1) $2 Cr_2O_7^{-2} + 3C° + 16H^+ \rightarrow 4 Cr^{+3} + 3CO_2 + 8H_2O$
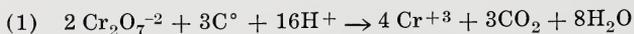
The amount of dichromate reduced by reaction with organic carbon is then estimated by titration of residual dichromate with an iron (II) solution in the presence of a suitable oxidation-reduction indicator. In calculating the organic carbon content of soil when using the rapid titrimetric methods it is assumed that the organic carbon has an average oxidation state of zero and an equivalent weight of 3.

The rapid titrimetric methods have gained wide acceptance since first proposed by Schollenberger (7) and subsequently modified by Walkley and Black (11), Smith and Weldon (8), and Mebius (5) because of their speed and ease. However, the dichromate-titration methods

have several deficiencies which may give spurious results in some soils. Chloride and iron (II) ions in soil are oxidized by dichromate during carbon analysis resulting in positive errors. Higher oxides of manganese may react with iron (II) ions during titration of residual dichromate causing negative errors in soil carbon content. Another potential source of error rests with the fact that the carbon in some soils may have an average oxidation state greater or less than O. Widespread use of the rapid titrimetric methods for analysis of soils in the eastern portion of the U.S. suggest that the errors listed above are negligible for Indiana soils. However, it should be noted that dichromate-titrimetric procedures using a short period of heating or no external heat during digestion (4,7,8,11) require a correction factor to account for incomplete oxidation of organic carbon during analysis. The appropriate correction factor must be determined for each class of soils. No correction factor is required for procedures such as that of Mebius (5) which have sufficient heating to oxidize all of the carbon in soils.

Carbon analysis procedures involving measurement of CO_2 evolved during wet-or dry-combustion of soil samples are not subject to the errors and interferences common to rapid titration methods. However, the wet-oxidation technique described by Allison (1) and the modified Van Slyke-Folch procedures (3,10) are very tedious and time consuming, whereas the dry combustion procedures using automated high temperature induction furnaces (9) require very expensive equipment.

Many soil research laboratories have a requirement to determine the organic carbon content of soil samples. Where finances permit and sample numbers justify the expenditure, use of an automated instrument such as a Leco 70-Second Carbon Analyzer (9) may be the most desirable technique for organic carbon analyses. However, for those laboratories unable to justify the purchase of an automated system, a rapid dichromate-titration technique is most preferable because manual wet-or dry-combustion techniques involving measurement of CO_2 liberated from soils are much too time consuming for routine analysis. The objective of this work was to develop and evaluate a rapid dichromate-titration procedure which quantitatively recovers organic carbon from diverse soils and which did not require a correction factor to account for incomplete oxidation of organic carbon during digestion. The basic approach used was to modify the organic carbon procedure of Mebius (5) such that heating of the soil sample-$K_2Cr_2O_7$-H_2SO_4 mixture was carried out in Folin-Wu nonprotein nitrogen (NPN) tubes placed in an aluminum block. In this manner 60 samples could be digested simultaneously and a large number of samples could be analyzed each day.

Materials and Methods

The soil samples used were those described by Nelson and Sommers (6). The samples represented a wide range in organic matter contents and textures. All samples were ground to < 100-mesh before analysis.

The procedure described below for determination of organic carbon involves digestion of soil samples in pyrex Folin-Wu N.P.N. tubes

(Kimax 47125 or Corning 7940) placed in an aluminum heating block. The heating block was constructed as described by Blanchar et al. (2) but was modified by completely enclosing the block with an asbestos board to prevent temperature gradients from developing when the block was heated in a fume hood. A Lindberg Hevi-Duty (Model H-2) electric hotplate was used in conjunction with the heating block.

Method for Determining Organic Carbon

Reagents

Concentrated H_2SO_4—Specify gravity 1.84

Potassium Dichromate Solution (1.00N)—Dissolve 49.024 g of $K_2Cr_2O_7$ (dried at 140°C) in 800 ml of distilled water and dilute to a volume of 1 liter. This solution is the primary standard for the procedure.

Ferrous Ammonium Sulfate Solution (0.20N)—Dissolve 78.390 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 50 ml of conc. H_2SO_4 and dilute to 1 liter with distilled water.

Indicator Solution—Dissolve 0.1 g of N-phenylanthranillic acid and 0.1 g of Na_2CO_3 in 100 ml of water.

Procedure

Weigh an amount of soil (<100-mesh) containing not greater than 8 mg of carbon (usually 100 to 500 mg) into a clean, dry Folin-Wu tube, add 5 ml of 1.00N $K_2Cr_2O_7$ solution and 7.5 ml of conc. H_2SO_4. Place the digestion tube in a preheated (150°C) aluminum block and heat at 150 ± 5°C for exactly 30 minutes. Remove the digestion tube from the aluminum block and allow the samples to cool for 30 minutes at room temperature. Quantitatively transfer the contents of the tube to a 125 ml Erlenmeyer flask and titrate the sample with ~ 0.2N ferrous ammonium sulfate using 0.3 ml of the N-phenylanthranillic acid solution as an indicator. The color change at the end point is from violet to bright green and is very rapid. An illuminated background is recommended for ease in observing the end point and the titration should be performed using a variable speed magnetic stirrer and teflon coated stirring bar.

Each set of samples should be accompanied by an unheated reagent blank and a reagent blank which is heated at the same time as the samples. The unheated blank is used to standardize the ferrous ammonium sulfate solution. The difference in titration value between heated and unheated blanks is used to correct all sample titration values for the amount of dichromate consumed by spontaneous decomposition during the heating process.

Computation of the organic carbon content of soil is performed as follows: (i) subtract sample titration values from the titration value for the heated blank (B-T), (ii) correct the resulting (B-T) value for self-decomposition of dichromate by dividing the difference in titration value for unheated and heated blanks by the titration value for the unheated blank, multiplying the correction factor (normally 0.04 to 0.08) by the (B-T) value, and adding the product to the (B-T) value.

The resulting value, labelled "A", is proportional to the amount of organic carbon present in the soil, (iii) complete calculation of the organic content using equation 2.

$$(2) \quad \% \text{ organic C} = \frac{(A) \; (N \text{ of ferrous solution}) \; (0.003) \; (100)}{(\text{sample weight, g})}$$

The calculation of results assumes that the equivalent weight of organic carbon in soil is 3. Previous research has shown that this assumption is valid for most agricultural soils having well stabilized organic matter. The potassium dichromate solution is the primary standard for the method and care should be taken in its preparation. However, the potassium dichromate solution is quite stable and it may be stored at room temperature indefinitely. Self-decomposition of dichromate during heating is very dependent upon the conditions of digestion. Therefore it is recommended that digestion tubes be dry before use to eliminate differences in volume, that the heating temperature be closely controlled (150 $\pm$ 5°C), and that the time of heating be accurately monitored. The ferrous ammonium sulfate solution oxidizes slowly and thus must be standardized each time it is used.

Results and Discussion

The H_2SO_4:water ratio and the milliequivalents of dichromate used in digestion of soil are those recommended by Mebius (5) after studies of the effects of these factors on carbon analysis of soil. Other factors which may influence the quantitative recovery of organic carbon in soil by the tube digestion method include temperature of heating, length of the heating period, and sample size. Table 1 gives data on the effect of heating temperature on the amount of organic carbon recovered from soils. When compared to the values obtained by the Allison procedure it is evident that heating samples at 130°C gave slightly low recoveries of organic carbon, whereas heating samples at 170°C gave high recoveries. Heating samples at 150°C gave organic carbon values which were very close to those obtained by reference methods. Thus, a heating temperature of 150°C was chosen as the standard. Further studies revealed that any heating temeprature between 140°C to 155°C gave satisfactory recovery of organic carbon in soils.

TABLE 1. *Effect of heating temperature on organic carbon values obtained by the proposed method.**

Soil	Allison Method	Temperature of heating, °C		
		130	150	170
		organic C content, %		
Romney _____	3.80	3.58	3.68	4.01
Romney subsoil _____	1.22	1.13	1.23	1.16
Zanesville _____	1.55	1.53	1.55	1.69
Ave. _____	2.19	2.08	2.16	2.29

* Samples were heated for 30 minutes.

The effects of varying the length of the heating period on recovery of organic carbon in soils is given in Table 2. When compared to values obtained by the Allison procedure it is apparent that a heating period of 15 minutes is insufficient for quantitative recovery of organic carbon in soils. There was little difference in organic carbon values obtained for samples which were heated for 30, 45, or 60 minutes. A heating period of 30 minutes was chosen as standard to maximize the efficiency of analysis.

TABLE 2. *Effect of heating time on organic carbon values obtained by the proposed method.**

Soil	Allison Method	Heating time (min.)			
		15	30	45	60
		Organic C content (%)			
Romney _____	3.80	3.45	3.68	3.72	3.74
Romney subsoil _____	1.22	1.16	1.23	1.24	1.23
Zanesville _____	1.55	1.49	1.55	1.52	1.59
Ave. _____	2.19	2.03	2.15	2.16	2.18

* Temperature of heating was 150°C.

Table 3 gives data on the effects of sample size on the amounts of organic carbon recovered from soils by the tube digestion technique. The proposed procedure gave the same value for organic carbon content of Romney subsurface and Zanesville soils over a sample size range of 200 to 500 mg. A range in sample size from 150 to 300 mg was suitable for the Romney soil, however, the organic carbon value obtained from a 400 mg sample of Romney soil was low due to incomplete oxidation of carbon in the larger sample. Incomplete oxidation occurred because the 400 mg sample contained much in excess of 8 mg of organic carbon, the upper limit expressed in the procedure. Sample size has no effect upon the organic carbon values obtained by the proposed procedure so long as the total amount of carbon in the sample to be analyzed does not exceed 8 to 10 mg.

The precision of the tube digestion technique was evaluated for three soils by performing duplicate analyses on at least five different

TABLE 3. *Effect of sample size on organic carbon values obtained by the proposed method.*

Sample size	Soil		
	Romney	Romney subsoil	Zanesville
	Organic C (%)		
150 _____	3.68	—	—
200 _____	3.71	1.23	1.55
300 _____	3.64	1.22	1.52
400 _____	3.43	1.21	1.51
500 _____	—	1.21	1.52

days. The results (Table 4) show that the standard deviation of the proposed method varied from 0.052 to 0.108% C and the coefficient of variation (CV) was 2.97 to 4.33%. By contrast it has been reported that the CV for determination of organic carbon in soils by the Mebius procedure averaged 1.2% (5), by the Allison procedure 0.65 to 6.45% (9), and by an automated dry combustion technique 0.65 to 6.25% (9). Therefore, it appears that the proposed procedure is sufficiently precise for routine estimation of organic carbon in soils.

TABLE 4. *Precision of the proposed method.*

Soil	No. of analyses	Range	Mean	SD	CV
		------------ Organic C (%) ------------			%
Romney _____	11	3.49–3.80	3.63	0.108	2.97
Romney subsoil _____	14	1.10—1.31	1.20	0.052	4.33
Zanesville _____	12	1.41—1.60	1.53	0.055	3.59

A comparison of the values obtained when ten soils were analyzed for organic carbon by five widely used procedures is given in Table 5. It is apparent that the value obtained using the proposed procedure agree very well with those obtained with the Allison (1) and Van Slyke-Folch (3, 10) methods. Both the Allison and Van Slyke-Folch procedures are widely accepted as giving quantitative recovery of organic carbon in soils. Therefore, it can be presumed that the proposed method is also giving quantitative values for organic carbon in soils. As compared to the Allison method, the Mebius procedure (5) gave slightly higher organic carbon values for three soils and a slightly

TABLE 5. *Comparison of organic carbon contents of soils as determined by several methods of analysis.*

Soil		Method of analysis+				
Type*	Depth	Proposed	A	M	WB	VS
	cm	----------- Organic C content (%) ---------				
Houghton muck _____	0-15	17.42	17.42	18.72	19.87	—
Davidson sic _____	0-15	4.04	4.03	4.09	2.31	—
Romney sicl _____	0-15	3.63	3.80	3.51	3.77	3.69
Romney sicl _____	15-30	1.23	1.22	1.29	1.10	1.19
Maumee sal _____	0-15	2.20	2.32	2.21	2.60	2.37
Chalmers sil _____	0-15	1.91	1.89	1.89	1.93	1.94
Chalmers sil _____	15-30	0.87	0.87	0.88	0.80	0.86
Zanesville sil _____	0-15	1.53	1.55	1.61	1.59	1.51
Russell sil _____	0-15	1.10	1.16	1.15	1.05	—
Plainfield lsa _____	0-15	0.76	0.75	0.88	0.81	—
Ave. _____		3.47	3.50	3.62	3.58	—

* Sic, silty clay; sicl, silty clay loam; sal, sandy loam; sil, silt loam; lsa, loamy sand.

+ A, method of Allison (1); M, method of Mebius (5); WB, method of Walkley and Black (11); and VS, method of Van Slyke and Folch (10).

low value for one soil. The Walkley-Black method (11) gave quite variable results as compared to other methods. The organic carbon values obtained by the Walkley-Black procedure were significantly larger than Allison values for two soils and significantly smaller than Allison values for two of the soils analyzed. The results indicate that the proposed procedure gives quantitative recovery of organic carbon in soils and that it provides a more accurate estimate of carbon content than other widely used titrimetric methods.

The proposed procedure is simple, rapid, precise, and appears applicable for routine determination of organic carbon in soils having diverse properties. The proposed procedure has an important advantage over currently used methods for carbon analysis by allowing simultaneous digestion of 60 samples with simple and inexpensive equipment. It is estimated that a technician will be able to analyze approximately 120 samples per 8-hour working day. Thus, the time and cost per sample analyzed by the proposed procedure is very low.

Literature Cited

1. ALLISON, L. E. 1960. Wet-combustion apparatus and procedure for organic and inorganic carbon in soil. Soil Sci. Soc. Amer. Proc. 24:36-40.

2. BLANCHER, R. W., REHM, and A. C. CALDWELL. 1965. Sulfur in plant materials by digestion with nitric and perchloric acid. Soil Sci. Soc. Amer. Proc. 29:71-72.

3. BREMNER, J. M. 1949. Use of the Van Slyke-Neil manometric apparatus for the determination of organic and inorganic carbon in soil and of organic carbon in soil extracts. Analyst 74:492-498.

4. BREMNER, J. M. and D. J. JENKINSON. 1960. Determination of organic carbon in in soil I. Oxidation by dichromate of organic matter in soil and plant material. J. Soil Sci. 11:394-402.

5. MEBIUS, L. J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120-124.

6. NELSON, D. W. and L. E. Sommers. 1972. A simple digestion procedure for estimation of total nitrogen in soils and sediments. J. Environ. Quality 1:423-425.

7. SCHOLLENBERGER, C. J. 1927. A rapid approximate method for determining soil organic matter. Soil Sci. 24:65-68.

8. SMITH, H. W. and M. D. WELDON. 1941. A comparison of some methods for determination of soil organic matter. Soil Sci. Soc. Am. Proc. 5:177-182.

9. TABATABAI, M. A. and J. M. BREMNER. 1970. Use of the Leco automatic 70-second carbon analyzer for total carbon analysis of soils. Soil Sci. Soc. Amer. Proc. 34:608-610.

10. VAN SLYKE, D. D. and J. FOLCH. 1940. Manometric carbon determination. J. Biol. Chem. 136:509-541.

11. WALKLEY, A. and I. A. BLACK. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.