provide comparable data to established methods and use relatively inexpensive equipment.

## ORGANIC CARBON

### Introduction

Carbon is the chief element present in soil organic matter, comprising from 48 to 58% of the total weight. Therefore, organic C determinations are often used as the basis for organic matter estimates through multiplying the organic C value by a factor. For many years the Van Bemmelen factor of 1.724 was used based on the assumption that organic matter contains 58% organic C. However, a number of studies have shown that the proportion of C in soil organic matter is highly variable for a range of soils and there is no factor appropriate for all soils. If a factor must be selected for converting organic C concentrations of organic matter contents, values of 1.9 and 2.5 for surface and subsoils, respectively, are most appropriate (Broadbent, 1953). The factor varies not only from soil to soil but also between horizons in the same soil. This finding suggests that it is most appropriate to determine and report the organic C in a soil rather than convert the analytically determined organic C value to organic matter content through use of an approximate correction factor.

Organic C may be determined by: (i) analysis of a soil for total C and inorganic C and subtraction of the inorganic C concentration for the total C content, (ii) a total C determination on the sample after destruction of inorganic C, and (iii) oxidation of organic C compounds by $Cr_2O_7^{2-}$ and subsequent determination of unreduced $Cr_2O_7^{2-}$ by oxidation-reduction titration with $Fe^{2+}$ or by colorimetric methods. Table 34–2 summarizes the principles, advantages, and disadvantages of several methods for determination of organic C in soils. All current methods have inherent problems associated with them, and the investigator should use the method most applicable for the soils to be analyzed and the required accuracy of the results.

In this section, procedures are described for the determination of organic C in both calcareous and noncalcareous soils based on the difference between total C and inorganic C concentrations. Two methods also are given for organic C estimations based on destruction of inorganic C compounds prior to total C determinations. In addition, two rapid dichromate oxidation procedures are described. The Walkley and Black (1934) method that oxidizes organic C through heat-of-dilution of $H_2SO_4$ is given because it is simple, rapid, widely used, and requires minimal equipment even though the results obtained cannot be considered quantitative. Many soil testing and soil survey personnel have need for a method that gives an approximate organic C concentration. A tube digestion techniques (Nelson & Sommers, 1975) that involves extensive heating of the chromic acid-soil mixture is given because it is quantitative, rapid, and represents the best combination of digestion reagents, heating procedure, and titration reagents of the modern dichromate methods. Dichromate procedures are widely used in soil investigations because of their simplicity and rapidity compared with wet or dry com-

Table 34–2. Comparison of methodologies used for determination of organic C in soils.

| Method | Principle | Advantages | Disadvantages |
|---|---|---|---|
| Difference between total C and inorganic C | Total C and inorganic C are determined on separate samples: organic C = total C – inorganic C | Useful if total C and inorganic C are routinely determined | Two separate analyses are required, total C determination requires special equipment, organic C calculated by difference has some inherent error |
| Determined as total C after removal of inorganic C | Total C is determined in soil sample after removal of inorganic C with an acid pretreatment: organic C = total C | Accurate if dolomite is absent from soil | Not all dolomite in soil may be removed by acid treatment, specialized equipment needed. |
| Dichromate oxidation without external heat | Dichromate oxidizes organic C to $CO_2$ in acid medium; amounts of $Cr_2O_7^{2-}$ reduced is quantitatively related to organic C present; not all organic C in samples is oxidized when external heat is omitted, and a correction factor is required | Very rapid and simple, no special equipment needed | Incomplete oxidation of organic C necessitates use of a correction factors, which often results in erroneous values; chloride, $Fe^{2+}$, and $MnO_2$ interfere with method; it assumes soil organic C has an average valence of 0; variable recovery of C from carbonized materials |
| Dichromate oxidation with external heat | This is the same as the dichromate method above except that all organic C in the sample is oxidized, and no correction factor is required | Rapid and simple, complete oxidation of organic C occurs | Chloride, $Fe^{2+}$, and $MnO_2$ interfere with method; some specialized equipment is needed; it assumes soil organic C has an average valence of 0; variable recovery of C in carbonized materials |

bustion. However, the rapid $K_2Cr_2O_7$ methods are subject to interference by oxidizable or reducible soil constituents such as $Cl^-$, $Fe^{2+}$, and $MnO_2$.

## Organic Carbon as Calculated from Total Carbon Determinations

Methods previously described for total C are basic for many of the procedures used to determine organic C in soils. However, soils may contain both organic and inorganic C and, thus, total C analysis procedures recover both forms of C. In noncalcareous soils and soils not recently limed, the total C can be considered to be organic C. With calcareous or recently limed soils, organic C may be estimated as the difference between total C and inorganic C concentrations.

### Organic Carbon in Noncalcareous Soils

Prepare soil samples, and conduct a total C determination by dry or wet combustion using titrimetric, gravimetric, volumetric, infrared, or thermal conductivity techniques to quantitate evolved $CO_2$ as described in "Total Carbon." Report the total C determined as percentage organic C in the sample (i.e., total C = organic C).

### Organic Carbon in Calcareous Soils

Prepare soil samples, and conduct a total C determination on the sample by dry or wet combustion techniques as described in "Total Carbon." Determine inorganic C on a separate sample by one of the quantitative methods described in Chapter 15 (Loeppert & Suarez, 1996). Calculate the percentage organic C in the sample from the relationship

$$\text{organic C, \%} = \text{\% total C} - \text{\% inorganic C} \qquad [7]$$

## Wet and Dry Combustion Techniques for Direct Measurement of Organic Carbon in Calcareous Soils

In contrast to noncalcareous soils, inorganic C must be removed from calcareous or recently limed soils before the analysis if wet or dry combustion techniques are used to directly measure the organic C present.

Inorganic C is conveniently removed before wet combustion by pretreating the sample contained in a digestion flask with a mixture of dilute $H_2SO_4$ and ferrous sulfate ($FeSO_4$). The $FeSO_4$ is added to the mixture to minimize oxidation and decarboxylation of organic matter by added $H_2SO_4$ or by $MnO_2$ present in soil (Allison, 1960). After pretreatment, the digestion flask containing soil is transferred to the combustion train, and a total C determination is carried out as described in "Total Carbon by Wet Combustion."

Inorganic C removal is generally more difficult before determination of organic C by dry combustion techniques. Treatment of soil at room temperature with sulfurous acid ($H_2SO_3$) followed by heating to remove excess $H_2SO_3$ is normally used to decompose inorganic C compounds (Piper, 1942, p. 221–222; Bremner, 1949); however, several difficulties are apparent with the procedure.

Little destruction of organic matter occurs during room temperature treatment of samples with $H_2SO_3$, but some decarboxylation is possible as the sample is heated (Bremner, 1949). It is difficult to decide when all inorganic C has been removed and when $H_2SO_3$ treatment should be discontinued. It is doubtful that dolomite is completely decomposed by the relatively mild $H_2SO_3$ treatment employed (Allison, 1965). Nommik (1971) suggested that inorganic C may be effectively removed from soil samples by treatment with a metaphosphoric acid solution for 30 min at room temperature and 30 min at 130°C. However, Nommik's procedure has not been evaluated with a variety of soils.

### Test for Presence of Inorganic Carbon

Place finely ground soil on a spot plate, and moisten with a few drops of water. Add 4 *M* HCl dropwise to the wetted sample, and observe any effervescence. Allow sufficient time for dolomite to react (~5 min). If inorganic C is absent from the soil, proceed with organic C (total C) analysis as per the section on "Total Carbon." If inorganic C is present or the test is not definitive, proceed as described below.

### Pretreatment Prior to Wet Combustion

#### *Special Apparatus*[6]

#### *Reagents*

1. Digestion reagent for carbonates ($H_2SO_4$-$FeSO_4$): Dissolve 57 mL of concentrated $H_2SO_4$ and 92 g of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in 600 mL of deionized water, cool, and dilute to 1 L.
2. Potassium dichromate, reagent grade, pulverized.
3. Other reagents as described in "Reagents" under "Wet Combustion Method."

#### *Procedure*

Prepare soil samples as described in "Procedures" under "Wet Combustion Method." Transfer a sample of known water content and containing 20 to 40 mg of C (but not more than 2 g of soil) to the flask used for the wet combustion apparatus (e.g., a 100-mL Kjeldahl digestion flask or standard taper round bottom). Using 3 mL of the $H_2SO_4$-$FeSO_4$ digestion acid, wash down any soil that adheres to the neck of the flask. Place the flask in a rack or beaker, and allow the sample to digest at room temperature with occasional turning of the flask for at least 20 min or until effervescence appears to cease. Then hold the flask upright over a flame 2 cm high, and boil the contents slowly for 1.5 min to destroy any remaining carbonate. Rotate the flask continuously during boiling to avoid excessive frothing. Allow the sample to cool.

Insert a long-stemmed funnel into the flask, and add 2 g of pulverized $K_2Cr_2O_7$. Immediately connect the flask to the reflux condenser (Fig. 34–4), and

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[6] See the special apparatus listed in "Special Apparatus" under "Wet Combustion Methods."

proceed with the determination of organic C as directed in "Procedure" under "Wet Combustion Method" beginning with the third sentence.

Report the C present in the pretreated sample as percentage organic C.

*Comments*

The 3 mL of 1 *M* (2N) $H_2SO_4$-5% $FeSO_4$ used in this procedure replaces the 3 mL of distilled water used in the total C procedure described in "Procedure" under "Wet Combustion Method." Three mL of this reagent adds 3 millimoles (6 meq) $H^+$, which will neutralize 0.3 g of $CaCO_3$ (i.e., 15% $CaCO_3$ in a 2-g soil sample). An appreciable excess of acidity must be present to ensure complete decomposition of carbonates. Rather than using >3 mL of the 1 *M* reagent for soils containing more than ~10% $CaCO_3$ equivalent, it is preferable to use 3 mL of a 1.5 *M* or even a 2 *M* $H_2SO_4$-5% $FeSO_4$ reagent.

### Pretreatment Prior to Dry Combustion

*Special Apparatus*[7]

*Reagents*

1. Sulfurous acid, approximately 5%: Bubble $SO_2$ through distilled water until a saturated solution is obtained. Keep the bottle well stoppered to prevent rapid loss of $SO_2$.
2. Sodium hydroxide (NaOH), pellets.

*Procedure*

Transfer a soil sample that passes through a 100- or 140-mesh (106–150 µm) sieve (see "Comments" under "Medium-Temperature Resistance Furnace Method") and of known water content to a nonporous combustion boat that has been previously ignited and cooled. Based on an estimate of inorganic C present, treat the sample with an excess of a 5% $H_2SO_3$ solution. After several hours, remove the water and excess $H_2SO_3$ by leaving the boat overnight in an evacuated desiccator containing NaOH pellets. Repeat the treatment until evolution ceases on addition of $H_2SO_3$.

Proceed with the determination of organic C by one of the dry combustion methods (see "Medium-Temperature Resistance Furnace Method" or "High-Temperature Induction Furnace Method"). Report the C present in the pretreated samples as percentage organic C.

## Organic Carbon in Soil Extracts

### Special Apparatus

See the special apparatus listed in "Special Apparatus" under "Wet Combustion Method."

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[7] See the special apparatus listed in "Special Apparatus" under "Medium-Temperature Resistance Furnace Method" and "Special Apparatus" under "High-Temperature Induction Furnace Method."

### Reagents

See the reagents listed in "Reagents" under "Wet Combustion Method."

### Procedure

Place an aliquot of the extract (10 to 50 mL, depending on the organic C content) in a 100-mL Klejdahl digestion flask, and add 1 mL of the $H_2SO_4$-$FeSO_4$ reagent. Immerse the bulb of the flask in boiling water, and direct a stream of dry, dust-free air onto the surface of the liquid in the flask. Reduce the volume of solution in the flask to 3 mL or less. Add five or six glass beads and 1 g of $K_2Cr_2O_7$ to the flask, and proceed with the determination of organic C as directed in "Procedure" under "Wet Combustion Method."

### Comments

Drying of extracts is best accomplished in 100-mL flasks of the Kjeldahl type. A 2-L beaker conveniently holds four flasks.

## Rapid Dichromate Oxidation Techniques

### Introduction and Principles

Schollenberger (1927) first proposed that the organic matter in soil may be oxidized by treatment with a hot mixture of $K_2Cr_2O_7$ and $H_2SO_4$ according to Eq. [8].

$$2\ Cr_2O_7^{2-} + 3\ C^0 + 16\ H^+ = 4\ Cr^{3+} + 3\ CO_2 + 8\ H_2O \qquad [8]$$

After the reaction, the excess $Cr_2O_7^{2-}$ is titrated with $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and the $Cr_2O_7^{2-}$ reduced during the reaction with soil is assumed to be equivalent to the organic C present in the sample. It must be emphasized that all methods based on determination of $Cr_2O_7^{2-}$ remaining or $Cr^{3+}$ formed assume that C in soil organic matter has an average valence of zero. Although most dichromate oxidation procedures described since the original Schollenberger method have involved chromic acid solutions or mixtures of concentrated $H_2SO_4$ and aqueous $K_2Cr_2O_7$ solutions (Table 34–3), the use of other oxidants has been proposed. Degtjareff (1930) suggested that a mixture of $H_2O_2$ and chromic acid be used to oxidize organic matter. However, Walkley and Black (1934) conclusively established that the addition of $H_2O_2$ to chromic acid procedures gave fictitiously high values for organic C because $H_2O_2$ reduced $Cr_2O_7^{2-}$ in acid solution. Edson and Mills (1955) suggested that organic C be oxidized by $Cl_2$ (1% solution) and residual $Cl_2$ determined colorimetrically by reaction with *o*-tolidine ($C_{14}H_{16}N_2$). The intensity of yellow color was proportional to organic C oxidized. Others have suggested that organic C in aqueous extracts of soil can be determined by oxidation with a Mn(III)-pyrophosphate complex (Bartlett & Ross, 1988). The loss of color from Mn(III) is proportional to the amount of organic C oxidized. Tinsley (1950) and Kalembasa and Jenkinson (1973) proposed that the chromic acid mixture used to oxidize organic C compounds be 3 and 1.5 *M* (9 and 4.5 *N*), respec-

Table 34–3. Characteristics of dichromate methods for determining organic C in soils.

| | Digestion reagent concentrations | | | | | |
|---|---|---|---|---|---|---|
| Method | $K_2Cr_2O_7$ | $H_2SO_4$ | $H_3PO_4$ | Ratio of $H_2O$/acid | Digestion conditions | Reported precision |
| | ——— | N | ——— | v:v | | CV, % |
| Schollenberger (1927) | 0.058 | 18 | -- | ∞ | Tube heated by flame at 175°C for 90 s | 1.4–1.9 |
| Tyurin (1931) | 0.066 | 9 | -- | 1.00 | Flask with funnel boiled at 140°C for 5 min | 8.5 |
| Walkley-Black (1934) | 0.055 | 12 | -- | 0.50 | Flask with no external heat, max. temp is 120°C | 1.6–4.2 |
| Anne (1945) | 0.027 | 11 | -- | 0.46 | Flask with condensor refluxed at 178°C for 5 min | 1.3 |
| Tinsley (1950)† | 0.027 | 7.2 | 3 | 0.67 | Flask with condenser refluxed for 2 h at 150°C | 0.8–3.1 |
| Mebius (1960) | 0.045 | 10 | -- | 0.42 | Flask with condensor refluxed for 30 min at 159°C | 1.2–1.8 |
| Kalembasa & Jenkinson (1973) | 0.033 | 9 | 1.67 | 0.067 | Flask with condenser refluxed for 20 min at 165°C | 0.8 |
| Nelson & Sommers (1975) | 0.066 | 10.8 | -- | 0.67 | Tube heated in block at 150°C for 30 min | 3.5 |
| Modified Mebius‡ | 0.033 | 10.8 | -- | 0.67 | Flask with condenser refluxed at 150°C for 30 min | 1.0–3.6 |
| Heanes (1984) | 0.055 | 12 | -- | 0.50 | Tube heated in block at 135°C for 30 min | 4.1 |
| Yeomans & Bremner (1988) | 0.066 | 10.8 | -- | 0.67 | Tube heated in block at 170°C for 30 min | 1.0–4.4 |
| Ciavatta et al. (19898) | 0.145 | 10.2 | -- | 0.77 | Special flask heated over flame at 1160°C for 10 min | 5.4 |
| Soon & Abboud (1991) | 0.066 | 10.8 | -- | 0.67 | Tube heated in block at 155°C for 30 min | 2.7 |

† Reagents used by Bremner and Jenkinson (1960a).

‡ As described by Nelson and Sommers (1982).

tively, with respect to $H_3PO_4$ (Table 34–3). There is no evidence, however, to suggest that oxidation mixtures containing $H_3PO_4$ are more efficient in oxidizing organic matter than $K_2Cr_2O_7$-$H_2SO_4$ mixtures. The oxidizing mixtures used in most published methods are between 0.0267 and 0.0583 *M* (0.16 and 0.35 *N*) in $K_2Cr_2O_7$ and 7.5 and 12.5 *M* (15 and 25 *N*) in $H_2SO_4$ (Table 34–3). However, Tyurin (1931) Tinsley (1950), Nelson and Sommers (1975) and Yeomans and Bremner (1988) used an aqueous $H_2SO_4$-water mixture that was 0.145 *M* (0.87 *N*) in $K_2Cr_2O_7$. Schollenberger (1927) used concentrated $H_2SO_4$ (~18 *M* or ~36 *N*) as the solvent for $K_2Cr_2O_7$.

Rapid dichromate oxidation techniques have employed heating times and temperatures that vary from no external heat to extensive boiling of chromic acid mixtures. Schollenberger (1927) suggested that the soil-$H_2SO_4$-$K_2Cr_2O_7$ mixture be heated in a Pyrex test tube over a flame until the solution temperature reached 175°C at which time heating was discontinued. Later investigators realized that the time and temperature of heating were critical and must be standardized to insure that a constant proportion of soil organic matter was oxidized and that a consistent amount of dichromate was thermally decomposed during the digestion. Degtjareff (1930), Tyurin (1931), Schollenberger (1945) and Jackson (1958) suggested that the soil-chromic acid mixtures be heated for defined periods (5–10 min) in test tubes submerged in $H_2SO_4$ or oil baths maintained at prescribed temperatures (140–170°C).

Walkley and Black (1934), however, proposed that the heat of dilution of $H_2SO_4$ (120°C) was satisfactory for oxidizing 75% of the organic C in soils and that a correction factor could be used to account for incomplete digestion. Several investigators have found that an extended period of heating is required to obtain quantitative oxidation of soil organic C by chromic acid (Anne, 1945; Tinsley, 1950; Mebius, 1960; Kalembasa & Jenkinson, 1973; Heanes, 1984). High digestion temperatures (>145°C) lead to thermal decomposition of dichromate and resultant high blank values (Tinsley, 1950; Metson et al., 1979; Heanes, 1984). Clay minerals have been reported to catalyze the thermal decomposition of $Cr_2O_7^{2-}$ (Walkley, 1947) but a recent study has shown little thermal decomposition when high clay soils free of organic matter were heated with $K_2Cr_2O_7$-$H_2SO_4$ for 60 min at 125 or 145°C (Heanes, 1984). Digestion temperature is normally regulated by the ratio of water/$H_2SO_4$ in the mixture (Table 34–3) and the temperature rises as water vapor is lost during heating. Tinsley proposed that cold finger condensers fitted on Erlenmeyer flasks be used to prevent loss of water during digestion, whereas other investigators have used Erlenmeyer flasks fitted with Liebig condensers. Heating times employed in reflux methods have varied from 20 min to 2 h. Ciavatta et al. (1989) have recently proposed that a chromic acid mixture successfully oxidizes soil organic C when samples are heated in a 200 mL narrow-necked digestion flask by direct flame at 160°C for 10 min. The neck of the flask serves to reflux the digestion reagents.

Nelson and Sommers (1975) proposed that organic C could be determined by heating soil-chromic acid mixtures under reflux in 50 mL Folin-Wu nonprotein nitrogen tubes placed in an aluminum block on a hot plate. Heating time and digestion temperature recommended were 30 min and 150°C, respectively. Subsequently, a number of other similar tube digestion methods have been proposed for estimation of organic C all employing 100-mL tubes. Heanes (1984), Yeomans and Bremner (1988), and Soon and Abboud (1991) recommended heating for 30 min at 135°C, 170°C, and 155°C, respectively. Yeomans and Bremner (1988) and Soon and Abboud (1991) specify the same digestion reagents as Nelson and Sommers (1975), i.e., 5 mL of 0.167 *M* $K_2Cr_2O_7$ and 7.5 mL of concentrated $H_2SO_4$, whereas Heanes (1984) recommends 10 mL of 0.167 *M* $K_2Cr_2O_7$ and 20 mL of $H_2SO_4$. Tube digestion procedures have been reported to yield organic C values that approximate those from dry and wet combustion techniques.

Diphenylamine was the first oxidation-reduction indicator used for the titration of excess $Cr_2O_7^{2-}$ with $Fe^{2+}$ (Schollenberger, 1927, 1931, 1945; Allison, 1935). Later studies suggested that the diphenylamine end point could be improved by addition of $H_3PO_4$, NaF, or HF before titration (Schollenberger, 1931, 1945; Walkley & Black, 1934), and these substances were widely used in dichromate titrations. Peech et al. (1947) established that barium diphenylamine sulfonate (diphenyl-4-sulfonic acid) in combination with $H_3PO_4$ was as effective and more stable compared with diphenylamine ($C_{12}H_{11}N$) and has been used as an indicator in other procedures (Tinsley, 1950). Jackson (1958) recommended that *o*-phenanthroline ($C_{12}H_8N_2$) be used as an indicator in $Cr_2O_7^{2-}$ titrations because the color change (formation of the complex with $Fe^{2+}$) occurs at higher oxidation-reduction potential compared with diphenylamine. A mixture of *o*-phenanthroline and $H_3PO_4$ is normally used to give a good end point; however, the indicator has been successfully used without $H_3PO_4$ addition. A problem with *o*-phenanthroline is that the indicator tends to be absorbed by some suspended soil materials, thereby obscuring the color change at the end point. therefore, the diluted chromic acid-soil mixture is often passed through an acid fast filter paper on Büchner funnel before titration. Simakov (1957) proposed that *N*-phenylanthranilic acid ($C_{13}H_{11}HO$) be used as an indicator in $Cr_2O_7^{2-}$ titrations with $Fe^{2+}$. Mebius (1960) confirmed that *N*-phenylanthranilic acid gives a very sharp and clean end point and this compound is currently the indicator of choice for $Cr_2O_7^{2-}$ titrations.

Other methods of titration not involving oxidation-reduction indicators have been used to estimate reacted $Cr_2O_7^{2-}$. One approach is to add a slight excess of $Fe^{2+}$ to the $Cr_2O_7^{2-}$-$H_2SO_4$-soil mixture and then back-titrate the $Fe^{2+}$ with $KMnO_4$ (Smith & Weldon, 1941). In this titration procedure, the only reagent that requires standardization is $KMnO_4$ if the same amounts of $Fe^{2+}$ and $Cr_2O_7^{2-}$ are added to both samples and blanks. The end point in the titration of $Cr_2O_7^{2-}$ with $Fe^{2+}$ also may be estimated very accurately by monitoring the oxidation-reduction potential with platinum and calomel electrodes attached to a potentiometer (Raveh & Avnimelech, 1973). The end point of the titration involves a potential change of ~400 mV with 0.02 mL of titrant.

The amount of $Cr_2O_7^{2-}$ remaining after reaction with soil organic matter also may be estimated by colorimetry after removal of soil by filtration or centrifugation (Carolan, 1948). Perrier and Kellogg (1960) proposed that any possible interference of $Cr^{3+}$ in $Cr_2O_7^{2-}$ determination be eliminated by dilution and subsequent reaction of excess dichromate with s-diphenylcarbazide ($C_{13}H_{14}N_4O$) to yield a violet colored complex with an absorption maxima at 540 nm. Conversely, colorimetry has been widely used to determine the amounts of $Cr^{3+}$ formed from the reaction of $Cr_2O_7^{2-}$ with soil (Wilde, 1942; Graham, 1948; Carolan, 1948; Datta et al., 19862; Sinha & Prasad, 1970; Sims & Haby, 1971; DeBolt, 1974; Gupta et al., 1975; Baker, 1976; Heanes, 1984). The green color due to $Cr^{3+}$ is normally quantitated at wavelengths of 590 to 625 nm and the absorbance is usually related to organic matter concentrations in soil by a standard curve prepared from sucrose (Graham, 1948; Sims & Haby, 1971; DeBolt, 1974; Heanes, 1984). Baker (1976) used a probe colorimeter to measure $Cr^{3+}$ absorbance directly in the reaction vessel after centrifugation, thereby avoiding a transfer into a spectrophotometer cuvette. From a comparison of the methods that quantitate $Cr_2O_7^{2-}$ and those that determine $Cr^{3+}$, Metson (1965) concluded that measurement of $Cr^{3+}$ is the preferred procedure.

Dichromate methods that use heat of dilution or minimal heating do not give complete oxidation of organic compounds in soil although the most active forms of organic C are converted to $CO_2$. Walkley and Black (1934) found that on the average about 76% of the organic C in 20 soils was recovered by the heat of dilution procedure, and they proposed that a correction factor of 1.32 be used to account for unrecovered organic C. However, the actual recoveries of organic C from the soils tested varied from 60 to 86%. Schollenberger (1945) reported that the Walkley and Black procedure oxidized an average of 79% (range 70–86%) of organic C in soils he studied. Allison (1960) reviewed available information on the recovery of organic C in a wide variety of soils by the Walkley and Black procedure and showed that the average recovery with different groups of soils varied from 63 to 86% and that the correction factor varied from 1.16 to 1.59. Table 34–4 gives data on the correction factor found to be required for the Walkley and Black procedure in investigations carried out during the past 30 yr. Recoveries of organic C by the Walkley and Black technique were highly variable, and the correction factor appropriate for individual soils varied from 1.0 to 2.86. The average correction factor appropriate for a group of soils varied from 1.03 to 1.41. This data clearly show that $Cr_2O_7^{2-}$-$H_2SO_4$ methods that involve minimal heating give variable recovery of organic C from soils. An average correction factor found for a group of soils may be applicable to the "average" soil in the group but will give erroneous values for many soils in the group. Therefore, procedures such as the Walkley and Black should be considered to give approximate or semi-quantitative estimates of organic C in soil because of the lack of an appropriate correction factor for each soil analyzed. If an experimentally determined correction factor is not available for a particular groups of soils, the use of 1.3 as the factor appears most reasonable over a range of soils. Methods that involve extensive heating, such as those of Tinsley (1950), Mebius (1960), Nelson and Sommers (1975), Heanes (1964) or Yeomans and Bremner (1988) do not require a correction factor because all of the organic C in the soil is oxidized to $CO_2$. However, methods that involve minimal heating (e.g., Schollenberger, 1927; Tyurin, 1931) require a small correction factor (e.g., 1.15) to account for unoxidized organic C.

The rapid dichromate methods are subject to interferences by certain soil constituents that lead to spurious results with some soils (Walkley, 1947). Chloride, ferrous iron and higher oxides of Mn have been shown to undergo oxida-

Table 34–4. Correction factors for organic C in surface soils not recovered by the Walkley-Black method.

| Reference | Origin of samples | Number of soils studies | Organic C recovery, % |  | Average correction factor |
|---|---|---|---|---|---|
|  |  |  | Range | Average |  |
| Tinsley (1950) | England | 10 | 77–92 | 83.6 | 1.20 |
| Bremner & Jenkinson (1960a) | England | 15 | 27–92 | 84 | 1.19 |
| Kalembesa & Kenkinson (1973) | England & Wales | 22 | 46–80 | 77 | 1.30 |
| Orphanos (1973) | Cyprus | 12 | 69–79 | 75 | 1.30 |
| Richter et al. (1973) | Argentina | 12 | 79–87 | 83 | 1.20 |
| Nelson & Sommers (1975) | Indiana | 10 | 44–88 | 79 | 1.27 |
| Bornemisza et al. (1979) | Costa Rica | 50 | 69–81 | 75 | 1.33 |
| Rhodes et al. (1981) | Sierra Leone | 10 | 93–100 | 97 | 1.03 |
| Richardson & Bigler (1982) | North Dakota | 21 | 35–91 | 88 | 1.33†<br>1.13‡ |
| Heanes (1984) | Australia | 12 | 85–98 | 92 | 1.09 |
| Amacher et al. (1986) | Louisiana | 179 | 46–87 | 71 | 1.41 |
| Gillman et al. (1986) | Queensland | 450 | 65–95 | 76§<br>81¶ | 1.32<br>1.24 |
| Willet & Beech (1987) | Australia | 30 | 60–144 | 85 | 1.18 |
| Lowther et al. (1990) | Australia | 38 | 74–102 | 88 | 1.14 |
| Soon & Abboud (1991) | Alberta | 39 | 62–87 | 71.4 | 1.40 |

† Low C samples.
‡ Other samples.
§ Soils derived from basalt, alluvium, or beach sand.
¶ Soils derived from granite or metamorphic rocks.

tion-reduction reactions in chromic acid mixtures leading to incorrect values for organic C. The presence of significant amounts of $Fe^{2+}$ or $Cl^-$ in soil will lead to a positive error, whereas reactive $MnO_2$ in soil samples will result in a negative error and low values for organic C.

Chloride interferes with dichromate methods through the formation of chromyl chloride, as indicated in Eq. [3], which results in consumption of $Cr_2O_7^{2-}$. Chloride interference may be eliminated by washing the soil free of $Cl^-$ before analysis or by precipitating the $Cl^-$ as AgCl by addition of $Ag_2SO_4$ to the digestion acid (Walkley, 1947; Quinn & Salomon, 1964; Gupta et al., 1975). Alternatively, Walkley (1947) found that Eq. [9] may be used to correct organic C values for soils having $Cl^-/C$ ratio of $\leq 5.1$

$$\text{organic C in soil (\%)} = (\text{apparent \% C in soil}) - (\%\ Cl^-/12) \qquad [9]$$

It has recently been reported that $Ag_2SO_4$ addition did not eliminate $Cl^-$ interference in a low temperature tube digestion method and that an assay for $Cl^-$ coupled with stoichiometric correction for chromyl chloride loss is necessary for accurate estimates of organic C (Heanes, 1984).

When present in soil, $Fe^{2+}$ will be oxidized to $Fe^{3+}$ by $Cr_2O_7^{2-}$, as indicated in Eq. [10], resulting in a positive error in the analysis, i.e., giving high values for organic C content

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ = 2Cr^{3+} + 6Fe^{3+} + 7H_2O \qquad [10]$$

Appreciable $Fe^{2+}$ may be present in highly reduced soils, and errors may result when dichromate methods are applied to undried samples of anaerobic soils before drying (Lee, 1939). However, Walkley (1947) found that thorough air-drying of reduced soils before analysis resulted in oxidation of $Fe^{2+}$ to $Fe^{3+}$ and accurate determination of the organic C present. The amounts of $Fe^{2+}$ present in well-aerated soils are so small relative to the amounts of organic C present that no detectable interference is likely. Pyrite also is oxidized during treatment of soils with dichromate and samples containing pyrite sulfur concentrations >0.29% result in significant over estimation of organic C by the Walkley and Black method (Willett & Beech, 1987). Metallic iron ($Fe^{\circ}$) present in soil samples also may lead to positive interferences in dichromate methods, (Allison, 1935; Heanes, 1984). Therefore, care should be taken to ensure that soils are not ground with iron or steel equipment before analysis. The higher oxides of Mn (largely $MnO_2$) compete with $Cr_2O_7^{2-}$ for oxidizable substances when heated in an acid medium according to Eq. [11].

$$2MnO_2 + C^{\circ} + 2Mn^{2+} + 4H_2O \qquad [11]$$

Therefore, any reactive $MnO_2$ present will give a negative error when soils are analyzed by dichromate techniques. Although soils contain substantial amounts of $MnO_2$ and other higher oxides of Mn, Walkley (1947) and Heanes (1984) concluded that in most soils the quantity of reactive (reducible) oxides of Mn is small because only the freshly precipitated $MnO_2$ will take part in redox reactions. Even in highly manganiferous soils, only a small fraction of the $MnO_2$ present is able to compete with $Cr_2O_7^{2-}$ for oxidation of organic C compounds. Therefore, interference from $MnO_2$ is not thought to be a serious error in the vast majority of soils. In soils with large amounts of reactive $MnO_2$, Walkley (1947) suggested pretreatment of samples with the exact amount of $FeSO_4$ necessary to reduce the amount of reactive $Mn_2$ present prior to treatment with $K_2Cr_2O_7$ and $H_2SO_4$.

Other problems associated with dichromate methods involve assumptions about the average oxidation state of organic C in soils (i.e., equivalent weight of C) and recovery of highly reduced forms of organic C from soils. All dichromate methods assume that the organic C in soil has an average oxidation state of zero and an equivalent weight of 3 g per equivalent when reacted with dichromate according to Eq. [8] even though no studies have been conducted to evaluate this assumption. However, the fact that dichromate methods using extensive heating give organic C values similar to those obtained with wet or dry combustion where $CO_2$ is determined directly suggests that this assumption is reasonably correct.

Dichromate methods that involve little or no external heating give very poor recovery of organic C present in carbonized materials (e.g., charcoal, graphite, coal, coke, and soot). For example, Walkley (1947) found that the Walkley and Black method recovered only 2 to 11% of the organic C present in such materials. In a detailed study, Bremner and Jenkinson (1960b) found that the Walkley and Black method gave low recovery (0–57%) of organic C from carbonized materials, whereas methods involving external heat such as those of Tinsley gave substantial (64–104%) and variable recovery or organic C from such materials. Conversely, Heanes (1984) found that very little organic C in charcoal or coke was oxidized by a tube digestion procedure at 135°C. Other investigators have found that the Walkley and Black procedure completely recovers C in weathered coal seams, i.e., coal "blooms" (Kalisz & Sainju, 1991). These conflicting results suggest that recovery of organic C from carbonized materials is highly dependent upon the characteristics of the materials and digestion conditions (i.e., temperature, reagent concentrations). It is appropriate to conclude that dichromate methods cannot be used to quantitatively recover carbonized materials from soils or to discriminate between C in carbonized materials and C in soil organic matter because organic C recovery varies with type of carbonized material and time and temperature of heating of the chromic acid mixture. Therefore, unreliable results for organic C will be obtained if dichromate methods are applied to soils containing significant amounts of carbonized materials. Dry combustion methods are most appropriate for soils containing large amounts of elemental C.

### Walkley-Black Method

The Walkley-Black Method was described by Walkley (1946), Peech et al. (1947) and Greweling and Peech (1960).

#### *Reagents*

1. Potassium dichromate, 0.167 *M* (1 N): Dissolve 49.04 g of reagent-grade $K_2Cr_2O_7$ (dried at 105°C) in water, and dilute the resclution to a volume of 1000 mL.
2. Sulfuric acid, concentrated (not less than 96%): If $Cl^-$ is present in soil, add $Ag_2SO_4$ to the acid at the rate of 15 g per liter.
3. Phosphoric acid, concentrated.
4. *o*-Phenanthroline-ferrous complex, 0.025 *M*: Dissolve 14.85 g of *o*-phenanthroline monohydrate and 6.95 g of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in water. Dilute the solution to a volume of 1000 mL. The *o*-phenanthroline-ferrous complex is available under the name of Ferroin from the G. Frederick Smith Chemical Co. (Columbus, OH).
5. Barium diphenylamine sulfonate: Prepare a 0.16% aqueous solution. This reagent is an optional substitute for no. 4.
6. Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) solution, 0.5 *M* (0.5 N): Dissolve 140 g of reagent-grade $FeSO_4 \cdot 7H_2O$ in water, add 15 mL of concentrated sulfuric acid, cool the solution, and dilute it to a volume of 1000 mL> Standardize this reagent daily by titrating it against 10 mL of 0.167 *M* (1 N) potassium dichromate, as described below.

#### *Procedure*

Grind the soil to pass through a 0.5-mm sieve, avoiding iron or steel mortars. Transfer a weighed sample, containing 10 to 25 mg of organic C, but not in excess of 10 g of soil, into a 500-mL wide-mouth Erlenmeyer flask. Add 10 mL of 0.167 *M* (1 N) $K_2Cr_2O_7$, and swirl the flask gently to disperse the soil in the solution. Then rapidly add 20 mL to concentrated $H_2SO_4$, directing the stream into the suspension. Immediately swirl the flask gently until soil and reagents are mixed, then more vigorously for a total of 1 min. Allow the flask to stand on an insulated sheet for about 30 min. Then add 200 mL of water to the flask, and filter the suspension using an acid resistant filter paper (e.g., Whatman 540), if experience shows that the end point of the titration cannot otherwise be clearly discerned. Add three to four drops of *o*-phenanthroline indicator and titrate the solution with 0.5 *M* (0.5 N) $FeSO_4$. As the end point is approached, the solution takes on a greenish cast and then changes to a dark green. At this point, add the ferrous sulfate heptahydrate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background). Make a blank determination in the same manner, but without soil, to standardize the $K_2Cr_2O_7$. Repeat the determination with less soil if >75% of the dichromate is reduced.

Calculate the results according to the following formula, using a correction factor "*f*" = 1.30 or a more suitable value found experimentally

$$\text{Organic C, \%} = \frac{(\text{mL}_{\text{blank}} - \text{mL}_{\text{sample}})\,(M_{\text{Fe}^{2+}})\,(0.003)\,(100)}{\text{wt.} \bullet \text{water-free soil, g}} \times \text{f} \qquad [12]$$

### *Comments*

The coefficient of variation for the Walkley-Black procedure has been reported to vary between 1.6 and 4.2% (Table 34–3). Ferrous ammonium sulfate also is a suitable titrant for excess $Cr_2O_7^{2-}$ in conjunction with the Walkley-Black method. The Smith and Weldon (1941) modification involving complete reduction of $Cr_2O_7^{2-}$ with $Fe^{2+}$, and subsequent back-titration of excess $Fe^{2+}$ with $MnO_4^-$ solution also may be used to estimate unreacted $Cr_2O_7^{2-}$. Other oxidation-reduction indicators that have provided satisfactory results include barium diphenylamine sulfonate and N-phenylanthranilic acid. The amounts of $Cr_2O_7^{2-}$ reduced to $Cr^{3+}$ by reaction with soil organic matter also may be estimated colorimetrically or by potentiometric titration with a ferrous ammonium sulfate solution. Grinding samples to <0.2 mm has been shown to reduce sampling errors and the coefficient of variation even when relatively large sample sizes (1 g) are used (Metson et al., 1979). Heanes (1984) reported that reduction in particle size from 0.5 to 0.15 mm significantly increased recovery of organic C in 12 soils.

## Tube Digestion Method

### *Special Apparatus*

1. Pyrex digestion tubes (100 mL) sized for block digestor.
2. Block digestor: 40-tube Kjeldahl block digestor supplied by Technicon Instruments Corp., Tarrytown, NY, or Tecator Inc., Herndon, VA, or equivalent.

### *Reagents*

1. Potassium dichromate solution, 0.167 *M* (1.0 N)—dissolve 49.025 of $K_2Cr_2O_7$ (dried at 140°C) in 800 mL of distilled water and dilute the solution with water to a volume of 1000 mL in a volumetric flask. This is the primary standard for the procedure.

2. Concentrated sulfuric acid—specific gravity 1.84.
3. Ferrous ammonium sulfate solution 0.2 *M* (0.2 N)—Dissolve 156.8 g of ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] in 100 mL of concentrated sulfuric acid and dilute the solution with water to a volume of 2 L in a volumetric flask. This solution must be standardized daily because it undergoes slow oxidation.
4. Indicator solution—Dissolve 0.1 g of *N*-phenylanthranilic acid and 0.1 g of $Na_2CO_3$ in 100 mL of distilled water.

### *Procedure*

Weigh an amount of soil air dried and ground to <0.15 mm containing not greater than 8 mg of organic C (usually 100–500 mg) into a clean, dry digestion tube and add 5 mL of 0.167 *M* (1.0 *N*) $K_2Cr_2O_7$ solution and 7.5 mL of concentrated $H_2SO_4$. Place the tube in the digestion block preheated to 150°C for exactly 30 min. Remove the digestion tube from the block and allow the samples to cool for 30 min at room temperature. Quantitatively transfer the contents of the tube to a 125-mL Erlenmeyer flask and titrate the sample with 0.2 *M* (0.2 *N*) ferrous ammonium sulfate solution using 0.2 mL of the *N*-phenylanthranillic acid solution as the 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 25-mL burette calibrated at 0.1-mL intervals and a variable speed magnetic stirrer and teflon coated stirring bar.

Each set of soil samples should be analyzed with two unheated reagent blanks and two reagent blanks that are heated at the same time as the samples. The unheated blanks are used to standardize the ferrous ammonium sulfate solution. The difference in titration values between heated and unheated blanks is used to correct all sample titration values for the amount of dichromate consumed by thermal decomposition during the heating process.

Computation of the organic C content of soil is performed as follows: (i) subtract sample titration values ($mL_{soil}$) from the average titration value of the heated (boiled) blank ($mL_{bb}$), (ii) correct the resulting [$mL_{bb} - mL_{soil}$] value for thermal decomposition of dichromate by dividing the difference in average titration value for unheated and heated blanks by the average titration value for the unheated blank, multiplying the correction factor (normally 0.04–0.08) by the [$mL_{bb} - mL_{soil}$] value, and adding the product to the [$mL_{bb} - mL_{soil}$] value (Eq. [13]). The resulting value, labeled "A" is proportional to the amount of organic C present in the soil, (iii) complete the calculation of organic C content using Eq. [14]

$$A = (\mathrm{mL_{bb}} - \mathrm{mL_{soil}}) \frac{(\mathrm{mL_{ub}} - \mathrm{mL_{bb}})}{\mathrm{mL_{ub}}} + (\mathrm{mL_{bb}} - \mathrm{mL_{soil}}) \qquad [13]$$

where *ub* is unboiled blank and *bb* is boiled blank.

$$\text{Organic C, \%} = \frac{(A)\,(M_{\mathrm{Fe2+}})\,(0.003)\,(100)}{\text{wt. water-free soil, g}} \qquad [14]$$

### *Comments*

The coefficient of variation for the method has been reported as 3.5% (Nelson & Sommers, 1975). Coefficients of variation reported for other tube digestion methods have ranged from 1.1 to 4.4% (Heanes, 1984; Yeomans & Bremner, 1988; Soon & Abboud, 1991). The precision of the method can be improved by using a computer-aided automatic titration system (Yeomans & Bremner, 1988). Colorimetric analysis of $Cr^{3+}$ also can be used to estimate the amount of dichromate that has reacted with organic C during tube digestion (Heanes, 1984; Soon & Abboud, 1991). The potassium dichromate solution is the primary standard for the method and care should be taken in its preparation. This solution is quite stable and may be stored at room temperature indefinitely. The ferrous ammonium sulfate solution oxidizes slowly and thus must be standardized each time it is used. Small particle size reduces the sampling error and increases recovery of organic C. Heanes (1984) found that organic C values increased by about 2% as particle size was reduced from 0.5 to 0.15 mm.

Thermal decomposition of dichromate occurs at temperatures exceeding 136°C (Heanes, 1984) and the degree of decomposition is quite dependent upon the heating conditions. Therefore, it is recommended that the digestion tubes by dry before use to eliminate differences in acid/water ratio and that the heating temperature and time be accurately controlled. A variety of temperatures varying from 135 to 170°C have been recommended for tube digestion methods (Table 34–3). When thermal decomposition of dichromate is accurately taken into account with a heated blank, the four tube digestion methods have quantitatively determined organic C in a variety of soils.

Interferences present in the Walkley-Black procedure also are a problem with tube digestion methods. As a result of extensive heating, the tube digestion methods give complete recovery of organic C from soils and, thus, do not require a factor to account for incomplete oxidation of organic matter. Heanes (1984) reported that little organic C in charcoal and coke was recovered by the tube digestion procedure that he described.

The tube digestion technique can be used to estimate organic C in soil extracts by carrying out the digestion with 1 or 2 mL of extract and 4 or 3 mL of dichromate solution, respectively. It is essential that the acid/water ratio be maintained at 1.5 in the digest so the volume of dichromate solution must be reduced as the volume of extract is increased. Both heated and unheated blanks should be prepared using the same volume of blank extracting solution and the dichromate solution as that employed for the extracts.

The modified Mebius method described by Nelson and Sommers (1982) is recommended as an accurate and precise dichromate oxidation procedure for those investigators not having access to a block digestor. The major advantage of the tube digestion procedure is the decreased analysis time per sample because of the relatively large number of samples (40) that can be heated at one time.

## Comparison of Methods for Determining Organic Carbon

Most studies have shown that very good agreement is obtained when wet combustion, dry combustion, and Van Slyke-Folch (1940) methods are used to