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Abstract

The amount of soil organic carbon (OC) is a good indicator of a soil's productivity potential. Thus, OC analyses are routinely used to evaluate the soil's potential to supply N during a cropping season, or to assess the potential adsorption of a herbicide. Two methods are currently used to determine OC in soil. Both have some limitations for use in processing large numbers of samples for routine analyses. The dry combustion procedure requires expensive instrumentation, while the wet digestion procedure is rather lengthy and the disposal of Cr can be a problem. This study describes a new method for a relatively inexpensive, accurate, and rapid determination of OC in soil. Finely ground soil samples are equilibrated with an extracting solution of 0.25 M KOH and 0.05 M Na₂-EDTA (ethylenediaminetetraacetic acid) for 2 h at 85 °C. After cooling, absorbance of the extract was measured at 260 nm. An empirical equation is used to obtain an indirect estimate of soil OC. The empirical equation was developed from comparative analysis between the dry combustion and the proposed spectrophotometric method ($r^2 = 0.89$) by using a series of soils of different genesis and development. The method was further evaluated by analysis of samples from two soil profiles not used in the development of the equation, and the results were in close agreement with those obtained from dry combustion.

USE OF RAPID AND ACCURATE METHODS to estimate soil OC has spanned half a century (Salter, 1916; Walkley and Black, 1934; Carolan, 1948; Mebius, 1960; Nelson and Sommers, 1975). These methods employ either a dry combustion procedure where CO₂ is measured, or a wet digestion procedure that uses potassium dichromate (K₂Cr₂O₇) and concentrated H₂SO₄ where C is measured by titration, CO₂ adsorption or colorimetry. Advantages and disadvantages of these two approaches have been extensively discussed (Kalembasa and Jenkinson, 1973; Nelson and Sommers, 1982).

Both methods have certain serious limitations. The combustion procedure provides the best accuracy, but many laboratories do not have access to the instrumentation because of its high cost. Because of cost limitations, many laboratories use the wet digestion procedure, not only because of its simplicity in equipment needs, but also because it is more convenient than the combustion method for soils containing CaCO₃. Since dry combustion measures both inorganic and organic C, calcareous soils require an acid pretreatment for carbonate removal. On the other hand, the wet digestion method is more time consuming, creates a serious disposal problem due to chromium and the strong acid, and interfering ions can cause errors in titration or colorimetric determinations.

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The purpose of this investigation was to evaluate an alternative method for routine analysis of soil OC, especially in situations where exact values may not be required. Need for such determinations arises frequently in the case of applying fertilizer N based on mineralizable N from soil organic matter (OM), and in assessing herbicide persistence-sorption reactions with soil OM. The objectives of this study were to (i) evaluate and compare absorbance of selected base extractants against soil OC levels measured by dry combustion, and (ii) test the method on a different set of soil samples.

Materials and Methods

Method development focused on extractability of absorbing materials (as related to OC) from a range of soils with differing physical and chemical properties (Table 1). Five of the 11 soils were selected because of their range in OC, clay content, free lime, and total Fe content. Standard methods were used for the measurement of clay (hydrometer) (Gee and Bauder, 1986), free lime (Nelson, 1982), and total Fe (Olsen and Rosco, 1982). These factors are known to affect the absorbance in soil extracts. Parameters such as base extractant, temperature, time, and sample size were optimized for OC extraction and correlated with known OC values.

Soils were ground to pass through a 150 μ m sieve, and a 1-g soil sample was extracted with each of the extractants. Three soil extractants were evaluated for their effectiveness in extracting absorbing materials as an index of soil OC. The extractants and experimental conditions were as follows: (i) extract with 20 mL of 0.5 M NaHCO₃ (NaHCO₃) for 0.5 h, (ii) extract with 20 mL of 0.5 M KOH (KOH) for 2 h, and (iii) extract with 50 mL of a solution containing 0.25 M KOH and 0.05 M Na-EDTA (KOH-EDTA) for 2 h. Extractions were made at room temperature (25 °C) by shaking on a rotary shaker. The extract was filtered through Whatman no. 1 filter paper. Additional extractions with KOH-EDTA were made at 85 °C for time periods of 1, 2, and 4 h. The absorbance of the extracts was read at 260 nm (ultraviolet) and at 550 nm (visible) wavelengths.

After selecting the optimum time and temperature for the KOH-EDTA extractant, the effect of soil sample size and reproducibility were investigated. A soil sample (0.25, 0.50, or 1.00 g) was incubated with KOH-EDTA for 2 h at 85 °C. Extractions were replicated three times.

In order to compare differences among extractants, temperature, and soil sample size, a regression equation was developed to compare measured absorbance with dry combustion OC values for the specific soils used in each experiment. From these equations, the calculated absorbance values were converted to OC concentrations.

One-half to 1.00 g of well-mixed and finely-ground soil (5–35 g OC kg⁻¹) was weighed into a 100-mL volumetric flask. A larger sample size should be used for loamy sand and sandy loam soils, and a smaller sample size with loams, clays, and dark-colored soils. For low OC sandy subsoils (< 5 g C kg⁻¹), 1.5 g of soil can be used, and for soils suspected to be very high in OC (>20 g C kg⁻¹), 0.25 g can be used. Fifty mL of the extractant (0.25 M KOH and 0.05 M EDTA) was added to the soil, mixed, and incubated at 85 °C for 2 h. After 5 to 10 min of open incubation (to eliminate buildup of gases), glass stoppers were loosely inserted into the flasks to minimize volume loss. After 2 h, the flasks were removed from the incubator and their contents mixed. After the flasks had cooled to room temperature, extracts were filtered and absorbance read at 260 and 550 nm. A 2-cm glass cell was used at 550 nm and a 1-cm quartz cell was used at 260 nm. Because of the high sensitivity at 260 nm, a dilution was normally required in order to keep the absorbance readings

Table 1. Physical and chemical properties of selected soils.

Soil	Lab no.	Classification	pH (1:1 H ₂ O)	CaCO ₃	Total Fe	Texture		
						%		
					g kg ⁻¹			
Ascalon sandy loam	1	Fine-loamy, mixed mesic Aridic Argiustoll	6.2	0	14.8	68	20	12
Cecil sandy loam	2	Clayey, kaolinitic, thermic Typic Kanhapludult	4.8	0	9.0	76	15	9
Duroc loam	3	Fine-silty, mixed, mesic Pachic Haplustoll	7.3	<5	19.6	41	46	13
Haverson loam	4	Fine-loamy, mixed (calcareous), mesic Ustic Torrifuvent	7.9	83	20.6	35	39	26
Campana loamy sand	5	Fine-loamy, mixed, thermic Aquic Xerochrept	6.2	0	9.2	85	9	6
Latahco silt loam	6	Fine-silty, mixed, frigid Argiaquic Xeric Argialboll	6.1	0	25.2	12	65	23
Nunn clay loam	7	Fine, montmorillonitic, mesic Aridic Argiustoll	7.4	<5	23.8	33	42	25
Palouse silty clay loam	8	Fine-silty, mixed, mesic Pachic Ultic Haploxeroll	5.8	0	32.2	12	61	27
Promise clay	9	Very-fine, montmorillonitic, mesic Udic Chromustert	6.4	0	31.4	10	40	50
Redfeather sandy loam	10	Loamy-skeletal, mixed Lithic Cryoboralf	5.2	0	48.6	64	27	9
Vona sandy loam	11	Coarse-loamy, mixed, mesic Ustollic Haplargid	6.5	0	14.8	79	12	9

<1 (usually a 10-fold dilution). With all dilutions, a reagent blank was diluted by the same amount as the extract to maintain the same solution matrix. In most cases, absorbance at 550 nm could be read without dilution.

In order to evaluate extractants, temperature, soil sample size and OC contents, and sensitivity at two wavelengths, the absorbance values were calculated on a basis of 1 g of soil and an initial volume of 50 mL. Appropriate dilutions are required when absorbance values are >1.

Eleven soils were extracted by this method for calibration of OC absorbance against values obtained from dry combustion (Table 1). For validation of the method, two independent soil profiles were evaluated. All samples were replicated three times.

For standard values, OC was measured by a dry combustion procedure (Nelson and Sommers, 1982) by using a Carlo Erba¹ NA 1500 analyzer (Carlo Erba Strumentazione, Milan, Italy). Calcareous soils were pretreated with excess sulfuric acid to remove inorganic C. Samples were dried and three replicate samples of 20 to 30 mg (weighed to 0.1 mg) were combusted.

Regression analyses were made between absorbance values at both wavelengths against OC values from the dry combustion. The resulting empirical equation was used to estimate OC in samples from two soil profiles not used for the development of the equation. A Student's *t*-test was used to compare OC values obtained by the proposed method with those from dry combustion.

Results and Discussion

The intensity of the absorbance from base extraction of soil is influenced primarily by aromatic humic substances (Schnitzer, 1971). Methods based on color extraction assume humates account for greater than 90% of the total organic matter in soils. Thus, these colorimetric methods are more amenable to soils with humified OM as opposed to soils with fresh organic matter inputs; however, other factors can also affect absorbance, both chemically and physically. A serious chemical interference is the prevalence of the Fe³⁺ species (Moore, 1985). Also differential extinction coefficients from the many classes of organic compounds could contribute differentially to absorbance. On the other hand, clay and CaCO₃ can physically protect the

Table 2. Organic C (OC) determined from absorbance measurements (260 and 550 nm) of soil extracts, using three different extractants, after a 2-h incubation. Values are means of three samples.

Soil	OC by		NaHCO ₃		KOH		KOH-EDTA	
	dry	Temperature	260	550	260	550	260	550
	g kg ⁻¹	°C	nm	nm	nm	nm	nm	nm
Ascalon	7.8	25	6.8	5.8	13.5	20.0	9.2	8.4
		85						7.2
Haverson	10.2	25	14.3	17.7	4.1	9.2	9.9	8.8
		85						9.3
Campana	2.0	25	3.2	3.0	2.1	2.1	2.5	7.3
		85						4.1
Promise	35.4	25	29.3	31.4	8.1	22.0	30.9	36.8
		85						35.7
Redfeather	20.1	25	26.2	16.4	90.0	95.0	25.2	14.2
		85						24.3

organic matter from base extractions unless certain pretreatments are introduced (Stevenson, 1982).

Even though our intent was to obtain very good correlation between absorbance and OC content, we felt that the method had to be simple and easy to perform. Thus, Savory and Bowman (1986) first tested the Olsen bicarbonate extraction procedure and found a high correlation for grassland soils ($r^2 = 0.67$). Since bicarbonate extraction is routinely used for the determination of available P, this approach has the advantage that OC can be estimated from an existing extract. The extractant, however, was found to be too weak for high clay and high organic matter soils such as the Promise clay (Table 2). Potassium hydroxide alone was a poor extractant for the calcareous soil (Haverson, Table 2), and overestimated OC in an Fe-rich acid soil (Redfeather, Table 2). The KOH-EDTA extractant was the most efficient since the EDTA was able to chelate metal cations thus disrupting clay-metal-organic matter bridges. Additional heat (25 vs. 85 °C), and a 2-h incubation gave best recoveries of relative to known OC values (Table 2). The 1-h extraction was not enough for clay soils, and the 4-h extraction did not provide a decided advantage over the 2-h incubation (data for 1 and 4 h not shown).

The effects of sample size were tested with three soils (Table 3). The soils ranged in OC contents from 2 g

¹ Trade names are included in the text as a convenience to the reader and do not constitute any preferential endorsement of these products more than other similar products.

Table 3. Organic C (OC) determined from absorbance measurements (260 and 550 nm) of soil KOH-EDTA extracts after a 2-h incubation. Values represent the means of three samples with CV in parentheses.

Soil	OC by dry combustion g kg ⁻¹	Sample size g	g kg ⁻¹	
			260 nm	550 nm
Haverson	10.2	0.25	9.2 (2.6)	9.6 (1.0)
		0.50	8.9 (2.4)	9.5 (1.6)
		1.00	8.3 (2.9)	9.4 (3.3)
Campana	2.0	0.25	4.1 (4.4)	7.5 (5.7)
		0.50	4.1 (4.2)	7.5 (3.5)
		1.00	4.3 (1.8)	7.6 (3.3)
Promise	35.4	0.25	37.6 (3.2)	32.1 (3.0)
		0.50	35.2 (4.2)	32.2 (3.3)
		1.00	36.2 (4.4)	33.1 (4.7)

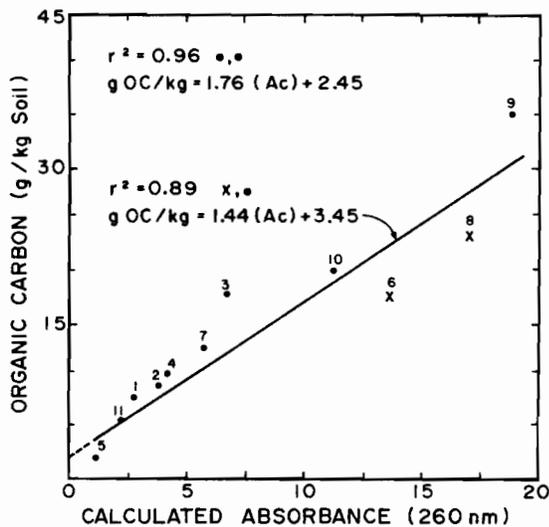


Fig. 1. Relationship between soil organic C determined by dry combustion and calculated absorbance (Ac) measured from KOH-EDTA (85 °C) soil extracts.

kg⁻¹ (Campana) to 34 g kg⁻¹ (Promise). Linearity between sample size and absorbance was quite good. Coefficients of variation for samples run on a single day were usually <5%. Aggregate values for runs on different days were higher, but rarely exceeded 8%.

The proposed method was used with 11 soils (Fig. 1). The correlation at 260 nm ($r^2 = 0.89$, $P = 0.001$) was far superior to that at 550 nm ($r^2 = 0.74$, $P = 0.01$). This correlation was greatly improved without the Latahco and Palouse soils ($r^2 = 0.96$ at 260 nm and $r^2 = 0.89$ at 550 nm). Extracts from these two soils contained more absorbing materials relative to their OC content than the other nine soils. The r^2 values (including Latahco and Palouse) however, still represent excellent correlations considering the soils were taken from different geographic areas, and represented different genesis and development (5 different soil orders).

A Keith silt loam (Aridic Argiustoll) and a calcareous Promise clay profile series were used to test both empirical equations developed from results in Fig. 1. The term Ac is the calculated absorbance of the soil extract. For these two soil profiles, soil OC amounts obtained by dry combustion and absorbance (9 soil

Table 4. Comparison of KOH-EDTA (260 nm) and dry combustion methods for the determination of soil organic C (OC).

Soil	Soil depth cm	CaCO ₃ g kg ⁻¹	Dry combustion g kg ⁻¹	KOH-EDTA g kg ⁻¹	
				A†	B‡
Keith sil	0-10	0	14.7	15.0	13.7
	10-30	2.2	11.1	12.3	12.5
	30-60	27.8	8.7	9.6	9.9
	60-120	69.0	5.5	7.0	7.7
	120-180	68.6	2.8	3.2	4.7
	180-240	73.2	1.2	2.8	3.7
Promise c	0-18	24.6	13.5	12.8	12.0
	18-46	59.7	7.9	8.3	8.5
	46-76	47.2	5.4	5.8	5.9
	76-114	164.6	3.2	3.5	4.1

† Calculated from empirical equation $OC(g\ kg^{-1}) = 1.76(Ac) + 2.45$ (9 soils), where Ac is the calculated absorbance of the soil extract.

‡ Calculated from empirical equation $OC(g\ kg^{-1}) = 1.44(Ac) + 3.45$ (11 soils).

equation) were closely related ($r^2 = 0.98$) and not significantly different (t test, $P = 0.05$) (Table 4). Slope of the equation was 0.93 with an intercept of 1.13. Comparative OC values obtained from the empirical equation derived from all 11 soils are also given in Table 4. Even though there was no significant difference between methods when comparing the two soils at all depths, the spectroscopic method overestimated the OC from the low layers (120-240 cm) of the Keith soil where OC concentrations were very low. The method is not sensitive enough for soils below 5 g OC kg⁻¹ because zero absorbance readings would give a value of 2.5 g OC kg⁻¹. These low OC soils could present a problem for the proposed method, and may require some sample size adjustments for adequate absorbance or the use of lower OC standards. The use of lower standards would shift the intercept value towards zero (note cluster of samples, Fig. 1, with OC values below 15 g kg⁻¹ soil).

There still remains the question of standard curve development in a particular laboratory. Since the method was developed primarily for soil testing groups, these laboratories or even research laboratories may want to use their own standard soils, and further calibrate their test to a particular range of soils or OC values. While our empirical equation may serve as a guide, each laboratory should develop their own correlations based upon their instrumentation and other analytical techniques. Because of the day-to-day differences, it is recommended that two or three standard soils be run with each batch of unknowns.

References

- Carolan, R. 1948. Modification of Graham's method for determining soil organic matter by colorimetric analysis. *Soil Sci.* 66:241-247.
- Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. p. 383-411. *In* A. Klute (ed.) *Methods of soil analysis*. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Kalambasa, S.J., and D.S. Jenkinson. 1973. A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soils. *J. Sci. Food Agric.* 24:1085-1090.
- Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. *Anal. Chim. Acta* 22:120-124.
- Moore, T.R. 1985. The spectrophotometric determination of dissolved organic carbon in peat waters. *Soil Sci. Soc. Am. J.* 49:1590-1592.
- Nelson, R.E. 1982. Carbonate and gypsum. p. 181-197. *In* A.L. Page et al. (ed.) *Methods of soil analysis*. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

- Nelson, D.W., and L.E. Sommers. 1975. A rapid and accurate procedure for estimation of organic carbon in soil. *Proc. Indiana Acad. Sci.* 84:456-462.
- Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 539-579. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Olson, R.V., and Roscoe Ellis, Jr. 1982. Iron. p. 301-312. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Salter, R.M. 1916. A rapid method for the accurate determination of total carbon in soil. *Ind. Eng. Chem.* 8:637-639.
- Savory, D.J., and R.A. Bowman. 1986. Estimation of soil organic carbon using UV spectrophotometric analysis of NaHCO_3 extractions. p. 214. *In* *Agronomy abstracts.* ASA, Madison, WI.
- Schnitzer, M. 1971. Characterization of humic constituents by spectroscopy. p. 60-95. *In* A.D. McLaren and J. Skujins (ed.) *Soil biochemistry.* Vol. 2. Marcel Dekker, New York.
- Stevenson, F.J. 1982. *Humus chemistry.* John Wiley & Sons. New York.
- 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.