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Colorimetric Determination of Organic Carbon in Soils by Dichromate Digestion in a Microwave Oven

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Abstract: External heating of soil samples following addition of dichromate is a commonly used method for the determination of organic carbon (C). We have successfully standardized microwave oven as a source of external heating for reliable and quick determination of organic C in soil samples by colorimetric method. Ten soils varying in C content were collected from agricultural fields differing in physico-chemical and other characteristics. The method involves: I) addition of 8 mL of conc. H_2SO_4 and 5 mL of $2N$ $K_2Cr_2O_7$ solution to soil samples containing *ca* 5 mg C and placed in 100 mL glass beakers, ii) heating the treated samples in a microwave oven with rotating plate, iii) dilution of the contents of the beaker to 50 mL with distilled water, iv) determination of optical density of the samples at 590 nm, v) calculations for C content against a glucose standard (5 mg C mL^{-1} solution; 1.25 g glucose 100^{-1} mL) treated like the samples. The newly developed method gives values of C lower than those obtained with the conventional colorimetric method and Walkley-Black method. However, almost perfect correlations existed between the values obtained by the three methods. Regression equations thus developed can conveniently be used for the accurate measurement of organic C in soil samples. Time of heating in microwave oven that varied from 45 to 120 sec did not have a significant bearing on the amount of C determined. Hence, heating for 60 sec can conveniently be adopted for routine analysis. The proposed method is not only quick but highly economical in terms of energy used in heating the samples.

Key words: Colorimetric method, dichromate digestion, microwave digestion, organic C, Walkley-Black method

INTRODUCTION

Quantification of soil organic matter is generally made by determining carbon © content using dry or wet oxidation methods. A comprehensive account of methods used for C and organic matter determination vis-à-vis their reliability/reproducibility and costs involved has been provided by Nelson and Sommers[1]. However, different labs have adopted methods according to the needs and economic feasibility. Of the different methods available, the one described by Walkley-Black[2] and Walkley[3] is simple and requires only the commonly available laboratory supplies. This method employs dichromate oxidation (without external heating) approach and involves determination of $Cr_2O_7^{-2}$ used in oxidizing organic C present in the sample by titrimetric or colorimetric method[4]. In either case, relatively expensive chemicals are required. Rhiem and Ulrich[5] proposed the use of 8 mL of concentrated H_2SO_4 and 5 mL $2N$ $K_2Cr_2O_7$ for samples containing *ca* 5 mg C and heating the samples for 90 min at 110°C followed by measurement of optical density of diluted and centrifuged sample at 590 nm. Nelson and Sommers[6] also used dichromate oxidation but reduced the time of external heating to 30 min at 150°C; optical density is measured at 600 nm. Several other combinations of temperature and duration of heating have also been proposed[1]. The objective of present study was to evaluate microwave as a source of external heating of dichromate treated soil samples with a view to hasten and economize the process of C determination in soil samples.

MATERIALS AND METHODS

Soils used in the study were collected from 0-15 cm of agricultural fields at different locations and chosen to provide variation in organic and inorganic ($CaCO_3$) C content. Physico-chemical analyses (Table 1) of the air-dried and sieved (<2 mm) samples were performed using standard procedures.

For Walkley-Black method, 2 g samples of soils in triplicate were treated with potassium dichromate and sulfuric acid followed by titration and calculations for C

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Table 1: Phycio-chemical properties of soils

Soil	pH*	EC* (dS m^{-1})	Total N (%)	$CaCO_3$ (%)	clay (%)	silt (%)	sand (%)
1	7.8	1.42	0.041	2.0	26.5	19.7	53.8
2	7.4	1.41	0.069	1.5	28.6	19.3	52.1
3	8.1	2.90	0.072	5.0	36.6	23.3	40.1
4	8.3	1.51	0.040	8.1	14.1	5.8	80.1
5	8.1	1.43	0.054	6.1	15.8	10.2	74.0
6	8.6	1.45	0.022	4.5	12.7	10.7	76.6
7	8.2	1.33	0.036	9.2	18.6	14.6	66.8
8	8.2	1.46	0.033	12.3	20.9	16.7	62.4
9	8.2	1.64	0.045	9.1	16.9	14.7	68.4
10	8.0	1.66	0.041	5.0	28.8	20.7	20.5

*, Measured in saturation extract

content as described by Nelson and Sommers[1]. Colorimetric methods of Nelson and Sommers[1], Rhiem and Ulrich[2] involve external heating of soil samples (containing *ca* 5 mg C) for 30-90 min in a conventional oven or using other heating arrangements. In the proposed modification, 8 mL of concentrated H_2SO_4 and 5 mL of 2*N* $K_2Cr_2O_7$ were added to multiple 1 g portions of soils taken in 100 mL beakers. Triplicate samples together with 1 mL aliquots of glucose standard (5 mg C mL^{-1}; 1.25 g glucose 100^{-1} mL) were heated in the microwave oven with rotating plate for 45, 60, 90 or 120 sec. For comparison, triplicate samples containing 1 g soil, 8 mL concentrated H_2SO_4 and 5 mL of 2*N* $K_2Cr_2O_7$ were heated in an oven at 110°C for 90 min with intermittent shaking[4]. After heating in either way, the samples were diluted to 50 mL, centrifuged for 10 min at 4000 rpm and subjected to measurement of Optical Density (OD) at 590 nm. Calculations for C content were made as: mg C in the sample = [mg C in glucose standard/OD of glucose standard] x OD of glucose standard.

Standard deviation, % variance and coefficient of correlations were calculated using MS EXCEL software, while MSTAT was used for regression analyses.

RESULTS AND DISCUSSION

Carbon content of the 10 soils measured by Walkley-Black[3] method ranged between 1.45 and 6.05 mg g^{-1} soil averaging 3.71 mg g^{-1} soil and showing % variance of 39 (Table 2). Colorimetric method[4] involving external heating of chromic acid containing samples at 110°C for 90 min in a conventional oven yielded values almost comparable to those obtained with standard Walkley-Black method; coefficient of correlations being 0.99. Regression equation developed for the two methods was: y = 0.0375+1.072x; where, y and x are the values obtained with colorimetric and Walkley-Black method, respectively (Fig. 1).

When microwave radiation was used as a source of external heating, the values of organic C content of soils were consistently lower than those obtained following heating in a conventional oven but the two sets of data showed a perfect positive correlation (r = 1.00) (Fig. 1). The time of heating (45-120 sec) in microwave oven did not have a significant bearing on the quantification of organic C in different soils; the average for all soils ranged from 2.63 to 2.70.

Table 2: Carbon content of soils (mg g^{-1} soil) determined by different methods

	W and B (Original)*	Colorimetric normal heating$^{\Psi}$	Colorimetric, microwave heating$^{\varphi}$			
			45 sec	60 sec	90 sec	120 sec
Soil	M_1**	M_2	M_3	M_4	M_5	M_6
1	2.50	2.56	1.97	1.96	2.00	2.17
2	6.05	5.02	4.71	4.73	4.88	4.75
3	5.45	4.95	3.98	3.82	3.83	4.11
4	3.23	3.21	2.60	2.62	2.87	2.70
5	5.62	4.62	3.86	4.26	4.17	3.83
6	1.45	1.31	1.10	1.00	1.12	1.05
7	2.96	2.47	1.84	1.95	1.84	1.81
8	3.02	2.87	1.91	2.01	2.05	1.92
9	2.86	2.63	1.73	1.81	1.86	1.71
10	3.95	3.61	2.57	2.77	2.41	2.66
Average	3.7	3.4	2.6	2.7	2.7	2.7
SD	0.4	1.4	1.2	1.2	1.2	1.2
Variance (%)	38.7	40.5	44.7	44.8	44.8	44.7
Coefficient of correlations (R^2)						
M_1		1.00	0.99	1.0	0.99	0.99
M_2			1.00	0.99	0.99	1.00
M_3				1.00	1.00	1.00
M_4					1.00	1.00
M_5						1.00

*, As described by Nelson and Sommers[1]

$^{\Psi}$, As described by Rhiem and Ulrich[5] with external heating of samples in a conventional oven

$^{\varphi}$, As described by Rhiem and Ulrich[5] but with external heating of samples in a microwave rather than conventional oven

**, Different methods as described in the row above M_1-M_6

Reliability of microwave heating was apparent from almost perfect correlation (r = 0.99) between C content of different soils measured with either of the heating methods and for all the 4 heating durations (Fig. 1). Regression equation obtained was: y = 0.67+1.01x; where y and x are the values of C determined by heating in conventional (90 min) and microwave (45 sec) oven, respectively; co-efficient of correlations being 0.982. Similarly, a highly significant correlation was obtained between the C values obtained by microwave method and Walkley-Black method and a regression equation: y = 0.39+1.266x was obtained (y and x being values of C measured by the use of Walkley-Black method and microwave method, respectively). Co-efficient of correlations was 0.986 in this case. The results suggest that microwave heating can conveniently be used for a reliable quantification of organic C in soil samples. Some other studies showed that the method is equally good for plant and liquid samples and overcomes the problems arising from interference by inorganic C present in soil as carbonates.

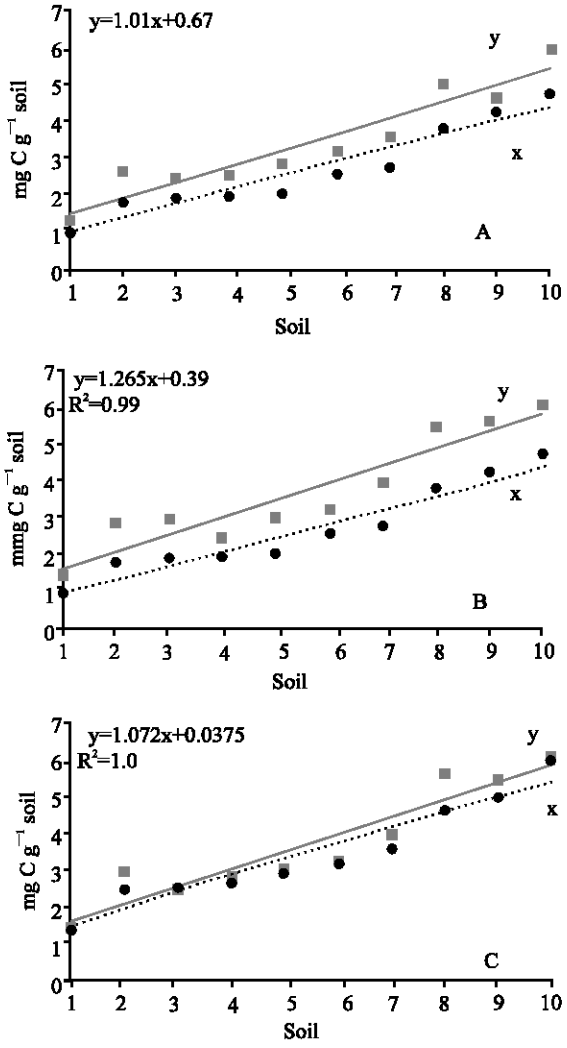

Fig. 1: Statistical relationships between the three methods of determining organic C in soil samples. A, colorimetric method using microwave versus conventional oven for heating chromate treated sampes; B, colorimetric method using microwave versus Walkley-Black method; C, colorimetric method using conventional oven versus Walkley- Black method

Results of present study demonstrate that microwave heating can replace heating in a conventional oven thereby leading to a significant reduction in the time and cost (in terms of equipment prices and consumption of electricity) of analysis. This method is also a perfect replacement of Walkley-Black method and gives realistic values for the later when regression equation developed in the present study is applied for the calculation of organic C in soil samples. Hence, the method is not only economical but more accurate and less time consuming.

REFERENCES

1. Nelson, D.W. and L.E. Sommers, 1996. Total Carbon, Organic Carbon and Organic Matter. In: Sparks D.L. *et al.* (Ed.) Methods of soil analysis. Part 3. Chemical Methods. SSSA Book Ser. 5. SSSA, Madison, WI. pp: 961–1010.
2. Walkley, A. and I.A. Black, 1934. An examination of the Degtjarref method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.
3. Walkley, A., 1947. A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci., 63: 251-263.
4. Carolan, R., 1948. Modification of Graham's method for determining soil organic matter by colorimetric analysis. Soil Sci., 66: 241-247.
5. Rhiem, H. and B. Ulrich, 1954. Quantitative colorimetrisch Bestimung der organischen substanz im Boden. Landwirthschaft Forschung, 6: 173-176.
6. Nelson, D.W. and L.E. Sommers, 1975. A rapid and accurate method for estimating organic carbon in soil. Proceedings Indiana Academy of Sciences, 84: 456-462.