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A Reevaluation of the Chromic Acid Colorimetric Procedure for Soil Organic Carbon

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ABSTRACT

Two methods are currently used to measure soil organic carbon (SOC); one based on measuring CO₂ evolution after high temperature combustion of the sample and the other on measuring dichromate reduction after organic carbon (OC) oxidation. The former method is the more accurate, but requires correction for soils with free calcium carbonate; the latter uses a hazardous heavy metal, dichromate (Cr 6+), but is easier when done colorimetrically (Cr 3+ measured at 625 nm) and is less expensive. If we could minimize the hazard and health concerns associated with the hexavalent chromium (Cr 6+), which is required in excess for complete SOC oxidation, and assure adequate accuracy, this method may be more acceptable especially where samples are few, and free calcium carbonate may be present. A methodology is presented where the amount of soil sample (0.1 to 0.5 g) and dichromate used in the analysis (2.5 mL of 0.167M dichromate) is small. The unreacted dichromate after analysis, is further reduced with glucose or other carbon (C) source to produce the less toxic Cr 3+, which in small quantities, can be discarded to landfills once properly precipitated and neutralized. Accuracy

of the colorimetric procedure when compared to the CN Analyzer was adequate for samples with less than 1.3% SOC [2% soil organic matter (SOM)], amounts typical for cultivated soils of the Central Great Plains. For samples above 1.3% SOC (>2% SOM), accuracy was improved by reducing sample size so 2.0 to 5.5 mg of OC was contained in the sample.

INTRODUCTION

Methods to estimate soil organic matter from the determination of SOC are numerous (Salter, 1916; Walkley and Black, 1934; Mebius, 1960; Nelson and Sommers, 1982; Synder and Trofymow, 1984). These methods measure SOC by either a high temperature dry combustion procedure of the sample where carbon dioxide (CO_2) output is quantified, or by a wet digestion procedure that uses potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and concentrated (18M) sulfuric acid (H_2SO_4) where SOC is measured by titration of the unreacted Cr 6+ to Cr 3+, by colorimetry and absorbance of Cr 3+, or by CO_2 adsorption and weight. Advantages and disadvantages of these two approaches are discussed by Kalembasa and Jenkinson (1973), Nelson and Sommers (1982), and Tiessen and Moir (1993).

The traditional method in soil science for many years has been the classic Walkley-Black (1934) procedure. The method provides a good estimate of SOM where levels are less than 3.0% SOM, and where free calcium carbonate (CaCO_3) may be present. One of the more rapid and easy modifications of the Walkley-Black procedure is the use of colorimetry to quantify the reduced hexavalent dichromate to the green trivalent chromium. All these modifications, however, still retain the problems associated with the original procedure such as incomplete oxidation of complex humic C, and trivalent chromium production from chloride oxidation in saline soils (Nelson and Sommers, 1982).

In spite of its ease, the chromic acid method is in disfavor because of the use of the hexavalent chromium ion (Cr 6+) which poses some serious health hazard, and to some extent, because of the generation of large amounts of strong sulfuric acid waste. No longer do municipalities permit the disposal of heavy metals and strongly acidic solutions in their sewer systems. The hexavalent Cr heavy metal is reported to cause cancer, and the method is only adequate when this ion is in excess at the end of the SOC wet digestion.

The objective of this report was to present data to improve accuracy and to show that the colorimetric modification of the method has a role where samples are few, budget is low, calcium carbonate exists, and where we can minimize the hazards both to the analysts and for disposal. The main objection to the procedure can be addressed by utilizing less of the dichromate reagent with smaller soil sample size, and by reducing the hazardous residual hexavalent chromium (Cr 6+) to the less hazardous trivalent chromium (Cr 3+) which can be neutralized and precipitated prior to disposal.

TABLE 1. Selected physical and chemical characteristics of 6 test soils.

| Soil Series | Classification | pH | Silt | Clay |
|--------------|---|-----|------|------|
| Ascalon s 1 | Fine-loamy, mixed, mesic aridic argiustolls | 6.3 | 20 | 10 |
| Haverson 1 | Fine-loamy, mixed (calcareous) mesic ustic torrifluvents | 7.8 | 20 | 18 |
| Platner si 1 | Fine, montmorillonitic, mesic aridic paleustolls | 6.5 | 25 | 20 |
| Rago si 1 | Fine, montmorillonitic, mesic pachic argiustolls | 6.6 | 20 | 18 |
| Vona s 1 | Coarse-loamy, mixed, mesic Ustollic Haplargids | 6.2 | 15 | 10 |
| Weld si 1 | Fine, montmorillonitic, mesic aridic paleustolls | 6.4 | 20 | 18 |

MATERIALS AND METHODS

The chromic acid method using colorimetry for quantification was reevaluated for accuracy and safety. Six different soil series from the Central Great Plains (Table 1) and two non-calcareous soils with known SOM content (standards) from the University of Missouri were used for SOC method evaluation. Results from the CN Analyzer were used for comparison with the chromic acid procedure. Three soils from among these were selected to evaluate OC in soil profiles containing free calcium carbonate. Soil pH (1:1 in water) and texture (hydrometer) were conventionally determined (McLean, 1982; Gee and Bauder, 1986). For samples without free lime, a dry combustion method (Carlo Erba C-N-S 1500 Series 2 analyzer) was used as the standard for assessing the chromic acid method.

For the CN Analyzer C analysis, oven-dried 2-mm screened samples were further ground to a powder-like consistency. This was accomplished by placing 4 cylindrical iron rods (50 mm length, 4 mm diameter) in 100-mL stoppered glass milling bottles with about 10 g of representative soil sample. Bottles were placed on a rotating shaker for 24 h. Since only a very small sample size is used for the CN Analysis (about 15 to 20 mg), thorough mixing and replications are recommended for this procedure to increase precision and accuracy.

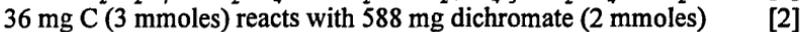
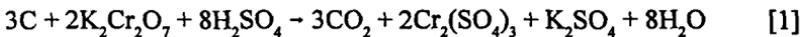
A modification of the Heanes (1984) chromic acid procedure was used in the determination of SOC. Three different soil sample sizes (0.10, 0.25, and 0.50 g) were evaluated with 2.5 mL of 0.167M $K_2Cr_2O_7$, and 5 mL of 18M (concentrated) sulfuric acid. Dichromate from an amber bottle and 18M sulfuric acid were delivered from calibrated repipets to minimize analyst contact with chemicals,

TABLE 2. Organic C recovery of synthetic substrates with the chromic acid procedure (glucose-C as standard).

| Substrate | % OC | mg OC added | mg OC recovered |
|-----------------------------|------|-------------|-----------------|
| Dextrose | 40.0 | 4.6 | 4.6 (100%) |
| Ca Phytate | 8.4 | 2.8 | 2.9 (104%) |
| Ca PhyLate | 8.4 | 5.3 | 5.4 (102%) |
| Na2EDTA | 32.2 | 5.6 | 5.3 (95%) |
| Tryptophane | 64.7 | 4.2 | 4.0 (95%) |
| Humic acid (Sodium salt) | 40.3 | 3.6 | 3.7 (103%) |

and to reduce manual pipetting. The different sample sizes were placed into volumetric tubes with calibration at 25, and 50 mL. Sample with dichromate and sulfuric acid was vortexed for 10 seconds and then placed on a preheated block at 135°C for 30 minutes. Samples were then cooled, made to 25-mL volume, mixed again, and cooled. Supernatant was poured off, centrifuged at 10,000 rpm (radius=10 cm), and the reduced dichromate (Cr 3+) read colorimetrically at 625 nm against glucose-carbon standards. More complex synthetic OC substrates (Table 2) were also used to determine efficiency of oxidation against glucose-C. Samples were read directly with a sipper assembly from the centrifuge tubes for soils, or directly from the calibrated tubes for standards and synthetic substrates. Water blanks were read between each sample to prolong life of sipper assembly (continuous strong acid would degrade the rubber component).

Unreacted Cr 6+ in the acidic waste was treated with dextrose to complete the reaction of unreacted Cr 6+ to Cr 3+, the less toxic of the two. After colorimetric Cr 3+ determination of the extract, waste from the centrifuge tubes is poured into an Erlenmeyer flask, or other appropriate container, solid dextrose is added, and flask is placed on block at 135°C until green color (Cr 3+) is observed according to Equations [1] and [2].



Thus, about 19 mg dextrose per sample (7.53 mg C for 123 mg K₂Cr₂O₇ in 2.5 mL of dichromate) is required stoichiometrically, but dextrose is added in excess. If dextrose is not available, most discarded soft paper products could serve the same function.

RESULTS AND DISCUSSION

Properties for test soils (Table 1) show an adequate representation of soils common to the Central Great Plains. These soils once cultivated generally contain

less than 1.2% OC (<2% SOM) in the surface 15 cm. Since there are rarely clay loams in the surface, SOM content is relatively low (Nichols, 1984) and less physical protection of SOM occurs (Schimel, 1986). Precisely because of this more labile status of the SOM that the chromic acid procedure can be a good estimate of SOM in most cases.

Although in existence for over half a century, the Walkley-Black procedure was always considered an approximation method for soil organic matter. Oxidation conditions (heat, time, acid-dichromate ratio, sample size) were never considered adequate for complete oxidation of OC. Thus, correction factors were determined for each soil type or horizon from more quantitative methods. Heanes (1984), however, introduced an improved chromic acid method where greater oxidation recoveries were obtained, and the colorimetric determination was relatively simpler than the more tedious titration method where end-point, if done visually, was difficult to detect.

The colorimetric procedure requires a C source (glucose in this case) as standard. It assumes that the C in SOM behaves relatively the same as the C in glucose in terms of ease of oxidation. While this is difficult to prove, quantitative recoveries equal to the CN Analyzer were obtained with other more complex synthetic OC substrates with glucose as standard (Table 2). Even though tryptophane [usually used in nitrogen (N) recovery studies] and a commercial salt of humic acid were completely oxidized, protection by clay and its effects on recovery were not studied.

Organic carbon results using the chromic acid procedure for the three sample sizes were compared to the CN Analyzer results (Table 3). Since an important objective was to reduce the amount of dichromate in the procedure (2.5 mL as opposed to 10 mL in most routine procedures), a soil sample size consistent with this volume for adequate reduction of dichromate was desired. From a theoretical consideration, the 2.5 mL of 0.167M $K_2Cr_2O_7$ will oxidize 7.53 mg of OC (from Equation [2]). Since Cr 6+ must remain in excess it is recommended that total OC in the sample not exceed 5.5 mg. This was borne out by the studies on recoveries with synthetic organic substrates and with the various soil sample sizes.

There appeared to be a definite range within which efficiency of oxidation was complete. In most instances, the 0.5 g sample was too large for the amount of dichromate (88% average recovery of OC). This value would have been 92% without the high standard soil (2.84% OC). The 0.25 g sample appeared adequate in most instances (100%). The 0.10 g soil sample appeared adequate for higher SOC samples as shown with standard soil number two, but for lower OC soils, average recovery was 117%. Whether this excessive recovery was due to other simultaneous oxidations (Cl oxidation for instance) was not investigated. For the suggested maximum OC (5.5 mg) in the sample, a soil weight of 0.25 g is only adequate for samples with 2.0% or less OC which was exceeded by standard soil no. 2 (2.8%). While sample sizes of 0.25 or 0.10 g may appear small for adequate accuracy, it must be remembered that the CN Analyzer uses only 0.025 g. Thus, adequate mixing for a representative sample is crucial for both methods.

TABLE 3. Effect of soil sample size on oxidation of organic carbon with the chromic acid procedure for 0-5 cm surface soils (average of 4 replications).

| Soil | CN Analyzer | Sample size (g) chromic acid | | |
|-------------------------|-------------|------------------------------|-------------|-------------|
| | | 0.10 | 0.25 | 0.50 |
| ----- % Organic C ----- | | | | |
| Weld 1 | 1.04±0.03 | 1.17 (112%)* | 1.00 (96%) | 0.88 (89%) |
| 2 | 1.40±0.04 | 1.56 (111%) | 1.35 (96%) | 1.26 (90%) |
| 3 | 0.84±0.02 | 1.00 (119%) | 0.89 (106%) | 0.79 (90%) |
| 4 | 0.57±0.02 | 0.71 (124%) | 0.64 (112%) | 0.54 (95%) |
| Platner 1 | 0.76±0.02 | 1.00 (132%) | 0.83 (109%) | 0.78 (103%) |
| 2 | 0.91±0.03 | 1.15 (126%) | 0.90 (99%) | 0.86 (95%) |
| Std 1 | 1.34±0.02 | 1.50 (112%) | 1.33 (99%) | 1.20 (90%) |
| 2 | 2.84±0.05 | 2.80 (98%) | 2.45 (86%) | 1.60 (56%) |
| Average (% recovered) | | (117%) | (100%) | (88%) |

* (%) represents % recovery from CN Analyzer values.

TABLE 4. Carbon and N values using CN Analyzer and organic carbon (OC) values for chromic acid procedure for soils with and without free lime (average of 3 replications).

| Soil | Depth (cm) | CN Analyzer | | | Chromic acid | |
|----------|---------------|---------------|-------|------|--------------|-------------------|
| | | OC | N | C/N | OC | CaCO ₃ |
| | | ----- % ----- | | | % | |
| Haverson | 00.0-15.0 | 1.61 | 0.090 | 17.9 | 0.82 | yes |
| Platner | 00.0-07.5 | 1.17 | 0.080 | 14.7 | 0.78 | yes |
| Platner | 07.5-15.0 | 1.33 | 0.069 | 19.2 | 0.68 | yes |
| Platner | 00.0-07.5 | 0.86 | 0.082 | 10.5 | 0.82 | no |
| Platner | 07.5-15.0 | 1.06 | 0.062 | 16.2 | 0.63 | yes |
| Vona | 00.0-07.5 | 0.71 | 0.068 | 10.4 | 0.68 | no |
| Ascalon | 00.0-07.5 | 0.63 | 0.068 | 9.2 | 0.64 | no |
| Rago | 00.0-07.5 | 1.21 | 0.100 | 10.2 | 1.19 | no |
| Rago | 07.5-15.0 | 0.68 | 0.059 | 11.4 | 0.69 | no |

For the prescribed procedure it appeared that accuracy was achieved when about 25 to 75% of the dichromate was used (reduced). Thus, for samples with <0.6% SOC (<1.0% SOM), it is recommended that the sample size be 0.5 g, but for samples with >2.0% SOC (>3.5% SOM), 0.1 g is recommended.

As discussed before, CN Analyzers measure all CO₂ produced during high-temperature combustion regardless of source (organic and inorganic). Since carbonate content has no effect on dichromate reduction, the chromic acid procedure is not affected. Table 4 shows data for SOC determined by CN Analyzer and chromic acid procedures for soils with and without carbonates. While there are procedures to measure both inorganic and organic sources of CO₂, where SOM content is low, and lime content is high, precision and accuracy for SOC can be compromised. As shown by the CN Analyzer data, when soil samples have not been checked for lime, C to N ratios, and position in the profile, can alert the analyst to a potential problem. Carbon to N ratios exceeding 15, and a lower horizon showing higher SOC content than a surface horizon, together, can indicate the presence of free carbonates, and an excessive SOC value.

Dextrose or any easily oxidized source of C (table sugar) or paper can be used to reduce the excess dichromate that remain after wet digestion. The strong acid can be neutralized with base and the Cr precipitated as Cr(OH)₃, and if acid is used in large quantities, can be neutralized with lime or commercial cement. Under these conditions, in most instances if the level of chromium used is not great, the waste (Cr 3+) can be discarded to landfills.

With the precautions to minimize hazards from use and disposal, the chromic acid method deserves consideration under some conditions. It can be used for screening purposes or for greater accuracy once conditions are optimized; it can be used where sample numbers are small, and where lime may confound results with methods that measure CO₂ evolution.

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