

Procedure for the Determination of Permanganate Oxidizable Carbon

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Overview:

This procedure describes a technique for the determination of permanganate oxidizable carbon (POXC) in soil samples. This procedure is synonymous with the 'Active Carbon' method described by Weil et al. (2003) and is adapted with help from the Glover lab (Land Institute) and the Barbercheck lab (Pennsylvania State University).

Instrumentation and Materials:

- Spectrophotometer capable of reading absorbance at 550 nm
- Weighing balance capable of accurately weighing ~2.50g of soil to two decimal places (0.01 g)
- pH meter calibrated for measurement in the range of ~6.0-8.0 pH and NaOH for pH adjustment
- Oscillating (or horizontal) shaker capable of at least 240 oscillations per minute (or 120 rpm)
- Magnetic stir plate and stir bars
- Adjustable 10 mL pipettor and tips
- Adjustable 100-1000 μ L pipettor and tips
- (2) Adjustable bottle-top dispensers fitted to a bottle of deionized water and calibrated to deliver 18.0 mL and 49.5 mL
- 50 mL disposable polypropylene centrifuge tubes with caps (Falcon tubes)
- Laboratory glassware for reagent preparation and waste collection
- Labeling supplies such as permanent markers and tape
- Reagent grade Potassium Permanganate (KMnO_4 ; FW=158.03 g mol⁻¹)
- Reagent grade Calcium Chloride, Dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; FW=147.01 g mol⁻¹)
- Soil standard (pulverized, homogenous soil as lab reference sample)
- Timer capable of tracking time for two and ten minute intervals

I. Reagent Preparation:

KMnO_4 Stock Solution 0.2 M (makes 1 liter):

1. Weigh 147 g of CaCl_2 and place in a 1000 mL beaker. Add approximately 900 mL of deionized water. Add a stir bar to the beaker, place on a magnetic stir plate and stir until completely dissolved (no heating necessary).
2. Transfer to a 1000 mL volumetric flask or graduated cylinder. Bring to volume with deionized water.
3. Weigh 31.60 g of KMnO_4 into a 1000 mL beaker and add approximately 900 mL of the CaCl_2 solution. Place on the magnetic stir plate with gentle heat and stir until dissolved

completely. Note: Dissolution may be very slow, and due to the very dark color of this solution, it may be necessary to decant some of the solution to check for undissolved KMnO_4 .

- Once dissolution is complete, place the probe from a calibrated pH meter into the solution (with continued stirring) and measure the pH. Adjust the pH to 7.2 by adding 0.1 *N* NaOH, 1 drop at a time. Since only a few drops will be needed, allow the pH reading to stabilize between additions of NaOH (endpoint approaches rapidly). Once the pH is adjusted, pour the solution into a 1000 mL volumetric flask or graduated cylinder and bring the volume to 1000 mL with the CaCl_2 solution. Transfer to a brown glass bottle and store in a dark place. If stored as indicated, the stability of this solution is believed to be 3-6 months.
- The amount of KMnO_4 solution prepared may be adjusted depending on total number of samples analyzed. One soil sample will use 2.0 mL of 0.2 *M* KMnO_4 .

II. Standard preparation:

Four standard concentrations (0.005, 0.01, 0.015 and 0.02 *M*) will be prepared from the KMnO_4 stock solution. The standard preparation involves first making a standard stock solution and then diluting each standard stock solution to a final working standard. The following materials will be needed:

- 50 mL disposable polypropylene centrifuge tubes
- Adjustable 1.0-10.0 mL pipettor and tips
- Adjustable 100-1000 μL pipettor and tips
- Adjustable bottle-top dispensers fitted to a bottle of deionized water and calibrated to deliver 49.5 mL

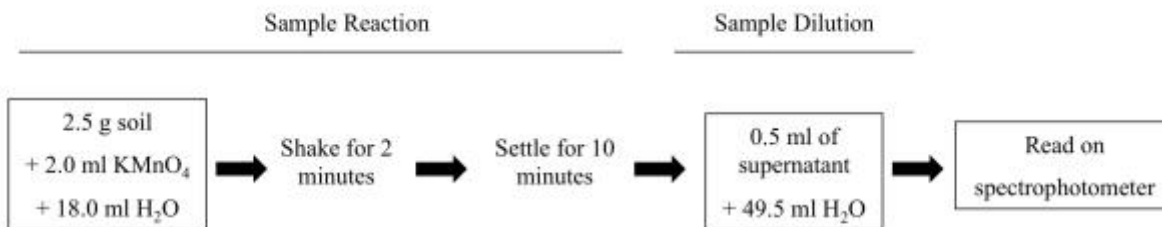
- Part 1- Standard Stock Solutions:** Use the table below to prepare standard stock solutions. These stock solutions can be prepared in centrifuge tubes or in small brown glass bottles and used for three days (in glass and in the dark) to prepare working standards.

Concentration	Volume of KMnO_4 stock solution	Volume of deionized water
0.005 <i>M</i>	0.25 mL	9.75 mL
0.01 <i>M</i>	0.5 mL	9.5 mL
0.015 <i>M</i>	0.75 mL	9.25 mL
0.02 <i>M</i>	1.0 mL	9.0 mL

- Part 2- Dilution Step:** Dilute each standard stock solution to a working standard by adding 0.5 mL of each stock solution to 49.5 mL of deionized water in 50 mL centrifuge tubes. These tubes now contain the working standards and should be prepared fresh daily.

III. Sample Preparation:

Sample preparation involves a two part process: a sample reaction and sample dilution, as illustrated below.



A soil standard and solution standard are prepared in the same manner as the unknown samples. The **soil standard** serves as a laboratory reference sample. It is recommended to pulverize and homogenize a large batch of air-dried soil for long-term use. The soil standard allows for a quality control check across POXC analyses performed on different batches, over multiple days, or with different reagents. The **solution standard** serves as another quality control reference. It is prepared in the same manner as the unknown soil samples, but without the soil. The solution standard will reveal if reagents or lab ware have been contaminated with oxidizing agents or carbon.

It is important that the timing of each step be consistent, particularly the shaking and settling times. The permanganate will continue to react with soil as long as it remains in contact. Hence, working quickly with small batches of 10 samples or less is advised.

The following materials will be needed:

- (2) 50 mL disposable polypropylene centrifuge tubes with caps for each sample
- Adjustable 1.0-10.0 mL pipettor and tips
- Adjustable 100-1000 μ L pipettor and tips
- (2) Adjustable bottle-top dispensers fitted to bottles filled with deionized water and calibrated to deliver 18.0 mL and 49.5 mL
- Labeling supplies such as permanent markers and tape
- Oscillating shaker capable of at least 240 oscillations per minute (or 120 rpm) and fitted with a lidded box that will hold at least ten 50 mL centrifuge tubes
- Timer capable of tracking time for two and ten minute intervals
- Soil standard (pulverized, homogenous soil as lab reference sample)

Sample Reaction

1. Label two 50 mL centrifuge tubes for each sample. Weigh 2.50 grams (± 0.05 g) of air-dried soil into one of the centrifuge tubes (may be done in advance). A soil standard (2.50 g of known pulverized soil) should also be prepared. Place the other set of tubes aside.
2. Add 18.0 mL of deionized water to each of the centrifuge tubes containing the soil. Using the 1.0-10.0 mL pipettor, add 2.0 mL of 0.2 M KMnO_4 stock solution to each tube.

3. Prepare a solution standard by made by adding 18.0 mL of deionized water and 2.0 mL of 0.2 M KMnO₄ stock solution to a tube (no soil) and process in the same manner as the unknown soils.
4. Working quickly, cap tubes tightly and hand-shake each tube vigorously for 2 seconds to assure soil dispersion within the solution.
5. Place tubes on shaker and shake at 240 oscillations per minute for 2 minutes.
6. After 2 minutes, remove samples from shaker and swirl or shake the tube vigorously to ensure that there is no soil clinging to the sides or cap of the tube. At this point, remove caps to avoid further disturbance of soil after settling. Place the samples in a dark area and allow soil to settle for ten minutes. Settling time is a critical step so a timer is essential.

Sample Dilution

1. While samples are settling, add 49.5 mL of deionized water to the second set of centrifuge tubes (may be done in advance).
2. Once the ten minute settling period has passed, quickly transfer 0.5 mL of supernatant (avoiding any particulate matter) to the second tube containing 49.5 mL of water. Note: This step should be performed as quickly as possible as the permanganate will continue to react with soil as long as it remains in contact.
3. Cap second set of tubes and invert to mix. These are the final sample solutions for analysis. They are stable for up to 24 hours if stored in the dark.

Reading Samples on Spectrophotometer

1. This method has been shown to perform well on both single cuvette machines and 96-well plate reading spectrophotometers. If available, a 96-well plate reader is recommended to save time (see steps 2-5 below).
2. Clear polystyrene flat-bottom cell culture plates (or equivalent) work well, so more expensive UV-transparent plates are not necessary.
3. It is recommended to replicate all standards on a plate, including deionized water blanks. Running each standard three or more times and taking the average typically yields good results.
4. Determine and record the absorbance (optical density) of standards and unknowns at 550 nm using spectrophotometer software.
5. Subtract out average of deionized water blanks from all absorbance values (if not automatically performed by software). The intercept of the standard curve should be very close to zero.

Calculating Mass of POXC for Unknown Soil Samples

1. The amount of carbon oxidized is a function of the quantity of permanganate reduced. Consequently, the higher the POXC values the lower the absorbance (intensity of the color of the solution).
2. Use the following equation to determine POXC, after Weil et al. (2003):

$$\text{POXC (mg kg}^{-1}\text{ soil)} = [0.02 \text{ mol/L} - (a + b \times \text{Abs})] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L solution/ Wt})$$

Where: 0.02 mol/L = initial solution concentration

a = intercept of the standard curve

b = slope of the standard curve

Abs = absorbance of unknown

9000 = milligrams of carbon oxidized by 1 mole of MnO_4 changing from $\text{Mn}^{7+} \rightarrow \text{Mn}^{4+}$

0.02 L = volume of stock solution reacted

Wt = weight of air-dried soil sample in kg

Example Calculation:

Construct standard curve with the following values:

Y-axis (Molarity of stock KMnO_4 standards)*	0.005	0.01	0.015	0.02
X-axis (Abs values from spectrophotometer)	0.1000	0.1984	0.3034	0.3966

*Note: The standard curve should use the molarity of the stock standards, and not the working standards, since the stock standards represent the actual concentration (0.02 M KMnO_4) used to react with the soil.

This produces the regression line: $y = 0.0502x - 0.00004$; $R^2 = 0.999$

Unknown sample absorbance: 0.3087; unknown sample soil weight: 2.48 grams

$$\begin{aligned} \text{POXC (mg kg}^{-1}\text{ soil)} &= [0.02 \text{ M} - (-0.00004 + (0.0502 \times 0.3087))] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L solution/ } 0.00248 \text{ kg}) \\ &= 329.75 \text{ mg POXC kg}^{-1}\text{ soil} \end{aligned}$$

Clean-up and Disposal

Leaving the centrifuge tubes capped but on the bench top for a week or more will allow the permanganate to completely react with the soil and lose all purple pigmentation. Liquid can then be safely disposed of down the sink and tubes with soil thrown out or cleaned and reused. The second dilution of samples and standards contains very little KMnO_4 and may be safely flushed down the drain with copious amounts of water. However, check with your environmental health and safety department to ensure compliance with your department's procedures.

Reference:

Weil RR, Islam KR, Stine MA, Gruver JB, Samson-Liebig SE. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture* 18:3–17.

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