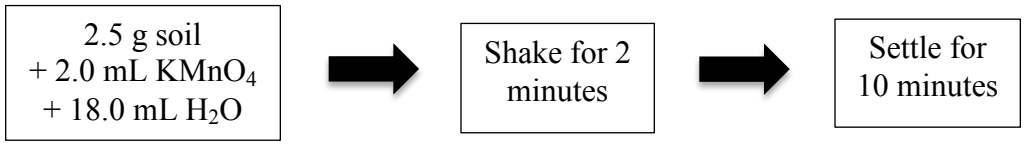
Procedure for the Determination of Permanganate Oxidizable Carbon

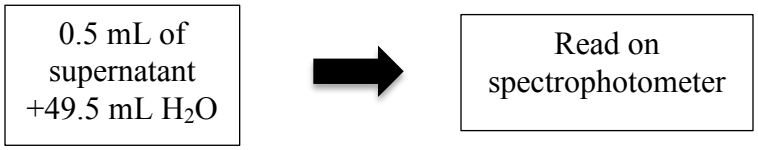
Procedure Overview:

This document describes a procedure 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). Soils that are air-dried and ground to <2 mm are typically used.

Sample Reaction



Sample Dilution



Instrumentation and Materials:

$KMnO_4$ Stock Solution Preparation

- Reagent grade Potassium Permanganate ($KMnO_4$; FW=158.03 g mol^{-1})
- Reagent grade Calcium Chloride, Dihydrate ($CaCl_2 \cdot 2H_2O$; FW=147.01 g mol^{-1})
- Magnetic stir plate and stir bars
- Laboratory glassware for reagent preparation and waste collection
- Brown laboratory glassware for reagent storage

Standard Preparation

- 50 mL disposable polypropylene centrifuge tubes with caps (Falcon tubes)
- (2) Adjustable bottle-top dispensers fitted to a bottle of deionized water and calibrated to deliver 18.0 mL and 49.5 mL
- Adjustable 10 mL and 100-1000 µL pipettor and tips

Sample Reaction and Dilution

- 50 mL disposable polypropylene centrifuge tubes with caps (Falcon tubes)
- Analytical balance capable of weighing to two decimal places
- Soil checks (pulverized, homogenous soil as lab reference samples)
- (2) Adjustable bottle-top dispensers fitted to a bottle of deionized water and calibrated to deliver 18.0 mL and 49.5 mL
- Adjustable 10 mL and 100-1000 µL pipettor and tips
- Oscillating (or horizontal) shaker capable of at least 180 oscillations per minute
- Timer capable of tracking time for two and ten minute intervals

Sample Quantification

- Clear polystyrene flat-bottom cell culture 96-well plates
- Adjustable 30-300 µL pipettor and tips
- Spectrophotometer capable of reading absorbance at 550 nm

Detailed Procedure:

I. 0.2 *M* $KMnO_4$ Stock Solution Preparation (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 slow and due to the dark color of this solution, it may be necessary to decant some of the solution to check for undissolved $KMnO_4$.
4. The original protocol (Weil et al., 2003) included a step to adjust the pH to 7.2. Gruver (2015) reported a rapid drop in stock solution pH within days after preparing and reported no differences of POXC values with manipulated pH levels of stock solutions. We have also measured this pH drop over time and therefore no longer recommend pH adjustments to the $KMnO_4$ stock solution.
5. Pour the solution into a 1000 mL volumetric flask or graduated cylinder and bring the volume to 1000 mL with the $CaCl_2$ solution.
6. Transfer to a brown glass bottle and store in a dark place. This stock solution can be used for up to 6 months.
7. 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* $KMnO_4$.
8. To make 4 liters of stock solution, add 588 g of $CaCl_2$ with 126.4 g of $KMnO_4$ to 3716 ml of deionized water and stir for 10-15 minutes until completely dissolved.

II. Standard Preparation:

Four solution standards (0.005, 0.01, 0.015 and 0.02 *M*) are prepared from the $KMnO_4$ stock solution. The standard preparation involves first making a standard stock solution and then diluting to a final working solution standard.

1. **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 *M*	0.25 mL	9.75 mL
0.01 *M*	0.5 mL	9.5 mL
0.015 *M*	0.75 mL	9.25 mL
0.02 *M*	1.0 mL	9.0 mL

2. **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 Reaction:

1. Label two 50 mL centrifuge tubes for each sample. One will be the **reaction tube**, the other the **dilution tube**. Weigh 2.50 g (± 0.05 g) of air-dried soil into the reaction tube (may be done in advance). Place the dilution tubes aside.
2. Soil checks should be prepared in the same manner as the unknown soils and serve as laboratory reference samples. It is recommended to pulverize and homogenize a large batch of air-dried soil for long-term use. The soil checks allow for a quality control check across POXC analyses performed on different batches, over multiple days and with different reagents.
3. Add 18.0 mL of deionized water to each of the reaction 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.
4. Working quickly, cap tubes tightly and place horizontally on a shaker at 180 oscillations per minute for 2 minutes ("low" setting on Eberbach reciprocal shaker).
5. After 2 minutes, remove samples from shaker and invert the tubes vigorously to ensure that there is no soil clinging to the sides of the tube. Next, remove caps to avoid further disturbance of soil after settling. Allow soil to settle for ten minutes. Settling time is a critical step so a timer is essential.
6. It is important that the timing of each step be consistent, particularly the shaking and settling times. The permanganate will continue to react as long as it remains in contact with the soil. Hence, working quickly with small batches of 10 or less samples is advised.

IV. Sample Dilution:

1. While samples are settling, add 49.5 mL of deionized water to the dilution 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) from the reaction tube to the corresponding dilution 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 dilution 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.

V. Sample Quantification:

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 (as outlined 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 two or more times and taking the average typically yields good results. Using a BioTek Epoch platereader, our percent error between wells averages 0.5%. Therefore analytic replicates (multiple wells containing same sample) are not used.
4. Dispense 200 microliters of each standard and unknown samples into each well of the 96-well plate.

5. Determine and record the absorbance (optical density) of standards and unknowns at 550 nm using spectrophotometer software. Sample absorbance has a broad spectrum and reports of using different wavelengths (e.g., 540 nm) have yielded results consistent with 550 nm.
6. Subtract out average of deionized water blanks from all absorbance values. The intercept of the standard curve should be very close to zero.

VI. 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):

POXC (mg kg^{-1} soil) =

$$[0.02 \text{ mol/ L} - (a + b \times \text{Abs})] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L rx solution/ Wt})$$

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

POXC (mg kg^{-1} soil) =

$$[0.02 \text{ } M - (-0.00004 + (0.0502 \times 0.3087))] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L rx solution/ } 0.00248 \text{ kg})$$

= **329.75 mg POXC kg^{-1} soil**

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 can be 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 institution's procedures.

References:

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