# SOP: Permanganate Oxidizable Carbon (POXC)

## **Overview**:

This standard operating procedure (SOP) describes a protocol for a potassium permanganate ($KMnO_4$) oxidation of organic matter to characterize the pool of soil organic matter that is thought to be "biologically active (Culman et al., 2021), though recent research in model systems demonstrates that POXC is sensitive to polyphenols and lignin (Woodings and Margenot 2023; Christy et al., 2023). Dilute oxidations of organic matter using $KMnO_4$ are common in soil organic matter characterization (Matsuda and Schnitzer, 1972; Lefroy et al., 1993; Blair et al., 1995), but recent implementations have focused on its potential as a rapid and inexpensive soil health indicator. This method was originally proposed by Weil et al. (2003), but most current implementations use the modification by Culman et al. (2012). The procedure requires $KMnO_4$ and $CaCl_2$ reagents and a spectrophotometer. Soils that are ground to pass a < 2 mm sieve are recommended (Hurisso et al., 2018; Pulleman et al., 2020; Wade et al., 2020).

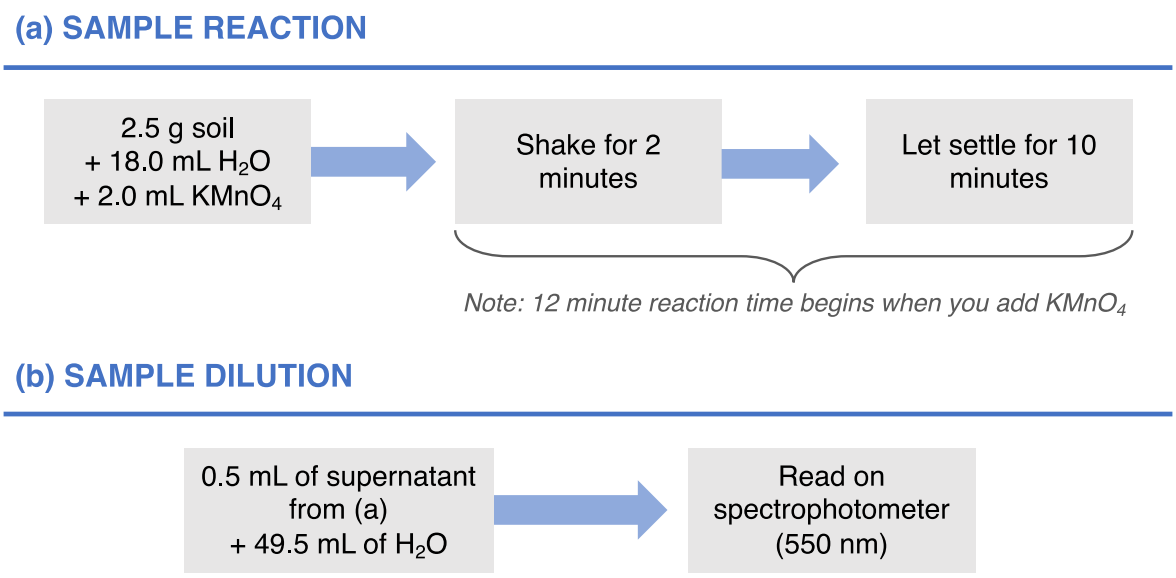


Figure 1. Overview of POXC protocol and the two overall steps of (a) sample reaction and (b) sample dilution.

## **Safety:**

All standard safety protocols and online safety training via UIUC Division of Research Safety (DRS) are required.
Personal protection (PPE) for this procedure include:
Eye Protection: $KMnO_4$ can cause severe eye irritation or burns. No eye covering is required given proper safety protocols.

Body Protection: $KMnO_4$ reacts with organic substances and will stain skin and clothing a dark brown. A lab coat is recommended.
Hand Protection: $KMnO_4$ will cause skin irritation and slight discoloration. No additional PPE is required, although gloves are recommended for those with sensitive skin.
Particularly hazardous substances: None.
Specific details on these substances are incorporated in the **Detailed Procedure** below.

## Instrumentation & Consumables:

**$KMnO_4$ 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
- Adjustable bottle-top dispensers (i.e. dispensette) fitted to bottles of deionized water, calibrated to deliver 18.0 mL and 49.5 mL
- Adjustable 10 mL pipettor and tips
- Adjustable 100-1000 µL pipettor and tips

**Sample Reaction and Dilution**
- 50 mL disposable polypropylene centrifuge tubes with caps
- Analytical balance capable of weighing to two decimal places
- Soil checks (pulverized, homogenous soil as lab reference samples)
- 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 pipettor and tips
- Adjustable 100-1000 µL pipettor and tips
- Oscillating (or horizontal) shaker capable of at least 120 oscillations per minute. *Note: shaking frequency is positively related to POXC values.*
- Timer capable of tracking time for two- and ten-minute intervals

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

## Detailed Procedure:

I. **$KMnO_4$ Stock Solution (makes 1 L of 0.2 M stock solution)**
   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.

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 $KMnO_4$.*
   *Note: the original protocol* (Weil et al., 2003) *included a step to adjust the pH to 7.2. However, both Gruver* (2015) *and Culman et al.* (2020) *report that the pH adjustment lasted < 24 hours. Therefor pH adjustment to the $KMnO_4$ stock solution is no longer recommended.*
4. Pour the solution into a 1000 mL volumetric flask or graduated cylinder and bring the volume to 1000 mL with the $CaCl_2$ solution.
5. Transfer to a brown glass bottle and store in a dark place.
   *Note: This stock solution can be used for up to 6 months if kept in the dark and refrigerated.*
6. 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 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. 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.

| Standard Stock Solution concentration | Volume of $KMnO_4$ stock solution | Volume of DI water |
|---|---|---|
| 0.005 M | 0.25 mL | 9.75 mL |
| 0.010 M | 0.50 mL | 9.50 mL |
| 0.015 M | 0.75 mL | 9.25 mL |
| 0.020 M | 1.00 mL | 9.00 mL |

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

sieved, air-dried soil into the reaction tube (may be done in advance). Place the dilution tubes aside.
*Note: at least two analytical replicates (i.e., two separate 2.5 g of soil subjected to oxidation) should be performed for each soil sample.*

2. Soil checks should be prepared in the same manner as the unknown soils and serve as laboratory reference samples.
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 120 oscillations per minute for exactly 2 minutes.
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 or the cap. Next, remove caps to avoid further disturbance of soil after settling. Allow soil to settle for exactly 10 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*. This step may be done in advance.
2. Once the 10-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. This will create a 1:100 dilution.
3. Cap dilution tubes and invert once 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 ($F_{1,10} = 1.7$, $p = 0.225$) (Wade et al., 2020). If available, a 96-well plate reader is recommended to save time (as outlined below).
2. It is recommended to replicate all standards on a plate 2-3 times, including deionized water blanks. Running each standard two or more times and taking the average of the replicates typically yields better results.

3. Dispense 200 µL of each standard and unknown samples into each well of the 96-well plate.
4. 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.
5. Subtract out average of deionized water blanks from all absorbance values. The intercept of the standard curve should be very close to zero.

## VI. Clean up and Disposal

Leaving the reaction 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.

## VII. Calculations

The amount of $Mn^{7+}$ reduced is inversely proportionate to the concentration of $MnO_4^-$ at the end of the reaction step (2 min shake + 10 min settle). The more $Mn^{7+}$ reduced, the lower the absorbance and the lower the intensity of the purple color. The most recent recommendations for POXC suggest that it is most appropriately reported as mol $MnO_4^-$ reduced $kg^{-1}$ soil (usually in mmol) (Wade et al., 2021; Woodings and Margenot 2023). The calculation is as follows:

*Equation:*

$$mol\ MnO_4^-\ reduced\ kg^{-1}\ soil = \frac{\left[0.02\ mol\ L^{-1} - \left(a + \left(b\ \times\ Abs_{adj}\right)\right)\right] \times\ 0.02\ L\ solution}{mass}$$

Where:

0.02 mol $L^{-1}$ is the initial concentration of $MnO_4^-$ in the *reaction tube*

*a* is the intercept of the standard curve

*b* is the slope of the standard curve

$Abs_{adj}$ is the adjusted absorbance of the unknown sample (raw absorbance – the average of the three deionized water blanks)

0.02 L is the volume of solution in the *reaction tube*

*mass* is the mass of air-dried soil in the *reaction tube* (in kg)

Notably, this equation does not assume that 9000 mg C $mol^{-1}$ $MnO_4^-$ can be applied as a conversion factor between $MnO_4^-$ reduction and C oxidation. This value incorrectly assumes that $Mn^{7+}$ reduces to $Mn^{4+}$ and that $C^0$ oxidizes to $C^{4+}$ (Gruver, 2015; Wade et al., 2021; Woodings and Margenot 2023), which was incorrectly reported in the original protocol by Weil et al. (2003) as oxidation from $Mn^{7+}$ to $Mn^{2+}$. Additional discussion on the oxidation reduction stoichiometry can be found in Woodings and Margenot 2023.

## Example calculation:

1. Construct standard curve

| x-axis *(Molarity of stock $KMnO_4$ standards)** | Y-axis *(Blank-adjusted absorbances from spectrophotometer)* |
|---|---|
| 0.005 | 0.1000 |
| 0.010 | 0.1984 |
| 0.015 | 0.3034 |
| 0.020 | 0.3966 |

*Note that the standard curve should use the molarity of the stock standards and not the working standards, since the stock standard represent that concentration that reacts with the soil in the *reaction tubes*.

The resulting standard curve is: $Y = 0.05024x - 0.00004$; $R^2 = 0.9995$

2. Calculate Unknown sample value (mol $MnO_4^-$ reduced $kg^{-1}$ soil)

   *If:* the adjusted absorbance of the unknown sample = 0.3087 and the mass of the unknown soil sample = 2.48 g

   *Then:* mol $MnO_4^-$ reduced $kg^{-1}$ soil

   = [0.02 M – (-0.00004 + (0.05024 x 0.3087))] x 0.02 L / 0.00248 kg soil

   = 0.0365 mol $MnO_4^-$ reduced $kg^{-1}$ soil or 36.54 mmol $MnO_4^-$ reduced $kg^{-1}$ soil

3. Calculate Unknown sample value (mg C $kg^{-1}$ soil)
   *If:* the unknown sample = 0.0365 mol $MnO_4^-$ reduced $kg^{-1}$ soil

   *Then:* mg C $kg^{-1}$ soil
   = 0.0365 mol $MnO_4^-$ reduced $kg^{-1}$ soil x (9000 mg C oxidized $mol^{-1}$ $MnO_4^-$)
   = 328.6 mg C $kg^{-1}$ soil

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## **Citation**:

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