Active Carbon

Purpose and Justification:

Active Carbon (also known as Permanganate-Oxidizable Carbon or POXC) measures the portion of soil organic matter that can serve as a readily available food and energy source for the soil microbial community, thus helping to maintain a healthy soil food web. To measure Active Carbon, soil is reacted with a potassium permanganate solution which has a deep purple color. As the solution oxidizes, it loses some of its color. This loss of color upon reaction is directly proportional to the amount of Active Carbon in the soil sample, which is determined by using a spectrophotometer and calibrated against standards of known concentration.

Background / References:

This method is intended as a rapid and repeatable approach to estimating a labile portion of soil organic carbon, which is sensitive enough to detect different management practices across soil types and climatic zones. An alkaline, $KMnO_4$ solution oxidizes easily accessible soil carbon fractions, with greater diminishment of purple color – due to conversion of Mn(VII) to Mn(II) – corresponding to increasing concentrations of "active carbon", measured as absorbance at 550 nm. Our testing is geared primarily to agronomic soils, therefore we test the whole soil fraction (all soil passing 2 mm) and use 2.5 g samples, which provides a suitable detection range and consistent results for soils with less than 10% soil organic matter.

Weil, R., Islam, K. R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E. *Estimating active carbon for soil quality assessment: A simple method for laboratory and field use*. Amer. J. Alternative Agric. 18(1):3-17 (2003).

Stiles, Cynthia A., et al. "Validation testing of a portable kit for measuring an active soil carbon fraction." *Soil Science Society of America Journal* 75.6 (2011): 2330-2340.

Wade, J., et al. "Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils." Geoderma 366 (2020) 114235

Objective:

Duplicate soil samples are air dried to constant weight, shaken with 0.02 $KMnO_4$ solution, allowed to settle, diluted and absorbance measured at 550nm.

NOTE: ***For quarantined soils, see labeled procedures in italics at the bottom of each section.***

Materials and Equipment:

Potassium Permanganate ($KMnO_4$)
Calcium Chloride Dihydrate ($CaCl_2$)
0.5M Potassium Hydroxide Solution (KOH) for pH adjustment
0.1M Hydrochloric Acid (HCl) for pH adjustment
Distilled H_2O
50 mL Falcon tubes w/ caps
Falcon tube racks (24 tubes per rack)
Bottle-top solution dispenser (50 mL)
pH meter and buffered calibration solutions
Analytical balance (3 digit)
Colorimeter (w/ 550 nm setting)
Kimwipes ®
100-1000 µL pipettor and disposable tips
10 mL pipettor and disposable tips
Repeater pipettor with 50 mL reservoir tip
Platform shaker
Stop watch
Stir plate
Stir bar
1000 mL volumetric flasks, beakers and graduated cylinder
Amber bottle
Plastic Bin
Ethyl Alcohol, 70%
Autoclave Bags

Procedure:

Safety Considerations and Protocols:

1. When working with dry soil a mask should always be worn.
2. When preparing the stock solution a mask, nitrile gloves and eye protection should always be worn.
3. This procedure should be done on the lab bench top.
4. When using the stock solution nitrile gloves, a lab coat, and eye protection should be worn. The solution, while dilute, is an oxidizer and will stain skin and clothes.
5. Be aware of locations of PPE, first-aid kit and contact info for EHS and Lab management.

Sterilization solutions approved for use with Quarantined soil:
Bleach-10% Bleach solution within a labeled spray bottle must be left on contaminated equipment for ½ hour before rinsing.
Ethyl Alcohol 70% within a labeled spray bottle must be left on contaminated equipment for 30 minutes before rinsing.

Additional Quarantine Protocols:

1. ***In the event of spilling quarantine soil, the soil should be swept up using a hand broom and dustpan and disposed of in an autoclave bag. The hand broom and dustpan as well as any surfaces contaminated with Q soil should be heavily sprayed down with 70% ethanol. The alcohol should be left on surfaces for 30 minutes.***
2. ***Always work within a secondary container when working with quarantined soil.***

Preparation of 0.2M $KMnO_4$ stock solution

1. Dissolve 147.01g $CaCl_2$ dihydrate in ~750 mL distilled water in a volumetric flask. Dissolve completely, using stir plate with stir bar or by hand shaking. Once the solid $CaCl_2$ is dissolved, bring the flask to 1000 mL with distilled water (final concentration, 1M)

Note: $CaCl_2$ is included in the stock solution as a flocculating agent, which is intended to eliminate the need for centrifugation. The reaction of $CaCl_2$ with water is an exothermic process. It will warm the solution as it dissolves, reducing the need for a stir plate.

2. In a separate volumetric flask, add ~750 mL of the 1M $CaCl_2$. Add 31.61 g $KMnO_4$ to the solution and bring the flask to 1000 mL total volume with the 1M $CaCl_2$ solution. Allow to dissolve completely (about one hour or up to overnight), covering solution and stir plate with an opaque box or paper bag to protect it from as much light as possible.
3. Ensure that the pH meter is properly calibrated.
4. Measure solution pH (final pH should be 7.2).
5. Depending on pH measure, make a dilute (~0.1M) acid or base solution using

HCl or KOH. Using a pipettor, slowly add acid or base, while monitoring pH, until constant at 7.2.

6. The solution is light sensitive - add to an opaque bottle, label and date. Solution should remain stable for 3-6 months. Store cold in the refrigerator.

*Standard curve (**see Figure 1 below** for schematic of methodology)*

1. Ensure that the colorimeter is set to 550 nm and zero with distilled water.
2. Dispense 45 mL distilled water into each of three Falcon tubes.
3. Add additional distilled water to the tubes in the following volumes:
   - tube 1, 3.75 mL
   - tube 2, 2.50 mL
   - tube 3, 0.00 mL
4. Then add 0.2M $KMnO_4$ to the tubes in the following volumes:
   - tube 1, 1.25 mL
   - tube 2, 2.50 mL
   - tube 3, 5.00 mL

   Final concentrations of 50 mL $KMnO_4$ solutions are now 0.005M, 0.01M, 0.02M. Cap and shake for 10 seconds.
5. Dispense 20 mL distilled water into 9 Falcon tubes – three for each standard solution.
6. Add 0.202 mL of each standard to each respective triplicate set. Cap and shake for 10 seconds.
7. Read and record the absorbance of each triplicate standard, filling the cuvette with one volume of standard and cleaning the outside with a Kimwipe to remove any liquid or smudges before each reading.

*Measuring Active Carbon in Soil Samples (see **Figure 2 below** for laboratory schematic)*

1. Soil samples should have previously been air dried to constant weight and sieved past 2 mm (see procedure CSH 01). ***Do all laboratory procedures inside a plastic tub and sterilize any laboratory equipment that comes in contact with quarantined soil.***
2. Each soil sample is run in duplicate, requiring 2 opaque Falcon tubes for the reaction and 2 clear Falcon tubes for dilution and reading. Typically, 24 samples per rack are run (11 duplicate soil samples and 1 duplicate quality control).
3. Dispense 20 mL distilled water into 2 clear Falcon tubes for each soil sample that will be tested and set aside.
4. Into opaque Falcon tubes, measure two 2.5 g replicates for each soil sample. (±.005g)
5. Dispense 0.2M $KMnO_4$ solution into a beaker in small amounts as needed (~50 mL volumes) and cover with an opaque container to block light.
6. In sequence, add 18 mL distilled water to each opaque tube containing soil. Then, in same sequence, begin redox reaction by adding 2 ml of 0.2M $KMnO_4$ to each tube using the repeating pipettor. Note the time required to smoothly move from tube one through tube 24 (about 1 minute). Cap tightly.
7. Place tubes and rack on the shaker at 120 rpm, start stopwatch and allow to shake

for 2 minutes.

8. After 2 minutes (do not stop stopwatch), remove samples from the shaker and 'slosh' solution in tubes to ensure that soil is not stuck to the cap or top of the tube. Uncap tubes. On bench-top, allow settling and reaction to continue for a further eight minutes.
9. After 10 minutes of total reaction time, remove 0.2 mL from each reaction tube and transfer to a Falcon tube with 20 mL distilled water. **Note**: dispensing this 0.202 mL aliquot from the reaction tube into 20 mL distilled water is a 100x dilution; *this ends the reaction*. The time spent moving from tube one to tube 24 should be nearly one minute (see step 6). A consistent rate of delivery keeps the reaction time at 10 minutes for all of the samples in the set.
10. After all reactions have been stopped, cap diluted sample tubes and shake by hand for 10 seconds.
11. Read and record absorbance as described above.
12. **Note:** Repeat duplicates with a coefficient of variance in absorbance greater than 10%. Use all four replicates for the average active carbon for these samples.
13. Clean all materials (particularly colorimeter cuvette) using distilled water.

Note: Avoid pouring waste soil and permanganate solution down the drain. The vials used in the reaction containing soil and permanganate solution, the residual stock solution on the repeating pipettor, and in any beakers used should be rinsed into a waste glass container. All waste permanganate solution and soil remaining in vials after testing is collected and stored in glass containers until it is disposed of by a professional waste disposal service. ***Sterilize all quarantine laboratory equipment that encounters quarantined soil with disinfection solution. Sterilize all liquid that encounters soil with 10% bleach solution for 30 minutes.***

Calculations:

The bleaching (loss of purple color; reduction in absorbance) of the $KMnO_4$ is proportional to the amount of oxidizable C in the soil sample. It is assumed that 1 mol MnO_4 is consumed (reduced from Mn^{7+} to Mn^{2+}) in the oxidation of 0.75 mol (9000 mg) of C.

Standard curve:

Concentration = a + b * (absorbance). Determine the slope (b) and y-intercept (a) of a linear regression equation with concentration as the dependent variable (y) and absorbance as the independent variable (x).

Active carbon:

Active C (mg/kg) = [0.02 mol/L - (a + b * absorbance)] * (9000 mg C/mol) * (0.02 L solution/0.0025 kg soil).

Where:

0.02 mol/L is the initial solution concentration,

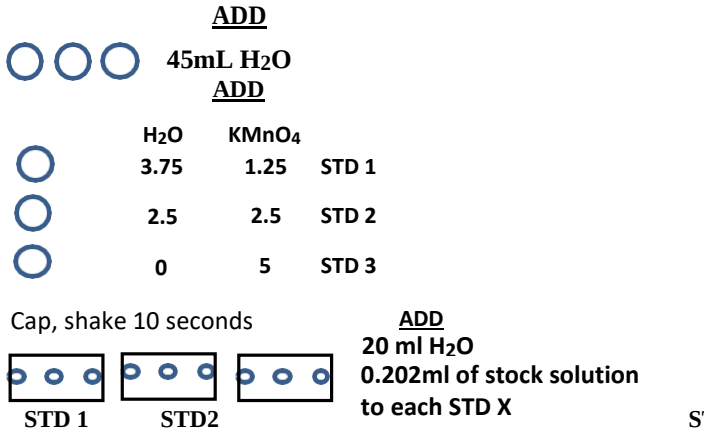
(a + b * absorbance) is the post-reaction concentration, calculated from the standard curve

9000 mg of C (0.75 mol) is assumed to be oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{2+},

0.02 L is the volume of $KMnO_4$ solution reacted, and 0.0025 kg is the weight of soil used.

Figure 1. Schematic of bench top protocol for $KMnO_4$ dilution for development of a standard curve in the Active Carbon test. Three standards are created and are diluted in triplicate.

Active Carbon Standards Diagram



Standard Curve data

Conc	Abs	target
0.005		0.105
0.005		
0.005		
0.01		0.210
0.01		
0.01		
0.02		0.415
0.02		
0.02		

Figure 2. Laboratory schematic of Active Carbon testing procedure/data collection.

Duplicate readings for each sample (11 samples and 1 quality control sample = 24 tubes) in each rack

ADD

Add to each tube **2.5g sample** (remember to duplicate)

Add to each tube **18ml H_2O**

Finish filling all tubes, THEN add **2ml $KMnO_4$.** Cap quickly. Put rack on shaker. Start stopwatch. Shake for 2 mins on shaker at 120rpm. Keep stopwatch running. Uncap tubes, wait 8 more minutes for 10 minute total reaction time.

WHILE SHAKING, prepare 20 more tubes with **20 mL H_2O**

End reaction by adding **0.202 mL of reaction solution** to tube with 20 mL H_20. Just rinse pipette tip between samples.

Cap diluted sample, shake 10 seconds, read absorbance.

ID	Rep	Abs.
	1	
	2	
	1	
	2	
	1	
	2	
	1	
	2	

Quality Control/ Standards:

The Quality Control (QC) standard soil (Lima silt loam, 0-6" depth, air dried to constant weight, sieved past 2mm) is run in a set of 18 replicated samples to determine the reproducibility of the test.

Table 1 below lists the descriptive statistics from the 2020 standard soil Quality Control (QC) experiment. Each sample has two replicate measurements and a mean is calculated. The grand mean of this QC set plus and minus two standard deviations is used as a criteria for determining the range of acceptability for any data set. For the two QC replicates in any sample set, their mean must fall within the acceptable range.

From Figure 2 above a data sheet can be created which inserts a routine position in each set of samples for this QC standard sample. If this standard result falls outside the expected range, the entire data set is rejected and is re-run.

Table 1. Lima silt loam soil standard descriptive statistics for the Active Carbon test

ID	Active C (mg/kg)
1	681.8
2	717.8
3	671.1
4	660.3
5	665.2
6	654.4
7	706.0
8	646.6
9	604.7
10	650.1
11	648.0
12	658.7
13	725.3
14	764.1
15	711.1
16	760.6
17	711.1
18	725.3
19	667.5
20	625.8
21	723.1
22	684.9
23	671.0
24	639.7
25	664.0
26	716.1
27	667.5
28	664.0
29	691.5
30	702.2
31	730.6
32	698.6
33	744.7
34	737.7
35	680.7
36	684.2

Mean	687.7
Standard Error	6.3
Median	683.0
Standard Dev.	37.7
Range	159.5
Minimum	604.7
Maximum	764.1
Count	36
mean	**687.7**
mean MINUS 2 SD	**612.2**
mean PLUS 2 SD	**763.1**