

Standard Operating Procedure

Soil Inorganic Carbon by Modified Pressure Calcimeter

Scope

This document outlines the measurement of inorganic carbon (IC) concentrations in mineral soil samples by pressure calcimeter. This method is very well described in Fomesbeck, Boettinger and Lawley, (2013). The measurement procedure is described in general terms, as apparatus-specific operations will vary. Soil sample preparation is the same as for [total carbon and nitrogen by dry combustion](#), and the same ground sample can be used for both analyses. Inorganic C concentration should be reported on an oven-dry (105 °C) soil mass basis.

Equipment

125 mL Wheaton serum bottle
Two-prong, gray butyl rubber septum
Aluminum tear off seal, 20 mm
Bottle seal crimper, 20 mm
Bottle top dispenser
2.5 L bottle
5 mL syringe with a 22-gauge, 1 in. (2.54 cm) long hypodermic needle
18-gauge, 1 in. (2.54 cm) hypodermic needle
Pressure transducer, 0-15 PSIG range, 0.03 to 5.03 V DC
Multimeter
24 V AC transformer
Chemical resistant tubing, 0.25 in. inner diameter ($\frac{1}{4}$ "), 0.375 in. outer diameter
0.6 μm particulate filter
Weigh boat

Reagents

Calcium carbonate, CaCO_3 reagent grade, dried @105 °C
3 M hydrochloric acid for fizz test
6 M hydrochloric acid containing 3% $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ by weight
Deionized (DI) water
Silica sand, lab-grade
Soapy water solution (~30 mL of commercial dishwashing liquid mixed into 1 L water)

Procedure

Part 1) Identification of samples requiring inorganic carbon testing.

Soils should be air-dried and ground to pass through a 2 mm sieve as described in the total carbon standard operating procedure. If the soil sample does not have a pH analysis, that is ok, a simple fizz test works on any soil. We suggest a prior pH reading to reduce labor in a high throughput situation.

1. OPTIONAL. Analyze soil pH with the 1:2 H₂O method (one part soil to two parts water by weight). Chose only samples with pH greater than 6.5 for the fizz test.
2. Perform a fizz test on the dried and ground soil sample by dropping 1N HCL from a dropper bottle into a small (quarter-sized soil sample). Observe visually and by sound, to see if the soil reacts to the acid. Record the level of effervescence as “None”, “Slight”, “Moderate”, or “Strong” effervescence. Samples with “None” do not require the inorganic carbon test as part of soil organic carbon determination.
3. For samples with “Strong”, “Moderate”, and “Slight” effervescence, weigh a 1.00, 2.00, or 10.00 g sample (Table 1) and proceed with Part 2. (Note: samples containing inorganic carbon in the form of dolomite [CaMg(CO₃)₂] may not fizz upon addition of hydrochloric acid. If dolomite is suspected based on soil parent material or amendment history, proceed assuming “Slight” effervescence).

Table 1: Amount of soil to use based on effervescence level and associated masses of CaCO₃ standards. These standards can be made in batches ahead of time and used according to the reference level of each soil sample to be measured for inorganic carbon.

Effervescence Class	Amount of dried ground sample (g)	CaCO ₃ Standards (g)
Strong Effervescence	1	0.020, 0.040, 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00
Moderate Effervescence	2	0.025, 0.05, 0.10, 0.20, 0.50, and 1.00
Slight Effervescence	10	0.025, 0.05, 0.10, 0.20, 0.50, and 1.00 [§]

[§]Lab-grade silica sand should be mixed with carbonate standards to achieve a total mass of 10.00 g to match the volume of soil in the 10.00 g samples.

Part 2) Pressure calcimeter configuration

The setup for this measurement requires creating a pressure calcimeter. This setup is inexpensive, straightforward and requires little maintenance. The pressure calcimeter needs a multimeter, pressure transducer, filter, and hypodermic needle (Figure 1). Parts and specs for building the calcimeter can be found in [Sherrod et al. \(2002\)](#) and briefly described below.

The pressure transducer is connected to a power supply (24 V DC) with 14-gauge wire. The digital voltmeter is wired inline to monitor transducer output. Attached to the base of the pressure transducer

is 20 cm of clear laboratory tubing (0.25 in. inner diameter) connected to an 18-gauge hypodermic needle with a particle filter (0.6 μm for removing particles from carrier gas) spliced in the middle of the tubing to prevent any reflux from reaching the pressure transducer. A 125 mL Wheaton serum bottle (Wheaton Science Products, Millville, NJ) serves as the reaction vessel for soils.

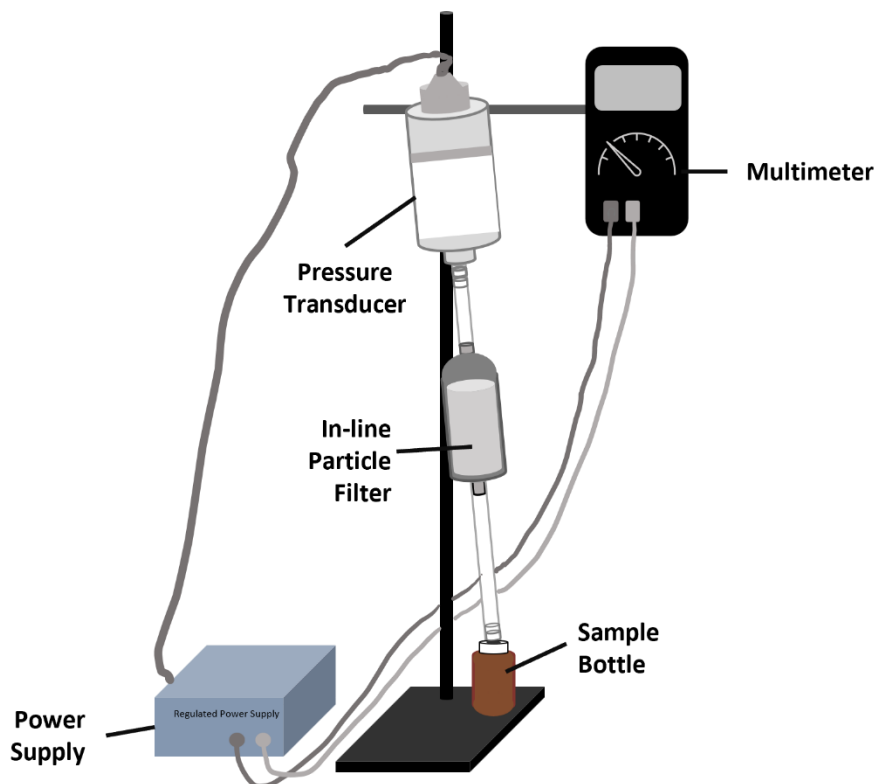


Figure 1. Setup of a modified pressure calcimeter.

Part 3) Procedure for creating a calibration curve for sample quantification

- 1) Group samples by effervescence level for streamlining the following steps.
- 2) Homogenize each sample and transfer the correct weight of soil based on effervescence level (Table 1) to tared weigh boat. Record weight to 0.0001 g and transfer to a numbered serum bottle. You may choose to analyze many samples (30 to 90) in a batch.
- 3) Include a set of control samples that have been created with the corresponding level of effervescence of the samples being analyzed and CaCO_3 concentration (Table 1), at the beginning, at an interval of every 30 samples, and at the end of the samples. Record the weights of the controls.
- 4) For slight effervescing samples, weigh approximately 0.50 g CaCO_3 after the last control and record the weight to 0.0001 g. Then add lab-grade silica sand to the weigh boat from Step 3 until the final weight is 10 g and transfer to a numbered serum bottle.
- 5) Include three empty serum bottles as blanks at the beginning of the batch and one at the end. The readings from the blanks will be included later when generating the standard curve.

- 6) Weigh and record CaCO_3 weights (g) for standards corresponding to the correct effervescence level (Table 1).
- 7) Using a bottle top dispenser, add 5 mL DI water to each bottle (including standards). Soil should be completely moistened.
- 8) Insert into each serum bottle a two-leg stopper, ensuring that one leg is lined up with the bottle number.
- 9) Cap with aluminum tear-off cap and secure using crimper, ensuring that the cap will open to the left while looking at the bottle number, on all bottles.
- 10) Swirl contents.
- 11) Peel the center of the cap to the left exposing the septum.
- 12) Place bottles in fume hood, and using a syringe, inject 4 mL of acid reagent into the serum bottle. Insert the needle into the septum through the “front leg” to reduce the chance of leakage.
- 13) Remove the needle quickly to reduce loss of pressure.
- 14) After each injection, turn the serum bottle 90 degrees to signify the next sample.
- 15) Using a transfer pipet, place a drop of the soapy water solution on the injection site to identify a leak. If bubbles form, reweigh and repeat steps beginning with Step 2.
- 16) Swirl bottles after injection and then every hour after for a total reaction time of 6 hr.
- 17) Turn on the multimeter at least 15 min before reading pressures.
- 18) After 6 hr, insert the needle connected to the pressure transducer into the septum through the “back leg”. Start with the blanks, standards, and then read and record samples.
- 19) Start all readings with the meter set to 2000 mV DC. If the meter reading rises then shows only lines, move the meter setting to 20 V DC for the higher range.
- 20) Reading will stabilize in less than 10 seconds. Record the reading.
- 21) After all samples have been read, insert the venting needle to release any remaining pressure.
- 22) Remove aluminum caps and septum and pour contents with rinsing into a 3-gallon plastic bucket placed in a sink.
- 23) Neutralize content by slowly adding sodium bicarbonate until effervescence stops.
- 24) Plot the weights of the standards as X, and their corresponding voltages as Y, including the mean voltage of the 4 blanks whose CaCO_3 weight is 0 g. The resulting equation will then be applied to the unknowns.
- 25) The generated curve should be linear and appear in the form of $y = bX + a$ where b is the slope of the line and a is the intercept.
- 26) Determine the concentration of unknown samples using the linear regression equation generated in the previous step by solving for X (CaCO_3 g).
- 27) Calculate the ratio of air-dried (AD) to oven-dried (OD) soil weights and multiply by the CaCO_3 weight calculated in Step 26. Multiple by 100 to derive % CaCO_3 .
- 28) Multiple % CaCO_3 by 0.12 to determine % inorganic carbon.

Calculations

1. $AD/OD = (\text{mass of air-dry soil and tin} - \text{mass of empty tin}) / (\text{mass of oven-dry soil and tin} - \text{mass of empty tin})$. Commonly referred to as a moisture correction. If the sample submitter requests that this value be included in their data report, round to a minimum of three decimal places (e.g., 1.028).
2. $Y = bX + a$
where:
Y = voltage from unknown sample
b = slope from linear regression equation
X = CaCO_3 (g) from unknown sample
a = intercept from linear regression equation
3. $\% \text{CaCO}_3 = (\text{CaCO}_3 \text{ (g)}/\text{sample weight (g)}) \times AD/OD \times 100$
4. $\% \text{Inorganic carbon} = (\% \text{CaCO}_3) \times 0.12$

QA/QC

1. The standards should create a very straight line, if there is noise around the line this gives you an idea of the quality of the standards and if there are leaks in the system. If the points about the line make a nonlinear trend, check the standards, and assess possible temperature gradients (remember ideal gas law $PV = nRT$).
2. Make sure there is no extrapolation, sometimes the fizz test will over or underestimate carbonate concentration in a soil sample. If the pressure of the soil sample is outside the range of your calibration line, redo that sample with the appropriate set of standards.
3. Report the R^2 and RMSE of the calibration line used to determine soil inorganic carbon.
4. After every 20 samples, run a duplicate analysis on one of the preceding 20 samples selected at random. Include data for QC duplicates in final data report. The difference in IC concentration between duplicate readings of the same sample should be less than 20% of the average of the two readings.
5. In addition to instrument-specific calibrations, which may not use soil materials, analysis of a minimum of four soil standard reference materials is recommended every six months.

References

Sherrod, L.A., G. Dunn, C.A. Peterson, and R.L. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calimeter method. *Soil Sci. Soc. Am. J.* 66:299-305. doi:10.2136/sssaj2002.0299

Fonnesbeck, B.B., J.L. Boettinger, J.R. Lawley, 2012. Improving a simple pressure-calimeter method for inorganic carbon analysis. *Soil Sci. Soc. Am. J.* 77:1553-1562. doi 10.2136/sssaj2012.0381

NOTE

This SOP was developed by SHI, for SHI communication, and developed after the references listed above. For any specific questions, contact erieke@soilhealthinstitute.org