Standard Operating Procedure for TEMPEST Fe-OM Experiments

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# Purpose

To describe the various lab procedures and analyses such as using the glovebox, preparing samples for the Shimadzu TOC-L analyzer, preparing samples for the Aqualog (CDOM), preparing samples for Ferrozine assay, size fractionating colloids via centrifugation, and washing anoxic/oxic soil samples. We intend to streamline procedures and produce a method that can be easily reproduced.

# Scope

We wrote this procedure for Connor O’Loughlin’s winter/spring SULI project. This experiment was a branch off an experiment conducted to investigate how solutions of varying ionic strength act as a mechanism for DOC leeching in upland forest soils. This project focused on one ionic strength and investigated if Fe-OM complexes were a significant factor in DOC release in upland forest soils and whether changing redox conditions influenced the magnitude of DOC release as well as the temporal release. Samples were size fractionated to observe the colloidal fragments of the leachate.

# Vials and samples

* 3 replicates per treatment
  + 2 treatments: Oxic and Anoxic
* 1 salinity treatment
  + 25% ASW (diluted from 35% stock solution)
* 3 size fractions
  + >1 mm
  + 1-0.45 mm
  + 0.45-0.1 mm
* 2 blanks
  + 1 25% ASW blank
  + 1 DI water blank
* There will be 8 vials to start with and 6 will need soil.

# 35% ASW recipe and procedure

This recipe and procedure were taken from:

*PREPARATION OF ARTIFICIAL SEAWATER1*. (n.d.). <https://doi.org/10.4319/lo.1967.12.1.0176>

1. Add Gravimetric Salts to a (insert container type) with about 2/3 the mass of water needed.
   * 1. Add the water volumetrically since water has a density of 1 g/mL (total water contribution will be about **957 grams**).
     2. I just went down to 900 grams so it would be an even 600 and 300 mL split then added the extra 43 grams/mL gravimetrically till I reached 1 kg.
   1. NaCl: 23.926 grams
   2. Na2SO4: 4.008 grams
   3. KCl: 0.677 grams
   4. NaHCO3: 0.196 grams
   5. H3BO3: 0.026 grams
   6. NaF: 0.003 grams
   7. KBr: 0.098 grams
      1. H3BO3 and NaHCO3 can be left hydrous. The other salts need to be prepared anhydrous (dried and then weighed).
2. In another (insert container type) add the volumetric salts to the other 1/3 of water
   * 1. Add the water volumetrically since water has a density of 1 g/mL (@ 25°C (total water contribution will be about **957 grams**)).
   1. MgCl x 6H2O: **20.333 grams** to make 100 mL or 1.0 M solution.
      1. We need **53.27 mL** of this stock solution added to the ASW.
   2. CaCl2 x 2H2O: **14.703 grams** to make 100 mL of 1.0 M solution.
      1. We need **10.33 mL** of this stock solution added to the ASW.
   3. SrCl2 x 6H2O**: 1.332 grams** to make 50 mL of 0.1 M solution.
      1. We need **0.09 mL** of this stock solution added to the ASW.
3. **ATTENTION!** In order to avoid CaCO3, CaSO4, SrCO3, or SrSO4 from precipitating from the solution we had to separate the gravimetric salts and volumetric salts and make two separate solutions.
   1. Combine both solutions while stirring.
      1. Use a stir plate and stir bar.
4. In the end we should have 1 kg or 1 liter of 35% ASW that we will dilute to 25% ASW during the initial ASW wash.
5. I think I will need about 3 L just to be safe. 2 will be deoxygenated and 1 will be oxygenated

# Centrifugation Key

This section of the SOP is a reference for time and RCF speeds used to centrifuge for the size fractions in section 3.

Table

Description automatically generated

Note: We used the 15,000 RCF and 9-minute method to fractionate for 0.45-0.1 mm. The 17,000 RCF was added for reference.

Initially, the method was 22,040 RCF for 6 minutes. This is fine for the Sorvall ST Plus Series centrifuge since it has an upper RCF limit of 25,830 x g (lower limit is 300 RCF) but the centrifuge tubes have an upper limit of 15,000 x g.

To adjust we used a ratio calculated from the RCF to adjust for time. Example calculations below:

Normally you would calculate these values using Stokes law because this relationship is not linear like this ratio would suggest. Therefore, these adjusted times are at best estimates. Differences are more dependent upon K factor of the rotor and the sediment coefficient. The above calculation is a good rule of thumb that can be used for solutions that do not have unusual characteristics like high viscosity.

# 3-Day Anoxic Pretreament

1. 50 mL centrifuge tubes (8 ct) and the soil will be placed into the glove box for anaerobic conditioning.
   1. Weigh out 5g of soils and place into clean and labeled 50 mL tubes.
2. Stock ASW and DI water should also be placed inside the glove box to remove oxygen.
   1. May need a stir plate and stir bar. If needed, we may purge with N2 gas.
3. The deoxygenated DI water will be used for the subsequent DI washes that involve anoxic samples samples.
   1. May need a stir plate and stir bar. If needed, we may purge with N2 gas.
4. This will need to be done in advance before “Day 1” of experiment.

For my purposes, I will need everything to be starting the pretreatment cycle on Friday so it can run through the weekend and Monday will be “Day 1”.

# Setting up the Glove box

Read the SOP for operation of the glove box and how to place/remove items from the glove box.

* Make sure there are 2 stir plates in the glove box.
  + These will be used to stir the ASW and DI water for the anoxic treatments.
* Make sure the vacuum chamber was properly working.
  + I got an error that the purge sequence was halted because of something. I suspect it is because the N2 tank was not hooked up to it.
* There is an oxygen detector in the upper lefthand portion of the glovebox. After opening and closing the vacuum chamber, check this to make sure there is no oxygen present in the atmosphere.
  + If there is oxygen detected, turn on the catalysts and wait before opening any containers that should not be exposed to oxygen.

# Day 1: First wash

## Reagents needed

* 35 mL X 7 vials = 245 mL 25% ASW needed.
* 714.29 mL 35% ASW needed for dilution.
  + Dispense into clean (insert container) and dilute with DI water to 1L to make 25% ASW.
  + This will need to be deoxygenated.

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* **Need 8 Clean 50 mL tubes.**

## Procedure

1. Fill the 6-50 mL tubes containing the anoxic treated soils with 35 mL of 25% ASW (deoxygenated).
   * Dilute in the tube. 25 mL of 35% ASW and 10 mL of DI H2O.
2. Fill an additional tube with 35 mL DI water and another tube with 35 mL 25% ASW for blanks.
3. Remove the samples and place onto shaker table. Record Time and wait 24 hours.

# Day 2: Second Wash and 1st centrifugation

## Reagents needed

1. DI water
2. 25% ASW. 735 mL needed.
   1. Some will need to be deoxygenated.
3. Deoxygenated DI water
4. Conc. HCl to stabilize Fe (II) for Ferrozine

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* The 6 soil containing tubes will undergo centrifugation and decanting into new 50 mL tubes.
  + Need 18 clean 50 mL tubes for centrifugation.
  + +2 for the blanks
* **Need 20 clean 50 mL tubes in total.**

## Procedure

1. Stop the shaker table and remove samples.
2. Anoxic samples should be placed into the glovebox, along with new tubes to collect the wash.
   1. Pre-determined oxic samples and blanks can be exposed to O2 at this point.
3. Decant the wash into clean 50 mL tubes.
4. Refill with 35 mL of DI water
   1. Deoxygenated DI for anoxic samples.
5. Return to the shaker table ASAP! Record time.
6. Start centrifuging the samples to collect fractions.
7. MAKE SURE TO DECANT EACH CENTRIFUGE CYCLE IN A NEW TUBE. Then we will keep the old tube and refill that with more solution to resuspend the pellet at the bottom.
   1. Pellets will need to be resuspended between each run with 25% ASW.
      1. Deoxygenated for anoxic treatments.
   2. 15,000 RCF x g for 9 minutes the dispense into clean and labeled tube.
      1. The blanks will also be run at this speed.
   3. 810 RCF x g for 8 minutes then dispense into clean and labeled tube.
   4. 300 RCF x g for 4.5 minutes then dispense into clean and labeled tube.
8. Sub Sample for ferrozine, ICP, CDOM, and DOC
   1. 2 mL for ferrozine. Will need to use 0.2 mL of Conc. HCl and 2.0 mL of sample to stabilize the Fe (II).
   2. 10 mL for for ICP-MS.
   3. 9 mL for CDOM (pooled across replicates so we only need 3 from each replicate).
   4. 9 mL for DOC.
9. Take chemical measurements.
   1. pH
   2. Eh
   3. Conductivity

# Day 3: Third wash and 2nd centrifugation

## Reagents needed

1. DI water
2. Deoxygenated DI water
3. Conc. HCl to stabilize Fe (II) for Ferrozine

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* The 6 soil containing tubes will undergo centrifugation and decanting into new 50 mL tubes.
  + Need 18 clean 50 mL tubes for centrifugation.
  + 2 for the blanks
* **Need 20 clean 50 mL tubes in total.**
* Need 20 clean 2 mL centrifuge tubes for ferrozine assay.
* Need 40 clean vials for CDOM and DOC

## Procedure

1. Stop the shaker table and remove samples.
2. Anoxic samples should be placed into the glovebox, along with new tubes to collect the wash.
3. Decant the wash into clean 50 mL tubes.
4. Refill with 35 mL of DI water
   1. Deoxygenated DI for anoxic samples.
5. Return to the shaker table ASAP! Record time.
6. Start centrifuging the samples to collect fractions.
   1. Pellets will need to be resuspended between each run with 25% ASW.
      1. Deoxygenated for anoxic treatments.
   2. 15,000 RCF x g for 9 minutes the dispense into clean and labeled tube.
      1. The blanks will also be run at this speed.
   3. 810 RCF x g for 8 minutes then dispense into clean and labeled tube.
   4. 300 RCF x g for 4.5 minutes then dispense into clean and labeled tube.
7. Sub Sample for ferrozine, ICP, CDOM, and DOC
   1. 2 mL for ferrozine. Will need to use 0.2 mL of Conc. HCl and 2.0 mL of sample to stabilize the Fe (II).
   2. 10 mL for for ICP-MS.
   3. 9 mL for CDOM (pooled across replicates so we only need 3 from each replicate).
   4. 9 mL for DOC.
8. Take chemical measurements.
   1. pH
   2. Eh
   3. Conductivity

# Day 4: Fourth wash and 3rd centrifugation

## Reagents needed

1. DI water
2. Deoxygenated DI water
3. Conc. HCl to stabilize Fe (II) for Ferrozine

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* The 6 soil containing tubes will undergo centrifugation and decanting into new 50 mL tubes.
  + Need 18 clean 50 mL tubes for centrifugation.
  + 2 for the blanks
* **Need 20 clean 50 mL tubes in total.**

## Procedure

1. Stop the shaker table and remove samples.
2. Anoxic samples should be placed into the glovebox, along with new tubes to collect the wash.
3. Decant the wash into clean 50 mL tubes.
4. Refill with 35 mL of DI water
   1. Deoxygenated DI for anoxic samples.
5. Return to the shaker table ASAP! Record time.
6. Start centrifuging the samples to collect fractions.
   1. Pellets will need to be resuspended between each run with 25% ASW.
      1. Deoxygenated for anoxic treatments.
   2. 15,000 RCF x g for 9 minutes the dispense into clean and labeled tube.
      1. The blanks will also be run at this speed.
   3. 810 RCF x g for 8 minutes then dispense into clean and labeled tube.
   4. 300 RCF x g for 4.5 minutes then dispense into clean and labeled tube.
7. Sub Sample for ferrozine, ICP, CDOM, and DOC
   1. 2 mL for ferrozine. Will need to use 0.2 mL of Conc. HCl and 2.0 mL of sample to stabilize the Fe (II).
   2. 10 mL for for ICP-MS.
   3. 9 mL for CDOM (pooled across replicates so we only need 3 from each replicate).
   4. 9 mL for DOC.
8. Take chemical measurements.
   1. pH
   2. Eh
   3. Conductivity

# Day 5: Final wash and 4th centrifugation

## Reagents needed

1. DI water
2. Deoxygenated DI water
3. Conc. HCl to stabilize Fe (II) for Ferrozine

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* The 6 soil containing tubes will undergo centrifugation and decanting into new 50 mL tubes.
  + Need 18 clean 50 mL tubes for centrifugation.
  + 2 for the blanks
* **Need 20 clean 50 mL tubes in total.**

## Procedure

1. Stop the shaker table and remove samples.
2. Anoxic samples should be placed into the glovebox, along with new tubes to collect the wash.
   1. Pre-determined oxic samples and blanks can be exposed to O2 at this point.
3. Decant the wash into clean 50 mL tubes.
4. Refill with 35 mL of DI water
   1. Deoxygenated DI for anoxic samples.
5. Return to the shaker table ASAP! Record time.
6. Start centrifuging the samples to collect fractions.
   1. Pellets will need to be resuspended between each run with 25% ASW.
      1. Deoxygenated for anoxic treatments.
   2. 15,000 RCF x g for 9 minutes the dispense into clean and labeled tube.
      1. The blanks will also be run at this speed.
   3. 810 RCF x g for 8 minutes then dispense into clean and labeled tube.
   4. 300 RCF x g for 4.5 minutes then dispense into clean and labeled tube.
7. Sub Sample for ferrozine, ICP, CDOM, and DOC
   1. 2 mL for ferrozine. Will need to use 0.2 mL of Conc. HCl and 2.0 mL of sample to stabilize the Fe (II).
   2. 10 mL for for ICP-MS.
   3. 9 mL for CDOM (pooled across replicates so we only need 3 from each replicate).
   4. 9 mL for DOC.
8. Take chemical measurements.
   1. pH
   2. Eh
   3. Conductivity

# Day 6: Final centrifugation

## Reagents needed

1. DI water
2. Deoxygenated DI water
3. Conc. HCl to stabilize Fe (II) for Ferrozine

## Vials

* 3 Reps
* 2 treatments
  + Oxic
  + Anoxic
* 1 Salinity
* 1 Size
* 6 Samples total (+2 for the blanks)
* The 6 soil containing tubes will undergo centrifugation and decanting into new 50 mL tubes.
  + Need 18 clean 50 mL tubes for centrifugation.
  + 2 for the blanks
* **Need 20 clean 50 mL tubes in total.**

## Procedure

1. Stop the shaker table and remove samples.
2. Anoxic samples should be placed into the glove box along with new tubes needed to collect the wash.
3. Decant the wash into clean 50 mL tubes.
4. Start centrifuging the samples to collect fractions.
   1. Pellets will need to be resuspended between each run with DI water.
      1. Deoxygenated for anoxic treatments.
   2. 15,000 RCF x g for 9 minutes the dispense into clean and labeled tube.
      1. The blanks will also be run at this speed.
   3. 810 RCF x g for 8 minutes then dispense into clean and labeled tube.
   4. 300 RCF x g for 4.5 minutes then dispense into clean and labeled tube.
5. Sub Sample for ferrozine, ICP, CDOM, and DOC
   1. 2 mL for ferrozine. Will need to use 0.2 mL of Conc. HCl and 2.0 mL of sample to stabilize the Fe (II).
   2. 10 mL for for ICP-MS.
   3. 9 mL for CDOM (pooled across replicates so we only need 3 from each replicate).
   4. 9 mL for DOC.
6. Take chemical measurements.
   1. pH
   2. Eh
   3. Conductivity

# Day 8: Setting up DOC run 1

## Procedure

1. Aliquot samples for DOC analysis
   1. 9 mL needed.
2. Run first set of DOC samples
   1. About 2/3rds
   2. Pool day 8 samples across treatment?
   3. Should be about 60 samples.

# Day 9: First CDOM set

## Procedure

1. Aliquot samples into new tubes
   1. Pool samples (replicates)
   2. About 9 mL in total and 3 mL from each replicate.
2. Run on Aqualog for CDOM

# Day 10: Final DOC run

## Procedure

1. Run final set of DOC samples
   1. Should be about 40 samples.