

ANALYSIS OF PLANT MATERIAL FOR N, P AND CATIONS: WET DIGESTION TECHNIQUE FOR BLOCK DIGESTOR

****IMPORTANT: CHEMICALS IN THIS PROCEDURE ARE HIGHLY TOXIC!****
****BEFORE BEGINNING THIS PROCEDURE, READ MSDS SHEETS FOR ALL CHEMICALS!****

Introduction

The block digestion method is based on one developed by Parkinson and Allen (1975) and has been reliable in other laboratories. Samples are digested on a block digester, and are diluted to a known volume in calibrated glass digestion tubes. The block digestion method has proven preferable to dry ashing since the oxidation of organic material is carried out in solution in an acid medium which prevents formation of complex insoluble silicates. Sulfuric acid and hydrogen peroxide are used as oxidants, lithium sulfate raises the boiling point and acts as an additional alkali metal in analyzing for potassium, and selenium acts as a catalyst.

Advantages:

- No volatilization losses
- Rapid and easy for routine analyses
- One solution is used for N, P, K, Ca, and Mg

Nitrogen and phosphorus are determined colorimetrically by flow injection analysis (N-ammonia-salicylate complex; P-phospho-molybdenum complex). Potassium, calcium, and magnesium are determined by atomic absorption spectrophotometry. Lanthanum is used as a releasing agent for phosphorus interference on calcium and magnesium. Manganese, sodium, and zinc may also be determined in this digest, although manganese and zinc may be difficult to detect with the small sample size used.

Materials

- Block digester system and digestion tubes marked at 50 ml.
- Acid-resistant re-pipet dispenser calibrated to deliver 3.0 ml.
- Manual pipettor calibrated to deliver 1.5 ml.
- Vortex mixer and paper towels.
- Polyethylene bottles, wide mouth, 60 ml capacity, for storage of digested samples.
- Analytical balance (four places, 0.0001 g)
- 1 L Erlenmeyer flask, 500 and 100 ml graduated cylinders, 100 ml beakers, 1L amber glass bottle (with chemical "safety coat", if possible).
- Rubber gloves
- Full face shield
- Lab coat
- Reflux funnels (glass funnels melted shut at the tip)
- Fume hood (handle ALL chemicals in fume hood)

Chemicals ****READ MSDS SHEETS BEFORE HANDLING. WEAR ALL SAFETY GEAR, INCLUDING LAB COAT, FACE SHIELD, AND RUBBER GLOVES (not disposable latex gloves) ****

- Lithium sulfate, - $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$, 14.0 g.
- Selenium dioxide, - SeO_2 , 0.59 g.
- Hydrogen peroxide, 30% - H_2O_2
- Sulfuric acid, concentrated (18 M), - H_2SO_4 , 420 ml
- De-ionized, distilled water - (DDW or DI water)

Reagent Preparation

Digestion mix for approximately 4 runs, 46 tubes each (prepare prior to digestion).

NOTE: Rubber gloves (not disposable latex gloves), full face shield, and lab coat must be worn during reagent preparation and should be done under the fume hood.

In a fume hood, weigh out 14.0 g $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ and add to Erlenmeyer flask. Add 80 ml DI water to dissolve lithium sulfate. Mix contents by swirling flask. Weigh out 0.59 g SeO_2 and add to flask, swirling gently to mix. Measure 420 ml H_2SO_4 with 500 ml graduated cylinder and transfer to flask carefully by pouring acid slowly down inside of flask to minimize mixing of acid and water. Add a stirrer to the flask. Place a beaker slightly larger than mouth of flask upside down over flask mouth and place flask in a washtub half-full with water and ice. Place washtub with flask on a mechanical stirring plate and start stirrer slowly. Let stir until mix is cool and transfer digest mix to acid-resistant amber glass bottle with re-pipet dispenser. Acid digest mix will keep indefinitely if stored in dark refrigerator.

DOUBLE BATCH (enough for 6 runs): 28.0g Li_2SO_4
160 mL water
1.18g SeO_2
840mL H_2SO_4

Procedures

Sample preparation:

1. Plant material should be oven-dried (70° C) and ground to pass a 1 mm screen. Samples should be re-dried for a minimum of 24 hours at 70° C and stored in a dessicator or air-tight container.
2. Using a four-place scale, weigh out 0.2 g sample into a weigh boat and transfer to a digestion tube. Record sample ID, tube number, and weight to the nearest 0.0001 g.
3. The last two samples should be: (1) 0.2g of longleaf standard and (2) blank (no sample)

Digestion: (see appendix B for detailed instructions on operation of the LACHAT Block Digestor).

1. Turn on block digestor (requires a long pre-heating time). It should be set for the following :
2. 200 Degrees/ 30 minutes:: 340 Degrees/ 120 minutes.
3. Transfer racks of sample tubes to acid fume hood. Remove air from digest mix re-pipet dispenser by pumping plunger several times into a spare clean beaker. (Transfer mix back to digest bottle later.) Add 3.0 ml of digest mix to each tube by gently lifting and depressing plunger once, with a slow, even stroke. With paper towel folded and placed on vortex mixer's platform, place each tube on mixer and let solution mix until all suspension in tube has charred. Check bottom of each tube to insure all of the mix has charred and does not remain stuck at tube bottom.
4. Beginning with back of tube rack and working forward (to minimize exposure to reaction gases), add 1.5 ml hydrogen peroxide to each tube with manual pipettor. Mixture will boil vigorously upon peroxide addition; let it boil until calm and then carefully mix each tube on paper-toweled vortex. *Let tubes stand until reaction has completed and solution is stable.* (If, after peroxide addition, mixture boils over side of tube, rinse outside of tube into waste container for selenium/sulfuric acid and dry with paper towel; place paper towel in separate hazardous-waste container).

5. Transfer tubes to digest block preheated to 200 ° C (it may not reach 200 ° C exactly; a temperature of “about” 200 is acceptable to start the run). Press green “Start” button (first two lights should light-green light comes on). After 30 minutes, machine will beep continuously; press “Start” again to start second half of digest (120-minute section; two more lights should light). When temperature reaches 300-310 ° C, place glass reflux funnels on tubes. When machine is finished heating, it will beep for ~10 seconds and then shut itself off. Total time on the block is approximately 2 ½ hours. Digestor will then cool to temp 1.

Dilution and sample storage:

1. Remove funnels from tubes; place in soapy water washtub. In fume hood, remove tubes from block and place carefully on blue heat-resistant rack for cooling (10-15 minutes). Slowly and carefully add DI water to each tube to 20 ml calibration mark (*solution should bubble and fizz; water is being added to acid*). Then bring tubes to 50 ml volume with DI water. (Note: if necessary to move samples to another hood for dilution, use cart to move tube rack and cover with parafilm to eliminate inhalation of fumes while making transfer.)
2. In fume hood, mix solutions by completing the following procedure: Transfer contents of tube into labeled polyethylene storage bottle, pour back into tube while vortexing (on *LOW*), and then pour sample carefully BACK into bottle. Triple rinse digest tubes into waste container with small amount of water before placing in soapy water washtub. (Note: Solution may be stored for several months at room temperature; be sure bottles are well marked and identifiable.)
3. Cleaning: Wipe all counters and fume hood surface with damp paper towel. Place towel and any other spill cleanup absorbants into separate hazardous-waste container for off-site hazardous waste disposal. (check with Lab Manager). Rinse tube rack and clean all glassware carefully and properly.

Appendix A – supplemental safety information

Handling:

Material Safety Data Sheets (MSDS) are available for the chemicals used in the digestion procedure. The MSDS notebooks are located on the safety reference shelf in the hall of the laboratory building. You are encouraged to become familiar with the chemicals you are using. The MSDS sheets, provided by the chemical manufacturers, supply basic information regarding any hazards or risks associated with the use and/or exposure to these chemicals.

Disposal:

Disposal of digest solution should be in an appropriately labeled, high-density polyethylene container. Labeling should include references to sulfuric acid and selenium contents. If wastes are removed off-site by a private contractor, they do not need to be neutralized. (Check with the Lab Manager for direct and supplemental instructions.)

Appendix B – operation of Lachat Block Digestor

TO PREPARE BLOCK:

1. Spray with Silicone at least every 4th run
 2. Heat for 2 ½ hours at 250°C
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OPERATION:

1. General operation of the block digester can be reviewed in the *User's Guide for the BD-26 Block Digestor (Lachat Instruments)*. For this procedure, the operation of the digester is fairly simple and straight-forward. To begin, make sure that the block is plugged in and the circuit-breaker switch is in the **UP/ON/I** position.
2. Switch the **ON/STDBY** switch to **STDBY** (standby is the “off position”). Set the controller thumb-wheel switches to the following settings:

Temp 1	Time 1	Temp 2	Time 2
200 °C	030 min	340 °C	120 min
3. Turn the **ON/STDBY** switch to **ON**. The first light (yellow) should light. The Current Block Temp display should read room temperature (20 to 30 degrees C). The block is ready for use when the Current Block Temp display shows between 195-205 °C and the second light (red) switches on.
4. When the tubes are ready and placed in the block, press the green **START** button. The 30 minute “Time 1” period will begin and the third light (green) should light. At the end of the 30 minute “Time 1” period, the machine will continuously beep at a high pitch.
5. Press the **START** button; this starts the 120 minute “Time 2” period. The fourth light (yellow) should light and the machine should stop beeping. The block will begin heating to 340 °C.
6. When the temperature reaches 300-310 °C, place the reflux funnels on the tubes, starting from the back of the block. Once the final 340 °C temperature is reached, the fifth light (green) should light (it may take a minute or so).
7. Once the 120 minute “Time 2” period has elapsed, the machine will beep for 10 seconds and the sixth light (red) should light, and all the other lights should turn off. The machine will start decreasing in temperature but will remain “on” (temp 1). Remove tubes from fume hood and let cool for 10-15 minutes.
8. Switch the block to **STDBY**.