PREPARATION OF SEDIMENTS FOR TOTAL KJELDAHL NITROGEN AND TOTAL PHOSPHORUS ANALYSES

1. SCOPE AND APPLICATION

1.1. This method details the digestion of sediments for Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP). Digestates produced by this method are suitable for analysis of TKN using the Seal AQ2 discrete analyzer (see DEP SOP NU-101) and TP using the Seal AQ2 discrete analyzer (see DEP SOP NU-084). For analytical details such as calibration ranges and method detection limits (MDLs) see the relevant analysis SOP.

1.2. The tests covered by this SOP are S-TKN and S-TP.

1.3. The organic and inorganic forms of nitrogen (excluding nitrite and nitrate) and phosphorous are converted to ammonium and orthophosphate respectively, through hydrolysis and nitrogen reduction.

1.4. Applicable matrices are soils/sediments.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method numbers 351.2, Rev. 2.0 (1993) and 365.4 (Issued 1974).

2.2. A digestion solution containing mercuric sulfate, potassium sulfate and sulfuric acid is added to the samples to accomplish the conversion of organic nitrogen and phosphorus to their inorganic forms. This conversion takes place while the samples are being heated in a digestion block. Mercuric sulfate acts as a catalyst. Potassium sulfate raises the boiling temperature of the digestion, and increases the conversion rate of the organic forms to their inorganic forms.

2.3. Interferences: Nitrate in concentrations greater than 10 mg N/L can interfere with the digestion of TKN. The interference can be either negative or positive. For any samples with a known nitrate concentration of 10 mg N/L or higher the TKN result should be qualified with a J qualifier or the sample diluted sufficiently (by weighing out a smaller amount of sample) to decrease the nitrate concentration to below 10 mg N/L PRIOR to digestion.

3. EQUIPMENT AND SUPPLIES

Note: All volumetric glassware listed in this SOP (except digestion tubes) is Class A.

3.1. 50- well AI Scientific (AIM 600) and Seal BD50 digestion blocks.

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3.2. 50 mL graduated digestion tubes, etched at 12.5 mL and 25 mL

3.3. Vortex mixer

3.4. Boiling beads

3.5. Metal rack/cooling rack

3.6. Parafilm

3.7. Graduated cylinder

3.8. 100, 200 mL, 1, 2 L beakers

3.9. 250 mL, 500 mL, 1, 2 L volumetric flasks

3.10. Variable Eppendorf pipettes, dispenser

3.11. Weigh boats

3.12. MadgeTech TC101A single-channel data logger with Type K thermocouple, or MadgeTech Quadtemp four-channel data logger with Type K thermocouple

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water.

Note: Before preparing reagents or standards, or running samples, please read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

Note: All reagent and standard preparations must be documented in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and also documented in the sediment prep worksheet in LIMS. If available, a Certificate of Analysis for each purchased standard and reagent is maintained in the REAGENT_PREP folder on the Nutrients drive at \\FLDEP1\DEAR\Labs\LAB3\Nutrients\REAGENT_PREP\S-TKN_TP_PREP_CofA. . All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals:

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4.1.1. Deionized (DI) Water

4.1.2. Potassium Sulfate, K_2SO_4, certified A.C.S.

4.1.3. Mercuric oxide, Red, certified A.C.S.

4.1.4. Sulfuric acid, H_2SO_4 conc., certified A.C.S.

4.1.5. Hydrochloric acid, HCl

4.1.6. Nitric acid, HNO_3

4.1.7. Standard Reference Material (soil ALTQC)

4.2. Reagents:

4.2.1. **0.2 % Sulfuric Acid Solution (Diluent):** Add 4 mL of concentrated sulfuric acid (4.1.4.) to 800 mL of DI water in 2 L volumetric flask. Dilute to mark and invert to mix. The diluent is used for method blanks (MBLKs), laboratory fortified blanks (LFBs) and sample dilutions. Holding time, 30 days.

4.2.2. **Mercuric Sulfate Solution (Catalyst):** Add 65 mL of DI water to a beaker. While stirring, add 10 mL of concentrated reagent grade sulfuric acid (4.1.4.) and 8.0 g of red mercuric oxide. Stir until dissolved. Pour mixture into a 100 mL volumetric flask and dilute to volume with DI water. Invert flask 3 times to mix. Holding time, 60 days.

4.2.3. **Mercuric Sulfate Digestion Solution:** Under a well-ventilated fume hood in a large graduated beaker, slowly and carefully add 400 mL of concentrated sulfuric acid to 1.4 L of DI water while stirring with a magnetic stirrer. (Caution: This solution will get very hot due to the exothermic nature of this reaction. Add the acid in increments while allowing the mixture to cool some between additions). Next, add 266 g of K_2SO_4 (4.1.2.) in small increments and 50 mL of the mercuric sulfate solution (4.2.2.) to the sulfuric acid solution with constant mixing using a magnetic stirrer. Let solution stir under the hood until it reaches room temperature (approx. 30 min.). Pour into a 2 L volumetric flask and bring to volume with DI water. Cap and invert 5 times to mix. Pour into the digestion solution bottle (amber glass bottle) and put on hot/stir plate at stirring speed 4 and temperature between 1 and 2 until ready to be used. Holding time, 30 days.

4.2.4. **Acid solutions for cleaning digestion tubes:** The following solutions are needed only in case the dishwasher cannot be used and the tubes have to

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be cleaned manually. The detailed procedure for cleaning tubes is given in DEP SOP NU-046.

- **1:2 Hydrochloric acid:** Fill a designated squeeze bottle halfway with DI water. Under a hood, fill up the rest of the squeeze bottle with concentrated HCl (4.1.5.). Put a gloved finger over the tip of the bottle and invert several times over the sink. Remove finger, holding the bottle in a sink, and allow excess to drain.
- **1:2 Nitric acid:** Fill a designated squeeze bottle halfway with DI water. Under a hood, fill up the rest of the squeeze bottle with concentrated HNO_3 (4.1.6.). Put a gloved finger over the tip of the bottle and invert several times over the sink. Remove finger, holding the bottle in a sink, and allow excess to drain.

4.3. Standard and spiking solutions:

Note: The TP calibration standards and CCV/CHCK standard are not digested prior to analysis. Refer to DEP SOPs NU-084 for preparation details.:

4.3.1. **TKN/ammonium stock standard 1000 mg N/L:** Prepared as described in DEP SOP NU-092. Holding time, 180 days.

4.3.2. **TP/orthophosphate stock standard 1000 mg P/L:** Prepared as described in DEP SOP NU-084. Holding time, 180 days.

4.3.3. **TKN working calibration standards:** First, fill several 500 mL volumetric flasks with approximately 250 mL of fresh DI water (4.1.1). Prepare standards by pipetting the amounts of 1000 mg N/L stock standard (4.3.1) specified in the table below into these 500 mL volumetric flasks. Add 1 mL conc. Sulfuric acid (4.1.4) to each flask to acidify to 0.2% final volume, as to mimic the sulfuric acid preservation of the samples. The following standards are to be digested with every TKN soil digestion. Holding time, 30 days.

Standard Conc. (ppm)	Volume of 1000 mg N/L (4.3.1) (mL)	Final Volume (mL)	Comments
1.0	0.50	500	
1.5	0.75	500	PQL
2.5	1.25	500	
5.0	2.5	500	
10	5.0	500	CCV1
20	10	500	
25	12.5	500	High std

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4.3.4. **TP PQL standard (PQCK2) (TP: 0.06 mg P/L)**: Prepared as described in DEP SOP NU-084. Holding time, 30 days.

4.3.5. **TKN/TP spiking stock solution (TKN: 150 mg N/L, TP: 50 mg P/L)**: Prepared as described in DEP SOP NU-084. This solution is used to prepare LFBs and matrix spikes (MS). Spike code "S3". Holding time, 180 days.

4.3.6. **ALTQC standard:** The quality control standard (4.1.7.) is prepared according to the manufacturer's instructions. The supplier (Environmental Resource Associates) differs from the supplier of the calibration standards. The stock ALTQC standard may be diluted appropriately (by weighing out a smaller amount of soil standard) so that the concentrations fall within the calibration range. For the concentration and expiration date see the manufacturer's certificate.

Note: S-TP is analyzed with matrix matched (non- digested) standards. These are prepared by the analyst post digestion (NU-084), using the mercuric digestion solution (4.2.3). Should a tray only require S-TP tests, do not digest any TKN calibration standards.

5. SAMPLE PREPARATION (SEE DEP SOP NU-033)

Note: While preparing the samples any extra sample waste not containing mercury can be discarded down the sink. Any samples/solutions containing digestion solution that need to be discarded are to be collected in a hazardous waste container labeled "Mercury Waste".

5.1. Sediment samples are stored upon receipt in the Nutrient refrigerator at ≤ 6°C (not frozen) located in the receiving room. Sediment samples have a holding time of 28 days from collection. Sediment samples containing more than 20 % solids are ground and homogenized prior to digestion according to DEP SOP MT-064. Samples with less than 20 % solids are prepared taking an aliquot directly from the original sample bottle.

5.2. Samples with TKN concentrations above 20 mg N/L and TP concentrations above 10 mg P/L must be redigested using a smaller amount of sample. Do not weigh less than 0.01 g for a particular sample unless instructed by a supervisor.

5.3. Preparation procedure for sediment samples:

5.3.1. Remove all standards necessary for digestion from the refrigerator and allow them to come to room temperature (organic spiking solution (4.3.5.), PQCK2 (4.3.4.), and TKN working calibration standards (solution (4.3.3.)

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5.3.1.1. Pour standards into digestion tube up to the 25 mL mark, and add 5 mL of the mercuric oxide digestion solution (4.2.3.) to each tube. Add three boiling beads.

5.3.2. To setup a tray for digestion the following is required:

- One ALTQC standard (4.3.6.) for each tray.
- One PQL level standard for and one for TP (PQCK2) standard (4.3.1.) for each tray.
- Seven TKN calibration standards (4.3.3) for each tray with any S-TKN tests.
- One check standard for TKN (CCV1)). More can be added, if necessary.
- One MBLK per batch (10 samples).
- One LFB per batch; spiked at a concentration of 4/12 ppm TP/TKN.
- One matrix spike and a sample in duplicate and/or a matrix spike duplicate per batch.
- Two blanks (diluent 4.2.1)

5.3.3. All MBLKs, LFBs, and samples dilutions are prepared using the 0.2 % H_2SO_4 diluent solution (4.2.1.).

5.3.4. One quality control sample is chosen randomly for each batch to evaluate accuracy (from matrix spike) and precision (from matrix spike and/or sample in duplicate). This sample may be changed if upon observation it does not seem to be representative of the rest of the samples e.g. very different texture or color. In such a case you may treat this sample as a separate batch and continue with the sample preparation.

5.3.5. Create a new digestion prep worksheet which can be found under the prep ID “351.2/365.4” in the Prep Worksheet Manager module of LIMS. Complete the upper part of the sheet, which contains some general information including: worksheet and prep IDs, prep analyst’s name, SOP (NU-048), spiking solution unique ID number and units, start and completion dates and times of the digestion, digestion block equipment ID, balance ID, reagent unique ID numbers, prep comments, temperature logger ID, and review information.

Update the sample grid table below the general information section. Add spike codes and amounts for spiked samples, serial numbers for the standard solutions used, sample wet weights, reagent amounts, and sample comments (if needed). Make sure test IDs and other auto-filled information such as standard names, matrix, % solid, and reprep info for

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samples are correct and updated. Check that sample dry weight calculations are correct. Prep batch IDs are assigned in the worksheet. Ask a supervisor to review the worksheet.

5.3.6. Make sure enough clean digestion tubes are available for samples and QC.

5.3.7. Label all the clean empty digestion tubes with samples ID's and QC labels.

5.3.8. Sample weighing procedure:

- Before the sample portion is weighed out, the sample must be thoroughly homogenized using a clean stainless-steel spatula and mixing the sample well.
- Check the maintenance book of the balance to confirm that the balance calibration was checked on the day you intend to use it. If it was not done perform balance calibration checks (see DEP SOP CM-011 for detailed instructions). Record the balance ID in the prep worksheet.
- Place a small polystyrene weighing boat onto an analytical balance and tare the balance so that it reads 0.0000 g. Using a clean wooden applicator, carefully transfer enough homogenized wet sample to the weighing boat to yield 0.2000 g +/- 0.01 g of material. Record the weight under "sample wet weight" on the sediment sample digestion worksheet, and check the % solid information and calculated sample dry weight for each sample. Transfer the sample to the tube. Rinse the weighing boat with 0.2 % sulfuric acid (4.2.1.) into the digestion tube to ensure all sample is added to the tube.

Note: Make sure that NO sample has spilled onto the balance outside of the weigh boat. When weighing replicate portions of sample for the duplicates and spikes, take time to make sure that the sample weights are close together. It is very important that all weights be measured and recorded accurately.

- Slowly add 0.2 % sulfuric acid solution to wash the sediment down the sides of the tube into the tube bottom.

5.3.9. LFBs and matrix spikes are prepared at a concentration of 4/12 ppm TP/TKN (Spike code S3) by adding 2 mL of spiking TP/TKN solution (4.3.2.) to a 25 mL sample aliquot for matrix spikes or to diluent (4.2.1.) for LFBs. A change in spike levels may be necessary for later digestions. See your supervisor if you have any questions regarding the spike level.

5.3.10. Fill the digestion tubes with 0.2 % sulfuric acid (4.2.1.) up to the 25 mL mark, and add 5 mL of the mercuric oxide digestion solution (4.2.3.) to each tube.

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5.3.11. Sediment samples tend to "bump" more than water samples. Add three boiling beads to prevent this.

5.3.12. Place the tubes in the digestion block. For sediment samples, the initial part of the digestion at lower temperatures occurs for a longer time than for water samples. Set the block controller to Program 4 which ramps the temperature up to 180 °C at a rate of 1 °C/min, then holds at 180 °C for 90 minutes. The temperature is then ramped up to 270 °C at a rate of 5 °C/min and held for 60 minutes. The final temperature ramp is up to 385 °C at 5 °C/min, and held for 210 minutes. The digestion may be performed overnight.

5.3.13 One spot on the block is chosen at random to place a tube filled with sand and insert the thermocouple of the data logger system (3.12.). This system is used for temperature recording during the digestion process. The data logger program is started in advance using the MadgeTech4 software, as follows:

a. Plug data logger into the USB cable at the PC with the MadgeTech4 software installed.

b. Open the MadgeTech4 software. The data logger ID will appear in the “Connected Devices” panel of the software interface. Click on the ID once to highlight it.

c. In the Device tab menu bar, click Quick Start (this will use the most recent device settings, visible in the Connected Devices panel). When the data logger has been properly started, the status will read “Running”.

d. Unplug the data logger from the USB interface cable, and attach to the appropriate thermocouple cable outside the hood where the digestion block sits.

e. After the digestion is complete (see section 5.3.14.), unplug the thermocouple cable and repeat steps a and b, above. Click “Stop” in the Device tab.

f. Click “Download” to open the temperature report. Use the worksheet ID/tray number to name the temperature file in the dialog box that opens, and to save the report on the Chemistry drive: \\FLDEP1\DEAR\Labs\LAB5\Chemistry\InorganicPrep\WRKSHTS\Templates\CURRENT\Nutrients\TKN-HighTP\TKN-TPSOIL\Temperature Logs.

Print, initial, and date the report for the analyst to include with the run folder. Make sure the temperature report includes the unique ID’s of the data logger and thermocouple system.

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g. Close the MadgeTech4 software, and recharge the data logger battery if necessary.

5.3.14. After the digestion is complete, remove the digestion tubes carefully from the heating block and let samples cool to room temperature, approximately 15-20 minutes. If samples are still yellow or contain black particles they should be redigested using less sediment. The volume of the sample at this point may not exceed 10 mL. At times crystalline materials may be formed upon cooling. CAUTION! The digestate at this point contains concentrated sulfuric acid and salts and may liberate heat and splatter upon dilution. Point the digestion tube away from yourself and bring the volume carefully back up to 25 mL with fresh DI water. Cover the tube with parafilm or a lid. Mix the contents thoroughly by using a vortex device. While vortexing the sample, be sure to tilt the tube to facilitate the mixing process.

5.3.15. When the sediment sample digestions are complete, make sure that the digestion sheet has been reviewed by a supervisor, then complete the worksheet. Label the sample rack with the worksheet/tray ID.

6. SAMPLE ANALYSIS/QUANTITATION AND DATA ARCHIVAL

Please refer to the applicable analysis DEP SOPs NU-101 and NU-084.

7. QUALITY CONTROL (SEE DEP SOPs NU-038 AND NU-043)

A digestion batch is defined as a set of 10 samples or less from the same matrix group. Matrices S-FRHWTRSD, S-OTHER, SEDIMENT, S-SOIL etc. can be grouped together as one batch. If a sample or group of samples is visibly different from the rest, they should be batched separately.

7.1. Digested PQL (TKN) and PQCK2 (TP) - one of each per tray.

7.2. Digested ALTQC - one per tray.

7.3. Digested MBLK - one per batch.

7.4. Digested LFB - one per batch.

7.5. One matrix spike (for accuracy) and a matrix spike duplicate and/or a sample duplicate (for precision) - one per batch.

7.6. For method performance and acceptance criteria for QC measures and corrective action for handling out-of-control or unacceptable data refer to DEP SOPs NU-038 and NU-043 as well as the applicable analysis SOPs mentioned in 1.1.

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8. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

8.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-health-and-safety-plan).

8.2. Safety glasses, gloves and lab coats must be worn at all times whilst handling samples and reagents.

8.3. Read the MSDS information for safe reagent and sample handling. In particular be aware of the hazardous nature of the digestion solution.

8.4. The solution used to digest samples contains mercury, which is a hazardous waste and toxic. All sample waste that contains digestion solution (i.e. prior to or after digestion) is collected in hazardous waste containers labeled as 'Mercury Waste'.

8.5 Do not store more than 5 full waste containers in the laboratory at any given time. Label each full container with the date before storage. Full containers cannot be stored for more than 3 working days at the temporary storage location in the laboratory. After that the waste must be taken to the waste disposal room.

9. REFERENCES

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

9.1. EPA Method 351.2, Rev. 2(1993).

9.2. EPA Method 365.4.

9.3. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

9.4. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

9.5. DEP SOP NU-041, Reagent Preparation Procedure.

9.6. DEP SOP NU-042, Standard Preparation Procedure.

9.7. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

9.8 DEP SOP NU-046, Procedure for TKN/TP/LLTP glassware cleaning.

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9.9. DEP SOP MT-064, Homogenization and Pulverization of Solid Samples (Specific to the Retsch PM100 Planetary Ball Mill).

9.10. DEP SOP NU-101, Determination of Total Kjeldahl Nitrogen in Soils using the Seal AQ2 Discrete Analyzer.

9.11. DEP SOP NU-084, Determination of Total Phosphorus in Soils using the AQ2 Discrete Analyzer.

9.12. DEP SOP NU-092, Determination of Total Kjeldahl Nitrogen in Water using the Seal AQ2 Discrete Analyzer.

9.13 DEP SOP CM-011, Gravimetric Analysis: Analytical Weight and Balance Calibration

9.14. Quality Manual, Bureau of Laboratories, FDEP.

9.15. Laboratory Health and Safety Plan.

APPENDIX OF SIGNIFICANT CHANGES

04/27/2010 The SOP was amended to account for the use of this SOP for the preparation of sediment samples only.

04/04/2012 Details were added for standard preparation, QC requirements, digestion sheets, digestion temperature program and waste disposal.

03/23/2015 Information about creating and completing prep worksheets in the Prep Worksheet Manager module in LIMS and the use of the Data logger system was added to Section 5.

02/21/2018 SOP edited to reflect new standard preparation procedure for the analysis of TKN on the AQ2 instrument. Waste storage and disposal procedure clarified in section 8.

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