DETERMINATION OF TOTAL KJELDAHL NITROGEN IN SOILS USING THE SEAL AQ2 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. The SEAL AQ2 Discrete Analyzer is used for the determination of total Kjeldahl nitrogen (TKN) in soils. The calibration range is from the method detection limit (MDL) of 62 mg N/kg (0.50 mg N/L) to 3125 mg N/kg (25 mg N/L) TKN. The range is dependent on the percent solids of the sample and may be extended by reducing the amount of sample prepared for analysis. The practical quantitation limit (PQL) is 190 mg N/kg (1.5 mg N/L).

1.2. This SOP is based on EPA method 351.2, Rev. 2.0 (1993). The test covered by this SOP is S-TKN.

2. SUMMARY OF THE METHOD

2.1. Prior to analysis, mercuric sulfate catalyzed digestion converts free ammonia and organic nitrogen compounds to ammonium sulfate (see DEP SOP NU-048).

2.2. Ammonium reacts with salicylate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

2.3. Interferences: The working buffer eliminates precipitation of calcium and magnesium hydroxides. Samples that are turbid must be filtered prior to determination. High nitrate concentrations (>10 mg N/L) interfere with the TKN preparation procedure. If interference is suspected samples are diluted prior to digestion.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – SEAL AQ2 Discrete Analyzer.

3.2. SEAL BD50 digestion block (for sample preparation)

3.3. Glassware and pipettes: Class A type volumetric (vol.) glassware flasks and pipettes.

Stock Reagents:	4-500mL Volumetric flask
	1-100mL Volumetric flask
	3-500mL Clear Plastic bottle
	1-500mL Nalgene bottle
	1-100mL Nalgene bottle
Working Reagents:	1-500mL Volumetric flask
	2-100mL Volumetric flask
	6- AQ2 Reagent Wedge
	1-500mL Clear plastic bottle
Misc.:	analytical balance
	heated magnetic stirrer and stir bar
	spatulas for weighing out reagents
	variable Eppendorf type pipettes

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polypropylene syringe 5 mL
0.45 um pore syringe filter
vortex apparatus
vacuum filtration apparatus

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, read the safety notes in the safety/hazardous waste section of this SOP and the MSDS's for the chemicals used in this analysis.

Note: All reagent preparations must be documented in the Reagent and Standard Logsheet. This excel sheet is located in the TKN-Hg folder in the REAGENT_PREP folder on the Nutrients drive, and needs to be updated whenever a reagent is newly prepared. 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 documented in the prep worksheet in LIMS or in the Reagent and Standard Logsheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard and reagent is maintained in the \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\TKN-Hg\Certificates of Analysis folder . 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:

4.1.1. Ammonium Chloride, NH_4Cl

4.1.2. Glycine, $C_2H_5NO_2$

4.1.3. Glycerophosphoric Acid Disodium Salt, Pentahydrate, $C_3O_{11}H_{17}PNa_2*5H_2O$

4.1.4. Ethylenediaminetetraacetic acid, disodium salt dihydrate (EDTA)

4.1.5. DI Water, ammonia-free

4.1.6. Sodium Salicylate, $NaC_7H_5O_3$

4.1.7. Sulfuric Acid, concentrated H_2SO_4

4.1.8. Sodium Hydroxide, NaOH

4.1.9. Sodium Hypochlorite, NaOCl (approx. 8.25 % NaOCl)

4.1.10. Sodium Nitroferricyanide, $Na_2Fe(CN)_5NO*2H_2O$

4.1.11. Sodium Potassium Tartrate, $KOCO(CHOH)_2COONa*4H_2O$

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4.1.12. Sodium Phosphate, Dibasic Heptahydrate, $Na_2HPO_4*7H_2O$

4.1.13. Standard Reference Material (SRM) purchased from ERA (soil ALTQC)

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

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

4.1.16. Hydrochloric acid, HCL (only used if tubes need to be cleaned manually)

4.1.17. Nitric acid, HNO_3 (only used if tubes need to be cleaned manually)

4.2. Reagents:

4.2.1. **Stock sodium hydroxide, 20 % solution** - In a 250 mL vol. flask, add 50g NaOH (4.1.8.) to approx. 100 mL DI water while stirring slowly. Bring to volume with DI water. Do not filter. **Holding time, 180 days**.

Note: Solution will become hot as sodium hydroxide is added to water. Use a plastic tray containing water as a water bath to cool the NaOH solution.

4.2.2. **Stock buffer** - Using a 1 L beaker and magnetic stirrer, add 127.0g sodium phosphate dibasic heptahydrate (4.1.12.) and 10 g NaOH (4.1.8.) to approx. 250mL DI water. Stir until dissolved, approx. 10 min. Transfer to a 500 mL vol. flask and bring to volume with DI water. Store at room temperature. **Holding time, 60 days**.

Note: Sodium phosphate may have hardened inside the container please break down to as small of particulates as possible before attempting to dissolve. Also, Sodium phosphate may fall out of stock buffer solution and need to be gently warmed and mixed, leaving a stir bar in the bottle can aid with reducing contamination.

4.2.3. **Stock Sodium potassium tartrate, 10 % solution** - Dissolve 50 g of sodium potassium tartrate (4.1.11.) in approx. 250 mL of DI water. Add 3-5 pellets of NaOH (4.1.8.) and a few boiling beads, boil gently for 45 minutes. Cover, and after the solution has cooled down to room temperature, bring it to volume in a 500 mL vol. flask. Adjust the pH to that of DI water (approx. pH 6-7) with conc. H_2SO_4 (4.1.8.). **Holding time, 180 days**.

4.2.4. **Working/Stock Salicylate/Nitroferricyanide:**

4.2.4.1. **Stock Alkaline Sodium Salicylate Solution:** Using a 1 L beaker and magnetic stirrer apparatus, dissolve 75 g sodium salicylate (4.1.6.), 10 g sodium hydroxide (4.1.8.) in approx. 350 mL DI water. Mix for a few minutes, transfer to a 500 mL vol. flask and bring to volume. Transfer to 500 mL Nalgene bottle. **Holding time, 60 days.**

Note: Use caution when weighing out sodium salicylate; it may have a small particle size and be dusty.

4.2.4.2. **Stock sodium nitroferricyanide dihydrate:** Using a 100 mL flask dissolve 3 g of Sodium nitroferricyanide dihydrate in

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approx. 50 mL of DI water. Mix for a few minutes and bring to volume. Transfer to 100 mL Nalgene bottle. **Holding time, 30 days.**

4.2.4.3. **Working Color (Salicylate/Nitroferricyanide Solution):** In a AQ2 reagent wedge place 0.25 mL of Stock sodium nitroferricyanide dihydrate. Add 40 mL of Alkaline sodium salicylate stock solution into the wedge and using pipette mix solution until homogenized (3-5 times). Keep this solution out of direct light either in AQ2 or covered. **Holding time, 3 days.**

4.2.5. **Working Buffer** – Always combine the reagents in the stated order. Use a clean and empty 100 mL vol. flask. Add 20 mL of stock buffer solution (4.2.2.) to 50 mL of stock sodium potassium tartrate (4.2.3.) and mix. Add 3.2 mL of 20 % NaOH solution (4.2.1.) to the mixture. Dilute to the mark with DI water. **Holding time, 3 days**.

4.2.6. **Working Hypochlorite** - To a 100 mL vol. flask containing approx. 50 mL DI water, add 14.54 mL hypochlorite (8.25 % NaOCl, 4.1.9.). Bring to volume. **Holding time, 3 days**.

4.2.7. **Alkaline EDTA-** To a 500 mL flask with approximately 200 mL of DI water add 2.5 g of EDTA and 10 g of sodium hydroxide pellets. Stir to dissolve and fill to volume with DI water. **Holding time, 60 days**.

4.3. Standard and spiking solutions:

4.3.1. **Calibration Standards and Quality Control Standard** - Refer to DEP SOP NU-048 for the preparation of all standard solutions. The prepared ALTQC (designated as ERAQC#) may be diluted appropriately (by weighing out a smaller amount of soil standard) so that the concentration falls within the calibration range.

4.3.2. **Blank** (Diluent) - 0.2 % sulfuric acid (digested according to DEP SOP NU-048).

5. SAMPLE HANDLING AND PREPARATION

For sample collection, preservation, shipment, storage, and preparation instructions details see DEP SOP NU-048.

6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

Note: • Digested solutions containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer. If there is particulate matter in the digestate the sample should be diluted and re-digested.

• Samples with organic N concentrations above 20 mg N/L must be diluted prior to digestion to report results without a J qualifier.

• Observe routine maintenance as recommended by the instrument manufacturer and

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enter any maintenance performed in the maintenance logbook.

6.1. Read the SEAL AQ2 operations manual before proceeding.

6.2. Initial Preparation:

6.2.1. Select digested trays to be analyzed and view preparation worksheet in LIMS.

6.2.2. Vortex samples thoroughly and check sample tubes to make sure all samples are present with no extras. Discard expired reagents and re-prepare if necessary.

Note: Some samples may need to be placed in a bath of warm but not boiling water for 1-5 minutes to fully homogenize.

6.2.3. Complete preparation worksheet in LIMS Prep Worksheet Manager. While in the manager save prep sheet as an excel XML file so that the worksheet can be later insert into AQ2 run schedule.

6.3. Reagents, Controls, and Standard Loading

6.3.1. Fill six separately labeled AQ2 reagent wedges with the following:

- Working Buffer (4.2.5.) position 4
- Working Color (4.2.4.3) position 5
- Working Hypochlorite (4.2.6) position 6
- Alkaline EDTA (4.2.7) position 8
- CCV1 position 10
- CCB position 11

6.3.2. Reagent Location - The reagent tray, with plastic wedge-shaped containers is located on the right side of the injector arm. It holds all reagents and controls (CCV and CCB) in the assigned locations.

6.3.3. Empty old sample cups into the hazardous waste bottle and dispose of all the old sample cups. Perform any other maintenance, e.g., replacing the drying pad, pump tubing, cuvettes, or lamp as needed.

6.4. Calibration:

6.4.1 The AQ2 is manually calibrated using the calibration standards prepared in DEP SOP NU-048.

6.4.2. The standards in the calibration are as follows:

STD	**Conc. (PCU)**	**Source**
S1	0.0	Digested Blank (4.3.2.)
S2	1.0	See NU-048
S3	1.5 (PQL)	See NU-048
S4	2.5	See NU-048

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S5	5.0	See NU-048
S6	10 (CCV1)	See NU-048
S7	20	See NU-048
S8	25 (High Std)	See NU-048
S0	0.0	Digested Blank (4.3.2.)

6.5. Analysis Setup/Sequence Setup:

6.5.1. Turn on the AQ2 Analyzer (large button on the right side at the back of the instrument) and the computer. To start the software, double-click on the AQ2 Icon on the screen. The username is '**Instrument 1'** and the password is '**password**'.

6.5.2. Check the DI water reservoir and the waste containers. Replace all used reaction segments.

6.5.3. In the software, go to the 'Settings' menu and select maintenance. Zero the reaction segment counter. Perform maintenance and record in maintenance book.

6.5.4. In the 'settings' menu, select the daily start-up option.

6.5.5. Setup of the analytical run - Click on the scheduling icon on the left side of the screen. Choose **Reagent Set 2**. Select a free tray and name the run using the format STKNYYYYMMDD001K. For the first run on instrument 1 on 02/24/2018 the name of the run would be TKN20180224001K.

6.5.6. The sequence screen should appear. **Starting in Cup 2**, type the sample sequence in the 'Sample ID' column. Include at least 7 blank "C" cups at the end of the run to avoid a known timing issue affecting the final CCB/CCV results (see the Seal AQ2 Method EPA-111-A, Rev. 4 for more information).

6.5.7. The preparation worksheet manager will give the order of the sample tubes. A run should be ordered as follow starting in cup 2:

- **Calibration Standards**
  - **STANDARD 1 (1-8, 0)**
- **CCV1** and **CCB** (digested blank, 4.3.2.)
  - Checks can be poured manually on the tray or scheduled to auto bracket every ten cups on the run.
- **ERAQC#_Tray #**
- **PQL**
- **MBLK_Prep batch**
- **LFB**
- **Sample Batch (copied from previously saved excel XML file of the prep worksheet)**

6.5.8. Once the sample IDs are typed into the run, highlight the typed lines and

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select “auto-fill” and click the **TKN25** test. The test should appear in the requested test column to the right of the sample ID’s. Click the ‘save’ icon. Click on the ‘print’ icon to print the bench sheet.

Cup	Type	Sample ID	Sample Details	Requested Tests
1				
2	S1	STANDARD 1		TKN25
3	S2	STANDARD 2		TKN25
4	S3	STANDARD 3		TKN25
5	S4	STANDARD 4		TKN25
6	S5	STANDARD 5		TKN25
7	S6	STANDARD 6		TKN25
8	S7	STANDARD 7		TKN25
9	S8	STANDARD 8		TKN25
10	S0	STANDARD 0		TKN25
11	U1	C C V		TKN25
12	U2	C C B		TKN25
13	U3	ERAQCSOIL6_1234		TKN25
14	U4	PQL		TKN25
15	U5	MBLK_P33123		TKN25
16	U6	LFB		TKN25
17	U7	1902123		TKN25
18	U8	1902123_O1_A		TKN25
19	U9	1902123_O1_B		TKN25
20	U10	1902124		TKN25
21	U11	C C V		TKN25
22	U12	C C B		TKN25

6.5.9. When pouring samples, rinse once with sample first. Do not fill sample cups past the line in the cup.

6.5.10. Once the trays are loaded, double-click on the ‘Run’ icon on the left side of the screen. A dialog box will appear. Select the tray that contains the sequence to be analyzed. Click ‘OK’. Another dialog box will appear. Check the “Auto-Standardize” box, and the “Perform Water Baseline”, then click ‘OK’. (If the analysis should use the previous calibration do not check the auto-standardize option.) Select ‘OK’ on the following dialog box.

6.6. Quantitation/Calculations:

6.6.1. The instrument will give the analysis results for TKN in mg N/L.

6.6.2. The LIMS QC-Manager calculates results for the soils in mg N/kg and recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and/or spikes. For general calculations including RPDs and spike recoveries see Section 5.9 of the FDEP Quality Manual.

6.6.3. The calibration standards are prepared according to DEP SOP NU-048. The correlation coefficient for the calibration is calculated by the instrument software.

6.7. Post-Run Procedure:

6.7.1. After the run has finished, print the AQ2 Test Parameters Report from the ‘Settings’ menu on the top of the screen.

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6.7.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.7.3. Click on the 'Accept All' Icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the naming convention described in 6.4.5. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder located in \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\TKN_Soils\

6.7.4. Open the file in excel and save as text, tab delimited (*.txt).

6.7.5. Upload into LIMS and save file as STKNyyyymmdd001K.

7. DATA ARCHIVAL

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (S-TKN), filename/s (STKNyyyymmdd001K), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, reagent logsheet, digestion temperature data logger report, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. SEAL AQ2 Discrete Analyzer data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\TKN_COP\.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to DEP SOPs NU-038 and NU-043.

8.2. The 10 mg N/L working check standard (CCV) and the MBLK (method blank)/MDL (digested blank, 4.3.2.) are run every 10 samples and at the beginning and end of each run. Acceptance limits for the MBLK and MDL are < 0.44 and > - 0.44 mg N/L. The check standard recovery acceptance limits are 85 - 115 %.

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8.3. Each tray of digested samples contains a digested PQL level standard (PQL) and a digested ALTQC standard (ERAQC#, 4.3.4.). Acceptance limits for the recovery of the PQL are 70 - 130 %. Refer to the manufacturer's Certificate of Analysis for the acceptance limits of the soil ALTQC.

8.4. A method blank (MBLK), a laboratory fortified blank (LFB), a duplicate sample and/or duplicate matrix spikes are digested and analyzed for every batch of 10 samples (see DEP SOP NU-048). Acceptance limits for the LFB and matrix spike recoveries for soils and sediment samples are 80 - 120 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results above the PQL.

8.5. A new calibration is performed at the beginning of every analytical run. The working calibration range is verified every time a new calibration is performed. The correlation coefficient for every component reported must be equal to or larger than 0.995. The acceptance limits for the recoveries for each calibrant above the PQL are 90 - 110 %. For general information, see the FDEP Bureau of Laboratories Quality Manual, Appendix B, Section 7.0, Technical Requirements and for the linear calibration range see Section 7.1.1, 'Protocol for Determining the Test Method Range of Applicability'.

8.6. Every day samples are analyzed a new calibration has to be performed at the beginning of the analysis and the calibration must be verified (8.5.). Consecutive runs on one day may use the same calibration. To verify the calibration range the recoveries for calibration standards above the PQL must be 90 -110 %.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

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

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

9.3. Read the MSDS information for safe reagent and sample handling.

9.4. The solution used to digest samples contains mercury, which is hazardous and toxic. All waste from the instrument, digested samples, and standards made with digestion solution should be collected in bottles clearly labeled as hazardous waste.

9.5. 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.

10. 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.

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10.1. EPA method 351.2, Rev. 2.0 (1993).

10.2. Quality Manual, Bureau of Laboratories, FDEP.

10.3. Laboratory Health and Safety Plan.

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

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

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

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

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

10.9. DEP SOP NU-048, Preparation of Sediments for Total Kjeldahl Nitrogen and Total Phosphorus Analyses.

10.10. DEP SOP NU-095, Determination of Ammonia in Water by Gas Diffusion Segmented Flow Analysis

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

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

10.13. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

Appendix of Significant Changes

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