DETERMINATION OF AMMONIA IN KCL EXTRACTED SEDIMENTS USING THE LACHAT QUIKCHEM AUTOANALYZER

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1. SCOPE AND APPLICATION

1.1. Sediments and soils are extracted for exchangeable ammonia, using a 2 M KCl extraction technique.

1.2. The calibration range is from the method detection limit (MDL) 0.025 mg N/L to 1.0 mg N/L. The range may be extended by dilution. The practical quantitation limit (PQL) is 0.050 mg N/L. The results are then converted to mg/kg. The test covered by this SOP is S-NH3.

2. SUMMARY OF THE METHOD

2.1. This method is based on Lachat method 12-107-06-1-A. Sediments are mixed with 2 M KCl, shaken and allowed to settle. The acidified KCl extract is then filtered (see DEP SOP NU-052).

2.2. The KCl extract is analyzed for ammonia using a Lachat flow injection analyzer equipped with an autosampler. Ammonia reacts with phenol, hypochlorite and nitroprusside to form indophenol blue. The absorbance of indophenol blue at 630 nm is measured; this absorbance is directly proportional to the concentration of ammonia in the sample.

2.3. Interferences - Color and turbidity may interfere with the absorbance readings. Samples containing particulate matter must be filtered. Color correction (6.7.) is performed to correct for interference due to background absorbance.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation - Lachat QuickChem 8500 Auto-Analyzer. Injection time, cycle times and pump tubing sizes can be found in Lachat Method No. 12-107-06-1-A.

3.2. Glassware and pipettes:

- Various analytical pipettes for stock/working standard preparation.
- 100 mL, 200 mL, 1 L and 2 L volumetric flasks (Class A) for reagents and standards preparation.
- 500 mL, 1 L and 2 L beakers for reagents preparation.
- 10 mL borosilicate test tubes.

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- 15 mL centrifuge tubes for sample dilutions and spiking.

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 standard and reagent preparations should be documented in the NU-RGTSTD Reagent and Standard Logsheet located on the network at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\NH3\NH3 KCL\. This sheet needs to be updated whenever a reagent or standard is newly prepared. All reagent and standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent used for the extraction and analysis are generated in the module and documented in the prep worksheet in LIMS or in the logsheet submitted with each analysis folder. The Certificate of Analysis for the SRM is kept in a binder in C307. If available, a Certificate of Analysis for each purchased reagent is also maintained in the REAGENT_PREP folder on the Nutrients drive. 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. Liquefied phenol, 88 % C_6H_5OH

4.1.2. Sodium hydroxide, NaOH

4.1.3. Sodium nitroprusside, $Na_2Fe(CN)_5NO.2H_2O$

4.1.4. Disodium ethylenediaminetetraacetate dihydrate, EDTA, $C_{10}H_{14}N_2Na_2O_8 .2H_2O$

4.1.5. Ammonium chloride, NH_4Cl

4.1.6. Sulfuric acid, concentrated, H_2SO_4

4.1.7. Sodium hypochlorite, 4 – 6 % NaOCl (Fisher purified grade), or Clorox

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4.1.8. Potassium chloride, KCl

4.1.9. Standard Reference Material (SRM) purchased from Environmental Resource Associates (Nutrients in soil, cat #542)

4.2. Reagents:

4.2.1. 2 M KCl Solution (acidified carrier) - Add 700 mL deionized (DI) water to a 1 L beaker. Add 150 g of KCl (4.1.8.) and acidify with 2 mL H_2SO_4 (4.1.6.). Transfer to a 1 L volumetric flask and dilute to the mark. Mix well to dissolve. Alternatively, you may acidify an aliquot of the extraction solution (DEP SOP NU-052, 4.2.1.), for use as carrier. The carrier is also used for un-extracted blanks: continuing calibration blanks (CCBs), the MDL, and filter check blanks. Store at room temperature. Holding time: 90 days.

4.2.2. Buffer (EDTA Solution 5 %) - Buffer is made with 50.0 g of EDTA (4.1.4.) and 14 g of NaOH (4.1.2.) in 1 L of DI water. Store at 10 ºC or cooler. Holding time, 30 days.

4.2.3. Phenolate - Dissolve 88 mL of 88 % liquefied phenol (4.1.1.) into 600 mL DI water. While stirring, slowly add 32 g NaOH (4.1.2.). Transfer solution to a 1 L volumetric flask, dilute to the mark and invert three times. Store in a dark bottle and refrigerate. Holding time, 7 days (see note below).

Note: Phenol is harmful if inhaled or if it contacts the skin. Phenolate is prepared in a hood while wearing gloves.

Note: A batch of phenolate is used for no longer than 7 days. Phenolate reagent turns darker over time. Before running, the color of the phenolate must be checked. If dark amber, it must not be used; if medium or light amber, it may be used. Rinse any glassware, which came in contact with phenol, twice with 0.5 M NaOH before rinsing with DI water (phenol does not readily dissolve in water).

4.2.4. Hypochlorite - Mix 200 mL 4-6 % sodium hypochlorite (4.1.7.) with 200 mL DI water. Store refrigerated. Holding time, 3 days.

4.2.5. Nitroprusside - Dissolve 3.5 g sodium nitroprusside (4.1.3.) in 1 L DI water. Store in a dark bottle and refrigerate. Holding time, 30 days.

4.3. Standards:

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4.3.1. Stock 1000 mg N/L for preparation of 100 mg N/L (4.3.2.) and 20 mg N/L (4.3.3.) working stock standard - Dissolve 3.819 g dried NH_4Cl (4.1.5.) in 800 mL DI water. Acidify with 2 mL H_2SO_4 (4.1.6.). Dilute to 1 L and mix. This solution is also used for spiking (high level spike at 5 mg N/L, spike code S2). Holding time, 180 days.

4.3.2. Working stock 100 mg N/L for standard preparation - To a 100 mL flask containing about 50 mL DI water, add 10 mL (using a volumetric pipette) of 1000 mg N/L (4.3.1.). Acidify with 0.2 mL H_2SO_4 (4.1.6.) and dilute to 100 mL. Mix well. This solution is also used for spiking (low level spike at 0.5 mg N/L, spike code S1, and the PQL (PQCK2) at 0.050 mg N/L). Holding time, 90 days.

4.3.3. Working stock 20 mg N/L for standard preparation - To a 100 mL flask containing about 50 mL DI water, add 2 mL (using an analytical pipette) of 1000 mg N/L stock (4.3.1.). Acidify with 0.2 mL H_2SO_4 (4.1.6.) and dilute to 100 mL. Mix well. Holding time, 30 days.

4.3.4. Calibration standards - Prepare calibration standards at the following levels: blank, 0.050, 0.10, 0.25, 0.50 and 1.0 mg N/L. Make up in acidified 2 M KCl solution (4.2.1.), in a 100 mL volumetric flask. Holding time, 30 days.

STD	Conc. (mg N/L)	Vol. of 20 mg N/L Std (4.3.3.)	Vol. of 100 mg N/L Std (4.3.2.)	Final Volume
C1	0.00	0.00 mL		100 mL (BLK)
C2	0.05	0.25 mL		100 mL (PQL)
C3	0.10		0.10 mL	100 mL
C4	0.25		0.25 mL	100 mL
C5	0.50		0.50 mL	100 mL (CHCK)
C6	1.0		1.0 mL	100 mL (CHCK4)

4.3.5. SRM standard (4.1.9.) - Prepare according to the manufacturer's instructions. The supplier (Environmental Resource Associates) differs from the supplier of the calibration standards. The SRM may be diluted appropriately (by weighing out a smaller amount of soil standard) so that the concentrations falls within the calibration range. For the concentration and expiration date please see the manufacturer's certificate.

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5. SAMPLE PREPARATION

5.1. For sample collection, preservation, shipment, storage, and preparation instructions details see DEP SOP NU-052.

5.2. Samples and extracts are stored at ≤ 6°C (not frozen) while awaiting analysis. Samples for ammonia analysis should be well sealed to prevent loss of analyte through volatilization.

5.3. All samples must be extracted and analyzed within 28 days of collection.

5.4. Pre-filter the aliquot to be analyzed using a syringe filter with a 0.45 µm nominal pore size. Also, analyze a filtered blank and check standard with this type of syringe filter.

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

6.1. Remove samples, standards and reagents from refrigerators. Carrier is 2 M KCl solution (4.2.1.). Sparge the hypochlorite, buffer, nitroprusside and carrier with helium for 5 minutes. Do not sparge the phenolate.

6.2. Refer to DEP SOP NU-066 (section 6.1.) for general instrument start up and operation.

An example run sequence includes:

- **Calibration – six calibrants including a blank (4.3.4.)**
- **CCB (Carrier, 4.2.1.) and CHCK (4.3.4.) – these cups are scheduled to bracket every 10 samples on the run. An additional carrier cup may be scheduled between the 10th sample and the bracketing CCB and CHCK.**
- **PQCK2 (4.3.2.)**
- **MDL (Carrier, 4.2.1.)**
- **ERAQCNH3SOIL# (SRM, 4.3.5.)**
- **CHCK4 (4.3.4) – this cup is followed with a Carrier (4.2.1.) cup to check carryover**
- **CHCK_F and MBLK_F – filtered blank (4.2.1.) and CHCK (4.3.4.), see section 5.4 above**
- **Batched samples with extracted MBLKs and LFBs, and including sample duplicates, duplicate low level spikes, and one high level spike – check prep worksheet for batching information and prep batch IDs (see DEP SOP NU-052 for**

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batching information). A carrier cup is added after the high level spike.

- **CCB and CHCK – final bracket**

6.3. Locate the manifold for Ammonia. Check the condition of the tubing on the manifold. If worn and/or flat, the tubing should be changed.

6.4. Start flushing the instrument with DI water. The ammonia method will automatically set the heater to 60 ºC (heater takes approximately 15 min to reach temperature). Flush with DI water for approximately 10 min. Flush with reagents 5-10 min. Calibrate the instrument with the 5 working standards and blank (4.3.4.).

6.5. After an acceptable calibration is obtained (the calibration plot of absorbance vs. concentration is linear and must have a correlation coefficient of > 0.995), analysis may begin.

6.6. Peak shapes must be normal and free of air peaks.

6.7. Color Correction - Samples with background absorbance at the analytical wavelength may interfere. Color correction is used to eliminate sample background interference.

6.7.1. Any sample at or above the MDL displaying color should be run for color correction. At the end of the run pull out the hypochlorite line and put it in the water beaker. Wait approximately 5 min until system equilibrates. Meanwhile, set up a new tray starting with 5 blanks, the samples to be run for color correction, followed by 5 more blanks at the end. Then start the run as usual.

6.7.2. Color correction calculations – Open an Excel spreadsheet template found under: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\color corrections. Enter all blank concentrations from the color correction run (Sample ID: MBLK), sample ID of samples that required color correction, their respective raw value (A) and color correction value (B). An average blank concentration ($MBLK_{Avg}$) is calculated from the 10 blanks ran before and after the samples (6.7.1.) and the color correction is calculated as follows:

Color correction = $A – (B – MBLK_{Avg})$

Subtract only if the color correction value B is at or above the MDL. Color corrected results are manually entered into the QC Manager.

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6.8. Post-run procedure:

6.8.1. Exporting Files:

6.8.1.1. Save file as NHyymmddM# (where M indicates the Lachat 1 instrument)

6.8.1.2. Select Run Tab in the Run Properties window.

6.8.1.3. Select *Export Data to File* button.

6.8.1.4. In the QC Manager, select the Instrument: Nutrients: Lachat 1.

6.8.1.5. Convert the file and save as raw data.

6.8.2. Printing Data:

6.8.2.1. Select *Tools* then *Custom Report*

6.8.2.2. Select *Print* button. (Font size may be changed.)

6.8.2.3. Calibration info will print along with data.

7. QUANTITATION

7.1. The instrument will give the analysis results for ammonia in mg N/L. The QC-manager converts the results to mg/kg. The concentration of analyte in mg/kg dry weight is calculated as follows:

A = (filtrate analysis results (mg/L)) × (final vol (0.1 L)) × (Dilution Factor)

B = (wt. of sample extracted (kg)) × (% solids as decimal fraction)

Analyte concentration (mg/kg) = A/B

7.2. The QC-Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see Section 5.9 the FDEP Quality Manual.

8. DATA ARCHIVAL

8.1. 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.

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The folder is labeled with the test (S-NH3), filename/s (NHyymmddM#), and initials of the analyst.

8.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, Reagent and Standard Logsheet including the prep worksheet ID(s) associated with the extraction, color correction calculations spreadsheet (if color correction was required), 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.

8.3. 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.

8.4. Lachat instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\FIA\sediments\S-NH3\.

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

9.1. 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.

9.2. Each tray of extracted samples contains a PQL (PQCK2, 4.3.2.) and a certified standard reference material (SRM, 4.3.5.). Acceptance limits for the PQL recovery are 70 to 130 %. Acceptance limits for recovery of the SRM standard are specified in the Certificate of Analysis provided by the manufacturer.

9.3. A blank and a check standard (CHCK, 0.50 mg N/L) are run every 20 cups. The blank result must be less than the MDL. Acceptance limits for the MBLKs, MDL, and CCBs are < 0.025 and > - 0.025 mg N/L. The check standard recovery acceptance limits are 85 to 115 %.

9.4. A method blank (MBLK), laboratory fortified blank (LFB, 0.5 mg N/L), sample in duplicate and two low-level spikes (0.5 mg N/L) and one high-level spike (5 mg N/L) are extracted and analyzed for every batch of 20 samples. Acceptance limits for the LFB and matrix spike recoveries are

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80 - 120 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results over the PQL. One filtered blank and one filtered mid range standard must be analyzed per calibration, if any samples on that calibration are filtered.

Note: Blanks and LFBs are prepared and extracted in the same way as samples, using Ottawa sand as the matrix (see DEP SOP NU-052).

9.5. Linear Calibration Range (LCR) - See Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear calibration must be equal to or larger than 0.995. The % error calculated for each calibrant should not exceed ± 10 % for all calibrants above the PQL and ± 30 % at the PQL level.

9.6. Every day samples are analyzed a new calibration is performed at the beginning of the analysis and the LCR must be verified (9.5.). Consecutive runs on one day may use the same calibration.

10. MAINTENANCE

10.1. Carry out routine maintenance as recommended by Lachat and document any maintenance performed in logbook.

10.2. The manifold is cleaned with 1 M NaOH every 14 days or before storage to remove phenolate buildup.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

11.1. Excess sample, buffer, nitroprusside and hypochlorite may be poured down the sink, flushing with plenty of water.

11.2. Waste from the manifold is collected in a 2.5 or 4 L bottle labeled "Phenol Waste". The waste produced from the manifold while running contains approximately 1 L of phenolate reagent per 6.5 L of waste. This waste, along with excess phenolate reagent, is disposed of into the phenolate waste drum within 3 days after the collection bottle has been filled. Excess phenolate reagent must be diluted before or during transfer into the drum as follows: 1 L phenolate to 5.5 L DI water.

11.3. Rinse from glassware that contacted phenol is combined with manifold waste.

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11.4. Refer to the MSDS for information about chemicals used in this analysis.

11.5. **Phenol is harmful if inhaled or if it contacts the skin.** Phenolate is prepared in a hood while wearing gloves. Cover the open phenolate reagent bottle with parafilm. Check the manifold for leaks and make sure the waste tubing is inserted deep into the collection funnel. Cover the phenolate waste container with parafilm.

11.6. Avoid breathing NaOCl fumes.

11.7. Safety glasses, gloves and lab coats should be worn at all times during analysis.

11.8. When dumping waste in the waste drum always wear a gas mask, gloves, safety glasses/goggles and lab coat.

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

12. 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.

12.1. Lachat QuickChem Method No. 12-107-06-1-A.

12.2. EPA Method 350.1.

12.3. Quality Manual, Bureau of Laboratories, FDEP.

12.4. Laboratory Health and Safety Plan.

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

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

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

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

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

12.10. DEP SOP NU-052, KCL Extraction of Nitrate/Nitrite and Ammonia From Soils and Sediments.

12.11. DEP SOP NU-066, Determination of Nitrate and Nitrite in Water Using the Lachat QuickChem 8500 Autoanalyzer.

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

Appendix of Significant Changes

08/05/2010 Updated section 7 to include more detailed operational instructions and clarifications.

04/27/2012 Working MDL set to 0.025 mg N/L.

06/14/2012 The color correction calculation procedure was described in more details.

07/09/2015 Data archival information was clarified in Section 8.

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