II. DIAGNOSTIC METHODS FOR SOIL AND ENVIRONMENTAL MANAGEMENT

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Chapter 6

Nitrate and Exchangeable Ammonium Nitrogen

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6.1 INTRODUCTION

Inorganic N in soils is predominantly in the form of nitrate (NO_3) and ammonium (NH_4). Nitrite is seldom present in detectable amounts, and its determination is normally unwarranted except in neutral to alkaline soils receiving NH_4 and NH_4-producing fertilizers (Keeney and Nelson 1982). Soil testing laboratories usually determine NO_3 to estimate available N in agricultural soils, while laboratories analyzing tree nursery and forest soils often determine both NO_3 and NH_4.

There is considerable diversity among laboratories in the extraction and determination of NO_3 and NH_4. In addition, incubation methods (both aerobic and anaerobic) have been used to determine the potentially mineralizable N (see Chapter 46) and nitrogen supply rates using ion exchange resins (see Chapter 13).

Nitrate is water-soluble and a number of solutions including water have been used as extractants. Exchangeable NH_4 is defined as NH_4 that can be extracted at room temperature with a neutral K salt solution. Various molarities have been used, such as 0.05 *M* K_2SO_4, 0.1 *M* KCl, 1.0 *M* KCl, and 2.0 *M* KCl (Keeney and Nelson 1982). The most common extractant for NO_3 and NH_4, however, is 2.0 *M* KCl (e.g., Magill and Aber 2000; Shahandeh et al. 2005).

The methods of determination for NO_3 and NH_4 are even more diverse than the methods of extraction (Keeney and Nelson 1982). These range from specific ion electrode to manual colorimetric techniques, microdiffusion, steam distillation, and continuous flow analysis. Steam distillation is still sometimes employed for ^{15}N; however, for routine analysis automated colorimetric techniques using continuous flow analyzers are preferred. Segmented flow analysis (SFA) and flow injection analysis (FIA) are continuous flow systems that are rapid, free from most soil interferences, and very sensitive.

The methods for the most commonly used extractant (2.0 *M* KCl) and SFA methods for the determination of NO_3 and NH_4 are presented here. The FIA methods often use the same chemical reactions but with different instruments (e.g., Burt 2004). The steam distillation methods for determination of NO_3 and NH_4 have not been included, since they have not changed much over the last several years. Detailed description of these methods can be found elsewhere (Bremner 1965; Keeney and Nelson 1982).

6.2 EXTRACTION OF NO_3-N AND NH_4-N WITH 2.0 *M* KCl

6.2.1 Principle

Ammonium is held in an exchangeable form in soils in the same manner as exchangeable metallic cations. Fixed or nonexchangeable NH_4 can make up a significant portion of soil N; however, fixed NH_4 is defined as the NH_4 in soil that cannot be replaced by a neutral K salt solution (Keeney and Nelson 1982). Exchangeable NH_4 is extracted by shaking with 2.0 *M* KCl. Nitrate is water-soluble and hence can also be extracted by the same 2.0 *M* KCl extract. Nitrite is seldom present in detectable amounts in soil and therefore is usually not determined.

6.2.2 Materials and Reagents

1. Reciprocating shaker.
2. Dispensing bottle.
3. Erlenmeyer flasks, 125 mL.
4. Nalgene bottles, 60 mL.
5. Filter funnels.
6. Whatman No. 42 filter papers.
7. Aluminum dishes.
8. Potassium chloride (2.0 *M* KCl): dissolve 149 g KCl in approximately 800 mL NH_3-free deionized H_2O in a 1 L volumetric flask and dilute to volume with deionized H_2O.

6.2.3 Procedure

A. Moisture determination

1. Weigh 5.00 g of moist soil in a preweighed aluminum dish.

2. Dry overnight in an oven at 105°C.
3. Cool in a desiccator and weigh.

B. Extraction procedure

1. Weigh (5.0 g) field-moist soil (or moist soil incubated for mineralization experiments) into a 125 mL Erlenmeyer flask. In some instances air-dried soil may also be used (see Comment 1 in Section 6.2.4).
2. Add 50 mL 2.0 *M* KCl solution using the dispensing bottle. (If the sample is limited, it can be reduced to a minimum of 1.0 g and 10 mL to keep 1:10 ratio.)
3. Carry a reagent blank throughout the procedure.
4. Stopper the flasks and shake for 30 min at 160 strokes per minute.
5. Filter through Whatman No. 42 filter paper into 60 mL Nalgene bottles.
6. Analyze for NO_3 and NH_4 within 24 h (see Comment 3 in Section 6.2.4).

6.2.4 COMMENTS

1. Significant changes in the amounts of NO_3 and NH_4 can take place with prolonged storage of air-dried samples at room temperature. A study conducted by the Western Enviro-Agricultural Laboratory Association showed that the NO_3 content of soils decreased significantly after a 3-year storage of air-dried samples at room temperature (unpublished results). Increases in NH_4 content have also been reported by Bremner (1965) and Selmer-Olsen (1971).
2. Filter paper can contain significant amounts of NO_3 and NH_4 that can potentially contaminate extracts (Muneta 1980; Heffernan 1985; Sparrow and Masiak 1987).
3. Ammonium and NO_3 in KCl extracts should be determined within 24 h of extraction (Keeney and Nelson 1982). If the extracts cannot be analyzed immediately they should be frozen. Potassium chloride extracts keep indefinitely when frozen (Heffernan 1985).
4. This method yields highly reproducible results.

6.3 DETERMINATION OF NO_3-N IN 2.0 *M* KCl EXTRACTS BY SEGMENTED FLOW ANALYSIS (CADMIUM REDUCTION PROCEDURE)

6.3.1 PRINCIPLE

Nitrate is determined by an automated spectrophotometric method. Nitrates are reduced to nitrite by a copper cadmium reductor coil (CRC). The nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This couples with *N*-1-naphthylethylenediamine dihydrochloride to form a reddish purple azo dye (Technicon Instrument Corporation 1971).

6.3.2 MATERIALS AND REAGENTS

1 Technicon AutoAnalyzer consisting of sampler, manifold, proportioning pump, CRC, colorimeter, and data acquisition system.

2 CRC—activation of CRC (O.I. Analytical 2001a)—Refer to point 5 in this section for CRC reagent preparation. This procedure must be performed before connecting the CRC to the system. Do not induce air into CRC during the activation process (see Comment 6 in Section 6.3.5 regarding the efficiency of the CRC).

a. Using a 10 mL Luer-Lok syringe and a 1/4″-28 female Luer-Lok fitting, slowly flush the CRC with 10 mL of deionized H_2O. If any debris is seen exiting the CRC, continue to flush with deionized H_2O until all debris is removed.

b. Slowly flush the CRC with 10 mL of 0.5 *M* HCl solution. Quickly proceed to the next step as the HCl solution can cause damage to the cadmium surface if left in the CRC for more than a few seconds.

c. Flush the CRC with 10 mL of deionized H_2O to remove the HCl solution.

d. Slowly flush the CRC with 10 mL of 2% cupric sulfate solution. Leave this solution in the CRC for approximately 5–10 min.

e. Forcefully flush the CRC with 10 mL of NH_4Cl reagent solution to remove any loose copper that may have formed within the reactor. Continue to flush until all debris is removed.

f. The CRC should be stored and filled with deionized H_2O when not in use.

Note: Solution containing Brij-35 should not be used when flushing or storing the CRC.

Note: Do not allow any solutions other than deionized H_2O and reagents to flow through the CRC. Some solutions may cause irreversible damage to the reactor.

3 Standards

a. Stock solution (100 μg NO_3-N mL^{-1}): dissolve 0.7218 g of KNO_3 (dried overnight at 105°C) in a 1 L volumetric flask containing deionized H_2O. Add 1 mL of chloroform to preserve the solution. Dilute to 1 L and mix well.

b. Working standards: pipet 0.5, 1.0, 1.5, and 2.0 mL of stock solution into a 100 mL volumetric flask and make to volume with 2.0 *M* KCl solution to obtain 0.5, 1.0, 1.5, and 2.0 μg NO_3-N mL^{-1} standard solution, respectively.

4 Reagents

a. Dilute ammonium hydroxide (NH_4OH) solution: add four or five drops of concentrated NH_4OH to approximately 30 mL of deionized H_2O.

b. Ammonium chloride reagent: dissolve 10 g NH_4Cl in a 1 L volumetric flask containing about 750 mL of deionized H_2O. Add dilute NH_4OH to attain a pH of 8.5, add 0.5 mL of Brij-35, dilute to 1 L, and mix well. (*Note*: it takes only two drops of dilute NH_4OH to achieve the desired pH.)

c. Color reagent: to a 1 L volumetric flask containing about 750 mL of deionized H_2O, carefully add 100 mL of concentrated H_3PO_4 (see Comment 2 in Section 6.3.5) and 10 g of sulfanilamide. Dissolve completely. Add 0.5 g of *N*-1-naphthyl-ethylenediamine dihydrochloride (Marshall's reagent), and dissolve. Dilute to 1 L volume with deionized H_2O and mix well. Add 0.5 mL of Brij-35. Store in an amber glass bottle. This reagent is stable for 1 month.

5 Reagents for CRC

a. Cupric sulfate solution (2% w/v): dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in approximately 900 mL of deionized H_2O in a 1 L volumetric flask. Dilute the solution to 1 L with deionized H_2O and mix well.

b. Hydrochloric acid solution (0.5 *M*): carefully add 4.15 mL of concentrated HCl to approximately 70 mL of deionized H_2O in a 100 mL volumetric flask (see Comment 2 in Section 6.3.5). Dilute to 100 mL with deionized H_2O and mix well.

6.3.3 PROCEDURE

1 If refrigerated, bring the soil extracts to room temperature.

2 Shake extracts well.

3 Set up AutoAnalyzer (see Maynard and Kalra 1993; Kalra and Maynard 1991). Allow the colorimeter to warm up for at least 30 min.

4 Place all reagent tubing in deionized H_2O and run for 10 min.

5 Insert tubing in correct reagents and run for 20 min to ensure thorough flushing of the system (feed 2.0 *M* KCl through the wash line).

6 Establish a stable baseline.

7 Place the sample tubing in the high standard for 5 min.

8 Reset the baseline, if necessary.

9 Transfer standard solutions to sample cups and arrange on the tray in descending order.

10 Transfer sample extracts to sample cups and place in the sample tray following the standards.

11 Begin run.

12 After run is complete, rerun the standards to ensure that there has been no drifting. Reestablish baseline.

13 Place tubing in deionized H_2O, rinse and run for 20 min before turning the proportioning pump off.

6.3.4 CALCULATION

Prepare a standard curve from recorded readings (absorption vs. concentration) of standards and read as $\mu g\ NO_3\text{-}N\ mL^{-1}$ in KCl extract. Results are calculated as follows:

$$NO_3\text{-N in moist soil } (\mu g\ g^{-1}) = \frac{NO_3\text{-N in extract } (\mu g\ mL^{-1}) \times \text{volume of extractant (mL)}}{\text{Weight of moist soil (g)}} \tag{6.1}$$

$$\text{Moisture factor} = \frac{\text{Moist soil (g)}}{\text{Oven-dried soil (g)}} \tag{6.2}$$

$$NO_3\text{-N in oven-dried soil } (\mu g\ g^{-1}) = NO_3\text{-N in moist soil } (\mu g\ g^{-1}) \times \text{moisture factor} \tag{6.3}$$

There are data collection software packages associated with the data acquisition systems and these will automatically generate calculated concentration values based on intensities received from the colorimeter and inputs of the appropriate information (e.g., sample weight, extract volumes, and moisture factor).

6.3.5 COMMENTS

1 Use deionized H_2O throughout the procedure.

2 *Warning*: Mixing concentrated acids and water produces a great amount of heat. Take appropriate precautions.

3 All reagent bottles, sample cups, and new pump tubing should be rinsed with approximately 1 *M* HCl.

4 Range: 0.01–2 $\mu g\ NO_3\text{-}N\ mL^{-1}$ extract. Extracts with NO_3 concentrations greater than the high standard (2.0 $\mu g\ NO_3\text{-}N\ mL^{-1}$) should be diluted with 2.0 *M* KCl solution and reanalyzed.

5 Prepared CRCs can be purchased from various instrument/parts supplies for SFA systems. Previously, the method called for preparation of a cadmium reductor

column. However, preparation was tedious and time consuming and cadmium granules are no longer readily available.

6 Reduction efficiency of the CRC (O.I. Analytical 2001a).

a. In the CRC, nitrate is reduced to nitrite. However, under some conditions, reduction may proceed further with nitrite being reduced to hydroxylamine and ammonium ion. These reactions are pH-dependent:

$$NO_3 + 2H^+ + 2e \rightarrow NO_2 + H_2O \tag{6.4}$$

$$NO_2 + 6H^+ + 6e \rightarrow H_3NOH + H_2O \tag{6.5}$$

$$NO_2 + 8H^+ + 6e \rightarrow NH_4^+ + 2H_2O \tag{6.6}$$

At the buffered pH of this method, reaction 6.4 predominates. However, if the cadmium surface is overly active, reaction 6.5 and reaction 6.6 will proceed sufficiently to give low results of nitrite.

b. If the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite. This condition is defined as poor reduction efficiency.

c. To determine the reduction efficiency, run a high-level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. The reduction efficiency is calculated as given below.

$$PR = (N_3/N_2) \times 100 \tag{6.7}$$

where PR is the percent reduction efficiency, N_3 is the nitrate peak height, and N_2 is the nitrite peak height.

d. If the response of the nitrite is as expected but the reduction efficiency is less than 90%, then the CRC may need to be reactivated.

7 The method includes NO_3-N plus NO_2-N; therefore, samples containing significant amounts of NO_2-N will result in the overestimation of NO_3-N.

8 The method given in this section outlines the configuration of the Technicon AutoAnalyzer. However, the cadmium reduction method can be applied to other SFA and FIA systems.

6.3.6 PRECISION AND ACCURACY

There are no standard reference samples for accuracy determination. Precision measurements for NO_3-N carried out for soil test quality assurance program of the Alberta Institute of Pedology (Heaney et al. 1988) indicated that NO_3-N was one of the most variable parameters measured. Coefficient of variation ranged from 4.8% to 30.4% for samples with 67.3 ± 3.2 (SD) and 3.3 ± 1.0 (SD) μg NO_3-N g^{-1}, respectively.

6.4 DETERMINATION OF NH_4-N IN 2.0 *M* KCl EXTRACTS BY SEGMENTED FLOW AUTOANALYZER INDOPHENOL BLUE PROCEDURE (PHENATE METHOD)

6.4.1 Principle

Ammonium is determined by an automated spectrophotometric method utilizing the Berthelot reaction (Searle 1984). Phenol and NH_4 react to form an intense blue color. The intensity of color is proportional to the NH_4 present. Sodium hypochlorite and sodium nitroprusside solutions are used as oxidant and catalyst, respectively (O.I. Analytical 2001b).

6.4.2 Materials and Reagents

1 Technicon AutoAnalyzer consisting of sampler, manifold, proportioning pump, heating bath, colorimeter, and data acquisition system.

2 Standard solutions:

a. Stock solution #1 (1000 μg NH_4-N mL^{-1}): in a 1 L volumetric flask containing about 800 mL of deionized H_2O dissolve 4.7170 g $(NH_4)_2SO_4$ (dried at 105°C). Dilute to 1 L with deionized H_2O, mix well, and store the solution in a refrigerator.

b. Stock solution #2 (100 μg NH_4-N mL^{-1}): dilute 10 mL of stock solution #1 to 100 mL with 2.0 *M* KCl solution. Store the solution in a refrigerator.

c. Working standards: transfer 0, 1, 2, 5, 7, and 10 mL of stock solution #2 to 100 mL volumetric flasks. Make to volume with 2.0 *M* KCl. This will provide 0, 1, 2, 5, 7, and 10 μg NH_4-N mL^{-1} standard solutions, respectively. Prepare daily.

3 Complexing reagent: in a 1 L flask containing about 950 mL of deionized H_2O, dissolve 33 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot H_2O$) and 24 g of sodium citrate ($HOC(COONa)(CH_2COONa)_2 \cdot H_2O$). Adjust to pH 5.0 with concentrated H_2SO_4, add 0.5 mL of Brij-35, dilute to volume with deionized H_2O, and mix well.

4 Alkaline phenol: using a 1 L Erlenmeyer flask, dissolve 83 g of phenol in 50 mL of deionized H_2O. Cautiously add, in small increments with agitation, 180 mL of 20% (5 *M*) NaOH. Dilute to 1 L with deionized H_2O. Store alkaline phenol reagent in an amber bottle. (To make 20% NaOH, dissolve 200 g of NaOH and dilute to 1 L with deionized H_2O.)

5 Sodium hypochlorite (NaOCl): dilute 200 mL of household bleach (5.25% NaOCl) to 1 L using deionized H_2O. This reagent must be prepared daily, immediately before use to obtain optimum results. The NaOCl concentration in this reagent decreases on standing.

6 Sodium nitroprusside: dissolve 0.5 g of sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot 2H_2O$) in 900 mL of deionized H_2O and dilute to 1 L. Store in dark-colored bottle in a refrigerator.

6.4.3 PROCEDURE

Follow the procedure (6.3.3) outlined for NO_3-N (see Kalra and Maynard 1991; Maynard and Kalra 1993).

6.4.4 CALCULATION

The calculations are the same as given in 6.3.4.

6.4.5 COMMENTS

1 Use NH_4-free deionized H_2O throughout the procedure.

2 All reagent bottles, sample cups, and new pump tubing should be rinsed with approximately 1 *M* HCl.

3 Range: 0.01–10.0 μg NH_4-N mL^{-1} extract. Extracts with NH_4 concentrations greater than the high standard (10.0 μg NH_4-N mL^{-1}) should be diluted with 2.0 *M* KCl solution and reanalyzed.

4 It is critical that the operating temperature is 50°C ± 1°C.

5 The method given in this section outlines the configuration of the Technicon AutoAnalyzer (Technicon Instrument Corporation 1973). However, the phenate method can be applied to other SFA and FIA systems.

6.4.6 PRECISION AND ACCURACY

There are no standard reference samples for accuracy determination. Long-term analyses of laboratory samples gave coefficient of variations of 21%–24% for several samples over a wide range of concentrations.

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