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## **Soil Inorganic Nitrogen Nitrate Nitrogen (Colorimetric Method)**

### **1. Application**

In this procedure, nitrogen in the form of the nitrate ion ( $\text{NO}_3^- \text{N}$ ) is extracted from the soil with water and measured colorimetrically after reaction with phenoldisulphonic acid.

### **2. Summary of Methods.**

Water is used to extract  $\text{NO}_3^- \text{N}$ , using 1 part soil to 5 parts water. Colloids are precipitated with  $\text{Ca}^{++}$ , and soluble organics are removed with activated charcoal. After filtration, an aliquot of extract is reacted with phenoldisulphonic acid. The  $\text{NO}_3^- \text{N}$  forms a blue-colored complex, which is analyzed with a colorimeter.

### **3. Safety**

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis. In this procedure, fuming sulfuric acid is used to prepare the phenoldisulphonic acid.

### **4. Interferences**

Principles interferences are chloride and soluble organic compounds. Chloride is precipitated with  $\text{Ag}_2\text{SO}_4$ . Colored organic compounds are co-precipitated with  $\text{Cu}(\text{OH})_2$  by the addition of  $\text{CuSO}_4$ , followed by  $\text{Ca}(\text{OH})_2$ .

### **5. Apparatus and Materials**

- 5.1 Soil scoop calibrated to contain 10 g of light-colored silt loam.
- 5.2 Erlenmeyer flask, 125- ml
- 5.3 Graduate cylinder, 50-ml, 100-ml
- 5.4 Oscillating shaker
- 5.5 Measuring scoop, ½ tsp
- 5.6 Beaker, 150-ml
- 5.7 Funnel tubes
- 5.8 Hotplate
- 5.9 Pipette, 10-ml
- 5.10 Medicine dropper, 3-ml
- 5.11 Burette, 50-ml
- 5.12 Colorimeter or spectrophotometer
- 5.13 Colorimeter tubes, matched

## 6. Reagents.

- 6.1  $\text{CuSO}_4$  solution, saturated: Add 210 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to 100 ml of water.
- 6.2  $\text{Ag}_2\text{SO}_4$  solution, saturated: Add 10 g of  $\text{Ag}_2\text{SO}_4$  to 100 ml of water.
- 6.3  $\text{Ca}(\text{OH})_2$ : finely ground powder
- 6.4  $\text{MgCO}_3$ : finely ground powder
- 6.5 Activated charcoal: Heat in a muffle furnace at  $500^\circ\text{C}$  for 1 hour to remove  $\text{NO}_3^-$ .
- 6.6 Phenoldisulphonic acid: Dissolve 83 g pure phenol in 500 ml of concentrated  $\text{H}_2\text{SO}_4$ . Dissolve until clear. (Check the  $\text{H}_2\text{SO}_4$  for  $\text{NO}_3^-$  contamination by dropping several crystals of phenol in several ml of the acid. The solution must remain clear.) Add a 1-pint bottle of fuming  $\text{H}_2\text{SO}_4$ . (Use the fume hood!) Place in a boiling water bath for two hours. Store in an amber bottle in a dark cabinet. **This reagent is extremely corrosive.**
- 6.7  $\text{NH}_4\text{OH}$ , 1:1: Mix equal volumes of concentrated  $\text{NH}_4\text{OH}$  and distilled water.
- 6.8 Stock standard nitrate solution, 500 ppm N: Dissolve 3.60 g  $\text{KNO}_3$ , dried at  $105^\circ\text{C}$ , in water and dilute to 1 liter with water.
- 6.9 Dilute standard nitrate solution, 20 ppm N: Dilute 20 ml of 500 ppm N to 500 ml with water.

## 7. Methods

- 7.1 Place a 10-g scoop of soil into a 125-ml Erlenmeyer flask.
- 7.2 Add 50 ml of water by means of a graduate cylinder.
- 7.3 Add 2 drops of  $\text{Ag}_2\text{SO}_4$  and 3 drops of  $\text{CuSO}_4$ .
- 7.4 Shake 10 min on an oscillating shaker (or 30 min intermittently by hand).
- 7.5 Add  $\frac{1}{2}$  tsp of  $\text{Ca}(\text{OH})_2$ ; shake thoroughly by hand and let stand 10 minutes.
- 7.6 Decant about 30 ml of the suspension into a 150-ml beaker.
- 7.7 Add  $\frac{1}{2}$  tsp of  $\text{MgCO}_3$  and swirl.
- 7.8 Add  $\frac{1}{2}$  tsp of activated charcoal; shake by hand and let stand 2 to 3 minutes.
- 7.9 Filter into funnel tubes.
- 7.10 Wash the 150-ml beakers employed in steps 7.6 – 7.9.
- 7.11 Pipette 10 ml of filtrate into the same 150-ml beaker, and evaporate to dryness on a hotplate. The temperature of the hotplate should not be high enough to permit spattering as the solution approaches dryness. The sample must be completely dry.
- 7.12 Cool; then add 3 ml of phenoldisulphonic acid rapidly to the residue in the beaker. Use a rapid delivery medicine dropper calibrated to deliver 3 ml. The reagent should flood the bottom of the beaker rapidly to prevent formation and loss of volatile nitrogen oxides.
- 7.13 Swirl; let stand until the residue is dissolved and the solution is clear.
- 7.14 Carefully add approximately 20 ml of distilled water.
- 7.15 Cool.
- 7.16 With a 50-ml burette in a fume hood, carefully add 1:1  $\text{NH}_4\text{OH}$  until full yellow color develops and then 3 ml in excess (approximately 15 ml total).

- 7.17 Transfer the sample to a 100-ml graduate cylinder and dilute to 99 ml with water. Mix the solution by pouring back-and-forth from cylinder to beaker several times. (A small amount of solution will remain as a film in the beaker. Also, a graduate cylinder is calibrated “to deliver” rather than “to contain” a given volume. A 100-ml graduate cylinder will contain slightly more than 100 ml, the excess being retained as a film on the cylinder walls when the cylinder is emptied. To compensate, the cylinder is filled to only 99 ml. A volumetric flask should be used for precise work.)
- 7.18 Determine the  $\text{NO}_3^- \text{N}$  using a colorimeter at 420 nm. Zero the colorimeter with a reagent blank.
- 7.19 Prepare a standard curve by evaporating the volumes of 20 ppm  $\text{NO}_3^- \text{N}$  solution indicated in the table below to dryness in a 150-ml beaker, and proceed with steps 7.12 above.

$\text{NO}_3^- \text{N}$  equivalents of different volumes of standard 20 ppm  $\text{NO}_3^- \text{N}$  solution.

Vol. of 20 ppm $\text{NO}_3^- \text{N}$ soln.	Final conc. of $\text{NO}_3^- \text{N}$	$\text{NO}_3^- \text{N}$ equiv- alent in soil*
ml	ppm	ppm
0	0.0	0
1	0.2	5
2	0.4	10
3	0.6	15
4	0.8	20
5	1.0	25
7	1.4	35
10	2.0	50

\*  $\text{NO}_3^- \text{N}$  equivalent in soil using wt. of soil and solution volumes indicated in Methods.

## 8. Calculations

$$\begin{aligned} \text{ppm } \text{NO}_3^- \text{N in soil} &= \text{ppm } \text{NO}_3^- \text{N in final solution} \times \frac{50 \text{ ml}}{10 \text{ g}} \times \frac{100 \text{ ml}}{10 \text{ ml}} \\ &= \text{ppm } \text{NO}_3^- \text{N in final soln.} \times 50 \end{aligned}$$

## 9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable  $\text{NO}_3^- \text{N}$  content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

## **10. Reporting**

Results are reported as ppm of nitrogen in the form of nitrate  $\text{NO}_3^- \text{N}$  in soil.