Practical Work n°3:

Determination of Nitrate Concentration in Soil by the Sodium Salicylate Method

1. Introduction

Nitrate (NO_3^-) is an important form of nitrogen in soil and represents a readily available source of nitrogen for plants. The determination of nitrate concentration provides useful information about soil fertility and nitrogen availability.

2. Objective

Determination of nitrate concentration in a soil sample by the sodium salicylate spectrophotometric method.

3. Principle

In the presence of sodium salicylate, nitrate ions react to form a yellow-colored sodium paranitrosalicylate compound. The intensity of the yellow color is proportional to the nitrate concentration in the sample.

The absorbance of the developed color is measured using a spectrophotometer at a wavelength of 410 nm.

A series of nitrate standards of known concentration is used to establish a calibration curve. The nitrate-nitrogen concentration in the soil extract is then determined from this calibration curve.

4. Materials, Reagents and Solutions

4.1. Materials and Equipment

-Soil sample

-2 mm sieve

-Analytical balance

-Erlenmeyer flask or extraction bottle

-Mechanical shaker

-Filter paper and funnel

-Beakers

-Heating device or water bath

-Spectrophotometer

-Cuvettes

-Pipettes

-Volumetric flask

4.2. Reagents and Solutions

- **Nitrate-nitrogen stock standard solution (50 mg/L NO_3-N)**

-Dissolve 0.0815 g of potassium nitrate (KNO_3) in a 1000 mL volumetric flask.

-Make up to volume with distilled water.

-Homogenize thoroughly.

- **Potassium chloride extraction solution (KCl, 2 M)**

-Dissolve 149 g of KCl in a 1000 mL volumetric flask.

-Make up to volume with distilled water.

- **Sodium salicylate solution (1%)**

-Dissolve 1 g of sodium salicylate in 100 mL of distilled water.

-Prepare fresh daily.

- **Sodium hydroxide solution**

-Prepare using 200 g NaOH and 50 g EDTA.

-Make up to 1000 mL with distilled water.

-Store in a polyethylene bottle.

- **Concentrated sulfuric acid (H_2SO_4)**
- **Acetic acid**

5. Experimental Protocol

A. Preparation of the Calibration Standards

In a series of numbered beakers, successively introduce the reagents, mixing after each addition:

Tube No.	0	1	2	3	4	5
$C_{NO_3\text{-}N}$ mg/L	0	1	2	3	4	5
Initial volume (mL)	0	2	4	6	8	10
Acetic acid (mL)	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water (mL)	10	8	6	4	2	0
Final volume (mL)	10					

B. Preparation of the Reagent Blank

-Transfer 10 mL of distilled water into a suitable vessel.

-Add 1 mL of 1% sodium salicylate solution and mix thoroughly.

-Evaporate the solution to dryness at approximately 75–80 °C. Allow the residue to cool.

-Add 1 mL of concentrated sulfuric acid and allow to stand for 10 minutes.

-Add 15 mL of 2 M KCl. Add 10 mL of sodium hydroxide solution and mix thoroughly.

-Allow the yellow color to develop for 15 minutes.

C. Soil Sample Preparation and Nitrate Extraction

-Weigh 10 g of air-dried soil that has been ground and passed through a 2 mm sieve.

-Transfer the soil into an Erlenmeyer flask or extraction bottle.

-Add 50 mL of 2 M KCl extraction solution.

-Shake the suspension for 30 minutes using a mechanical shaker.

-Filter the suspension using filter paper and a funnel.

-Collect the clear filtrate for nitrate determination.

-Transfer 10 mL of the soil extract into a suitable vessel.

-Add 1 mL of 1% sodium salicylate solution and mix thoroughly.

-Evaporate the solution to dryness at approximately 75–80 °C and allow the residue to cool.

-Dissolve the residue by adding 1 mL of concentrated sulfuric acid.

-Allow the mixture to stand for 10 minutes.

-Add 15 mL of 2 M KCl.

-Add 10 mL of sodium hydroxide solution and mix thoroughly.

-Allow the color to develop for 15 minutes (the development of a yellow color indicates the presence of nitrate).

-Set the spectrophotometer to a wavelength of 410 nm.

-Use the reagent blank to zero the spectrophotometer.

-Measure the absorbance of the calibration standards.

-Measure the absorbance of the soil extract.

-Plot absorbance versus nitrate-nitrogen concentration (mg/L) to construct the calibration curve.

-Determine the nitrate-nitrogen concentration of the soil extract from the calibration curve.

6. Calculation of Nitrate Concentration

-The nitrate concentration in the soil is calculated from the nitrate-nitrogen concentration obtained from the calibration curve.

The following equation is used :

$$\mathbf{NO_3^- \text{ concentration (ppm)} = C_1 \times 5 \times 4.427}$$

Where :

-C_1 = nitrate-nitrogen concentration obtained from the calibration curve (mg/L or ppm)

-5 = extraction/dilution factor associated with the soil extraction procedure

-4.427 = conversion factor from nitrate-nitrogen (NO_3-N) to nitrate (NO_3^-)

The factor 4.427 is based on the molecular mass relationship :

$$\mathbf{NO_3^- / N = 62 / 14 = 4.427}$$