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Spectrophotometric Determination of Available Nitrate in Plant and Soil

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Research Paper

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ABSTRACT

The available nitrate was determined by nitration of salicylic acid under highly acidic conditions absorbs maximally at 410nm in basic solution (pH>12). Absorbance of the chromophore is directly proportional to the amount of nitrate –N present. Ammonium .nitrite and chloride do not interfere.

Introduction

Soil is the natural medium in which the roots of most plants grow. There are three important nutrients required by plants .Nitrogen and phosphorous are required in appreciable quantities, while sulphur in least quantity. Available nitrogen is taken up by plant roots in the form of NO_3^- &NH_4^+.The available form of nitrogen are very water soluble and move rapidly through the soil profile with rainfall and irrigation[1,2].

Instruments and Reagents

UV-Spectrophotometer, pH meter

Preparation of soil samples [3-6]

1. Soil samples were collected from different areas of Chhattisgarh.
2. Samples were collected from different places by using auger at different depths (from 0-100cm.).The auger was screwed to the desired depth and the sample was withdrawn.
3. Soil samples were transferred to plastic bags and were labeled.
4. In the lab, the samples were air-dried; grass and any external objects were removed.

5. Sieving was done by mechanical sieving apparatus which consist of different sizes of meshes (10.0>0.075mm) after rolling the samples to break down the large masses of the soil particles.
6. The sieved samples (>2.0mm) were preserved in labeled bags.

Extraction of the samples

1. 50gm. of each of the soil samples were weighed transferred to 250ml stopper conical flask and was shaken with exactly 50ml of distilled water(1:1ratio).
2. After shaking, the equilibration was taken place by leaving the samples for 30minutes.
3. The samples were filtered into Buchner funnels by using filter papers Whatman no. 42.
4. In the case of turbid filtrates, they were centrifuged by using 3000 cycle/min. centrifuge for 5min.

Preparation of plant samples:- Freeze the plant and crush it. Grind the plant tissue in a blender. After that boil upto 10-15minutes and cool it .Use 1:1 ethanol and water mixture to extract the nitrate in fresh plants. If the plant tissue have high level of nitrate then dilute the sample before analysis.

Standard stock solution 0.25g/l NO3-N (250mg/L,250 µg/ml):- Dissolve 1.805gm KNO_3 in 600ml distilled water. Ensure all KNO_3 is dissolved, make upto the mark with distill water,mix and store in a suitably labelled plastic bottle.

Salicyclic acid –H_2SO_4 :- Dissolve 5gm. of salicylic acid in 100ml of concentrated H_2SO_4. The Salicyclic acid –H_2SO_4 should be made fresh every week and stored in a brown bottle. Nitrate standards should be stored at 4^0C.

2N NaOH:- In a 250ml beaker dissolve 40.0gm. of NaOH pellets in 100ml of distill water. Transfer to a 500ml volumetric flask and make up to 500ml with distill water.

Blanks :- A blank of 0.25ml extractant (or H_2O) for each sample with normal reagents is normally sufficient. For pigmented samples a separate blank may be required for each sample. This blank shall consists of the extract, 0.8ml of Conc. H_2SO_4 (minus salicyclic acid) and 19ml of 2N NaOH.

Procedure :-Pipette out aliquot (0.25ml) of extract or standard into a 50ml of Erlenmyer flask and then mix thoroughly with 0.8ml of 5%(W/V) salicyclic acid in Conc. H_2SO_4.After 20 minutes at room

temperature, add 19ml of 2N NaOH to raise the pH above12.Cool the sample at room temperature and measure the absorbance at 410nm.

Result and discussion

The pH of soil samples analysed was in the range of 5-6.This range is more suitable for absorption of NO_3-N in soil. 1-2% moisture content is suitable for absorption of micronutrients .Colour of the complex in both soil and plant samples was found tobe at pH 12 .

Conclusion

Present work is the analysis of nitrate in soil samples of residential, cultivable and industrial areas and plants (flowering and non flowering).Our method is very simple, highly selective, reproducible and relatively inexpensive.

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