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BUREAU OF INDIAN STANDARDS

DRAFT FOR COMMENTS ONLY

भारतीय मानक *मसौदा*

मृदा में नाइट्रोजन और नाइट्रोजन यौगिकों का निर्धारण

(*आइ एस 14684 पहला पुनरीक्षण*)

Draft ***Indian Standard***

DETERMINATION OF NITROGEN AND NITROGENOUS COMPOUNDS IN SOILS

(*First Revision of IS 14684*)

ICS 65. 080

Soil Quality and Fertilizers Sectional Committee, FAD 07

Last date of comments: 16 February 2025

FOREWORD

(*Adoption clauses will be added later*)

Nitrogen is one of the most essential nutrients required for successful crop production. It promotes vegetative growth of the plants. Several Indian soils are deficient in nitrogen content. This standard was published in 1999 to provide appropriate analytical procedure for determination of nitrogen and nitrogenous compounds in soils. This standard would help various research organizations and those engaged in organized farming including plantation and forestry crops.

In this revision, the test method for determination of nitrite nitrogen and available nitrogen by alkaline $KMnO_4$ have been incorporated.

In reporting the result of a test made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'

1 SCOPE

This standard prescribes the methods for the determination of total nitrogen and nitrogen compounds in soils.

2 REFERENCES

The Indian Standard listed below contains provisions which through reference in this text, constitutes provision of this standard. At the time of publication, the edition indicated was valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
IS 1070 : 2023	Reagent Grade Water – Specification (*fourth revision*)

3 DETERMINATION OF NITROGEN IN SOIL (EXCLUDING NITRATE)

3.1 Apparatus

3.1.1 *Macro-Kjeldahl Digestion Flask* – 500 or 800 ml.

3.1.2 *Macro-Kjedahl Digestion Unit*

3.1.3 *Macro-Kjeldahl Distillation Apparatus*

3.1.4 *Vacuum Pump*

3.1.5 *Mechanical Shaker*

3.1.6 *Hot Air Oven*

3.2 Reagents

3.2.1 *Sulphuric Acid* – Concentrated.

3.2.2 *Potassium Sulphate* – Analytical reagent grade.

3.2.3 *Copper Sulphate* ($CuSO_4.5H_2O$) – Analytical reagent grade.

3.2.4 *Sodium Hydroxide Solution* – 40 percent (*m*/*v*) solution in reagent grade water, kept in a tightly stoppered pyrex glass bottle.

3.2.5 *Mixed Indicator Solution* – Dissolve 0.5 g bromocresol green and 0.1 g methyl red in 95 percent (*m*/*v*) methanol.

3.2.6 *Boric Acid* – 2 percent (*m*/*v*) aqueous solution containing 5 ml of mixed indicator solution

per litre. The *p*H of the solution shall be adjusted to 5.0.

3.2.7 *Standard Sulphuric Acid Solution* – 0.1 N.

3.2.8 *Salicylic Acid: Sulphuric Acid Mixture* – Dissolve 25 g salicylic acid in one litre of concentrated sulphuric acid.

3.2.9 *Sodium Thiosulphate* ($Na_2S_2O_3.5H_2O$) – Analytical reagent grade.

3.2.10 *Potassium Chloride Solution (1 N)* – Dissolve 74.5 g potassium chloride analytical reagent grade, in one litre distilled water.

3.2.11 *Magnesium Oxide* – Analytical reagent grade (Heavy grade).

3.2.12 *Devarda's Alloy* – It is a mixture of Copper 50 percent (*m*/*m*), Aluminium 45 percent (*m*/ *m*) and Zinc 5 percent (*m*/*m*) and is available commercially (Lab grade or Reagent grade).

3.3 General

The Kjeldahl procedure is generally employed for determination of total nitrogen and Kjeldahl's wet digestion distillation procedure for determination of total nitrogen involves two steps, namely:

a) *Digestion* – Heating the sample with sulphuric acid containing potassium sulphate, and copper sulphate as catalyst, thus oxidising the organic matter and converting organic nitrogen into ammonium nitrogen; and

b) *Distillation* – Distilling the digest with excess of above 40 percent solution of sodium hydroxide to recover ammonia in excess of standard boric acid which is thereafter titrated against standard acid.

3.3.1 *Procedure*

Take 10 g of dry soil sample passed through 2 mm sieve into a 500 ml or 1000 ml Kjeldahl flask and add 10 g of digestion mixture comprising of potassium sulphate: copper sulphate 10:1 or 10:0.5; (*m*/*m*). Add 30 ml (6 ml/g of organic matter in soil) of concentrated sulphuric acid and heat the flask continuously avoiding excessive frothing by controlling heat. Raise the temperature to about 380 °C and digest till the contents of the flask are clear. Continue digestion for about 15 min and after complete digestion allow the flask to cool and add 100 ml reagent grade water.

To determine ammoniacal nitrogen liberated by digestion, place a 500 ml Erlenmeyer flask containing 50 ml of boric acid under the condenser of distillation apparatus so that the end of the condenser is below the surface of the boric acid. Hold the distillation flask. Cool, dilute and digest at 45 °C angle. Add glass beads and pour about 150 ml of sodium hydroxide solution (*see* **3.2.4**), down the neck of the flask so that the alkali reaches the bottom of the flask without mixing appreciably with the digest. Attach the flask quickly to the distillation apparatus, mix the contents thoroughly by gentle swirling and heat. Control the heating to prevent sucking by back of boric

acid and to minimize frothing during distillation. Check that the flow of cold water through the condenser is sufficient to keep the temperature of the distillate less than 35 °C. Collect about 150 ml of distillate, rinse the condenser with water and stop distillation. Determine total ammoniacal nitrogen in the distillate by titration with standard sulphuric acid. The colour change at end point of titration is from green to pink.

3.3.2 Calculation

Calculate total nitrogen in ammoniacal form, percent by mass of soil taken for the test by using the following formula:

1 ml of 0.1 N standard sulphuric acid = 0.001 4 g of nitrogen.

4 ESTIMATION OF TOTAL NITROGEN INCLUDING NITRATE FORM

Take 10 g of soil sample in a Kjeldahl flask, add 35 ml of salicylic acid sulphuric acid mixture (*see* **3.2.7** and **3.2.8**) and mix well. Allow the mixture to stand overnight, add 5 g of sodium thiosulphate *(see* **3.2.9**) and heat the flask till frothing subsides. Cool the flask, add 10 g of digestion mixture comprising of potassium sulphate: copper sulphate (*see* **3.2.2** and **3.2.3**) 10:1 or 10:0.5 (*m*/*m*) and complete the digestion. Determine ammoniacal nitrogen including nitrate by distillation as described in **3.3.1** and **3.3.2**.

5 DETERMINATION OF AMMONIACAL, NITRITE AND NITRATE NITROGEN AND NITRITE NITROGRN IN SOIL EXTRACTS

5.1 General

Ammoniacal nitrogen in solutions containing glutamine or other alkali labile organic nitrogen compounds may be estimated quantitatively from the ammoniacal nitrogen liberated by distillation of these solutions with a small amount of magnesium oxide and determining ammoniacal nitrogen titrimetrically. The solution is then cooled, powdered Devarda's alloy is added and redistilled to recover ammoniacal nitrogen formed as a result of reduction of nitrite and nitrate forms of nitrogen.

5.2 Procedure

5.2.1 *Extraction of Inorganic Nitrogen from Soils*

Take about 50 g of fresh, wet soil sample in an Erlenmeyer flask, add 250 ml 1 N acidified potassium chloride solution, stopper the flask and shake on a mechanical shaker for one hour. Filter the contents of the flask using a vacuum pump or using a funnel and Whatman No.1 filter paper or equivalent.

Transfer the filtrate to a 1000 ml Kjeldahl digestion flask and add about 2 to 4 g magnesium oxide carefully. Place a 250 ml Erlenmeyer flask containing 20 ml of 2 percent boric acid (*see* **3.2.6**) indicator solution under the condenser, the tip being placed below the boric acid level in the flask. Connect the Kjeldahl flask to the condenser and distill by heating. Collect about 40 to 45 ml of distillate and stop distillation. Titrate the ammonia against standard sulphuric acid. 1 ml of 0.02 N standard sulphuric acid is equivalent to 0.000 28 g of nitrogen.

5.2.2 *Determination of Nitrite and Nitrate Nitrogen*

Cool the flask after distillation (*see* **5.2**) carefully add 1 to 2 g of powdered Devarda's alloy and immediately connect the flask to the condenser. Distill ammonia by heating the flask as described in (*see* **5.2**). Titrate the ammonia against standard sulphuric acid. 1 ml of 0.02 N standard acid is equivalent to 0.000 28 g of nitrogen.

NOTE – To determine ammoniacal, nitrite and nitrate forms of nitrogen, fresh and moist sample is used. Determine the moisture content of the soil by drying a suitable aliquot in an air-oven at 105 °C for 6 h. Cool, weigh and calculate moisture content, percent by mass. Correct the result of inorganic nitrogen content by compensating for the moisture.

6 DETERMINATION OF NITRITE NITROGEN

6.1 General

The sensitivity and accuracy of the method used for colorimetric determination of NO_2^- are greatest when measurements are made at 540 nm (the wavelength having maximal molar absorptivity for NO_2^-) and the color system obeys Beer's law up to at least 120 μg of NO_2^--N/litre when measurements are performed at this wavelength using a light path of 1 cm. Photometric measurements are usually performed at 540 nm or with green color filters exhibiting maximal transmittance in the range from 520 to 550 nm, using cells having light paths of 1 to 5 cm. A 1 cm light path is suitable for measurement of up to 6 µg of NO_2^--N in a 50 ml volume, and a 5 cm path can be used for measurements of up to 1.25 µg of NO_2^--N/50 ml.

6.2 Apparatus

6.2.1 Spectrometer, providing a light path of 1 cm and capable of absorbance measurements at a wavelength of 520 nm.

6.3 Reagents

a) *Potassium chloride (KCl) solution* – 2 M
b) *Diazotizing reagent* – Dissolve 0.5 g of sulfanilamide in 100 ml of 2.4 N hydrochloric acid (HCl). Store the solution in a refrigerator at 4 °C.
c) *Coupling reagent* – Dissolve 0.3 g of N-(1-naphthyl)-ethylenediamine hydrochloride in 100 ml of 0.12 N hydrochloric acid (HCl). Store the solution in an amber bottle in a refrigerator at 4 °C.
d) *Standard nitrite (NO_2)* solution – Dissolve 0.247 g of sodium nitrite ($NaNO_2$) in water, and dilute the solution a volume of 1000 ml in a volumetric flask. If pure, dry $NaNO_2$ is used, this solution contains 50 μg of NO_2^--N/ml. Store the solution in a refrigerator at 4 °C.

6.4 Procedure

6.4.1 *Preparation of Filtered Soil Extract* – Collect dry soil samples and pass through a 2 mm sieve. Prepare soil extract by mixing soil and water or extractant in 1:5 ratio (e.g., 5 g soil and 25 ml water or extractant). Shake for 1 min. Allow the sample to settle for 5 min or filter it using filter paper and funnel.

6.4.2 *Analysis of Extract* – Pipette an aliquot (usually 2 ml) of the extract into a 50 ml volumetric flask, and add water to make the total volume about 45 ml. Add 1 ml of the diazotizing reagent

[*see* **6.3 b)**], and mix the solution. After 5 min, add 1 ml of the coupling reagent [*see* **6.3 c)**]. Mix the solution, and allow it to stand for 20 min. Then make the solution to volume, mix it thoroughly, and measure its color intensity at 540 nm against a reagent blank solution.

Determine the NO_2^--N content of the sample by reference to a calibration curve plotted from the results obtained with samples containing 0, 1, 2, 3, 4, 5, and 5 μg of NO_2^--N. To prepare this curve, dilute 20 ml of the standard NO_2^--N solution [*see* **6.3 d)**] to 1000 ml with water in a volumetric flask, and mix the solution thoroughly. Then add 0, 1, 2, 3, 4, 5 and 6 ml volumes of this diluted standard solution to 50 ml volumetric flasks, and measure the absorbances obtained by the procedure described for analysis of the extract.

7 DETERMINATION OF AVAILABLE NITROGEN BY ALKALINE $KMnO_4$

7.1 General

The available N in a soil is a fraction of the total N which can easily be absorbed by the plants. The organic forms of N like proteins, amino acids and amino sugars gradually become available to the plants for absorption after their decomposition, depending upon their nature of combination and the soil conditions. Therefore, a method taking this reserve of N into account gives a better index of N supplying capacity of soil. The alkaline potassium permanganate ($KMnO_4$) method of available N determination in soil is widely used. The $KMnO_4$ is a mild oxidizing agent and extracts the relatively easily utilizable N fractions of organic N.

7.2 Principle

A known weight of the soil is mixed with alkaline potassium permanganate ($KMnO_4$) solution and distilled using Kjeldahl distillation unit. The organic matter present in soil is oxidized by the nascent oxygen liberated by $KMnO_4$ in the presence of NaOH and the released NH_3 is condensed and absorbed in the known volume of H_3BO_3 with mix indicator to form ammonium borate, followed by titration with a standard acid (H_2SO_4).

7.3 Apparatus

a) *Kjeldahl's distillation assembly* - manual, semi-automatic and automatic
b) *Measuring cylinder* – 100 ml
c) *Pipette* – 5 ml/10 ml
d) *Burette* – 25 ml/50 ml
e) *Conical flasks* – 100/125 ml
f) *Kjeldahl distillation flask* – 250 ml

7.4 Reagents

a) *$KMnO_4$ solution* (0.32 percent) – Dissolve 3.2 g of $KMnO_4$ in distilled water and make final volume to 1 litre in a volumetric flask.
b) *Sodium hydroxide (NaOH) solution* (2.5 percent) – Dissolve 25 g NaOH in 900 ml distilled water and make final volume to 1 litre in a volumetric flask.

c) *Liquid paraffin/wax* – glass beads
d) *Standard H_2SO_4 solution* (0.02 N)
e) *Mixed indicator* – Dissolve 0.099 g of bromocresol green and 0.066 g of methyl red in 100 ml of 95 percent ethanol.
f) *H_3BO_3 solution* (2 percent) – containing 20 ml mixed indicator per 1 litre and adjusting the solution *p*H 4.5 with dilute NaOH or HCl

7.5 Procedure

Weigh 5 g of soil sample after drying and grinding, and transfer it to the Kjeldahl distillation flask. Moisten the sample with distilled water and attach the flask to the distillation unit and other sides of hose. Keep 20 ml of 2 percent H_3BO_3 [see **7.4 f)**] with mixed indicator [see **7.4 e)**] in 150 ml conical flask. Add 25 ml each of 0.32 percent $KMnO_4$ [see **7.4 a)**] and 2.5 percent NaOH solution [see **7.4 b)**]. The sample is heated by passing steam at a steady rate and the liberated NH_3 is absorbed in 20 ml of 2 percent H_3BO_3 containing mixed indicator solution kept in a 150 ml conical flask. With the absorption of NH_3, the pinkish colour turns to green. Nearly 50 ml of distillate is collected in about 10 min. Titrate the green colour distillate against 0.02 N H_2SO_4 till the colour changes to original shade (pinkish/ wine red colour, around *p*H 4.5).

Simultaneously, run a blank sample (without soil). Note the blank and sample titration readings (ml) and calculate the available N in soil as given in **7.6**.

7.6 Calculation

1 ml of 1 N H_2SO_4 = 14 mg of N = 0.014 g of N

Available N in soil (kg ha^{-1}) =

$$\frac{\text{T (sample reading} - \text{blank reading)} \times \text{Normality of acid} \times 0.014 \times \text{soil weight of one ha (kg)}}{\text{Soil sample weight (g)}}$$

$$= \frac{T \times 0.02 \times 0.014 \times 2.24 \times 10^6}{5} = \text{T} \times 125.44$$

where,

T = actual volume, ml, of standard H_2SO_4 used for the sample during titration.

Note - Weight of one hectare furrow slice of soil (15 cm depth) is considered as 2.24×10^6 kg.

7.7 Interpretation of Results

Soil rating for available N (kg ha^1): <271 (Low); 271-543 (Medium); >543 (High).

8 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be used in tests.

NOTE - 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.