Food and Agriculture Organization of the United Nations

Standard operating procedure for soil nitrogen

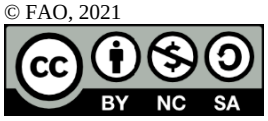
Kjeldahl method

GLOBAL SOIL PARTNERSHIP

Standard operating procedure for soil nitrogen Kjeldahl method

Food and Agriculture Organization of the United Nations
Rome, 2021

Required citation:
FAO. 2021. *Standard operating procedure for soil nitrogen - Kjeldahl method.* Rome.



SOIL NITROGEN
Kjeldahl method

VERSION HISTORY

N°	Date	Description of the modification	Type of modification
01	18 January 2021	All comments by RESOLANs and reviewers to the draft SOP were addressed	Finalization of the SOP
02			
03			
04			
Etc.			

Modify by	Revision	Approved Date	Validated Date
GLOSOLAN SOP Tech. W.G. Global leader: Miriam Ostinelli, Argentina	By the Review Panel	18 January 2021	18 January 2021

Contents

1. Brief introduction to Kjeldahl nitrogen

Nitrogen (N) is considered the most important nutrient in agriculture due to its key role in the formation of proteins, DNA, RNA, etc. Soil nitrogen is present in organic or inorganic forms. The organic fraction constitutes most of the N in the soil (more than 95 percent of the total N) and is mainly composed of plant and microbial remains. The inorganic fraction (less than 5 percent of the total N) comprises ammonium ions (NH_4^+), nitrate (NO_3^-) and very little nitrite (NO_2^-).

The organic fraction of N is a measure of the nutrient reserve in the soil and an indicator of its ability to release N through mineralization, so the analysis of N in soils dedicated to agricultural activity is useful for making decisions about the management of N nutrition in crops.

Monitoring N dynamics in soils is also important from an environmental perspective, since NO_3^- is one of the main pollutants in groundwater and surface waters, whilst nitrous oxide as a by-product of NO_3^- application is a contributor to poor air quality and Greenhouse Gas emissions.

The analysis method used to measure soil N will define the fraction of N to be quantified.

The organic N plus a fraction of inorganic N, which is in the form of NH_4^+, is commonly called total N (for the high percentage that both fractions represent) or Kjeldahl N (KjN), and are measured using the Kjeldahl method. This method includes a wet digestion of the soil sample to mineralize the N to NH_4, which will be distilled and measured.

The inorganic forms of N (NH_4^+, NO_3^- and NO_2^-) are generally determined by distillation or colorimetry of a soil extract, but N-NO_3 can also be measured by potentiometric methods.

The advantages of this method are based on its robustness, low initial cost of the equipment and extensive application. However, there are disadvantages in using this method compared to others, for example, the use of potentially hazardous reagents that require consideration for safe operation are utilized, alongside the generation of hazardous waste and on-going cost of consumables, which can be mitigated via modified protocols that use lower quantities of reagents. Another limitation of this method is that it measures organic N and mineral N in the form of NH_4^+, therefore, to measure the total N, the mineral N in the form of NO_3- must be measured separately.

2. Scope and field of application

This standard operating procedure (SOP) describes, in general terms, the quantification of the KjN content in soil samples.

The method allows the quantification of N in the form of NH_4^+ and organic N. Nitrate and nitrite are not included. Compounds N-bonding (N-N, N-O and heterocycles, especially pyridine) are not digested entirely. For most soils, the Kjeldahl method achieves a good estimate of the total N content of the soil.

A typical limit of detection is 0.01% N, and a typical limit of quantification is 0.03% N, however, this varies with the characteristics of the equipment used.

This SOP is applicable to all types of soils.

3. Principle

This procedure involves the digestion of a soil sample, the distillation of the NH_3 produced and its quantification by titration (or colorimetry).

The soil is digested in concentrated sulfuric acid, in the presence of a catalyst mixture that allows the boiling temperature to be regulated, until complete dissolution and oxidation. The organic N contained in the sample is oxidized to ammonium (as ammonium sulphate).

The NH_4^+-N in the digest is quantified by collection of the NH_3 liberated by steam distillation of the digest with alkali and titrated with a volumetric acid solution.

If the quantification of nitrates is required, some modifications can be included that enable the reduction and transformation of this anion into ammonium, for example, with the addition of salicylic acid (not included in this SOP).

4. Apparatus

Usual laboratory equipment and:

4.1. Balance to three decimal points - 0.001 g.

4.2. Analytical balance to four decimal points - 0.0001 g.

4.3. Digestion unit or block-digester, suitable for digestion of samples with sulphuric acid at 390 °C ± 10 °C, with fume extraction system.

4.4. Kjeldahl digestion tubes, of nominal volume 100 mL, suitable for digestion unit.

4.5 Distillation unit, with steam generator.

4.6. Burette, graduated with appreciation 0.01 mL. Alternatively, an automatic burette or automatic titrator can be used (connected to a pH-meter or not).

4.7. Magnetic stirrer.

If it is necessary to apply the macro method, the appropriate equipment and materials will be used.

5. Materials

All reagents shall be of analytical grade. Use water of grade 2 complying with ISO 3696.

5.1. Copper sulfate pentahydrate ($CuSO_4.5H_2O$)

5.2. Potassium sulphate (K_2SO_4).

5.3. Sulfuric acid, $\rho(H_2SO_4) = 1.84$ g cm^{-3}

Note: Alternatively, hydrochloric acid, $\rho(HCl) = 1.19$ g cm^{-3}, can be used.

5.4. Boric acid (H_3BO_3)

5.5. Sodium hydroxide (NaOH)

5.6. Bromocresol green (unless a pH titrator is used)

5.7. Methyl red (unless a pH titrator is used)

5.8. Ethanol 96% (unless a pH titrator is used)

5.9. Catalyst mixture. Grind and thoroughly mix 6g of copper sulfate pentahydrate ($CuSO_4.5H_2O$) (5.1) and 94 g of potassium sulfate (K_2SO_4) (5.2).

The $CuSO_4.5H_2O$ is ground and the K_2SO_4 is added, grinding and mixing, until achieving a uniform color mixture. It is stored in a covered plastic container, to protect it from moisture.

Note: If commercially available, catalyst tablets with the same catalyst may be used.

If the reaction is too long (in a particular type of soil), it is possible to add another component to the catalyst mixture, for example, titanium dioxide (TiO_2). in the same proportion as $CuSO_4.5H_2O$ (6g).

5.10. Boric acid solution, 2%: Dissolve 20.0 g ± 0.1 g of H_3BO_3 (5.4) in 800 mL of preheated water at a temperature lower than 60 ºC. Cool to room temperature. Adjust the pH from 4.8 to 5.0 by carefully adding sodium hydroxide, c (NaOH) = 0.1 M (mol L^{-1}) (5.14) or sulfuric acid (H_2SO_4), c (H +) = 0.1 M (mol L^{-1}) (5.15) as required. Finally, bring to final volume of 1000 mL with water.

Note: Alternatively, for use with automated distillation equipment and in laboratories that process large numbers of samples, the boric solution can be prepared in larger volume and with the addition of the indicator solution.

For this, 400g ± 0.1 g of H_3BO_3 (5.4) are dissolved in 19 L of preheated water at a temperature lower than 60 ºC. In parallel, 0.50 g of bromocresol green and 0.35 g of methyl red are dissolved in 500 mL of ethanol. This last solution is added to the H_3BO_3 solution, cooled to room temperature. Adjust the pH from 4.8 to 5.0 by carefully adding sodium hydroxide, c (NaOH) = 0.1 M (mol L^{-1}) (5.14) or sulfuric acid (H_2SO_4), c (H +) = 0.1 M (mol L^{-1}) (5.15) as required. Finally, bring to final volume of 20L with deionized water with continuous stirring.

5.11. Sodium hydroxide, c (NaOH) = 10 M (mol.L^{-1}): Dissolve 400 g of NaOH (5.5) in 800 mL of water, refrigerating conveniently. Cool to room temperature and bring to final volume of 1000 mL with water.

5.12. Sulphuric acid (H_2SO_4), c (H+) = 0.01 M (mol.L^{-1}) concentration should be checked with standardization if prepared in the laboratory.

Note: Alternatively, hydrochloric acid (HCl), c (H+) = 0.01 M (mol.L^{-1}) can be used instead of sulphuric acid.

5.13. Mixed indicator. Dissolve 0.10 g of bromocresol green (5.6) and 0.07 g of methyl red (5.7) in 100 mL ethanol (96%) (5.8). The indicator mixture has a range of pH variation that goes from approximately pH 4.8 (pink) to 5.5 (emerald green)

5.14. Sodium hydroxide, c (NaOH) = 0.1 M (mol.L^{-1}): Dilute sodium hydroxide solution (5.11) in a volumetric flask (1 mL and bring to final volume of 100 mL with deionized water).

5.15. Sulphuric acid (H_2SO_4), c (H+) = 0.1 M ($mol.L^{-1}$): Dilute concentrated sulphuric acid (5.3) in a volumetric flask (0.28 mL and bring to final volume of 100 mL with water). Always add acid to water!

5.16. Ammonium sulfate ($(NH_4)_2SO_4$)

5.17. For data quality and batch checks, the use of a soil Reference Material (RM) with a known KjN content and homogeneity is recommended.

5.18. Substances with known content of N to control the whole procedure. This may be Ethylenediaminetetraacetic acid (EDTA), acetanilid, l-asparaginacid, sulfanilacid or other amino acids with known N content. The ammonium sulphate ($(NH_4)_2SO_4$) is used to distillation control.

5.19. Control sample or Internal Reference Material (IRM) suitable for the samples to be analyzed.

6. Health and safety

This procedure involves the use of hazardous chemicals. Refer to laboratory safety guidelines or Material Safety Data Sheet (MSDS) before proceeding.

6.1. Personnel safety

Personal protection elements. Safety glasses, gloves and lab coats must be worn when handling any chemicals and samples.

Take necessary precautions when handling high-temperature equipment. Follow the manufacturer's safety guidelines when operating the equipment.

Hygiene: Wash hands and clean other exposed areas with mild soap and water after using all chemical reagents.

6.2. Chemical hazard

Sulphuric acid: Keep away from naked flames/heat. Carry out operations in a fume hood with exhaust/ventilation. Do not discharge the waste into the drain. Never dilute by pouring water into the acid. Always add the acid to the water!!!

All titrations and handling of chemicals to be undertaken in a fume hood.

7. Sample preparation

The sample size varies from grams (g) to milligrams (mg), depending on the KjN of the sample. The smaller the sample size, the finer the grinding should be to achieve sample homogenization. A representative portion of the pretreated soil sample (dried and sieved to 2 mm) should be porphyrized until the entire fraction passes through a sufficiently fine mesh sieve.

In general, if a mass greater than 1 g is weighed, a soil sample sieved by 2 mm can be used, but if a mass less than 1 g is used, it is recommended that the sample be sieved through a 0.5 mm mesh or less.

It must be ensured that the milling equipment and the sieves do not introduce contamination to the samples.

8. Procedure

8.1. Digestion

8.1.1. Weigh (4.1) 0.2-1.0 g air-dry soil (adjust if necessary, see guideline recommended from Table 1) into a 100-mL digestion tube (4.4.). Record the exact mass with an appreciation of 0.001 g.

Table 1. Recommended weight of sample for analysis

Weight (g)	KjN (approximately) (%)	OC (approximately) (%)	Color
0.2	0.5	5	Black, dark gray, dark brown
0.5	0.2	2	brown - dark brown, gray - dark gray
1.0	0.1	1	Brown

8.1.2. Add about 1-2 g catalyst mixture (5.9), 3-5 mL concentrated H_2SO_4 (in the fume hood) (5.3), and swirl carefully until the acid is thoroughly mixed with the sample. Place tubes in the block-digester (4.3) and slowly increase temperature setting to 390°C ± 10°C, ensuring that the temperature of the solution does not exceed 410°C.

Note: The K_2SO_4 in the catalyst allows the digestion temperature to be increased to an optimal value between 380ºC and 400ºC. Temperatures below 360°C produce incomplete digestions and above 420 °C losses occur.

Boil until the digestion mixture becomes clear and then continue digestion for at least ten minutes, taking care that the acid is not completely consumed.

The boiling time may be different and depends on the characteristics of the sample, but the solution must be clear at the end of the boiling.

Note: For most soils, a digestion time of 30-60 minutes is enough.

Each batch of 20-40 samples should contain at least two reagent blanks (no soil), and one or more chemical standard, control sample (IRM) or CRM.

Note: If you use traditional digestion equipment and it is necessary, you can add glass beads or some pumice granules to reduce foaming.

Boil the mixture gently so that the H_2SO_4 condenses in approximately the lower third of the tube.

The amount of soil and reagents added can be changed in the described ratio.

In soils with very little N, the macro version of the Kjeldahl method may be appropriate.

8.1.3. Lift the tube rack out of the block-digester, carefully place on a rack holder, and let tubes cool.

8.1.4. Slowly, while stirring, add about 20 mL of water to the tubes and cool. This allows the suspension of insoluble material in the solution.

If tube contents are solidified and do not dissolve, heat the tubes again until the precipitate dissolves, then cool with tap water.

If an automatic distillation unit is used with the capacity to dispense the water prior to the beginning of the distillation process, it is recommended not to allow the digests to cool completely.

8.2. Distillation and titration

8.2.1. Adjusting the distillation conditions - Distillation Blank

- Add 20 mL of H_3BO_3 solution (5.10) to an Erlenmeyer flask. Place the flask under the tip of the condenser, with the tip touching the surface of the solution.
- Place a clean, empty tube in the Distillation Unit (4.5) and add 20 mL of water and 20 mL of NaOH solution (5.11).
- Distill about 100 mL of condensate (the amount for quantitative results depends on the dimensions of the apparatus), rinse the end of the condenser.

  When steam distillation is used, a distillation rate of up to approximately 25 mL min^{-1} is applied to keep the temperature of the distillate below 22 °C.
- Add a few drops of indicator (5.13) to the distillate.
- Titrate with H_2SO_4 solution (c (H+) = 0.01 $mol.L^{-1}$) (5.12) to the end point of the indicator. Recorded the volume of the acid used (mL)

  When using a potentiometric titration, the endpoint of the titration should be at about pH = 5. In this case, the end point of the titration corresponds to the pH of the indicator turn.

  This blank distillation procedure should be repeated at least three times before distilling the reagent blanks, IRM, and samples.

  The volume of H_2SO_4 solution 0.01 $mol.L^{-1}$ (5.12) used for the titrations of blanks must be higher than 0.05 mL and lower than 0.30 mL. If the acid consumption is outside this range, the pH of the boric acid solution should be adjusted until obtaining a positive blank that meets this requirement, taking into account that:
- If the consumption of sulfuric acid (5.12) is lower than 0.05mL, the pH of the H_3BO_3 solution must be increased using NaOH solution (0.1 $mol.L^{-1}$) (5.14).
- If the consumption of sulfuric acid (5.12) is higher than 0.30 mL, the pH of the H_3BO_3 solution must be decreased using H_2SO_4 solution (0.1 $mol.L^{-1}$) (5.15).

8.2.2 Distillation control

This control ensures that there are no losses in the distillation equipment, therefore, it is necessary to carry it out before each sample run.

- Approximately 0.0100 g of $(NH_4)_2SO_4$ (5.16) is weighed in triplicate, recording the exact mass.
- The procedure indicated in point 8.2.1 is applied, maintaining the volume of distillate.

  Instead an ammonium solution can be used, containing approximately 2 mg of N in the 20 mL aliquot. This solution must be prepared the same day of the distillation control.
- The N recovered from distillation (Nr) is calculated using the formula included in Calculation.
- The N contained in the mass of added $(NH_4)_2SO_4$ is calculated:

$$N_{(NH4)2SO4} = \frac{m \times 28.013 \times p}{132.14}$$

Where,

$N_{(NH4)2SO4}$ is the N contained in the mass of $(NH_4)_2.SO_4$ used, in milligrams.
m is the mass of $(NH_4)_2.SO_4$ used, in milligrams.
p is the factor that considers the purity of $(NH_4)_2.SO_4$ used for the control (% $w.w^{-1}$). The exact value of purity is obtained from what is reported on the certificate or on the label of the reagent container, since it varies with its quality.
28.013 is the mass of the N contained in one millimole of $(NH_4)_2SO_4$, in milligrams
132.14 is the mass of one millimole of $(NH_4)_2SO_4$, in milligrams

The recovery percentage of N is calculated.

$$\%R = \frac{Nr \times 100}{N_{(NH4)2SO4}}$$

Where,

% R is the recovery percentage of N
Nr is the N recovered in the distillation stage, in milligrams
$N_{(NH4)2.SO4}$ is the N contained in the mass of $(NH_4)_2.SO_4$ used, in milligrams.

The results of the verifications must be recorded and compare the result with the recovery target (in percentage), which is predefined for the usual range of work - the system is usually considered adequate when the percentage of N recovery is in the range of 95 percent to 105 percent.
If the distillation control fails, the cause of the failure must be identified and corrective actions must be identified and undertaken to ensure confidence in the control samples and

associated samples, with repeat analyses to ensure the results are acceptable. Otherwise the analysis cannot be continued.

8.2.3. Distillation and Titration of blank test (reagent blanks), IRM or CRM and samples

The procedure indicated in point 8.2.1 is applied, maintaining the volume of distillate for reagent blanks, IRM or CRM and samples.

The acid consumption (mL) is recorded in the blanks test, in control samples (IRM), in CRM and in the soil samples.

The distillates are titrated with H_2SO_4 solution until the indicator changes color or the end of the potentiometric titration is reached.

As specified, at least two blank tests must be included in each series and the average blank value is used for subsequent calculations.

Blank test results should be kept constant over time. The cause of any recorded variation must be identified. The control chart of blank values is a good means to monitor system performance.

8.3. Spectrometric quantification

Some equipment providers offer automatic distillation with spectrometric instead of potentiometric detection of the titration end-point. One of the alternative methods for spectrometric quantification (Indo-phenol blue method) is presented in Appendix I.

9. Calculation

The content of nitrogen, (N), in milligrams (mg) of N per gram (g) of soil, is calculated using the next formula.

$$N = \frac{(V_1 - V_0) \times c(H+) \times MN}{m}$$

Where:

N It is the concentration of nitrogen in the soil, in milligrams of N per gram of soil (or grams of N per kilogram of soil);

V_1 is the volume, in millilitres (mL), of the H_2SO_4 solution used in the titration of the sample

V_0 is the volume, in millilitres (mL), of the H_2SO_4 solution used in the titration of the blank test (***V0*** is the average blank value)

c(H+) is the concentration of H+ in the H_2SO_4 solution in millimoles per millilitre (e.g. if 0.01 M H_2SO_4 solution is used, so c(H+) = 0.02 M)

MN is the molar mass of N, in milligrams (mg) per millimole (it is 14.0067)

m is the mass of test sample, in grams (g)

Report N using the International Units System as: milligrams of N per gram of soil ($mg.g^{-1}$) or grams of N per kilogramm of soil ($g.kg^{-1}$). It is also possible to report results in percentage of N (%N), for this, the result in $mg.g^{-1}$ or $g.kg^{-1}$ must be divided by ten.

Results should be reported based on air dry soil or oven dry soil (40°C).

The number of decimals reported must conform to the conventional rules of maintaining 3 digits:

- values greater than 100, no decimal reported;
- values between 10 and 100, 1 decimal (0.1) reported; and
- values less than 10, 2 decimals (0.01) reported.

10. Quality assurance/Quality control

To check performance of the method, sample replicates, CRM, substances with known content of N, control samples (IRM) and blanks should be incorporated at regular intervals in each test batch. The number and frequency of the control is dependent on the type of sample and the characteristics of the equipment and the working conditions, but it is recommended to include at least two reagent blanks and one or more chemical standard or control sample (IRM) for each batch of 20-40 samples.

In small batches, it is recommended to include daily blanks, calibrations and controls.

10.1. Precision test

- At least five percent of the samples in a test batch must be replicates, ensuring at least one duplicate sample if the batch is small.
- Calculate the percent relative standard deviation (% RSD) to determine precision.

$$\%\ RSD = \frac{s}{\bar{x}} \times 100$$

Where: s = standard deviation of the replicate result

$\bar{x}$ = mean

- Compare the result with the previously specified precision.

Acceptance requirements for precision testing should be defined by the equipment used, environmental conditions and other testing factors, as well as by the specifications or requirements for the use of information and agronomic criteria.

If the precision test fails, the cause of the failure must be identified and corrective or preventive actions must be developed and recorded.

10.2. Trueness test

10.2.1. **Recovery test**

- Perform triplicate analysis of Certified Reference Material of the analysed matrix (soil) (CRMs) or an Internal Reference Material (IRM), in accordance with the present SOP.

This test is necessary for the operational qualification of the equipment, when there is a problem with the other controls, or if the equipment has been repaired.

The CRM should cover the range of KjN typically found in test samples.

To assess equipment performance, this procedure should be replicated with different levels of KjN. Different levels can be selected by using CRM with different concentrations of KjN or by simply weighing different masses of the same CRM.

All CRMs must be stored and used as directed by the manufacturer's label.

Besides to CRM, chemical substances with known content of N are used to control the digestion and distillation unit, to control the whole procedure.

- Calculate the percent recovery based on the equation below.

$$\% \text{ Recovery} = \frac{\text{mean of observed values}}{\text{true value}} \times 100$$

- Compare the result with the recovery target (in percentage), which is predefined for the usual range of work.

The recovery target must be defined for the usual range of work. The definition should consider the working conditions. It should also consider the specifications or requirements for the given use of the information and for any agronomic criteria. The recovery can also be considered acceptable if it is within the confidence interval reported for the certified value of CRMs.

If the recovery test fails, the cause of the failure must be identified and corrective or preventive actions must be developed.

10.2.2. **Interlaboratory comparison**

At least once a year the laboratory must participate in interlaboratory proficiency test.

If the result obtained is questionable or unsatisfactory, it is necessary to carry out an evaluation, identify the root cause of the problem, and develop corrective and preventive actions.

10.3. Control chart

- Perform the replicate analysis of a control sample or an IRM in a test batch of samples;
- Plot the result in a control chart;
- Monitor the results.

If results are out of specified limits (or tend to be so), an evaluation must be made. The cause of the non-compliance must be identified to develop corrective and preventative actions.

11. References

Bremner, J.M. 1996. Nitrogen-total. In *Methods of soil analysis, Part 3, Chemical methods*. (Ed. D.L. Sparks), pp. 1085-1121. Nº 5. Soil Science Society of America Book Series. Soil Science Society of America, Inc. and American Society of Agronomy Inc., Madison, Wisconsin, USA.

Eurachem. 2014. *The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics*. Second Edition.

ISO 11261:1995. 1995. *Soil quality - Determination of total nitrogen – Modified Kjeldahl method.*

12. Appendix I. Spectrometric quantification (indo-phenol blue method)

12.1. Brief introduction and principle

The method reported by Berthelot (1859) is based on the formation of a blue product by reacting ammonia (obtained from samples that have been decomposed and distilled before the reaction) with sodium phenate and sodium hypochlorite in the presence of acetone.

The indophenol blue method has good sensitivity (with an absorbance maximum at 630 nm, but the spectrum from 600 to 650 nm is considered acceptable). The method is thought to be quick and reliable if the procedures are carefully followed and is considered to have very good sensitivity. The method can achieve a precision of ± 2% or higher, whereas the Nessler method achieves a precision of ± 3% (Allen et. at., 1974).

12.2. Apparatus

Common laboratory equipment includes:

12.2.1. A water bath (maintaining a temperature of 40±0.5° C);

12.2.2. Spectrophotometer that can read at a wavelength of 630nm;

Note. You can also use the equipment listed in item 4.1 through item 4.5.

12.3. Materials

All reagents shall be of analytical grade. Use water of grade 2 complying with ISO 3696.

12.3.1. Ammonium chloride

12.3.2. Sodium hydroxide

12.3.3. Sodium phenolate

12.3.4. Sodium potassium tartrate (Rochelle salt)

12.3.5. Sodium nitroprusside

12.3.6. Sodium hypochlorite solution (5% available Cl)

12.3.7. Chloroform (chloroform is toxic!)

12.3.8. Nitrogen standard.

Stock solution (1000 $mg.L^{-1}$). Dissolve 1.9100 ± 0.0001 g NH_4Cl (dry: at 110^{O}C until constant weight is obtained) in 400 mL of water, add 2 mL of chloroform (as a preservative) and dilute to 500 mL.

Working solution (10 $mg.L^{-1}$). Make working solution by diluting the stock solution 100-fold. Prepare this standard shortly before use.

12.3.9. Sodium hydroxide, c (NaOH) = 10 M (mol.L-1) : Dissolve 400.00 ± 0.01 g of NaOH (5.5.) in 800 mL of water, refrigerating conveniently. Cool to room temperature and dilute to final volume of 1000 mL with water.

12.3.10. Sodium phenolate reagent. Dissolve 50.00 ± 0.01 g phenol in 250 mL 10 M NaOH (12.3.2) and dilute with water to 400 mL.

12.3.11. Sodium potassium tartrate reagent. Dissolve 50.00 ± 0.01 g in 400 mL of water and dilute with water to 500 mL.

12.3.12. Sodium nitroprusside solution. Dissolve 0.16 ± 0.01 g sodium nitroprusside in 80 mL of water and dilute with water to 100 mL.

12.3.13. Certified Reference Material (CRM) with known KjN content. Soil samples with certified KjN content.

12.3.14. Substances with known content of N to control the whole procedure. This may be Ethylenediaminetetraacetic acid (EDTA), acetanilid, l-asparaginacid, sulfanilacid or other amino acids with known N content. The ammonium sulphate ($(NH_4)_2SO_4$) is used for distillation control.

12.3.15. Control sample or Internal Reference Material (IRM) suitable for the samples to be analyzed.

Note: Make sure that you strictly comply with the health and safety recommendations described in item 6.

12.4. Procedure

12.4.1. Digestion and Distillation

Perform the digestion according to item 8.1.

Perform the distillation according to item 8.2, measuring the final volume (100 mL) safely, in a volumetric flask (without adding any indicators and without titrating).

12.4.2. Spectrometric quantification.

Use 50 mL volumetric flasks.

12.4.2.1 To make the calibration curve add 0 to 10 mL of the working solution (12.3.8) to obtain a range from 0.01 to 0.1 mg of N (considering that each mL of working solution contains 0.01 mg of N). Add up water to 10 mL (or another volume corresponding to the aliquot used for the samples) to each of the standards.

12.4.2.2. Add 10 mL aliquot (or another convenient volume) of the distilled sample;

12.4.2.3. Add 10 mL of water to each volumetric flask (this step should be performed both for the samples and for the standards);

12.4.2.4. Add 8.00 ± 0.01 mL of sodium potassium tartrate (12.3.11) to each flask (this step should be performed both for the samples and for the standards);

12.4.2.5. Add 1.00 ± 0.01 mL of sodium nitroprusside solution (12.3.12.) (this step should be performed both for the samples and for the standards);

12.4.2.6. Add 2.00 ± 0.01 mL of sodium phenolate reagent (12.3.10.) and stir (this step should be performed both for the samples and for the standards);

12.4.2.7. Add 1.00 ± 0.01 mL of sodium hypochlorite solution (12.3.6.) (this step should be performed both for the samples and for the standards);

12.4.2.8. Dilute it with water up to the volumetric mark and incubate in a water bath at a temperature of 40.0°C ± 0.5º C for 20 min (carefully monitor the temperature and time), then cool to controlled room temperature under the same conditions for both the samples and the standards (this step should be performed both for the samples and for the standards).

12.4.2.9. Measure absorbance/transmittance at 630 nm.

12.5. Calculations.

The content of nitrogen, (N), in milligrams (mg) of N per gram (g) of soil, is calculated using the following formula:

$$N = \frac{(a - b) \times 100}{aliquot \times m}$$

Where:

N It is the concentration of nitrogen in the soil, in milligrams of N per gram of soil (or grams of N per kilogram of soil);

a is the mass of nitrogen in the aliquot of the distilled sample, obtained by the calibration curve, in milligrams (mg),

b is the mass of nitrogen in blank of the calibration curve, in milligrams (mg),

100 is the total distilled volume, in millilitres (mL),

aliquot is the volume of the aliquot (taken from the total distilled volume), in milliliters (mL),

m is the mass of test sample, in grams (g)

Report N using the International Units System as: milligrams of N per gram of soil ($mg.g^{-1}$) or grams of N per kilogram of soil ($g.kg^{-1}$). It is also possible to report results in percentage of N (%N), for this, the result in $mg.g^{-1}$ or $g.kg^{-1}$ must be divided by 10.

Results should be reported based on air dry soil or oven dry soil (40°C).

The number of decimals reported must conform to the conventional rules of maintaining 3 digits:

- values greater than 100, no decimal reported;
- values between 10 and 100, 1 decimal (0.1) reported; and
- values less than 10, 2 decimals (0.01) reported.

Note: Perform Quality assurance/Quality control as described in item 10.

References

Allen, S.E., Grimshaw, H. M., Parkinson, J.A. & Quarmby, C. 1974. *Chemical analysis of ecological materials*. Blackwell Scientific Publications.

Berthelot, M. 1859. Repertoire Chimie Pure Applique, 1, 284.

13. Appendix II.—Acknowledgments

GLOSOLAN would like to thank Ms. Miriam Mabel Ostinelli for leading the harmonization of this SOP, and the following members of the working group who harmonized information and drafted the SOP: Ms. Špela Velikonja Bolta, working group leader for Europe and Eurasia, and Mr. Vladimir Metodiev Ilinkin. GLOSOLAN would also like to thank the experts who were part of the Review Panel and who ensured the finalization of the SOP, all the laboratories that provided inputs for the harmonization of this method, all the experts who technically reviewed this SOP, and Mr. Michael Watts from the British Geological Survey, United Kingdom for the technical proofreading of the document.

14. Appendix III.—List of authors

Leading author:

- Ms. Miriam Mabel Ostinelli, Laboratorio del Instituto de Suelos CIRN-CNIA-INTA**, Argentina**

Main authors (in alphabetical order):

- Ms. Špela Velikonja Bolta, Agricultural Institute of Slovenia, **Slovenia**
- Mr. Vladimir Metodiev Ilinkin - Central Laboratory, Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", **Bulgaria**

Members of the Review Panel (in alphabetical order):

- Ms. Ágnes Nagy, Food Chain Safety Centre Non-profit Ltd., Soil Conservation Laboratory, Velence, **Hungary**
- Ms. Hana'a Abdel Burezq, Soil Chemistry Laboratory, Soil Physics Laboratory, **Kuwait**
- Ms. Miriam Mabel Ostinelli, Laboratorio del Instituto de Suelos CIRN-CNIA-INTA**, Argentina**
- Mr. Sadeq Jaafar Hasan Dwenee, Soil Chemical Analysis Lab, Soil and Water Resources Center, Agricultural Researches Directorate, Ministry of Science and Technology, **Iraq**

15. Appendix IV.—Contributing laboratories

GLOSOLAN thanks the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for the soil nitrogen Kjeldahl method. This information was used as a baseline for the global harmonization.

From the Asian region:

- Soil Research Directorate, Agriculture Research Institute of Afghanistan, Ministry of Agriculture Irrigation and Livestock, **Afghanistan**
- Bangladesh Soil Resource Development Institute, **Bangladesh**
- Royal University of Agriculture, **Cambodia**
- ICAR-Indian Institute of Soil Science, **India**
- Indonesian Soil Research Institute, **Indonesia**

- Department of Agricultural Land Management, **Lao People's Democratic Republic**
- Soil and Agrochemistry Laboratory, School of Agroecology, Mongolian University of Life Science, **Mongolia**
- Department of Agricultural Research, **Myanmar**
- Soil Science Division, NARC, **Nepal**
- Regional Soils Laboratory, Integrated Laboratory Division, Department of Agriculture, Regional Field Office 3, **Philippines**
- Laboratory Services Division - Bureau of Soils and Water Management, **Philippines**
- Horticultural Crops Research and Development Institute, Department of Agriculture, **Sri Lanka**
- Laboratory of Soil Science, Dep. of Plant and Soil Sciences, Fac. Of Agriculture, Chiang Mai University, **Thailand**
- Soils Fertilizers Research Institute, **Viet Nam**

From the Pacific region:

- DES - Chemistry Centre, **Australia**
- Fiji Agriculture Chemistry Laboratory, **Fiji**
- Sugar Research Institute of Fiji, **Fiji**
- Institute of Applied Sciences, University of the South Pacific, **Fiji**
- Laboratoire des Moyens Analytiques Nouméa, **New Caledonia**
- University of the South Pacific, Alafua Campus, **Samoa**
- Scientific Research Organisation of Samoa, **Samoa**

From the Near East and North African region:

- Soil and water Institute, **Islamic Republic of Iran**
- Ministry of Science and Technology, Directorate of Agricultural Researches, Soil and Water Resources Centre, **Iraq**
- National Agricultural Research Center, **Jordan**
- Kuwait Institute for Scientific Research, **Kuwait**
- Lebanese Agricultural Research Institute, **Lebanon**
- General Commission for Scientific Agricultural Research, **Syrian Arab Republic**
- Land & Water Research Centre, **Sudan**
- Natural Resources/ Land Use, Conservation and Production Administration Central Laboratory, **Sudan**
- Institut National de Recherches en Génie Rural, Eaux et Forêts / Laboratoire de Recherche Valorisation des Eaux Non Conventionnelles, **Tunisia**

From the African region:

- International Institute of Tropical Agriculture (IITA), **Cameroon**
- Institut de Recherches Agronomique et Forestière, **Gabon**
- CSIR-Soil Research Institute, **Ghana**
- Laboratoire National d'Analyse de Sols, des Engrais, des Végétaux et Eaux du Services National des Sols, **Guinea**

- Kenya Agricultural & Livestock Research Organization (KALRO), **Kenya**
- Laboratoire Sol, Eau, Plante et Engrais, IRAN- Centre Régional de la Recherche Agronomique, **Niger**
- National Soil Testing Laboratory Complex, **Nigeria**
- National Soil, Fertilizer and Water Laboratory, Kaduna, **Nigeria**
- Laboratoire des Sols, Eau, Végétaux et Engrais, Institut Togolais de Recherche Agronomique, **Togo**
- Zambia Agriculture Research Institute, **Zambia**
- University of Zambia, **Zambia**
- Zimbabwe Sugar Association Experiment Station, **Zimbabwe**
- Fertilizers, Seed and Grain, **Zimbabwe**

From the European region:

- Axe Echanges Eau-Sol-Plantes, GxABT, Liege University, **Belgium**
- REQUASUD, **Belgium**
- Central Laboratory, Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", **Bulgaria**
- Forest Research Institute-BAS, **Bulgaria**
- Central Institute for Supervising and Testing in Agriculture, **Czechia**
- Scientific-Research Centre of Agriculture, **Georgia**
- Food Chain Safety Centre Non-profit Ltd., **Hungary**
- Mediterranean University AGRARIA Department, **Italy**
- Latvian State Forest Research Institute "Silava", **Latvia**
- State Plant Protection Service, Agrochemical Laboratory, **Latvia**
- University “St. Kliment Ohridski”-Bitola Scientific Tobacco Institute – Prilep, **North Macedonia**
- Laboratório de Solos e Fertilidade da Escola Superior Agrária de Castelo Branco, **Portugal**
- National Research and Development Institute for Soil Science Agrochemistry and Environment – Department for physical and chemical analysis*,* **Romania**
- Kmetijski Inštitut Slovenije/Agricultural Institute of Slovenia, **Slovenia**
- Soil and Fertilizer Laboratory, Dept of Soil Sci and Plant Nutrition, Faculty of Agriculture, Ankara University, TURKEY (SOFREL-TR), **Turkey**

From the Eurasian region:

- Laboratory of Instrumental Soil Research Methods of the National Scientific Center «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», **Ukraine**

From Latin America:

- Laboratorio del Instituto de Suelos CIRN – CNIA - INTA**, Argentina**
- Centro de Estudios Ambientales Integrados, Facultad de Ingeniería, Universidad Nacional de la Patagonia San Juan Bosco, **Argentina**

- Laboratorio de Química de Suelos-LABOSUELOS-UASD, **Dominican Republic**
- Ministry of Agriculture, Natural Resources and Rural Development (MARNDR), **Haiti**
- Colegio de Postgraduados (LABFER-CPM), **México**
- Laboratorio de Suelo y Agua Comandante Fidel Castro Ruz, Instituto Nicaragüense de Tecnología Agropecuaria (INTA - Nicaragua), **Nicaragua**
- Instituto de Investigación Agropecuaria de Panamá (IDIAP), **Panama**
- Ministerio de Ganadería Agricultura y Pesca (MGAP)- Laboratorio de Caracterización de Suelos, **Uruguay**
- Instituto Venezolano de Investigaciones Científicas, **Venezuela**

From North America:

- None

The Global Soil Partnership (GSP) is a globally recognized mechanism established in 2012. Our mission is to position soils in the Global Agenda through collective action. Our key objectives are to promote Sustainable Soil Management (SSM) and improve soil governance to guarantee healthy and productive soils, and support the provision of essential ecosystem services towards food security and improved nutrition, climate change adaptation and mitigation, and sustainable development.

GLOSOLAN
GLOBAL SOIL LABORATORY NETWORK

GLOSOLAN is a Global Soil Laboratory Network which aims to harmonize soil analysis methods and data so that soil information is comparable and interpretable across laboratories, countries and regions. Established in 2017, it facilitates networking and capacity development through cooperation and information sharing between soil laboratories with different levels of experience. Joining GLOSOLAN is a unique opportunity to invest in quality soil laboratory data for a sustainable and food secure world.

Thanks to the financial support of

PHOSAGRO®

CB3642EN/1/03.21