
**Soil quality — Determination of nitrate,
nitrite and ammonium in field-moist soils
by extraction with potassium chloride
solution —**

Part 2:

**Automated method with segmented flow
analysis**

*Qualité du sol — Dosage des nitrates, des nitrites et de l'ammonium
dans des sols bruts par extraction avec une solution de chlorure de
potassium —*

Partie 2: Méthode automatisée avec analyse en flux segmenté



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14256-2 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

ISO 14256 consists of the following parts, under the general title *Soil quality — Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution*:

- *Part 1: Manual method*
- *Part 2: Automated method with segmented flow analysis*

Soil quality — Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution —

Part 2: Automated method with segmented flow analysis

1 Scope

This part of ISO 14256 describes an automated method for the determination of nitrate, nitrite and ammonium in a 1 mol/l potassium chloride extract of field-moist soil samples.

This part of ISO 14256 is applicable to all types of soils homogenized by suitable methods.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Principle

The homogenized soil samples are extracted by means of 1 mol/l potassium chloride solution. The concentrations of the inorganic nitrogen compounds nitrate, nitrite and ammonium in the extracts are determined using automated spectrophotometric methods.

NOTE The method of determination specified in this part of ISO 14256 is based on segmented flow analysis systems. Continuous flow analysis systems may also be appropriate.

4 Reagents

Use only reagents of recognized analytical grade.

4.1 Water, of grade 2 in accordance with ISO 3696, having a specific conductivity not higher than 0,2 mS/m at 25 °C.

4.2 Ammonium chloride, (NH₄Cl).

4.3 Potassium chloride, KCl.

- 4.4 Sodium citrate dihydrate**, $C_6H_5Na_3O_7 \cdot 2 H_2O$.
- 4.5 Sodium hydroxide**, NaOH.
- 4.6 Potassium nitrate**, KNO_3 .
- 4.7 Sodium nitrite**, $NaNO_2$.
- 4.8 Sodium salicylate**, $C_7H_5O_3Na$.
- 4.9 Sulfanilamide**, $C_6H_8N_2O_2S$.
- 4.10 Ammonium sulfate**, $(NH_4)_2SO_4$.
- 4.11 Sodium potassium tartrate tetrahydrate**, $C_4H_4KNaO_6 \cdot 4 H_2O$.
- 4.12 Hydrochloric acid**, $c(HCl) = 1 \text{ mol/l}$.
- 4.13 Phosphoric acid**, $w(H_3PO_4) = 85 \%$.
- 4.14 N-(1-naphthyl)ethylenediamine dihydrochloride**, $C_{12}H_{16}N_2Cl_2$.
- 4.15 Sodium nitroprusside dihydrate**, $Na_2[Fe(CN)_5NO] \cdot 2 H_2O$.
- 4.16 Sodium dichloroisocyanurate**, $C_3Cl_2N_3NaO_3 \cdot 2 H_2O$.
- 4.17 Polyoxyethylene lauryl ether** ¹⁾ (30 %), wetting agent.
- The wetting agent solution should not be stored longer than 6 months.
- 4.18 Coarse cadmium powder** (particle size 0,3 mm to 0,8 mm).
- 4.19 Potassium chloride solution (I)**, $c(KCl) = 1 \text{ mol/l}$.
- Dissolve 74,55 g of potassium chloride (4.3) in water (4.1). Make up with water (4.1) to 1 l.
- 4.20 Potassium chloride solution (II)**, $c(KCl) = 2 \text{ mol/l}$.
- Dissolve 149,10 g of potassium chloride (4.3) in water (4.1). Make up with water (4.1) to 1 l.
- 4.21 Ammonium hydroxide solution**, $w(NH_4OH) = 3 \%$.
- 4.22 Copper(II) sulfate solution**, $\rho(CuSO_4) = 20 \text{ g/l}$.
- 4.23 Ammonium stock solution**, $\rho(NH_4-N) = 200 \text{ mg/l}$.

In a volumetric flask of 1 l, dissolve 0,943 9 g of ammonium sulfate (4.10) in water (4.1). Make up with water (4.1) to 1 l.

NOTE Stored in a refrigerator at 4 °C, this solution is stable at least one month.

1) Polyoxyethylene lauryl ether is available commercially under the name Brij-35. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4.24 Nitrate stock solution, ρ (NO₃-N) = 375 mg/l

Dissolve 2,708 3 g of potassium nitrate (4.6) in water (4.1). Make up with water (4.1) to 1 l.

NOTE Stored in a refrigerator at 4 °C, this solution is stable at least six months.

4.25 Nitrite stock solution, ρ (NO₂-N) = 300 mg/l.

Dissolve 1,479 g of sodium nitrite (4.7) in water (4.1). Make up with water (4.1) to 1 l.

NOTE Stored in a refrigerator at 4 °C, this solution is stable at least six months.

4.26 Sodium nitroprusside solution

Dissolve 1 g of sodium nitroprusside dihydrate (4.15) in 1 l of water (4.1).

4.27 Sodium dichloroisocyanurate solution

Dissolve 2 g of sodium dichloroisocyanurate (4.16) and 25 g of sodium hydroxide (4.5) in 1 l of water (4.1).

4.28 Buffer solution (I)

Dissolve, in a volumetric flask of 1 l, 25 g of ammonium chloride (4.2) in some water. Add 12,5 ml of ammonium hydroxide solution (4.21) and 1 ml of wetting agent (4.17). Make up to the mark with water (4.1) and mix.

The pH of this solution should be between pH 6 and pH 8.

4.29 Buffer solution (II), pH = 5,2

In a glass vessel of 1 l, containing about 500 ml of water (4.1), dissolve 24 g of sodium citrate dihydrate (4.4) and 33 g of sodium potassium tartrate tetrahydrate (4.11). Adjust the pH of the solution to pH 5,2 by adding hydrochloric acid (4.12) (about 20 ml are needed). Transfer the solution in a volumetric flask of 1 l. Fill up to the mark with water. Add 1 ml of wetting agent (4.17).

4.30 Cd-Cu reducing agent

Swirl approximately 5 g of cadmium powder (4.18) during 1 min with about 30 ml of hydrochloric acid (4.12). Wash with water until acid-free. Then add about 50 ml of a copper(II)sulfate solution (4.22) and swirl during 3 min. Wash at least ten times with water to remove any flocculated copper. Store the Cd-Cu reducing agent in a dark place.

4.31 Colour reagent (I)

In a 1 l volumetric flask already containing about 800 ml of water (4.1), dissolve 80 g of sodium salicylate (4.8) and 25 g of sodium hydroxide (4.5) and make up to the mark with water.

4.32 Colour reagent (II)

In a 1 l volumetric flask, add 150 ml of concentrated phosphoric acid (4.13) to 0,5 l of water (4.1). Add 0,5 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride (4.14) and swirl until dissolved. Then dissolve 10 g of sulfanilamide (4.9) in this mixture and make up to the mark with water.

5 Apparatus

Standard laboratory apparatus, and in particular the following.

5.1 Balances, of accuracy 0,1 g and 0,001 g.

5.2 Polyethylene bottles of nominal volume 500 ml, with screw caps, or other suitable containers which neither absorb nitrite or ammonium nor release it into solution.

5.3 Shaking machine, end-over-end shaker, frequency 40 min⁻¹.

5.4 Centrifuge, capable of holding tubes of nominal volume 100 ml.

5.5 Polyethylene centrifuge tubes, nominal volume 100 ml.

5.6 Glass vessels, Erlenmeyer type, nominal volume 100 ml.

5.7 Segmented flow analysis (SFA) system for total content of nitrate and nitrite, consisting of sampler, pump, reduction column, nitrate analysis unit, photometer and recorder. The flow diagram of an appropriate SFA system is given in Figure 1.

NOTE This flow diagram may require adaptation, depending on the system used.

5.8 Segmented flow analysis (SFA) system for content of nitrite, consisting of sampler, pump, nitrite analysis unit, photometer and recorder. The flow diagram of an appropriate SFA system is given in Figure 2.

NOTE This flow diagram may require adaptation, depending on the system used.

5.9 Segmented flow analysis (SFA) system for content of ammonium, consisting of sampler, pump, ammonium analysis unit, photometer and recorder. The flow diagram of an appropriate SFA system is given in Figure 3.

NOTE This flow diagram may require adaptation, depending on the system used.

5.10 Cu-Cd reduction tube, consisting of U-shaped glass tubing (or as recommended by the manufacturer), about 15 cm long and with internal diameter of 2 mm, provided with ferrules for connection to the SFA tubing.

NOTE It is possible to purchase such tubes filled with cadmium from the SFA system manufacturer, or to make them (see 4.28, 4.30 and 7.2.2).

6 Sampling

6.1 Handling of the soil samples

The samples shall be protected from warming during the sampling procedure.

Transportation to the laboratory shall be organized in such a way that no warming occurs. Transportation in a cool box is recommended, especially in late spring and summer.

If the samples are analysed within three days, it is sufficient to store them at 4 °C. Otherwise, they should be stored at -20 °C (deep-frozen), which enables storing for several weeks, without any significant change in the content of mineral nitrogen.

When the content of mineral nitrogen is determined in deep-frozen soil samples, the temperature and the duration of the thawing process shall be controlled. The samples can be thawed at room temperature, if they are homogenized and extracted within 4 h after beginning of thawing. Thawing at 4 °C is also possible, but the thawing period should not exceed 48 h.

The time period necessary for thawing is greatly influenced by the size of the sample. It is recommended that the soil sample be as small as practicable for the analysis before deep-freezing.

6.2 Laboratory sample

The laboratory sample is made by homogenizing the original, or the deep-frozen and thawed, soil sample. Homogenizing can be done by mixing thoroughly the total soil sample, either by hand or using a mechanical mixer, which ensures that soil aggregates are divided into particles less than 5 mm in diameter.

Use rubber gloves when mixing the soil by hand.

Use part of the laboratory sample to determine the water content in accordance with ISO 11465.

If soil samples cannot be analysed within the time periods given above for fresh soil samples, and therefore have to be deep-frozen, it is recommended to homogenize them before freezing and to freeze only the amount of soil which is necessary for analysis (see 7.1).

7 Procedure

7.1 Extraction

7.1.1 General

The field-moist soil is extracted with 1 mol/l potassium chloride solution (4.19) at a 1:5 ratio (mass concentration) at $20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. After 1 h of extraction, the solution is centrifuged for the determination of the inorganic nitrogen compounds in the supernatant solution. The extraction is performed at a nearly constant temperature of $20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, because the amount of extractable ammonium nitrogen is influenced by the temperature during the extraction.

In other climatic conditions, a different constant temperature may be chosen. The extraction temperature used should be mentioned in the test report.

Centrifugation is preferred because most filter papers either absorb ammonium or may be contaminated. When the extracts are filtered, the filter papers should be stored at least one week in a desiccator over concentrated sulfuric acid or a drying medium containing sulfuric acid. The first 20 ml of the filtrate shall be discarded.

Alternatively, clear supernatant solutions may be obtained by letting the soil settle for 4 h at a temperature not exceeding $4\text{ }^{\circ}\text{C}$.

7.1.2 Procedure

Weigh out 40,0 g of the laboratory sample in the polyethylene container (5.2). Add 200 ml of potassium chloride solution (4.19) at a temperature of $20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. Shake mechanically (5.3) for 1 h at $20\text{ }^{\circ}\text{C}$. Decant about 60 ml of the extract suspension into centrifuge tubes (5.5) and centrifuge (5.4) for 10 min at about 3 000 g. Decant the supernatant solution into glass vessels (5.6) and measure the content of nitrate, nitrite and ammonium, as described in the following procedures (7.2, 7.3 and 7.4, respectively) using the relevant aliquots.

Also perform a blank test by adding only the potassium chloride solution (I) (4.19) to the polyethylene bottle (5.2).

Measurement of the relevant nitrogen fractions should be performed immediately, or at the latest one day after the extraction. If this is not possible, the extracts should be stored in a refrigerator at temperatures not higher than $4\text{ }^{\circ}\text{C}$, for one week at most.

NOTE Most manufacturers of flow analysers offer systems in which three analysis units can be installed, in order to measure the content of nitrate, nitrite and ammonium at the same time. If such a system is used, the stock solutions (see 4.24, 4.25 and 4.23) and the solutions of the calibration series of nitrate, nitrite and ammonium can be prepared together in the same flask, so that each solution contains both nitrogen compounds at the relevant concentration.

7.2 Determination of the sum of nitrate and nitrite

7.2.1 General

In a segmented flow analysis (SFA) system, nitrate in the extracts is reduced to nitrite by passing copperized cadmium powder (4.30). Nitrite originally present in the soil and those produced by reduction form a diazo compound in acid medium after the addition of sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride (4.14) (Griess-Ilosvay reagent). Its absorbance is measured at a wavelength of 543 nm.

Under aerobic conditions, the content of nitrite in soils is negligible. In this case, the determination of the content of nitrite is not necessary, and the method gives the content of nitrate directly.

7.2.2 Preparation of the Cd-Cu reduction tube

Fill the U-shaped column (5.10) with buffer solution (I) (4.28), taking care not to introduce air bubbles. Introduce the activated (copperized) cadmium powder (4.30) with the aid of a funnel on both sides of the column. Apply vibration now and then to pack the powder. Fill the column to 5 mm from the top and seal the ends with a small plug of glass wool.

The column is now ready for use and can be placed in the SFA system.

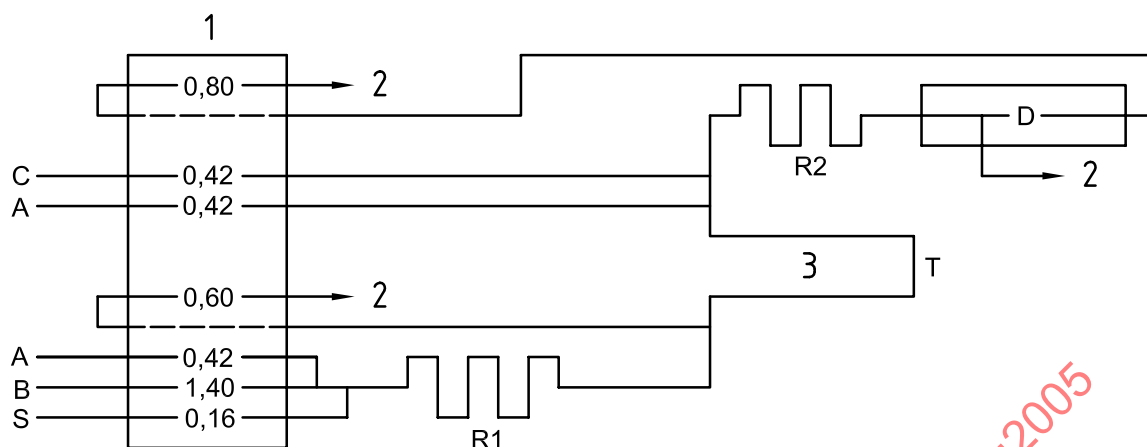
NOTE To lengthen the lifetime of the Cd-Cu reducing agent, a copper wire can be installed at the entrance of the Cd-Cu reduction tube and renewed if necessary.

7.2.3 Procedure

Pipette 0 ml, 1,0 ml, 2,0 ml, 3,0 ml and 4,0 ml of the nitrate stock solution (4.24) into 250 ml volumetric flasks, add 125 ml of the potassium chloride solution (II) (4.20) and make up to the mark with water (4.1). This calibration series can be used up to one week when the flasks are stored in a refrigerator. It has the following NO₃-N concentrations:

0 mg/l, 1,50 mg/l, 3,00 mg/l, 4,50 mg/l and 6,00 mg/l.

Use the centrifugates obtained in accordance with 7.1.1. Place the soil extracts, the blank extract and the calibration series in the automatic sampler. Operate the segmented flow system, for example according to the flow diagram given in Figure 1 or according to the manufacturer's specification. Plot the calibration curve and record the nitrogen concentrations.



Key

1 pump, ml/min

2 wastes

3 nitrate reduction

S soil extract solution

A segmenting air

B buffer solution (I) (4.28)

C colour agent (II) (4.32)

R1 reaction coil 1, length = 75 cm (10 turns), internal diameter = 1,5 mm

R2 reaction coil 2, length = 150 cm (20 turns), internal diameter = 1,5 mm

T reduction tube, U-shaped, length = 15 cm, internal diameter = 2 mm, filled with copperized cadmium powder (4.30)

D detector, measurement cell 10 mm, filter 543 nm

Figure 1 — Example of a segmented flow analysis system for the determination of nitrogen as nitrate plus nitrite (7.2)

7.3 Determination of nitrite

7.3.1 General

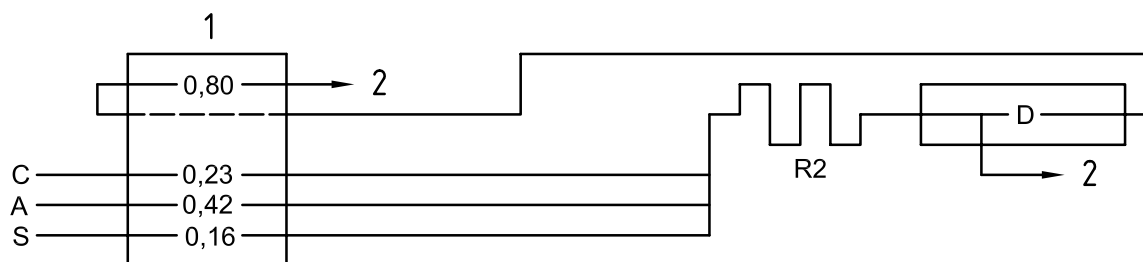
In a segmented flow analysis (SFA) system, nitrite forms a diazo compound in acid medium after the addition of sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride (Griess-Ilosvay reagent). Its absorbance is measured at a wavelength of 543 nm.

7.3.2 Procedure

Pipette 0 ml, 1,0 ml, 2,0 ml, 3,0 ml and 4,0 ml of the nitrite stock solution (4.25) into 250 ml volumetric flasks, add 125 ml of the potassium chloride solution (II) (4.20) and make up to the mark with water (4.1). This calibration series can be used during one week maximum, if the flasks are stored in a refrigerator. It has the following NO₂-N concentrations:

0 mg/l, 1,20 mg/l, 2,40 mg/l, 3,60 mg/l, and 4,80 mg/l.

Use the centrifugates obtained in accordance with 7.1.1. Place the soil extracts, the blank extract and the calibration series in the automatic sampler. Operate the segmented flow system, for example according to the flow diagram given in Figure 2 or according to the manufacturer's specification. Plot the calibration curve and record the nitrogen concentrations.

**Key**

1 pump, ml/min

2 wastes

S soil extract solution

A segmenting air

C colour reagent (II) (4.32)

R2 reaction coil 2, length = 150 cm (20 turns), internal diameter = 1,5 mm

D detector, measurement cell 50 mm, filter 543 nm

Figure 2 — Example of a segmented flow analysis system for the determination of nitrogen as nitrite (7.3)

7.4 Determination of ammonium

7.4.1 General

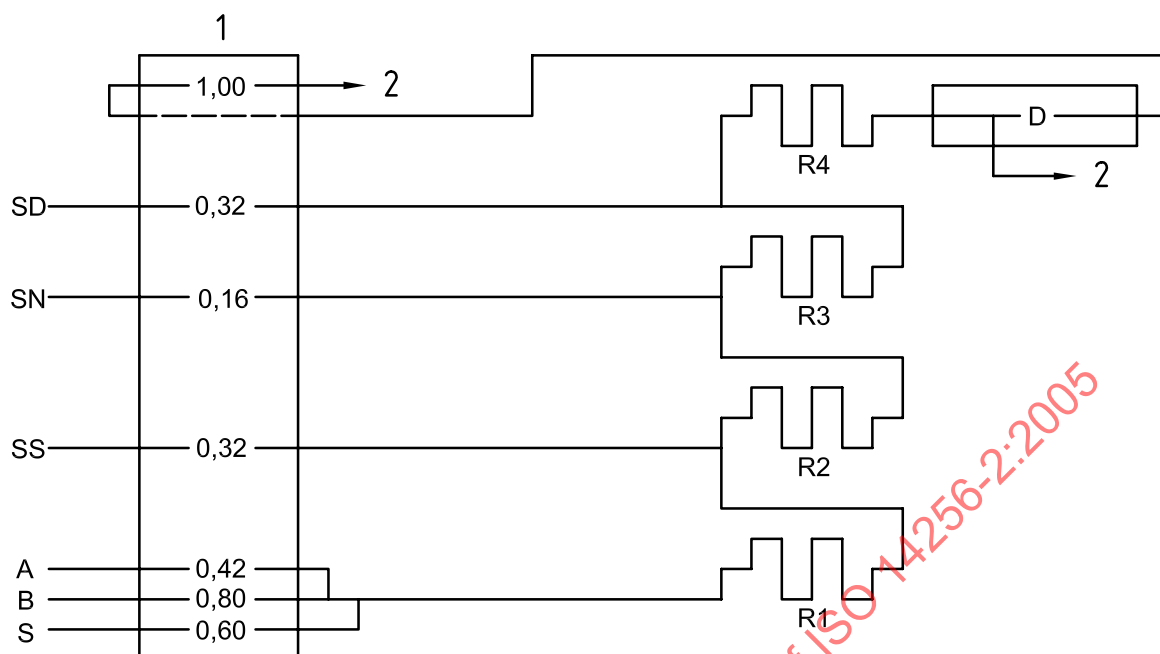
The determination of ammonium ions is carried out using an SFA system and is based on the Berthelot reaction, in which a phenol derivative (here salicylate, 4.8) forms an indophenol in the presence of ammonium and sodium dichloroisocyanurate (4.16) under catalytic action of sodium nitroprusside (4.15). In alkaline medium, the indophenol thus formed has a green-blue colour, the absorbance of which is measured at a wavelength of 660 nm.

7.4.2 Procedure

Pipette 0 ml, 0,5 ml, 1,0 ml, 1,5 ml, and 2,0 ml of the ammonium stock solution (4.23) into 250 ml volumetric flasks, add 125 ml of the potassium chloride solution (II) (4.20) and make up to the mark with water. This calibration series can be used during one week maximum, if the flasks are stored in a refrigerator. It has the following $\text{NH}_4\text{-N}$ concentrations:

0 mg/l, 0,40 mg/l, 0,80 mg/l, 1,20 mg/l, and 1,60 mg/l.

Use the centrifugates obtained in accordance with 7.1.1. Place the soil extracts, the blank extract and the calibration series in the automatic sampler. Operate the segmented flow system, for example according to the flow diagram given in Figure 3 or according to the manufacturer's specification. Plot the calibration curve and record the nitrogen concentrations.

**Key**

1 pump, ml/min

2 wastes

S soil extract solution

A segmenting air

B buffer solution (II) (4.29)

SS colour reagent (I) (4.31)

SN sodium nitroprusside solution (4.26)

SD sodium dichloroisocyanurate solution (4.27)

R1, R2, R3 reaction coils, length = 40 cm (5 turns), internal diameter = 1,5 mm

R4 reaction coil, length = 280 cm (35 turns), internal diameter = 1,5 mm, with a constant temperature of 40 °C

D detector, measurement cell 30 mm, filter 660 nm

Figure 3 — Example of a segmented flow analysis system for the determination of ammonium (7.4)**8 Calculation and expression of results****8.1 Nitrogen as nitrate plus nitrite**

The mass fraction of both nitrogen forms, nitrate and nitrite, in the soil, $w[(\text{NO}_3 + \text{NO}_2)\text{-N}]$, expressed in milligrams nitrogen per kilogram of oven-dry soil, is calculated according to Equation (1):

$$w[(\text{NO}_3 + \text{NO}_2)\text{-N}] = (a - b) \cdot \left[\frac{V_{\text{ES}}}{m_{\text{S}}} \cdot \left(1 + \frac{w_{\text{W}}}{100} \right) + \frac{w_{\text{W}}}{100} \right] \quad (1)$$

where

a is the mass concentration of $(\text{NO}_3 + \text{NO}_2)\text{-N}$ in the soil extract, in milligrams per litre;

b is the mass concentration of $(\text{NO}_3 + \text{NO}_2)\text{-N}$ in the blank extract, in milligrams per litre;

V_{ES} is the volume of extracting solution (usually 200 ml);

m_S is the mass of wet soil used for extraction (usually 40 g);

w_W is the mass fraction (as a percentage) of water content on the basis of the oven-dry soil, determined in accordance with ISO 11465.

8.2 Nitrogen as nitrite

The mass fraction of nitrite in the soil, $w(\text{NO}_2\text{-N})$, expressed as milligrams per kilogram of oven-dry soil, is calculated according to Equation (2):

$$w(\text{NO}_2\text{-N}) = (a - b) \cdot \left[\frac{V_{ES}}{m_S} \cdot \left(1 + \frac{w_W}{100} \right) + \frac{w_W}{100} \right] \quad (2)$$

where

a is the mass concentration of $\text{NO}_2\text{-N}$ in the soil extract, in milligrams per litre;

b is the mass concentration of $\text{NO}_2\text{-N}$ in the blank extract, in milligrams per litre;

V_{ES} is the volume of extracting solution (usually 200 ml);

m_S is the mass of wet soil, used for extraction (usually 40 g);

w_W is the mass fraction (as a percentage) of water content on the basis of the oven-dry soil, determined in accordance with ISO 11465.

8.3 Nitrogen as ammonium

The mass fraction of ammonium nitrogen in the soil material, expressed in milligrams per kilogram of oven-dry soil, $w(\text{NH}_4\text{-N})$, is calculated according to Equation (3):

$$w(\text{NH}_4\text{-N}) = (a - b) \cdot \left[\frac{V_{ES}}{m_S} \cdot \left(1 + \frac{w_W}{100} \right) + \frac{w_W}{100} \right] \quad (3)$$

where

a is the mass concentration of $\text{NH}_4\text{-N}$ in the soil extract, in milligrams per litre;

b is the mass concentration of $\text{NH}_4\text{-N}$ in the blank extract, in milligrams per litre;

V_{ES} is the volume of extracting solution (usually 200 ml);

m_S is the mass of moist soil, used for extraction (usually 40 g);

w_W is the mass fraction (as a percentage) of water content on the basis of the oven-dry soil, determined in accordance with ISO 11465.

9 Test report

The test report shall contain the following information:

- a) a reference to this part of ISO 14256 (ISO 14256-2:2005);