Chapter 5

Determination of mineral nutrients (N, P, K.....)

I. Nutrients:

With the exception of carbon (C), all nutrients are taken up by the plant from the soil solution. They are divided into two categories (quantitative classification):

Nitrogen (N): It is the driving force of plant growth. It represents 1 to 4% of plant dry matter. It is taken up from the soil in either nitrate (NO3-) or ammonia (NH4+) form. It combines with compounds from the plant's carbohydrate metabolism to produce amino acids and proteins. As the essential component of proteins, it plays a role in the main processes of plant development and yield determination. An adequate supply of nitrogen to the plant is also important for the absorption of other nutrients.

Phosphorus (P): It constitutes 0.1 to 0.4% of the dry matter and plays a crucial role in energy transfer. It is therefore essential for photosynthesis and other chemical and physiological processes in plants. It is vital for cell differentiation and the development of young tissues from which plant growth originates. Most cultivated and uncultivated soils are deficient in phosphorus or have high phosphorus fixation, thus limiting its bioavailability.

Elemental phosphorus is highly chemically reactive and is not found in its pure form in nature. It is always combined with other elements. Most of these compounds are unavailable to plants because they are insoluble.

Potassium (K): It represents 1 to 4% of the plant's dry matter and has multiple functions. It activates more than 60 enzymes (chemical substances that govern life). It thus plays a vital role in the synthesis of carbohydrates and proteins. K improves the plant's water supply and increases its tolerance to drought, frost, and salinity. Plants well-nourished with potassium are less susceptible to disease.

I. NITROGEN DETERMINATION

Nitrogen can be present in water in both mineral and organic forms.

In general, when it comes to natural waters, mineral forms are by far the most important.

A number of terms need to be clarified:

Total **Nitrogen**

Total nitrogen includes all nitrogenous forms, both mineral and organic.

KJELDAHL **Nitrogen**

Kjeldahl nitrogen refers to that found in the form of organic nitrogen compounds and ammonium. It therefore does not include oxidized nitrogen compounds such as nitrates and nitrites, nor certain other forms like oximes, hydrazine, and heterocycles.

The expression "KJELDAHL nitrogen" originates from the name of the person who developed the universal method used to measure the nitrogen fractions concerned.

Mineral **nitrogen**

Mineral nitrogen consists of ammonia, nitrites, and nitrates.

Organic nitrogen

Organic nitrogen is essentially formed by proteins, polypeptides, urea, and amino acids.

Ammoniacal *nitrogen*

Ammoniacal nitrogen represents nitrogen in the form of $NH4^{+}$

1. Relationship between the various nitrogen fractions

Given the definitions above, the following relationships exist between the different nitrogen fractions:

$$\text{Total}^{\text{ nitrogen =}}\ \text{Organic}^{\text{ nitrogen +}}\ \text{Mineral}^{\text{ nitrogen}} \qquad (1)$$

$$\text{N KJELDAHL = organic N + N NH4+} \qquad (2)$$

$$\text{Mineral nitrogen = N NH4}^{+}\text{ + N NO2- + N NO3- (3)}$$

Equation (2) thus allows us to determine organic nitrogen from the measurement of KJELDAHL nitrogen and ammoniacal nitrogen.

Indeed, we have: Organic N = KJELDAHL N – NH4+ N (4)

Note: *The expression "total nitrogen" is sometimes used to define what actually corresponds to "Kjeldahl nitrogen." This is a bad habit, a source of regrettable errors.*

2. Manipulation

It consists of performing the KJELDAHL titration of nitrogen, then that of ammoniacal nitrogen, and deducing the organic nitrogen using the following relationship:

Organic N = N KJELDAHL – N NH4+

2. Kjeldahl nitrogen determination

2.1. Principle

1. Mineralization : Organic nitrogen is mineralized as ammonium sulfate by the combined action of sulfuric acid and mineralization catalysts. The reaction scheme is as follows:

$$\text{Organic nitrogen + H2SO4 + catalysts} \longrightarrow \text{NH4}^{+}\text{ + H2, }^{\text{SO4-}}$$

2. Distillation: The $NH4^{+\text{ ions}}$ resulting from this mineralization, as well as those that pre-existed in the water, are then transformed into ammonia by a caustic soda solution.

$$\text{NH4}^{+}\text{ + NaOH} \longrightarrow \text{NH3 + H2O}$$

3. Titration: The ammonia is then carried by a vapor stream to a trapping solution where it can be titrated by simple acidimetry.

$$\text{NH3 + H2O} \longrightarrow \text{NH4OH}$$

$$2\ NH_4OH + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

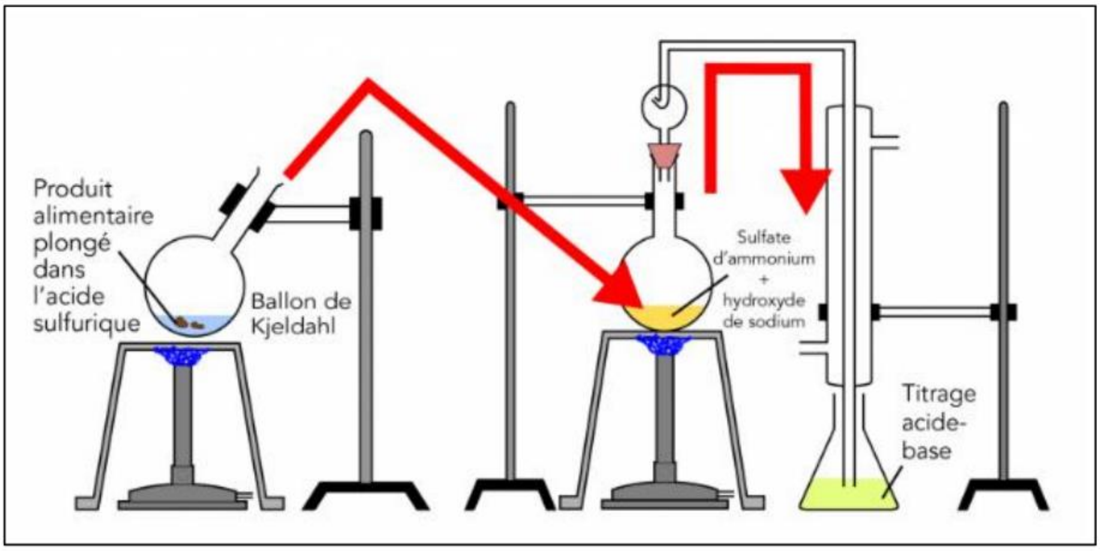

Figure: Kjeldahl nitrogen dosage

2.2. Products, equipment and instrumentation

Products

Mineralization catalyst ;

acid (hazardous product);

solution , antifoaming agent;

0.1 N sulfuric acid solution ;

Caustic soda solution at 300 g/l (hazardous product);

Colour indicator .

Equipment

Mineralization tubes ;

25 ml burette ;

10 and 25 pipettes ;

100 ml graduated ;

10 and 100 ml test tubes ;

Beakers ;

Glass beads ;

Magnetic stirrer .

Instrumentation

Distillation unit

Digester

2.3. Operating Procedure

Switch the digester on immediately upon arrival at the laboratory.

2.3.1. Sample Preparation

Three test portions of the same sample will be prepared simultaneously.
Introduce successively into each mineralization tube:

Approximately 3 g of catalyst;

100 ml of sample in a graduated cylinder ;

10 ml of concentrated sulfuric acid, in a graduated cylinder and with care ;

A few drops of anti-foaming agent; a

few glass beads.

2.3.2. Mineralization

Place the tubes in the digester chambers ;

Connect the tubes to the manifold;

 Turn on the water pump connected to the manifold;

Adjust the rheostat so that boiling is not excessive;

increase the heating power as soon as the water has disappeared and practically only the acid remains;

Allow mineralization to occur for 45 m from the appearance of white fumes;

off the digester after this period of time;

Grasp the collector with the clamp provided for this purpose and carefully place the tubes on the rack;

Allow to cool then remove the collector;

again if it appears, by gently adding a little distilled water.

Caution: *This dangerous operation involves adding water to a concentrated acid. Therefore, it is essential to direct the tube in a direction that poses no risk to you or those around you.*

2. 3.3. Distillation

The device must be started up in the presence of the monitor who will make any necessary adjustments, and strictly following the order of the following sequences:

Preparation *for distillation*

Open the water tap and check that the flow rate is approximately 11/min;

Turn the device on;

Check that the intermediate vessel is empty; if not, open the drain valve and close it.

when the operation is performed;

Place one or two drops of phenophthalein in each of the sample tubes;

Position a tube on its support and ensure that the PTFE flexible tube is immersed at least 1 cm in the solution;

, add a little water, but do so with caution;

Seal the tube with the connecting cap and place the tube on the tilting platform;

Place an Erlenmeyer flask containing 25 ml of boric acid (test tube) on the receiving stand;

Introduce a few drops of colored indicator into the Erlenmeyer flask ;

Add the necessary amount of distilled water so that the tube is in contact with the boric acid solution;

Gently introduce approximately 50 cm3 of tap acid sodium hydroxide marked "NaOH"; Close the tap.

Note: *Generally, the solution in the distillation tube turns pink, the color of phenophthalein in a basic medium. This color may disappear initially, then reappear during the distillation. If the amount of sodium hydroxide added is insufficient, the medium will remain acidic and the pink color will not appear!*

Distillation *itself*

- Start the distillation by operating the tap marked “distillation”;

Observe that the solution contained in the Erlenmeyer flask quickly turns green;

Collect approximately 100 to 200 ml of distillate;

Remove the Erlenmeyer flask;

Rinse the hose with a jet of water from a wash bottle and collect the rinse water ;

Close the "distillation" tap;

Observe , in the following seconds, the aspiration of the contents of the distillation tube into the intermediate vessel;

Remove the distillation tube with an asbestos glove and place it on its rack;

Activate the "drain" valve to remove the contents of the intermediate vessel;

Rinse the PTFE hose, the device is ready for a new distillation.

2.1.3.4. Dosage

Plan

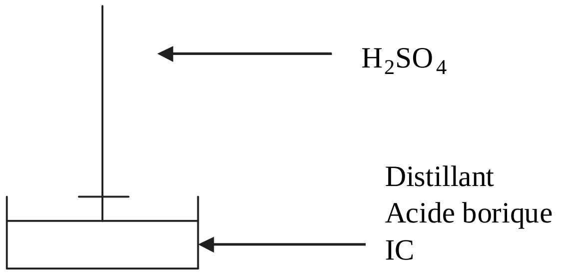

Figure 1: Ammonia dosing device

Protocol

Titrate the contents of the Erlenmeyer flask with 0.1 N H2SO4 until the green solution returns to its initial color;

Note V1 as the volume of acid used;

Empty and rinse the Erlenmeyer flask;

Introduce 25 ml of boric acid (color indicator) into the Erlenmeyer flask, and proceed as follows.

dosage only for the distillate;

V0 the volume of acid used to obtain the color change, V0 corresponds to the ammonia possibly supplied by the boric buffer;

Note (V1 – V0) represents the volume of acid used to neutralize the only ammonia released by the sample after mineralization.

Expression *of results*

We have: N1 * (V1 – V0) = N2 * V2

$$N_2 = \underline{\qquad\qquad} N_1 * ({}_V V_{13} - Vo)$$

With: (V1 – V0) = volume of acid required for neutralization;

N1 = normality of the acid;

V2 = volume of the test sample (100 ml);

N2 = normality of the ammonia solution.

Nitrogen in mg/l is then expressed as T2 mg/l = N2.1000.14

II. PHOSPHATE DETERMINATION

Principle:

Phosphate ions (PO_4^{3-}) react in an acidic medium with ammonium molybdate to form a blue phosphomolybdic complex after reduction by ascorbic acid. We will therefore perform a spectrophotometric assay in the visible range at a wavelength of 720 nm. First, a standard curve is prepared, and its absorbance is measured to plot a calibration curve. Then, the absorbance of the water sample is measured to determine its phosphate ion concentration by comparison with the calibration curve.

Preparation of the stock solution S_0:

- Accurately weigh out 0.439 g of dry potassium dihydrogen phosphate (KH_2PO_4) . Place this in a 1 L volumetric flask to prepare, by dissolution, a solution S **with a** concentration C = 3.23 $\times 10^{-3}$ mol·L^{-1} · Indeed, n

$$n = \frac{m}{M(KH_2PO_4)} = \frac{0,439}{136,1} = 3,23.10^{-3}\,\text{mol}$$

Top up with distilled water to the calibration mark. Shake well to homogenize the solution.

solution 1/10 : using a volumetric pipette, transfer 10.0 mL of the prepared solution S into a 100 mL volumetric flask, then fill to the mark with distilled water. Shake well to homogenize the solution.

- Repeat this operation (dilution by 1/10 of the already diluted solution). This yields a solution S ₀ with a concentration C ₀ = 3.23 x 10⁻⁵ mol/L, or a mass concentration of phosphorus C ₀ = 1 mg P/L. The mass concentration of phosphorus is expressed here in mg P/L (milligrams of phosphorus per liter of solution). This refers to the mass of phosphorus present per liter of solution. Indeed, 1.0 mg of phosphorus corresponds to

$$n = \frac{m}{M(P)} = \frac{1,0.10^{-3}}{31} = 3,23.10^{-5}$$

mole of phosphorus atoms.

Since 1 mole of potassium dihydrogen phosphate contains 1 mole of phosphorus atoms, the molar concentration of the solution S_0 is indeed C_0 = 3.23 10^{-5} mol.L^{-1} .

Preparation of the standard range:

Use the following table to prepare 5 daughter solutions, labeled S1 to S5 , of concentration C' in 5 numbered test tubes. V0 is the volume of solution S0 to be introduced (using a micropipette), and Vwater is the volume of distilled water to be added (using a burette).

Solution	S_1	S_2	S_3	S_4	S_5
C' (mg P/L)	0.1	0.2	0.3	0.4	0.5
V_0 (mL)	0.5	1.0	1.5	2.0	2.5
Water volume (mL)	4.5	4.0	3.5	3.0	2.5

We obtain a color scale.

Plotting the calibration curve:

- Add 1.0 mL of the reagent whose preparation is indicated below, to the contents of each test tube.
- Shake well. Wait approximately 15 minutes for the blue color to stabilize.
- Measure the absorbance A of each solution for a filter with a wavelength λ = 720 nm (red filter). (Remember to "reset the filter" between each measurement.)
- Plot the curve illustrating the variations of absorbance A as a function of concentration C'.

Reagent preparation:

- 148 mL of concentrated sulfuric acid in a 1 L flask. Adjust with distilled water.
- 12 g of ammonium molybdate in 250 mL of distilled water.
- 0.291 g of double tartrate of antimony and potassium in 100 mL of 4 N sulfuric acid.
- Mix the three solutions in a 2 L flask and adjust with distilled water.
- Dissolve 1 g of ascorbic acid in 50 mL of the molybdate solution thus prepared.
- A yellow coloration forms.

Analysis of river water:

- First, the water taken from the river must be filtered using a Büchner funnel (suction filtration).

- Introduce 5.0 mL of filtered river water into a test tube + 1.0 mL of reagent and measure the absorbance as before.
- Use the previously plotted curve to determine the mass concentration of phosphorus in the analyzed water.

Security :

KH_2PO_4 : Precautions for use, S22-24/25

Reagent: corrosive

Using the spectrophotometer:

1. Connect the power cord and turn on the device; the green indicator light will illuminate.
2. Place the filter corresponding to the chosen wavelength □ in its housing.
3. Place the **reference solution** (here: distilled water) in the test tube compartment and briefly press the **R** (Reference) push button. The red indicator light will illuminate and the display should show .00A (meaning that the absorbance A of the solution is zero).
4. Remove the reference solution and insert the test tube containing the solution whose absorbance you want to measure into the slot. Press and then release the **T** (Test) button. The indicator light will illuminate again, showing the absorbance of the solution at the specified wavelength.

Important note: it is necessary to redo the "blank" (setting with distilled water) before each measurement for greater accuracy.

III. POTASSIUM DETERMINATION

1. Principle

Potassium from the soil, easily soluble and immediately available to plants, extracted by water (H2O10-Ex method) is measured by flame atomic absorption spectrometry, at a wavelength of 769.9nm.

2. Execution

Appliances and utensils

(A) Atomic absorption spectrophotometer (AAS) with specific lamp. Air/acetylene mixture, oxidant, weak flame, blue.

(B) Various laboratory glassware.

(C) Balance (accuracy 0.1 g).

Reagents

(1) Demineralized water (H_2O , conductivity < 5 µS/cm).

(2) Fuming hydrochloric acid, (HCl).

(3) Lanthanum(III) chloride heptahydrate ($LaCl_3.7H_2O$)

(4) Lanthanum chloride solutions:

(4a) 2% lanthanum chloride solution Weigh 53.2g (C) of lanthanum chloride (3) into a 1000ml volumetric flask, add 40ml of hydrochloric acid (2), stir and fill to the mark with water (1)

(4b) 0.2% w/v lanthanum chloride solution: Transfer 100 ml of solution (4a) into a 1000 ml volumetric flask, fill to the mark with water (1)

(5) Ready-to-use standard potassium solution at 1000 mg K/L

(6) Calibration solutions:

2 % $LaCl_3$ (3a), make up to 100 ml with H_2O (1) = 0.0 mg K/L

(6b) 0.25 ml of standard solution (5), 10 ml of 2% $LaCl_3$ (4a), make up to 100 ml with H_2O (1) = 1.0 mg K/L

(6c) 0.5 ml of standard solution (5), 10 ml of 2% $LaCl_3$ (4a), make up to 100 ml with H_2O (1)

= 2.5 mg K/L

(6d) 0.1 ml of standard solution (5), 10 ml of 2% $LaCl_3$ (4a), make up to 100 ml with H_2O (1)

= 5.0 mg K/L

(6e) 1.0 ml of standard solution (5), 10 ml of 2% $LaCl_3$ (4a), make up to 100 ml with H_2O (1)

= 10.0 mg K/L

(6f) 2.0 ml of standard solution (5), 10 ml of 2% $LaCl_3$ (4a), make up to 100 ml with H_2O (1) = 20.0 mg K/L

Operating procedure

Pipette 9.0 ± 0.5 ml of the extract (H2O10-Ex method) into a test tube, add 1 ml of 2% $LaCl_3$ (4a). Determine the potassium concentration on the atomic absorption spectrophotometer (A) at a wavelength of 769.9 nm.

Remarks :

- *Transferring the extract into the test tube – without the use of pipettes, etc. – reduces the risk of contamination. An accuracy of ±0.5 ml in filling influences the result by ±0.5%.*

- *Depending on the type of appliance, it is possible to operate in absorption or emission mode. On some appliances, it is recommended to position the burner at an angle to reduce the curvature of the calibration line.*

If dilution is required, use the 0.2% LaCl3 solution (4b) .

3. Calcul

Extraction: 10 g of dry fine earth in 100 ml of extraction agent Calculation: content in mg/kg of dry fine earth.

a = concentration of K in mg/L in the 1:1.11 diluted extracts

mg K/kg of dry fine earth = a [mg/L] • dilution factor x extraction volume [L] / weight [kg] = a • 1.11 • 0.1/0.01

= a • 11.1

Note: If dilution is required, take this into account for the calculation.

4. Result

mg K/kg of dry soil; one decimal place.