

## Protocols

### Method for determination of soil organic matter

#### Materials:

1. o-phenanthroline indicator
2. 0.4mol/L potassium dichromate-sulfuric acid solution
3. potassium dichromate standard solution  $c(1/6 K_2Cr_2O_7)=0.2000\text{mol/L}$
4. Ferrous sulfate standard solution
5. Silicon dioxide
6. Soil samples

#### Methods:

1. After air drying, the soil samples are sieved by 0.28mm, and then put into a grinding bottle for later use.
2. Take 0.5g of the air-dried sample in a test tube, add 10mL of 0.4mol/L potassium dichromate-sulfuric acid solution accurately and shake well.
3. Boil the test tube filled with the sample in a silicone oil bath at 170°C for 5 ± 0.5min
4. After boiling, cool for a while and transfer the solution to a conical flask. Wash with distilled water for 3 times, and add the washing liquid into the conical flask.
5. The total volume of the solution in the conical flask should be controlled at 60 – 80mL, and 3-5 drops of the o-phenanthroline indicator should be added. Use ferrous sulfate standard solution Titrate the remaining potassium dichromate. The color change process of the solution is from orange to blue-green, and then to brown-red, that is, to reach the endpoint.
6. Two or three blank calibrations must be made for the determination of each batch of samples at the same time. Take 0.500g powder silica instead of the sample, and other steps are the same as the sample determination, and take the average value.

#### Calculation:

The calibration method of ferrous sulfate standard solution

Absorb 20mL of potassium dichromate standard solution, put it into a 150mL triangular bottle, add 3mL of concentrated sulfuric acid, and 3-5 drops of o-phenanthroline indicator, titrate with ferrous sulfate solution and calculate the concentration of ferrous sulfate standard solution according to the consumption of ferrous sulfate solution.

$$c_2 = \frac{c_1 \cdot V_1}{V_2}$$

$c_2$ - The concentration of ferrous sulfate standard solution, mol/L;

$c_1$ - The concentration of potassium dichromate standard solution, mol/L;

$V_1$ -The volume of absorbed potassium dichromate standard solution, mL;

$V_2$ -The volume of ferrous sulfate solution consumed during titration, mL.

Soil organic matter content  $X$ , calculated by formula

$$X = \frac{(V_0 - V)c_2 \times 0.003 \times 1.724 \times 100}{m}$$

$X$ -Soil organic matter content,%

$V_0$ -The volume of ferrous sulfate standard solution consumed in blank titration, mL;

$V$ -The volume of ferrous sulfate standard solution consumed when measuring samples, mL;

$c_2$ -Concentration of ferrous sulfate standard solution, mol/L;

0.003-The molar mass of 1/4 carbon atoms, g/mol;

1.724- Coefficient converted from organic carbon to organic matter;

$m$ -Mass of dried sample, g.

## Method for determination of soil water content

### Materials:

Soil samples

### Methods:

1. air-dried soil samples are crushed and screened by a 1mm sieve for later use.
2. Accurately weigh the quality of the aluminum plate. Weigh about 2g of sample and put it in an aluminum tray, and accurately record the initial mass.

3. Bake in a soil moisture quick tester for about 20min, cool to room temperature, and record the final weight.

**Calculation:**

$$\text{water content, \%} = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

$m_0$ -Aluminum plate quality, g;

$m_1$ -Quality of aluminum plate and soil sample before drying, g;

$m_2$ -Quality of aluminum plate and soil sample after drying, g.

**Method for determination of soil total nitrogen**

**Materials:**

1. Soil samples
2. 0.01mol/L hydrochloric acid
3. 10mol/L sodium hydroxide solution
4. Boric acid 2%(W/V)
5. Mixed indicator: bromocresol green and methyl red
6. Mixed catalyst ( $\text{K}_2\text{SO}_4$  :  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  : Se =100;10:1)

**Methods:**

1. Weigh about 1.0g of air-dried soil sample (passing through 0.25mm sieve) (record accurately). Send the soil sample to the bottom of a dry Kjeldahl bottle, add a small amount of deionized water (about 0.5 – 1mL) to wet the soil sample, add 2.0g accelerator and 5mL concentrated sulfuric acid, and shake well.
2. Place the Kjeldahl flask on a 300W variable temperature electric furnace and heat it with a small fire. When the reaction in the bottle eases (about 10 – 15min), strengthen the firepower to keep the boiled upper liquid slightly boiling, and the heated part does not exceed the liquid level in the bottle, so as to prevent the ammonium salt from being decomposed by heat due to the high temperature of the bottle wall, resulting in nitrogen loss. The best boiling temperature is that sulfuric acid vapor condenses and refluxes at the upper 1/3 of the bottleneck. After the digestion solution and the upper grains are all gray and slightly green, continue to digest for 1 hour. Decocting, cooling, and distilling. While cooking the soil sample, make two blank determinations, except that no soil sample is added, other operations are the same as those when determining the soil sample.

- Before distillation, check whether the distillation unit leaks air, and wash the pipeline through the distillate of water. After the digestion solution is cooled, transfer the digestion solution into the distiller quantitatively with a small amount of deionized water, and wash the Kjeldahl flask with water for 4 ~ 5 times (the total water consumption does not exceed 30 – 35mL). Add 5mL 2% of boric acid-indicator mixture into a 150mL conical flask and place it at the end of the condenser tube. Then, 20mL of 10mol/L sodium hydroxide solution was slowly added into the distillation chamber, and steam was introduced for distillation. When the distillate volume was about 50mL, the distillation was finished.
- Titrate the distillate from blue-green to just red-purple with 0.01mol/L hydrochloric acid standard solution. Record the volume (mL) of acid standard solution used.

#### Calculation:

$$\text{soil total nitrogen}(\%) = \frac{(V - V_0)c_2 \times C_H \times 0.014}{m} \times 100$$

$V_0$ -The volume of acid standard solution used in titration of test solution, g;

$V_1$ -The volume of acid standard solution used when titrating blank, g;

$C_H$ -concentration of acid standard solution, mol/L;

0.014-millimolar mass of nitrogen atom;

$m$ -mass of dried soil sample, g.

#### Method for the determination of available phosphorus

##### Materials:

- Sodium bicarbonate
- Sodium hydroxide 50%(m/V) solution
- Ammonium molybdate
- Sulfuric acid
- Potassium antimonyl tartrate [K (SbO) C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·1/2H<sub>2</sub>O] :0.30%(m/V) solution
- ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>, levorotatory, specific rotation+21 ~+22)
- Potassium dihydrogen phosphate
- Extractant (0.50mol/L NaHCO<sub>3</sub>, pH=8.5). Dissolve 42.0g of sodium bicarbonate in about 800mL of water, dilute it to 1L, and adjust it to pH 8.5 with sodium hydroxide solution.

9. Molybdenum antimony stock solution: *10.0g* ammonium molybdate is dissolved in *300mL* of water at about *60°C* and cooled. Another *181mL* of sulfuric acid is slowly injected into about *800mL* of water, stirred evenly, and cooled. Then dilute sulfuric acid is injected into ammonium molybdate solution, and stirred at any time; Then *100mL* of potassium antimony oxide tartrate solution is added; Finally, dilute it to *2L* with water and put it in a brown bottle.
10. chromogenic reagent: *0.50g* ascorbic acid is dissolved in a *100mL* molybdenum antimony stock solution.
11. phosphorus standard stock solution ( $c(P) = 100\text{mg/L}$ ): weigh *0.4394g* of potassium dihydrogen phosphate dried at *105°C*, dissolve it in about *200mL* of water, add *5mL* of sulfuric acid, transfer it to a *1L* volumetric flask, and use water to constant volume. The reserve solution can be stored for a long time;
12. phosphorus standard working solution ( $c(P) = 100\text{mg/L}$ ): accurately dilute phosphorus standard stock solution by 20 times with leaching agent.

#### Methods:

1. weigh *2.50g* of air-dried soil sample passing through *1mm* sieve, put it into a dry *150mL* conical flask, add *50.0mL* of *25.1°C* leaching agent, plug it with a rubber stopper, shake it on a reciprocating shaker for *30.1min* at a liquid temperature of *105°C*, and immediately filter it into the dry *150mL* conical flask with phosphorus-free filter paper.
2. Quantification of phosphorus infiltrate on the day of leaching soil samples, suck *10.00mL* of filtrate and put it into a dry *50mL* conical flask, add *5.00mL* of chromogenic reagent, and shake slowly to make carbon dioxide escape. Then add *10.00mL* of water, shake well, and exhaust the carbon dioxide. After standing at room temperature higher than *15°C* for *30min*, use a *1cm* optical path colorimetric tank to measure the absorbance at *880nm*, and use the blank solution (*10.00mL* leaching agent instead of soil filtrate, the same as above) as the reference solution to adjust the zero point of the spectrophotometer.
3. While measuring soil samples, absorb *0,1.50,2.50,5.00,10.00,15.00,20.00* and *25.00mL* of phosphorus standard working solution, put them into *50mL* volumetric flasks, and use the extractant to make the volume constant. The concentration of phosphorus in this standard series solution is *0,0.15,0.25,0.50,1.00,1.50,2.00,2.50mg/L P*, *10.00mL* of each standard series solution is absorbed, and the color is developed as above, and the absorbance of the series solution is measured. Then draw a calibration curve or calculate a linear regression equation of two variables with the phosphorus concentration of the standard series solution as the abscissa and the corresponding absorbance as the ordinate.

### Calculation:

Available phosphorus content of soil  $X$ (P,mg/kg) is calculated according to the following formula:

$$X = c \times 20$$

$c$ - The concentration of phosphorus in soil filtrate obtained from calibration curve or regression equation,mg/kg P;

20- ratio of liquid to soil during extraction

### Method for determination of soil total sodium

#### Materials:

1. Soil samples
2. nitric acid ( $\rho_{20} = 1.42\text{g/mL}$ )
3. Perchloric acid ( $\rho_{20} = 1.67\text{g/mL}$ )
4. Hydrofluoric acid (not less than 40%)
5. 3mol/L hydrochloric acid solution
6. 20g/L boric acid solution
7. 2mol/L nitric acid solution
8. Standard sodium stock solution: 1000mg/L

#### Methods:

1. Sample digestion: 0.5000g air-dried soil passing through a 0.149mm pore size sieve was weighed to the accuracy of 0.0001g g. Carefully put it into a polytetrafluoroethylene crucible, where 15mL of nitric acid and 2.5mL of perchloric acid were added. The sample was placed on an electric heating plate covered with asbestos cloth and boiled to a slight boiling point in a fume hood. When nitric acid was completely exhausted and part of the perchloric acid decomposed into a large amount of white smoke and the sample became a paste, it was removed and cooled.
2. With a pipette and hydrofluoric acid 5mL, add perchloric acid 0.5mL. heating after silicate decomposition, continue to heat to more than hydrofluoric acid, and perchloric acid was driven out, stop the white smoke, remove the cooling.
3. Add 3 mol/L hydrochloric acid solution 10mL, continue to heat to the residue dissolved (such as residue dissolved incomplete, the solution should be evaporated to dryness, and then add hydrofluoric acid 3 ~ 5 ml, perchloric acid

0.5mL, continue to digest), remove the cooling, add 20g/L boric acid solution 2mL, water quantitative into 250mL volumetric flask, volume, this is the soil digestion solution. At the same time prepare a reagent blank solution as above.

4. Accurately absorb 1000 mg/L sodium standard stock solution 10 mL, respectively into 100mL volumetric flask, diluted with water volume, this is 100mg/L sodium standard working solution. According to the linear detection range of calcium, magnesium, and sodium by the instrument used, 100 mg/L sodium standard working solutions were diluted with water to form the following standard series solutions.
5. Then, 0, 5, 10, 20, and 30 mL of 100 mg/L sodium standard working solution were separated into five 100mL volumetric flasks, and 10 ml of 2mol/L nitric acid solution was added, respectively, to volume with water. This standard series contains 0, 5, 10, 20, and 30 mg/L sodium.
6. The sodium was determined by the flame photometric method. A certain amount of soil digestion solution was taken and diluted with water until the concentration of sodium ion was equivalent to the concentration range of sodium standard series solution. This was the soil solution to be tested. Before volume determination, 10ml of 2mol/L nitric acid solution was added into the sodium test solution to make the acidity of the soil test solution reach 0.126%–0.200%, and then the solution was determined according to the instrument instruction.
7. Adjust the zero point of the instrument with the solution with the sodium concentration of zero in the standard series of solutions, and determine the flame photometric values of the sodium solution to be tested and the blank solution.

#### Calculation:

$$\text{total sodium, g/kg} = c \times \frac{V_1}{m(1-H)} \times \frac{V_3}{V_2} \times 10^{-3}$$

$c$ -concentration of test solution from the working curve of soil sodium, mg/L

$V_1$ -digestion liquid volume, mL

$V_2$ -digestion liquid suction, mL

$V_3$ - liquid volume, mL

$m$ - sample weight, g;

$10^{-3}$ —conversion factor from mg/L concentration unit to g/kg

$1 - H$ —conversion factor for converting air-dried soil to dry soil

$H$ —the percentage of water content in air-dried soil

## Method for determination of soil pH

### Materials:

1. Soil samples
2. Buffer solution for calibrating pH meter.

Buffer solution 1, pH 4.00 at 20°C. Dissolve 10.21g potassium hydrogen phthalate ( $\text{C}_8\text{H}_5\text{O}_4\text{K}$ ) and dilute it to 1000 mL water.

Buffer solution 2, pH 9.22 at 20°C. Dissolve 3.80g sodium borate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) and dilute it to 1000 mL water.

### Methods:

1. take a small number of representative samples, which should be no less than 5 mL. Put the sample in the sample bottle, and add water 5 times the volume of the sample. Shake the suspension continuously for  $(60 \pm 10)$  min, use a mechanical oscillator or mixer, and then let it stand for more than 1 h, but not more than 3 h. During the standing period after oscillation, air should be prevented from entering the sample bottle.
2. Adjust the pH meter concerning the manufacturer's instructions. In the range of  $(20 \pm 2)^\circ\text{C}$ , use buffer solution and calibrate the pH meter according to the requirements of the manufacturer's instructions.
3. At the temperature of  $(20 \pm 2)^\circ\text{C}$ , immediately measure pH in the suspension at the beginning of stirring. The stirring speed should be selected so that soil particles can be distributed in the suspension relatively evenly, but air cannot be entrained. When the pH value is stable, it can be read, and the recorded value is accurate to two decimal places.