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Soil quality – Determination of soil microbial biomass – Part 2: Fumigation – extraction method

This Swedish Standard consists of the English version of the International standard ISO 14240-2: 1997, Soil quality – Determination of soil microbial biomass – Part 2: Fumigation-extraction method

This part of ISO 14240 specifies a method for the estimation of microbial biomass of soils by measurement of total extractable organic biomass material mainly from fresh killed microorganisms. The method is also applicable to estimation of microbial nitrogen and microbial ninhydrin-reactive nitrogen in soil, but this part of ISO 14240 describes only the measurement of extractable organic carbon. The fumigation-extraction method is applicable to aerobic and anaerobic soils over the whole range of soil pH.

Swedish Standards corresponding to documents referred to in this Standard are listed in ”Catalogue of Swedish Standards”, issued by SIS.

Markundersökningar – Bestämning av markens mikrobiella biomassa – Del 2: Fumigering – extraheringsmetod

Denna standard utgörs av den engelska versionen av ISO 14240-2:1997, Soil quality – Determination of soil microbial biomass – Part 2: Fumigation-extraction method

I denna del av ISO 14240 beskrivs en metod för uppskattning av den mikrobiella biomassan genom att mäta totalt extraherbart organiskt material huvudsakligen från biomassa av nyligen avdödade mikroorganismer. Metoden kan också användas för uppskattning av mikrobiellt kväve och mikrobiellt ninhydrinreaktivt kväve i jord men denna del av ISO 14240 beskriver enbart bestämning av extraherbart organiskt kol. Fumigering - extraheringsmetoden är användbar för aeroba och anaeroba jordar inom hela pH-intervallet för jordar.

I SIS ”Katalog över svensks standard”, framgår vilka av de publikationer som omnämns i denna standard som har fastställts som svensk standard och som har översatts till svenska.

ICS 13.080.00

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Introduction

Soil consists of both living and nonliving components which exist in a complex and heterogeneous environment. Soil microflora is responsible for the degradation of organic matter, stability of aggregates and most nutrient cycling which occurs in soils. The purpose of determining the microbial biomass of soils is to allow assessment of the continued maintenance of soil fertility, the potential ability to degrade added organic materials, and the effects of added materials on the natural microbial population.

Soil quality — Determination of soil microbial biomass —

Part 2:
Fumigation-extraction method

1 Scope

This part of ISO 14240 specifies a method for the estimation of microbial biomass of soils by measurement of total extractable organic biomass material mainly from freshly killed microorganisms. The method is also applicable to the estimation of microbial nitrogen and microbial ninhydrin-reactive nitrogen in soil, but this part of ISO 14240 describes only the measurement of extractable organic carbon. The fumigation-extraction (FE) method is applicable to aerobic and anaerobic (water-logged, paddy) soils over the whole range of soil pH. Biomass can be also measured in soils containing actively decomposing substrates and soils supersaturated with potassium sulfate solution.

NOTE — Chloroform fumigation also affects soil fauna. The contribution of carbon from these organisms is generally small (< 5 %) and can usually be neglected.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 14240. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 14240 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10381-6:1993, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory.*

ISO 10694:1995, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis).*

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method.*

3 Definition

For the purposes of this part of ISO 14240, the following definition applies.

3.1 soil microbial biomass
mass of intact microbial cells in a given soil

NOTE — This parameter can be estimated from the measurement of the carbon or nitrogen content of these cells or by the measurement of their ability to mineralize an added carbon source. Dead cells and cell fragments may be detected when carbon or nitrogen analysis is used but only intact cells will be detected when respiration is measured.

4 Principle

Through fumigation of the soil sample, intact microbial cells are lysed and the microbial organic matter released. Nonliving soil organic matter is not seriously affected by such fumigation. Soil samples are fumigated with chloroform for 24 h. The organic carbon extracted using 0,5 mol/l potassium sulfate is determined in fumigated and unfumigated samples, and the difference in extracted organic carbon is used to determine microbial biomass carbon. The method is usually termed fumigation-extraction (FE).

5 Materials and reagents

5.1 Soils

Guidance for the collection, handling and storage of soil (ISO 10381-6) shall be followed, as far as applicable. Sieve the soil samples at approximately 40 % water-holding capacity (WHC) if homogeneous samples are required.

Determine the soil WHC in accordance with annex A.

The water content of samples shall be higher than 30 % WHC to ensure uniform chloroform distribution and effective fumigation. Take care to avoid smearing and compaction of wet soil in this method. Samples of waterlogged soils do not have to be dried prior to analysis.

5.2 Reagents

Reagents of recognized analytical grade shall be used, including:

5.2.1 Silicone grease (medium viscosity).

5.2.2 Ethanol-free chloroform.

WARNING — In the presence of light, ethanol-free chloroform degrades rapidly to form phosgene gas ($COCl_2$) which is odourless and highly toxic.

5.2.3 Potassium sulfate solution, $c(K_2SO_4)$ = 0,5 mol/l (ρ = 87,135 g/l).

5.2.4 Soda lime.

6 Apparatus

6.1 Room, or incubator, capable of being maintained at (25 ± 2) °C.

6.2 Implosion-protected desiccator.

6.3 Filter paper[1].

6.4 Glass beakers.

6.5 Petri dishes.

6.6 Polyethylene bottles, of capacity 250 ml.

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1) Whatman No. 42, Schleicher & Schüll 595 1/2, Machery & Nagel 261 G 1/4, are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 14240 and does not constitute an endorsement by ISO of these products.

6.7 Vacuum line (water-pump or electric pump).

6.8 Horizontal or overhead shaker.

6.9 Freezer, operation at (−15 to −20) °C.

6.10 Antibumping granules.

7 Fumigation and extraction

7.1 Fumigation

Line the desiccator (6.2) with moist filter paper (6.3) for fumigation of the soil samples.

Weigh at least three samples of moist soil (5.1), each containing a mass equivalent to 25 to 50 g of oven-dry soil, into glass beakers (6.4) or Petri dishes (6.5) and place them in the desiccator with a beaker containing 25 ml of ethanol-free chloroform (5.2.2) and a few antibumping granules (6.10), and a beaker with soda lime (5.2.4). Evacuate the desiccator until the chloroform has boiled vigorously for approximately 2 min. Close the vacuum tap on the desiccator and incubate in the dark (6.1) at (25 ± 2) °C for 22 h to 24 h.

If there is insufficient soil available, use smaller samples provided the ratio of soil mass to volume of extractant is kept the same (1:4). In soils containing more than 20 % organic material (as determined according to ISO 10694), increase the ratio soil:extract above 1:4 (to a maximum of 1:30 for soils containing 95 % organic matter, e.g. L-layer) in order to obtain sufficient extracted matter. Record the mass of soil used.

After fumigation is completed, remove the beaker containing the chloroform and the filter paper from the desiccators. Remove the chloroform vapour from the soil by repeated evacuation (6 times for 2 min each) in the desiccator. The samples are ready for extraction.

Place three unfumigated, moist control samples (50 g dry mass) into polyethylene bottles (6.6) and immediately extract with 200 ml of the potassium sulfate (5.2.3), as indicated in 7.2.

7.2 Extraction

To extract organic carbon, transfer the soil quantitatively to polyethylene bottles (6.6), add 200 ml of potassium sulfate (5.2.3), shake the bottles on a horizontal shaker (6.8) at 200 r/min for 30 min or an overhead shaker at 60 r/min for 45 min and filter the extracts through a folded filter paper (6.3). Extract the unfumigated controls and filter the extracts in the same way.

If not analyzed at once, store the extracts of fumigated and unfumigated soil samples in the freezer (6.9) at between −15 °C and −20 °C. Homogenize frozen extracts before use, after thawing at room temperature.

NOTES

1 A white precipitate occurs during storage of the potassium sulfate soil extracts (especially if the samples are frozen) because they are usually supersaturated with calcium sulfate ($CaSO_4$). It is unnecessary to dissolve this excess $CaSO_4$ because it does not interfere with any of the analytical procedures described in this method.

2 Cell membranes of young, living roots are also affected by chloroform fumigation. In soils containing large amounts of living roots, the pre-extraction procedure given in annex B should be used.

8 Determination of carbon in the extracts

The carbon content of microorganisms in a soil sample is determined analytically and can be used to make comparisons between different soil samples. Where a figure for actual microbial biomass is required, then such analyses are multiplied by a conversion factor derived from experiments correlating a known cell mass to carbon analysis after fumigation-extraction. All conversion factors used are related to this initial factor.

Determine the carbon content in the extracts using either dichromate oxidation (8.1) or instrumental analysis (8.2).

8.1 Microbial biomass carbon by dichromate oxidation

8.1.1 Principle

In the presence of strong acid, organic matter is oxidized and Cr(VI) reduced to Cr(III). The amount of dichromate left is back-titrated.

8.1.2 Additional reagents

8.1.2.1 Potassium dichromate, $c(K_2Cr_2O_7)$ = 0,0667 mol/l (19,6125 g dry potassium dichromate/litre water).

WARNING — Because of the hazardous nature of potassium dichromate, great care should be taken in its use and eventual disposal.

8.1.2.2 Phosphoric acid, (H_3PO_4), ρ= 1,71 g/ml.

8.1.2.3 Sulfuric acid, (H_2SO_4), ρ = 1,84 g/ml.

8.1.2.4 Iron(II) ammonium sulfate, titration solution, $c[(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O]$ = 0,040 mol/l.

Iron(II) ammonium sulfate (15,69 g) is dissolved in distilled water, acidified with 20 ml of the sulfuric acid (8.1.2.3) and made up to 1 000 ml with distilled water.

8.1.2.5 1,10-phenanthroline sulfate complex solution, 0,025 mol/l.

8.1.2.6 Acid mixture: two volumes sulfuric acid (8.1.2.3) mixed with one volume phosphoric acid (8.1.2.2).

8.1.3 Additional apparatus

8.1.3.1 Liebig condenser (water-cooled).

8.1.3.2 250 ml round-bottom flask.

8.1.3.3 Burette, volume 10 ml, graduated in 0,05 ml divisions.

8.1.3.4 Pipette, volume 2 ml.

8.1.4 Procedure

Add 2 ml of the potassium dichromate solution (8.1.2.1) (P_D) using the pipette (8.1.3.4) and 15 ml of the acid mixture (8.1.2.6) to 8 ml of filtered extract (7.1) (P_S) in a 250 ml round-bottom flask (8.1.3.2). Gently reflux (8.1.3.1) the whole mixture for 30 min, allow to cool and dilute with 20 ml to 25 ml water, added through the condenser to rinse it.

Digest duplicate blanks containing 8 ml of the potassium sulfate solution (5.2.3) in the same way.

NOTE — These digested blanks are also known as "refluxed blanks".

Determine the excess dichromate by back-titration (8.1.3.3) with the iron(II) ammonium sulfate titration solution (8.1.2.4), using a few drops of the 1,10-phenanthroline-iron(II) sulfate complex solution (8.1.2.5) as an indicator.

8.1.5 Calculation of results

Calculate the extractable organic carbon using equations (1) and (2).

$$C\ (\mu g / ml) = [(V_H - V_S) / V_C] \times MP_D \times E \times 1000 / P_S \quad \ldots (1)$$

where

V_S is the volume titrant (8.1.2.4) consumed by the sample, in millilitres;

V_H is the volume titrant (8.1.2.4) consumed by the refluxed blank, in millilitres;

V_C is the volume titrant (8.1.2.4) consumed by the unrefluxed blank, in millilitres;

M is $c(K_2Cr_2O_7)$, in moles per litre;

P_D is the added volume of $K_2Cr_2O_7$ solution, in millilitres;

P_S is the added volume of sample, in millilitres;

E is 3{conversion of organic C[C] to CO_2[C(+IV)]}.

$$C\ (\mu g / g\ \text{dry soil}) = C\ (\mu g / ml) \times (P_K/D_W + S_W) \quad \ldots (2)$$

where

P_K is the mass of extractant, in grams;

D_W is the dry mass of sample (determined according to ISO 11465), in grams;

S_W is the soil water (grams of water/grams of dry soil) (determined according to ISO 11465).

Calculate the biomass carbon, B_C, using equation (3).

$$B_C = E_C / k_{EC} \quad \ldots (3)$$

where

E_C = (mass of organic C extracted from fumigated soils) − (mass of organic C extracted from unfumigated soils);

k_{EC} = 0,38.

NOTE — The factor k_{EC} was calculated by relating the results of the fumigation-incubation method and fumigation-extraction method (12 soils).

8.2 Microbial biomass carbon by spectrometric carbon analysis

8.2.1 Principle

In the presence of potassium persulfate ($K_2S_2O_8$), extractable soil organic carbon is oxidized to carbon dioxide, and measured using infrared (IR) or ultraviolet (UV) spectrometric detection.

8.2.2 Additional reagents

8.2.2.1 Potassium persulfate ($K_2S_2O_8$).

8.2.2.2 Phosphoric acid (see 8.1.2.2).

8.2.2.3 Sodium polyphosphate [$(NaPO_3)_n$], extra pure.

8.2.2.4 Potassium persulfate reagent

Dissolve 20 g of potassium persulfate (8.2.2.1) in 900 ml of distilled water, acidify the solution to pH 2 with phosphoric acid (8.2.2.2) and make up to a final volume of 1 000 ml with distilled water.

8.2.2.5 Sodium polyphosphate reagent

Dissolve 50 g of sodium polyphosphate (8.2.2.3) in 900 ml of distilled water, acidify the solution to pH 2 with phosphoric acid (8.2.2.2) and make up to a final volume of 1 000 ml with distilled water.

8.2.3 Additional apparatus

8.2.3.1 Automatic carbon analyzer with IR detection[1] or continuous-flow system with colorimetric detection[2].

For the purposes of this part of ISO 14240, the determination of microbial biomass carbon is based on UV-activated persulfate oxidation of organic carbon.

8.2.4 Procedure

For the automated UV-persulfate oxidation method, mix 5 ml of the potassium sulfate soil extract (7.1) with 5 ml of sodium polyphosphate reagent (8.2.2.5). Any precipitate of $CaSO_4$ in the soil extracts is dissolved by this procedure. The potassium persulfate reagent (8.2.2.4) is automatically fed into the UV oxidation chamber, where the oxidation to CO_2 is activated by UV light. The resulting CO_2 is measured by IR absorption or UV spectrophotometric detection.

8.2.5 Calculation of results

Calculate the extractable organic carbon (C) using equation (4).

$$C\ (\mu g / g\ \text{dry soil}) = [(V \times D_v) - (B \times D_B)] \times (P_k / D_w + S_w) \quad \ldots (4)$$

where

V is the C (µg / ml) of sample;

B is the C (µg / ml) of blank;

D_v is the dilution of sample with sodium phosphate, in millilitres;

D_B is the dilution of blank with sodium phosphate, in millilitres;

P_k see equation (2);

D_w see equation (2);

S_w see equation (2).

Calculate the biomass B_C using equation (5).

$$B_C = E_c / k_{EC} \quad \ldots (5)$$

where

E_c = (organic C extracted from fumigated soils) − (organic C extracted from unfumigated soils);

k_{EC} = 0,45.

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[1] Dohrman DC 80, Maihak Tocor 4, are examples of suitable equipment available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

[2] Skalar, Perstorp Alpkem, are examples of suitable equipment available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.