SVENSK STANDARD
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Markundersökningar – Bestämning av markens mikrobiella biomassa –
Del 2: Fumigering – extraheringsmetod (ISO 14240-2:1997)

Soil quality – Determination of soil microbial biomass –
Part 2: Fumigation-extraction method (ISO 14240-2:1997)

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Europastandarden EN ISO 14240-2:2011 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 14240-2:2011.

Denna standard ersätter SS-ISO 14240-2, utgåva 1.

The European Standard EN ISO 14240-2:2011 has the status of a Swedish Standard. This document contains the official version of EN ISO 14240-2:2011.

This standard supersedes the Swedish Standard SS-ISO 14240-2, edition 1.

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Denna standard är framtagen av kommittén för Karaktärisering av avfall, mark och slam, SIS/TK 535.

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 14240-2

June 2011

ICS 13.080.30

English Version

Soil quality - Determination of soil microbial biomass - Part 2: Fumigation-extraction method (ISO 14240-2:1997)

Qualité du sol - Détermination de la biomasse microbienne du sol - Partie 2 : Méthode par fumigation-extraction (ISO 14240-2:1997)

Bodenbeschaffenheit - Bestimmung der mikrobiellen Biomasse von Böden - Teil 2: Fumigations-Extraktionsverfahren (ISO 14240-2:1997)

This European Standard was approved by CEN on 3 June 2011.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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Ref. No. EN ISO 14240-2:2011: E

Foreword

The text of ISO 14240-2:1997 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 14240-2:2011 by Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2011, and conflicting national standards shall be withdrawn at the latest by December 2011.

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The text of ISO 14240-2:1997 has been approved by CEN as a EN ISO 14240-2:2011 without any modification.

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.

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.