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Soil quality — Determination of soil microbial biomass —

Part 2: Fumigation-extraction method

*Qualité du sol — Détermination de la biomasse microbienne du sol —
Partie 2: Méthode d'extraction par fumigation*

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Foreword

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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.

International Standard ISO 14240-2 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 14240 consists of the following parts, under the general title *Soil quality — Determination of soil microbial biomass*:

- *Part 1: Substrate-induced respiration method*
- *Part 2: Fumigation-extraction method*

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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, $c(\text{K}_2\text{SO}_4) = 0,5 \text{ mol/l}$ ($\rho = 87,135 \text{ g/l}$).

5.2.4 Soda lime.

6 Apparatus

6.1 Room, or incubator, capable of being maintained at $(25 \pm 2) ^\circ\text{C}$.

6.2 Implosion-protected desiccator.

6.3 Filter paper¹⁾.

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.