INTERNATIONAL STANDARD

ISO 14240-1

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Soil quality — Determination of soil microbial biomass —

Part 1:
Substrate-induced respiration method

Qualité du sol — Détermination de la biomasse microbienne du sol —

Partie 1: Méthode par respiration induite par substrat

Reference number
ISO 14240-1:1997(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 14240-1 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 organic materials, and the effects of added materials on the natural microbial population.

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Soil quality — Determination of soil microbial biomass —

Part 1:
Substrate-induced respiration method

1 Scope

This part of ISO 14240 specifies a method for estimating the active aerobic, heterotrophic microbial biomass in aerated agricultural and mineral soils.

Determination of the effects of chemicals on biomass is outside the scope of this part of ISO 14240.

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 10390:1994, *Soil quality — Determination of pH.*

ISO 11277:—[1)], *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation following removal of soluble salts, organic matter and carbonates.*

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

3 Definitions

For the purposes of this part of ISO 14240, the following definitions apply.

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.

3.2 soil respiration rate
volume of carbon dioxide released per unit mass of soil per unit time

1) To be published.

4 Principle

Soil is amended with a series of increasing concentrations of glucose until a maximum respiration rate is reached (usually within the first hour of the experiment). From this rate, known as the maximum initial respiration rate, the active biomass can be estimated.

5 Test conditions

The biomass determination shall be performed at a constant temperature of (22 ± 1) °C and at a constant moisture content. The soil water content shall be the same as that existing in the environment from which the soil sample was taken.

NOTE — The factor used to convert the respiration rate into a biomass figure (see clause 9) was calculated using a temperature of (22 ± 1) °C.

6 Reagents and materials

6.1 Soil, for which the following soil characteristics shall be determined:

a) Physical properties
- — particle size distribution, in accordance with ISO 11277;
- — water content during the incubation period, in accordance with ISO 11465.

b) Chemical properties
- — pH of soil, in accordance with ISO 10390, or determined in KCl or $CaCl_2$ solution;
- — organic matter content, in accordance with ISO 10694.

The recommendation for collection, handling and storage of soil (ISO 10381-6) shall be followed as far as is applicable.

6.2 Finely powdered D-glucose

6.3 Fine quartz sand, with a particle size of 0,1 mm to 0,5 mm, or talcum powder, for mixing with the glucose (6.2).

7 Apparatus

The usual laboratory apparatus and equipment is required, together with the following items:

7.1 Ceramic mortars, for grinding glucose (6.2) with sand or talcum powder (6.3).

7.2 Electric hand mixer.

7.3 **Equipment to measure the rate of carbon dioxide (CO_2) release** from soil samples at regular intervals. This can be achieved by automatic infrared gas analysis, gas chromatography or any other suitable method.

8 Procedure

8.1 Determination of optimum glucose concentration

Add an excess of glucose (6.2) to the test soil (6.1) to determine the glucose concentration at which CO_2 evolution is observed. Ensure that the amount of glucose added does not cause inhibitory effects, e.g. the development of adverse osmotic conditions.

Prepare sufficient (at least five) samples for testing a range of glucose concentrations.

NOTE 1 For example, the range of glucose concentrations used for arable soils would be from 500 mg/kg to 6 000 mg/kg.

Thoroughly grind the glucose in the mortar (7.1) with the quartz sand or talcum powder (6.2) at a ratio of 1:5 and then mix with the soil.

NOTE 2 The size of the soil sample depends on the quantity of soil available, the microbial activity of the soil, and the method used for CO_2 determination.

Measure the rate of evolution of CO_2 from each soil sample using the equipment selected (7.3) every hour for at least 6 h. At this stage replication is not necessary.

Determine the glucose concentration at which maximum CO_2 evolution is observed.

8.2 Determination of microbial biomass

Carry out the procedure described in 8.1 in replicate (at least triplicate) using only the glucose concentration at which the maximum rate of CO_2 evolution occurred.

NOTE — It is also possible to determine biomass using the procedure given in 8.1 if sufficient replication is used.

9 Calculation of results

Calculate the biomass, using the lowest CO_2 evolution rate obtained after the start of the measurements, with the equation below:

$$X = 40R + 0{,}37$$

where

X is the concentration of soil microbial carbon, in milligrams per kilogram;

R is the rate of CO_2 evolution, in millilitres per kilogram per hour.

NOTE — The factor 40 in the above formula is obtained from the correlation between respiration rate and the amount of soil microbial biomass measured by the fumigation and incubation method in annex A of reference [5].

10 Test report

The test report shall contain the following information:

a) test soil characteristics (see 6.1);

b) information on the test procedure, i.e. methodology used, equipment and apparatus used (see clause 8);

c) raw data, figures and/or tables of the results of the analyses.

Annex A
(informative)
Bibliography

[1] ISO 10694:1995, *Soil quality — Determination of organic and total carbon after dry combustion.*

[2] ISO 11260:1994, *Soil quality — Determination of effective cation exchange capacity and base saturation using barium chloride solution.*

[3] ISO 11266:1994, *Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions.*

[4] ANDERSON, J.P.E. and DOMSCH, K.H. A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol. Biochem.*, **10**, 1978, pp. 215-221.

[5] JENKINSON, D.S. and POWLSON, D.S. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol. Biochem.*, **8**, 1976, pp. 209-213.

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