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Soil quality — Determination of abundance and activity of soil microflora using respiration curves

Qualité du sol — Détermination de l'abondance et de l'activité de la microflore du sol à l'aide de courbes de respiration

Reference number
ISO 17155:2012(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17155 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 17155:2002), which has been technically revised.

Soil quality — Determination of abundance and activity of soil microflora using respiration curves

1 Scope

This International Standard specifies a test method for determining the activity of active aerobic, heterotrophic microbial biomass in soils. This method is applicable to the monitoring of soil quality and to the evaluation of the ecotoxic potential of soils and soil materials. It is also applicable for soils sampled along contamination gradients in the field and to soils that are contaminated experimentally in the field or in the laboratory.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

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

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

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

ISO 14238, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
basal respiration rate
R_B
constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time without substrate addition

NOTE See Figure 1 for a typical basal respiration curve.

3.2
substrate-induced respiration rate
R_S
constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time shortly after addition of a carbon substrate

NOTE 1 See Figure 1 for a typical substrate-induced respiration curve.

NOTE 2 If glucose is used as a carbon substrate, microbial biomass can be determined from the substrate-induced respiration rate (see ISO 14240-1[1]).

3.3
respiratory activation quotient
Q_R
basal respiration rate divided by substrate-induced respiration rate

$$Q_R = \frac{R_B}{R_S} \quad (1)$$

3.4
specific growth rate
μ
exponent representing respiration rate per unit of time during the exponential phase of growth

NOTE See Equation (3).

3.5
time to the peak maximum
$t_{peakmax}$
time from addition of substrate to the maximum respiration rate

NOTE 1 See Figure 1.

NOTE 2 The time to the peak maximum also reflects the viability of the growing organisms.

3.6
cumulative CO_2 evolution or O_2 consumption
C_R
total area bounded by the line of the soil respiration rate curve to the time axis from time of the addition of substrate to the time of peak maximum ($t_{peakmax}$)

NOTE See Figure 1.

3.7
soil material
material composed of excavated soil, dredged materials, manufactured soils, treated soils or fill materials

4 Principle

The CO_2 production or O_2 consumption (respiration rate) from unamended soils as well as the decomposition of an easily degraded substrate (glucose + ammonium + phosphate) is monitored regularly (e.g. every hour). From the CO_2 production or O_2 consumption data, the different microbial parameters (basal respiration, substrate-induced respiration, respiratory activation quotient, $t_{peakmax}$, C_R) can be calculated.

5 Reagents

5.1 Glucose, $C_6H_{12}O_6$.

5.2 Potassium dihydrogenphosphate, KH_2PO_4.

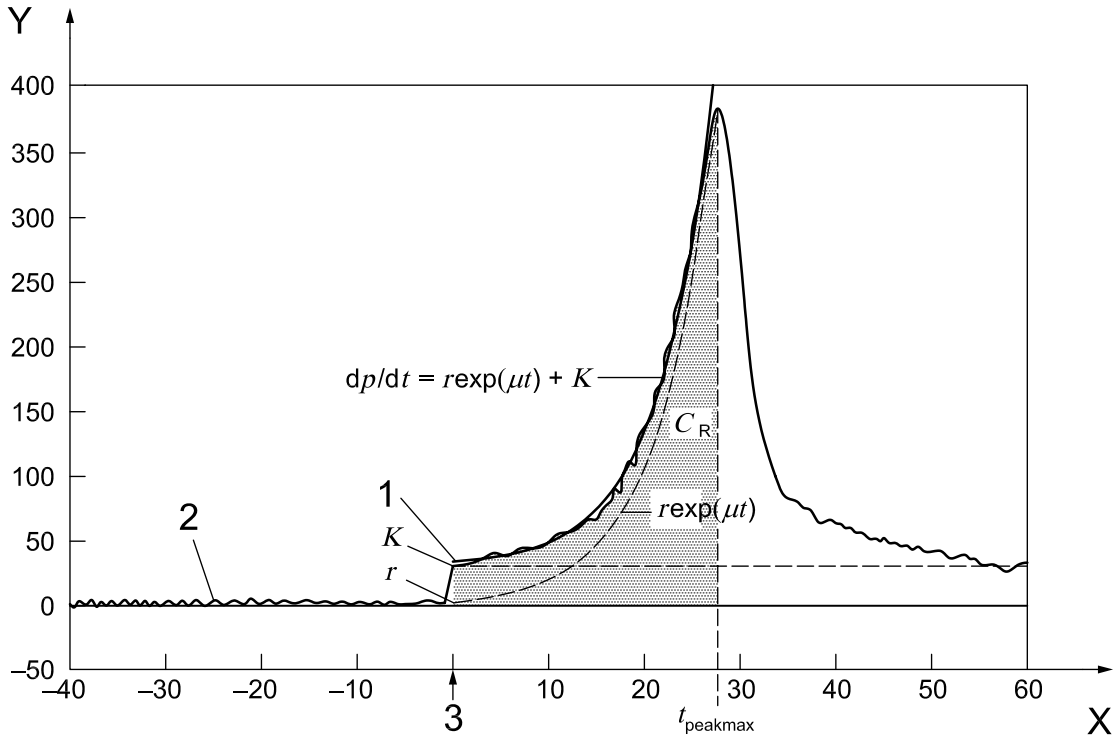
5.3 Diammonium sulfate, $(NH_4)_2SO_4$.

5.4 Substrate, consisting of a mixture of 80 g of glucose (5.1), 13 g of diammonium sulfate (5.3), and 2 g of KH_2PO_4 (5.2), which is thoroughly ground and mixed in a mortar.

6 Apparatus

Ordinary laboratory equipment and 6.1.

6.1 Respirometer for continuous measurement of CO_2 evolution or O_2 consumption, maintained at a constant temperature (preferably 20 °C). Suitable examples of equipment are given in ISO 16072.[2]



Key

X	t	h	time
Y	R	µg g^{-1}_{dm} h^{-1}	CO_2 or O_2 respiration rates
	C_R		cumulative CO_2 evolution or O_2 consumption
	dp/dt		rate of product formation after substrate addition
	K		respiration rate of K-strategist at the time of substrate addition
	r		respiration rate of r-strategist at the time of substrate addition
	$t_{peakmax}$		time to the peak maximum
	μ		specific growth rate
1	R_S		substrate-induced respiration $R_S = K + r$ (at $t = 0$)
2	R_B		basal respiration
3			substrate addition

Figure 1 — Soil respiration rate before and after addition of an easily degraded substrate

7 Sampling

7.1 Sample quantities

Choose the size of the soil samples taking into account the apparatus (6.1) used, the organic matter content of the samples (7.3) and the soil needed for sample characterization (7.3). It is recommended that at least three replicates per sample be measured.

7.2 Sampling and storage

The recommendations in ISO 10381-6 for collection, handling and storage of soil samples shall be followed.

7.3 Soil sample characteristics

Soil samples generating soil respiration curves can be obtained from mineral, organic, polluted, and unpolluted soils. Determine the following characteristics for each soil sample:

— particle size distribution in accordance with ISO 11277;

— water content in accordance with ISO 11465;

— water-holding capacity in accordance with Annex A of ISO 14238:2012;

— pH in accordance with ISO 10390;

— organic matter content in accordance with ISO 10694.

8 Procedure

8.1 Test

Pre-incubate moist soil samples (preferably 40 % to 60 % of maximum water holding capacity or 0,01 MPa to 0,03 MPa suction pressure) at 20 °C for 3 d to 4 d before the beginning of the measurement. Measure the basal respiration of the sub-samples first. Measure the respiration rates until constant rates are obtained.

After measuring the basal respiration, add 10 mg of the substrate (5.4) per gram soil (dry mass) and mix homogeneously with a spatula into the soil samples. If the organic matter content is >5 %, add 0,2 g of the substrate per gram humus (see References [4][5]).

8.2 Toxicity testing

In principle, testing the influence of chemicals should also be possible with the method. Up to the time of publication, there is only scarce experience available in the literature.

To determine the influence of chemicals on the abundance and activity of soil microorganisms, a soil with low content of organic carbon (mass fraction between 0,5 % and 1,5 %). Particles of size <20 µm should not exceed 20 % mass fraction in order to provide a high degree of bioavailablity.

The effect of chemicals on the soil microbial activity can be determined as follows. Using a range-finding test, determine the concentration range in which chemicals would be likely to have an effect on this activity. Test a single, microbiologically active soil at five concentrations in a logarithmic series, including a blank control, in triplicate (e.g. 0, 1, 3,2, 10, 32, and 100 times the lowest concentration). Use the test procedure specified in 8.1. Using this simple test design, dose–response relationships can be established

Before the start of the test, the test chemical may be added to the soil in one of the following ways:

— in aqueous solution (depending on the solubility in water);

— in an organic solution using a water-miscible solvent (depending on the solubility in the solvent);

— mixed with a solid, e.g. coated on quartz sand (prior to mixing with the soil).

If the test chemical is added in the form of an organic solution, keep the amount of the solvent to the minimum (<1 %) necessary for the application of the compound. Furthermore, take into account the possible toxicity (e.g. by including a further control for testing the toxicity of the solvent) and biodegradability of the solvent used.

NOTE Long-term effects of chemicals can be detected by using different incubation times (weeks or months). A comparison of C_R (see 3.6) of the unamended control and the chemical-treated soil samples has been shown to be very sensitive to chemical influences.

9 Calculation

9.1 Microbial parameters

9.1.1 Basal respiration

Calculate the basal respiration, R_B, as the average of the hourly respiration rates during a stable period.

9.1.2 Substrate-induced respiration

Calculate the substrate-induced respiration, R_S, as the average of the values shortly after the substrate addition when the respiration is fairly constant. A minimum of three hourly measurements should be used to calculate the average.

Alternatively, R_S can be calculated according to Equation (2):

$$R_S = r + K \tag{2}$$

where

R_S is the substrate-induced respiration;

r is the respiration rate of r-strategist;

K is the respiration rate of K-strategist immediately after substrate addition.

As proposed in Reference [14] (see Figure 1), the respiration of the non-growing microorganisms, K, and the growing microorganisms, r, is derived by curve fitting using Equation (3):

$$\frac{dp}{dt} = r \exp(\mu t) + K \tag{3}$$

where

$\frac{dp}{dt}$ is the rate of product formation after substrate addition;

p is the accumulated amount of CO_2 evolved or O_2 consumed in a dry mass of soil, in micrograms per gram per hour;

t is the time after addition of substrate, in hours;

μ is the specific growth rate.

NOTE Substrate-induced respiration, R_S, can be used for the estimation of microbial biomass in soils. According to Equation (4), R_S can be converted into C_{mic}(SIR).

$$C_{mic}(\text{SIR}) = 20,6 R_S + 0,37 \tag{4}$$

where

R_S is the substrate-induced CO_2 respiration in micrograms per gram per hour;

C_{mic}(SIR) is the microbial biomass in micrograms per gram.

Close correlations ($R^2_{xy} = 0,84$ to 0,97) exist between C_{mic}(SIR) estimated according to this International Standard and C_{mic} estimated according ISO 14240-1[1] (see Annex B). The correlation variables depend on soil texture and substrate concentrations used.

9.1.3 Respiratory activation quotient

Calculate the respiratory activation quotient, Q_R, by dividing basal respiration rate by substrate-induced respiration rate according to Equation (5).

$$Q_R = \frac{R_B}{R_S} \tag{5}$$

where

R_S is the substrate-induced respiration;

R_B is the basal respiration rate;

Q_R is the respiratory activation quotient.

9.1.4 Specific growth rate

The specific growth rate, μ, can be calculated according to Equation (3).

9.1.5 Time to the peak maximum

The time to the peak maximum, $t_{peakmax}$, can be calculated as the time between substrate addition and the maximum respiration rate.

9.1.6 Cumulative CO_2 evolution or O_2 consumption

The effect of a chemical on μ can be combined by determining the cumulative CO_2 evolution or O_2 consumption from the point in time of the addition of substrate to the point in time of the maximum respiration in the blank control, (i.e. $t_{peakmax}$ in Figure 1). It is essentially equivalent to the hatched area in Figure 1.

For each concentration, the cumulative CO_2 evolution or O_2 consumption, C_R, is measured to the $t_{peakmax}$ in the control blank. A plot of C_R versus the logarithmic concentration of the test substance often gives an S-shaped curve from which EC_{10} and EC_{50} can be estimated.

NOTE This test is carried out under conditions stimulating microbial growth. However, a soil respiration inhibition test can also be carried out at low, growth-limiting concentrations of ^{14}C-labelled acetate followed by $^{14}CO_2$ determination (see Reference [9]).

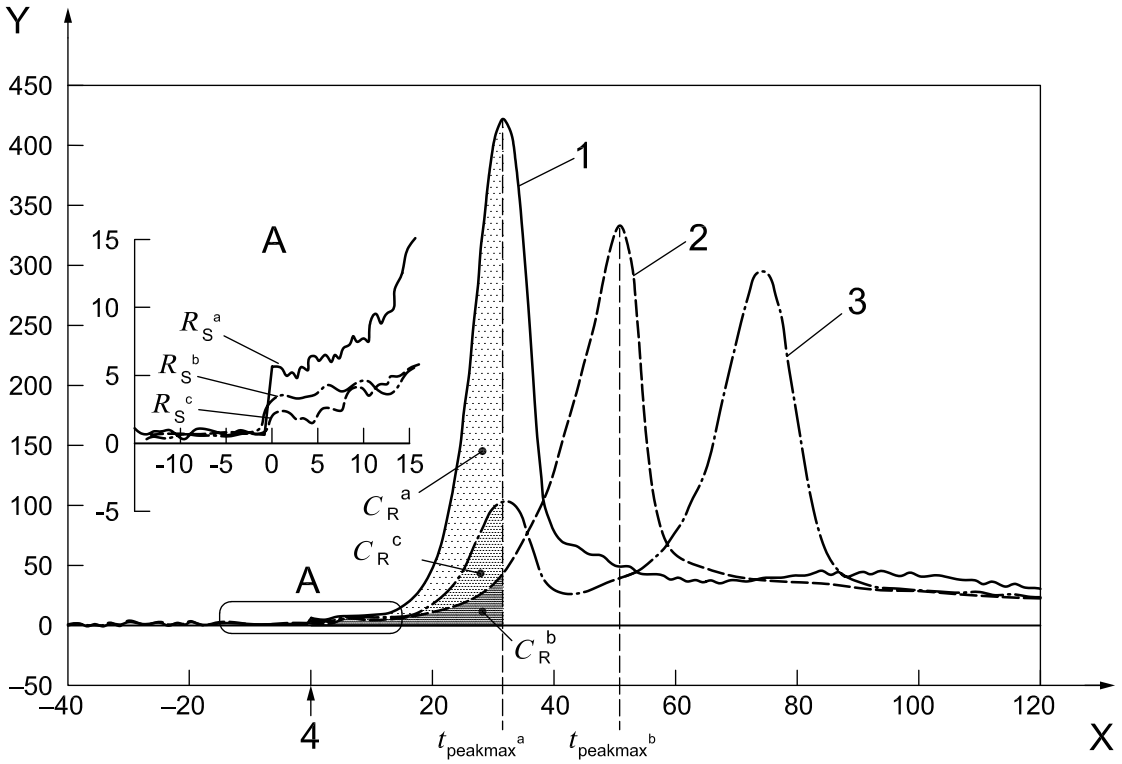
9.2 Interpretation of data

9.2.1 Evaluation of the ecotoxic potential of soils

Polluted soils often show higher respiratory activation quotients and longer time to the peak maximum, $t_{peakmax}$, than unpolluted soils (see Figure 2). By comparing all these parameters with those obtained from unpolluted soils of similar physical and chemical properties, contamination of soils or soil materials can be detected. Respiratory activation quotients $Q_R > 0{,}3$ (mineral soils arable, grassland), $Q_R > 0{,}4$ (mineral forest soils) and $Q_R > 0{,}6$ (organic layers L, Of, Oh) and $t_{peakmax} > 50$ h are indicative for polluted materials (see References [7] and [8]).

Moreover, polluted samples often do not show any logarithmic increase of respiration rates after addition of substrate and/or formation of double peaks (see Figure 2). Double peak formation is caused by a short-term or selective toxic effect of a contaminant. In particular, slowly growing fungi with the marker 18:2δ9,12 seem to be responsible for the formation of a second respiration maximum (Reference [15]).

NOTE Double peaks can also occur in unpolluted samples. Reasons for this phenomenon are growth of fungi due to suboptimal (high) water contents.



Key

X	t	h	time
Y	R	µg g^{-1}_{dm} h^{-1}	CO_2 respiration rate
	C_R		cumulative CO_2 evolution or O_2 consumption
	R_S		substrate-induced respiration
	$t_{peakmax}$		time to the peak maximum
	1		unpolluted soil (control), Reference [4]
	2		Cu-polluted soil (190 mg kg^{-1}_{dm}), Reference [5]
	3		TNT-polluted soil (50 mg kg^{-1}_{dm}), Reference [6]
	4		substrate addition

a Reference [1].

b Reference [2].

c Reference [2].

Figure 2 — Soil respiration curves of an unpolluted and two polluted soils

9.2.2 Additional criteria for the interpretation of the results from soils deliberately contaminated with chemicals

Theoretically, when a chemical is added to soil, the micro-flora can respond in four different ways, of which the two intermediate are most likely to occur.

— Death: the substance is very toxic. The respiration decreases rapidly, but if the toxic substance is removed, either by degradation or evaporation, the survivors can decompose the dead biomass and the respiration is temporarily as high or higher than before the addition of the substance, but the biomass remains low for a considerable time (see Reference [10]).

— Intolerance: the toxicity of the test substance is moderate. Sensitive species are replaced by more resistant ones. The decomposition of soil organic matter becomes less efficient and less biomass is formed (see Reference [11]). The activity and vitality of the microorganisms might also be reduced.

— No observed effect: the toxicity of the test substance is small. If some species are affected they are replaced by others that are as effective as the original flora (see Reference [12]). CO_2 from the slow degradation of an organic test substance can possibly mask a reduced degradation of soil organic matter.

— Enhancement: the test substance is a suitable substrate for at least some of the soil organisms. Respiration is increased until the test substance is consumed (see Reference [13]). The biomass and vitality of the growing organisms are also increased.

The responses of intolerance or no observed effect to a chemical are the most likely to occur.

10 Test report

The test report shall include the following information:

a) general:

— soil collection, treatment, incubation, including date collected, length of storage, temperature of storage,

— test substance: chemical identification data (chemical testing),

— soil characteristics (see also 7.3):

  — particle size distribution in accordance with ISO 11277

  — water content in accordance with ISO 11465

  — water-holding capacity in accordance with Annex A of ISO 14238:2012;

  — pH in accordance with ISO 10390

  — organic matter content in accordance with ISO 10694;

b) test conditions:

— date and place of sampling,

— date of start and end of the test,

— temperature,

— incubation time with the test substance before microbial measurements,

— concentrations tested or range of concentrations in test area compared to background levels,

— solvent used to add the test substance;

c) results:

— list of the microbial parameters for each sub-sample,

— mean values for each sample,

— plot of log of concentration of test substance vs. microbial parameters, EC_{10} and EC_{50},

— regression of these relationships.

Annex A
(informative)

Results of a laboratory ring test carried out in Germany

A laboratory ring test using four different contaminated soils (see Table A.1) was carried out in Germany. Results obtained from CO_2 and O_2 measurements are given separately, because in contaminated soils there is no constant ratio of CO_2/O_2. Extreme values were eliminated according to DIN 38402-42.[3]

Table A.1 — Chemical and physical properties and contaminant concentrations of soils

Parameter	Soil			
	1	2	3	4
	Textural class			
	loam	sand	sand	sand
WHC_{max} [ml/kg]	511,8	347,7	241,8	268,5
pH	7,3	7,3	7,5	5,6
C_{org} [%]	4,7	8,5	5,9	1,7
N_t [%]	0,38	0,27	0,20	0,16
Σ Nitrotoluenes [mg/kg]	336,3	n.d.	n.d.	n.d.
16 PAHs [mg/kg]	77,0	60	21	2268
Mineral oil [mg/kg]	<50,0	43 660,1	8 798,9	<50
As [mg/kg]	5,4	3,6	12,1	<3
Cd [mg/kg]	6,4	29,6	9,3	<3
Cr [mg/kg]	65,9	2 005,1	320,3	94,9
Cu [mg/kg]	104,4	10 921,3	367,8	10,0
Ni [mg/kg]	29,2	5 894,2	578,5	6,5
Pb [mg/kg]	820,8	1 082,2	547,5	10,3
Zn [mg/kg]	764,7	5 065,3	577,6	26,6
n.d.: not determined				

Table A.2 — Basal respiration rates, R_B

Parameter	Soil				Soil			
	1	2	3	4	1	2	3	4
	CO_2 measurement				O_2 measurement			
Mean values, CO_2 or O_2 [µg g^{-1} h^{-1}]	2,08	5,27	5,20	3,37	0,79	7,77	5,17	2,74
Coefficient of variation [%]	66	53	37	50	27	16	10	29
Number of participating laboratories	8	8	7	8	11	11	11	11
Number of laboratories considered	8	8	7	8	10	10	10	10

Table A.3 — Substrate-induced respiration rate, R_S

Parameter	Soil				Soil			
	1	2	3	4	1	2	3	4
	CO_2 measurement				O_2 measurement			
Mean values, CO_2 or O_2 [µg g^{-1} h^{-1}]	8,45	10,72	12,11	6,14	5,84	11,38	8,83	14,39
Coefficient of variation [%]	41	65	28	43	84	40	48	98
Number of participating laboratories	8	8	7	8	10	11	11	11
Number of laboratories considered	8	8	6	8	9	10	10	11

Table A.4 — Respiratory activation quotient, Q_R

Parameter	Soil				Soil			
	1	2	3	4	1	2	3	4
	CO_2 measurement				O_2 measurement			
Mean values	0,21	0,58	0,32	0,60	0,20	0,78	0,71	0,46
Coefficient of variation [%]	43	47	44	45	65	36	32	65
Number of participating laboratories	8	8	7	8	10	10	10	11
Number of laboratories considered	7	8	6	8	9	10	10	11

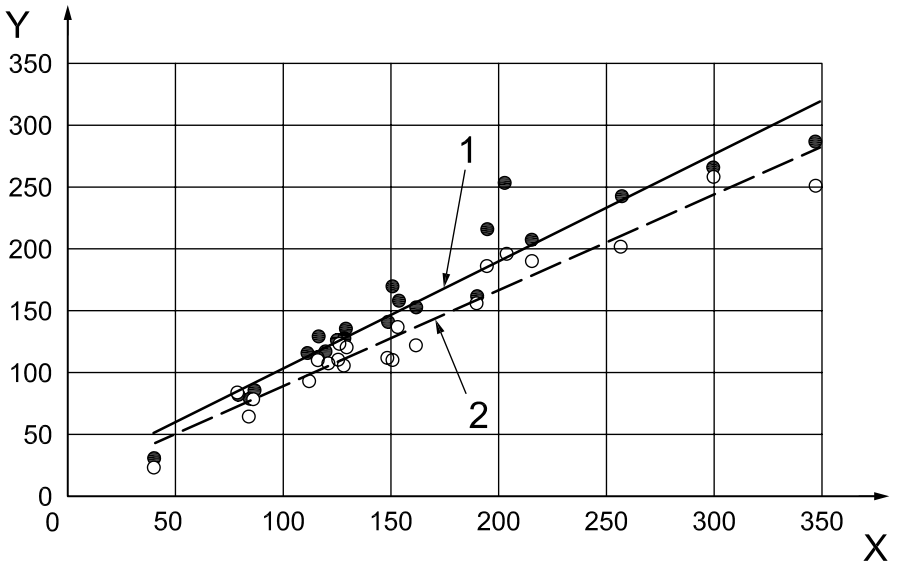
Table A.5 — Time to the peak maximum, $t_{peakmax}$ (for double peaks, only the second peak was considered)

Parameter	Soil				Soil			
	1	2	3	4	1	2	3	4
	CO_2 measurement				O_2 measurement			
Mean values [h]	55	71	61	47	65	69	54	60
Coefficient of variation [%]	38	26	23	37	23	43	43	16
Number of participating laboratories	7	6	7	7	7	7	6	7
Number of laboratories considered	7	6	7	7	7	7	6	7

Annex B
(informative)

Comparison of microbial biomass determination by respiration curve measurement (this International Standard) and substrate-induced respiration (ISO 14240-1[1])

Comparison of C_{mic} determined using glucose (SIR according to ISO 14240-1[1]) or using the substrate following this International Standard (NPKS + glucose) at recommended or reduced concentrations. The recommended substrate (5.4) contents in soil are: a) 10 mg g^{-1} with humus content <5 %; 0,2 g g^{-1} with humus content >5 %.



Key

X	C_{mic}(SIR) in soil	mg kg^{-1}	ISO 14240-1[1]	
Y	C_{mic} in soil	mg kg^{-1}	this International Standard	
1	substrate content in soil, 3,6 mg g^{-1}	$y = 0{,}85x + 16{,}77$	$R^2_{xy} = 0{,}91$	
2	substrate content in soil, 10 mg g^{-1}	$y = 0{,}76x + 11{,}54$	$R^2_{xy} = 0{,}94$	

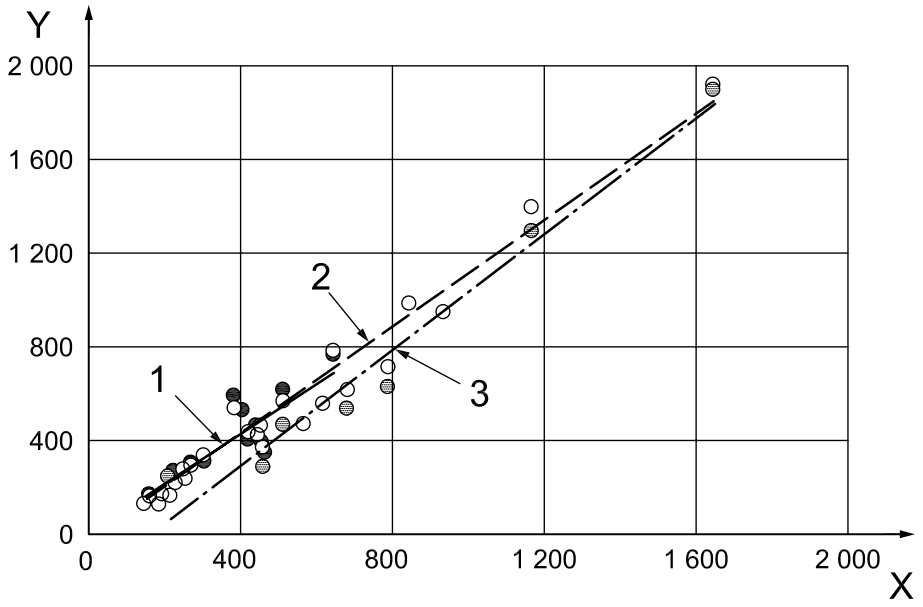
Figure B.1 — Relationship between microbial biomass determined using glucose as substrate in soil (3 mg g^{-1}) [C_{mic}(SIR) (ISO 14240-1[1])] and microbial biomass using 3,6 or 10 mg g^{-1} [C_{mic} (this International Standard)] in arable and grassland soils with a sandy texture

Results – sandy soils:

— good correlation ($R^2_{xy} > 0{,}9$);

— tendency for lower values using substrate according to this International Standard (slope values of 0,85 and 0,76);

— with increasing substrate concentration (3,6 mg g^{-1} soil to 10 mg g^{-1} soil) C_{mic} values are reduced.



Key

X	C_{mic}(SIR) in soil	mg kg^{-1}	ISO 14240-1[1]
Y	C_{mic} in soil	mg kg^{-1}	this International Standard

1	substrate content in soil, 3,6 mg g^{-1}	$y = 1{,}02x + 11{,}12$	$R^2_{xy} = 0{,}84$
2	substrate content in soil, 10 mg g^{-1}	$y = 1{,}14x - 45{,}15$	$R^2_{xy} = 0{,}97$
3	substrate content in soil, 0,2 g g^{-1}	$y = 1{,}22x - 192{,}47$	$R^2_{xy} = 0{,}96$

Figure B.2 — Relationship between microbial biomass determined using glucose as substrate in soil (3 mg g^{-1}) [C_{mic}(SIR) (ISO 14240-1[1])] and microbial biomass using glucose as substrate in soil 3,6 mg g^{-1}, 10 mg g^{-1} or as substrate in organic matter 0,2 g g^{-1} [C_{mic} (this International Standard)] in arable and grassland soils with a silty, loamy or clay texture

Results – silty, loamy or clay soils:

— good correlations ($R^2_{xy} > 0{,}8$);

— tendency for equal or higher values using substrate according to this International Standard (slope values of 1,0 to 1,2);

— at very high substrate concentration in organic matter (0,2 g g^{-1}), C_{mic} values are reduced especially at lower expected values.

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