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Soil quality — Determination of dehydrogenase activity in soils —

Part 1: Method using triphenyltetrazolium chloride (TTC)

Qualité du sol — Détermination de l'activité des déshydrogénases dans les sols —

Partie 1: Méthode au chlorure de triphényltétrazolium (CTT)

Reference number
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Foreword

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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.

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ISO 23753-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 23753 consists of the following parts, under the general title *Soil quality — Determination of dehydrogenase activity in soils*:

— *Part 1: Method using triphenyltetrazolium chloride (TTC)*

— *Part 2: Method using iodotetrazolium chloride (INT)*

Introduction

The soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulfur and phosphorus cycles. Dehydrogenases, as respiratory chain enzymes, play a major role in the energy production by organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme system of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in the soil.

Soil quality — Determination of dehydrogenase activity in soils —

Part 1:
Method using triphenyltetrazolium chloride (TTC)

1 Scope

This part of ISO 23753 specifies a method for determining the dehydrogenase activity in soil using 2,3,5-triphenyltetrazolium chloride (TTC).

It is not applicable for determining the dehydrogenase activity in the upper layers (L, F, H horizons) of forest humus forms with low microbial activity (e.g. mor), or in soils showing reducing properties (e.g. waterlogged soils).

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 for the assessment of aerobic microbial processes in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 11259, *Soil quality — Simplified soil description*

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

3 Principle

TTC solution is added to a soil sample and the mixture is incubated at 25 °C for 16 h. The triphenylformazan (TPF) released is extracted with acetone and determined by photometry at a wavelength of 485 nm.

NOTE 1 The method is based on a modified version of the method reported in Reference [3].

NOTE 2 Other extraction liquids than acetone may be used (e.g. ethanol, acetone-CCl_4 mixture). According to Reference [1], methanol is not suitable because the extraction efficiency is influenced by the soil and the soil water content.

In the case of soil having reducing characteristics (e.g. waterlogged soil), dehydrogenase activity should not be used as a measure of the biological activity in the soil [2]. Abiotic components, such as iron(II) compounds or sulfides, can reduce TTC.

NOTE 3 Organic solvents can extract excessive amounts of humic substances from humus rich soils (e.g. L, F, H horizons, mouldy peat), giving blank values (X_{LP}) which do not differ from measured values (X_{VP}) in soils with low microbial activity.

4 Reagents and materials

4.1 Soil

Take and prepare soil samples as specified in ISO 10381-6. If samples which have been sieved in the fresh state cannot be analysed immediately, they may be kept for up to three months at 4 °C. Determine the dry matter content of the sample in accordance with ISO 11465.

The storage can affect the enzyme activity and hence dehydrogenase activity of samples with different storage times should not be compared.

4.2 Hydrochloric acid, c(HCl) = 1 mol/l.

4.3 Tris buffer solution, c = 0,1 mol/l.

Dissolve 12,11 g of tris(hydroxymethyl)aminomethane in 600 ml of distilled water and adjust the pH value to

a) 7,8 for acidic soil (pH less than 6);

b) 7,6 for neutral soil (pH 6 to 7);

c) 7,4 for carbonate-rich soil (pH greater than 7);

using 1 mol/l hydrochloric acid and make up to 1 000 ml with distilled water. Determine pH value in accordance with ISO 10390.

4.4 Substrate solution (TTC)

The optimum substrate concentration is generally between 0,1 % and 2 % TTC, depending on soil textural class and humus content. Determine the soil texture in accordance with ISO 11259.

Dissolve 2,3,5-triphenyltetrazolium chloride in tris buffer solution (4.3). The solution may be stored for one week at 4 °C in the dark.

Depending on the humus and clay content of the sample, use the following substrate concentrations:

a) sandy soil, slightly humic and slightly clayey soil: 0,1 % to 0,5 % TTC;

b) loam, humic and loamy soil: 0,6 % to 1,0 % TTC;

c) clayey and humic soil: 1,0 % to 2,0 % TTC.

4.5 Analytical grade acetone

4.6 Triphenylformazan (TPF) solutions

4.6.1 TPF stock solution, dissolve 1 000 mg of triphenylformazan (TPF) in acetone (4.5) to make up 100 ml.

4.6.2 TPF working solution, dilute 1,0 ml of stock solution (4.6.1) to 100 ml with acetone (4.5).

4.6.3 TPF calibration solutions

Pipette 0 ml, 1,0 ml, 2,0 ml, 5,0 ml and 10,0 ml respectively, of the solution specified in 4.6.2 into a series of five test tubes and make the volume up to 30 ml with acetone (4.5). The solutions obtained have TPF concentrations of 0 µg/ml, 3,33 µg/ml, 6,67 µg/ml, 16,7 µg/ml and 33,3 µg/ml respectively.

Since TTC and TPF are light-sensitive, the solutions should be protected from exposure to light throughout the analysis.

5 Apparatus

5.1 Photometer

5.2 pH meter

5.3 Test tubes

For example, 2 cm diameter and at least 30 ml capacity (test portion 5 g, see also Clause 6).

5.4 Incubator, capable of being set to (25 ± 1) °C.

5.5 Suitable conical flasks, volumetric flasks, pipettes and funnels

5.6 Test tube shaker

5.7 Fluted filter, with slow filtering action (90 s to 100 s).

6 Procedure

Weigh 5,00 g portions of naturally moist soil into each of four test tubes (5.3). Add 5 ml of substrate solution (4.4) to three samples (complete samples). Pipette 5 ml of tris buffer solution (4.3) instead of the substrate solution to the blank sample. Shake each tube, seal the tubes with rubber stoppers and incubate them at 25 °C for 16 h. To extract the triphenylformazan formed, add 25 ml of acetone (4.5) to the samples and allow them to stand for 2 h in the dark. Shake the samples after adding the extractant, after 1 h, and again after 2 h. Then filter the samples in semidarkness using a fluted filter.

Within 1 h, measure the absorbance of the filtrates against the calibration curve zero by photometry at a wavelength of 485 nm.

According to Reference [1], optimum conditions are given at a soil/solution ratio of 1:1. There is no linear correlation between test portion and TTC reduction if the same test tubes are used. If the test tube diameter is fitted to the test portion, a linear correlation between TTC reduction and the mass of test portion is obtained. Optimum test tube diameters are 1,5 cm for 2 g of soil, 2 cm for 5 g of soil and 2,5 cm for 10 g of soil.

For L, F, H horizons, soil portions of 0,5 g or 1 g and a test tube of 2 cm diameter are recommended [4].

7 Calculation

Determine the dehydrogenase activity (based on dry soil) from the calibration curve and the following equation:

$$a = \frac{(\overline{\rho}_{cs} - \overline{\rho}_{bs}) \times V \times 100}{m \times DM \times t}$$

where:

a is the dehydrogenase activity in µg/g of dry soil per hour;

$\overline{\rho}_{cs}$ is the complete sample average TPF concentration, in µg/ml;

$\overline{\rho}_{bs}$ is the average TPF concentration of the blank sample, in µg/ml;

V is the solution volume (= volume of substrate or buffer solution + volume of extractant), in ml;