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Second edition
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Soil quality — Determination of dehydrogenases 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

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality,* Subcommittee SC 4, *Biological characterization*.

This second edition cancels and replaces the first edition (ISO 23753-1:2005), which has been technically revised. The main changes compared to the previous edition are as follows:

— a new Clause 5 “Limitations” has been added;

— in Clause 6, reagents and their preparation have been updated to new results [e.g. use of less toxic solvent (ethanol), substrate concentration of 60 mmol/l of TTC, concentration of Tris buffer of 100 mmol/l at pH 7,6, incubation time of 6 h];

— Tables 1 and 2 have been added;

— Clause 10 "Validity criteria" has been added;

— a new Annex A “Results of modified parameters” has been added;

— Clause 2 “Normative references” and the Bibliography have been updated.

A list of all the parts in the ISO 23753 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The soil microflora is responsible for the decomposition and conversion of organic substances, carbon, nitrogen, sulfur and phosphorus cycles, soil aggregates stability and as a food source for microbivores. Dehydrogenases, as intracellular enzymes and respiratory chain components of the microbial cells, play a major role in the production of energy by organisms. They oxidize organic compounds by transferring electrons to an acceptor (e.g. NAD^+). Dehydrogenases are essential components of the enzyme system of microorganisms. Dehydrogenases activity can therefore be used as an indicator of biological redox systems and as a measure of the viable and physiologically active soil microbial community.

Microbial oxidative activity in soil is linked to respiratory activity, which could be approached with the determination of dehydrogenases activity. Basal and induced respiration in soil could be affected by soil management, practices and contamination.

Soil quality — Determination of dehydrogenases activity in soils —

Part 1:
Method using triphenyltetrazolium chloride (TTC)

1 Scope

This document specifies a method for determining the activity of dehydrogenases enzymes in soil using 2,3,5-triphenyltetrazolium chloride (TTC).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

TTC solution is added to a soil sample and the mixture is incubated at 25 °C ± 1 °C for 6 h. The triphenylformazan (TPF) released is extracted with ethanol and quantified by spectrophotometry at a wavelength of 485 nm.

NOTE 1 The method is based on a modified version of the method reported in Reference [2].

NOTE 2 Other extraction liquids than ethanol can be used (e.g. acetone).

5 Limitations

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

— Abiotic components, such as iron(II) compounds or sulfides can reduce TTC and consequently interfere with the measurement of dehydrogenases activity.