Soil quality - Determination of dehydrogenases activity in soils - Part 2: Method using iodotetrazolium chloride (INT) (ISO 23753-2:2019)

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NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 23753-2:2019 sisaldab Euroopa standardi EN ISO 23753-2:2019 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 23753-2:2019 consists of the English text of the European standard EN ISO 23753-2:2019.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 20.03.2019.	Date of Availability of the European standard is 20.03.2019.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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English Version

Soil quality - Determination of dehydrogenases activity in soils - Part 2: Method using iodotetrazolium chloride (INT) (ISO 23753-2:2019)

Qualité du sol - Détermination de l'activité des déshydrogénases dans les sols - Partie 2: Méthode au chlorure de iodotétrazolium (INT) (ISO 23753-2:2019)

Bodenbeschaffenheit - Bestimmung der Dehydrogenaseaktivität in Böden - Teil 2: Verfahren mit Iodotetrazoliumchlorid (INT) (ISO 23753-2:2019)

This European Standard was approved by CEN on 4 February 2019.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN ISO 23753-2:2019) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with Technical Committee CEN/TC 444 “Test methods for environmental characterization of solid matrices” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2019, and conflicting national standards shall be withdrawn at the latest by September 2019.

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

This document supersedes EN ISO 23753-2:2011.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 23753-2:2019 has been approved by CEN as EN ISO 23753-2:2019 without any modification.

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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).

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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 23753-2:2005), 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. concentration of Tris buffer of 100 mmol/l at pH 7,6, incubation time between 4 h to 6 h);

— new 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;

— references in Clause 2 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. Dehydrogenase 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 2: Method using iodotetrazolium chloride (INT)

1 Scope

This document specifies a method for determining activity of dehydrogenases in soil, using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT)[1]-[5]. As the INT reduction is less sensitive to O_2, the method is more robust than the TTC-method described in ISO 23753-1.

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

INT solution is added to a soil sample and the mixture is incubated at 25 °C ± 1 °C for 4 h to 6 h depending of soil uses (agricultural or forest soil for exemple). The iodonitrotetrazolium formazan (INTF) released is extracted with acetone 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 [1].

NOTE 2 Acetone is used as extractant and samples are not extracted to completion.

5 Limitations

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

— For upper layers (L, F, H horizons) of forest humus forms[6] or soils showing high organic matter[7], this method gives very low and variable values.