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Interested committees:

Title: Draft BS EN ISO 23611-1 Soil quality — Sampling of soil invertebrates. Part 1: Hand-sorting and extraction of earthworms.

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Introduction

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Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	



DRAFT International Standard

ISO/DIS 23611-1

Soil quality — Sampling of soil invertebrates —

Part 1: Hand-sorting and extraction of earthworms

Qualité du sol — Prélèvement des invertébrés du sol —

Partie 1: Tri manuel et extraction des vers de terre

ICS: 13.080.05; 13.080.30

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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 on 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 the following URL: www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 23611-1:2018), which has been technically revised. The main changes are:

- addition of a definition for ecological categories of earthworms;
- addition of a section on the preparation of composite samples for community DNA metabarcoding;
- removal of the informative [Annex C](#) on the modified TSBF method;
- removal of the outdated informative Annex E with examples of earthworm monitoring programmes (including presentation of their results).

A list of all parts in the ISO 23611 series can be found on the ISO website.

Introduction

This Standard has been originally drawn up since there was a growing need for the standardization of terrestrial zoological field methods. Such methods, mainly covering the sampling, extraction and handling of soil invertebrates, are necessary for the following purposes:

- biological classification of soils including soil quality assessment^{[9][19][30][33][45]};
- terrestrial bio-indication and long-term monitoring^{[2][15][17][39][44]};
- evaluation of the effects of chemicals on soil animals in the field^[29].

Data for these purposes are gained by standardized methods since they can form the basis for far-reaching decisions (e.g. whether a given site should be remediated or not). In fact, the lack of such standardized methods is one of the most important reasons why bio-classification and bio-assessment in terrestrial (i.e. soil) habitats has so far relatively rarely been used in comparison to aquatic sites.

Since it is neither possible nor useful to standardize methods for all soil organisms, the most important ones have been selected. In this document sampling of earthworms is described.

Originally, the methods described in this document were developed for taxonomical and ecological studies, investigating the role of earthworms (macrofauna) in various soil ecosystems. These animals are without doubt the most important soil invertebrates in temperate regions.^{[21][23][35]} This does not necessarily imply that earthworms are less important in tropical soils. In fact, there is wide evidence that earthworms may be as important as or even more important than the abundant termites and ants in many locations of the tropical region.^[22] Since Darwin (1881) (see Reference ^[11]), their influence on soil structure (e.g. aeration, water holding capacity) and soil functions like litter decomposition and nutrient cycling is well-known.^[14] Due to their often very high biomass they are also important in many terrestrial food-webs.

In earlier versions of this document the chemical formalin was recommended as extraction fluid. Since then, evidence increased that formalin has properties of concern, mainly in terms of human toxicity. According to the European Chemicals Agency (ECHA) chemical database, this chemical is labelled as carcinogenic and suspected to be mutagenic (Categories Carc. 1B and Muta. 2).^[13] In addition, negative effects on non-target organisms (including soil microorganisms, mesofauna and plants) have been reported (e.g. see Reference ^[10]). Therefore, this substance has been replaced.

Due to the growing reservations against the use of formalin, several alternatives have been studied. In Reference ^[46] allyl isothiocyanate (AITC) was tested for its effectiveness as a chemical expellant for sampling earthworms. AITC is a natural breakdown product of glucosinolates in many Cruciferae, i.e. it is the component imparting the sharp taste of mustard. In the ECHA chemical database, properties of concern 'skin sensitising' and 'respiratory sensitising' are listed.^[12] It is also toxic and hazardous to the aquatic environment. Hence, although less harmful than formalin, AITC shall only be used with appropriate safety precautions.

Some studies have been performed in which the extraction efficiency of formalin and AITC were compared at the same sites and dates. According to Reference ^[27] no differences were found in numbers or biomass of earthworms extracted at crop sites when using either formalin or AITC as extractant. Also, no interaction was found on the sampling sites between the extractant and the site, indicating that no site-specific differences were observed in extraction efficiency of the extractants. When plotting the correlation between worm numbers extracted with AITC versus formalin in a Bland-Altman graph (a common way to compare a gold-standard method to an alternative method in the medical sciences), no significant bias of the AITC method as compared to the formalin method was found, indicating the similarity/exchangeability of the two methods^[32].

Basic information on the ecology of earthworms and their use as bioindicators in the terrestrial environment can be found in the references listed in the Bibliography.