



**International
Standard**

ISO 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

**Third edition
2026-06**

Sample Document

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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 description of ecological categories of earthworms;
- addition of information related to live identification of earthworms;
- revised description for determination of biomass;
- addition of a section on the preparation of composite samples for community DNA metabarcoding;
- revision of [Annex A](#) on other methods of earthworm sampling;
- removal of the former 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.

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

This document was originally drawn up because of the 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][24][36][39][51]}
- terrestrial bio-indication and long-term monitoring;^{[2][16][18][45][50]}
- evaluation of the effects of chemicals on soil animals in the field.^[35]

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 been used relatively rarely 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, the 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.^{[27][29][41]} This does not necessarily imply that earthworms are less important in tropical soils. In fact, there is wide evidence that earthworms can be as important as or even more important than the abundant termites and ants in many locations of the tropical region.^[28] Since Darwin (1881) (see Reference ^[11]), their influence on soil structure (e.g. aeration, water holding capacity) and on soil functions (e.g. 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 has 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 ^[52] 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. The ECHA chemical database provides an overview of the hazard classification and labelling and of properties of concern.^[12] Hence, although not currently labelled as carcinogenic like formalin, AITC must 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 ^[33] 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 earthworm 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 and exchangeability of the two methods.^[38]

Earthworms can be divided into three main ecological categories: endogeic, epigeic and anecic.^[6] Endogeic species vary greatly in size, with little or no pigmentation, are geophagous, live in the soil and rarely rise to the surface, creating galleries with little connectivity to the soil surface. Epigeics tend to have smaller sizes, are dorsally or completely pigmented, live on or near the soil surface or in places with accumulation of organic matter (composters, bromeliads, rotten trunks, epiphytic soils), ingest little soil and produce basically organic casts. Anecics often move to the soil surface, have dorsal pigmentation especially in the anterior part of the body, are usually large in size and produce vertical galleries and accumulations of

organo-mineral casts (often with higher organic matter contents than the ingested soil) on the surface of the soil at the entrance of their galleries. These categories are evident in temperate regions. But, especially in tropical and subtropical regions, there are blends of categories, such as “epi-endogeic”^[4], and the anecic category is not as common as in the temperate region, with a predominance of endogeics especially in agroecosystems^[15].

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.

The sampling design of field studies in general is given in ISO 18400-101^[20] and ISO 23611-6^[23], and guidance on the determination of effects of pollutants on earthworms in field situations is given in ISO 11268-3¹⁾. These aspects can vary according to the national requirements or the climatic and regional conditions of the site to be sampled.

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1) This document will be replaced by an Organisation for Economic Co-operation and Development (OECD) document (either a Test Guideline or Guidance Document) currently under development (OECD project no. 2.47 ‘New Test Guideline on Determination of Effects on Earthworms in Field Studies’).

Soil quality — Sampling of soil invertebrates —

Part 1: Hand-sorting and extraction of earthworms

1 Scope

This document specifies a method for sampling and handling earthworms from field soils as a prerequisite for using these animals as bioindicators (e.g. to assess the quality of a soil as a habitat for organisms).

This document is applicable to all terrestrial biotopes in which earthworms occur. This document does not apply to semi-terrestrial soils (i.e. soils that are partly aquatic, such as bogs, beaches, marshes, stream margins) and it can be difficult to use under extreme climatic or geographical conditions (e.g. in high mountains). Methods for other soil organism groups, such as micro-arthropods and enchytraeids (mesofauna), are covered in other parts of the ISO 23611 series.

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 10390, *Soil, treated biowaste and sludge — Determination of pH*

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

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

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

ISO 11465, *Sludge and solid environmental matrices — Determination of dry residue or water content and calculation of the dry matter fraction on a mass basis*

3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

earthworm

megadrile soil-inhabiting invertebrate belonging to the order Crassicitellata (class Clitellata, phylum Annelida) and classified as soil macrofauna

Note 1 to entry: The length of adult individuals can vary from a few centimetres to more than 1 m.