SLOVENSKI STANDARD
oSIST prEN ISO 23611-1:2017

01-januar-2017

Kakovost tal - Vzorčenje nevretenčarjev v tleh - 1. del: Ročno razvrščanje deževnikov in njihova ekstrakcija (ISO/DIS 23611-1:2016)

Soil quality - Sampling of soil invertebrates - Part 1: Hand-sorting and extraction of earthworms (ISO/DIS 23611-1:2016)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 1: Handauslese und Extraktion von Regenwürmern (ISO/DIS 23611-1:2016)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 1: Tri manuel et extraction des vers de terre (ISO/DIS 23611-1:2016)

Ta slovenski standard je istoveten z: **prEN ISO 23611-1**

ICS:

13.080.30	Biološke lastnosti tal	Biological properties of soils

oSIST prEN ISO 23611-1:2017 **en,fr,de**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 23611-1

ISO/TC **190**/SC **4** Secretariat: **AFNOR**

Voting begins on: **2016-11-16**

Voting terminates on: **2017-02-07**

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

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 23611-1:2016(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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.

ISO 23611-1 was prepared by Technical Committee ISO/TC 190, *Soil quality,* Subcommittee SC 4*, Biological methods.*

This second edition cancels and replaces the first edition (ISO 23611-1:2006), which has been technically revised.

ISO 23611 consists of the following parts, under the general title *Soil quality — Sampling of soil invertebrates:*

— *Part 1: Hand-sorting and extraction of earthworms*

— *Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)*

— *Part 3: Sampling and soil extraction of enchytraeids*

— *Part 4: Sampling, extraction and identification of free-living stages of terrestrial nematodes*

— *Part 5: Sampling and extraction of soil macro-invertebrates*

— *Part 6: Guidance for the design of sampling programmes with soil invertebrates*

Introduction

This part of ISO 23611 has been drawn up since there is a growing need for the standardisation 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[25], [31], [39];

— terrestrial bio-indication and long-term monitoring[11], [14], [33];

— evaluation of the effects of chemicals on soil animals (ISO 11268-3).

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 standardised 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 part of ISO 23611 sampling of earthworms is described.

Originally, the methods described in this International Standard were developed for taxonomical and ecological studies, investigating the role of earthworms in various soil ecosystems. These animals are without doubt the most important soil invertebrates in temperate regions and, to a lesser extent, in tropical soils[30], [16], [18]. Since Darwin (1881) [see 8], their influence on soil structure (e.g. aeration, water holding capacity) and soil functions like litter decomposition and nutrient cycling is well-known[10]. Due to their often very high biomass they are also important in many terrestrial food-webs.

In the previous version of this International Standard the chemical formalin was recommended as extraction fluid. However, within the last years evidence increased that formalin does have critical properties, mainly in terms of human toxicity. In December 2012, the Risk Assessment Committee (RAC) of the European Chemicals Agency (ECHA) stated that there is sufficient scientific evidence to classify this chemical as "probably carcinogenic for humans (Category Ib). In addition, negative effects on non-target organisms (including soil microorganisms, mesofauna and plants) have been reported (e.g. [7]). Therefore, this substance had been replaced.

Due to the growing reservations against the use of formalin several alternatives have been studied. In [40] there was tested allyl isothiocyanate (AITC) 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. According to the European Chemical Agency (ECHA), there is no regulatory action in the context of human or environmental risk assessment considered, i.e. there is no concern regarding its use.

Within the last years, some studies have been performed in which the extraction efficiency of formalin and AITC were compared at the same sites and dates. According to [22] there were not found differences in numbers or biomass of earthworms extracted at crop sites when using either formalin or AITC as extractant. In a recent unpublished review [see 28] there were not found significant differences in earthworm numbers/biomass when comparing the efficiency of the two extraction chemicals. Also no effect was found for the interactions extractant 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 vs. formol 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.

Soil quality — Sampling of soil invertebrates —

Part 1:
Hand-sorting and extraction of earthworms

1 Scope

This part of ISO 23611 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).

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.

This part of ISO 23611 applies to all terrestrial biotopes in which earthworms occur. The sampling design of field studies in general is specified in ISO 10381-1 and guidance on the determination of effects of pollutants on earthworms in field situations is given in ISO 11268-3. These details can vary according to the national requirements or the climatic/regional conditions of the site to be sampled (see also Annex C).

This part of ISO 23611 is not applicable for semi-terrestrial soils and it can be difficult to use under extreme climatic or geographical conditions (e.g. in high mountains). Methods for some other soil organism groups, such as collembolans, are covered in other parts of ISO 23611.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — 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, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

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

3.1
earthworm
megadrile soil-inhabiting earthworms (length of adult individuals: few centimetres to more than 1 m) belonging to the order Oligochaeta (class Clitellata, phylum Annelida)

EXAMPLE Species of the families Lumbricidae (Holarctic), Glossoscolecidae (Latin America), Eudrilidae (Africa) or Megascolecidae [Asia, North America (Pacific Coast)].

3.2
peregrine species
earthworms occurring in many regions world-wide today, usually introduced by man

Note 1 to entry: Well-known examples of peregrine species are several lumbricid species like Aporrectodea caliginosa (originally coming from Eurasia, but now living also in the Americas and Australia) or the pan-tropical species Pontoscolex corethrurus (probably coming from Northern Brazil and/or the Guyanas).

Note 2 to entry: See Reference [18].

3.3
clitellum
ring or saddle of epidermal thickening only in mature worms which is near the anterior and eventually forms the cocoon

4 Principle

Earthworms at a certain site are sampled from the soil by using a combination of two different methods:

— hand-sorting animals from a certain area (e.g. 0,25 m^2) or volume (e.g. 50 cm × 50 cm × 20 cm);

— extraction of worms from the soil by applying AITC.

The former method is known for about 100 years while the new extraction fluid was proposed about 15 years ago[6],[40]. After extraction, the earthworms are fixed and transported to the laboratory. There they are preserved in a way that they can be stored in a collection indefinitely (e.g. for taxonomical purposes). In addition, the determination of the biomass of earthworms is described. Finally, abundance and biomass values can be recalculated to area (usually 1 m^2) or, more rarely, volume parameters.

NOTE 1 Alternative methods can be useful under special circumstances (e.g. electrical extraction), but cannot be recommended as a general procedure (see Annex A)

NOTE 2 The sampling of earthworms is often included in much broader monitoring programs, trying to cover the whole soil fauna or parts of it (e.g. the macrofauna). The design of such programs is not included in this part of ISO 23611.

NOTE 3 Some hints for the taxonomy of peregrine (occurring in many regions world-wide) earthworms, mainly belonging to the family Lumbricidae, are given in Annex B.

5 Reagents

5.1 Formalin [formaldehyde solution 4 % (volume fraction)].

5.2 Allyl-isothiocyanate (AITC) [synthetic grade (about 94 % to 97 % (volume fraction))].

5.3 Ethanol, [70 % (volume fraction)].

6 Apparatus

Use standard laboratory equipment and the following.

6.1 Plastic vessels, capacities 250 ml and 500 ml, for storing the worms.

6.2 Rubber gloves.

6.3 Forceps.

6.4 Piece of thick plastic sheeting, 1 m^2 to 2 m^2.

6.5 Spade or shovel.

6.6 Dissecting microscope, with low magnification (10 to 40 times).

6.7 Balance, weigh range from 0,01 g to 200 g.

6.8 Water-can, preferably 20 l, with water (20 l per sampling plot).

6.9 Watering can.

6.10 Pencil, **notebook, water resistant marker, labels** that go in the vessel.

6.11 Thermometer, e.g. for measuring air temperature.

6.12 Drying cabinet, for soil moisture determination.

7 Procedure

7.1 Sampling of the earthworms

7.1.1 General

Sampling of earthworms is done by a combination of two different methods: hand-sorting and AITC extraction. Based on several comparative studies, the combination of a physical and a chemical method is clearly recommended in the various reviews on earthworm ecology, independent from the type of chemical expellent (e.g. references [9], [10], [18]).

Sampling should be done at times of the year when the animals are not forced by the environmental conditions (i.e. low soil moisture and/or high temperatures) into diapause (i.e. are not reacting to AITC). In temperate regions, such unfavourable sampling times are winter and, in particular, midsummer periods [18]. Earthworms sampled from the same plot, but sampled under the two different methods, should be stored in individual plastic vessels. After the end of the sampling process, the excavated and examined soil is returned to the original sampling plot. In some cases, it is appropriate to use only one of the two methods; e. g. when no deep-burrowing animals are occurring at a given site, AITC extraction is not necessary. On the other hand, at sites where giant earthworms are living (parts of South America, South East Asia and Australia), hand-sorting is not useful [26]. A very similar method, known as modified TSBF method, is particularly suitable for tropical regions (see Annex C).

NOTE Usually the earthworms are determined after preservation, but if the species spectrum of a sampling site is well known, worms can also be determined alive [35] (see also Annex B).

In case the collected earthworms are to be used for further analysis or testing, e. g. for biomarker measurements or for use in bioassays, storage or incubation of the worms in a small portion of soil from the sampling site is recommended. In the case of AITC extraction, rinsing the worms in tap water is needed before incubation in soil.