

# SVENSK STANDARD

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### **Markundersökningar – Provtagning av marklevande ryggradslösa djur – Del 2: Provtagning och extraktion av ”microarthropods” (Collembola and Acarina) (ISO 23611-2:2006)**

### **Soil quality – Sampling of soil invertebrates – Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina) (ISO 23611-2:2006)**

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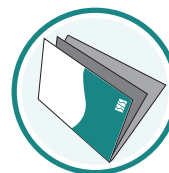
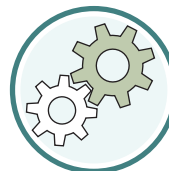
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Denna standard ersätter SS-ISO 23611-2:2006, utgåva 1.

The European Standard EN ISO 23611-2:2011 has the status of a Swedish Standard. This document contains the official version of EN ISO 23611-2:2011.

This standard supersedes the Swedish Standard SS-ISO 23611-2:2006, edition 1.

**Förhållandet till övriga delar under samma huvudtitel - Utdrag ur Förord i ISO 23611-2:2006/  
Relations to other parts under the same general title - Extract from the Foreword of  
ISO 23611-2:2006**

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

- Part 1: *Hand-sorting and formalin 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*

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EUROPEAN STANDARD

**EN ISO 23611-2**

NORME EUROPÉENNE

EUROPÄISCHE NORM

July 2011

ICS 13.080.30; 13.080.05

English Version

**Soil quality - Sampling of soil invertebrates - Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina) (ISO 23611-2:2006)**

Qualité du sol - Prélèvement des invertébrés du sol - Partie 2 : Prélèvement et extraction des micro-arthropodes (Collembola et Acarina) (ISO 23611-2:2006)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 2: Probenahme und Extraktion von Mikroarthropoden (Collembolen und Milben) (ISO 23611-2:2006)

This European Standard was approved by CEN on 17 June 2011.

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## **Foreword**

The text of ISO 23611-2:2006 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 23611-2:2011 by Technical Committee CEN/TC 345 “Characterization of soils” 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 January 2012, and conflicting national standards shall be withdrawn at the latest by January 2012.

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### **Endorsement notice**

The text of ISO 23611-2:2006 has been approved by CEN as a EN ISO 23611-2:2011 without any modification.

## Introduction

This part of ISO 23611 has been drawn up since there is a growing need for the standardization of sampling and extraction methods of soil micro-arthropods. These methods are needed for the following purposes:

- biological classification of soils including soil quality assessment (e.g. References [31], [32], [35], [41], [45], [46]);
- terrestrial bioindication and long-term monitoring (e.g. References [1], [7], [17], [40], [42]).

Data collected by standardized methods can be more accurately evaluated allowing more reliable comparisons between sites (e.g. polluted versus non-polluted sites, changes in land-use practices).

From the several micro-arthropod groups, Collembola and Acarina are the most studied in soil ecology. Their relevance for the soil system comes from their high abundance and diversity, and also from their role in key biological processes. Collembola and Oribatid mites act mainly as catalysts in organic matter decomposition [4], [20], whereas predacious mites may act as webmasters in soil food webs [9]. These characteristics, allied to a widespread taxonomic knowledge, allowed their use as study organisms in several research programmes dealing with the impacts of forest practices (e.g. References [12], [13], [14], [15], [18], [19], [21], [22], [23], [25], [26], [27], [28], [29], [30], [31], [33], [34], [37], [38], [39]) or crop management practices (e.g. [6], [11], [16], [24]). These features make them suitable organisms to be used as bio-indicators of changes in soil quality, especially due to land-use practices and pollution [43].



# Soil quality — Sampling of soil invertebrates —

## Part 2:

## Sampling and extraction of micro-arthropods (Collembola and Acarina)

### 1 Scope

This part of ISO 23611 specifies a method for sampling, extracting and preserving collembolans and mites from field soils as a prerequisite for using these animals as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms).

Basic information on the ecology of micro-arthropods and their use can be found in the references listed in the Bibliography.

The sampling and extraction methods of this part of ISO 23611 are applicable to almost all types of soils. Exceptions may be soils from extreme climatic conditions (hard, frozen or flooded soils) and other matrices than soil, e.g. tree trunks, plants or lichens. For the sampling design of field studies in general, see ISO 10381-1.

Methods for some other soil organism groups such as earthworms are covered in other parts of ISO 23611.

This part of ISO 23611 does not cover the pedological characterization of the site which is highly recommendable when sampling soil invertebrates. ISO 10390, ISO 10694, ISO 11272, ISO 11274, ISO 11277, ISO 11461 and ISO 11465 are more suitable for measuring pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity.

### 2 Terms and definitions

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

#### 2.1

##### **micro-arthropods**

group which is defined by its small size (range size from 100 µm to a few millimetres) making up a significant part of the below-ground food web in many terrestrial ecosystems

NOTE This group is mainly composed by mites (Acarina), springtails (Collembola), Protura, Diplura, garden centipedes (Symphyla), Pauropoda, small centipedes and millipedes, and insects and their larvae from several orders (Diptera, Coleoptera, etc.).

### 3 Principle

Soil samples are collected in the field using a split corer. Soil cores are placed in plastic tubes (or plastic bags) and transported to the laboratory. Afterwards, Collembola and Acarida are rapidly (within a few days) extracted by behavioural methods, using a MacFadyen apparatus, and preserved for future identifications [7], [40]. In addition, preparation techniques are also described. Finally, abundance values can be recalculated related to area (usually 1 m<sup>2</sup>), volume or weight (usually 1 kg).

NOTE Alternative methods for extraction can be used under special circumstances. Flotation methods (e.g. the heptane flotation method) can be used in clay or loamy soils and a Kempson extractor is advisable in the case litter is sampled [40].

## 4 Test materials

### 4.1 Biological material

Collembola (springtails) are small wingless hexapods (from 150 µm up to 9 mm length), having a distinctive head with a pair of antennae, without true compound eyes, with six abdominal segments and three pre-genital appendages in the abdomen. In the first segment, there is the ventral tube (or colophore) that is used for adhering to smooth surfaces. The name Collembola comes from this structure (from Greek *colla* = glue and *embolon* = bar). In the third segment, there is the *tenaculum*, that holds the jumping apparatus on its normal position. This jumping appendage, the *furcula* (or spring), when it exists, is located in the fourth segment. Springtails live in litter and soil, and have very distinctive life forms. They belong to the class Collembola, and can be separated into 18 families [17].

Soil mites are small chelicerate arthropods related to spiders (length from 150 µm up to < 5 mm), living in soil and litter, and also presenting very distinctive life forms. They belong to the class Arachnida, subclass Acarida, and can be separated into four groups: Cryptostigmata (Oribatida), Mesostigmata (Gamasida), Prostigmata (Trombidiformes) and Astigmata.

NOTE Some hints for the taxonomy of springtails and mites are given in Annex A.

### 4.2 Reagents

Unless otherwise specified, use only reagents of good quality and distilled water.

**4.2.1 Propan-2-ol**, 80 % (volume fraction).

**4.2.2 Formalin** [formaldehyde solution 40 % (volume fraction)].

**4.2.3 Acetic acid**.

**4.2.4 Phenol**, C<sub>6</sub>H<sub>5</sub>OH, crystalline (carbolic acid).

**4.2.5 Hydrogen chloride**, *c*(HCl) from 8 mol/l to 10 mol/l.

**4.2.6 2,2,2-Trichloro-1,1-ethanediol** (chloral hydrate).

**4.2.7 1,2,3-Trihydroxypropane** (glycerine).

**4.2.8 von Törne fixative**, used to preserve the extracted animals and composed by Propan-2-ol (80 %), formalin (40 %) and glacial acetic acid (a volume fraction 10:0,3:0,03).

**4.2.9 Nesbitt clearing medium**, used to clear mite specimens composed of chloral hydrate (80 g), distilled water (50 ml) and concentrated hydrogen chloride (5 ml).

**4.2.10 Lactophenol solution**, used to clear mite specimens composed of lactic acid (10 ml), crystals of phenol (3,6 g) and distilled water (5 ml).

**4.2.11 2-Hydroxypropanoic acid** (lactic acid), to clear and observe micro-arthropod specimens, especially oribatid mites under the microscope.

**4.2.12 Ethanol**, 70 % to 75% (volume fraction), used for fixation and preservation (in this case, also in combination with glycerine, 10:1).

**4.2.13 Hoyer's medium**, used to mount Collembola specimens composed of distilled water (50 ml), gum-arabic (30 g), chloral hydrate (200 g) and glycerine (20 ml).

## 5 Apparatus

Use standard laboratory equipment and the following.

**5.1 Measuring tape.**

**5.2 Collecting flasks.**

**5.3 Wash bottle.**

**5.4 Forceps, pipette, fine painting brush, fine needles.**

**5.5 Petri dishes.**

**5.6 Stereomicroscope.**

**5.7 Microscope**, with phase or interference contrast is preferable.

**5.8 Microscopic slides**, with excavated area in the centre, and **lamellae**.

**5.9 Electrical heating plate.**

**5.10 Plastic vials.**

**5.11 Ceramic heating elements.**

**5.12 Pencil, notebook, water resistant marker, labels.**

**5.13 Split corer**

Sampling device made of stainless steel or aluminium (40 cm long and e.g. 5,6 cm diameter may be used; the size and diameter should not differ considerably from these numbers in order to maintain comparable conditions), used to collect soil cores (samples). It can be composed of two independent parts that fit together along the corer main axis or it can consist of one tube. On the top, it has a handle and on the bottom, a cutting edge.

**5.14 MacFadyen apparatus**

High-gradient (multiple) device used to extract micro-arthropods from soil samples. The principle is to create an artificial temperature gradient between the canister where the sample is placed (hot) and the collecting device below (cold), inducing a negative thermotactic (at the same time a positive hygrotactic, negative phototactic and positive skototactic) behaviour on the animals that, by this way, leave the soil sample.

**5.15 Plastic tubes**, with caps (5 cm diameter, 5 cm long), or **plastic bags**, for storing the soil samples.

**5.16 Kempson extractor**, in the case litter is sampled.

**5.17 Sample frame**, 25 cm × 25 cm × 15 cm, made of stainless steel and with sharpened edges, to sample animals from the litter layer.

NOTE For details concerning the equipment in 5.13 to 5.17, see References [7] and [40].