
**Soil quality — Method for testing
effects of soil contaminants on the
feeding activity of soil dwelling
organisms — Bait-lamina test**

*Qualité du sol — Méthode pour tester les effets des contaminants
du sol sur l'activité alimentaire des organismes vivant dans le sol —
Test Bait-lamina*

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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 WTO principles in the Technical Barriers to Trade (TBT), see the following URL: [Foreword — Supplementary information](#).

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

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Introduction

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis [38]. In addition to laboratory tests with individual species from important organism groups (e.g. earthworms, enchytraeids, or collembolans), functional tests can be used to assess the activity of the whole soil organism community *in situ* in the field and, thus, the habitat function of soil. They are potentially useful for the evaluation of the effects of chemicals on the soil [1][11][12][26][28], as well as for the long-term monitoring of the biological quality of soils (see Annex A)[16][18][34]. Data for these purposes are gained by standardised 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 functional methods have been so far relatively rarely used for the assessment of contaminated soils or for soil monitoring purposes.

The bait-lamina test is an approach which is used to measure the feeding activity of soil organisms *in situ* [23][35][36].

The important advantage of the bait-lamina method is its simplicity. Minimal training, special skill, or equipment is necessary. In contrast to the measurement of other functional parameters, like organic matter breakdown determined in a litterbag test [22][25][27], the bait-lamina method does not disturb the soil substrate, needs only short exposure periods (few days up to few weeks), and is rapidly evaluated.

In temperate regions, the applicability and usefulness of the bait-lamina test for the comparison and assessment of the influence of land use or chemicals on the feeding activity of soil communities has been demonstrated several times [2][14][28][31]. In addition, bait-lamina tests have also been applied in the tropics [13][15][30].

In addition to measures such as microbial respiration or litter decomposition, feeding activity is one of several functional parameters applicable for the assessment of the biological status of soils [7][13]. Recently, the bait-lamina test was used successfully in several case studies at which an environmental risk assessment for polluted soils was performed [8][21]. As a result of these works, the bait-lamina test was included into the tool box for site-specific risk assessment [e.g. the TRIAD approach (see ISO 19204)]. In fact, it became part of the recommended battery of tests for the ecological risk assessment of soils in Great Britain [9]. In addition, in the context of a European soil monitoring programme, the method was recommended for this purpose as well by the members of an EU Working Group [4].

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Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test

1 Scope

This International Standard specifies a technique for determining the effects of anthropogenic impacts (e.g. substances) in the context of the prevailing environmental conditions on the feeding activity of soil organisms in the field. In addition, the use of this method for monitoring the biological quality of soil is described (see [Annex A](#)). The breakdown of organic matter by soil invertebrates and microorganisms is a crucial process that determines important soil functions such as nutrient availability for plants and the maintenance of soil fertility. In addition, decomposing plant litter provides habitats and food for a wide range of organisms, thus supporting biodiversity and ecosystem services [33][34].

This International Standard is applicable to all soils in which soil organisms are active. The use of the bait-lamina test is independent from whether there is a litter layer or not. The sampling design of field studies in general is specified in ISO 23611-6 (see also Reference [20]). The design can vary according to the aim of the study as well as conditions (e.g. soil properties, contamination, etc.) of the site to be investigated.

This International Standard is not applicable for semi-terrestrial or very shallow soils. It can be difficult to use it under extreme climatic or geographical conditions (e.g. in high mountains).

2 Normative references

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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 11074, *Soil quality — Vocabulary*

ISO 23611-6, *Soil quality — Sampling of soil invertebrates — Part 6: Guidance for the design of sampling programmes with soil invertebrates*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23611-6, ISO 11074, and the following apply.

3.1

exposure time

period in which the bait-lamina strips are exposed in the field, usually in the mineral soil ([3.2](#))

EXAMPLE In temperate zones: 10 d to 20 d; in tropical areas: 4 d to 8 d.

3.2

soil

topsoil with or without a *litter layer* ([3.3](#))

3.3

litter layer

layer of mainly dead plant organic material present on the surface of the topsoil, which is composed of debris in different stages of decomposition

Note 1 to entry: Examples are leaves in orchards or forests or “turf” derived from permanent grass grown in meadows [5].

3.4

contaminant

substance or agent present in the *soil* (3.2) as a result of human activity

3.5

contaminated site

site with areas of high concentrations of substances hazardous to *soil* (3.2) and *soil functions* (3.7)

Note 1 to entry: In many countries, contaminated sites are registered publicly as a consequence of a specific legislation.

3.6

soil water content

mass of water evaporating from the *soil* (3.2) when dried to constant mass at 105 °C divided by the dry mass of the soil and multiplied by 100

3.7

soil functions

description of the significance of *soils* (3.2) to man and the environment

Note 1 to entry: Important soil functions in the context of this International Standard include the following:

- control of substance and energy cycles as compartment of ecosystems;
- basis for the life of plants, animals, and man.

3.8

biodiversity

variability among living organisms from all sources, and the ecological complexes of which they are part

Note 1 to entry: This includes diversity within species, between species, and of ecosystems.

3.9

ecosystem services

benefits that humans recognise as obtained from ecosystems that support, directly or indirectly, their survival and quality of life

Note 1 to entry: These include provisioning, regulating, and cultural services that directly benefit people and the supporting services needed to maintain the direct services.

4 Principle

In this functional test, the feeding activity of soil animals is measured. The term “bait lamina” refers to small perforated plastic strips that are inserted into the soil. An artificial mixture or natural organic material (= bait) is exposed to soil organisms (e.g. earthworms, Collembola, Diplopoda, Enchytraeidae) in the apertures of the strip. In principle, the loss of the bait material is assessed by counting the empty apertures of the bait-lamina strips after a certain exposure time. The number of empty apertures (i.e. areas from which the bait material has been removed) as well as their vertical distribution along the strip are evaluated, and it is assumed that this reflects the feeding activity of soil animals.

5 Reagents and material

5.1 Bait material

Organic material used as bait for soil organisms, e.g. a mixture of cellulose powder (70 %, preferably of nutrient quality), finely ground wheat bran (25 %), and activated carbon powder (5 %).

5.2 Reagents

5.2.1 Hydrochloric acid (10 %), for cleaning of bait-lamina strips at contaminated sites (see [7.2](#)).

6 Apparatus (equipment)

6.1 Bait-lamina strips

Plastic strips (PVC, of grey colour) of 120 mm × 6 mm × 1 mm, which have a pointed tip at the lower end. In the lower part (85 mm) of each strip 16 bi-conical apertures of 1,5 mm diameter are drilled, which are 5 mm apart from each other (see also [Figure 1](#)). The apertures should be numbered, starting with number 1 (soil depth = 0,5 cm) and ending with number 16 (soil depth = 8,0 cm).

Bait-lamina strips are commercially available, either with bait-filled or with empty apertures.

6.2 Sharp tool

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Tool formed like a thin screw-driver, long nail file or a barbecue skewer which is used to drill a small slit into the soil in which the individual bait-lamina strips is inserted when the soil is too dry or compact.

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7 Procedure <https://standards.iteh.ai/catalog/standards/sist/8b4aeb72-5345-4d96-99b0-28e5f8857b4c/iso-18311-2016>

7.1 General

When testing the effects of soil contaminants on the feeding activity of soil dwelling organisms, usually the bait strips are exposed at the potentially contaminated sites as well as at a reference site. The latter is defined as a site which in terms of soil properties, land use, or climatic conditions does not differ from the site to be assessed, except that it is not contaminated.

NOTE The use of the bait-lamina test for monitoring the biological status of soils is described in [Annex A](#).

The test should be performed at times of the year where soil organisms are active (i.e. when unfavourable environmental conditions such as low soil water content and/or extremely high temperatures do not drive them into diapause). In detail, a soil temperature of 5 °C to 15 °C is recommended [8]. The soil water content should be sufficient for invertebrate activity (i.e. >20 %). Environmental conditions such as climate or soil water content are expected to strongly influence the results [24], therefore the method should preferably be applied for comparing the biological activity between closely situated plots (e.g. contaminated sites versus reference areas). When performing this test in the field, the site should be characterized (e.g. concerning climate such as precipitation, temperature, and land use). However, such a characterization is not covered by this International Standard.

In addition, pH (ISO 10390), particle size distribution (ISO 11277) dry bulk density (ISO 11272), organic carbon content (ISO 10694), nitrogen content (ISO 13878) actual soil moisture (ISO 11461 or ISO 11465), and water holding capacity (ISO 11268-2) shall be measured. These parameters should be determined in the same soil layer (i.e. the uppermost 8 cm of the mineral soil) or, if applicable, separately in the uppermost mineral soil layer, the organic horizons, and the litter layer, in which the bait-lamina strips are exposed. These parameters have to be determined once at the beginning of the test (exception: soil moisture, this should be measured also at the end of the test).

If the study is performed at a contaminated site, it is recommended to determine the identity and concentration of the main contaminants.

7.2 Preparation of the bait-lamina strips

In the lower part (85 mm) of each bait-lamina strip 16 bi-conical apertures of 1,5 mm diameter are drilled at 5 mm intervals (see also [Figure 1](#)). These apertures are the place where the bait is filled-in. Their bi-conical form secures the stability of the bait-material inside the apertures. Different mixtures can be used as bait-material, but the following mixture is recommended: cellulose (70 %), finely ground wheat bran (25 %), and activated carbon (5 %). The latter component's black colour facilitates the differentiation between the bait material and soil particles when assessing the feeding activity at the end of the test. It is very important to grind the bran as fine and homogenous as possible. Therefore, it is recommended to sieve the grounded bran (mesh size: 0,25 mm). By doing so, not only the homogeneity of the bait-material but also its stability in the apertures of the bait-lamina strips is improved.

The three components of the bait material shall be well mixed before water is added. The amount of water cannot be given in detail but it should be enough in order to create a paste. Bait material shall be sufficiently moist to hold together and fill the apertures of the plastic strip. Too much water can lead to cracking upon drying causing the bait to fall out of apertures. Both, too dry and too wet baits, are not stable. This work shall be performed by hand (see [Figure 2](#)). After air-drying of the strips, they shall be visually inspected. All baits which show holes, small fissures, or ruptures shall be treated with the paste of bait-material again until optically all baits are intact. The whole procedure does secure that the bait-material remains intact inside the apertures of the plastic strips, in particular when they are inserted into the soil. Prepared strips may be stored under dry conditions in plastic bags for up to six months. Before starting a test, their integrity (no mould, holes, fissures, or ruptures in the baits) shall be checked.

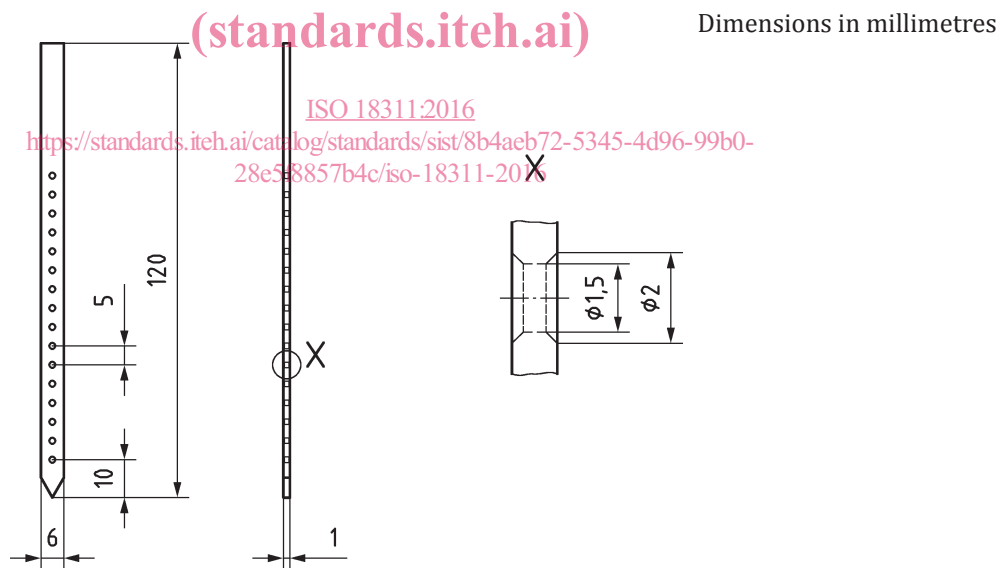


Figure 1 — Schematic view of individual bait-lamina strips^[24]



Figure 2 — Three individual materials (left: activated carbon; above: finely ground wheat bran; below: cellulose) and the mixture of the three components at right: the bait

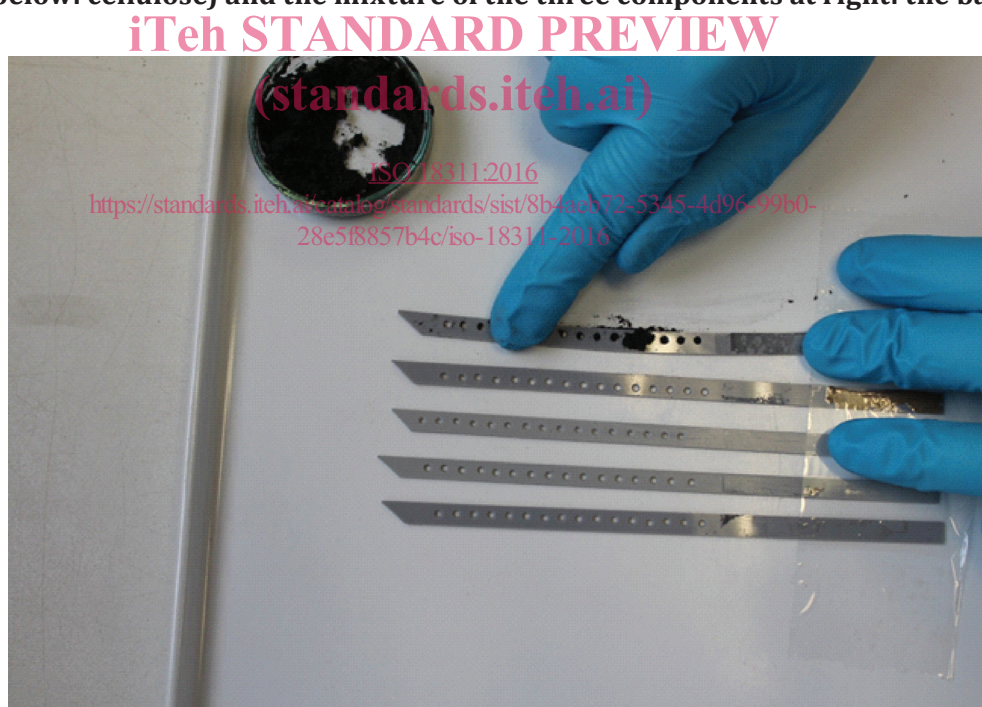


Figure 3 — Filling of bait-material into the bait-lamina strips

7.3 Experimental design and test performance

The prepared bait-lamina strips are exposed in the soil for an appropriate period of time, depending mainly on the climate, the soil properties, and the composition and abundance of the soil organism community at the test site. On average, the exposure time lasts 10 d to 20 d in temperate zones (e.g. References [8] and [12]) and 4 d to 8 d (partly 14 d) in tropical areas, e.g. Reference [30]. However, there are experiences that these numbers can differ considerably: e.g. at coniferous forest sites in Germany, with an acid soil with little macro-fauna, an exposure time of 49 d was used [29], and since