
**Soil quality — Guidance on the choice
and evaluation of bioassays for
ecotoxicological characterization of
soils and soil materials**

*Qualité du sol — Lignes directrices relatives aux choix et
à l'évaluation des essais appliqués pour la caractérisation
écotoxicologique des sols et des matériaux de type sol*

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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 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 the following URL: www.iso.org/iso/foreword.html. (standards.iteh.ai)

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

This second edition cancels and replaces the first edition (ISO 17616:2008), which has been technically revised. The main changes compared to the previous edition are as follows:

- definitions for “soil use” and “ecosystem service” [10] have been added to [Clause 3](#);
- end points of ecotoxicity tests (e.g. mortality, reproduction, growth, genotoxicity, and other functional activities), as well as the overall principles and application of test batteries have been clarified in [Clause 4](#);
- sub-chronic toxicity tests have been added;
- [Figure 1](#) was revised;
- [Tables 1](#) and [2](#) (test batteries for retention and habitat function assessment, respectively) have been revised (test categories, test organisms added/deleted, references updated).

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

The characterization of contaminated soils can be based on strategies considering chemical analyses and/or bioassays. ISO 15799 provides guidance on the selection of experimental methods for the assessment of the ecotoxic potential of soils and soil materials (e.g. excavated and remediated soils, refills, embankments) with respect to their intended use or re-use, and possible adverse effects on aquatic and soil dwelling organisms.

An assessment strategy giving instructions for the choice and evaluation of test results is hence proposed. The evaluation of the bioassays outcome is based on empirically-derived critical dilution levels that take into account the sensitivity of the test system and the intended use/re-use of the site under investigation. This approach intends to contribute to an effective and comparable assessment within the ecotoxicological characterization of contaminated soil or soil materials^[1]. The test systems included in this approach are not mandatory and may be replaced or accomplished by other test methods. Nevertheless, the selected test systems have proved to appropriately characterize contaminated soils and soil materials with respect to their ecotoxic properties^{[2],[3]}, both towards aquatic and terrestrial organisms, the latter being responsible for maintaining essential soil functions.

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Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

1 Scope

This document is one of the family of standards (ISO 15799, ISO 19204) providing guidance on the characterization of soils and soil materials in relation to their retention and habitat functions and uses. It is appropriate to use it in conjunction with the two other standards in this family. It provides guidance on the choice and evaluation of tests applied for ecotoxicological characterization of soils and soil materials. Recommendations for test strategies with respect to the protection of ground and surface waters and the maintenance of the habitat function of soil are included. The tests recommended represent a minimum test battery that can be complemented by additional tests, or even be replaced by others, according to the intended uses or protection goals envisaged. The effect values indicated in this document do not refer to regulation but represent the lowest level at which an adverse effect is considered likely to occur.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

3.1 Assessment

3.1.1

soil-related assessment

assessment of the ecotoxic potential of *soils* (3.2.1), soil substrates and *soil materials* (3.2.2) based on chemical analyses, biological tests and field inventories (monitoring) such as that mentioned in the TRIAD approach^[4]

Note 1 to entry: TRIAD means an assessment approach based on a combination of chemical (i.e. residue analysis), ecotoxicological (i.e. laboratory ecotoxic tests) and ecological (i.e. monitoring) data.

3.1.2

risk

expression of the probability that an adverse effect on *soil* (3.2.1) functions will occur under defined conditions and the magnitude of the consequences of the effect occurring

3.1.3

LID-value

lowest-ineffective-dilution value

lowest value of the dilution factor above which the test gives an ecotoxicological relevant reduction (e.g. 20 % inhibition of luminescence)

EXAMPLE A LID 8 corresponds to a dilution of soil extract of 1 : 8 (i.e. 1 part of soil extract: 7 parts of dilution water).

Note 1 to entry: The exact definitions are given in the standard of the respective bioassay. According to ISO 13829^[5] for the umu-test, it is the "DLi value" (explanation in Annex D). According to ISO 16240^[6] 3.4, it is the "decisive D_{\min} value".

Note 2 to entry: For further information on results expression and interpretation based on LID values see Annex A of this document.

3.2 Types of soil and other soil materials

3.2.1

soil

upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and organisms

[SOURCE: ISO 15799:2019, 3.1.1]

3.2.2

soil material

material coming from *soil* (3.2.1) and displaced and/or modified by human activity, including excavated soil, dredged materials, manufactured soils, remediated treated soils or fill materials

[SOURCE: ISO 17402:2008, 3.16]

3.3 Terms relating to Soil characteristics

3.3.1

habitat function

ability of *soils* (3.2.1)/ *soil materials* (3.2.2) to serve as a habitat for microorganisms, plants, soil-living animals and their interactions (i.e. biocenose)

[SOURCE: ISO 15799:2019, 3.2.1]

3.3.2

retention function

ability of *soils* (3.2.1)/ *soil materials* (3.2.2) to adsorb pollutants in such a way that they cannot be mobilised via the water pathway and translocated into the food chain

Note 1 to entry: The habitat and retention functions include the following soil functions according to ISO 11074:2015^[7], 3.3.31:

- control of substance and energy cycles as components of ecosystems;
- basis for the life of plants, animals and man;
- carrier of genetic reservoir;
- basis for the production of agricultural products;
- buffer inhibiting movement of water, contaminants or other agents into the ground water.

[SOURCE: ISO 15799:2019, 3.2.2]

3.3.3**contaminant**

substance or agent present in the *soil* (3.2.1) as a result of human activity

Note 1 to entry: There is no assumption in this definition that harm results from the presence of the contaminant.

Note 2 to entry: See also pollutant (3.2.3) and potentially harmful substance (3.2.5) definitions in ISO 15799:2019

[SOURCE: ISO 15176:2002^[9], 3.2.6, modified — Note 2 to entry added.]

3.3.4**pollutant**

substances, which due to their properties, amount or concentration cause negative impacts on the *soil* (3.2.1) function or soil use

Note 1 to entry: Adapted from ISO 15176:2002^[9] 3.2.7.

3.4 Land and sites**3.4.1****re-use**

useful and harmless utilisation of *soil materials* (3.2.2)

Note 1 to entry: In the context of this document re-use means the transfer of soil materials to another location for use in agriculture, horticulture, forestry, gardens, recreational areas and construction sites.

[SOURCE: ISO 15176:2002^[9], 3.4.1; ISO 15799:2019, 3.3.1]

3.4.2**soil use**

use of the *ecosystem services* (3.4.3) that *soil* (3.2.1) provides

3.4.3**ecosystem service**

service that is (directly or indirectly) provided by an ecosystem to benefit people

[SOURCE: Based on Millennium Ecosystem Assessment]

4 Principles and applications of test batteries

The sensitivity of organisms (e.g. bacteria, plants, animals) to toxicants may vary significantly from one species to another. Thereby, it is admitted that only the results of several ecotoxicity tests can give a clear indication of the toxic effects of soil or soil materials. As such, the combination of ecotoxicity tests, defined as a battery, shall include organisms belonging to various trophic levels, several biological responses or end points (e.g. mortality, reproduction, growth, genotoxicity, as well as other functional activities), in order to take into account the variability of species sensitivity within the studied compartment.

The ecotoxicity tests included in batteries should at least have the following characteristics:

- sensitivity;
- practicability;
- compliance with standardized methods;
- high cost efficiency;
- representativeness of the soil ecosystem and/or of the selected application scenario (i.e. habitat or retention functions).

Depending on the use of the soil or soil material (e.g. the agricultural use of waste) or the protection goal (i.e. habitat or retention functions), the applied test battery can differ (see Clause 5 in ISO 15799 for guidance on test selection criteria)^[1]. Nevertheless, the selected tests should allow the identification of the most sensitive trophic level(s) and give information on the toxic effects induced by solid samples.

The evaluation of results from the ecotoxicological tests should take into consideration the application purposes (further details on the field of application in ISO 15799, Clause 4), which can broadly be for:

- monitoring and control of the success of soil treatment (off-site, on-site, in situ) (see 5.1),
- assessment of soil / soil material quality or contamination effects according to its use or re-use (see 5.2).

Irrespective of the application purpose, the ecotoxicological characterization of soils and soil materials depends on the soil use/re-use and soil functions requiring protection^[1], as aforementioned. Overall, it can essentially rely on the:

- assessment of mobile and bioavailable potentially harmful substances, in cases where the soil/soil material (see 3.2.1 and 3.2.2) can affect the ground and/or surface water, as well as in cases where pollutants are added to soils (e.g. agricultural use of wastes like sludge, composts, etc.) (i.e. **retention function**), and/or
- assessment of the ecotoxic potential of soils and soil materials (see 3.2.1 and 3.2.2) and possible adverse effects on soil-dwelling organisms (i.e. **habitat function**).

5 Testing strategy and interpretation of test results according to the use and re-use of soils / soil materials and soil functions

5.1 Monitoring of soil treatment success

For evaluating the efficiency of soil treatments, the procedure below may be followed at different stages:

- a) before the treatment – perform the ecotoxicological assessment of the soil sample(s) using a test battery (5.2);
- b) monitoring during the soil treatment process – perform a simple test selected from the battery above [e.g. the most sensitive and practical test used among the battery applied in 5.1 a)];
- c) at the end of the treatment – perform a new assessment with the same test battery used in 5.1 a), in order to judge the success of the treatment.

5.2 Assessment of the ecotoxic potential of soils / soil materials

5.2.1 General

If soils or soil materials are assessed with respect to their intended use or re-use, the tests (see ISO 15799) appropriate to evaluate their quality regarding the retention (see Table 1) and/or habitat functions (see Table 2) should be applied. A strategy for the assessment of the ecotoxicological characterization of soils and soil materials is proposed in Figure 1. The chemical characterization of soils should always be conducted to increase the reliability of interpretations of the ecotoxicological results obtained upon the selected test battery.

The test battery usually includes a set of acute and chronic/sub-chronic toxicity tests. If acute toxicity is detected, it is not necessary to perform other tests. On the other hand, if no acute effect is detected, chronic/sub-chronic toxicity and genotoxicity test(s) shall be conducted.

The assessment of soil and soil materials may be influenced by the collection, handling and storage methods followed. Thereby, standardized procedures should be conducted in accordance with ISO 18400-206^[11].

5.2.2 Choice of test battery and evaluation of test results for assessing retention function

5.2.2.1 Acute and chronic/sub-chronic assays for assessing the ecotoxic potential of soil/soil materials

In a first approach, acute and sub-chronic tests using luminescent bacteria, algae and daphnids (see [Table 1](#)) are recommended to assess the retention function of soils through the testing of soil/soil material eluates. Depending on the legal requirements, other standardized bioassays may be selected (see Table 1 or Annex A of ISO 15799 for choosing additional assays).

For the assessment of effects, toxicity criteria are also given in [Table 1](#). The toxicity criteria are provided as LID-values, percentages of inhibition or mortality. If these values are exceeded, then an ecotoxicological potential is highlighted, thereby indicating that soil pollutants are soluble in water, bio-available, and can be transported via the water path. If at least one positive test result is obtained, the use of the soil or soil material is limited, or the requirements of remediation are not fulfilled (see [Figure 1](#)).

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