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# Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials

*Qualité du sol — Lignes directrices relatives à la caractérisation écotoxicologique des sols et des matériaux du sol*



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# Foreword

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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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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 15799:2003), which has been technically revised. The main changes compared to the previous edition are as follows:



— standardized forms of recommended test systems in Annex A have been amended and updated (e.g. ISO 20963 deleted and ISO 18311, ISO 18187 added).

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

Most of the existing ecotoxicological test methods (biotests) that are being internationally harmonized were developed to describe the ecotoxic potential of a test substance when added to a soil/soil material. These methods can be used with some modifications for the ecotoxicological characterization of soils and soil materials with respect to their function depending on the intended use. For substances with properties resulting in toxic effects, biotests are a complement to conventional chemical analysis. Results from chemical analysis can be used for ecotoxicological assessments based on information on the substances identified, including properties of the chemicals, e.g. their bioaccumulation potential. This information is often scarce (if it exists at all) and it does not include possible interactions (synergy/antagonism) between chemicals and the complex soil matrix. Furthermore, an exhaustive identification and quantification of substances is impractical. Therefore, ecotoxicological testing of soils can be used for investigating the potential toxicity of complex chemical mixtures. The extrapolation from laboratory tests to field conditions requires adequate consideration of important environmental factors within the test conditions and the selection of suitable ecotoxicological end points.

# Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials

## 1 Scope

This document is one of a family of International Standards providing guidance on soils and soil materials in relation to certain functions and uses including conservation of biodiversity. It applies in conjunction with these other standards. It 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 and possible adverse effects on aquatic and soil dwelling organisms.

NOTE This is a reflection of the maintenance of the habitat and retention function of the soil. In fact, the methods listed in this document are suitable for usage in a TRIAD approach, i.e. for an ecological assessment of potentially contaminated soils (see ISO 19204).

This document does not cover tests for bioaccumulation.

The ecological assessment of uncontaminated soils with a view to natural, agricultural or horticultural use is not within the scope of this document. Such soils can be of interest if they can serve as a reference for the assessment of soils from contaminated sites.

The interpretation of results gained by applying the proposed methods is not in the scope of this document.



## 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 Types of soil and other soil materials

**3.1.1**
**soil**
upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and organisms

[SOURCE: ISO 11074:2015, 2.1.11, modified — The definition has been slightly modified and the Note 1 to entry has been deleted.]

**3.1.2**
**soil material**
material which includes *excavated soil* (3.1.3), dredged materials, manufactured soils, treated soils and fill materials

**3.1.3**
**excavated soil**
any natural material excavated from ground including top-soil, sub-soil, altered parent rock and parent rock itself

Note 1 to entry: Excavated soil typically arises during construction works.

[SOURCE: ISO 15176:2002, 3.1.5]

**3.1.4**
**standard soil**
field collected soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

Note 1 to entry: An example for standard soils is "Eurosoils" (see Reference [28]).

## 3.2 Terms relating to soil characteristics

**3.2.1**
**habitat function**
ability of *soils* (3.1.1)/*soil materials* (3.1.2) to serve as a habitat for microorganisms, plants, soil living animals and their interactions (biocenoses)

**3.2.2**
**retention function**
ability of *soils* (3.1.1)/*soil materials* (3.1.2) to adsorb *pollutants* (3.2.3) 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:

— 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.

**3.2.3**
**pollutant**
substance which due to their properties, amount or concentration cause impacts on the soil function or soil use

Note 1 to entry: See also *contaminant* (3.2.4) and *potentially harmful substance* (3.2.5).

[SOURCE: ISO 11074:2015, 3.4.18, modified — Wording has been slightly modified and Note 1 to entry has been added.]

**3.2.4**
**contaminant**
substance or agent present in the *soil* (3.1.1) 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: see also *pollutant* (3.2.3) and *potentially harmful substance* (3.2.5).

[SOURCE: ISO 15176:2002, definition 3.2.6, modified — The wording in the Note 1 to entry has been slightly modified.]

**3.2.5**
**potentially harmful substance**
substance which when present in sufficient concentration or amount may be harmful to humans or the environment

Note 1 to entry: It may be the result of human activity [*contaminant* (3.2.4)] or naturally occurring.

[SOURCE: ISO 15176:2002, 3.2.8, modified — A Note 1 to entry has been added.]

### 3.3 Land and sites

**3.3.1**
**re-use**
useful and harmless utilisation of soil materials

Note 1 to entry: In the context of this International Standard, 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, 3.4.1]

## 4 Field of application

### 4.1 Soils and areas of soil use where ecotoxicity tests should be considered

Ecotoxicity tests should be considered in the following soils and areas of soil use:

— Assessment of the ability of a soil to sustain a natural biocenosis or agriculture.

— Assessment of the combined ecotoxicity of all bioavailable contaminants present in soils or soil materials.

— Assessment of the ecotoxicity of potentially harmful substances in cases where the soil/soil material can affect the ground and surface water.

— Identification of soils or soil materials (refills, embankments) with a low degree of contamination usually within a depth of 1 m, which can remain at the site without further treatment.

— Detection of potential ecotoxicity which could not be traced by chemical analysis.

— Monitoring and control of the success of soil treatment (off-site, on-site/*in situ*).

— Monitoring and control of soils/soil materials, which have been decontaminated and are to be applied at the surface.

### 4.2 Soils and areas of soil use where ecotoxicological tests are not necessary

Provided that groundwater contamination can be excluded, ecotoxicological testing is not necessary in the following cases.

— Contaminated soils which are classified as hazardous waste or can be characterized clearly by chemical/analytical parameters. In such cases, ecotoxicological testing may be useful for a final investigation after remediation and for process control during biological remediation.

— Commercially/industrially used areas with no prospect of horticultural/agricultural use.

— Soil materials or backfilled materials in an area which is to be effectively sealed by covering with buildings or other forms of low permeability cover such as concrete or tarmacadam or asphalt.

# 5 Selection of tests according to the use/re-use of soils and soil materials and soil functions

## 5.1 Use of ecotoxicity tests

Toxicants can affect different species (and in some cases genotypes) present within ecosystems at different concentrations. The ideal approach for the precise ecotoxicological characterization of the soil toxicity is to use a battery of tests with several species belonging to different taxonomic and trophic groups to avoid false-negative results due to an adaption of a test system (genotypic shift) to a specific contaminant as compared to uncontaminated soils. Studies using field or semi-field investigations are rarely carried out and may be very expensive.

The ideal scheme can be rendered more practicable by the adoption of simpler testing strategies and the application of safety factors to the results obtained. If, however, testing is performed on one species or function only, the high diversity in the sensitivity of species to toxicants will result in a high level of uncertainty. It is therefore recommended to test at least a microbial process, a species from the plant kingdom, and one from the animal kingdom, usually a saprophagous/detritivorous species. If more than one animal species are tested, a predatory species should be included in the test battery. The minimum number of species to be tested depends on the regulations to which the test strategy shall comply. This document only gives the basic principles for their use. Further considerations to the selection of tests using soil organisms are given in 5.3.

## 5.2 General criteria for selection of tests

Criteria for the selection of ecotoxicity tests were established in the context of hazard assessment and classification of chemicals. These criteria should also apply for the ecotoxicological characterization of contaminated soils. Criteria reviewed were scientific validity, ecological significance, practicability and acceptability (see References [27] and [28]).

Basic requirements which test protocols shall fulfil in order to be laid down in International Standards include reproducibility, statistical validity, general acceptance and performance.

The importance of a criterion is relative to the specific situation. Decisions have to be made between which criteria are most important or tests which may have to be modified by more practical considerations, such as easy culturing of test organisms in the laboratory or the availability of life stages required for a test throughout the year.

The test methods recommended (see Annex A) in this document were originally designed for hazard assessment of chemicals and were in most cases internationally harmonized e.g. by OECD, EU or ISO. In most of them provisions have been made to adapt the test design for the purposes within the scope of this document. In addition, the selection of ecotoxicological test methods for the assessment of soils/soil materials depends on their intended use/re-use and on the soil functions to be protected, in particular the retention and habitat functions.

Table 1 gives an example of a decision scheme based on the relevant function.

**Table 1 — Relevance for ecotoxicological testing to the intended re-use of the soil**

| Re-use of soils | Soil function | | |
|---|---|---|---|
| | Retention function | Habitat function | |
| | Aquatic organisms | Plant growth | Soil biocenoses |
| | Detection of biological effects | | |
| Below sealed areas | low[a] | low | low |
| Commercially and industrially used un-sealed areas | high | low | low |
| Landfill covering | high | high | low |
| Green areas, parks and recreation areas | high | high | high |
| Areas used in horticulture or agriculture | high | high | high |

[a] Applies only to the unsatured soil zone.

## 5.3 Considerations for the examination of soil functions

### 5.3.1 Retention function

Transport via water of soluble, colloidal or particle fractions play a dominant role in the risk assessment of contaminated soils. This is true not only because water can mobilize contaminants, but also because contaminants and metabolites in the water phase potentially have a severe effect on microorganisms, plants and soil fauna.

Aqueous eluates (for preparation see Clause 6, ISO 18772, EN 14735) are useful for testing ecotoxic effects on organisms exposed via the water mediated transport. It should be taken into account that substances mobilized via water can be subjected to different types of changes, such as metabolism or hydrolysis when transported into the groundwater and from there into surface waters, and that their concentrations are reduced by dilution. Moreover, substances can be mobilised over time due to environmental changes (e.g. pH, chemical and biological transformation). Eluates can serve as early indicators for the contamination of pore and ground water prior to the exposure of surface and drinking water.

With these aspects, the investigation of groundwater and eluates is of utmost importance regardless of the proposed soil use.

For ecotoxicology tests working with aqueous soil extracts and aquatic test organisms it shall be considered that nutrient ions and compounds are easily dissolved in water (at least easier than hydrophobic pollutants) and can substantially interfere during the test.

### 5.3.2 Habitat function

#### 5.3.2.1 General

The suitability of the soil for living organisms can best be examined by means of test methods which are selected to include organisms and processes representative of different taxonomic and ecological groups.

#### 5.3.2.2 Soil material used as control for bioassays on solid matrices

As a general principle in ecotoxicological testing, any end point measured in a treatment is compared with the one measured in the control(s).

In order to evaluate the suitability of the soil for soil-dwelling organisms, it is a prerequisite to compare the contaminated soil or soil material with a control material, which may also be used for preparing dilution series with the contaminated sample.

Several types of control material can be used:

— an uncontaminated soil with comparable pedological properties to the sample being tested;

— an inert material (e.g. quartz sand);

— a certified natural soil (e.g. standard soil);

— a standardized artificial soil (see ISO 11268-1 and ISO 11268-2, ISO 11267).

The choice between these control materials depends entirely on the aims of the ecotoxicological assessment, the type of biological test being carried out and the requirements of the test organism. This recommendation cannot be generalized for all biological tests. Adding sand to a soil or a soil material can create a compact mixture which is incompatible with the growth and development of many organisms (e.g. plant growth tests). It is preferable to use a more complex control material (such as artificial soil) for dilution where this would have the advantage of reproducing more closely the natural environment of the organisms and even if it may interact with pollutants. Placing an organism in a medium which does not match the most important characteristics of its natural habitat may cause stress.

— If a dose-response curve is needed, one of the control materials mentioned above may be used to dilute the contaminated substrate.

— If the aim is to classify each sample of soil or soil materials in terms of ecotoxicity hazard, it is preferable to use an inert material (e.g. quartz sand) which will not interact with the pollutants present in the sample, and whose composition and granulometry can be rigorously standardized.

The requirements of the control material shall take into account the different soil uses and the type and origin of the soil (e.g. undisturbed soil, refilling material, excavated soil, remediated soil). Nutrient deficiency, as well as physical conditions, can cause differences in plant growth and animal behaviour that need not necessarily be caused by the pollutant situation and the hazard potential.

— If the aim is to evaluate the ecotoxicity of a soil or soil material sample from a contaminated site, the preferred method would be to use an uncontaminated control material that is similar to the sample being tested.

— If the aim is to evaluate the ecotoxicity of soils or soil materials which may be re-employed for certain specific uses, the preferred method would be to use as a control material any material which may in future be mixed with soil or soil material.



#### 5.3.2.3 Soil as substrate (medium) for soil microorganisms

The soil microflora comprises on average 80 % of the mass of organisms living in soil. In combination with the microfauna, the main functions of the microflora are the decomposition and degradation of complex organic substances to easily available nutrients thereby maintaining the natural substance cycles of carbon, nitrogen, phosphorus and sulfur.

Substrate-induced respiration provides an indicator of the microbial population density.

Nitrifying bacteria, which are responsible for the oxidation of ammonium to nitrite and from nitrite to nitrate, are a very sensitive group of microorganisms. Decreased nitrification need not necessarily lead to significant changes in the ecosystem but can be used as a sensitive indicator for the inhibition of an essential soil process.

The purpose of determining the microbial biomass or other microbial processes in soils is to allow assessment of the continued maintenance of soil fertility, the potential ability to degrade organic compounds, and the effects of added materials on the soil microbial community.

#### 5.3.2.4 Soil as substrate for plant growth

After microorganisms, plant roots constitute the largest biological surface in soil. Their contact area with soil particles is increased by the presence of root hairs and mycorrhizal associations (VA-mycorrhiza with cultivated plants and additional ectomycorrhizal with woody plants).

 

As with the other bioassays proposed, tests with higher plants are designed to assess the bioavailability and effects of pollutants detected or not detected by chemical analysis respectively. By applying a test period of at least 14 days, short-term changes in the soil by the test plant itself are included.

The accumulation of pollutants in plants, their metabolism and their effects on consumers are not investigated in these tests. They do not apply to the assessment of soil fertility and productivity.

#### 5.3.2.5 Soil as substrate for soil-inhabiting fauna

Soil animals generally fulfil the following four functions:

— mechanical activities (drainage, aeration, mixing, mechanical comminution);

— chemical changes (enhanced availability of nitrate and phosphate from excrements and accelerated formation of clay-humus complexes, after the substrate has passed the gut);

— biological changes (distribution of microorganisms in the soil matrix, synergistic effects through stimulation of microbial activity and organic matter decomposition);

— significant links in the food web.

Short-term and long term tests are available for examination of the effects of pollutants on soil fauna. For testing the habitat function, characterization by sub-lethal test parameters is particularly recommended.

Since a single test method cannot adequately represent the vast number of very diverse invertebrates, a test battery should be used. When selecting the individual test species, the following criteria should be considered:



— trophic level: e.g. saprophagous and predatory species should be included;

— taxonomic/physiological groups: in order to cover the biodiversity of soil communities, at least representatives of Annelida and Arthropoda have to be selected;

— size class/exposure pathway: species of the micro-, meso- and macrofauna do not only represent various size classes but also different life-styles and therefore exposure routes (e.g. pore water versus food uptake);

— ecological role: at least soil-dwelling and litter-inhabiting species are important to be considered.

Only internationally standardized methods should be used.

## 6 Sampling, transport, storage and sample preparation

Before soil quality is assessed by any of the methods proposed, soil samples need to be collected from the site under investigation (see ISO 23611-6). Soil sampling should be carried out by trained personnel with sufficient knowledge of sampling, handling of samples and safety measures at contaminated sites and sampling locations. The sampling strategy and handling should be determined by the site to be investigated, the kind of contamination and the aim of the biological tests (e.g. quantities of soil samples can vary between 100 g and 100 kg depending on the tests selected).

Record all data concerning sampling, transport and sample preparation. Instructions on the design of sampling programmes, sampling techniques, safety, investigations of natural, cultivated, urban and industrial sites and on the collection, handling and storage of soil for the assessment of biological functional and structural end points in the laboratory are given in ISO 18400-206. For the preparation of eluates for testing the retention function with aquatic test methods ISO 18772 and EN 14735 are recommended.

## 7 Limitations of proposed biotests for soils/soil materials

Biological test systems are only suitable to a limited extent for volatile pollutants. Other methods should be developed for this purpose. Similarly the impact of organic contaminants, which are easily degradable under aerobic conditions, may be detected incompletely by the methods described. In this case alternative methods for sampling and sample preparation should be applied.

The proposed terrestrial and aquatic test methods in A.1 and A.2 were developed to assess the ecotoxic potential of chemicals. The characterization of soils or soil eluates was not their primary goal. Therefore, the methods shall be adapted to the specific requirements of soil and site assessment.