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

*Qualité du sol — Lignes directrices pour l'évaluation des essais
appliqués dans le domaine de la caractérisation écotoxicologique des
sols et des matériaux du 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.

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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.

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Introduction

The characterization of contaminated soils may be based on strategies considering chemical analysis 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, and possible adverse effects on aquatic and soil dwelling organisms. An assessment strategy giving instructions for the choice and evaluation of bioassays, and for the evaluation of the test results including 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, is proposed. This approach is based on the results of a research program and intends to contribute to an effective and comparable risk assessment within the ecotoxicological characterization of contaminated soil. The test systems included in this approach are not mandatory and may be replaced or accomplished by other test methods. Nevertheless, the test systems selected have proved to characterize contaminated soils appropriately with respect to their ecotoxic properties towards aquatic and terrestrial organisms maintaining the 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 International Standard is one of a series providing guidance on the characterization of soils and soil materials in relation to their retention and habitat function and uses. It should be read in conjunction with the other standards in this series. 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 may be accomplished by additional tests, or even be replaced by others, according to the intended uses or protection goals envisaged. The effect values indicated in this International Standard do not refer to regulation, but represent the lowest level at which a response is supposed to result in an adverse effect.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6341, *Water quality — Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) — Acute toxicity test*

ISO 8692, *Water quality — Freshwater algal growth inhibition test with unicellular green algae*

ISO 11074:2005, *Soil quality — Vocabulary*

ISO 11267, *Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants*

ISO 11268-1, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 1: Determination of acute toxicity using artificial soil substrate*

ISO 11268-2, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 2: Determination of effects on reproduction*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of chemicals on the emergence and growth of higher plants*

ISO 11348 (all parts), *Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test)*

ISO 13829, *Water quality — Determination of the genotoxicity of water and waste water using the umu-test*

ISO 15176:2002, *Soil quality — Characterization of excavated soil and other soil materials intended for re-use*

ISO 15685, *Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation*

ISO 15799:2003, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

ISO 15952, *Soil quality — Effects of pollutants on juvenile land snails (Helicidae) — Determination of the effects on growth by soil contamination*

ISO 16240, *Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome test (Ames test)*

ISO 16387, *Soil quality — Effects of pollutants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction and survival*

ISO 17155:—¹⁾, *Soil quality — Determination of abundance and activity of soil microflora using respiration curves*

ISO 17402:—¹⁾, *Soil quality — Guidance for the selection and application of methods for the assessment of bioavailability in soil and soil materials*

ISO 20079, *Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) — Duckweed growth inhibition test*

ISO 20665:—¹⁾, *Water quality — Determination of chronic toxicity to Ceriodaphnia dubia*

ISO 20666:—¹⁾, *Water quality — Determination of the chronic toxicity to Brachionus calyciflorus in 48 h*

ISO 20963, *Soil quality — Effects of pollutants on insect larvae (Oxythyrea funesta) — Determination of acute toxicity*

ISO 22030, *Soil quality — Biological methods — Chronic toxicity in higher plants*

3 Terms and definitions

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

3.1 Assessment

3.1.1

soil-related assessment

assessment of the ecotoxic potential of soils, soil substrates and soil materials based on chemical analysis, biological tests and field inventories (monitoring) such as that mentioned in the TRIAD approach ^[9]

NOTE TRIAD means an assessment approach based on a combination of chemical (i.e. residue analysis), ecotoxicological (i.e. laboratory tests) and ecological (i.e. monitoring) data.

3.1.2

risk

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

1) To be published.

3.1.3**LID-value**

lowest-ineffective-dilution value

lowest value of the dilution factor (LID) for 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.

NOTE 1 The exact definitions are given in the standard of the respective bioassay. According to ISO 13829 for the umu-test, it is the “ D_{Li} value”. According to ISO 16240, it is the “decisive D_{min} value”.

NOTE 2 For further information, see Annex A.

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

[ISO 11074:2005]

3.2.2**soil material**

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

[ISO 17402:—¹⁾]

3.2.3**topsoil**

upper part of a natural soil which is generally dark coloured and has a higher organic matter and nutrient content when compared to the subsoil below

3.3 Terms relating to soil characteristics**3.3.1****habitat function**

ability of soils/soil materials to serve as a habitat for microorganisms, plants, soil-living animals and their interactions (biocenoses)

[ISO 15799:2003]

3.3.2**retention function**

ability of soils/soil materials to adsorb pollutants in such a way that they cannot be mobilized via the water pathway and translocated into the food chain

[ISO 15799:2003]

3.3.3**contaminant**

substance or agent present in the soil as a result of human activity

cf. **pollutant** (3.3.4)

NOTE There is no assumption in this definition that harm results from the presence of the contaminant.

[ISO 15176:2002]

3.3.4

pollutant

substances, which due to their properties, amount or concentration cause impacts on the soil function or soil use

cf. **contaminant** (3.3.3)

NOTE Adapted from ISO 15176:2002. Also see the introduction to ISO 11074:2005.

3.4 Land and sites

3.4.1

re-use

useful and harmless utilization of soil materials

[ISO 15176:2002]

4 Principles and use of test batteries

Sensitivity of animal and plant communities to toxicants may vary significantly from one species to another. Thereby, it is therefore admitted that the results of several ecotoxicity tests can only give a clear view of toxic effects of soil or water samples.

This combination of ecotoxicity tests, defined as a battery, shall include organisms belonging to various trophic levels, several endpoints (acute toxicity, chronic toxicity, genotoxicity) as well as varied 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;
- standardized protocols;
- high cost efficiency.

These tests should allow the identification of the most sensitive trophic level and give information on toxic effects. The tests should also be representative of the soil ecosystem or at least representative of the selected scenario. According to the scenario (e.g. habitat function, agricultural use of waste), the applied test battery can differ.

Evaluation of results from ecotoxicological tests applies for

- monitoring and control of the success of soil treatment (off-site, on-site, *in-situ*),
- 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, and
- assessment of mobile and bioavailable potentially harmful substances, in cases where the soil/soil material can affect the ground and surface water and in cases where pollutants are added to soils and may enter the food chain, e.g. agricultural use of wastes (sludge, composts, etc.).

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 and control of the success of soil treatments

For assessing the success of soil treatments, the following procedure may be applied:

- a) ecotoxicological assessment of the soil sample with a test battery before the treatment (5.2);
- b) monitoring of the soil treatment with a simple test selected from the battery [e.g. the most sensitive and practical (5.1)];
- c) new assessment with the same test battery, in order to judge the success of the treatment (5.2).

5.2 Assessment of the ecotoxic potential of soils and soil materials with respect to their intended use

5.2.1 General

If soils or soil materials are assessed with respect to their intended use or re-use, tests (see ISO 15799) appropriate to assess the soil quality with respect to the retention function (see Table 1) and to the habitat function (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.

A test battery usually includes genotoxicity test(s), acute and chronic toxicity tests. If acute toxicity is detected, it is not necessary to perform other tests. Conclusions, indeed, can be drawn on the results of the acute toxicity test(s). On the other hand, if no acute effect is detected, chronic toxicity test(s) and genotoxicity test(s) shall be performed.

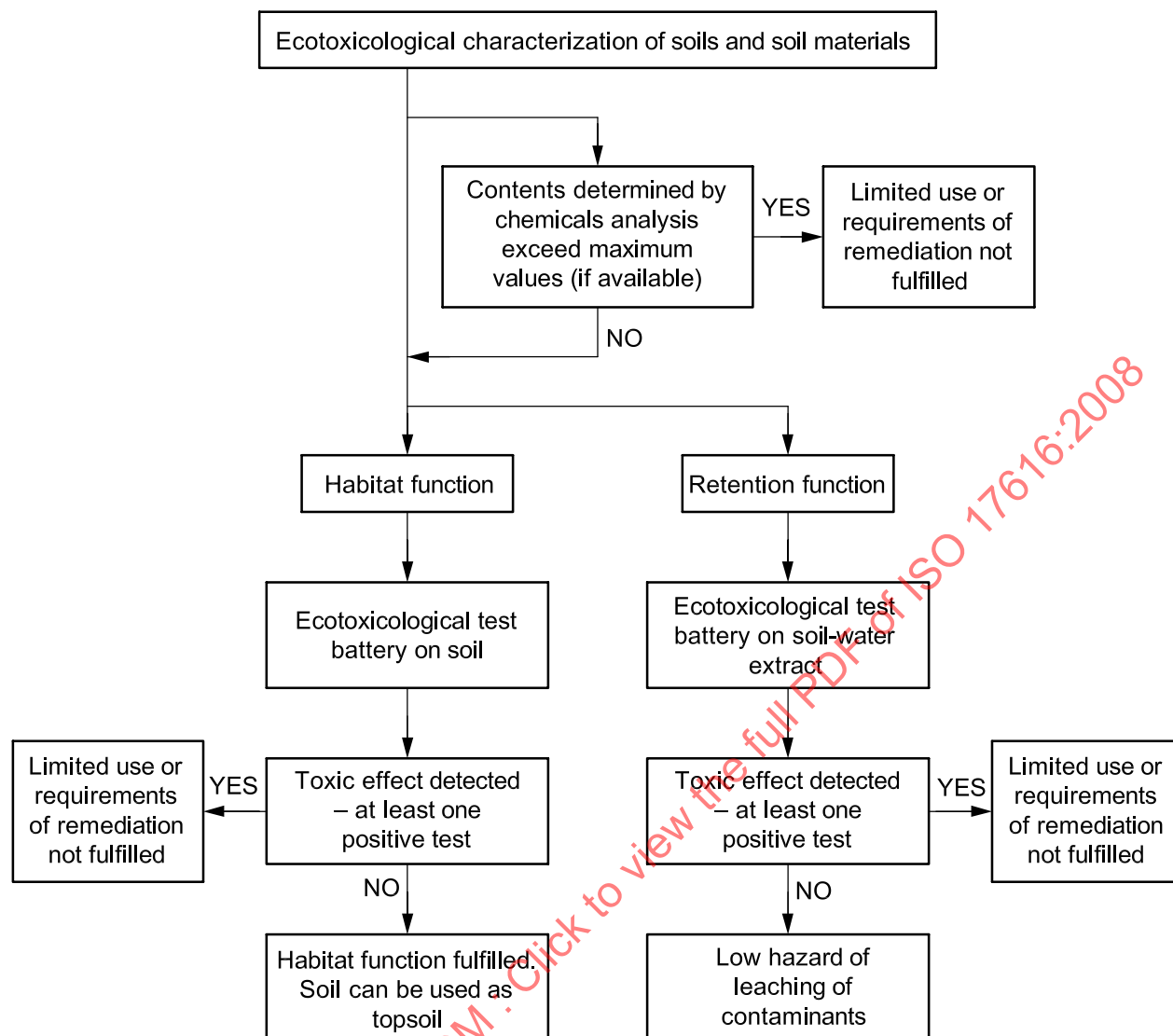


Figure 1 — Strategy for the assessment of the ecotoxicological characterization of soils and soil materials

5.2.2 Choice and evaluation of test results concerning the ecotoxicity of potentially harmful substances in cases where the soil/soil material can affect the ground and surface water (retention function)

Algae, luminescent bacteria and *Daphnia* (see Table 1) are recommended as test organisms in water extracts to assess the retention function of soil. Depending on the legal requirements, other standardized bioassays may be selected (see Table 1 or ISO 15799:2003, Annex A).

For the assessment of effects, toxicity criteria are also given in Table 1. If at least one positive test result is obtained, the use of the soil/soil material is limited or the requirements of remediation are not fulfilled. The toxicity criteria are given as percentages of inhibition or LID-values. The latter indicate, if exceeded, an ecotoxicological potential.

Nutrients extracted from soils (such as nitrates, phosphates) can interfere with the effects of algal growth inhibitors. Therefore, it shall be taken into account that toxicity of pollutants can be masked by substances favouring algal growth, and that soil composition shall be considered when analysing results.

Table 1 — Biological tests for the ecotoxicological assessment of the ecotoxic and genotoxic potential of water-extractable compounds in contaminated soils or soil materials

Test category	Trophic level	Test species	Reference	Endpoint	LID value ^{a, b}	Significant biological effect ^b
Acute tests	Decomposer	<i>Vibrio fischeri</i>	ISO 11348	Inhibition of light emission	LID > 8	20 % Inhibition
	Primary consumer	<i>Daphnia magna</i>	ISO 6341	Immobilization	LID > 4	20 % Inhibition
Chronic tests	Producer	<i>Lemna minor</i>	ISO 20079	Growth inhibition		25 % Inhibition
		<i>Pseudokirchneriella subcapitata</i>	ISO 8692	Growth inhibition	LID > 4	25 % Inhibition
	Primary consumer	<i>Ceriodaphnia dubia</i>	ISO 20665	Mortality and reproduction	—	20 % mortality 30 % Inhibition
		<i>Brachionus calyciflorus</i>	ISO 20666	Mortality and reproduction	—	20 % mortality 30 % Inhibition
Geno-toxicity tests	Decomposer	<i>Salmonella choleraesuis</i> subsp. <i>Choleraesuis</i>	ISO 13829	Induction rate of umuC gene	D _{Li} ≥ 3	—
		<i>Salmonella choleraesuis</i> subsp. <i>Choleraesuis</i>	ISO 13829	Induction rate of umuC gene in concentrated water extract 1:15	D _{Li} ≥ 3	—
		<i>Salmonella typhimurium</i> TA 98 and TA 100	ISO 16240	Induction rate of mutant colonies	Decisive D _{min} value ≥ 2	—
^a LID-values are relevant for a L/S ratio of 2:1.						
^b Toxicity criteria are established on the basis of testing a wide variety of contaminated and uncontaminated soils. It should be guaranteed that no false positive assessment is obtained [6], [12].						
NOTE Tests given in bold letters are part of a minimum test battery.						

Even if no effects are observed in the biological tests, transport of contaminants cannot be excluded completely. In those cases where effects are observed, soil pollutants are soluble in water, bio-available and may be transported via the water path.

5.2.3 Assessment of the genotoxic and mutagenic potential of soil samples

In addition to the toxic potential, the genotoxic potential should be assessed, as genotoxic effects may be present far below toxic threshold levels (for test systems see Table 2). A stepwise procedure, including tests of soil water extracts and 15-fold concentrated water extract with the Umu-test, is recommended. The concentrated water extract shall be tested additionally if no genotoxic effects were determined in the soil eluate.

If neither in the soil eluate nor in the concentrated eluate extract any genotoxicity was detected, but due to chemical analysis or former use of the site, it is suspected that water-extractable mutagenic substances are present, the assessment should be completed by the Ames-test. It is recommended to use the strains *Salmonella typhimurium* TA 98 and TA 100 with and without metabolic activation.

5.2.4 Choice and evaluation of test results concerning the ecotoxicity of potentially harmful substances in cases where the soil/soil material can affect soil organisms (habitat function)

The habitat function of soils can be characterized by the recommended tests in ISO 15799:2003, Annex A. Selected standardized tests that have been proven to be valid for application in the field of soil quality testing and relevant expressions of test parameters appropriate to be used for soil qualification purposes are proposed in Table 2. Other test methods not yet harmonized or sufficiently validated may be appropriate as well, and should be added supplementary to the proposed test battery or might replace others for economic or ecological reasons.

If there is one positive test result, a detailed assessment should be undertaken, considering the analytical data and physical-chemical soil properties, particularly the soil texture and pH value. If more than one of the terrestrial tests has positive effects, the habitat function of the soil is significantly restricted.

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