BRITISH STANDARD

BS ISO
17616:2008

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

ICS 13.080.05

BSI
British Standards

National foreword

This British Standard is the UK implementation of ISO 17616:2008.

The UK participation in its preparation was entrusted to Technical Committee EH/4, Soil quality.

A list of organizations represented on this committee can be obtained on request to its secretary.

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2008

ISBN 978 0 580 54123 0

Amendments/corrigenda issued since publication

Date	Comments

INTERNATIONAL STANDARD

ISO 17616

First edition
2008-03-15

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

Reference number
ISO 17616:2008(E)

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Foreword

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ISO 17616 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

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.

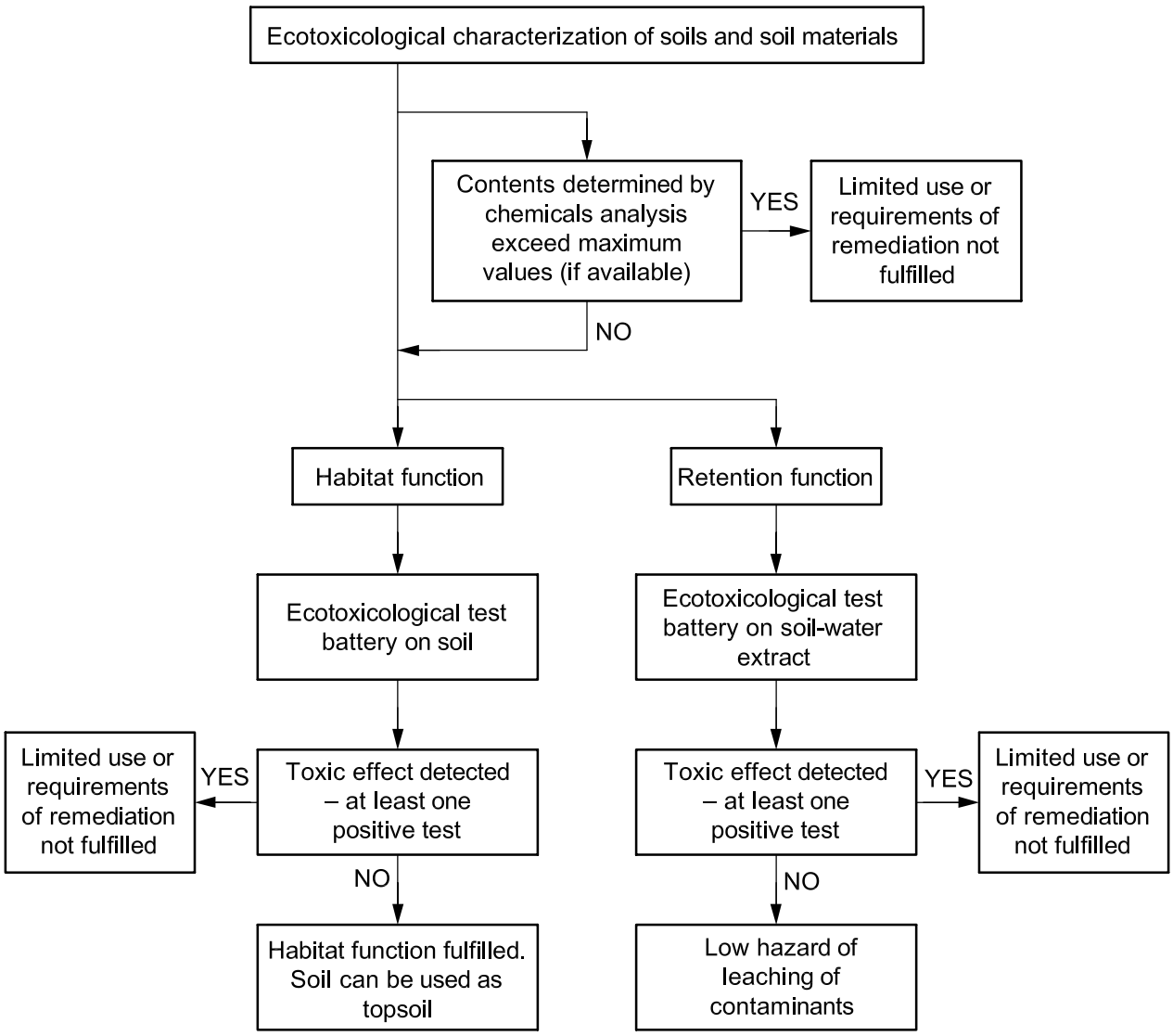

Figure 1 — Strategy for the assessment of the ecotoxicological characterization of soils and soil materials

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	***Vibrio fischeri***	ISO 11348	Inhibition of light emission	LID > 8	20 % Inhibition
	Primary consumer	***Daphnia magna***	ISO 6341	Immobilization	LID > 4	20 % Inhibition
Chronic tests	Producer	*Lemna minor*	ISO 20079	Growth inhibition		25 % Inhibition
		Pseudokirchneriella subcaptitata	ISO 8692	Growth inhibition	LID > 4	25 % Inhibition
	Primary consumer	*Ceriodaphnia dubia*	ISO 20665	Mortality and reproduction	—	20 % mortality 30 % Inhibition
		Brachionus calicyflorus	ISO 20666	Mortality and reproduction	—	20 % mortality 30 % Inhibition
Geno-toxicity tests	Decomposer	***Salmonella choleraesuis* subsp. *Choleraesuis***	ISO 13829	Induction rate of umuC gene	$D_{Li} \geqslant 3$	—
		Salmonella choleraesuis* subsp. *Choleraesuis	ISO 13829	Induction rate of umuC gene in concentrated water extract 1:15	$D_{Li} \geqslant 3$	—
		Salmonella typhimurium TA 98 and TA 100	ISO 16240	Induction rate of mutant colonies	Decisive D_{min} value $\geqslant 2$	—

[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.

Table 2 — Overview on relevant soil organisms and respective test systems considered in the test battery for the assessment of the habitat function of soils

Test category	Trophic level	Test species	Reference	Endpoint	Toxicity criteria
Acute tests	Primary producer	**Plant species**	**ISO 11269-2**	**Early growth inhibition**	Growth reduction > 30 %, or $G_m + SD < 0{,}9 \times G_{calc}$ where G_m is the determined growth in the mixture SD is the standard deviation of G_m G_{calc} is the calculated mean growth of the test and control soil $(G_{soil} + G_{control}) \times 2^{-1}$ [b]
	Primary consumer	***Eisenia fetida***	**ISO 11268-1**	**Mortality**	> 20 % mortality in the undiluted soil sample
		Oxythyrea funesta	ISO 20963	Mortality	> 20 % mortality in the undiluted soil sample
	Decomposer	**Soil microflora**	**ISO 17155**	**Inhibition of respiration**	Respiratory activation quotient (Q_R) > 0,3 Or lag times > 20 h and $t_{peak\ max}$ > 50 h at Q_R 0,2 to 0,3 [a] Material which is foreseen as an addition to soil (e.g. sewage sludge) is assessed by testing an environmentally relevant mixture of soil and test material. In this case, an effect criterion of 25 % inhibition of basal respiration with respect to untreated control soil is recommended.
			ISO 15685	**Ammonium oxidation**	Soils are assessed as positively affecting the nitrification process if the activity in the mixture with control soil deviates by more than 10 % of the average activities of both soils $A_m + SD < 0{,}9 \times A_{calc}$ where A_m is the determined activity in the mixture A_{calc} is the calculated mean activity of the test and control soil $(A_{soil} + A_{control}) \times 2^{-1}$ [a] Material which is foreseen as an addition to soil (e.g. sewage sludge) is assessed by testing an environmentally relevant mixture of soil and test material. In this case, an effect criterion of 25 % inhibition with respect to untreated control soil is recommended.
Chronic tests	Primary producer	*Brassica rapa* *Avena sativa*	ISO 22030	Growth and reproduction	Growth reduction > 30 %, reproduction rate reduced by > 50 % compared to the control
	Primary consumer	*Eisenia fetida*	**ISO 11268-2**	Reproduction inhibition	reproduction rate reduced by > 50 % compared to the control [b]
		Folsomia candida	**ISO 11267**	Reproduction inhibition	reproduction rate reduced by > 50 % compared to the control [b]
		Enchytraeus sp.	ISO 16387	Reproduction inhibition	reproduction rate reduced by > 50 % compared to the control [b]
		Helix aspersa	ISO 15952	Reproduction inhibition	reproduction rate reduced by > 40 % compared to the control [b]

[a] Toxicity criteria according to guidelines.

[b] Toxicity criteria 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.

Annex A
(informative)

Tests with soil eluate — Expression of results

For assessment, dilution values were defined, which, if exceeded, indicate a respective ecotoxicological potential. These “threshold levels” are prescribed in a manner similar to that of the threshold values in the fields of chemical analysis.

In the text, this scenario is described as “toxic” (LID-value: lowest ineffective dilution, i.e. dilution at which the effect is slightly below 20 % in the luminescence and algae test or slightly below 10 % in the test with daphnia; prerequisite: at least a dilution series using a factor of 2). If the respective LID-value was not reached, an ecotoxic potential of the soil via the water path could not be detected; this situation is described as “non-toxic”.

The toxicity criteria (LID $>$ 8 for *Vibrio fischeri* luminescence inhibition and LID $>$ 4 for the *Daphnia magna* survival test and the algal growth inhibition test, $D_{Li} \geqslant 3$ for the umu-test and decisive D_{min} value $\geqslant 2$ for the *Salmonella* microsome test) were discussed and set after testing large numbers of uncontaminated and contaminated soil samples and after ring testing [6]. One important aspect considered is that on the basis of these toxicity criteria soil samples that are definitely uncontaminated are not classified as “toxic”. For example, the German standard soil “Lufa 2.3” has an LID value of 8 in the *Vibrio fischeri* luminescence inhibition test [13].

NOTE Explanations are extracted from Reference [8]. Additional information is given in References [6] and [7].

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