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BSI Standards Publication

Soil quality — Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials (ISO 17402:2008)

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National foreword

This British Standard is the UK implementation of EN ISO 17402:2011. It is identical to ISO 17402:2008.

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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Qualité du sol - Lignes directrices pour la sélection et l'application des méthodes d'évaluation de la biodisponibilité des contaminants dans le sol et les matériaux du sol (ISO 17402:2008)

Bodenbeschaffenheit - Anleitung zur Auswahl und Anwendung von Verfahren für die Bewertung der Bioverfügbarkeit von Kontaminanten im Boden und in Bodenmaterialien (ISO 17402:2008)

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Foreword

The text of ISO 17402:2008 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 17402:2011 by Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2011, and conflicting national standards shall be withdrawn at the latest by December 2011.

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Endorsement notice

The text of ISO 17402:2008 has been approved by CEN as a EN ISO 17402:2011 without any modification.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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ISO 17402 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

Introduction

Laboratory and field studies have demonstrated that biological effects are not related to the total concentration of a contaminant in the soil. Instead, an organism responds only to the fraction that is biologically available (bioavailable) for that organism. This is particularly true in soils that undergo interaction of contaminant molecules with the soil, in such a way that the contaminant is not attainable anymore by the organism or is present in a non-available form (sometimes referred to as sequestration or irreversible sorption). The bioavailable fractions of contaminants are dependent on soil properties and various processes varying with time and on the biological receptors. The conservative approach of exposure assessment, as typically described in a regulatory context, assumes that the total concentration of a contaminant present in a soil or soil material is available for uptake by organisms, including man, and as such will overestimate the risks. Therefore, a risk assessment can be optimised by using an approach that is based on estimated exposure representing the available, effective concentration of the contaminant(s) and on (existing) intrinsic toxicity data.

This assumption is not new as, already in the last half of the nineteenth century, agronomists and soil scientists began to search for chemical methods to determine the concentration of individual plant-available nutrients in agricultural soils. The impetus for this search was the need for recommended nutrient additions to achieve maximum crop yield. Mulder [1] stated already in 1860: "*The unnecessary full analysis of soil to learn if it is fertile or not cannot be argued enough. The long and short of it is availability, which cannot be derived beforehand. The analysis shows what there is, agriculture must draw its own conclusions from that.*" Chemical methods were devised to reasonably predict the availability of inorganic ions necessary for plant development. Chemical partial extraction methods are now commonly used to evaluate available levels of nutrients in soils. Extraction methods have been optimised by correlating extraction results with response of susceptible crop species to the addition of fertilisers.

The concept of availability is nowadays applied to the risk assessment of contaminants and can be tailored to the specific protection goals. Depending on the intended use of a soil or soil material, soil characterisation for different purposes (e.g. assessment of habitat and retention functions, risk assessment and compliance with regulatory values) may include chemical testing and ecotoxicological testing with selected representative test organisms. These tests will, in many cases, be soil- or site-specific at a given point in time, and cannot be extrapolated to other soils or points in time where other factors may control bioavailability.

Bioavailability may be assessed in two complementary ways (see also Figure 1):

- Chemical methods (e.g. extraction methods) which determine the fraction of a well-defined class of contaminants available for defined specific biotic receptors or the mobility of the contaminants in the soil. Usually these chemical methods were developed to predict the amount of contaminants taken up by the organisms. Nevertheless, these analytically determined values can also be correlated with effects. In a routine assessment of soil quality, chemical measurements may replace biological testing, if a correlation between the resulting chemical values and effect or accumulation has been demonstrated.

- Biological methods which expose organisms to soil or soil eluates in order to monitor effects. If accumulation and/or effects (e.g. mortality, growth inhibition) are encountered, bioavailable contaminants are likely to be present even if they cannot be chemically identified. More knowledge on processes controlling bioavailability can close the still existing gap between chemical measurements and biological effects.

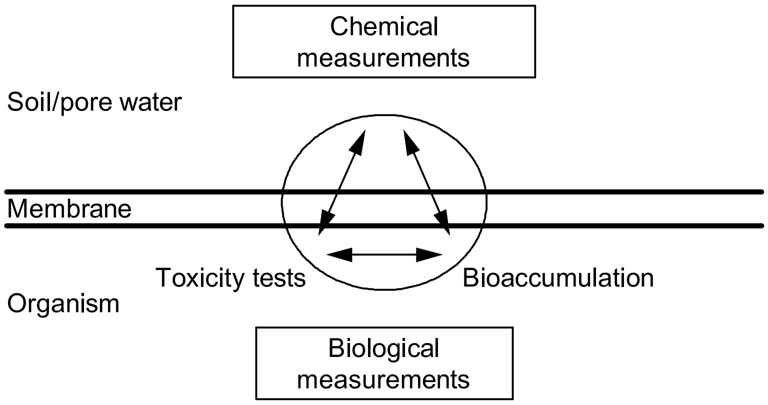


Figure 1 — Methods to assess bioavailability — Relation between chemical and biological assays and bioaccumulation

Under regulatory aspects of soil protection, the risk assessment should be based upon the same common concept with regard to determination/assessment of exposure and measurement/assessment of effects. Thus, existing concepts and derived trigger values based on total concentrations of pollutants in soils or soil materials can be transferred to the proposed concept based on the prediction of the bioavailable fraction by using the more accurate description of exposure. For instance, the translation of information on bioavailability into acceptable evaluations of “how clean is clean” (e.g. site-specific limits for regulating the extent to which the remediation of soil is required) is essential for establishing realistic risk assessments and the determination of proper endpoints for remediation.

A harmonised framework on bioavailability is considered in order to promote the development and introduction of workable standard methods to be used in soil and site assessment. In addition, methods for the estimation of bioavailable effective concentrations of contaminants according to the protection goals envisaged are required. These methods should preferably be described in International Standards and that standardization process should result in a limited set of established methods for the measurement of bioavailability [2]. As described in this International Standard, this process will not lead to one single method to measure bioavailability, because bioavailability depends on variables such as the contaminant, the target and the actual soil properties. Therefore, methods should not only use the word bioavailability but also refer to these variables (bioavailable *for*).

In this International Standard, requirements and guidance are given to select methods to assess bioavailability for different target species with regard to several classes of contaminants. Methods to assess bioavailability are not described in this International Standard. Reference is made to existing International Standards and additional principles of measurement, which may need to be worked out in these International Standards. As only a few standards exist, reference is also made to measuring principles. Guidance is also provided for further standardization of a method where promising first results are reported.

After a short description of methods (Clause 6), the pathways of a contaminant to the target organism are discussed (Clause 7). A summary of existing methods and promising methods that should be further developed is given in Clause 8. Clause 9 gives recommendations and includes the minimal requirements for application and further development.

Soil quality — Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials

1 Scope

This International Standard provides guidance for the selection and application of methods to assess bioavailability for the characterisation of contaminated soil and soil materials. This International Standard does not give a selection of the best applicable methods, but specifies boundary conditions and principles of methods to be used and gives the minimal requirements for the development of methods. The results obtained from such methods can be used as an estimate of bioavailability in a risk-assessment approach.

In this International Standard, when the term "soil" is only quoted for simplification, the broader term "soil and soil material" shall be considered.

The contaminants considered in this International Standard are metals, including metalloids, and organic contaminants, including organometal compounds. This International Standard is also applicable to metals originating from natural geological and pedological processes (natural pedo-geochemical content).

This International Standard can also be applied to sediments.

NOTE An assessment procedure based on the bioavailable fraction of the total amount of contaminants in the soil or soil material can contribute to the development of regulatory requirements of risk-based assessment procedures for soils.

According to the protection goals envisaged, applications of existing methods are recommended and their limitations discussed, with the intention of promoting the development and introduction of workable standard methods to be used in soil and site assessment. These methods are required to allow for the quantification of factors influencing bioavailability.

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 11074:2005, *Soil quality — Vocabulary*

ISO/TS 21268-1:2007, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 1: Batch test using a liquid to solid ratio of 2 l/kg dry matter*

3 Terms and definitions

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

3.1
bioavailability
degree to which chemicals present in the soil may be taken up or metabolised by human or ecological receptors or are available for interaction with biological systems

NOTE 1 Adapted from ISO 11074:2005.

NOTE 2 The concept of bioavailability is further explained in Clause 5.

NOTE 3 In ISO/TS 17924, a definition specific for human uptake through ingestion is given as the fraction of a substance present in ingested soil that reaches the systemic circulation (blood stream).

3.2
contaminant
substance or agent present in the soil as a result of human activity

[ISO 11074:2005]

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

3.3
critical body residues
CBR
internal concentration accumulated in a tissue, organ or all of the body that is correlated with an adverse effect

3.4
environmental availability
fraction of contaminant physico-chemically driven by desorption processes potentially available to organisms

NOTE 1 See also Figure 2.

NOTE 2 Environmental availability contains

1) an actual available fraction or the actual dissolved amount of pollutant at ambient conditions, or

2) a potentially available fraction, which is the maximum amount that can be released under (predefined) worst-case conditions. The potentially available fraction includes the actual available fraction.

3.5
environmental bioavailability
fraction of the environmentally available compound which an organism takes up through physiologically driven processes

NOTE See also Figure 2.

3.6
habitat function
ability of soil/soil materials to serve as a habitat for micro-organisms, plants, soil-living animals and their interactions (biocenosis)

[ISO 11074:2005]

3.7
leaching test
test during which a material is put into contact with a leachant under strictly defined conditions and some constituents of the material are extracted

[ISO/TS 21268-1:2007]

3.8
leachant
liquid used in a leaching test

[ISO/TS 21268-1:2007]

3.9
natural pedo-geochemical content
concentration of a substance in soils, resulting from natural geological and pedological processes, excluding any addition of human origin

[ISO 11074:2005]

NOTE In the background content [concentration of a substance in soil, resulting from natural geological and pedological processes, including diffuse source inputs (ISO 19258)], the natural pedo-chemical content is combined with the content resulting from diffuse pollution.

3.10
pollutant
substance or agent present in the soil (or groundwater), which due to its properties, amount or concentration causes adverse impacts on soil functions or soil use

NOTE Adapted from ISO 11074:2005.

3.11
potentially harmful substance
substance which, when present in a sufficient concentration or amount, may be harmful to humans or the environment

NOTE It may be present as a result of human activity or naturally.

3.12
receptor
potentially exposed person or part of ecosystem

3.13
retention function
ability of soils/soil materials to adsorb pollutants in such a way that they cannot be mobilised via the water pathway and translocated into the terrestrial food chain

[ISO 11074:2005]

NOTE In this International Standard, reversible adsorption and desorption processes are also considered.

3.14
sediment or subhydric soil
soil and its parent material beneath the surface water body

3.15
soil
upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and living organisms

NOTE 1 In a broader civil engineering sense soil includes top-soil and subsoils; deposits such as clays, silts, sands, gravels, cobbles, boulders and organic deposits such as peat; and materials of natural origin or of human origin (e.g. fills and deposited wastes).

NOTE 2 Adapted from ISO 11074:2005.

3.16
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

NOTE For the purposes of this International Standard, sediments are considered as soil material.

3.17
soil organisms
all organisms living completely, or during specific parts of their lifetime, in the soil or on the soil surface (including the litter layer) and which contribute to soil processes (e.g. nutrient cycling), including plants (including soil algae), microflora, invertebrate and (a few) vertebrate species

3.18
toxicological bioavailability
internal concentration of pollutant accumulated and/or related to a toxic effect

NOTE 1 See also Figure 2.

NOTE 2 This definition refers to internal concentrations in humans, mammals and other organisms.

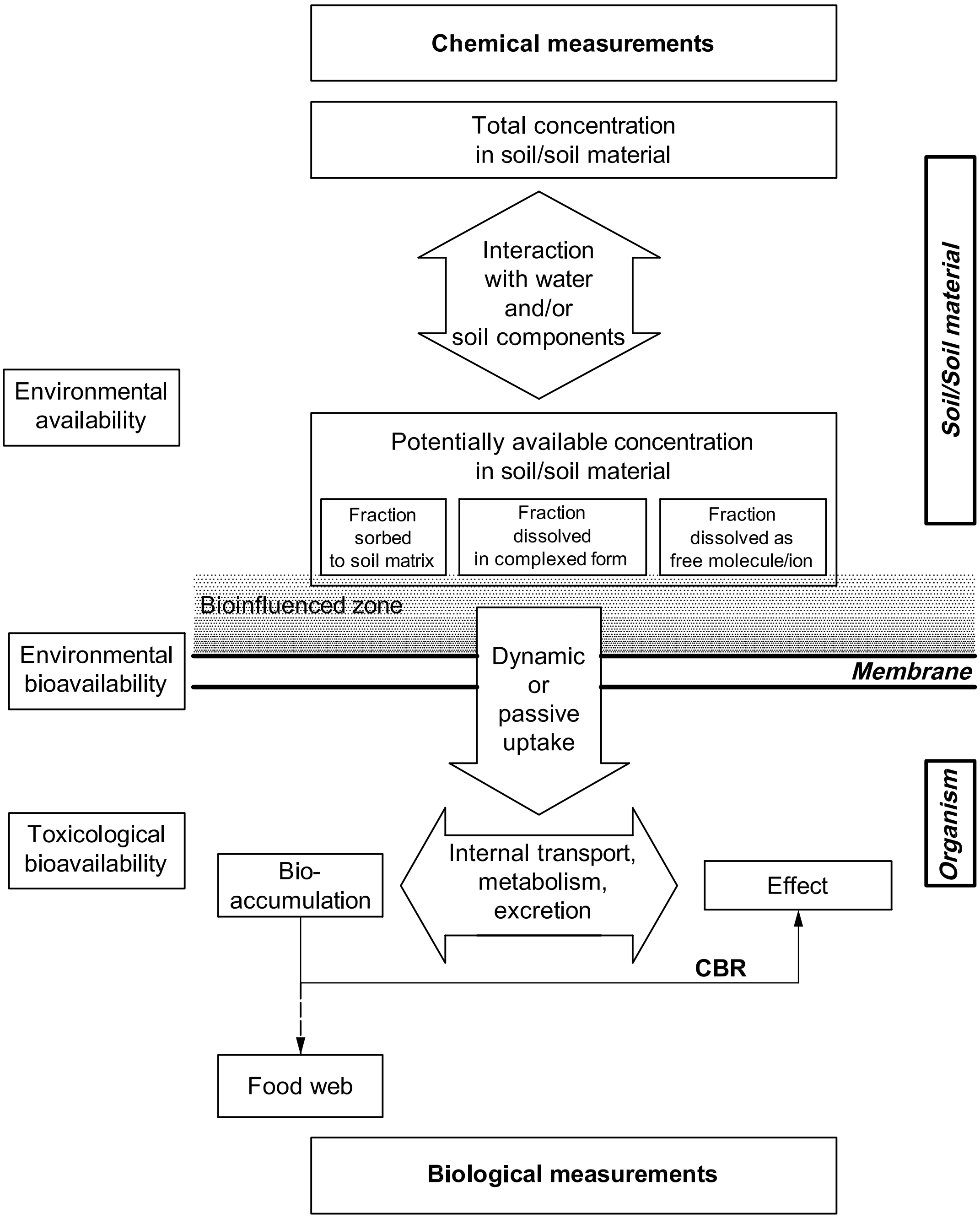


Figure 2 — From total concentration in soil to effect
(modified after Reference [7] in the Bibliography)

4 Bioavailability in relation to assessment of soil function

4.1 Soil functions and organisms to protect

Assessment of soil quality is relevant in a number of situations, for example, when contaminated soil and soil materials have to be evaluated for the degree of necessary clean-up, when excavated soil is to be reused for a specific purpose, or when land areas are to be evaluated with respect to proper land use. This assessment is very dependent on the availability of the contaminants in question, but other important factors are the presence or absence of a relevant type of receptor and its sensitivity to the contaminant. Bioavailability cannot be discussed without stating for whom the availability is relevant. In ISO 11074, the retention and habitat functions (see Clause 3) are distinguished. They include the following specific soil functions:

- 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 groundwater.

Apart from the retention and habitat functions and risk-assessment schemes derived, other scenarios, including the uptake of soil by humans (children's playgrounds) (see ISO/TS 17924) or by higher animals like grazing cattle, may be considered in an assessment procedure.

This can be described more specifically as follows:

- (bio)availability for different but defined target soil organisms or biotopes (habitat or retention);
- (bio)availability for organisms able to transform or mineralise the contaminant (habitat);
- (bio)availability for plant uptake (habitat);
- (bio)availability for human uptake (ingestion, inhalation, dermal contact) (habitat);
- (bio)availability for leaching processes (retention).

4.2 Risk assessment

Characterisations of bioavailability should be performed as a part of a risk and/or compliance assessment. A risk assessment comprises the following steps.

Step 1 The hazard identification: which in this context is the recognition of the potential of a substance to cause harm to human health or the environment.

Step 2 The exposure assessment: which is the process of establishing whether, and how much, exposure will occur between a receptor and a contaminated source. The contact time or exposure time is an important parameter for the exposure.

Step 3 The dose-response assessment: which is the characterisation of the relationship between the dose (exposure) of a chemical and the anticipated incidence of an adverse health or environmental effect in an exposed population. Dose-response assessments are typically carried out at national level as a part of the setting of criteria and are thus usually not undertaken as a part of the assessment of a specific site.

Step 4 Finally, and based on the above: the risk characterisation, a description of the nature and magnitude of a health or environmental risk. The description combines results of exposure assessment and hazard identification and describes the uncertainty associated with each step.

The exposure assessment (step 2) is the process wherein the intensity, frequency, and duration of the exposure of the receptor in question to a contaminant are estimated, and it comprises:

- source identification and characterization;
- identification of exposure routes;
- identification of relevant receptors/target groups;
- and, based on this, the actual exposure assessment.

Exposure assessments can be carried out in order to assess either the total exposure of a given receptor group (e.g. the population at risk) or the additional exposure from a given source or activity. Since, in this International Standard, only the risk resulting from the exposure to soil contaminants is addressed, it is limited to the direct contact of the target with soil. Other indirect effects like consumption of crops growing on contaminated land are part of an assessment procedure, but the bioavailability of contaminants in consumed crops is not a subject of this International Standard.

The actual exposure routes will depend on the site use. Both the actual and planned use of a contaminated area may be included in the assessment, as this may define which exposure routes are of relevance. Average, worst or reasonable-case exposure can be evaluated, and depending on the purpose of the exposure assessment, the data needs can differ for these situations.

The actual exposure time can differ between similar site uses due to differences in climate and actual site use patterns (e.g. number of days per week that the site is in use).

4.3 Protection goals

In soil assessment, the protection goal is a leading principle. The following protection goals can be distinguished.

- Human and higher animals:
  - humans;
  - grazing animals, e.g. cattle;
  - wildlife.
- Soil habitat function:
  - invertebrates;
  - soil micro-organisms;
  - vegetation;
  - food web (food web should be a protection goal, but as follows from this International Standard, it is not possible to have one single method to establish bioavailability for the whole foodweb).
- Soil retention function:
  - water organisms;
  - groundwater and surface water.

NOTE With regard to exposure in connection with groundwater, reference is made to ISO 15175. In ISO 15175, it is also considered that the mobile fraction of the total contaminant concentration controls groundwater exposure based on a similar hypothesis to that used in the bioavailability concept. Because the mobile contaminant fraction can also provide a good prediction of the bioavailable fraction for organisms in contact with the pore water, the mobile fraction is also described in this International Standard on bioavailability.

Depending on the potential use of the land — nature conservation, forests, pastures, arable land, paddy fields, horticulture, allotments, playgrounds, parks, below sealed areas, industrial areas or landfill covering — protection goals may differ. For instance, in playgrounds, human beings are the protection goals, while in nature conservation the goals mentioned under the habitat and retention function have to be considered. In industrial areas, it is usually most important to protect the groundwater (retention function). Defining the protection goals is a part of the assessment procedure and is not described in this International Standard.

5 Concepts of bioavailability

5.1 Definitions

The definition of bioavailability (3.1) is comprehensive, but is not explicit enough as a definition of bioavailability as a measurable quantity. Bioavailability depends on a specific target organism and specific contaminants and includes the following aspects: exposure time, transfer of contaminants from soil to organisms, their accumulation in the target organisms and the subsequent effects. For practical purposes, more specific definitions have been derived, for example as follows:

— bioavailability is the flux of contaminants to biota [3];

— bioavailability is the amount of chemicals in the soil that are present in forms and amounts that plants or other organisms can take up during the time they are alive [4];

— bioavailability is the rate at which a chemical compound can be transported to the specified biological population [5].

These definitions can be grouped into two categories, for example, the bioavailability defined in terms of flux or rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and that defined in terms of content ($\text{mol}\cdot\text{kg}^{-1}$). Theoretically, the content-based bioavailability is obtained by integrating the rate- or flux-based bioavailability over a period of interaction. In practice, it is difficult to directly measure the rate of processes, and the flux- or rate-based bioavailability is estimated from content-based bioavailability. Therefore, the content-based bioavailability is of primary importance from an evaluation point of view.

It should be realised that a content-based bioavailability is based on a net flux of the chemical to the target organism or organ. Bioavailability can conceptually be defined as a dynamic process, which can be described by the following three steps (Figure 2):

a) availability of the contaminant in the soil (e.g. environmental availability);

b) uptake of the contaminant by the organism (e.g. environmental bioavailability);

c) within the organisms, accumulation and/or effect of the contaminant (e.g. toxicological bioavailability) [6], [7].

Bioavailability is considered as soil- or organism-dependent but many organisms, as is the case for plants, may also modify soil characteristics (e.g. pH changes in the rhizosphere) making contaminants more or less available.

Because the total exposure of organisms depends on time, the available fraction is not a fixed fraction, but should be divided into more fractions or described as a continuum. The release of the contaminants (see Figure 3) depends on local environmental conditions (e.g. pH). The simplest approach is to define

— an actual available fraction or the actual dissolved amount at ambient conditions,

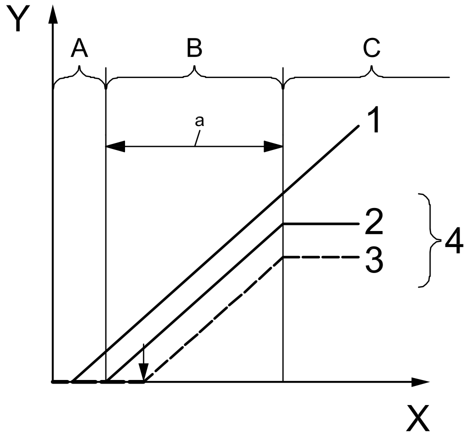
— a potentially available fraction, which is the maximum amount that can be released under (predefined) worst-case conditions, and

— a non-available fraction.

The potentially available fraction includes the actual available fraction but cannot exceed the total concentration. To establish the potential bioavailability, it is important to define the total system: soil, including the organisms, and environmental conditions that influence the fate of a contaminant in the soil or the activity of organisms. The subdivision in terms of actual and potential availability is important because it broadens the role of the pore water. Bioavailability covers not only the amount in the pore water, but may include the amount that desorbs during the time an organism is in contact with the soil. Regarding the organisms, a "bioinfluenced" zone could be defined [8]. This zone comprises the pore water and, depending on the organism, parts of the soil matrix. Consequently, the available concentration may have different values depending on the type of target organisms and time scale and, in turn, there could be numerous specific definitions (operational definition).

The bioavailability processes are also discussed in Reference [9] in the Bibliography. This description combines the conceptual and operational definition as used in this International Standard.

Bioavailability is also used to predict the amount of degradation of organic contaminants in a remediation process (see Annex A). With biodegradation, it is assumed that organisms degrade the contaminant. Bioavailability is used to predict a decrease of contaminant concentration. In a successful approach, the conceptual definition, the operational definition and the used tool should have relations as indicated in Annex A.



Key

X total concentration CBR

Y chemical response, bioaccumulation, biological response

A Zone A: the contaminant is detected, but there is no detectable effect

B Zone B: concentration-response curves parallel for chemical and biological assays: then the chemical assay constitutes a useful surrogate test

C Zone C: assays become non-linear and correlation is lost

1 chemical assay

2 bioaccumulation

3 biological effect

4 bioassay

[a] Possible correlation between biological/chemical; chemical assay = useful surrogate test.

Figure 3 — Response of chemical assays and bioassays as a function of the total concentration
(modified after Reference [8] in the Bibliography)

5.2 Links between bioavailability and biological effects and/or bioaccumulation

Whichever definition is used, the only direct way of measuring bioavailability for an organism to be protected is the use of that organism to measure the accumulation and/or effect (e.g. toxicological bioavailability). If this is not possible because of ethical (e.g. with higher organisms like human beings) or ecological (e.g. too many species in the ecosystem to be tested) reasons, one way could be to use surrogate species. They can then be used as indicators to study potential effects on and/or accumulation in the organism to be protected or the functional groups or total ecosystem. It should be kept in mind that, due to intra- and inter-species variations, the results obtained with the surrogate may not be extrapolated to the specific receptor to be protected.

As an example, if the protected organisms are human beings, the surrogate organism to assess toxicological bioavailability may be a pig, and a chemical test may also be developed in order to determine environmental availability. If one considers the protection of plants or soil invertebrates, surrogate species may be used (e.g. lettuce, earthworms) for which a chemical test may also be developed.

If environmental bioavailability (i.e uptake of the contaminant) for an organism or a group of organisms in a soil can be mimicked by a chemical process, then a chemical test can be used. The required condition to approximate environmental bioavailability with a chemical test which estimates the environmental availability is that a function exits between both in a statistical sense (Figure 3, Zone B). In general, since the rate of the mimicking chemical process is not the same to that of actual uptake processes, the time needed to estimate environmental availability is not the same (usually less time is necessary) compared to that for environmental bioavailability.

Figure 3 shows the relationship between chemical assays, biological effects and bioaccumulation. Only in Zone B do chemical and biological tests give parallel responses, meaning that the chemical method can be a surrogate of the biological one. The internal concentration which results from the uptake and accumulation can be used as a link to the observed effect, providing that the organism is not able to eliminate the contaminant. This concentration is defined as critical body residues [8].

As shown previously, we cannot have a general definition of bioavailability.

- For this International Standard, the content- or concentration-based bioavailability is of primary importance from the experimental point of view.
- Bioavailability is strongly linked to the exposure routes of an organism or a group of organisms, and to the habitat and feeding behaviour of these organisms.
- Therefore, correlations can be established between environmental availability obtained by chemical methods which mimic a specific exposure route (e.g. concentration of pollutants in pore water) and environmental bioavailability (e.g. bioaccumulation) or toxicological bioavailability obtained by observing effects in organisms (e.g. toxicity, reproduction).
- As these correlations also depend on substance properties, they may be restricted to substance classes (e.g. valid for substances with a certain range of water solubility).

ISO/TS 17924, the International Standard for the assessment of human exposure from ingestion of soil, also uses the term “bioaccessibility”, for the purposes of that standard defined as “proportional transfer of a substance into a human or ecological receptor”. It includes all physical, chemical and microbiological processes in the human body, from grinding in the mouth to precipitation in the intestine. The term bioaccessibility as included in the conceptual definition used in this International Standard would, in terms of ISO/TS 17924, refer to the amount available for uptake by the human intestine system.

NOTE In the literature, other definitions are also used. The environmental availability (upper part of Figure 2) is also referred to as bioaccessibility, as introduced by Semple et al. [10] to clarify which part of the soil/organism system was investigated. In addition to the accessibility, Reichenberg and Mayer [11] proposed to use chemical activity of the free ion or molecule as a complementary approach to explain environmental availability.

When considering bioavailability in an exposure assessment, the terms *absolute* and *relative* bioavailability are distinguished. In that context, the term absolute bioavailability is used to refer to the fraction of the applied dose that is absorbed by an organism and reaches a relevant target organ (and can never be greater than 100 %). Relative bioavailability refers to the comparison of bioavailability between two different sets of conditions, for example, from a soil sample vs. food or another matrix used in a toxicity study, and can be greater than or less than 1. This factor can be used in exposure assessments, for example, to assess the exposure by direct ingestion of soil, if the absolute bioavailability of the contaminant in the specific soil is expected to differ significantly from the absolute bioavailability in the matrix used when determining the toxicity value to which the exposure should be compared.

6 Description of methods to assess bioavailability

6.1 General

In most cases, the bioavailable fraction will be less than the total concentration as measured with traditional methods. Several chemical and biological methods have been developed independently already and some of them make use of common principles.

For a risk assessment, it seems more appropriate to determine the fraction of the pollutant that interacts with the water or another solution relevant in the context (see also Figure 2). Interaction can be direct equilibrium (actual availability) and equilibrium within the time frame of risk that is to be established (potential availability). This approach includes complex and interacting processes depending on soil properties, environmental and anthropogenic factors, such as pH, dissolved organic carbon (DOC) (concentration and quality), redox potential and specific properties of contaminants. The presence of dissolved organic matter, for instance, may lead to a higher amount of contaminants in the water phase (complexation). However, complexation in the water phase may strongly reduce the availability for the organism, and the measured concentration may not be equivalent to the fraction available. The processes can be described by means of extraction or leaching tests, which provide information on pollutant concentrations in extracts, and by establishing leaching profiles through routine measurements of pollutant concentrations in leachates. These may also include species analyses.

As already mentioned in Clause 4, bioavailability depends on the protection goals or receptors under consideration, the behaviour of the organisms under consideration, specific uptake mechanisms, accumulation, detoxification and excretion. Consequently, the consideration of bioavailability in ecotoxicological testing may lead to the following specific problems.

- Usually, in ecotoxicological testing (bioassays) bioavailability is not measured directly. Different endpoints of toxicity are considered, and various uptake mechanisms of a pollutant by an organism are included in the procedure. Nevertheless, for selected contaminants, the direct measure of internal concentrations during and/or at the end of a chronic exposure, together with the observed effects, can give a picture of bioavailability.

- The investigations have to be conducted with representative organisms, which live in different compartments of the soil and have different habitats, nutrients and/or feeding habits. As bioavailability is strongly linked to the exposure routes of organisms, several specific exposure routes need to be considered.

- In most cases, tests have been performed with one or a few soils. Though soil properties strongly influence bioavailability, a general soil assessment is only possible if the ecological requirements of the test species (e.g. pH, texture) concerning different soils are known [12].

As shown in Figures 1 and 2, bioavailability can be approached with chemical and biological methods. The fractions determined with these approaches are also referred to as environmental availability and toxicological bioavailability, respectively. In this International Standard, we use the term bioavailability for the fraction determined with both approaches.

6.2 Assessment of bioavailability using chemical test methods

The use of chemical tests is limited to known contaminants. As described already in the Introduction, the possible use of chemical methods is not restricted to existing International Standards. Promising chemical procedures that are proposed in this International Standard are still under development. The utility of these approaches, however, can only be considered adequately when the data they provide are correlated with results from biological testing of either accumulation or effect. Moreover, precision and sensitivity, as well as time and costs of chemical and biological assays, need to be compared. Although it can be expected that chemical tests will be less expensive, sufficiently precise and sensitive, this assumption needs to be verified. In addition, the duration of chemical assays and bioassay have to be compared to evaluate whether chemical testing is less time consuming as well. Also, suitable cost-effective reference materials or performance studies are needed, so that comparisons of results of standardized methods between different laboratories can be made.

There are already several methods to measure the availability of contaminants. In this International Standard, there are not technical details given for all of these methods. In general, the following chemical measures of bioavailability can be distinguished:

- water- based extractions;
- concentration in pore water;
- extraction from the water (use of an extra solid phase for exhaustive extraction of the water phase);
- adsorption tools that mimic behaviour of organisms;
- solvent-based extractions;
- weak solvent;
- short extraction or extraction with smaller energy input;
- weak acid or complexing agent (heavy metals) extractions.

The weakest extraction is the extraction with water. If the distribution between the solid soil and the water phase can be described by an adsorption curve (assuming equilibrium between the solid and the liquid phase), this procedure is suitable to describe direct effects. The partitioning between the solid phase and the soil solution is a major process to be considered, particularly with regard to the retention function. The solid/liquid partitioning of contaminants in the soil depends on factors such as:

- solubility of the contaminant;
- solubility-controlling factors of the water phase (pH, DOC, fine particles, ion composition);
- adsorption-controlling factors in soil (e.g. clay, organic matter, mineralogical composition, pH);
- slow exchange between micro- and macro-pores in the soils (diffusion, depending on properties such as pore size distribution, water content).

Typical methods used to estimate the solid/liquid partitioning of contaminants and their concentrations in pore water are leaching and/or extraction procedures, while models are increasingly successful to extrapolate results beyond laboratory conditions and calculate the specific chemical forms (speciation) of contaminants that may be bioavailable [13].

Even if organisms are mainly exposed via the pore water, the direct exposure by feeding on soil particles should be considered.

Partial extractions are empirically related to concentrations in an organism and/or effects. The procedures are empirical, and the final measurement endpoint techniques used are comparable with techniques applied to determine total substance concentrations, which facilitates integration into current laboratory practice. However, if knowledge of the principles of the extraction is insufficient, variations in the extraction procedures may strongly influence the results. Therefore, these methods are not suitable for standardization.

Metals in soils are mainly adsorbed into the components that determine the cation exchange capacity (organic matter, clay and Fe, Mn and Al oxides) or they occur as minerals, like carbonates. Their adsorption and/or precipitation behaviour is more or less reversible; it is strongly influenced by the pH.

Surface soils are mostly aerobic. Sediments, however, are anaerobic and low redox potentials are possible. Heavy metals in sediments are strongly bounded as carbonates and sulfides.

Hydrophobic organic contaminants are preferentially adsorbed onto the organic matter fraction. Organic matter not only includes the humic fraction, but also other carbon constituents of soils (e.g. soot, black carbon, kerogen) [14]. If the adsorption is reversible, the partition between water and soil can be described with the partition coefficient between water and the specified organic matter expressed as organic carbon) (K_{oc}). For several contaminants, this value is known or else can be derived from the K_{ow} (partition coefficient between octanol and water) or the water solubility. Totally reversible partitioning, however, only occurs with freshly added contaminants. Subsequent ageing results in a strongly reduced availability, which makes a prediction of the effects from the total concentration impossible.

For soluble contaminants, which adsorb weakly into organic matter, the pore water concentration may be suitable to explain internal concentrations and possible effects. For highly insoluble contaminants like polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB), it is generally difficult to measure pore-water concentrations directly. In this case, availability determined by chemical analysis has to be measured using a method suitable for extracting a certain portion of the substance from either the soil solution (actual availability) or the soil system (potential availability). Available methods are based on a mild extraction over a short period of time, or the use of adsorbents (three-phase system).

6.3 Assessment of bioavailability using ecotoxicological test methods

Due to organism-specific uptake systems and the ability of some organisms to change their immediate environment, total chemical analyses of contaminants in soil and soil materials do not provide information on the bioavailable fraction. Bioassays can be used to assess the effect of the bioavailable fraction of all pollutants present in a soil or soil material (see 7.4.2 and 7.4.3). Moreover, accumulation and eventually critical body residues may be determined to provide complementary data on the uptake of substances that are not metabolised in the organism. These data also give information on the amount of the contaminant available for bioaccumulation/biomagnification in the food web.

To obtain information about possible effects of pollutants on specific organisms and subsequent effects on the biocoenosis or the habitat function of soils, investigations with representative organisms, as outlined in Clause 5, are required. Organisms may colonise different regions of the soil, for example, larger pores, fissures, deeper soil regions such as burrow holes of earthworms, or top soils including the soil surface. As pollutants are not evenly distributed in the soil, but may be concentrated in organic matter particles, on the walls of macro-pores, earthworm burrows, etc., a refined exposure assessment should also take into consideration the habitat and feeding habits of specific organisms. Unequal distribution may lead to both higher and lower ecotoxicological effects, which could be determined in laboratory tests: higher if the test organism feeds in the polluted part, and lower if this part is not attractive for the organism or is actively avoided.

In ecotoxicological test methods, adverse effects can be observed at different levels/endpoints. Endpoints for measurement may be attributed to biological organization levels:

- the molecular level;
- the cellular level;
- the metabolic level (enzymatic activities or biomarkers);

- the individual level (accumulation, mortality, growth, reproduction, behavioural responses);
- the population level (abundance, diversity);
- the community level (species composition).

The parameters considered in the experiment determine the sensitivity of the test and have to be considered in the risk assessment.

7 Pathways related to soil quality (both organism and soil)

7.1 General

This International Standard covers contaminated soil and soil materials. Toxicity of the pure contaminant is not taken into account. If a pure contaminant (e.g. mineral oil) is present as a separate phase, other pathways than those considered in this International Standard can be responsible for effects on soil organisms.

7.2 Human

7.2.1 General

Human exposure from soil contamination is described in ISO 15800. The following exposure routes exist:

- soil ingestion;
- dermal contact;
- inhalative intake;
- groundwater used for the drinking water supply (in direct contact with the contaminant or water that has passed through contaminated soil).

Furthermore, it should be realised that other routes, like exposure through the food chain, can be responsible for effects. In this International Standard, only the routes directly related to the soil are considered. Intake by consumption of plants and consumption of products of grazing cattle are indirectly covered, because pathways to plants and cattle are part of this International Standard. The actual exposure routes will depend on the site use.

7.2.2 Soil ingestion

Soil ingestion by children happens through ingestion of dust, sucking of dirty fingers and by actual eating of soil. Distinction should be made between inadvertent and accidental intake and deliberate long-term persistent behaviour (Pica behaviour). In general, Pica behaviour should be regarded as a special case, not necessarily relevant for the actual assessment. Some young children go through a short period of exploratory soil ingestion.

Adults mainly ingest soil as dust, for example, in connection with gardening, and as soil on non-cleaned vegetables and fruit. In the case of the characterisation of a specific site, the actual behaviour should be taken into account.

7.2.3 Dermal contact

Skin contact with contaminated soil can be caused by dust reaching the skin through atmospheric deposition, or by playing or working with the soil. It should be noted that there is a distinction between skin contact in, for example, a private home and in the working place, since the latter is usually regulated by health and safety regulations. It should be noted that occupational exposures are not covered by this International Standard.

For an assessment of this route of exposure, the information needed is the available concentration for dermal contact of each substance in the soil. In the evaluation of soil contaminants in connection with skin contact, distinction should be made between contaminants that can be absorbed through the skin and substances potentially causing other effects, such as rashes or hypersensibility.

7.2.4 Inhalation of soil

Inhaled soil will be a fraction of the original soil and will be limited to the fine fraction. This fraction will also tend to contain most of the contaminants. Air may also contain fine particles of other origin. Therefore inhalation is not considered to be a special soil subject. The development of a standard covering air seems to be more appropriate.

7.2.5 Groundwater used for drinking water

Contaminants that leach from the soil to drinking water wells can be part of human exposure. Methods for evaluation of this exposure route are described in 8.2.5.

7.3 Exposure of higher animals

Intake by animals is usually mainly due to direct ingestion of soil from the surface layer or from soil adhering to forage. To a lesser degree, it is the result of consumption of fodder after plant uptake and accumulation. The degree of accumulation of the contaminants by animals will depend on the properties of the soil, the level of contamination and other components of the diet. The availability of contaminants in ingested soil for higher animals can be treated as comparable to that of ingestion by humans.

7.4 Exposure of soil organisms

7.4.1 General

Depending on their size (e.g. nematodes, for instance, cannot be present in the smallest pores of the soil) and on their feeding habits (e.g. different species of nematodes belong to different trophic levels and accordingly will have different feeding regimes, for example fungivores, phytophages), soil organisms will colonise different “regions” in soils (e.g. small pores, rhizosphere, specific structures such as drilosphere). Most of the organisms feed on water, soil and organic matter. Furthermore, as these organisms live in close contact with the soil, exposure through “dermal contact” and respiration of the soil atmosphere are also of concern. Thus, soil organisms may be exposed to contaminants through all these different routes, depending on their own biology. Depending on the soil type, the nature of contaminant and on the partitioning of the contaminant, some types of exposure will be predominant.

7.4.2 Exposure of soil micro-organisms

Soil micro-organisms (e.g. bacteria, fungi, algae) are organisms that require water for growth and reproduction. Thus, the main exposure will occur through the fraction of contaminants that interacts with water.

Studies on the bioavailability of pollutants to micro-organisms often consider toxicity resulting in a modification of structural (e.g. mortality, change in community) and functional (e.g. respiration, dehydrogenase activity, organic matter degradation) diversity [15].

Bacteria are present within colonies in soil or protected by clays; they often may not be exposed to the equilibrium solution activity of pollutants. Depending on their own size, soil micro-organisms are present in different soil pores. Depending on the type of measured effect, micro-organisms can be representative for the retention function or the habitat function.

Organic contaminants are often biodegradable and the extent and rate of degradation depends on the bioavailability for degrading organisms such as bacteria and fungi. The degradability is often used in remedial actions, as biodegradation will reduce the risks of contamination provided no new toxic compounds are created. If the bioavailability is low, high residual concentrations are often present in soil even when biodegradation occurs. Based on availability, it is possible to distinguish three different degradable fractions [16]:

- a rapidly degradable fraction which is degraded in the short term (days to a few months);
- a slowly biologically degradable fraction, where degradation may take months to years;
- a very slowly available fraction that needs several years or decades to be degraded.

Methods to measure the biodegradation shall be suitable to distinguish between these fractions.

7.4.3 Exposure of soil invertebrates (micro-, meso- and macro-fauna)

Soil invertebrates include micro-fauna (smaller than 0,02 mm, e.g. protista, nematodes), meso-fauna (from 0,02 mm to 4 mm, e.g. collembolans, mites, enchytraeids) and macro-fauna (larger than 4 mm, e.g. earthworms, predatory beetles, snails) [17]. They represent a wide variety of organisms with different sizes and feeding habits. Some live in the water phase in small pores and feed on micro-organisms, such as nematodes, whereas others live in the soil and feed on soil organic matter, such as earthworms, or on soil and plants, such as snails. Therefore, exposure routes are different for these different life forms.

In ecotoxicological tests, the effects generally assessed are survival, growth, behaviour and reproduction of selected species. Accumulation, for example, in earthworms [18], even if it does not result in a response, may affect organisms of the next level in the food web. In this context, the determination of accumulation is also important in order to obtain information on potential secondary effects of pollutants on soil invertebrates and consequently on the habitat function of soils. To increase the ecological relevance of ecotoxicological testing, additional multi-species investigations in terrestrial model ecosystems (TME) have been proposed [19].

Bioavailability may also be different, depending on the “region” of the soil to be evaluated (e.g. contaminants on the surface of clay materials may be more available than those absorbed in organic matter; the plant rhizosphere may play a role in the availability of the contaminants).

7.5 Exposure of plants

In general, the amount of contaminants taken up and accumulated by plants depends on the physico-chemical characteristics of the contaminant, the type of soil (including soil characteristics), the type of plant and the meteorological conditions. Uptake and translocation of soil contaminants may differ considerably between the species and even ecotypes, partially because different ecotypes may interfere with the soil in different ways (e.g. by changing physico-chemical and biological conditions in the rhizosphere). Toxicity of several metals to plants may also occur without any uptake, just by interaction with the membrane of root cells which induces a reduction of root elongation.

Plants may be contaminated through different pathways:

- active and passive uptake by roots;
- systemic translocation;
- uptake by leaves via volatilisation of contaminants (e.g. organic solvents, hydrocarbons, Hg, Se);

- adsorption on leaves of soil/dust particles (coming from the soil the plant is growing in);
- deposition on leaves of contaminants coming from diffuse sources.

When only the contamination through the roots is considered, it seems that plants can only absorb the amount of contaminant interacting with water. This therefore constitutes the most important parameter that defines the role of the soil.

Trace elements in the pore water are mostly absorbed by plant roots and then transported within the plant. This absorption may be passive (diffusion and mass flow linked to leaf transpiration) or active if the trace element is transported or accumulated by specific mechanisms (e.g. phytosiderophores, H^+-ATPase) [20].

The uptake and transport in the plant of organic contaminants is considered to be very low with lipophilic compounds (lg $K_{oc} > 3$). Usually, if they are present in plants, they originate from air pollution (adsorption on leaves) or from soil particles adsorbed both on aerial tissue and roots, even if some experiments have shown the possible transfer of such contaminants through roots [21]. Organic contaminants with lg K_{oc} of 1 to 2 (several solvents) can pass into the roots and can be transferred to the plant.

Bioavailability of contaminants for plants may be measured as part of a risk assessment for the plant community (habitat function) and/or for the contamination of the terrestrial food chains (production function). When regarding phytotoxicity, usually the reduction of emergence and growth of early stages are the most common measured parameters. If the endpoint is the contamination of the food chain, then the amount of contaminants in the parts of interest (i.e. fruits, roots, leaves) has to be measured. This last endpoint is generally the most often considered when deriving trigger and action values for crop cultivation.

8 Available methods to measure bioavailability

8.1 General

This clause starts with an overview (state of the art in 2005) of the chemical principles available and under development for the evaluation of bioavailability. The principles of chemical methods are described in relation to a specific pathway or specific goal and are summarized in a table. Ecotoxicological test methods are already standardized to a further extent, and an overview of available methods is given in the third part of this clause. In order to help with the selection of methods to be applied or to be defined, 9.2 describes the minimum requirements that shall be considered.

8.2 Chemical methods to measure environmental availability

8.2.1 General

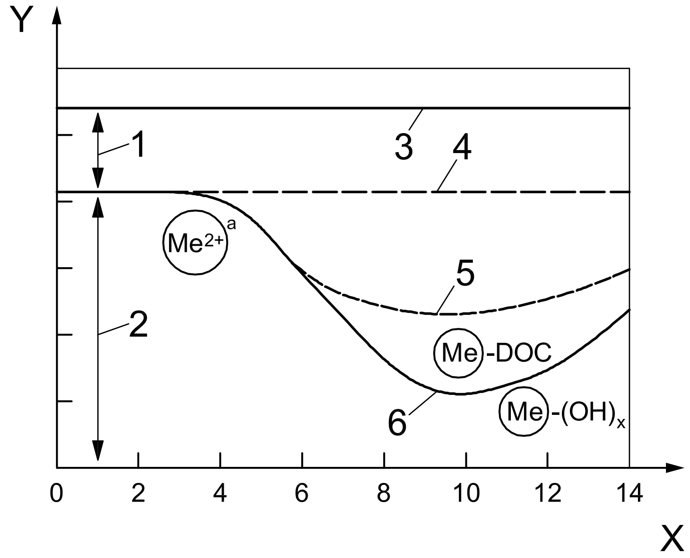
As shown in Figure 2, the release of contaminants from the soil matrix is a necessary first step towards the assessment of their bioavailability, as a substantial fraction of their total content is often unavailable. The release depends on (local) environmental parameters, particularly pH, and can be subdivided into

a) the potentially-available amount, for example the maximum amount that can be released under (predefined) worst-case conditions, and

b) the actual dissolved amount at ambient conditions.

Figure 4 shows how the solubility and release of metals is related to the pH at which the soil is exposed. Different pH domains are relevant for specific exposure scenarios. Very low pH values (pH 1 to pH 2) are relevant for ingested soil in the human stomach, while values between pH 4 and pH 8 span the typical range for soils. Higher values (pH 9 to pH 13) may occur for treatments such as cement stabilisation.

The relationships between the release of contaminants from the soil matrix and their bioavailability are complex and strongly dependent on the chemical forms, or “speciation”, in which the contaminant occurs. Figure 4 illustrates the example of a cationic metal. At a low pH, metals occur predominantly in their free ionic form (Me^{2+}), which is generally believed to be the major bioavailable metal species. From moderately acidic up to alkaline conditions, metals form strong complexes with DOC, particularly humic and fulvic acids. The bioavailability of these complexed species is believed to be much lower than that of the free metal ion. In addition, metals can also form (more labile and possibly bioavailable) complexes at intermediate to high pH with inorganic anions such as chloride, hydroxide and (bi)carbonate. Based on the free ion activity (FIA) concept, referred to above, models such as the biotic ligand model (BLM) have been developed and successfully used to describe metal bioavailability to a variety of (mainly aquatic) organisms [22]. This approach is increasingly applied to soil systems.



Key

X pH
Y log concentration

1 solid phase (matrix mineralogy)
2 aqueous phase
3 total concentration
4 potentially available
5 enhanced solubility by DOC
6 actual dissolved concentration

[a] Toxic cations.

Figure 4 — Schematic overview of typical relationships between the total composition, the potentially leachable amount or “availability” for leaching and the actual leaching at ambient conditions
(Modified after Reference [23] in the Bibliography)

In addition to direct analytical measurement, geochemical modelling can also be used to identify the specific chemical forms (speciation) of contaminants that may be bioavailable [13]. Plants and organisms may have regulation mechanisms to prevent uptake of specific contaminants, even when present in the bioinfluenced zone. Therefore, chemical methods (see 8.2.5) cannot always be used to predict the uptake by plants.

8.2.2 Methods for soil ingestion

To assess soil ingestion, the contaminant content taken into account is that resulting from physiologically based extraction procedures with acidic conditions simulating conditions in the stomach and the intestine. Such methods are described in ISO/TS 17924. After establishing the amount available for uptake, ingestion exposure factors have to be used for assessment.

8.2.3 Methods for dermal uptake

For modelling the percutaneous resorption of organic pollutants, modelling approaches have been developed as follows (for an overview see Reference [24] in the Bibliography):

- on the basis of the skin-permeability coefficients for pollutants in water environments (skin/water permeability coefficient, K_p);
- on the basis of octanol/water distribution coefficients and the Henry constant; both approaches suffer from a lack of data for validation.

As long as these methods are based on the total concentration, bioavailability is not included. If they are based on the concentration in the pore water, dermal uptake can probably be related to methods simulating the amount in the pore water.

8.2.4 Methods for plants

Uptake of contaminants by plants has been intensively studied for agricultural crops. Accumulation can be easily measured by analysing plant tissue. As experiments on transfers of contaminants from soil to plants are time consuming, it is crucial to be able to predict the uptake of contaminants by plants.

Plant uptake can (at least for some metals) be estimated via extraction tests utilising dilute, non-complexing neutral solutions or defined (acidic) pH solutions. All experience to date has shown that one of the best methods for soil extraction is to use diluted salt solutions (such as ammonium nitrate or calcium chloride).

For the assessment of the potential uptake of contaminants by plants, relevant soil parameters to be recorded may include the following: organic carbon fraction (f_{oc}), content (fraction) of clay particles (f_{cm}), cation exchange capacity of the soil (CEC), soil pH, soil bulk density, soil water content, organic carbon partition coefficient, K_{oc}, and redox potential. These parameters may be used to interpret and explain the amount of trace elements extracted.

Mathematical relations have been found that link different soil parameters (e.g. total trace element content, clay content, organic carbon content, CEC, distribution coefficients between solid phase and water and pH) to the amount of metals and organics accumulated in the plants [25], [26]. These studies have allowed the definition of models that are supposed to predict the transfer of trace elements from soil to plants. Care should be taken in generalising these models for the assessment of plant uptake, as they were developed for specific plants under specific conditions.

8.2.5 Methods for leaching from the solid phase to the soil solution

Leaching tests were developed to predict the transport of contaminants from the solid soil material to the water phase. Given the importance of pH on the solubility and release of (inorganic) contaminants, as illustrated in Figure 3, the pH-dependence leaching test (ISO/TS 21268-4) is being developed as a parametric basic characterisation test for the measurement of intrinsic leaching properties of inorganic contaminants in soil and soil materials. This test has also been demonstrated to provide the necessary input for geochemical models that can be used to predict the speciation of contaminants in both the solid matrix and pore water of the soil. The speciation includes partitioning of constituents between free and DOC-associated form as well as partitioning in the solid phase between particulate organic matter (POM), minerals, Fe-oxide and Al-oxide surfaces. In addition, a percolation test is suggested as a multi-parametric basic characterisation test to evaluate the combined effect of different parameters on contaminant release in a percolation-controlled environment. The percolation test (ISO/TS 21268-3) also allows for the simulation of pore water conditions by analysis of the first percolate fraction [at a liquid/solid (L/S) ratio of 0,1 l/kg]. Percolation tests are developed for permeable soils but are rarely applicable to sediments and swelling-shrinking clay soils. Furthermore, two batch leaching tests with L/S = 2 (ISO/TS 21268-1) and L/S = 10 (ISO/TS 21268-2) are available, which can be used for quick verification of leaching behaviour. All leaching methods in the ISO/TS 21268 series are applicable for the testing of inorganic parameters and, with specified precautions, also for organic contaminants. The limited solubility of organic contaminants and adsorption to equipment form the basis of these precautions. The eluates prepared from the above-mentioned tests are suitable for ecotoxicity testing. Provisions have been taken to accommodate the need for sometimes large volumes for testing. The choice of the leaching test method to be applied for specific purposes or questions is addressed in a guidance standard (ISO 18772).

8.2.6 Methods for biodegradation

The rapid degradable fraction can be measured using a biological test, measuring real degradation. Several weeks are however necessary for such a test. Chemical tests are much faster (about 1 day) and several chemical methods are already available to predict the rapid degradable fraction. Most methods have been developed for the biodegradation of PAH. Principles of the methods differ and three main groups can be distinguished [16]. Results of all these methods correlate with observed biodegradability, which is a condition for application.

- The Tenax method [27], [28], XAD method [29] and the cyclodextrin method [30] are based on release of the sorbed contaminant through the water phase to an adsorbent.

- Oxidation of unstable organic matter, in which contaminants are less strongly bounded (= more available) together with the contaminants adsorbed on that organic matter [31].

- An empirical approach making use of weak extraction methods to isolate easily extractable (= available) contaminants. Several methods and extractants are applicable, like supercritical fluid extraction (SFE) [32], acetic acid, methanol and surfactant solution.

All methods have to be empirically validated with real observed degradation; the first two mentioned groups of methods have a better theoretical basis. The last group is more empirical, for instance the extraction time is adjusted to give the proper result.

The prediction is only valid if no limiting conditions for biodegradation are present, for example the absence of oxygen for an aerobic degradation process or the presence of pesticides affecting the degradation capacity.

8.2.7 Methods for soil organisms

For organic contaminants, solid phase extractions have been shown to correlate with effects on soil-living organisms. Three main approaches can be distinguished.

a) Methods such as solid phase micro-extraction (SPME) can be used in water extracts or slurries and give a free dissolved amount (actual availability) [33].

b) Solid phase extraction in slurry, like the Tenax- or XAD-extraction already mentioned in 8.2.6. The amount measured is the amount that can desorb in a fixed time (approximately 20 h) which can also be described as the amount in equilibrium with the water phase (potential availability).

c) Extraction devices that are intended to mimic the organism (environmental bioavailabilty). These devices are placed in the soil or sediment to be investigated and act as a passive sampler that adsorbs contaminants within its immediate surroundings. Results after 4 days (use in sediment [34]) to 15 days (use in soil [35]) of passive sampling can be correlated with accumulation in the organisms to be mimicked. Examples of these systems are semi-permeable membrane devices (SPMD) and SPME.

As explained in 8.2.1, for heavy metals it is particularly important to know the free metal concentration. Several approaches are available for this purpose, such as the donnan membrane technique (DMT) [36] and differential gradients in thin films (DGT) [37]. In addition, a specific approach is developed to measure the speciation of heavy metals over redox gradients [38].

8.2.8 Available and promising chemical methods to measure bioavailability

Table 1 shows chemical methods that were in use in 2005. The methods simulate different fractions of the contaminant and can be related to a pathway. Using these two columns (columns 2 and 3) a method or principle can be selected. The following two columns give information on the calibration (measured values are related to concentrations in an organism and/or effects) status of the method. In the following column it is indicated whether the method is recommended for future use. Only the methods having a mechanistic/physiological background are recommended (+ in Table 1). Empirical methods are not advised to be further developed as international standards, despite the fact that several of these methods have been calibrated.

In Table 1, EDTA/DTPA are not recommended methods for heavy metals. Results can be correlated with effects, but comparable results are obtained using an acid extraction. Because an acid extract fits better in a mechanistic/physiological approach, the latter is chosen for further development. Similarly, mild extractions with water/solvent mixtures are not recommended for organic contaminants.

Table 1 — Chemical methods to estimate the bioavailable fraction

Approach	**Result simulates**	**Pathway to organisms**	**Method**	**Calibrated**		**Recommended**	**Limitations**
				Contaminant	Target		
Neutral extract	Amount in pore water	Direct contact with water	Neutral water extract ($NaNO_3$; KNO_3; NH_4NO_3; $CaCl_2$)	Various heavy metals	Soil organisms, plant uptake	+	NH_4NO_3 may reduce pH in low buffered soils $CaCl_2$ may reduce DOC concentrations, particularly in soils with low calcium content Poorly soluble compounds Depends on concentration of salt Difference in electrolyte concentration in sample and extract
		Direct contact with water	Extraction of pore water	Various heavy metals	Soil organisms, plant uptake	+	Pore water not available in dry soil Equilibration time if water is added
		Transport	Leaching test	Inorganic/ organic	Ground-water	+	Poorly soluble compounds
Separation by diffusion	Free metal concentration	Uptake	DMT, DGT	Inorganic	Soil organisms, plant uptake	+	Specialized equipment
Acid extraction	Potential soluble amount in water	Uptake	Acid extract (HNO_3)	Various heavy metals	Plant uptake after including soil characteristics	+	
		Transport	Leaching test	Inorganic	Ground-water	+	
		Uptake	Acid extract (pH of stomach or lower)	Pb	Screening, human ingestion	+	Gives a maximum of bioavailability, because pH in the intestine system is higher
Complexing agent	Potential amount soluble in water, competitive	Uptake, transport	EDTA, DTPA extract	Heavy metals	Plant uptake, groundwater	–	
Simulation of digestion	Oral uptake	Human uptake	Stomach and intestine system	Pb, Cd, Zn, Ni, Hg, PCB, PAH method comparison	Human	+	

Table 1 (*continued*)

Approach	Result simulates	Pathway to organisms	Method	Calibrated		Recommended	Limitations
				Contaminant	Target		
Adsorption to competitive adsorbant	Adsorbed amount in equilibrium with pore water	Contact with pore water	Adsorption to solid phase (Tenax, XAD, cyclodextrins)	PAH	Degrading organisms	+	
	Amount in pore water	Contact with pore water	Adsorption to solid phase (like SPME)	PAH, PCB, OCP	Uptake invertebrates	+	
Increasing solubility	Amount in equilibrium with pore water	Contact with pore water	Mild extraction using water/solvent mixtures	PAH	Degrading organisms	–	
Partial oxidation of organic matter	Amount of weakly bounded organic contaminants	Easily attainable by micro-organisms	Oxidation with persulfate	PAH	Degrading organisms	+	

+ promising

– not recommended

8.3 Ecotoxicological test methods to measure bioavailability

Ecotoxicological test methods applied to soil quality assessment may be selected with reference to the soil function potentially affected and to the intended use/re-use of soils and soil materials. Two major types of soil functions are identified in ISO 15799: the habitat and the retention function. Both functions can be characterised by the recommended tests in Annex A of ISO 15799:2003. Examples of standardized tests that have proved to be valid for application in the field of soil quality testing are proposed in Table 2.

Aspects considered in ISO 15799 with respect to soil functions include the following:

a) Retention function

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

- Aqueous eluates are useful for testing ecotoxic effects on organisms exposed via the water-mediated transport. Eluates may serve as early indicators for the contamination of pore, ground and surface water.

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

b) Habitat function

- For testing effects on the habitat function, characterisation by sublethal test parameters is particularly recommended. When selecting the individual test species, criteria such as trophic level, taxonomic/physiological groups, size, class/exposure pathway and the ecological role should be taken into consideration.

It is pointed out in ISO 15799 that substances mobilised via water may be subjected to different types of changes, for example, metabolism or hydrolysis when transported into the groundwater and from there into surface water, and that their concentrations are reduced by dilution. Moreover, substances may be mobilised over time due to environmental changes (e.g. pH, chemical and biological transformation). Even if no effects are observed in the biological tests, transport of contaminants cannot be excluded completely. Contaminants can also be transported due to bioturbation and transport of colloids. In those cases where effects are observed, soil pollutants are soluble in water, bioavailable and may be transported via the water path. Nutrients extracted from soils (such as nitrates and phosphates) may interfere with the effects of algal (and higher plant) growth inhibitors. Therefore, one shall be aware that toxicity of pollutants may be masked or increased depending on the amount of nutrients in the soil; therefore, soil composition should be considered when analysing results.

As the number of standardized test systems representing categories in terms of trophic levels, taxonomic/physiological groups, micro-habitat or exposure pathways is small compared to the taxa that are part of an ecosystem, it is obvious that bioavailability of contaminants will differ not only in relation with testing of the retention and habitat function but also with the categories the test system is representing.

Table 2 — International Standards for the determination of the toxicity of chemicals on soil organisms (laboratory tests)

Tests marked with an asterisk are validated for the assessment of soils.

Organism	**Measuring parameter**	**Habitat**	**Most important exposure route**		**Method**
			For soluble substance	For poorly soluble substance	
Soil function affected: Habitat function					
Micro-organisms	Nitrification, N-mineralization (incubation: 28 d) Ammonium oxidation Respiration Substrate-induced respiration Biomass (fumigation-extraction) Dehydrogenase activity	Water film of soil pores	Pore water	Pore water	ISO 14238 ISO 15685* ISO 17155* ISO 14240-1 ISO 17155* ISO 14240-2 ISO 23753-1 ISO 23753-2
Enchytraeids: *Enchytraeus albidus* (meso-fauna)	Reproduction	Water film of soil pores	Pore water	Pore water	ISO 16387*
Collembolans: *Folsomia candida* (meso-fauna)	Reproduction	Air-filled soil pores	Pore water	Pore water, air, feeding	ISO 11267*
Earthworms: *Eisenia fetida* (macro-fauna)	Mortality Reproduction Avoidance	Bulk soil	Pore water	Pore water, feeding, skin uptake	ISO 11268-1* ISO 11268-2* ISO 17512-1*
Higher plants: Mono-/dicotyledonous species	Emergence, growth	Bulk soil	Pore water	Pore water	ISO 11269-2*

Table 2 (*continued*)

Organism	Measuring parameter	Habitat	Most important exposure route		Method
			For soluble substance	For poorly soluble substance	
Soil function affected: Habitat function					
Juvenile land snails*:* *Helix aspersa aspersa*	Growth	Bulk soil	Pore water	Skin uptake, feeding	ISO 15952*
Oxythyrea funesta	Mortality				ISO 20963
Brassica rapa, Avena sativa	Growth, reproduction	Bulk soil	Pore water	Pore water	ISO 22030
Caenorhabditis elegans (Nematoda)	Growth, fertility, reproduction				ISO 10872 (ISO/TC147/SC5/ N0583)
Soil function affected: Retention function					
Vibrio fischeri: luminescent bacteria test	Inhibitory effect of water samples on the light emission	Surface water			ISO 11348-1 ISO 11348-2 ISO 11348-3
Desmodesmus subspicatus *Pseudokirchneriella subcapitata*	Growth inhibition	Surface water			ISO 8692
Daphnia magna Straus (cladocera, crustacea)	Inhibition of the mobility Reproduction and survival	Surface water			ISO 6341 ISO 10706
Ceriodaphnia dubia	Reproduction and survival	Surface water			ISO 20665
Brachionus calyciflorus	Reproduction	Surface water			ISO 20666

NOTE Table 2 mostly considers the assessment of bioavailability of contaminants through ecotoxicological tests (i.e. toxicological bioavailability). Even if it is not yet included in the protocols, it is also possible to measure the amount of contaminants in the exposed organism during or at the end of the test; together with information about the effects this will provide better information on the (environmental) bioavailability of contaminants (e.g. by determination of bioaccumulation factor, uptake and excretion rate). Such an approach is already under development [12], [39].

When the bioavailability of contaminants of soils coming from more or less frequently submerged soils/sediments is to be measured, some of the methods described in Table 2 may not be relevant. If conditions in the sediment (e.g. ionic strength, moisture content or redox) differ substantially from conditions found in soil, methods involving fresh water or marine organisms may be more suitable. For that reason, such methods have been listed in Annex B.

9 Requirements

9.1 General

Bioavailability can be assessed by different principles (see Clause 6). Before starting an assessment, the specific protection goal, depending on the intended use of the soil, shall be defined (target to be protected, level of protection, contaminants to be taken into consideration). This implies that methods have to be applied or developed for a specific goal. This clause gives the requirements for selection and application and also the requirements for development of new methods.

Although there are still substantial deficiencies in available methods for the determination of bioavailable fractions of contaminants, the available knowledge, as described in this International Standard makes it feasible to already include the concept of bioavailability in risk assessment. This concept aims to reach a more realistic and site-specific risk assessment. In addition it allows for a more cost effective approach in assessment procedures, where biological testing can be replaced by chemical measurements that have been demonstrated to predict the bioavailability of defined contaminants for a specific target organism under certain soil conditions. However, biological testing remains particularly valuable in situations of complex mixtures of contaminants that may be present in contaminated soils.

9.2 Requirements for selection and application

9.2.1 Requirements for selection

As previously discussed, chemical and ecotoxicological tests may be used to assess bioavailability. The first step is to decide whether a single test (chemical or ecotoxicological) may be enough to solve the problem or if both are needed. This will mainly depend on the contaminants to be evaluated and on the available methods. For bioaccumulating contaminants (e.g. trace elements, persistent organic pollutants) the most direct way to estimate bioavailability is to measure the amount of the contaminant in the organism to be protected. If this is not possible for ethical or technical reasons, then indirect methods such as chemical and ecotoxicological tests have to be used together, unless it has been demonstrated that a significant correlation exists between them. In that case, chemical tests may be used as a surrogate for biological tests. As less is known about non-bioaccumulating contaminants, chemical and ecotoxicological tests have to be conducted together to assess bioavailability.

When achieving this first step, to choose the relevant method, the following general requirements shall be met.

- The method to be used shall have a mechanistic/physiological basis.
- Unless the target itself changes the soil properties, a method for determination of the actual availability has to be conducted with a sample with unchanged soil properties and the method itself shall also not change the soil properties.
- For the retention function, methods based on the pore water concentration are preferred.
- For the habitat function also, the adsorbed amount in direct equilibrium with the pore water has to be considered, especially if the solubility of the contaminant is low and the concentration in pore water is difficult to measure (detection limit, adsorption onto equipment).
- If it is possible to describe the relation between the amount in the pore water and the amount in direct equilibrium with the pore water using soil properties and thermodynamic constants, it is possible to measure either the pore water concentration or the part in direct equilibrium.

For estimating the bioavailability of metals the following principles are recommended to select relevant methods.

- The actual available fraction for several soil organisms and for plant uptake and leaching from the solid phase to the pore water can be estimated by extraction and/or leaching procedures.

- As the bioavailability of complexed species is believed to be much lower than that of the free metal ion, it is recommended to directly measure the free metal concentration.

- The potentially available fraction for the above-mentioned targets can be determined by dilute acid extraction.

- If the potential and actual available fractions are known, availability at other pH-values can be calculated/estimated for the same soil.

- Oral uptake by humans can be simulated by physiologically based procedures encompassing the acidic conditions occurring in the stomach and the close to neutral conditions occurring in the intestine system.

NOTE 1 Depending on the situation, uptake via soil pore water cannot explain the total bioavailable fraction and other exposure routes can become important, for example, uptake via skin (direct soil contact) or via ingestion.

Specific controlling mechanisms for essential and non-essential metals have to be considered.

For estimating the bioavailability of organic pollutants, the following principles are recommended to select relevant methods:

- Actual available fractions for soil organisms, plants and leaching from the solid phase to the pore water can be estimated by measuring the substance concentration in pore water (for components having $\lg K_{\text{oc}}$ value < 3). For more hydrophobic compounds, methods such as SPME can be used to establish the amount in the water phase. Different materials are available, which are applicable depending on the adsorbing properties of the soil material, for instance the presence of soot [40].

- The potentially available fraction of contaminants ($\lg K_{\text{oc}} > 3$) can be estimated by a three-phase extraction (soil, solution and an excess of hydrophobic phase). Different phases (Tenax, cyclodextrin, XAD) can be used and will give comparable results.

- For poorly soluble substances ($\lg K_{\text{oc}} > 3$), other exposure routes may become important, for example uptake via skin (direct soil contact) or via ingestion.

NOTE 2 There is no validated method available yet for oral uptake by humans.

Different ecotoxicological methods can and should be used depending on the situation to be assessed. Considering the complexity of the ecosystem, usually a battery of tests has to be applied. In order to increase the efficiency of the testing efforts, the following criteria can be used to select the relevant methods.

- The soil function(s) to be protected determine the tests to be done: for example, whether it is the retention or the habitat function.

- For each soil function, the protection goal can be specified further (e.g. whether the focus is on soil organisms or plants).

- Before starting a test, it has to be secured that the test species is not negatively influenced by the actual test conditions, in particular soil properties.

- Also, the sensitivity of the test species to the specific contaminant has to be specified.

9.2.2 Requirements for application

When applying the selected relevant method, the following information is needed to interpret the results.

- In addition to the result of the specific measurement, the method used to establish bioavailability and the scope of the used method shall be reported.

- A key area for bioavailability methods is the sample pretreatment protocol. This aspect must be rigorously defined, as small changes in sample pretreatment can have very large effects on the results (e.g. particle size reduction, temperature variations, poor pH control).

- All factors having influence on the availability of the contaminant shall be reported. Soil characteristics related to the type of sample and type of contaminant are distinguished. For heavy metals, pH, organic matter content, clay content and DOC are important parameters. In specific situations, the redox potential (e.g. water-saturated soils and sediments) or electrical conductivity (saline soils) also influences the availability. The availability of non-polar organic contaminants is influenced by both the particulate organic matter content and by DOC (especially if $\lg K_{\mathrm{oc}} > 3$). If the organic contaminant has acid or basic properties, the other soil characteristics are of interest.

- When a soil extract is measured, a key issue for chemical assays is the final filtration/centrifugation step to remove all particulate and colloidal matter that would not penetrate the biological membranes of the relevant target organisms. If this is not achieved, then the reported results cannot be properly interpreted.

- If soil properties are changed by the target organism, this shall be included in the method and reported (e.g. oral intake, long-term pH changes of soil).

- The ecological relevance of the test results gained with the selected procedure must be presented (e.g. in case the method was developed for a temperate climate but is used for Mediterranean conditions).

- A report format, which includes minimum test requirements, should be added to the results.

9.3 Requirements for development

When developing new chemical methods to estimate bioavailability, the following list shall be taken into account.

- The methods shall have a mechanistic/physiological basis which shall be described in relation to the conceptual views underlying the endpoints for which the methods are designed.

- The scope of the method (soil types, organisms, properties of contaminants) should be clearly formulated.

- It is necessary to correlate the results of chemical methods and biologically measured effects.

NOTE It is possible that an effect is measured and no bioavailable contaminant is detected by routine chemical analysis. This case is outside the scope of this International Standard and further research is necessary.

- It can be necessary to pretreat the soil sample to be evaluated (e.g. reduce particle size, change the moisture, adjust or buffer the pH, filtrate or centrifuge the extract). These treatments may influence the measured bioavailability. The effect of each relevant treatment step shall be established during method development.

- It is recommended to concentrate on a limited number of methods with a potentially broad range of applications and to validate these methods in a round robin test.

- After establishing the ruggedness of a method, the method shall be validated in an inter-laboratory study. As part of the validation process, both reference soils as well as reference substances should be used.

For the development of new ecotoxicological methods to estimate bioavailability, test species should be selected according to the following criteria:

- taxonomic and physiological group in order to cover different life forms and exposure pathways (e.g. pore water and bulk soil);

- good knowledge of their ecological requirements, in particular with regard to soil properties;

- use of different endpoints measuring chronic (sublethal) effects (including behavior like avoidance) as well as the accumulation of a contaminant;
- broad sensitivity towards different classes of chemicals;
- ideally testable on different investigation levels (e.g. laboratory, microcosms);
- in the long-term, different regions (in addition to temperate conditions) have to be covered adequately;
- practicability in terms of resources and efforts (including the requirements of chemical analysis).

Annex A
(informative)

Bioavailability in relation to biodegradability

For bioremediation of degradable organic contaminants on a landfarm [16], the conceptual definition of bioavailability is:

Bioavailability is the amount of contaminant that can be transported (by diffusion or physical change of the adsorption site) to a site with a degrading micro-organism in a defined time period and that can enter the bio-influenced zone of this organism.

Because the time period may vary, the bioavailable fraction is not a fixed amount. In bioremediation, bioavailability for a specific process under defined conditions might be defined operationally as:

Bioavailability of the contaminant is the amount that is degraded on a landfarm in one year, under aerobic conditions and under specified climate conditions.

As a tool to predict the biodegradation, chemical measurements are used, which extract a certain amount from the sediment. Because the mechanisms of these methods are understandable, this tool is in agreement with the conceptual definition as illustrated in Figure A.1 [41].

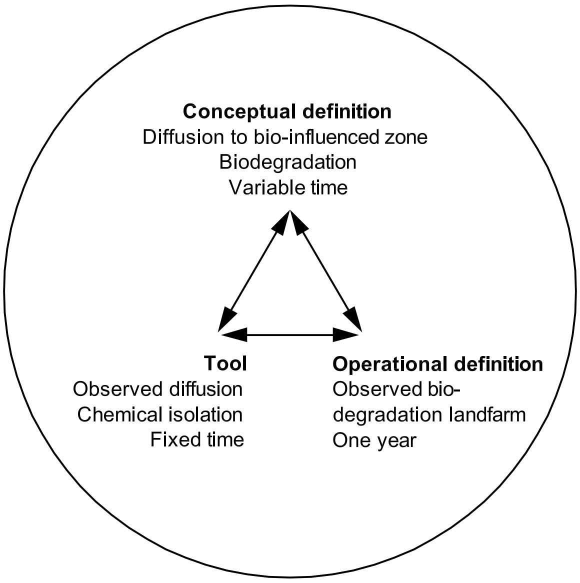


Figure A.1 — Example of a relation between conceptual and operational definition of bioavailability and the tool to estimate bioavailability in bioremediation (landfarming)

Bioavailability can also be used in relation to other assessment purposes. If it is used in the ecotoxicological sense, biodegradation in the operational definition as given above should be replaced by storage in the organism or effect on the ecosystem. Pathways can also be part of the definition. This will also change the operational definition and the tool. It is necessary to specify the conceptual and operational definition for every assessment purpose. This makes it possible to make a proper choice of tools to be used.

Annex B
(informative)

International Standards for the determination of the toxicity of chemicals on sediment organisms (laboratory tests)

Organism	Measuring parameter	Habitat	Most important exposure route		Method
			for soluble substance	for poorly soluble substance	
Function affected: Habitat function					
Corophium volutator and other marine species	Mortality	Marine and/or estuarine sediment	Pore water	Feeding	ISO 16712
Chironomus sp.	Survival/weight of larvae Emergence	Freshwater sediment	Pore water	Feeding	OECD TG 218
Lumbriculus variegatus	Reproduction/ Biomass	Freshwater sediment	Pore water	Feeding	OECD draft TG 225
Caenorhabditis elegans (nematodes)	Growth, fertility, reproduction	Freshwater sediment	Pore water		ISO 10872 (currently ISO/TC147/SC5/ N0583)
Function affected: Retention function					
Vibrio fischeri: luminescent bacteria test	Inhibitory effect of water samples on the light emission	Surface water			ISO 11348-1 ISO 11348-2 ISO 11348-3
Desmodesmus subspicatus *Pseudokirchneriella subcapitata*	Growth inhibition	Surface water			ISO 8692
Daphnia magna Straus (Cladocera, Crustacea)	Inhibition of the mobility Reproduction and survival	Surface water			ISO 6341 ISO 10706
Ceriodaphnia dubia	Reproduction and survival	Surface water			ISO 20665
Brachionus calyciflorus	Reproduction	Surface water			ISO 20666
Skeletonema costatum, Phaeodactylum tricornutum	Growth inhibition	Marine surface water			ISO 10253
Marine copepods *(Acartia tonsa, Tibse battagliai, Nitocra spinipes)*	Mortality	Surface water			ISO 14669
Amphipods	Mortality	Marine, water phase of sediments			ISO 16712

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[48] ISO 10872, *Water quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of* Caenorhabditis elegans (Nematoda)[1)]

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

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

[1)] Currently at the stage of a Committee Draft by ISO/TC 147/SC 5.

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

[52] 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*

[53] ISO 11348-1, *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria*

[54] ISO 11348-2, *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test) — Part 2: Method using liquid-dried bacteria*

[55] ISO 11348-3, *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test) — Part 3: Method using freeze-dried bacteria*

[56] ISO 14238, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

[57] ISO 14240-1, *Soil quality — Determination of soil microbial biomass — Part 1: Substrate-induced respiration method*

[58] ISO 14240-2, *Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method*

[59] ISO 14669, *Water quality — Determination of acute lethal toxicity to marine copepods* (Copepoda, Crustacea)

[60] ISO 15175:2004, *Soil quality — Characterization of soil related to groundwater protection*

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

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

[63] ISO 15800, *Soil quality — Characterization of soil with respect to human exposure*

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

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

[66] ISO 16712, *Water quality — Determination of acute toxicity of marine or estuarine sediment to amphipods*

[67] ISO 17155, *Soil quality — Determination of abundance and activity of the soil microflora using respiration curves*

[68] ISO 17512-1, *Soil quality — Avoidance test for testing the quality of soils and effects of chemicals on behaviour — Part 1: Test with earthworms (*Eisenia fetida *and* Eisenia andrei*)*

[69] ISO/TS 17924, *Soil quality — Assessment of human exposure from ingestion of soil and soil material — Guidance on the application and selection of physiologically based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil*

[70] ISO 18772, *Soil quality — Guidance on leaching procedures for subsequent chemical and ecotoxicological testing of soils and soil materials*

[71] ISO 19258, *Soil quality — Guidance on the determination of background values*

[72] ISO 20665, *Water quality — Determination of the chronic toxicity to* Ceriodaphnia dubia

[73] ISO 20666, *Water quality — Determination of the chronic toxicity to Brachionus calyciflorus in 48 h*

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

[75] ISO/TS 21268-2, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 2: Batch test using a liquid to solid ratio of 10 l/kg dry matter*

[76] ISO/TS 21268-3, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 3: Up-flow percolation test*

[77] ISO/TS 21268-4, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 4: Influence of pH on leaching with initial acid/base addition*

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

[79] ISO 23753-1, *Soil quality — Determination of dehydrogenase activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC)*

[80] ISO 23753-2, *Soil quality — Determination of dehydrogenase activity in soils — Part 2: Method using iodotetrazolium chloride (INT)*

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