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# Soil quality — Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent

*Qualité du sol — Disponibilité environnementale des composés organiques non polaires — Détermination de la fraction potentiellement biodisponible et de la fraction non biodisponible en utilisant un agent adsorbant fort ou un agent complexant*

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# Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

# Introduction

The solubility of most non-polar organic contaminants is limited and they are sorbed to the soil matrix. They may desorb and become available for organisms, which may result in an effect (toxicity, degradation or bioaccumulation). Not all sorbed (adsorbed and absorbed) contaminants will desorb and become available.

Extractions used in chemical analysis to measure the total concentration, release more contaminants from the soil than are available. It is however also possible that contaminants are so strongly bounded by the soil that they will not be released by chemical extraction. This strong sorption may also be caused by incorporation of the contaminant (or a degradation or reaction product of the contaminant) in the organic soil structure. The distribution of contaminants over sorption sites of varying sorption strength is not constant in time and contaminants will shift, with increasing contact time, to the stronger sorption sites.

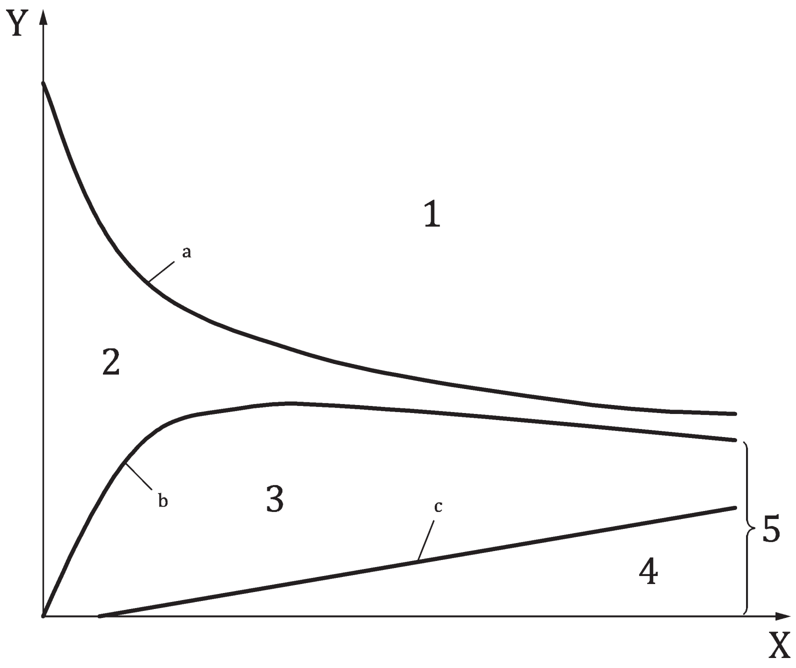
Figure 1 shows schematically the differentiation between:

— extractable residues that are also bioavailable (i.e. the potentially bioavailable fraction);

— residues that are extractable by harsher extraction methods but are non-bioavailable;

— residues that are neither extractable nor bioavailable.

If a degradable substance enters a soil, part of it will degrade over time (curve a). The area between curve a and c is extractable by exhaustive chemical procedures. For risk assessments, this part is considered as the “total concentration” for which values are defined in many regulations. However, only a part of this amount is bioavailable. The area between curves a and b is the bioavailable fraction and the area between curves b and c is the non-bioavailable fraction. The method described in this document enables the measurement of the potential bioavailable and the non-bioavailable fraction of a contaminant in soil.



**Key**

| | |
|---|---|
| X | time |
| Y | contaminant concentration |
| 1 | degradable |
| 2 | bioavailable |
| 3 | extractable, non-bioavailable |
| 4 | non-extractable: persistent residues |
| 5 | non-available fraction |

NOTE For curves a, b and c see description in text above.

**Figure 1 — Temporal changes in extractable/bioavailable fractions, extractable/non-bioavailable fractions and non-extractable/non-bioavailable fractions of a non-polar organic contaminant (modified from [1])**

In the scientific research to bioavailability a large number of definitions and concepts are in use, which reflect the discussion in the scientific world. However, a more clear and simple approach is necessary. In regulation, organic contaminants are either bioavailable or non-bioavailable. To support decisions, both should be measurable. Therefore, this document follows the approach of Ortega-Calvo et al. (2015) [2] as illustrated in Figure 2. In this approach all defined fractions are measurable as further explained in Clause 4.

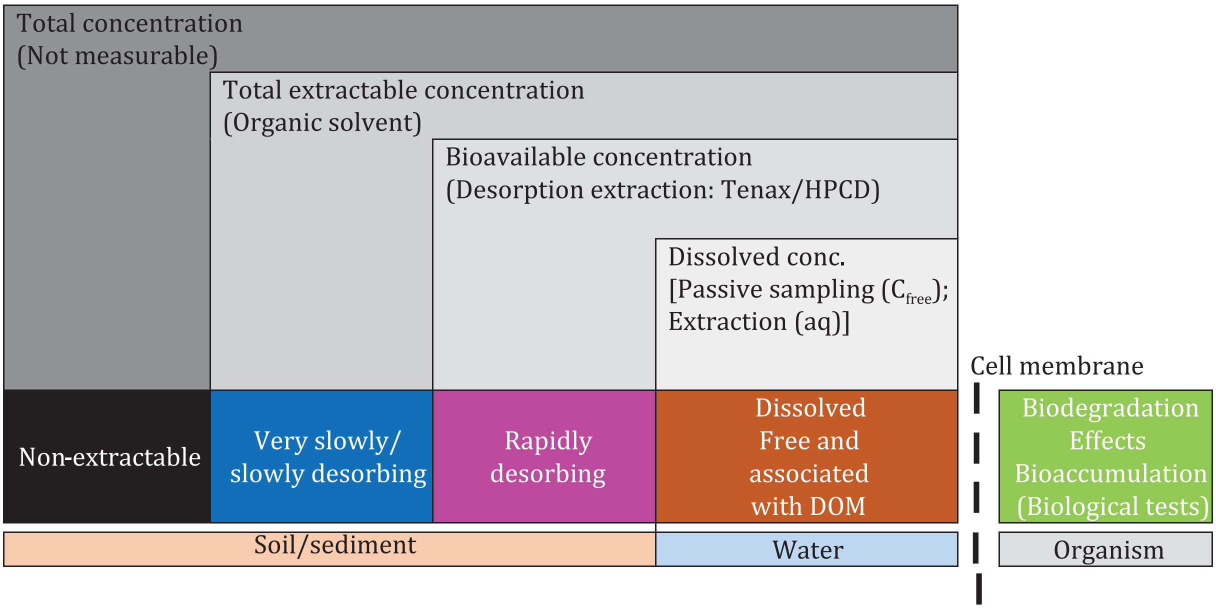


**Figure 2 — Measurement of bioavailability of organic chemicals: a simplified scheme for use in regulation[9]**

The colour boxes at the left of the biological membrane represent the distribution of pollutant molecules among four classes (non-extractable, very slowly/slowly desorbing, rapidly desorbing and water-dissolved) in soils and sediments. In the scheme in Figure 2, the bioavailable chemical is represented by the rapidly desorbing and dissolved concentrations. The chemical methods able to measure the pollutant present in each specific fraction are given in the grey boxes. The green box to the right of the cell membrane represents the processes that occur within the organism exposed to the pollutant. These biological processes can also serve as the basis for standard methods used for bioavailability measurements.

As presented in Figure 2, the bioavailable fraction can be measured using the method described in this document.

# Soil quality — Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent

## 1 Scope

This document specifies an extraction method to determine the bioavailable (potential and environmental available) fraction and the non-bioavailable fraction of a contaminant in soil using a "receiver phase" for an organic contaminant with strong sorbing or complexing properties, for example, Tenax®[1] or cyclodextrin, respectively.

NOTE 1 The bioavailable fraction is defined in ISO 17402 as environmental bioavailability.

The method is applicable for non-polar organic contaminants with an aqueous solubility of <100 mg/l. The method is applicable for soil and soil-like material including (dredged) sediments.

NOTE 2 The method is theoretically applicable to non-polar organic contaminants with an aqueous solubility of 1 000 mg/l. The method has been often applied for compounds with a much lower solubility ($K_{ow} > 3$) and less for compounds with a higher solubility. The applicability is therefore defined for compounds with an aqueous solubility of <100 mg/l.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *Soil quality — Vocabulary*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11074, ISO 17402 and the following apply.

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

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

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[1] Tenax® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

### 3.1

**potential bioavailable fraction**

amount of contaminant present in the matrix that can be released from the solid phase to the aqueous phase in a well-mixed water soil mixture and in presence of a receiving phase in a period of 20 h

Note 1 to entry: In ISO 17924 the term bioaccessibility is used, which is the fraction of a substance in soil or soil-like material that is liberated in (human) gastrointestinal juices and thus available for absorption. This document does not distinguish between bioavailability and bioaccessibility and uses the general term bioavailability. The concept of bioavailability as followed in this document is described in the introduction of this document.

## 4 Principle

This method gives an estimation of the potential bioavailable and non-bioavailable fraction of organic contaminants, i.e. the amount of the contaminant in the matrix that is potentially exchangeable with the aqueous phase; specifically that, which is adsorbed/complexed by Tenax®/cyclodextrin.

The extractable and non-bioavailable fraction of the contaminant left in the sample following the action of Tenax®/cyclodextrin can be subsequently measured with an exhaustive/harsh extraction technique (designed to measure the total concentration) and in this way the non-bioavailable fraction of the contaminant is assessed.

Thus, in numerical terms, the total contaminant concentration in a sample is the sum of the bioavailable concentration (established using a strong sorbent or complexing agent) and the non-bioavailable concentration (established using a subsequent harsh extraction method performed on the residue that is left after the matrix has been extracted using a strong sorbent or complexing agent):

$$c_{\text{tot,cont}} = c_{\text{bio}} + c_{\text{non-bio}} \tag{1}$$

where

$c_{\text{tot,cont}}$ is the total contaminant concentration;

$c_{\text{bio}}$ is the bioavailable concentration;

$c_{\text{non-bio}}$ is the non-bioavailable concentration.

The soil, soil-like material or sediment sample with particle size <2 mm is extracted with water containing a “receiver phase” for the organic contaminants. This phase is either a complexing agent (cyclodextrin) or a strong adsorbent [Tenax®]. The solubility of non-polar compounds is limited and in this method the receiver phase acts as an “infinite sink”. The measured amount, which is the amount that desorbs from the soil or soil-like material during 20 h, reflects the fraction of contaminant that can have effects on biotic systems and that can become mobile.

In the following step, the contaminants adsorbed are extracted from the receiver phase and determined by appropriate analytical methods. The amount of contaminants left in the soil residue, the non-bioavailable fraction, can be measured using a subsequent harsh/exhaustive extraction designed to measure the total concentration. Formula (1) can then be used to determine the total contaminant concentration in the sample (if desired).

NOTE 1 ISO 13859 and ISO 13876 are examples to measure the total concentration of respectively PAH and PCB in soil and soil-like materials.

NOTE 2 Formula (1) shows the relationship between the “total contaminant concentration” the “bioavailable concentration” and the “non-bioavailable concentration”. If two of the concentrations are known the third concentration can be calculated through the use of Formula (1). For example, by measuring the “total concentration” and the “non-bioavailable concentration”, the “bioavailable concentration” can be calculated. This is allowed with homogeneous materials. If it is not known whether a material is homogeneous and the bioavailable concentration is the concentration of interest, the bioavailable concentration needs to be measured.

## 5 Reagents

Reagents used shall be of suitable grade – analytical grade or higher– if not specified otherwise. The blank value of the reagents (including water) shall be negligible compared to the lowest concentration of organic contaminants to be determined.

**5.1 Demineralized water** or **deionized water** or **water of equivalent purity** ($5 < \text{pH} < 7{,}5$) with a conductivity of <0,5 mS/m in accordance with grade 3 specified in ISO 3696.

If biodegradation of the target compounds is to be expected, add sodium azide (5.2) to water to a final concentration of 0,2 g/l. This will minimize the biological degradation of the target compounds. If biodegradation is not to be expected, it is not necessary to add sodium azide. This is the case for some persistent target compounds, e.g. PCB.

NOTE With some soil samples it can be difficult to obtain a proper separation between the soil, aqueous phase and Tenax®. The use of 0,001 mol/l or 0,01 mol/l $CaCl_2$ (5.9) instead of water can improve this separation.

**5.2 Sodium azide**, [CAS No. 26628-22-8], $NaN_3$.

**WARNING — Attention is drawn to the hazard deriving from the use of the sodium azide which is acutely toxic.**

**5.3 Cyclodextrin**, (hydroxypropyl-$\beta$-cyclodextrin) of >97 % purity of a Food Grade, Medicine Grade or Pharmaceutical Grade.

NOTE Analytical Grade cyclodextrin is very expensive. For this method Food Grade has shown to be fit for purpose.

**5.4 Extraction solution of cyclodextrin**, dissolve 100 mmol (=146 g) of cyclodextrin (5.3) per litre of water (5.1).

NOTE If biodegradation of the target compounds is to be expected, add sodium azide (5.2) to this solution to a final concentration of 0,2 g/l. This will minimize the biological degradation of the target compounds. If biodegradation is not to be expected, it is not necessary to add sodium azide. This is the case for some persistent target compounds, e.g. PCB

**5.5 2,6-diphenyleneoxide polymer (Tenax® TA)**, 60 mesh to 80 mesh. See Annex A for the preparation and regeneration of Tenax®.

**5.6 Petroleum ether,** [CAS No. 8032-32-4], boiling range 40 °C to 60 °C.

**5.7 Ethanol**, [CAS No. 64-17-5], $C_2H_6O$.

**5.8 Acetone**, [CAS No. 67-64-1], $C_3H_6O$.

**5.9 Calcium chloride**, [CAS no.1035-04-8], $CaCl_{2.}$

**5.10 Sodium sulfate**, [CAS No. 231-820-9], $Na_2SO_{4.}$

## 6 Apparatus

Use the following equipment. All materials that come into contact with the sample (or reagents) shall not adsorb the contaminant of interest and shall not contaminate the sample. Glass and PTFE are suitable materials for most contaminants.

**6.1 Sieving equipment**, with 2 mm nominal screen size.

**6.2 Balance**, accuracy 0,01 g.

**6.3 Conical centrifuge tubes**, with screwing caps.

**6.4 Separation funnel**, of suitable size.

NOTE The hole in the tap of the separation funnel used for the Tenax® extraction needs to be large enough for the soil particles to leave the funnel. Otherwise no separation between Tenax® and the sample is possible.

**6.5 Shaking machine**, which limits breakdown of sample particles, e.g. an end-over-end shaker, capable of (20 ± 2) r/min or other mild agitation method or a horizontal movement shaker, capable to have 150 r/min to 180 r/min.

**6.6 Centrifuge**, capable to centrifuge the centrifuge tubes (6.3).

**6.7 Crushing equipment**, jaw crusher or cutting device.

**6.8 Appropriate glassware and plastic ware**.

**6.9 Kuderna Danish sample concentrator**.

**6.10 Collection vessel**.

**6.11 Folded filter**.

## 7 Procedure

### 7.1 Sample preparation

The sample shall be pretreated in the laboratory in accordance with ISO 14507, but with the following restrictions:

- Intensive pretreatment like grinding may have an effect on the environmental availability of contaminants, therefore grinding is not allowed.
- In general, the test portion to be prepared shall have a grain size less than or equal to 2 mm, but on no account the material shall be ground to reach this grain size.
- Remove stones, shells and any material not representative for the sample.
- Sieve the sample using a sieve (6.1). If necessary, press the material by hand through the sieve.

Instead of using ISO 14507, also freeze-drying according ISO 16720 may be used to pretreat the sample.

Some soils (e.g. peat and some sediments) are difficult to sieve. In these cases, remove stones, shells and material not representative for the sample (e.g. plant material) by hand and process the sample without sieving.

If the sample cannot be sieved at all because of its water content, reduce the water content until the laboratory sample can be sieved. In the case of drying, the drying temperature shall not exceed 30 °C.

If a rapidly degradable fraction is to be expected, reduction of water content by air drying is not allowed. In that case the original collected sample shall be forced by hand through a 2 mm sieve or the sample can be freeze-dried.