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Soil quality — Use of extracts for the assessment of bioavailability of trace elements in soils

Qualité du sol — Utilisation d'extraits pour l'évaluation de la biodisponibilité des éléments traces dans les sols

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Foreword

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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

As already mentioned in ISO 17402, 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. In the conservative approach of exposure assessment as typically described in a regulatory context, it is assumed that the total concentration of a contaminant present in a soil or soil-like material is available for uptake by organisms, including man, which 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.

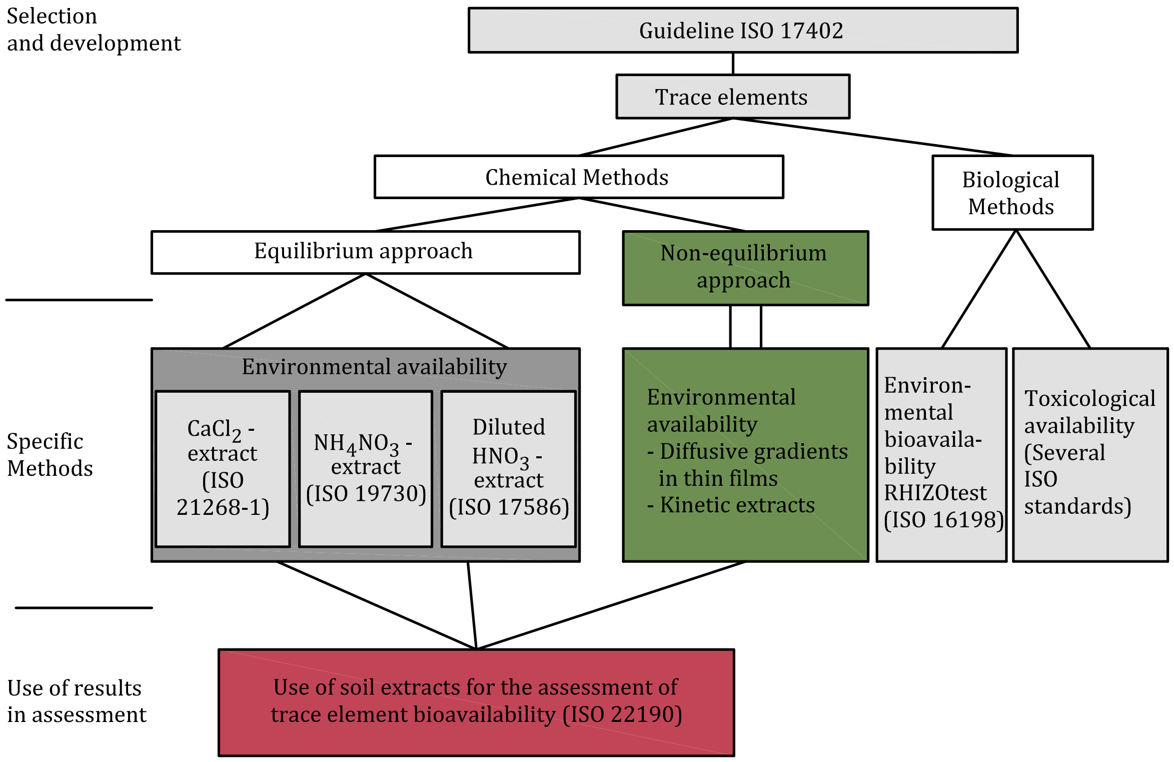
In standardization of methods for assessing the bioavailability of trace elements a framework of standards is used with the following layering of standards (see Figure 1). Starting point is ISO 17402 in which chemical and biological methods are distinguished and where guidance for selection of relevant methods is given. If a chemical method is to be used to establish environmental availability, there are the following possibilities:

a) Extractions based on equilibrium. For this approach standards are available or under development.

b) Method based on non-equilibrium. For this approach standards are not yet under development. If these standards become available they will also be included in this document (dashed line in Figure 1).

The methods referred to in this standard are all based on extraction. Extraction can be considered as a model to simulate the pore water concentration. The extraction methods give results that can be used in assessment and this standard gives guidance for that use.

The method for human bioaccessibility (ISO 17924) is not presented in Figure 1. It is an extraction method that simulates the human intestinal system and is specific for assessment of human risks.



Key

Red	this document
Grey	existing standards
Green	not yet available — for future development

Figure 1 — Layering of standards for bioavailability of trace elements (situation April 2018)

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, for regulatory purposes a more clear and simple approach is necessary. In a regulatory context, contaminants are either bioavailable or non-bioavailable. To support decisions, both should be measurable.

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

Soil quality — Use of extracts for the assessment of bioavailability of trace elements in soils

1 Scope

This document provides guidance on the use of chemical methods establishing the bioavailability of trace elements in soil and soil-like materials and to stimulate the use of bioavailability in assessments. The methods themselves are not subject of this document.

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 17402, *Soil quality — Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials*

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/

3.1
bioavailability
degree to which chemicals present in the soil can be absorbed or metabolised by a human or ecological receptor or are available for interaction with biological systems

Note 1 to entry: The concept of bioavailability is further explained in ISO 17402.

Note 2 to entry: This document follows the approach of Reference [20] as illustrated in Figure 2, in which all defined fractions are measurable as further explained in Clause 4.

Note 3 to entry: In ISO 17924 a definition specific for human uptake through ingestion is defined as the fraction of a substance present in ingested soil that reaches the systemic circulation (blood stream).

[SOURCE: ISO 11074:2015, 5.2.2, modified — Note 2 to entry was added and the following note to entry renumbered.]

3.2
environmental availability
fraction of contaminant physico-chemically driven by desorption processes potentially available to organisms

[SOURCE: ISO 17402:2008, 3.3]

3.3
environmental bioavailability
fraction of the environmentally available compound which an organism takes up through physiologically driven processes

[SOURCE: ISO 17402:2008, 3.5]

3.4
toxicological bioavailability
internal concentration of pollutant accumulated and/or related to a toxic effect

3.5
actual availability
concentration present in the soil pore water to which organisms are directly exposed.

Note 1 to entry: This definition refers to internal concentrations in humans, mammals and other organisms.

[SOURCE: ISO 17402:2008, 3.18]

3.6
potential availability
amount present in the soil sample (mg/kg) that can be released from the solid phase to the pore water within a specific time frame

3.7
bioaccessibility
fraction of a substance in soil or soil-like material that is liberated in (human) gastrointestinal juices and thus available for absorption or the amount available to cross an organism’s cellular membrane from the environment if the organism has access to the chemical

Note 1 to entry: See ISO 10390 for more information on the chemical.

[SOURCE: ISO 17924:2018, 3.2, modified — The definition was modified by adding "or the amount available to cross an organism’s cellular membrane from the environment if the organism has access to the chemical" and a Note 1 to entry was added.]

4 Background

4.1 General

4.1.1 Presence of trace elements in the soil matrix

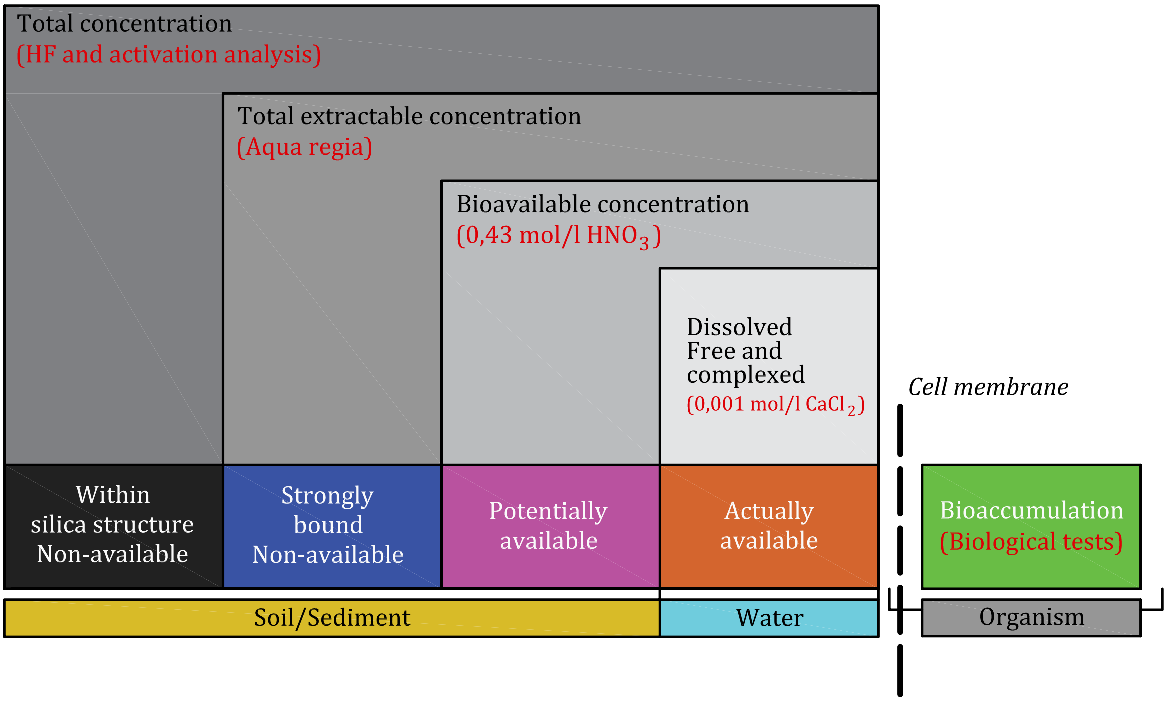
Because the total exposure of organisms depends on time, the available fraction is not a fixed fraction, but should be divided into multiple fractions or be described as a continuum. The release of the contaminants depends on local environmental conditions (e.g. pH). The simplest approach determines:

a) an actually available fraction or the actual dissolved amount at ambient conditions;

b) a potentially available fraction, which is the maximum amount that can be released to the soil pore water under (predefined) worst-case conditions. This can also be expressed as the reactive fraction;

c) a non-available fraction.

All together represent the total concentration. For environmental purposes it is generally accepted that the amount measured using aqua regia (see ISO 11466) represents the total concentration. In Figure 2 this is called the total extractable concentration. The ‘real’ total concentration also includes the amount within the silica matrix. To measure this amount an HF extraction should be included (see ISO 14869-3).



NOTE The coloured boxes at the left of the biological membrane represent the distribution of pollutant molecules among four classes (Within the silica structure, strongly bound, potential available and actual available) in soils and sediments. In the scheme in Figure 2, the bioavailable chemical is represented by the potential an actual available 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.

Figure 2 — Measurement of bioavailability: a simplified conceptual framework
(Source: Modified from ISO 8245[1])

The subdivision in terms of dissolved/actual and potential bioavailability is important, because it broadens the role of the pore water. Bioavailable is 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 "bio-influenced" zone could be defined[3]. This zone comprises the pore water and depending on the organism, parts of the soil matrix. Consequently, the available amount may have different values. Thus, there could be numerous bioavailabilities depending on the type of target organisms and time scale and, in turn, there could be numerous specific definitions (operational definition).

The bioavailability of trace elements for several organisms (flora and fauna) is regulated by the concentration and the speciation of trace elements in the water phase and the solid phase of soil (environmental availability). From a chemical point of view, this concentration can be expressed as (see ISO 17402):

a) The dissolved concentration and its chemical speciation at ambient conditions, which can be characterised as
    1) Free ion (activity);
    2) Total concentration dissolved, including inorganic and organic complexes.
b) The amount bound to the soil solid-phase that can re-supply the dissolved concentration when the latter is depleted during repetitive and ongoing uptake processes by organisms, for example the maximum amount that can be released under (predefined) worst-case conditions.

It is noteworthy that this document suggests methods, i.e. soil extracts, based on an equilibrium approach. Indeed, the time course of the different extraction methods is usually long enough to reach the equilibrium between trace elements in the soil solid-phase and the water phase. Consequently, these methods are only suitable for the assessment of the environmental bioavailability when this is driven by soil equilibria rather than by kinetic constraints (non-equilibrium approach).

4.1.2 Neutral extracts for measurement of actual availability (ISO 21268-1)

For regulatory purposes simple and cheap methods are required and a simple extraction that simulates the pore water quality is desirable. A neutral aqueous solution (i.e., limiting changes of the soil pH during extraction as much as possible) can be used for this purpose. The concentration of trace element measured in a neutral extract is assumed to reflect the concentration in the pore water [as well as ionic strength, temperature, pH, DOC (Dissolved Organic Carbon)]. These properties may show a variation during the year and can be influenced by external factors (e.g., rain, drought, addition of manure). Extraction of a soil sample with demineralised water may have impact on the soil. For the purpose of estimating the actual availability of trace elements, it is desirable to reduce the influence of external factors and to obtain data that are more independent of the time of sampling. Extraction procedures have been developed using aqueous solutions containing a fixed concentration of a specific salt (neutral extract) in order to simulate the soil pore water.

The stronger the extract (high ionic strength), the higher the amount of trace element released from the soil solid phase. On the other hand, the concentration of extracted DOC is also dependent on the choice of the neutral extract especially the concentration of divalent cations (Ca^{2+}) affects DOC. The ratio soil/extract also affects the DOC concentration[4]. With a higher amount of DOC extracted, a higher amount of extracted trace elements can be expected, especially trace elements with a high affinity for binding to DOC (e.g. Cu, Pb, Cr).

Originally 0,01 mol/l $CaCl_2$ has been applied as neutral extractant. For several soils this methods reflects the pore water concentration[5][6][7][8]. A concentration of 0,01 mol/l $CaCl_2$ is often higher than can be measured in the pore water and consequently 0,01 mol/l $CaCl_2$ can reduce DOC below concentrations in actual pore water, thereby having an effect on the amount of trace elements dissolved[9][10]. In this document, the 0,001 mol/l $CaCl_2$ extract (ISO 21268-1) is adopted as the currently most suitable soil extraction method, enabling an estimation of trace element concentration in the water phase with a result close to the actual pore water concentration. Results from this extraction can also be used in geochemical modelling of specific bioavailable trace element species in subsequent tiers of the risk assessment, as indicated in 4.2.

Although the 0,001 mol/l $CaCl_2$ extraction is adopted in this document as a general procedure to simulate the pore water concentration of trace elements, other neutral solutions have been shown to be suitable for specific purposes (see Annex A).

NOTE The use of a high salt concentration like 1 mol/l ammonium nitrate[40] as described in ISO 19730 and 0,01 mol/l $CaCl_2$ has a positive effect on reproducibility and repeatability (see validation of ISO 19730). Results have however a lower truthiness, because high salt concentrations do not simulate the pore water composition.

4.1.3 Acid extracts for measurement of potential availability (ISO 17586)

Strong acids like HNO_3 can be used as an estimate for the potential available fraction. It will be clear that the acid extraction has a very large impact on the composition of the solution. The obtained solution has no relevance anymore to the pore water. A strong acid is a stronger solvent and will give the amount sorbed on the CEC, but also the trace elements in acid soluble salts, which is the amount that comes potentially available. At pH 0,5 the potential available fraction is estimated and this pH is approached with 0,43 mol/l HNO_3. Non potential available trace elements included in the soil matrix are not extracted at pH 0,5. These are only extracted with a method for the total concentration like aqua regia. The difference between aqua regia and the acid extract are the non-available trace elements and therefore aqua regia is not suitable to estimate the available trace elements.

4.2 Tiered approach based on bioavailability

A hierarchy in test use (tiered approach) is promoted, in which stepwise more realistic and sophisticated tests and calculations are used for the determination of environmental availability in the framework of impact assessment. At higher Tiers, more site specific information is required. The following Tiered approach is advised when the bioavailability of trace elements is to be included in soil and site assessment. In this approach, results from a previous tier can always be used in the following Tiers:

a) **First Tier:** Measurement of potential environmental availability by using 0,43 mol/l HNO_3 (ISO 17586). In this first tier also basic soil properties like clay, organic matter and pH are measured, which makes it possible to make predictions of the actual environmental availability at this initial stage.

NOTE 1 In general, a (limited) number of total concentration measurements (aqua regia) will be necessary to test compliance with regulatory limit values. These data are not suitable for assessment of bioavailability and are, therefore, completed with measurements of the potential availability.

b) **Second Tier:** Measurement of actual environmental availability using 0,001 mol/l $CaCl_2$ (ISO 21268-1) and if necessary, application of general biological test. If risks are more specified it can be preferred to use 1 mol/l NH_4NO_3 (see ISO 19730) to predict plant uptake, leaching tests to predict mobility or to use specific biological tests like ISO 16198 for plant uptake. Modelling is already part of this Tier and may ask for specific measurements. Leaching procedures, such as ISO 12782-series and ISO 21268-series may be included within the assessment.

NOTE 2 The US EPA LEAF procedure[11] makes use of comparable leaching procedures and tiered approach.

c) **Third Tier:** Site specific measurement and site specific modelling. The measurement of human bioaccessibility can be part of this tear.

NOTE 3 There is no strict separation between the second and third Tier. Depending on the risks, a method can be part of the second or the third Tier.

In this approach the concept of bioavailability is already used in the first tier. Modelling is possible in an earlier stage. However, risk assessment, requires limit values for the potential available or accessible fractions. Having these, the step to a limit value for the actual available fraction will be small.

5 General procedure using an extract

The methods mentioned in this document are suitable for soils in contact with the atmosphere. They are not applicable for strongly reducing soil-like materials like sediments.

NOTE 1 If the methods are applied to reducing or anaerobic soils and sediments, the procedure has effect on the composition, for instance by oxidation of sulphide, thereby generally increasing the concentration that will be measured.

The following steps are standardized:

- **Pre-treatment:** Apply extraction procedures to untreated soil (see ISO 14507). During pre-treatment it is allowed to remove particles that are not representative. The test portion to be prepared shall have a grain size less than or equal to 2 mm. On no account shall the material be finely ground. If the laboratory sample cannot be crushed or sieved because of its water content, it is allowed, in this case only, to reduce the water content until the laboratory sample can be sieved. The drying temperature shall not exceed ambient temperature or 30 °C. Higher drying temperatures increase the DOC and consequently the amount of several dissolved trace elements[4].
- **Extraction procedure:** A specific amount of soil and extractant are shaken during a fixed period.
- **Measurement:** The concentrations of elements in the extracting solution are determined by appropriate analytical methods. Because the contaminant is often present at a low concentration, the use of blanks is necessary.