
**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

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.

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 of 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 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

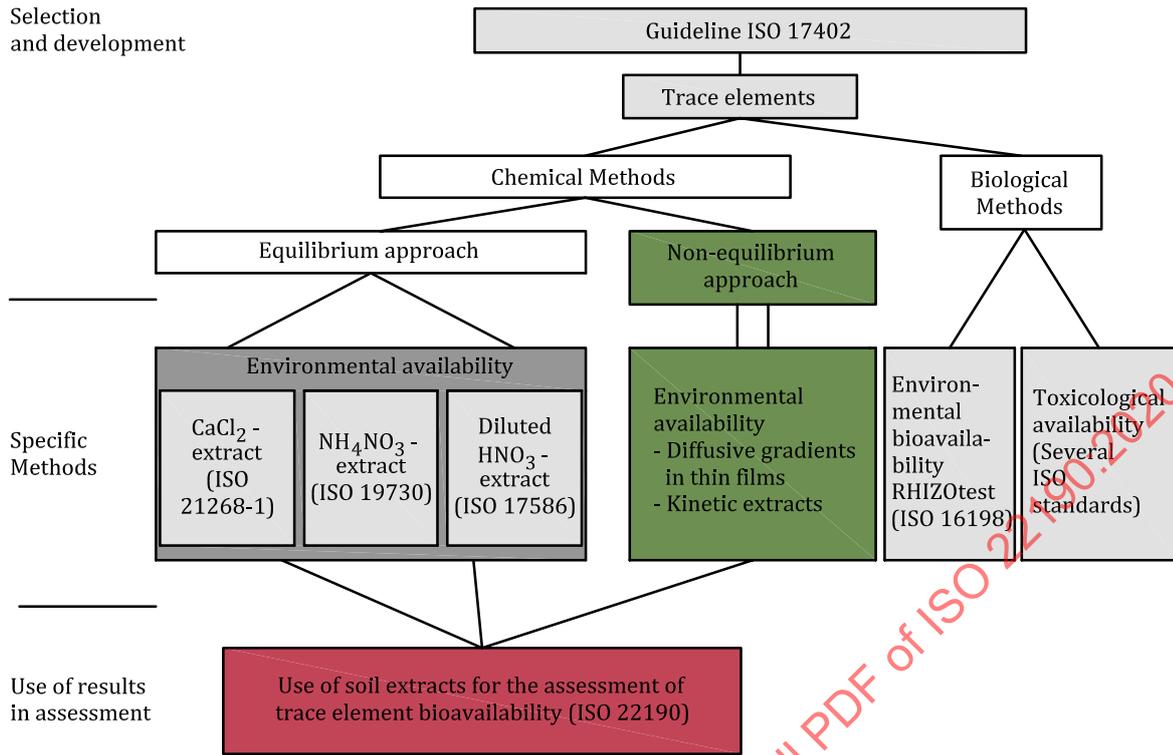
As already mentioned in ISO 17402, laboratory and field studies have demonstrated that biological effects are not related to the total concentration of a contaminant in the soil. Instead, an organism responds only to the fraction that is biologically available (bioavailable) for that organism. In the 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

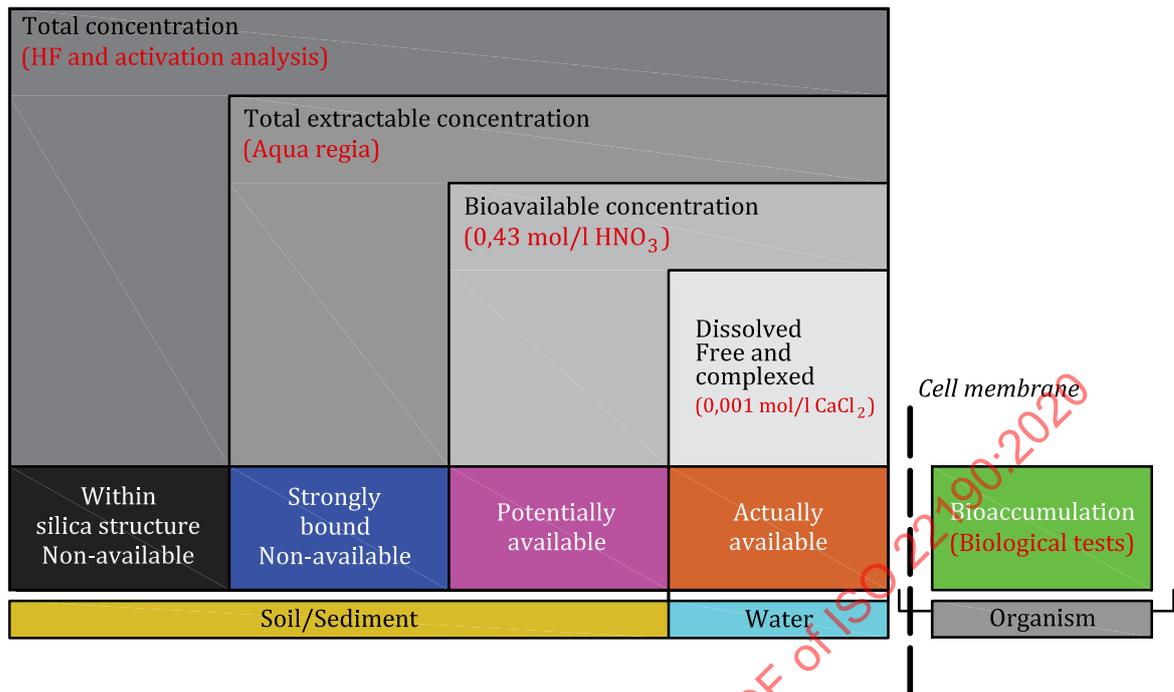
4.1 General

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 and 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 method 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 tier.

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.

An important precondition in ISO 17402 is that the method should have a mechanistic basis. Chemical interactions are important mechanisms that influence the concentration in the water phase. Therefore, measurement of only the trace element involved is not enough. The following parameters are also important:

- Soil:
 - pH (see ISO 10390);
 - clay (see ISO 11277);
 - organic matter (see ISO 10694 and ISO 12782-4);
 - Fe-/Al-oxides (see ISO 12782-1, ISO 12782-2 and ISO 12782-3).
- CaCl₂ extract:
 - pH (see ISO 10390);
 - dissolved organic matter (see ISO 8245, ISO 12782-5);
 - composition of macro parameters (see specific ISO water standards);
 - ionic strength;
NOTE 2 In most cases the salt in the extraction liquid determines the ionic strength. Saline soils may influence the ionic composition and thereby the ionic strength.
 - all other compounds that may form complexes with trace elements and are known to be present in the soil sample.

The soil parameters pH, clay and organic matter are already measured in the first tier. Specification of the organic matter and Fe-/Al-oxides are part of the second tier. The parameters in the CaCl₂ extract can only be measured if the extract is available and is therefore part of the second tier.

Having these parameters, it is possible to predict the actual availability from the potential availability using transfer functions (see [Annex B](#))^[12] or geochemical modelling. The application and use of different parameters in geochemical modelling is described in Reference [\[13\]](#) and ISO 17402.

Results of the methods are also useful for deriving soil quality standards^[15] and soil protection guidelines^[16].

6 Reporting

Results can be reported as concentration measured (mg/l) or based on the original soil (mg/kg).

For neutral extracts, the total amount extracted depends on the soil/extractant ratio. Therefore, results of neutral extracts shall be reported as concentration measured in the extract (mg/l).

The acid extract is stronger and extracts the potentially available amount. Therefore, reported results of acid extracts shall be based on the weight of the original soil sample (mg/kg).

Necessary equations for calculation are given in the specific standards.

7 Calibration

7.1 Introduction

The chemical extractions described in this document are used to establish the bioavailability of trace elements. The real bioavailability is characterized by uptake of trace elements reflecting in bioaccumulation or a toxic effect. To be used for specific trace elements and specific organisms, the

correlation between bioavailability, measured using a chemical extractant, and the effect on the specific organism has to be shown (calibration).

7.2 Applicability of soil extracts

Examples of calibration using 0,43 mol/l HNO_3 , 1 mol/l NH_4NO_3 and CaCl_2 are presented in [Annex A](#). This standard recommends the use of 0,001 mol/l CaCl_2 (ISO 21268-1) for measuring of the actual bioavailability and 0,43 mol/l HNO_3 extraction (ISO 17586) for measuring the potential bioavailability. The use of 0,001 mol/l CaCl_2 and an acid extraction do fit in a theoretical framework as described in this document. 1 mol/l NH_4NO_3 (see ISO 19730) can be used as a measure for the actual environmental availability to predict the uptake of trace elements by plants.

The number of investigations where 0,001 mol/l (rather than the classical 0,01 mol/l) CaCl_2 is used is still limited. Researchers are encouraged to use the approach presented in document, thereby supplying calibration results and improving applicability of the method.

NOTE Calibration results for 0,01 mol/l CaCl_2 are given in [Annex A](#).

7.3 Limits of soil extracts to estimate trace element bioavailability

Although soil extracts are simple and cheap methods to estimate trace element bioavailability, these methods cannot integrate all the processes involved in the control of trace element bioavailability to soil organisms.

Potential explanations of the discrepancies in the prediction of trace element bioavailability from soil extracts can be:

- a) Trace element bioavailability driven by non-equilibrium processes (i.e. kinetic constrains) in soil and requires use of a non-equilibrium approach to estimate trace element bioavailability (e.g. Reference [\[17\]](#));
- b) As described in ISO 17402 the bioavailability can be altered by soil organisms in the bio-influenced zone (e. g. the rhizosphere for plants or the drilosphere for earthworms), leading to an actual trace element bioavailability that drastically differs from that estimated by soil extracts performed on the bulk soil (i.e. non bio-influenced)[\[19\]](#). For human bioavailability (bioaccessibility) an extraction is used that simulates the intestine system;
- c) The bioavailability of trace elements for a range of biological species can greatly vary between species or even sub-species or cultivars for a given contaminated soil (see References [\[20\]](#) and [\[21\]](#)), which a single soil extract cannot mimic per se.

Consequently, soil extracts could not be correlated with trace element uptake by soil organisms in numerous studies. Situations in which the measuring results of soil extracts are not a proper predictor of trace element bioavailability are described for accumulation in plants (see References [\[19\]](#), [\[22\]](#) and [\[23\]](#)) and accumulation in soil organisms (see References [\[24\]](#), [\[25\]](#) and [\[26\]](#)). In these cases, the use of biological methods for a more direct assessment of trace element bioavailability is required.

Annex A (informative)

Calibration towards biological targets

The chemical extractions described in this document are used to establish the bioavailability of trace elements. The real bioavailability is characterized by uptake of trace elements reflecting in bioaccumulation or a toxic effect. To be used for specific trace elements and specific organisms, the correlation between bioavailability, measured using a chemical extractant, and the effect on the specific organism has to be shown (calibration).

In an extraction with a neutral salt the composition of the extraction solution should reflect the composition of the pore water. In an ideal situation, ionic strength, ionic composition and organic matter content and composition should be the same. This is not realistic for a standard method to estimate the actual environmental availability. A choice for a fixed composition has to be made that can be used for all soils

As mentioned, 0,01 mol/l CaCl_2 has been used and this method can predict the concentration in the water phase (see References [6], [7], [8] and [27]). This is correct if the original pore water has also a higher ionic strength. In an international context 0,01 mol/l is often too high, because rainwater composition is the starting point.

Making the choice for a fixed concentration means that it will be too high or too low for most soils. Too high means that the salt in the extraction solution will be responsible for an exchange with the trace elements on the cation exchange complex, giving a too high concentration of the trace element or it will reduce the organic matter content, thereby reducing the concentration of trace elements complexed by the organic matter (e.g. Cu). Too low means that the effect on the cation exchange complex is smaller and also the reduction on the dissolved organic matter. In calcium rich soils dissolution of calcium carbonate and/or calcium sulphate will compensate the low concentration.

Having this all in mind, a standard method can better choose for a low than a high concentration. In spite of this effect on the trace metal concentration, calibration results of 1 mol/l NH_4NO_3 , 0,01 mol/l CaCl_2 can be found in literature (see Table A.1). For assessment of the same soils, these calibrations can be used

Table A.1 — Calibration results of 1 mol/l NH_4NO_3 , 0,001 mol/l CaCl_2 , 0,01 mol/l CaCl_2 and 0,43 mol/l HNO_3 extraction

Extraction solution	Components*	Biological target	Endpoint considered for the calibration	Remarks	Reference
NH_4NO_3	Zn	Radish (<i>Raphanus sativus L.</i>)	Environmental bioavailability Uptake		[28]
NH_4NO_3	Cd, Tl, Zn, Ni	Kale (Tl, Zn), wheat (all), carrot (Zn, Tl), spinach (Cd, Ni, Tl), beet leaf (Zn) and lettuce (Cd, Tl, Zn)	Environmental bioavailability Uptake		[29]
HNO_3	Cd, Zn, Cu	Grass, maize, potatoes, wheat	Environmental bioavailability Uptake	Most reliable for Cd and Zn Include pH and CEC	[21]
HNO_3	Cd, Cu Pb and Zn	Human exposure	Human bioaccessibility SBET-method		[30]

Table A.1 (continued)

Extraction solution	Components*	Biological target	Endpoint considered for the calibration	Remarks	Reference
HNO ₃	Ag	Human exposure	Human bioaccessibility SBET-method		[31]
HNO ₃	Cd	Rice	Environmental bioavailability Uptake by rice	Include soil properties	[32]
HNO ₃ and CaCl ₂ 0,01 mol/l	Cd and Zn	<i>Thlaspi caerulescens</i>	Environmental bioavailability Uptake		[33]
HNO ₃ and CaCl ₂ 0,01 mol/l	Cd, Zn, Pb, Cu, Hg, As, Co, Ba, B, Cr, Mo, Ni, Sb, U	Agriculture	Environmental bioavailability Uptake	No relation for B, Cr, Mo, Ni, Sb, U	[34]
HNO ₃ and CaCl ₂ 0,01 mol/l	As, Cd, Ba, Cr, Cu, Ni, Pb, Zn	Agriculture	Environmental bioavailability Uptake		[35]
CaCl ₂ 0,001 mol/l	Zn	Inhibition of barley shoot growth	Toxicity		[63]
CaCl ₂ 0,01 mol/l	Cu	Yeast	Environmental bioavailability Uptake	Include pH	[36]
CaCl ₂ 0,01 mol/l	Ni	<i>Avena sativa</i>	Toxicological bioavailability Shoot production		[37]
CaCl ₂ 0,01 mol/l	Cd	12 genotypes in paddy rice fields	Toxicological bioavailability Root and grain bioaccumulation	Soil samples taken in dewatered fields before the harvest	[32]
CaCl ₂ 0,01 mol/l	Zn	Springtail (<i>Folsomida Candida</i>)	Toxicological bioavailability		[38]
CaCl ₂ 0,01 mol/l	Cd, Pb	Snail (<i>Helix aspersa</i>)	Toxicological bioavailability Concentration in the hepatopancreas	Include pH and organic matter. 0 d to 28 d exposure to spiked soils	[39]
CaCl ₂ 0,01 mol/l	Cd	Earthworms	Environmental bioavailability Uptake	Buffered at pH 7,2	[40]
CaCl ₂ 0,01 mol/l	Cd, Cu and to a lower extend As, Pb	Earthworms (<i>Eisenia Andrei</i>)	Toxicological bioavailability Bioaccumulation	3 weeks exposure, equilibrium between soil and body concentration assumed	[24]
CaCl ₂ 0,01 mol/l	As, Cd, Pb	Earthworms (<i>Eisenia Andrei</i>)	Toxicological bioavailability Bioaccumulation (internal concentrations in worms at steady state)	0 d to 63 d	[25]

Table A.1 (continued)

Extraction solution	Components*	Biological target	Endpoint considered for the calibration	Remarks	Reference
CaCl ₂ 0,01 mol/l	Cd, Pb	Oligochaeta (<i>Enchytraeus crypticus</i>)	Toxicological bioavailability Bioaccumulation	0 d to 35 d exposure.	[26]
CaCl ₂ 0,01 mol/l	Zn	Isopod (<i>Oniscus asellus</i>)	Toxicological bioavailability Total body concentration	1 d to 14 d	[41]

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Annex B (informative)

Examples of transfer functions

Transfer function:

$$\log\left[Q_{\text{soil}}/\{c_{\text{solution}}\}^n\right]=INT+a\times\log[SOM]+b\times\log[\text{clay}]-c\times\text{pH}+d[DOC]$$

where

c_{solution} is the actual available in water phase;

Q_{soil} is the concentration extractable using 0,43 mol/l HNO_3 ;

INT is the intercept obtained by multiple linear regression;

$[SOM]$ is the solid organic matter content, in percent (%);

$[\text{clay}]$ is the clay content, in percent (%);

$[DOC]$ is the dissolved organic carbon, in milligrams per litre (mg/l);

a, b, c are coefficients obtained by multiple linear regression;

n is the non-linearity term.

For the following trace elements transfer functions are available^[42]. The transfer functions are calibrated using Dutch soils, but reliable application has also been shown in Taiwan.

Table B.1 — Values for intercepts obtained by multiple linear regression (INT), a, b, c, d and n in transfer functions

Trace element	INT	a [SOM]	b [Clay]	c [pH]	d [DOC]	n	R^2
Cd	-4,75	0,61	0,26	0,29	-0,05	0,54	0,80
Cu	-2,61	0,60	0,12	0,23	-0,27	0,59	0,65
Pb	-2,38	0,95	0,22	0,07	-0,23	0,73	0,59
Zn	-4,23	0,47	0,43	0,37	-0,14	0,75	0,82

Using comparable functions it is possible to make a direct correlation with the effect for instance the uptake of trace elements by vegetation, thereby predicting the environmental bioavailability^[21]. Details are given in [Clause 7](#).