
**Soil quality — Parameters for geochemical
modelling of leaching and speciation of
constituents in soils and materials —**

**Part 5:
Extraction of humic substances from
aqueous samples**

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*Qualité du sol — Paramètres pour la modélisation géochimique de la
lixiviation et de la spéciation des constituants des sols et des matériaux —*

Partie 5: Extraction des substances humiques des échantillons aqueux
ISO 12782-5:2012

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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.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12782-5 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

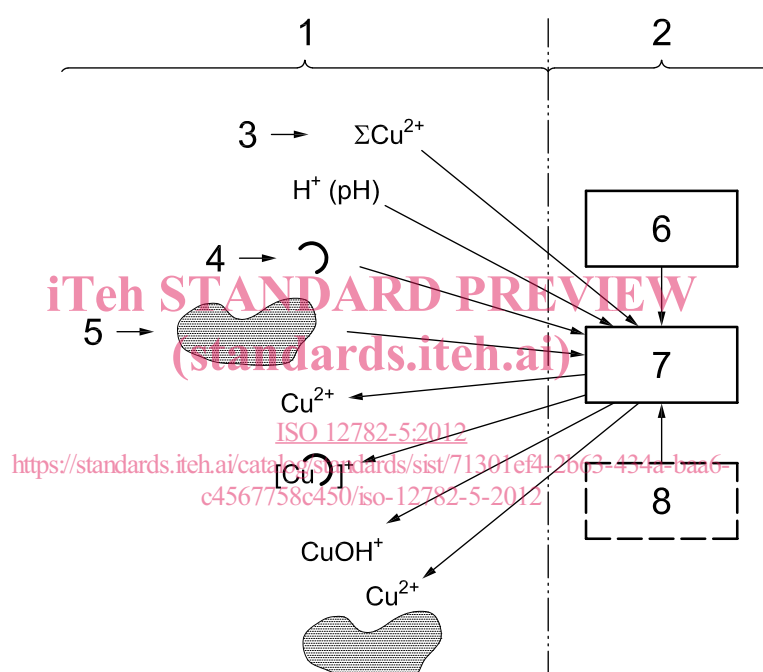
ISO 12782 consists of the following parts, under the general title *Soil quality — Parameters for geochemical modelling of leaching and speciation of constituents in soils and materials*:

- Part 1: Extraction of amorphous iron oxides and hydroxides with ascorbic acid
- Part 2: Extraction of crystalline iron oxides and hydroxides with dithionite
- Part 3: Extraction of aluminium oxides and hydroxides with ammonium oxalate/oxalic acid
- Part 4: Extraction of humic substances from solid samples
- Part 5: Extraction of humic substances from aqueous samples

Introduction

In addition to leaching procedures for subsequent chemical and ecotoxicological testing of soil and other materials including waste, predictive models are becoming indispensable tools in the environmental risk assessment of these materials. Models are particularly required when the results of laboratory leaching tests are to be translated to specific scenarios in the field, with regard to assessing the risks of both contaminant migration and bioavailability.

In the past few years, geochemical models have been shown to be valuable tools to be combined with the data obtained from characterization leaching standards, such as pH-dependence and percolation tests. These models have the advantage of being based on fundamental thermodynamic parameters that have a general validity. In order to enable extrapolation of laboratory leaching data to the mobility and/or bioavailability of a constituent in a specific field scenario, these models require additional input parameters for specific soil properties (see Figure 1).



Key

- 1 experiment
- 2 geochemical speciation modelling
- 3 available metal concentration
- 4 dissolved humic substances
- 5 reactive (solid) surfaces
- 6 database with stability constants
- 7 computer program
- 8 assumptions

Figure 1 — Relationships between experimental data, as obtained from laboratory leaching/extraction tests, and geochemical modelling of the speciation of a heavy metal in the environment (modified after M. Gfeller & R. Schulín, ETH, Zürich)

Characterization leaching standards provide information on the concentrations of the contaminant of interest as a function of, in particular, pH and liquid/solid (L/S) ratio. In addition, a more complete analysis of the leachates also provides information on the major ion composition and dissolved organic carbon (DOC), parameters that are particularly important for the chemical speciation of constituents through processes such as precipitation, complexation and competition for adsorption on reactive mineral and organic surfaces in the soil. As illustrated

in Figure 1, for the example of copper, geochemical modelling enables calculation of the metal distribution among these different chemical species in the system of interest. This provides necessary information for risk-assessment purposes, as these different chemical forms play distinct roles in the mobility and bioavailability of the metal in the soil. In addition to information obtained from the leaching standards (in their current state of development/definition), two additional types of information are required.

- a) The “available” (sometimes also referred to as “active” or “exchangeable”) concentration of the constituent in the solid phase, as opposed to the total concentration determined by acid destruction of the solid matrix. This “available” concentration can be obtained by leaching at low pH, a condition that can be obtained by extending the pH range in the pH-dependent leaching test (ISO/TS 21268-4) down to $\text{pH} \approx 0,5$ to $\text{pH} \approx 1$.
- b) The concentration of reactive organic and mineral surfaces in the soil, which constitute the major binding (adsorption) sites for most constituents in the soil matrix.

The major reactive surfaces that control the binding of constituents by sorption processes to the soil matrix are particulate organic matter and iron and aluminium (hydr)oxides. It is generally accepted that the reactivity of these mineral and organic surfaces can strongly vary as a function of their specific surface area/crystallinity [iron and aluminium (hydr)oxides] and composition (organic matter). When the results are intended to be used for the above-described purposes of geochemical modelling in conjunction with leaching tests, it is important that the methods be selective for reactive surfaces for which generic thermodynamic adsorption parameters are also available for the most important major and trace elements.

These reactive surfaces have been identified in soils, as well as in a variety of other materials for which the leaching of constituents is of relevance. It has been shown that the binding properties of these surfaces play a generic role in the speciation and leaching of constituents among these different materials. As an example, a similar geochemical modelling approach, using model input from the partial or complete ISO 12782 series, has been successfully applied to different soils^[4], amended soils^{[5][6]}, municipal incinerator bottom ash^[7], steel slag^{[8][9]}, bauxite residues^[10], and recycled concrete aggregate^[11]. Hence, the scope of the ISO 12782 series extends from soils to materials including soil amendments and waste materials.

This part of ISO 12782 aims to determine important reactive organic surfaces in soil and materials, for which generic thermodynamic adsorption parameters exist, i.e., humic and fulvic acids. The procedure is based on Reference [12], while generic thermodynamic adsorption parameters for humic and fulvic acids are available in References [13] and [14].

Thermodynamic parameters for adsorption models other than those used in References [13] and [14] are also available in the literature and may also be used to model the binding of constituents to humic and fulvic acids.

The method^[15] is based on a conventional isolation and purification method^[12] that is also used by the International Humic Substances Society (IHSS).

Soil quality — Parameters for geochemical modelling of leaching and speciation of constituents in soils and materials —

Part 5: Extraction of humic substances from aqueous samples

1 Scope

This part of ISO 12782 specifies a procedure to determine the concentration of humic substances in aqueous samples. These samples may be obtained as such or as eluates from leaching procedures applied to soil or other materials. Other materials also include waste. The content of humic substances can be used as input in geochemical models.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*
<https://standards.iteh.ai/catalog/standards/sist/71301ef4-2b63-434a-baa6-c4567758c450/iso-12782-5-2012>

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

dissolved organic carbon

DOC

sum of organically bound carbon present in water originating from compounds (including cyanate and thiocyanate) which will pass a membrane filter of pore size 0,45 µm

3.2

humic substance

HS

(partial) decomposition product from plant and animal tissue

NOTE 1 Humic substances form a series of relatively high-molecular-weight, brown-to-black-coloured substances formed by secondary synthesis reactions.

NOTE 2 The term is used as a generic name to describe coloured material or its fractions (e.g. humic and fulvic acids) obtained on the basis of solubility characteristics.

3.3

humic acid

HA

fraction of a humic substance that is not soluble in water under acidic conditions (pH <1 to 2) but is soluble at higher pH values

NOTE Humic acids are dark brown to black in colour.

3.4

fulvic acid

FA

fraction of a humic substance that is soluble in water under all pH conditions

NOTE 1 Fulvic acids remain in solution after removal of humic acid by acidification.

NOTE 2 Fulvic acids are light yellow to yellow-brown in colour.

3.5

hydrophilic organic carbon

Hy

organic carbon compound consisting of non-humic and humic-like substances

NOTE In this part of ISO 12782, Hy is essentially regarded as the extractable organic carbon fraction that is not identified as humic acid, fulvic acid or hydrophobic neutral organic carbon in accordance with the procedure specified in Clause 8. Hydrophilic organic carbon generally consists of molecules with a lower molecular weight and higher COOH/C ratios than humic acids and fulvic acids. Examples of compounds are: oxidized carbohydrates with carboxylic acid groups, low-molecular-weight carboxylic acids, and sugar phosphates.

3.6

hydrophobic neutral organic carbon

HON

difference between the amount of adsorbed fulvic acid and hydrophilic organic carbon and the amount of desorbed fulvic acid

NOTE Hydrophobic neutral organic carbon can include non-humic and humic-like compounds.

3.7

laboratory sample

sample intended for laboratory inspection or testing

[ISO 11074:2005]

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3.8

test sample

sample, prepared from the laboratory sample, from which the test portions are removed for testing or for analysis; this portion of material, resulting from the laboratory sample by means of an appropriate method of sample pretreatment, and having the size (volume/mass) necessary for the desired testing or analysis

NOTE Adapted from ISO 11074:2005.

3.9

test portion

analytical portion

quantity of material, of proper size, for measurement of the concentration or other property of interest, removed from the test sample

NOTE 1 The test portion may be taken from the primary sample or from the laboratory sample directly if no preparation of sample is required (e.g. with liquids), but usually it is taken from the prepared test sample.

NOTE 2 A unit or increment of proper homogeneity, size, and fineness, needing no further preparation, may be a test portion.

[ISO 11074:2005]

3.10

soil material

excavated soil, dredged material, manufactured soil, treated soil and fill material and other relevant materials, including soil amendments and waste materials

4 Principle

Specific dissolved organic carbon species are isolated based on defined operational conditions. Humic acids are precipitated at pH 1 and fulvic acids (and the hydrophobic organic neutral fraction) are adsorbed onto DAX-8 resin. The organics remaining in solution after resin addition are classified as hydrophilic organic substances. The DOC concentrations are measured after every step, from which the individual concentrations of humic and fulvic acids, hydrophobic organic neutrals, and hydrophilic organic substances, are calculated.

5 Apparatus

The following apparatus shall be used. All materials that come in contact with the sample (material or reagents) should not contaminate the compounds to be determined or adsorb the compounds of interest.

5.1 Balance, with an accuracy of 0,1 g.

5.2 Usual laboratory glass or plastic ware, rinsed in accordance with ISO 5667-3.

5.3 pH-meter, with a measurement accuracy of at least $\pm 0,05$ pH units.

5.4 End-over-end shaking machine (5 min^{-1} to 10 min^{-1}).

NOTE Other shaking methods can be used, provided that they can be shown to provide equivalent results.

5.5 Filtration apparatus, either a vacuum filtration device (between 2,5 kPa and 4,0 kPa) or a high-pressure filtration apparatus ($< 0,5 \text{ MPa}$). Cleaning is compulsory.

5.6 Filters, pore size $20 \mu\text{m}$, for use in the Büchner-funnel filtration device (5.7).

5.7 Büchner-funnel filtration device

5.8 Membrane filters, for the filtration device, fabricated from inert material with a pore size of $0,45 \mu\text{m}$. Filters shall be pre-washed with demineralized water in order to remove DOC.

5.9 Soxhlet extraction device.

5.10 Soxhlet extraction thimbles, glass-fibre extraction thimbles for Soxhlet extraction device (5.9).

5.11 Centrifuge, preferably at $3\,000g$. For other appropriate conditions, see Annex C.

5.12 Centrifuge bottles, e.g. polycarbonate, of capacity 250 ml, cleaned with distilled and demineralized water and diluted acid (HNO_3) before use.

5.13 Crushing equipment: jaw crusher or cutting device.

5.14 Sieving equipment, with a nominal screen size of 2 mm or 4 mm.

6 Reagents

The reagents used shall be of analytical grade and the water used shall comply with grade 3 in accordance with ISO 3696.

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

6.2 Potassium hydroxide, $c(\text{KOH}) = 0,1 \text{ mol/l}$ and 1 mol/l .

6.3 Hydrochloric acid, $c(\text{HCl}) = 0,1 \text{ mol/l}$ to 6 mol/l .

6.4 Sodium hydroxide, $c(\text{NaOH}) = 0,1 \text{ mol/l}$ to 5 mol/l .

6.5 Acetonitrile, (CH_3CN), suitable for liquid chromatography.

6.6 Methanol, (CH_3OH), suitable for liquid chromatography.

6.7 DAX-8 resin, e.g. Sigma-Aldrich¹⁾.

NOTE Various documented methods for HS isolation and purification make use of XAD-8 resin to adsorb HA and/or FA. This resin is no longer commercially available; therefore, the comparability of the substitute resin DAX-8 was tested. See Annex B for information.

6.8 Nitric acid, $c(\text{HNO}_3) = 0,1 \text{ mol/l}$.

7 Sampling

7.1 Laboratory sample

The laboratory sample shall consist of a volume of at least 50 ml.

7.2 Test sample

The samples shall be tested on filtered ($0,45 \mu\text{m}$) liquids. The test samples can be of diverse origins and should be stored in a refrigerator. To avoid changes in the samples during storage, NaN_3 ($0,1\%$ in solution) can be added.

Samples that have been spiked with NaN_3 should be processed in a fume hood. NaN_3 decomposes under acidic conditions, releasing toxic gasses.

7.3 Test portion

Based on eluate volume requirements for analysis, the test portion size shall be 50 ml (with a tolerance of $\pm 10 \%$).

8 Procedure

8.1 Preparation of DAX-8 resin

Clean every new batch of DAX-8 resin (6.7) to remove organic impurities with five $0,1 \text{ mol/l}$ hydrochloric acid (6.3) extractions (for 24 h). Renew the solution after each extraction. Repeat this cycle with $0,1 \text{ mol/l}$ sodium hydroxide (6.4). Then, clean the resin thoroughly by Soxhlet extractions (5.9) with acetonitrile (6.5) and methanol (6.6), each for 24 h. The cleaned resin is stored in methanol (6.6) until use.

Prior to use, remove the methanol by placing the DAX-8 resin (6.7) in a Büchner funnel (5.7) with a filter (5.6) and wash the resin under vacuum with water (6.1) that has a volume 20 times that of the resin. Subsequently, rinse the resin similarly with $0,1 \text{ mol/l}$ hydrochloric acid (6.3) having 10 times the resin volume.

NOTE It has been demonstrated^[15] that 250 g of DAX-8 resin (6.7) can be cleaned sufficiently by rinsing with 2 l of water (6.1) and 1 l of $0,1 \text{ mol/l}$ hydrochloric acid (6.3). This cleaning sequence can be used to obtain a DOC-free (DOC generally $< 2 \text{ mg C/l}$) and acidic (pH 1) resin.

1) DAX-8 resin from Sigma-Aldrich 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.

8.2 Determination of total humic acid (HA), fulvic acid (FA) and hydrophilic organic carbon (Hy) content in aqueous samples

Weigh the test portion (7.3) in a centrifuge bottle (5.12). Acidify the test portion (M_w) with 1 mol/l hydrochloric acid (6.3) to pH 1 to 2. Adjust the solution volume to 200 ml ($L/S = 10$) with 0,1 mol/l hydrochloric acid (6.3) and record the total volume of added hydrochloric acid (1 mol/l and 0,1 mol/l) (V_6). Close the centrifuge bottle, equilibrate the suspension by continuous shaking (5.4) for 1 h and centrifuge for 30 min at 3 000g or at appropriate centrifugation conditions as given in Annex C. Remove the supernatant (FAHyHON₁) from the residue by decantation into a 250 ml bottle (5.2) and record the water volume (V_7). Store the sample in a refrigerator until DAX-8 treatment (see below).

Neutralize the test portion that remains in the centrifuge bottle with 1 mol/l sodium hydroxide (6.4) to pH = 7,0. Add 0,1 mol/l sodium hydroxide under a N₂ atmosphere to a final volume of 200 ml ($L/S = 10$). Check that the final pH is ≥ 12 to ensure high HA solubility. If necessary, add 1 mol/l sodium hydroxide. Close the centrifuge bottle, equilibrate the suspension overnight by continuous shaking (5.4) and centrifuge the suspension for 30 min at 3 000g or at appropriate centrifugation conditions as given in Annex C. Remove the supernatant from the residue by decanting into a clean 250 ml centrifuge bottle (5.12) and record the volume of the decanted eluate (V_1).

Acidify the supernatant to precipitate HA by adding 6 mol/l hydrochloric acid (6.3) (V_2) while continuously stirring until a pH of 1,0 is reached. Allow the suspension to stand overnight and centrifuge for 30 min at 3 000g or under appropriate centrifugation conditions as given in Annex C. Remove the supernatant by decantation (V_8) into a 250 ml bottle (5.2). Store the solution (FAHyHON₂) in a refrigerator for DAX-8 treatment. Re-dissolve the HA that remains in the centrifuge bottle in 0,1 mol/l potassium hydroxide (6.2) (V_5) and analyse for DOC (DOC_{HA}).

Filter the stored solutions (FAHyHON_{1,2}) over a membrane filter (5.5, 5.8) and analyse DOC (Clause 10) ($DOC_{FAHyHON1,2}$). Transfer 50 ml ($V_{4,i}$) of the filtered solutions to (separate) 100 ml bottles (5.2) and add 10 g of moist DAX-8 (8.1) ($m_{DAX,i}$) to both samples. Equilibrate for 1 h by continuous shaking (5.4) and filter (5.6, 5.7) the suspensions. Analyse DOC (Clause 10) in both solutions ($DOC_{Hy1,2}$). In order to desorb FA, transfer the filtered resins to separate 50 ml bottles (5.2), add 20 ml of 0,1 mol/l potassium hydroxide (6.2) and equilibrate by continuous shaking for 1 h (5.4). Filter (5.6, 5.7) the suspensions and collect the filtrates in 100 ml bottles. Transfer the filtered resins to 50 ml bottles (5.2), add 20 ml of 1,0 mol/l potassium hydroxide (6.2) and repeat the desorption of FA in three additional steps (1 h each). The pH should be > 11 and can be adjusted with 1 mol/l potassium hydroxide (6.2) if necessary. Collect the four fractions of filtered potassium hydroxide in the same 100 ml bottle, record the total volume ($V_{9,10,i}$) and analyse DOC (Clause 10) ($DOC_{FA,i}$).

A schematic overview of the procedure is given in Annex A.

9 Eluate treatment and storage

Preserve the eluate sub-samples depending on the elements to be analysed and store them in accordance with the requirements in ISO 5667-3.

10 Analytical determination

Analyse the samples in accordance with ISO 8245.

11 Blank test

Perform a blank test to determine the DOC contribution from each batch of cleaned DAX-8 resin (8.1). Add 10 g of moist DAX-8 resin ($m_{DAX,BL}$) to 50 ml of 0,1 mol/l hydrochloric acid (6.3) ($V_{4,BL}$) after previous DOC analysis (Clause 10) (DOC_{BL1}). Allow the resin to settle for 5 min after 1 h of equilibration by continuous shaking (5.4), and measure DOC (Clause 10) (DOC_{BL2}).