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Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil-like materials —

Part 5: Batch test with forced aerobic or anaerobic conditions

Qualité du sol — Modes opératoires de lixiviation en vue d'essais chimiques et écotoxicologiques ultérieurs des sols et matériaux analogues au sol —

Partie 5: Essai en bâchée dans des conditions aérobies ou anaérobies forcées

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

A list of all parts in the ISO 21268 series can be found on the ISO website.

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

In various countries, tests have been developed to characterize and assess the substances which can be released from materials. The release of soluble substances upon contact with water is regarded as a main mechanism of release, which results in a potential risk to the environment during the use or disposal of materials. The intent of these tests is to identify the leaching properties of materials. The complexity of the leaching process makes simplifications necessary[1].

Not all of the relevant aspects of leaching behaviour can be addressed in one standard.

Tests to characterize the behaviour of materials can generally be divided into three categories addressed in ISO 18772 and EN 12920. The relationships between these tests are summarized as follows.

“Basic characterization” tests are used to obtain information on the short-term and long-term leaching behaviour and characteristic properties of materials. Liquid to solid ratios (L/S), leachant composition, factors controlling leachability, such as pH, redox potential, complexing capacity, role of dissolved organic carbon (DOC), ageing of material and physical parameters, are addressed in these defined tests.

“Compliance” tests are used to determine whether the material complies with a specific behaviour or with specific reference values. These tests focus on key variables and leaching behaviour previously identified by basic characterization tests.

“On-site verification” tests are used as a rapid check to confirm that the material is the same as that which has been subjected to the compliance test(s). On-site verification tests are not necessarily leaching tests.

The test procedure described in this method belongs to category a) “Basic characterization” tests.

NOTE 1 Volatile organic substances include the low molecular weight substances in mixtures such as mineral oil.

NOTE 2 It is not always possible to optimize test conditions simultaneously for inorganic and organic substances and optimum test conditions can also vary between different groups of organic substances. Test requirements for organic substances are generally more stringent than those for inorganic substances. The test conditions suitable for measuring the release of organic substances will generally also be applicable to inorganic substances.

NOTE 3 Within the category of organic substances, a significant difference in behaviour exists between the more polar, relatively water-soluble substances and apolar, hydrophobic organic substances (HOCs). In the latter case, mechanisms of release (e.g. particle-bound or dissolved organic carbon-bound) can be more crucial as well as sorption losses of soluble HOCs on different materials with which they come in contact (e.g. bottles, filters). The test and the results can be used for leaching of organic substances only with thorough consideration of the specific properties of the substances in question and the associated potential problems.

NOTE 4 For ecotoxicological testing, eluates representing the release of both inorganic and organic substances are needed. In this document, ecotoxicological testing is meant to include genotoxicological testing.

Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil-like materials —

Part 5:
Batch test with forced aerobic or anaerobic conditions

1 Scope

This document specifies a test with which in situ available concentrations of inorganic substances (such as heavy metals, arsenic and phosphorus) and organic substances in soil and soil-like materials can be simulated under forced aerobic and anaerobic conditions. The toxicity can then be estimated based on these available concentrations.

The test described in this document aims to measure the release of inorganic and organic substances from soil and soil-like material as well as to produce eluates for subsequent ecotoxicological testing. For ecotoxicological testing, see ISO 15799 and ISO 17616.

The eluate obtained can subsequently be characterized by physical, chemical and ecotoxicological methods in accordance with existing standard methods. The test is not suitable for substances that are volatile under ambient conditions.

This procedure is not applicable to materials with a dry-matter-content ratio lower than 33 %.

This test is mainly aimed at being used for routine and control purposes, and it cannot be used alone to describe all leaching properties of a soil. Additional leaching tests are needed for that extended goal. This document does not address issues related to health and safety. It only determines the leaching properties outlined in Clause 4.

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 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 7027-1, *Water quality — Determination of turbidity — Part 1: Quantitative methods*

ISO 10523, *Water quality — Determination of pH*

ISO 11271, *Soil quality — Determination of redox potential — Field method*

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

3 Terms and definitions

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

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

— IEC Electropedia: available ar https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
leaching test
test during which a material is put into contact with a *leachant* (3.2) under strictly defined conditions and some substances of the material are extracted

3.2
leachant
liquid used in a *leaching test* (3.1)

Note 1 to entry: For the purpose of this document, the leachant is specified in 8.2.2 and 8.2.3.

3.3
eluate
leachate
solution recovered from a *leaching test* (3.1)

3.4
liquid to solid ratio
L/S
ratio between the total volume of liquid (L in litres), which in this extraction is in contact with the sample, and the dry mass of the sample (S in kg of dry matter)

Note 1 to entry: L/S is expressed in litres per kilogram (l/kg).

3.5
dry matter content
w_{dm}
ratio, expressed in per cent, between the mass of the dry residue and the corresponding raw mass

Note 1 to entry: The mass of the dry residue shall be determined in accordance with ISO 11465.

3.6
water content
$w_{\mathrm{H_2O}}$
ratio, expressed in per cent, between the mass of water contained in the material as received and the corresponding dry residue of the material

Note 1 to entry: The basis for the calculation of the water content is the mass of the dry residue in this document, as specified in ISO 11465 (for the determination of the water content of soil).

3.7
laboratory sample
sample or subsample(s) sent to or received by the laboratory

3.8
test sample
sample, prepared from the *laboratory sample* (3.7), from which *test portions* (3.9) are removed for testing or analysis

3.9
test portion
quantity of material of appropriate size for measurement of the concentration or other properties of interest taken from the *test sample* (3.8)

Note 1 to entry: The test portion can be taken from the *laboratory sample* (3.7) directly if no pre-treatment of the sample is required, but usually it is taken from the test sample.

Note 2 to entry: A unit or increment of proper homogeneity, size and fineness, needing no further preparation, can be a test portion.

3.10
soil-like material
excavated soil, dredged materials, manufactured soils, treated soils and fill materials

3.11
redox potential
electrochemical potential reflecting the oxidation-reduction status of a liquid chemical system

Note 1 to entry: For the purpose of this document, the liquid chemical system is the soil solution.

3.12
aerobic
descriptive of a condition in which molecular oxygen is freely available

3.13
anaerobic
descriptive of a condition in which molecular oxygen is not available

4 Principle

The test portions, which originally or after suitable pre-treatment have a particle size less than or equal to 2 mm, are brought into contact with water containing a low concentration (0,001 mol/l) of calcium chloride. The method is based on the assumption that during the test period equilibrium is approached between the liquid and solid phases at an imposed (aerobic or anaerobic) redox condition. The redox condition in the test is not based on the condition of the material itself, but on a forced aerobic or anaerobic condition, reflecting the application of the material in a different redox environment. Examples are the application of originally anaerobic sediment in an aerobic soil environment, or the utilization or disposal of originally aerobic soil at anaerobic aquatic conditions (e.g. in former sand pits). After the test period the solid residue is separated from the liquid. The separation procedure may strongly influence the test results and shall be particularly stringent for organic substances. Further characteristics of the eluate can be carried out with methods that are suitable for monitoring water. The eluate obtained is also suitable for carrying out ecotoxicological tests.

The conditions under which leaching has taken place such as pH, conductivity, DOC and redox potential are recorded in the test report. If the material causes turbidity in the eluate this shall also be recorded in the test report.

NOTE 1 These parameters often control the leaching behaviour of soil and soil-like materials and are therefore important for the evaluation of the test results. DOC, in particular, is crucial in soil and soil-like materials for many inorganic and organic substances.

NOTE 2 The leachant is 0,001 mol/l $CaCl_2$ to minimize the mobilization of DOC caused by an ionic strength of the leachant which is too low.

The procedure described in this document is based on the more stringent test requirements for determining the release of organic substances and for subsequent ecotoxicological testing. If only the release of inorganic substances is to be measured, less stringent requirements may be adopted for some steps of the procedure.

5 Reagents

Reagents used shall be of analytical grade purity.

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

5.2 Calcium chloride ($CaCl_2 \cdot 2\ H_2O$), analytical grade.

5.3 Sodium azide (NaN_3), analytical grade.

5.4 Nitric acid (HNO_3), analytical grade, made to 0,1 mol/l rinsing solution.

5.5 Compressed air.

5.6 Nitrogen gas N_2.

5.7 Na-L-Lactate ($C_3H_5NaO_3$), analytical grade.

5.8 Organic solvent (acetone, analytical grade) **for rinsing and cleaning**, suitable for the material used for the bottle used and the type of analyte.

6 Apparatus

6.1 Borosilicate glass bottle, of high purity in accordance with ISO 5667-3, with a nominal volume of 1 l, having a cap of inert material, for example PTFE (polytetrafluoroethylene). Rinsing is compulsory; it shall be ensured that previously used bottles have no background level of analytes.

NOTE 1 If only inorganic parameters are analysed, alternative materials, such as HDPE/PP bottles, are appropriate, except for unpreserved samples for mercury analysis.

NOTE 2 To prevent organic compounds from degradation by light use a dark room, dark colored glassware or place a layer of aluminium-foil around the leaching equipment.

If boron analyses are necessary, any plastics bottles can be used, e.g. PTFE (polytetrafluoroethylene).

The volume of 1 l is selected in combination with the mass, m_D, of 100 g as specified in 7.4 in order to minimize head-space in the bottle at an L/S of 10 l/kg dry matter. In the case of materials with low density, deviation from this requirement can be necessary while still ensuring minimum headspace. This deviation shall be reported.

NOTE 3 Glass of high quality is considered adequate for both metals and organic substances, particularly, since the pH range usually covered in soil testing does not reach the conditions (pH > 10 and pH < 3) where glass itself can be partially dissolved. For ecotoxicity testing, eluates with both inorganic and organic substances are needed, which emphasizes the need to generate integrated eluates.

NOTE 4 Heat treatment of used glassware at 550 °C can be used to remove traces of analytes. However, this treatment has been shown to increase adsorption of organic substances from the air.

6.2 Measuring cylinders for determining volumes with an accuracy of 1 %.

6.3 Mechanical top stirrer.

6.4 PTFE stirring rod with two movable blades.

The blades of the stirring rod shall preferably be folded in so that they can be passed through the neck of the bottle (6.1). During stirring the blades shall be unfolded.

6.5 Gas washing bottle, with a nominal volume of 1 l of inert material.

NOTE Inert material such as glass, PTFE or PFA (perfluoroalkoxy alkane).

6.6 Airtight container with screw cap, with a nominal volume of large enough to accommodate the borosilicate glass bottle (6.1).

NOTE PTFE is a suitable material. Alternative materials can be used as long as they do not allow oxygen to enter.

6.7 End-over-end tumbler (5 min^{-1} to 10 min^{-1}) or **roller table,** rotating at about 10 min^{-1}.

Other shaking devices may be used, provided that they can be shown to provide equivalent results. These agitation devices are specified because excessive abrasion leading to significant particle size reduction shall be avoided.

6.8 Filtration apparatus, either a vacuum filtration device (between 2,5 kPa and 4,0 kPa) or a high-pressure filtration apparatus (<0,5 MPa).

Rinsing is compulsory. When semi-volatile substances are to be analysed, vacuum filtration shall not be used.

6.9 0,45 µm membrane filters, prerinsed or similarly cleaned (e.g. rinsed with 0,1 mol/l HNO_3 (5.4) and water (5.1) (only for analysis of inorganic substances).

The filters shall be chosen so as not to adsorb (or release) substances of interest.

NOTE This can be tested in preliminary experiments.

6.10 Glass fibre filters, with a degree of separation of 0,7 µm.

The filters shall be chosen so as not to adsorb (or release) substances of interest.

NOTE This can be tested in preliminary experiments.

6.11 Sieving equipment, with sieves of 2 mm nominal screen size.

NOTE Due to sieving, contamination of the sample can occur to an extent which affects the leaching of some substances of concern, e.g. chromium, nickel and molybdenum from stainless steel equipment or plasticisers from plastic sieves.

6.12 Centrifuge, operating at 20 000 *g* to 30 000 *g* using centrifuge tubes of FEP (fluorinated ethylene propylene) or tubes of an alternative material, which is inert with regard to both inorganic and organic substances and suitable for high-speed centrifugation.

NOTE Potential sorption of hydrophobic organic substances to the centrifuge tubes can be tested in preliminary experiments.

Alternatively, if a high-speed centrifuge is not available, a centrifuge operating at 2 000 *g* to 2 500 *g* using glass bottles may be used in combination with increased centrifugation time. Cooling shall be applied to maintain the desired temperature.

6.13 Sample container of fluorinated ethylene propylene (FEP) or tubes of an alternative material that is inert for both organic and inorganic substances.

6.14 Device for measuring electrical conductivity.

6.15 pH meter, in accordance with ISO 10523.

6.16 Redox potential meter, in accordance with ISO 11271.

6.17 Oxygen meter (e.g. ISO 17289).

6.18 Balance, with an accuracy of at least 0,1 g.

6.19 Sample splitter, for sub-sampling of laboratory samples (optional).

6.20 Turbidity meter, as specified in ISO 7027-1.

6.21 Crushing equipment, a jaw crusher.

NOTE Due to particle size reduction, contamination of the sample can occur to an extent which affects the leaching of some substances of concern, e.g. chromium, nickel and molybdenum from stainless steel equipment.

7 Sample pretreatment

7.1 Preparation of laboratory sample and specification of particle size

A representative laboratory sample of at least 2 kg (dry matter) is obtained (e.g. as described in ISO 18400-101, ISO 18400-104, ISO 18400-105, ISO 18400-202 and ISO 23909) and shall be stored in closed packages and at low temperatures (4 °C), in order to avoid unwanted changes in the material (see e.g. ISO 18400-105).

The test shall be carried out on soil or soil-like material sieved to < 2 mm (e.g. as described in ISO 11464). Oversized material of natural origin in the sample shall be separated and discarded. The type and amount of all discarded material shall be reported. If oversized material of anthropogenic origin is present and assumed to contain substances of interest, this part can be subject to alternative sample preparation or testing.

If the laboratory sample cannot be homogenized or sieved because of its water content, it is allowed in this case only to dry the laboratory sample (e.g. as described in ISO 11464). The drying temperature shall not exceed 30 °C.

NOTE 1 Sieving and drying at more than 30 °C, as well as crushing, can lead to a loss of semi-volatile substances (inorganic and organic) and can alter the leaching characteristics.

NOTE 2 Due to sieving, contamination of the sample can occur to an extent that affects the leaching of some substances of concern, e.g. chromium, nickel and molybdenum from stainless steel equipment or plasticizers from plastic sieves.

7.2 Preparation of test sample

Use a sample splitter (6.19) or apply coning and quartering to split the laboratory sample and obtain a test sample. The size of test sample required depends on the volume of eluate needed for the specific purpose and the subsequent chemical analysis and/or ecotoxicological tests to be carried out on the eluate.

NOTE 1 If needed for chemical analysis or ecotoxicological testing, larger volumes of eluate can be obtained by combining eluates from replicate tests after centrifugation (or filtration). Alternatively, larger volumes of eluate can also be produced in a single test, provided that the ratios in terms of L/S and minimum headspace are maintained.

NOTE 2 The required amount of the test sample depends on the particle size distribution of the soil to be analysed (see ISO 23909). The specified sample amount will generally be adequate. In specific cases, a smaller sample amount can be used, for instance, if for specific reasons less material is available, provided that the test can be carried out as specified in 7.2 to 7.4.

7.3 Determination of the dry matter content and of water content

The whole test sample, in accordance with the size criterion in 7.1, shall not be further dried. The water content of the test sample shall be determined on a separate test portion at (105 ± 5) °C. If the sample is air-dried prior to testing, the dry matter content w_{dm} of the air-dried sample shall be determined

as well. This shall be taken into account when adjusting the L/S. The dry mass of the sample shall be determined at (105 ± 5) °C, in accordance with ISO 11465, and the dry matter content calculated with Formula (1):

$$w_{dm} = 100 \times m_D / m_W \quad (1)$$

where

w_{dm} is the dry matter content, expressed in per cent (%);

m_D is the mass of the dried sample, expressed in kilograms (kg);

m_W is the mass of the undried sample, expressed in kilograms (kg).

The water content (w_{H_2O} in per cent) is calculated with Formula (2):

$$w_{H_2O} = 100 \times (m_W - m_D) / m_D \quad (2)$$

NOTE If volatile or unstable compounds are present in the soil sample, this gravimetric method cannot be used for accurate determination of the water content.

If, for reasons expressed in 7.1, the material was (partly) dried before sample splitting, the overall mass loss shall be taken into account.

7.4 Preparation of the test portion

Take, from the test sample, a test portion with an appropriate test portion size. Based on sample heterogeneity and eluate volume requirement for analysis, the test portion size shall be m_D = (100 ± 5) g [measured with an accuracy of 0,1 g (6.17) of dry mass (m_D) following Formula (3).

$$m = 100 \times m_D / m_{dm} \quad (3)$$

Use a sample splitter (6.18) or apply coning and quartering to split the sample.

NOTE Sample splitting or coning and quartering can lead to loss of semi-volatile substances (inorganic and organic).

In view of the minimum requirements of eluate volume for analytical purposes, it may be necessary to use a larger test portion and a correspondingly larger volume of leachant. This deviation from this document shall be specified in the test report.

If the test is performed on an air-dried sample, use $w_{dm,AD}$ instead of w_{dm} to determine the sample mass of the test portion.

8 Procedure

8.1 Temperature

This test shall be carried out at room temperature: (22 ± 3) °C.

For material that is very sensitive to biological degradation, performance of the test at reduced temperature (e.g. 4 °C) and preventing direct exposure to light will limit biological activity significantly. A reduced temperature may result in slower/lower release of organic substances and hence lower concentrations of these compounds in the eluates. If the test is modified in this way, this deviation shall be reported in the test report.

8.2 Preparation of leachants

8.2.1 General

The leaching procedure to be followed depends on the research question. If there are indications that by using the soil material the redox potential will change, for example upon oxidation of anaerobic sediment, the aerobic leaching procedure shall be followed. If for aerobic sediment an estimate is made of its leaching properties under anaerobic conditions, then the anaerobic leaching procedure shall be followed.

8.2.2 Calcium chloride solution, $c(CaCl_2)$ = 0,001 mol/l

Prepare a solution made to 0,001 mol/l $CaCl_2$ by dissolving 0,147 g $CaCl_2$ in water and dilute to 1 000 ml.

In special cases (i.e. measurement of Ca and/or chloride in the eluate are of interest or the sample exhibits an own salt load), water without addition of $CaCl_2$ can also be used. The leachant type used shall be reported in the test report.

NOTE 1 The application of demineralized water as leachant can induce higher turbidity and lower ionic strength in the eluate for some types of soils (e.g. high content of organic matter) and can cause increased concentrations of analytes adsorbed to colloids.

NOTE 2 For eluates that are not to be used for ecotoxicological testing, sodium azide (NaN_3) can be added to a resulting concentration of 0,1 % in order to reduce microbial degradation of organic substances. However, the addition of NaN_3 is known to only minimize biodegradation if a very high but in turn extremely poisonous concentration in the leachant is applied. Therefore, other appropriate measures can be considered to prevent/reduce biodegradation in the sample or collected eluate (e.g. application of γ-radiation to the sample, dark and air-conditioned room, shorter eluate collection periods). If only inorganic compounds are measured, the addition of NaN_3 is not appropriate.

8.2.3 Anaerobic calcium chloride solution, $c(CaCl_2)$ = 0,001 mol/l with 0,100 mol/l Na-L-Lactate

Add a stirrer bar to the calcium chloride solution (8.2.2) and place the bottle on a magnetic stirrer. To make this anaerobic (% O_2 < 1 %) pass nitrogen (5.6) through this solution, via a gas washing bottle (6.4) filled with water (5.1), for a minimum of 15 min.

By adding 0,100 mol/l Na-L-Lactate (5.7) as a readily degradable substrate for microorganisms in the calcium chloride solution the development of anaerobic conditions is accelerated.

NOTE When high residual amounts of Na-L-Lactate occur at the end of the test, and iron(hydr)oxides are not fully reductively dissolved, Na-L-Lactate may induce desorption of anionic substances such as arsenic or phosphate. The residual amount of Na-L-Lactate can be analyzed in the eluate (8.5), and the magnitude of this possible effect can be tested separately, for example, by performing ISO 21268-2 on the sample with and without addition of Na-L-Lactate.

8.3 Leaching step

8.3.1 Calculation to the quantity of leachant

Calculate the volume of leachant, in litres, following Formula (4):

$$V_L = \left[10 - w_{H_2O} / \left(\rho_{H_2O} \times 100\right)\right] \times m_D \quad (4)$$

where

V_L is the volume of leachant used, in litres (l);

m_D is the dry mass of the test portion, in kilograms (kg);

ρ_{H_2O} is the density of water, in kilograms per litre (usually taken as 1 kg/l);

w_{H_2O} is the water content for the test portion, in per cent (%).

8.3.2 Aerobic leaching procedure

Place the test portion with the total mass m corresponding to (100 ± 5) g of dry mass m_D in a bottle (6.1).

Depending on the particle size distribution, other test portions may be applied ensuring that a representative portion is used (see 7.1).

Use a measuring cylinder (6.2) to add the calculated quantity of aerobic leachant (8.2.1) to a bottle (6.1) containing the test portion. Ensure that the solid phase is mixed well with the liquid.

NOTE 1 Figures A.1 to A.3 in Annex A illustrate the operations of the aerobic leaching procedure.

Place the PTFE stirring rod (6.4) in the bottle by folding in the blades of the stirring rod and place the bottle with stirring rod under the mechanical top stirrer (6.3). Press the blades of the stirring rod onto the bottom of the bottle so that they fold out. The height of the stirrer blades in the bottle shall be selected such that they cannot touch the bottom.

Before use, the compressed air (5.5) shall first be passed through a water-filled gas washing bottle (6.5).

Stir the suspension with a mechanical top stirrer (6.3) for 14 days in the dark while the suspension is aerated with compressed air via a PTFE tube.

NOTE 2 A flow rate of 100 kPa is generally sufficient but depends on the number of parallel tests. Visual observation of air bubbles in the extraction solution can be used to set a sufficiently high flow rate.

Prevent the solids in the bottle from settling during stirring. At the end of the stirring time remove the bottle from the stirring set-up.

8.3.3 Anaerobic leaching procedure

Select the appropriate bottle size according to the test portion size. For m_D = 100 g, this means bottle sizes of respectively 1 000 ml.

Use a measuring cylinder (6.2) to add the calculated quantity of anaerobic leachant (8.3.1) to a bottle (6.1) containing the test portion. Ensure that the solid phase is mixed well with the liquid.

Before use, the nitrogen gas (5.6) shall first be passed through a water-filled gas washing bottle (6.5).

Check the oxygen content of the mixture with an oxygen meter (6.17) and if necessary pass extra nitrogen (5.6) through the mixture until the percentage of O_2 is < 1 %.

NOTE Figures A.4 to A.6 in Annex A illustrate the operations of the anaerobic leaching procedure.

Replace the air above the mixture with nitrogen by passing nitrogen gas (5.6) into the bottle. The bottle is then sealed.

Place the sealed bottle in a container with a screw cap (6.6) and saturate the air between the sealed bottle and the wall of the container with nitrogen by passing nitrogen gas (5.6) into the container. Then seal the container with the screw cap to make it airtight.

Place the airtight container for 14 days in an end-over-end-tumbler or roller table (6.7) at a rotation speed of 10 rotations per min in the dark.

8.4 Liquid/solid separation step

Allow the suspended solids to settle for (15 ± 5) min.

Transfer the supernatant to centrifuge tubes (6.12). The centrifugation containers shall be chosen so as not to adsorb (or release) analytes.

There are two options for solid-liquid separation.

a) Centrifuge the eluate for 30 min at 20 000 *g* to 30 000 *g* using a high-speed centrifuge (6.12, see also Annex C).

b) Centrifuge the eluate for 5 h at 2 000 *g* to 3 000 *g* in glass bottles using a lower-speed centrifuge (6.12).

Cooling shall be applied to maintain the temperature at (22 ± 3) °C (see 8.1).

NOTE 1 Based on Stoke's law, the results of both centrifugation methods are expected to be comparable. Other alternative combinations of centrifugation acceleration and time can be applied given comparable conditions are calculated related to the specification of the rotor (see guidance in Annex C).

Gentle braking of the centrifuge shall be applied in order to avoid resuspension. The deceleration time shall not exceed 20 min.

NOTE 2 In case lightweight substances (e.g. coaly particles) are still floating after centrifugation, a glass fibre filtration (6.10) can be applied to remove such particles or to reduce the turbidity.

After centrifugation, the eluate shall be transferred immediately to an appropriate container for measurement of pH and redox potential and stored for subsequent chemical analysis and/or ecotoxicological testing. In general, this eluate can be used for both analyses of inorganic and organic substances.

If only inorganic substances are measured, the centrifugation step can be omitted and the decanted eluate can be directly filtered using the appropriate membrane filters (6.9) and a vacuum or pressure filtration apparatus (6.8). When this filtration as specified is not possible in less than 1 h with a liquid flow rate of at least 30 ml/cm^2/h, a liquid-solid separation procedure, specific for the considered case, shall be applied. Report the details in the test report. This specific procedure shall not include the use of additives.

NOTE 3 For inorganic substances, it is often preferable to pre-centrifuge the eluate at 2 000 *g* to 3 000 *g* for 20 min before filtration using glass bottles with a screw cap and polytetrafluoroethylene inlay (or, if possible, using the leaching bottle directly) prior to filtration. Higher speed or longer time can also be applied (see Annex B).

NOTE 4 Such a specific liquid-solid separation procedure can include settling, prefiltration on coarser filter, centrifugation, filtration on large-size membrane filter, filtration at high pressure, filtration at increasing high pressure following a first period without pressure, etc.

Determine the volume of eluate V_E or record the volume of the aliquot used.

Measure immediately the redox potential (*E*h in mV), electrical conductivity (in mS/m) and pH of the eluate. Measurement of turbidity and DOC is highly recommended.

NOTE 5 Information on DOC concentration in the eluate is relevant both for release of inorganic substances, as well as for organic substances.

Under certain circumstances, particularly for alkaline eluates, it is recommended to measure the pH of the raw eluate prior to filtration or centrifugation, since these operations may change the pH of the eluate.

Proceed immediately with the eluate treatment, as specified in 8.5.

8.5 Further preparation of the eluate for analysis

If necessary, divide the eluate into an appropriate number of sub-samples and store them in accordance with the requirements in ISO 5667-3 for subsequent chemical analysis and/or ecotoxicological testing.

Since eluate for ecotoxicological tests shall not contain NaN_3 (see NOTE 2 in 8.2.2), microbial degradation of organic substances may occur during the test and during the period of eluate storage. Therefore, it is highly recommended to perform ecotoxicological tests on eluate containing organic substances as soon as possible after completion of the leaching test.

8.6 Blank test

Blank tests shall be carried out at regular intervals in order to check, as far as possible, how well the whole procedure is performed. A volume of leachant of 900 ml is submitted to the whole procedure, starting at 8.2.2 or 8.2.3 and using no soil sample.

The eluate of this blank test shall fulfil the following minimum requirements: in the eluate of the blank test, the concentration of each considered substance shall be less than 20 % of the concentration determined in the eluate of the tested material or less than 20 % of the concentration in the eluate of a limit value to which the measurement result is to be compared. The substances to be considered are all the substances which are to be determined in the eluate of the tested material.

If the above requirements are not fulfilled, it is necessary to reduce the contamination. The blank test results shall not be deducted from the results of the material leaching test.

The above provision does not take into account the sieving step, crushing step or the splitting step. In order to minimize the possible cross-contamination during these three steps, it is recommended to process a representative portion of the laboratory sample through the sieving device, the crushing device and through the splitting device and to discard such material thereafter. This provision does not cover the situation described in the note under 6.11.

9 Calculation

The concentrations of substances in the eluate are measured by appropriate analytical methods. They give concentrations in mg/l. The final result is a mass fraction, calculated on the basis of the leachant volume and the mass of the test portion used, in mg/kg dry matter.

Calculate the quantity of a substance leached from the material, based on the dry mass of the original material, from Formula (5)

$$A = \rho_{\text{subst}} \times \left\{ \left(V_{\text{L}} / m_{\text{D}} \right) + \left[w_{\text{H}_2\text{O}} / \left(\rho_{\text{H}_2\text{O}} \times 100 \right) \right] \right\} \tag{5}$$

where

A is the release of a substance at a L/S = 10 (mg/kg of dry matter);

ρ_{subst} is the mass concentration of a particular substance in the eluate (mg/l);

V_{L} is the volume of leachant used (l);

$w_{\text{H}_2\text{O}}$ is the water content as calculated in Formula (2);

m_{D} is the mass of the dried test portion (kg);

$\rho_{\text{H}_2\text{O}}$ is the density of water (usually taken as 1 kg/l).

10 Test report

The test report shall include the following details:

a) a reference to this document, i.e. ISO 21268-5:2023;

b) address of laboratory, name of responsible person;

c) any information necessary for the complete identification of the sample;

d) information on sample pretreatment;

e) water content;

f) type of leachant;

g) the used leaching procedure (aerobic or anaerobic);

h) centrifugation speed/force, time and type of vessels used, temperature readings;

i) detailed description of the filtration step and results of adsorption tests on the filters applied if hydrophobic organic substances are reported;

j) the test results including at least redox, pH, electrical conductivity, measured mass concentrations (mg/l), released quantities (mg/kg dry matter), and limit of detection for each substance;

k) the blank test results;

l) any details that are optional or any deviations from the specifications of this document, and any effects which may have affected the results.

11 Analytical determination

11.1 General

Since the analysis step is not included in the scope of this document, the analytical method applied together with the limit of quantification shall be reported in the test report.

11.2 Blank test information

The following shall be included in the test report:

— date of the last blank test performed;

— results of the blank test, including the substances considered for the tested material and the levels above which the results can be considered as valid, when compared with the measured concentrations, in mg/l.

Annex A
(informative)

Examples of aerobic and anaerobic extraction

A.1 Aerobic extraction

a) Fold the blades of the stirring rod in so that they can pass through the neck of the bottle as shown in Figure A.1.

Figure A.1 — Stirring rod with blades folded in

b) Press the blades of the stirring rod onto the bottom of the bottle so that they fold out again. The height of the stirrer blades in the bottle shall be selected such that they do not touch the bottom. See Figure A.2.

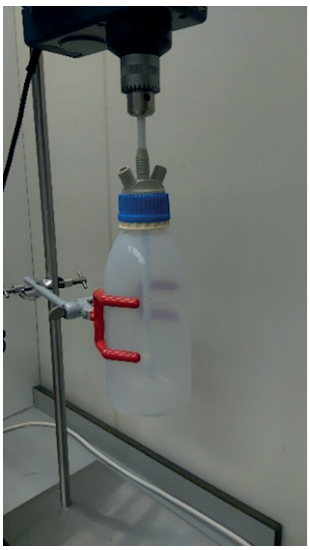

Figure A.2 — Stirring rod blades inside bottle

c) Aerate the suspension with compressed air via a Polytetrafluoroethylene (PTFE) tube as shown in Figure A.3.

Figure A.3 — Aeration of the suspension

A.2 Anaerobic extraction

a) Saturate the quantity of air above the extraction mixture with nitrogen as shown in Figure A.4.

Figure A.4 — Saturation of air with nitrogen above extraction mixture

b) Place the sealed bottle of extraction mixture in a container with a screw cap and saturate the free air with nitrogen as shown in Figure A.5.

Figure A.5 — Sealed bottle of extraction mixture in container with screw cap

c) Lay the screw cap on the container and saturate the free air with nitrogen for 15 min, then remove the nitrogen supply tube and seal the container with the screw cap as shown in Figure A.6.

Figure A.6 — Saturation of free air in container with nitrogen

Annex B
(informative)

Example of a specific liquid-solid separation procedure for soil samples

B.1 General

The original scope of leaching tests covers, in particular, solid substances containing larger amounts of dissolved salts. The general feasibility of these methods has limitations when the solubility of substances has to be determined in soil samples, in particular when, for example, oxidized, adsorbed or organically bound heavy metals are rather insoluble in those materials. Despite their low solubility, heavy metals are important from an environmental point of view. The lower the "pure" solubility of heavy metals in a contaminated soil sample, the higher is the relative influence of colloidal particle portions in eluate on the end result is.

Especially in the case of fine-textured soil samples that are rich in humus but poor in electrolytes, the filter cake produced during filtration exhibits very fine pores and less colloids pass through the membrane filter. Thus, the production of filter cake largely affects the "solubility" of heavy metals, a fact which is identified by this method. To obtain comparable results, it is necessary to stipulate the factors determining the height of the filter cake. In addition to sample-specific properties, the thickness of the filter cake is determined predominantly by the filter diameter and the volume of the eluate to be filtered. Absorption by the filter cake can be reduced when part of the extract solution is filtered.

B.2 Apparatus

B.2.1 Pressure filtration unit, for membrane filter (diameter 142 mm).

B.2.2 Membrane filter, of pore size 0,45 µm.

If another filter size is used, the volume to be filtered is modified according to the filter surface; an essential precondition is that the relationship between the volume to be filtered and the filter surface is complied with [relationship: about 1 l volume to 158 cm^2 filter surface (diameter 142 mm)].

B.2.3 Media-guiding material (in contact with extracts), in polytetrafluoroethylene.

B.3 Procedure

For sedimentation of the larger particles, allow the suspension to stand for 15 min after shaking.

Decant almost completely the supernatant liquid into a centrifuge tube or bottle device.

Apply centrifugation (30 min at 2 000 *g*).

Almost complete decant the supernatant liquid into the membrane pressure filter apparatus.

Apply, after 5 min of filtration without pressure, a pressure of 100 kPa to accelerate filtration. If after 15 min less than two thirds of the eluate have passed through the filter, increase the pressure to 200 kPa. If necessary, increase the pressure to a maximum of 350 kPa after 30 min. Continue the filtration until all the supernatant of centrifugation has passed through the filter. If the filtration is still incomplete after 2 h, stop the filtration, collect the incomplete filtrate and prepare it for analysis.