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Soil quality — Determination of some selected chlorophenols — Gas-chromatographic method with electron-capture detection

Qualité du sol — Dosage de certains chlorophénols — Méthode de chromatographie en phase gazeuse avec détection par capture d'électrons

Reference number
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Contents

Page

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 14154 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Soil quality — Determination of some selected chlorophenols — Gas-chromatographic method with electron-capture detection

WARNING — Chlorophenols are toxic and some are even carcinogenic. When handling samples containing chlorophenols, avoid skin contact. Use gloves and protective clothing. If large amounts of aerosols and dust particles are produced during the sampling, breathing protection may be necessary.

1 Scope

This International Standard describes the gas chromatographic determination of 15 chlorophenols (2,3-, 2,4- 2,5-, 2,6-, 3,4- and 3,5-dichlorophenol; 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6- and 3,4,5-trichlorophenol, 2,3,4,5- and 2,3,4,6-tetrachlorophenol and pentachlorophenol) in soil samples. This method can also be applied to other solid samples such as sediments and solid wastes.

This International Standard describes an acid-base liquid extraction, followed by acetylation and then liquid/liquid extraction. Determination of mass concentration is then carried out by gas chromatography and electron-capture detection.

This method is applicable to chlorophenols at the lowest mass concentrations ranging from approximately 0,01 mg/kg to 0,05 mg/kg depending on the component sensitivity and the quantity of sample used. In some cases complete separation of isomers cannot be achieved, in which case the sum is reported (for instance 2,4- and 2,5-dichlorophenols).

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 10381-1, *Soil quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

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

3 Terms and definitions

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

3.1
chlorophenol
aromatic hydroxy compound (phenol) carrying one to five chlorine atoms

4 Principle

The method described here is based on two steps. The first step includes a solid/liquid extraction: the chlorophenols are extracted from soil by a mixture of acetone-hexane at low pH. The second step is a purification step, based on successive extractions in basic and acidic aqueous media and hexane. Finally, the chlorophenols obtained in an aqueous carbonate solution are derivatized with acetic anhydride; the derivatives formed are extracted from this sample with hexane. The hexane fraction is analysed by gas chromatography with electron capture or mass detection.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

Gases for gas chromatography shall be of a purity as recommended by the gas chromatograph manufacturer.

Only commercially available certified standards of high purity shall be used.

5.1 **Water** of at least grade 1 as defined in ISO 3696:1987

5.2 **Ethanol**, C_2H_5OH, 99,5 % (mass fraction)

5.3 **Hexane**, C_6H_{14}

5.4 **Acetone**, CH_3COCH_3

5.5 **Hydrochloric acid**, HCl, concentrated, 37 % (mass fraction)

5.6 **Sodium hydroxide**, NaOH

5.7 **Potassium carbonate**, K_2CO_3

5.8 **Acetic anhydride**, $(CH_3CO)_2O$

5.9 **Sodium sulfate**, Na_2SO_4, anhydrous

Weigh portions of 2 g of sodium sulfate into test tubes with polytetrafluoroethylene (PTFE)-lined caps. Dry the test tubes at 500 °C ± 20 °C for 4 h ± 30 min without caps. Place them in a desiccator and let them cool. When cooled put on the caps and store the tubes at room temperature. Sodium sulfate can also be dried in larger portions and stored in a desiccator after cooling. Weigh portions of 2 g into tubes when needed.

5.10 **2,4,6-tribromophenol**, $C_6H_2Br_3OH$, (internal standard), CAS 118-79-6

5.11 **Chlorophenols** (standards)

— 2,3-dichlorophenol, $C_6H_3Cl_2OH$, CAS 576-24-9

— 2,4-dichlorophenol, $C_6H_3Cl_2OH$, CAS 120-83-2

— 2,5-dichlorophenol, $C_6H_3Cl_2OH$, CAS 583-78-8

— 2,6-dichlorophenol, $C_6H_3Cl_2OH$, CAS 87-65-0

— 3,4-dichlorophenol, $C_6H_3Cl_2OH$, CAS 95-77-2

— 3,5-dichlorophenol, $C_6H_3Cl_2OH$, CAS 591-35-5

— 2,3,4-trichlorophenol, $C_6H_2Cl_3OH$, CAS 15950-66-0

— 2,3,5-trichlorophenol, $C_6H_2Cl_3OH$, CAS 933-78-8

— 2,3,6-trichlorophenol, $C_6H_2Cl_3OH$, CAS 933-75-7

— 2,4,5-trichlorophenol, $C_6H_2Cl_3OH$, CAS 95-95-4

— 2,4,6-trichlorophenol, $C_6H_2Cl_3OH$, CAS 88-06-2

— 3,4,5-trichlorophenol, $C_6H_2Cl_3OH$, CAS 609-19-8

— 2,3,4,5-tetrachlorophenol, C_6HCl_4OH, CAS 4901-51-3

— 2,3,4,6-tetrachlorophenol, C_6HCl_4OH, CAS 58-90-2

— Pentachlorophenol, C_6Cl_5OH, CAS 87-86-5

5.12 Sodium hydroxide solution, NaOH, c(NaOH) = 0,1 mol/l

5.13 Sodium hydroxide solution, NaOH, c(NaOH) = 0,5 mol/l

5.14 Potassium carbonate solution, K_2CO_3, $c(K_2CO_3)$ = 0,1 mol/l

5.15 Potassium carbonate solution, K_2CO_3, $c(K_2CO_3)$ = 5,2 mol/l

5.16 Internal standard solutions

5.16.1 Stock solution

Prepare the stock solution of the internal standard by weighing 2,4,6-tribromophenol (5.10) and dissolving it in ethanol (5.2). A suitable concentration is given in Table A.1. Divide the stock solution into 5 ml bottles with tight caps, 1,5 ml in each bottle, and store at −18 °C.

Stock solutions are stable for at least half a year when stored in the dark at 4 °C. They are stable at least one year when stored at −18 °C.

NOTE 2,4-dibromophenol or 2,6-dibromophenol can also be used as internal standard.

5.16.2 Working solution

Prepare the working solution by diluting the stock solution (5.16.1) with water (5.1), and add a few drops of sodium hydroxide solution (5.13) to prevent precipitation. A suitable concentration of the internal standard working solution is given in Table A.1.

5.17 Standard solutions (see Table A.2)

5.17.1 Stock solutions

Weigh out each of the chlorophenol standards (5.11) into the same or into separate measuring flasks and dissolve them in ethanol (5.2). Divide the stock solution(s) into 5 ml bottles with tight PTFE-lined caps, 1,5 ml in each bottle, and store at −18 °C.

5.17.2 Working solutions

Dilute stock solution(s) (5.17.1) with distilled water to obtain a working solution, and add a few drops of sodium hydroxide solution (5.13) to prevent precipitation.

5.18 Acetone-hexane solution, 1:1 (volume fraction)

6 Apparatus

Ordinary laboratory apparatus and the following.

6.1 Standard laboratory **glassware**, appropriately cleaned and free of interfering compounds.

Do not use any kind of plastics containers, since the chlorophenols may adsorb to these; plastics materials may also contribute their impurities to the sample material.

6.2 **Capillary gas chromatograph**, preferably equipped with two electron-capture detectors and with the facility to connect two capillary columns to the same injection system or with two different injectors.

Results achieved with single-column GC-ECD systems should be confirmed by additional GC-ECD analysis using another column of different polarity or by GC-MS analysis.

6.3 **Capillary columns**, at least two, with stationary phases of different polarity.

6.4 **Ultrasonic bath**.

7 Sampling

WARNING — Soil contaminated with commercial mixtures of chlorophenols often contains impurities of polychlorinated phenoxyphenols (PCPPs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans, and polychlorinated biphenyls (PCBs) in minor concentrations. There is thus a contamination risk in the laboratory.

Take samples in accordance with ISO 10381-1 and pretreat the samples in accordance with ISO 14507.

Place the samples in glass bottles with PTFE caps. It is recommended to fill the bottles completely.

Store the samples in the dark in the laboratory either at −18 °C or at +4 °C. Chlorophenols can be subject to microbial conversion under certain conditions. It is recommended that samples be frozen if they are stored for more than 2 days.

8 Procedure

8.1 Test portion

Using a spoon or a spatula, mix the field-moist soil as well as possible. Take a soil sample of at least 10 g, with an accuracy of 0,01 g, directly from the bottle.

8.2 Dry matter content

Determine the dry matter content of the soil in a subsample from the same bottle in accordance with ISO 11465.

8.3 Blank sample

Treat blank samples exactly the same way as normal samples, but replace the test portion (8.1) by 10 g of representative sample matrix known to be free of chlorophenols.

8.4 Standard sample

Treat standard samples exactly the same way as normal samples, but replace the test portion (8.1) by 50 µl of standard working solution (5.17.2) and 10 g of representative sample matrix known to be free of chlorophenols.

8.5 Extraction

WARNING — Extreme care shall be taken through all steps of the extraction procedure, which shall be performed in a fume hood. All solvent wastes shall be collected and treated as hazardous waste.

Take a test portion (or blank or standard) and place it in a conical flask. Add 50 µl of internal standard working solution (5.16.2), 75 ml of acetone-hexane (5.18) and 0,5 ml of hydrochloric acid (5.5).

In case of high concentrations, a larger quantity of the internal standard should be added.

Sonicate the sample for 2 min every 10 min during 1 h. Allow the sample to settle, transfer the solution quantitatively to a separation funnel and extract twice with 40 ml of sodium hydroxide solution (5.12).

Some types of soil are acidic, contain carbonates or have a high buffer capacity. In these cases the amounts of acid and base added are not enough to reach pH values which are sufficiently low and high, respectively. If in doubt when chlorophenols are to be analysed in these types of soil, verify that the pH values are < 3 in the extraction step and > 12 after adding sodium hydroxide solution.

Collect the NaOH extract in a second separation funnel. Acidify the combined NaOH extracts with hydrochloric acid (5.5) to pH < 3, and extract twice with 50 ml of hexane (5.3). Collect the hexane extract in a third separation funnel. Finally, extract the hexane solution twice with 35 ml of potassium carbonate solution (5.14) and collect the potassium carbonate solutions in a fourth separation funnel.

8.6 Acetylation

8.6.1 Acetylation of samples

Acetylate the chlorophenols in the combined potassium carbonate solution (8.5) as follows. Add 1 ml of acetic anhydride (5.8) to the extract and shake the mixture vigorously for 2 min to release any carbon dioxide formed in the funnel. Let the mixture stand for 10 min while shaking occasionally, and then add 5 ml of hexane (5.3). Shake the funnel and let the two phases separate. Transfer as large a portion as possible of the hexane phase to a tube containing 2 g of Na_2SO_4 (5.9) for drying. After shaking, transfer the hexane solution to another vial with Na_2SO_4 and store at 4 °C. Analyse the chlorophenol samples as soon as possible (within 48 h) since the acetates are rather labile towards hydrolysis.

8.6.2 Acetylation of control samples

Take 50 µl of standard working solution and add it to a separation funnel with 100 ml of water (5.1). Add 2 ml of potassium carbonate solution (5.15) and shake the funnel. Add 1 ml of acetic anhydride (5.8) to the separation funnel and shake the mixture vigorously for 2 min to release any carbon dioxide formed in the funnel. Let the mixture stand for 10 min while shaking occasionally, and then add 5 ml of hexane (5.3). Shake the funnel and let the two phases separate. Transfer as large a portion as possible of the hexane phase to a tube containing 2 g of Na_2SO_4 (5.9) for drying. After shaking, transfer the hexane solution to another vial with Na_2SO_4 and store at 4 °C. Analyse the control samples as soon as possible (within 48 h).

8.7 Gas chromatographic analysis

Set up the gas chromatograph equipped with appropriate columns (6.3) according to the manufacturer's instructions. Optimize the gas flows to obtain sufficient separation. Ensure a stable condition. An example of gas chromatography conditions and temperature programme is given in Annex B.

8.8 Calibration

Use extracted and acetylated standard samples (8.4) as calibration solutions. Calibration solutions should be prepared in accordance with 8.4 by adding different volumes of working solution (5.17.2). For each compound, establish a separate calibration function consisting of at least five measurement points. It is permissible to examine several compounds in one calibration. A knowledge of the retention times of the compounds is

essential. The retention times can be determined by injecting separately standard solutions of each individual compound, analysed under defined analytical conditions.

The calibration function obtained for a particular compound is valid only for the established concentration range and the respective sample preparation method used. The calibration function depends on the operational conditions of the entire analytical system.

Calibration shall be based on peak height or area and on the response of the internal standard.

In general, the use of peak heights instead of peak areas is recommended.

Calculate the calibration function by regression using the ratio y_{ie}/y_{se} as a function of the ratio of ρ_{ie}/ρ_{se}, in accordance with Equation (1):

$$\frac{y_{ie}}{y_{se}} = a_{is} \cdot \frac{\rho_{ie}}{\rho_{se}} + b_{is} \tag{1}$$

where

y_{ie} is the measured response (dependent variable) of the compound i during calibration, depending on ρ_{ie};

y_{se} is the measured response (dependent variable) (signal area or signal height) of the internal standard s during calibration, depending on ρ_{se};

ρ_{ie} is the mass concentration (independent variable) of the compound i in the calibration solution, in micrograms per litre;

ρ_{se} is the mass concentration (independent variable) of the internal standard s in the calibration solution, in micrograms per litre;

a_{is} is the slope of the calibration curve of the ratio of the measured values y_{ie}/y_{se} as a function of the corresponding ratio of the mass concentration ρ_{ie}/ρ_{se} (corresponds to the substance-specific response factor, often referred to as f_i), nondimensional;

b_{is} is the axis intercept of the calibration curve on the ordinate, nondimensional.

9 Calculation

Calculate the mass $m_{i,j}$ of chlorophenol i in a test portion from column j, in micrograms, using Equation (2):

$$m_{i,j} = \frac{(y_{i,j} / y_{s,j}) - b_{is}}{a_{is}} \cdot m_{s,j} \tag{2}$$

where:

$y_{i,j}$ is the peak height or area of chlorophenol i from column j;

$y_{s,j}$ is the peak height or area of internal standard from column j;

$m_{s,j}$ is the mass of internal standard added to the sample, in micrograms;

b_{is} is as given in 8.8, see Equation (1);

a_{is} is as given in 8.8, see Equation (1).

Calculate the resulting amounts, $m_{i,\text{test}}$, as the arithmetic mean of the results obtained from both columns when two columns are used. If the higher result differs by more than 20 % from the lower result, use the lower result as the final result.

Convert the mass of each chlorophenol i in the test portion to the mass fraction of dry matter (mg/kg dry matter).

$$w_{i,\text{dry}} = \frac{m_{i,\text{test}} \cdot 100\%}{m_{\text{smpl}} \cdot w_{\text{dm}}} \tag{3}$$

where

$w_{i,\text{dry}}$ is the mass fraction of chlorophenol i , in milligrams per kilogram dry matter (mg/kg);

$m_{i,\text{test}}$ is the mass of chlorophenol i in test portion, in micrograms (µg);

m_{smpl} is the mass of the test portion, in grams (g);

w_{dm} is the mass fraction of dry matter of the soil, in percent (%);

Report the mass fractions of the substances to 2 significant figures.

10 Precision

This method has been tested in an interlaboratory test with 20 participating laboratories in the Nordic countries. The data were analysed according to ISO 5725-2 and the repeatability and the reproducibility were determined.

A summary of the data is given in Table C.1.

11 Quality assurance and control

Analyse a blank sample (8.3), a standard sample (8.4) and a control sample (8.6.2) with each series of samples. The first two samples show if any interferences have occurred during the extraction procedure, and the third sample shows the stability of the gas chromatograph.

If interfering peaks are detected in the blank sample (more than 10 % of the lowest measured value), then carry out systematic investigation to detect and eliminate the source of contamination.

Compare the concentration of the standard sample with the calibration curve. If the value is within the confidence interval of the corresponding value, it is permissible to use the calibration curve. If not, then establish a new calibration curve.

12 Test report

The following information shall be included in the test report:

a) all information necessary for identification of the sample;

b) a reference to this International Standard, including the year of publication;

c) the mass fraction of each chlorophenol, in milligrams per kilogram of dry matter, in accordance with Clause 9;

d) the time interval between the sampling and the extraction;

e) any disturbances observed during determination, for instance the presence of other peaks;

f) details of any operation not specified in this International Standard or regarded as optional, as well as any factor which may have affected the results.

Annex A
(informative)

Typical concentrations of standard solutions

Table A.1 — Internal standard

Internal standard	Stock solution	Working solution
2,4,6-tribromophenol	1,148 mg/ml	114,8 µg/ml

Table A.2 — Chlorophenol standards

Chlorophenol	Stock solution	Working solution
2,3-dichlorophenol	400 µg/ml	40 µg/ml
2,4-dichlorophenol	400 µg/ml	40 µg/ml
2,5-dichlorophenol	400 µg/ml	40 µg/ml
2,6-dichlorophenol	400 µg/ml	40 µg/ml
3,4-dichlorophenol	400 µg/ml	40 µg/ml
3,5-dichlorophenol	400 µg/ml	40 µg/ml
2,3,4-trichlorophenol	400 µg/ml	40 µg/ml
2,3,5-trichlorophenol	400 µg/ml	40 µg/ml
2,3,6-trichlorophenol	400 µg/ml	40 µg/ml
2,4,5-trichlorophenol	400 µg/ml	40 µg/ml
2,4,6-trichlorophenol	600 µg/ml	60 µg/ml
3,4,5-trichlorophenol	200 µg/ml	20 µg/ml
2,3,4,5-tetrachlorophenol	200 µg/ml	20 µg/ml
2,3,4,6-tetrachlorophenol	600 µg/ml	60 µg/ml
pentachlorophenol	1 mg/1 ml	100 µg/ml

Annex B
(informative)

Example of gas chromatographic conditions

The chromatographic separation using the calibration solutions can be optimized by applying the following guidelines.

Injector temperature:	250 °C
Oven temperature programme:	80 °C for 1,5 min
	80 °C to 140 °C, at 20 °C/min
	140 °C to 210 °C, at 2 °C/min
	210 °C to 270 °C, at 20 °C/min
	270 °C for 5 min
Detector temperature:	300 °C
Carrier gas:	helium
Gas flowrate:	20 cm/s to 30 cm/s
Columns:	Typical:
	length 25 m, internal diameter 0,22 mm and film thickness 0,33 µm, coated for example with chemically bonded methyl silicone or 5 % phenyl methyl silicone

Annex C
(informative)

Results of interlaboratory test

Table C.1 gives the results of interlaboratory test mentioned in Clause 10.

Samples A, B and C were prepared from authentic soil from a sawmill site in Finland contaminated with chlorophenols. Sample D was prepared by mixing known amounts of 16 different chlorophenols in water.

Table C.1 — Results

Chlorophenol	Sample	N	p	Expected level mg/l	Mean (mg/kg dry matter)	Mean mg/l	Recovery %	s_r mg/kg	CV_r %	s_R mg/kg	CV_R %
2,3-DCP	A	6	1								
	B	6	1								
	C	6	1								
	D	6	6	40		31,4	78,5				
2,4-DCP	A	8	7		0,029			0,007	25,6	0,009	31,6
	B	8	7		0,025			0,004	14,9	0,008	31,4
	C	8	6		0,362			0,030	8,2	0,250	69,2
	D	12	10	40		47,2	118,0	3,10	6,6	14,4	30,5
2,4/2,5-DCP	A	5	5		0,040						
	B	5	5		0,043						
	C	5	3		0,340						
	D	3	3	80		67,9	84,9				
2,5-DCP	A	1	0								
	B	1	0								
	C	1	0								
	D	2	2	40							
2,6-DCP	A	6	2		0,012						
	B	6	2		0,010						
	C	6	1								
	D	10	10	40		35,9	89,7	1,99	5,6	7,42	20,7
3,4-DCP	A	8	7		0,039			0,008	19,5	0,023	58,3
	B	9	6		0,037			0,027	72,0	0,028	76,1
	C	10	9		0,327			0,037	11,4	0,183	55,8
	D	10	9	40		31,8	79,4	2,74	8,6	5,96	18,8

Table C.1 — Results *(continued)*

Chlorophenol	Sample	N	p	Expected level mg/l	Mean (mg/kg dry matter)	Mean mg/l	Recovery %	s_r mg/kg	CV_r %	s_R mg/kg	CV_R %
3,5-DCP	A	7	5		0,011						
	B	7	5		0,023						
	C	7	5		0,118						
	D	7	7	40		35,1	87,8	1,97	5,6	2,79	7,9
2,3,4-TCP	A	5	2								
	B	5	3		0,011						
	C	7	7		0,066						
	D	9	9	40		32,5	81,2	1,64	5,1	5,44	16,8
2,3,5-TCP	A	6	1								
	B	6	2		0,005						
	C	6	3		0,075						
	D	6	62	40		31,0	77,6	1,16	3,7	5,17	16,7
2,3,6-TCP	A	5	2		0,019						
	B	5	4		0,027						
	C	7	8		0,264						
	D	8	9	40		46,8	116,9	2,56	5,5	22,5	48
2,4,5-TCP	A	12	9		0,029			0,005	15,6	0,013	45,6
	B	12	9		0,023			0,005	23,2	0,008	34,6
	C	11	10		0,281			0,029	10,4	0,149	53,0
	D	13	11	40		44,2	110,4	2,02	4,6	7,95	18,0
2,4,6-TCP	A	18	16		0,253			0,027	10,6	0,081	31,8
	B	17	15		0,083			0,014	17,0	0,034	40,8
	C	16	13		1,98			0,244	12,4	1,40	71,1
	D	17	14	60		53,4	89,0	2,80	5,2	9,26	17,3
3,4,5-TCP	A	7	7		0,043			0,024	55,5	0,040	95,1
	B	7	6		0,030			0,011	37,9	0,016	51,9
	C	7	6		1,64			0,136	8,3	0,851	51,9
	D	8	8	20		18,1	90,7	1,54	8,5	6,05	33,3
2,3,4,5-TCP	A	10	8		0,015			0,002	13,1	0,006	41,8
	B	10	7		0,021			0,020	94,4	0,019	90,2
	C	9	9		0,658			0,076	11,5	0,356	55,5
	D	11	10	20		19,5	97,6	0,855	4,4	3,92	20,1
2,3,4,6-TCP	A	19	17		2,33			0,266	11,4	1,12	47,9
	B	19	16		2,23			0,271	12,2	1,06	47,7
	C	19	17		193			38,4	19,8	110	56,9
	D	16	13	60		52,8	88,1	2,32	4,4	18,0	34,1