

## **Determination of Nutrients**

### **Determination of Ammonia, Nitrate and nitrite**

#### **Pretreatment and extraction for the determination of extractable ammonia, nitrate and nitrite**

### **Introduction**

This document is developed in the project 'Horizontal'. It is the result of desk study "Determination of total phosphorus and nitrogen and fractions" in the project and aims at the description of the standard for pretreatment and extraction of the determination of extractable ammonia, nitrate and nitrite in soil, sludge, biowaste and related waste. After discussion with all parties concerned in CEN and selection of a number of test methods described in this study will be developed further as a modular horizontal method and validated in the project 'Horizontal'.

Until now test methods determining properties of materials were often prepared in Technical Committees (TC's) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for each other product.. Consequently this resulted in a lot of duplicate work. To avoid such duplication of work for parts of a testing procedure often was referred to parts of test methods from other TC's. However following problems are often encountered while using references in this way: 1).The referenced parts are often not edited in a way that they could easily be referred to, 2). the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that they can be used for all materials and products or for all materials and products with certain specifications. Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in a test procedure and not the whole test procedure (from sampling to analyses) but only.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

This standard is a module, for determining nutrients in solid materials and liquids.

This module concerns with the extraction of ammonia, nitrate and nitrite in soil, sludge, biowaste and related waste .

The other horizontal modules that will be available in due time are to be found in the informative annex B which contains a brief overview of the modules that are or will be worked out in the project 'Horizontal.'

The texts of the chapters 1-11 are normative; annexes are normative or informative, as stated in the top lines of the annexes.

## **1 Normative references**

ISO 11464 Soil quality – Pretreatment of samples for physico-chemical analysis  
ISO 11465 Soil quality – Determination of dry matter and water content on a mass basis – gravimetric method  
EN 12880 Characterisation of sludge – Determination of dry residue and water content  
CEN/TC 292 WI 29292030 Characterisation of waste – Preparation of test portions from the laboratory sample  
prEN 14671 Characterisation of sludges – Pretreatment for the determination of extractable ammonia using 2 mol/l potassium chloride  
ISO 14256-1 Soil quality – Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution – part 1: manual method  
ISO 14256-2 Soil quality – Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution – part 2: automated method  
E DIN 19746 Soil quality – Determination of mineral nitrogen (nitrate and ammonium) in soil profiles (N<sub>min</sub> laboratory method)  
EN 11732 Water quality – Determination of ammonium nitrogen by flow analysis (CFA and FIA) and spectrometric detection  
ISO 5664 Water quality - Determination of ammonium: distillation and titration method  
EN ISO 13395 Water quality – Determination of nitrite nitrogen, nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection

## **2 Scope and working area**

This standard describes the pretreatment and extraction method for the nitrogen fractions ammonia, nitrate and nitrite in soil, sludge, biowaste and related waste. The extraction method is suitable for moist samples.

The determination of the nitrogen fractions can be done manually or by automated methods.

## **3 Principle**

An aliquot of the homogenized moist material is shaken for one hour with 2 mol/l potassium chloride solution at room temperature. The ratio of extractant to material is five to one. The extraction solution is filtered and the nitrogen fractions are analysed by flow injection analysis (FIA, EN 11732, ISO 7150-2) or continuous flow analysis (CFA, EN 14256-2, EN 11732, ISO 7150-2) or by manual methods as distillation and titration (ammonia, ISO 5664) or spectrophotometric method (ammonia, nitrate, nitrite, EN 14256-1, ).

## 4 Reagents

### 4.1 General

All reagents have to be of recognized analytical grade

### 4.2 Potassium chloride $c(\text{KCl}) = 2 \text{ mol/l}$

Dissolve 745,5 g of potassium chloride, dried at  $105^\circ \text{C}$ , in approximately 3 litres of water and dilute to 5 litres with water.

## 5 Apparatus

### 5.1 General

Usual laboratory equipment is needed

### 5.2 Analytical balance with an accuracy of 0,05 g

### 5.3 Extraction bottles

Wide necked glass or plastic bottles with secure stopper or caps, nominal volume 250 or 500 ml or other. The material must not adsorb ammonia, nitrate or nitrite or contaminated with this species.

### 5.4 Shaking apparatus

End-over-end shaker, frequency  $30 \text{ min}^{-1}$  to  $40 \text{ min}^{-1}$

### 5.5 Filter paper, free of nitrogen fractions

## 6 Calibration samples

To store calibration samples it is necessary to freeze them to avoid losses or changing of the nitrogen fractions. It is suitable to control the extraction with samples with known content of the nitrogen fractions.

To control the measuring, it is suitable to have stabilised filtrates of materials which run the process during measuring the test samples.

## 7 Pretreatment of test samples

All samples shall be pretreated according to the special standard in the fields of soil, sludge, biowaste and related waste.

The samples shall be analysed as soon as possible.

The samples can change composition through biological and/or chemical activity.

The samples shall be protected from being warmed up during the sampling procedure. The transportation to the laboratory shall be organised in such a way that no warming up occurs. Transportation in a cool box is recommended. If the samples are analysed within three days it is enough to store them at  $4^\circ \text{C}$ , otherwise they should be stored at  $-20^\circ \text{C}$  (deep-frozen), which enables storing for several weeks, without any significant change in the content of mineral nitrogen. It is advantageous to homogenise the moist sample, to divide it into the test sample before storing them at  $-20^\circ \text{C}$ .

When the content of mineral nitrogen is determined in deep-frozen samples, the temperature and the duration of the thawing process have to be controlled. The samples can be thawed at room temperature, if they are homogenised and extracted within 4 h after beginning of thawing. Thawing at  $4^\circ \text{C}$  is also possible, but the thawing period should not exceed 48 h.

Note 1: The deep frozen homogenised test sample is directly transferred to the extraction bottle and the potassium chloride solution if change in the content of the nitrogen fractions is to be suggested.

Note 2: Drying of the material, even rapid microwave drying will result in change of content especially of ammonia. Drying is not subject within this standard.

## **8 Working instructions**

Note 3: Homogeneity of the laboratory sample and the test sample is to be guaranteed

### **8.1 Extraction**

Transfer a known weight of the homogenised test sample (equal to 0,25 to 2,0 g dry mass) to an extraction bottle (5.3), add potassium chloride solution (4.2) in the ratio test sample (dry mass) to extraction solution of one to five (m/V). Replace the bottle cap and place the extraction bottle to the shaking apparatus (5.4). Shake the extraction bottle for 1 h at room temperature.

Note 4: The amount of test sample is related to the homogenising procedure. Take care, that the test sample is a homogeneous part of the collected sample and the laboratory sample.

### **8.2 Filtration**

Filter the extraction solution through the filter (5.5). Collect the filtrate for determination of the nitrogen fractions in a volumetric flask and fill up to volume with potassium chloride solution (4.2).

Note 5: The analysis of the nitrogen fractions shall be done as soon as possible. For the high concentration of potassium chloride avoids biological activity, the extracts shall be stored at 4 °C.

### **8.3 Blank test**

Carry out a blank test in the whole procedure as it is performed with the test sample without the test sample.

### **8.4 Measuring**

Measuring is done according to EN ISO 11732, ISO 14256-2, ISO 14256-1, ISO 5664, EN ISO 14911. State of the art is to use the flow injection analysis (FIA, reference method) or the continuous flow analysis (CFA, reference method), their description is presented in the standard EN ISO 11732 and ISO 14256-2

## **9 Calculations and expression of results**

The calculation is described in the mentioned standards in chapter 8.4. The results of extractable ammonium, nitrate or nitrite are expressed in g/kg dry mass. The dry mass is determined according to the related standard.

## **10 Validation and precision**

The extraction procedure is not validated. This has to be done for all materials in future. The determination of nitrogen species is validated in EN ISO 11732.

## **11 Test report**

The test report is formulated according to the special conditions of accreditation (EN 41001 or EN 17025).

### **Annex A**

(informative)

Validation and precision

To validate the extraction procedure only the following proposal is made:  
The test material is extracted with potassium solution in an amount to get sufficient extraction solution to distribute this solution to the participants of the round robin study. Additionally the organizer of the round robin study supplies the participants with calibration solutions for FIA or CFA. The participants do their own extraction and measure this solution together with the delivered solution against the delivered calibration solution and their own calibration solution. The statistical operation of these data can explain the contribution of the extraction and the measuring to the standard deviation or the uncertainty of result.

In the future the validation is done with three samples of different soils, three samples of different sludges, three samples of different biowaste and three samples of different related wastes with different contents of phosphorus each. These samples are moist samples.

The repeatability and the reproducibility are calculated from the results of the round robin studies with the factor 2,8 .

Investigation has to be done about the question of the concentration of the extraction solution, the extraction time and and the repetition of the extraction.

### **Annex B**

(informative)

Bibliography

Page, A.L. et al. (1982): Methods of soil analysis, Part 2, American Society of Agronomy and Soil Science of America, Madison, WI.

Lickfett, T., Merkel, D., von Hörstein, D., Przemeczek, E., Lücke, W. (1996):  
Konservierung von Nmin-Proben durch Mikrowellentrocknung? Agrobiol.Res. 49,  
169-178

ISO 11464 Soil quality – Pretreatment of samples for physico-chemical analysis

ISO 11465 Soil quality – Determination of dry matter and water content on a mass  
basis – gravimetric method

EN 12880 Characterisation of sludge – Determination of dry residue and  
watercontent

CEN/TC 22 WI 29292030 Characterisation of waste – Preparation of test portions  
from the laboratory sample

prEN 14671 Characterisation of sludges – Pretreatment for the determination of  
extractable ammonia using 2 mol/l potassium chloride

ISO 14256-1 Soil quality – Determination of nitrate, nitrite and ammonium in field-  
moist soils by extraction with potassium chloride solution – part 1: manual method

ISO 14256-2 Soil quality – Determination of nitrate, nitrite and ammonium in field-  
moist soils by extraction with potassium chloride solution – part 2: automated method

E DIN 19746 Soil quality – Determination of mineral nitrogen (nitrate and ammonium)  
in soil profiles (Nmin laboratory method)

EN 11732 Water quality – Determination of ammonium nitrogen by flow analysis  
(CFA and FIA) and spectrometric detection

ISO 5664 Water quality - Determination of ammonium: distillation and titration  
method

EN ISO 13395 Water quality – Determination of nitrite nitrogen, nitrate nitrogen and  
the sum of both by flow analysis (CFA and FIA) and spectrometric detection