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Organic and organo-mineral fertilisers — Detection of specific pathogens

ICS:

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European foreword

This document (CEN/prEN 17803) has been prepared by Technical Committee CEN/TC 260 “Fertilisers and liming materials”, the secretariat of which is held by DIN.

This is a working document.

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

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

Introduction

Regulation (EU) 2019/1009 [1] lays down the rules on the making available on the market of EU fertilising products and the specific safety and quality requirements for the defined product function categories (PFCs). Organic and organo-mineral fertilisers have been classified as PFC 1(A) and PFC 1(B). Inorganic fertilisers have been classified as PFC 1(C).

In this document the normative references of the test methods to be used for the detection of specific pathogens in organic, organo-mineral and certain inorganic fertilisers are defined in order to assess compliance with the related requirements in Regulation (EU) 2019/1009 [1].

1 Scope

This document is applicable to organic, organo-mineral and inorganic fertilisers which contain more than 1 % by mass of organic carbon, other than organic carbon from chelating or complexing agents, nitrification inhibitors, denitrification inhibitors or urease inhibitors, coating agents, urea or calcium cyanamide.

It is relevant for fertilising products which are classified as PFC 1(A), PFC 1(B) and certain fertilising products classified as PFC 1 (C), as long as the main function of the EU fertilising product is classified as PFC 1(A), PFC 1(B) and PFC 1(C) of Regulation (EU) 2019/1009 [1].

This document is also applicable to the blends of fertilising products where a blend is a mix of at least two of the following component EU fertilising products: fertilisers, liming materials, soil improvers, growing media, inhibitors or plant biostimulants, and where organic, organo-mineral or inorganic fertilisers as indicated above are the highest % in the blend by mass or volume, or in the case of liquid form by dry mass. If organic, organo-mineral or inorganic fertilisers as indicated above are not the highest % in the blend, the European Standard for the highest % of the blend applies. In case a blend of fertilising products is composed of components in equal quantity, or in case the component EU fertilising products used for the blend have identical formulations, the user decides which standard to apply.

This document specifies references to the methods for the:

- enumeration of *Enterococcaceae*;
- detection of *Salmonella* spp.;
- enumeration of *Escherichia coli*.

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.

EN 12944-1:1999,¹ *Fertilisers and liming materials - Vocabulary - Part 1: General terms*

EN 12944-2:1999,² *Fertilisers and liming materials - Vocabulary - Part 2: Terms relating to fertilisers*

EN 1482 (all parts), *Fertilisers and liming materials — Sampling and sample preparation*

prEN 17804, *Organic, organo-mineral and inorganic fertilisers — Enumeration of Enterococcaceae*

prEN 17780, *Organic, organo-mineral and inorganic fertilisers — Detection of Salmonella spp.*

prEN 17781, *Organic, organo-mineral and inorganic fertilisers — Enumeration of Escherichia coli*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1:1999¹ and EN 12944-2:1999² apply.

¹ As impacted by EN 12944-1:1999/AC:2000.

² As impacted by EN 12944-1:1999/AC:2000.

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

- IEC Electropedia: available at <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

3.1

laboratory sample

sample intended for laboratory inspection or testing

3.2

test sample

sample prepared from the laboratory sample (3.1) and from which test portions (3.3) will be taken

3.3

test portion

quantity of material taken from the test sample (or if both are the same, from the laboratory sample) and on which the test is carried out

3.7

initial suspension

primary dilution obtained after a weighed or measured test portion of the product under examination (or of a test sample prepared from the product) has been mixed with, normally, a nine-fold quantity of diluent

Note 1 to entry: A closer ratio between the diluent and the quantity of product is often not recommended because of possible inhibiting influences of the matrix.

3.8

further dilution

suspension or solution obtained by mixing a measured volume of the initial suspension (3.7) with an x-fold volume of diluent and by repeating this operation with further dilutions until a dilution series, suitable for the inoculation of culture media, is obtained.

Note 1 to entry: Ten-fold dilutions are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes.

4 Principle

The detection of specific pathogens in a fertilising product consists of a sampling procedure, a sample preparation procedure and a detection method that is specific for each pathogen. The sample preparation method in this document can also be used for other microorganisms.

5 Diluents

For standard laboratory practice, EN ISO 7218 [2] and EN ISO 11133 [3] can be used.

Composition of diluents and their preparation are specified in Annex A (normative).

When testing organo-mineral or inorganic fertilisers, the pH value of the substrate in solution should be determined in a preliminary test. Fertilisers with a high mineral content can significantly change the pH value of the initial suspension, which can negatively affect the viability of the microorganisms to be detected. In those cases, it is recommended to use a double-buffered phosphate buffer (A.2) or double-buffered peptone water (A.4) for the preparation of the initial suspension (8.3).

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications. Usual microbiological laboratory equipment as described in prEN ISO/DIS 7218 shall be used and, in particular, the following.

6.1 Equipment for dry sterilization (oven) and wet sterilization (autoclave).

6.2 Equipment for size reduction, which may include:

- scissors;
- tweezers;
- straight scalpels;
- knives;
- hammers (used with a sterile bag around the sample);
- saws;
- spatulas;
- mortar and pestle.

6.3 Homogenizing equipment, which may include:

- a magnetic stirrer;
- orbital shaker, with bottles or plastic bags (glass beads may be used in the case of viscous or thick materials);
- peristaltic blender (paddle blender), with sterile bags, preferable with a filter;
- laboratory mixer;
- vortex mixer (for dilutions).

6.4 Incubator(s), capable of maintaining a temperature of 44°C to 47°C.

6.5 Scales of the required range and accuracy for the different products to be weighed.

6.6 Water bath, capable of maintaining a temperature between 44 °C and 47 °C.

6.7 Cooling unit, adjustable to 5 °C ± 3°C.

6.8 pH-meter, capable of reading to the nearest 0,1 pH unit at 20 °C to 25 °C.

6.9 Sterile tubes, bottles, or flasks with caps, of appropriate capacity.

6.10 Pipettes or pipettor and sterile tips, of nominal capacities of 1 ml and 10 ml.

6.11 Volumetric pipettes, burettes or flasks, of appropriate capacity.

7 Sampling

Sampling shall be performed carefully, following the principles described in EN 1482 with the appropriate adaptations to account for the specificities of organic, organo-mineral and inorganic fertilisers, and for the microbiological quality of the samples.

It is important that the laboratory receives a laboratory sample which is representative and has not been contaminated, damaged or changed during transport or storage.

Liquid, semi-liquid and moist fertilisers shall be stored at $5\text{ °C} \pm 3\text{ °C}$ (6.7) after reception of the sample until testing. Solid fertilisers should be stored at $5\text{ °C} \pm 3\text{ °C}$ (6.7). Samples arriving at the laboratory in their original state can be stored for up to 5 days, but they shall be processed as soon as possible. Samples must not be frozen under any circumstances.

NOTE Fertilisers packaged in carton boxes or paper bags should not be put in the refrigerator to avoid problems caused by humidity.

8 Sample preparation prior to microbiological examination

8.1 General

The sample shall be prepared in accordance with the instructions provided in this document. It is recommended to also consult EN ISO 6887 [5] and EN ISO 18593 [7].

Sample preparation prior to microbiological examination consists of two consecutive steps. The second step depends on the intent of the subsequent microbiological examination, meaning enumeration or detection.

1. Preparation of the test portion (3.3) from the laboratory sample (3.1);
 - 2a. **For detection:** preparation of a first enrichment to obtain a distribution of microorganisms in the test portion as uniform as possible
 - 2b. **For enumeration:** preparation of an initial suspension to obtain a distribution of microorganisms in the test portion as uniform as possible.

8.2 Preparation of the test portion

The types of packaged materials sent to the laboratory can be in flexible packaging. This is to be removed or opened aseptically with scissors, knives or scalpels (6.2).

All operations before and after opening the packaged material shall be carried out under aseptic conditions, to avoid any external contamination.

NOTE There is no need to disinfect the packaging if the contents may be removed aseptically after opening without any risk of external contamination.

Pre-processing may be required before the test portion (3.3) is taken. These include:

- **For pre-shaped materials:** The test portion (3.3) is taken directly from the material, without “reconstitution” by addition of water.
- **For solid rigid materials:** Solid materials shall be reduced in size to obtain a representative test sample (3.2) under aseptic conditions (6.1). The dimension of the pieces shall not exceed approximately 2 cm in all dimensions.

Remove any incidental pieces (e.g. stones) with a sterile spoon or tweezers (6.2). Then homogenize for 1 min to 3 min on a paddle blender (6.3), on a laboratory blender, approximately 15 minutes on an orbital shaker or magnetic stirrer (6.3), or until a homogeneous suspension is obtained.

Table B.1 in Annex B provides examples of materials and recommended equipment, as well as a description of the operating procedure for sample preparation. The laboratory shall choose the most appropriate procedure depending on the characteristics of the material.

8.3 Preparation of the initial suspension

8.3.1 For detection (*Salmonella* spp.)

The initial suspension shall be prepared using buffered peptone water (BPW) (A.3) as diluent. Pre-warm the BPW to room temperature before use.

A test portion (3.3) of 25 g or 25 ml shall be weighed (6.5) or measured (6.11) and BPW (A.3) shall be added to yield a minimum ten-fold dilution (mass or volume). It is recommended to consult EN ISO 6887-1 [6] for this procedure.

NOTE It is recommended to check the pH of the initial suspension (6.8) and to correct it to a pH between 6 and 8 if needed [e.g., by using double-buffered peptone water (A.4) for the preparation of the initial suspension].

8.3.2 For enumeration (*Escherichia coli* and *Enterococcaceae*)

The initial suspension shall be prepared by weighing (6.5) a test portion (3.3) of 10 g (wet mass) inside a sterile plastic bag and adding 90 ml of phosphate buffer (A.1), pre-warmed to room temperature in an incubator (6.4) or a water bath (6.6).

The amount of sample shall adequately represent the material to be tested. In case of very coarse inhomogeneous material, the sample quantity should be increased.

The initial suspension shall be mixed in a paddle blender (6.3) for 1 min on high speed. If the sample material contains hard, sharp parts that can damage the bag, the sample shall be mixed manually first and then shaken an orbital shaker for 10 min.

NOTE 1 It is recommended to check the pH of the initial suspension (6.8) and to correct it to a pH between 6 and 8 if needed [e.g., by using double-buffered phosphate buffer (A.2) for the preparation of the initial suspension].

NOTE 2 If the initial suspension is used both for enumeration and for detection, adapt the amount of sample and follow the procedure under 8.3.1 [e.g., weigh (6.5) 26 g of sample into a sterile plastic bag and add 234 ml buffered peptone water (A.3)]. For the enumeration methods, proceed rapidly to the preparation of the appropriate decimal dilution(s) from the initial suspension, as nutritional substances in BPW could promote microbial growth.

9 Detection of specific pathogens

9.1 General

Relevant analytical methods for the detection of pathogens in organic and organo-mineral fertilisers are specified in 9.2 to 9.4.

9.2 Enumeration of *Enterococcaceae*

The enumeration of *Enterococcaceae* shall be performed in accordance with CEN/TS 17804:2022.

9.3 Detection of *Salmonella* spp.

The detection of *Salmonella* spp. shall be performed in accordance with CEN/TS 17780:2022.

9.4 Enumeration of *Escherichia coli*

The enumeration of *Escherichia coli* shall be performed in accordance with CEN/TS 17781:2022.

Annex A (normative)

Composition and preparation of diluents

A.1 Basic phosphate buffer

A.1.1 Composition

Peptone ^a	1,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O) [‡]	9,0 g
Potassium dihydrogen phosphate (KH ₂ PO ₄) [‡]	1,5 g
Water	1 000 ml
^a For example, enzymatic digest of casein. [‡] See A.2.	

Commercially available, ready-to-use diluent is suitable.

A.1.2 Preparation

Dissolve the components in water in flasks, bottles or test tubes (6.9), by heating if necessary.

If necessary, adjust the pH (6.8) to $7,0 \pm 0,2$ at 25 °C after sterilization.

A.2 Double-buffered phosphate buffer

This buffer contains twice the amount of the two buffer components (marked ‡ in A.1.1) with respect to the basic phosphate buffer.

Commercially available, ready-to-use diluent is suitable.

A.3 Buffered peptone water (BPW)

A.3.1 Composition

Peptone ^a	10 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O) [‡]	9,0 g
Potassium dihydrogen phosphate (KH ₂ PO ₄) [‡]	1,5 g
Water	1 000 ml
^a For example, enzymatic digest of casein. [‡] See A.4.	

Commercially available, ready-to-use BPW is suitable.

A.3.2 Preparation

Dissolve the components in water in flasks, bottles or test tubes (6.9), by heating if necessary.

If necessary, adjust the pH (6.8) to $7,0 \pm 0,2$ at 25 °C after sterilization.

A.4 Double-buffered peptone water

This buffer contains twice the amount of the two buffer components (marked ‡ in A.3.1) with respect to the buffered peptone water.

Commercially available, ready-to-use diluent is suitable.

Annex B
(informative)

Examples of product types with their proposed corresponding procedural steps for sample preparation

Table B.1 provides an overview of example materials, with the required equipment and a description for the operating procedure of the preparation of the sample.

Table B.1 – Description of different material types with corresponding pre-treatment, treatment and diluent ratios

Product classification	Product form	Product composition	Pre-treatment ^a	Treatment ^b	Diluent ratio ^c	Remarks
Solid organic fertiliser	3,5 mm pellets	Bio Garden fertiliser 6-3-3 containing feather meal, bone meal, vinasse potassium and cacao shells		Horizontal shaking machine	1:10	
Solid organic fertiliser	4 mm pellets	Chicken Manure Pellet, containing processed chicken manure				
Solid organic fertiliser	Pellets	Feather meal			[more liquid needed?]	
Solid organic fertiliser	Pellets	Bone meal		Paddle blender		
Solid organic fertiliser	Pellets	Pig hair meal			[more liquid needed?]	
Solid organic fertiliser	Granules	Horn/bone/hoof shavings (white)		Horizontal shaking machine		
Solid organic fertiliser	Chips	Horn shavings (black)	[something needed here, they are very hard]			
Solid organic fertiliser	Granules					
Solid organic fertiliser	Dry mixture	Mixture of plant-based materials, bone meal and feather meal		Paddle blender		
Solid organic fertiliser	Dry mixture	Mixture of plant-based materials, blood meal, sand and DDGS (distillers dried grains)				

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Solid organic fertiliser		Heat-treated straw-rich manure				
Liquid organic fertiliser		Liquid manure (suitable for FPR)		Paddle blender	1:10	
Liquid organic fertiliser		Liquid molasses		Paddle blender	1:10	
Liquid organic fertiliser		Liquid hydrolysed animal by-product		Paddle blender	1:10	
Solid organo-mineral fertiliser	2-5 mm broken pellets	Plant-mineral fertiliser 15-5-10, containing soy, corn gluten, DAP, ammonium sulfate, sodium chloride, iron sulfate and magnesium oxide				[whether there will be a problem with pH?]
Solid organo-mineral fertiliser	1-4 mm round granules	Lawn fertiliser 10-4-8 , containing chicken manure, meat meal, rapeseed meal and diammonium sulfate (DAP)				
Solid organo-mineral fertiliser						
Solid organo-mineral fertiliser		Bone meal with				
Solid organo-mineral fertiliser		Feather meal with				
Liquid organo-mineral fertiliser				Paddle blender		
Liquid organo-mineral fertiliser				Paddle blender		

Inorganic fertiliser with more than 1% C _{org}						[whether there will be a problem with pH?]
a Particles bigger than 2 cm shall be reduced in size by appropriate measures.						
b The choice of equipment for sample treatment will depend on the characteristics of the fertiliser, including particle size, hardness and sharpness. The paddle blender shall not be used for materials that could damage its plastic bags.						
c The dilution ratios mentioned are suggestions.						

Bibliography

- [1] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003
- [2] EN ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations (ISO 7218)*.
- [3] EN ISO 11133:2014³, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133)*
- [4] ISO 16649-2:2001, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*
- [5] EN ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination (ISO 6887)*
- [6] EN ISO 6887-1:2017, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1:2017)*
- [7] EN ISO 18593, *Microbiology of the food chain — Horizontal methods for surface sampling (ISO 18593)*

³ As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.