PD CEN ISO/TS 29843-2:2021



BSI Standards Publication

Soil quality — Determination of soil microbial diversity

Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee EH/4, Soil quality.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Qualité du sol - Détermination de la diversité microbienne du sol - Partie 2: Méthode par analyse des acides gras phospholipidiques (PLFA) en utilisant la méthode simple d'extraction des PLFA (ISO/TS 29843-2:2021)

Bodenbeschaffenheit - Bestimmung der Diversität von Bodenmikroorganismen - Teil 2: Verfahren mittels Phospholipidfettsäure(PLFA)-Analyse unter Verwendung des einfachen PLFA-Extraktionsverfahrens (ISO/TS 29843-2:2021)

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European foreword

This document (CEN ISO/TS 29843-2:2021) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with Technical Committee CEN/TC 444 “Environmental characterization of solid matrices” the secretariat of which is held by NEN.

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.

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Endorsement notice

The text of ISO/TS 29843-2:2021 has been approved by CEN as CEN ISO/TS 29843-2:2021 without any modification.

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 29843-2:2011), which has been technically revised.

The main changes compared to the previous edition are as follows:

— addition of specification for qualitative and quantitative analysis of PLFAs;

— use of BAME (qualitative) or FAME (quantitative) standards;

— use of GC-MS apparatus;

— precisions in 7.2 and 7.3;

— possibility to use commercial cartridges in addition to, or replacement of, home-made cartridges;

— update of bibliographic references.

A list of all the parts in the ISO/TS 29843 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.

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Introduction

Phospholipids are essential components of membranes of all living cells. Extracted from soil samples in fatty acid form (phospholipid fatty acids, PLFA) or ether-linked isoprenoid side chains (phospholipid ether lipid, PLEL), they provide quantitative and qualitative insights into the soil's viable/active microbial biomass. Cellular enzymes hydrolyse and release the phosphate group within minutes to hours following cell death[1]. The determination of total PLFA and PLEL provides a quantitative measure of the viable biomass of soil, i.e. microorganisms of all three primary domains of the biosphere (bacteria, archaea and microeukaryota). PLFA and PLEL can also allow for rough taxonomic differentiation within complex microbial communities[2],[3]. Each microbial species contains several fatty acids, with a total composition in PLFA subject to the environmental conditions[4]. The approach is performed to evaluate biomass and shifts in microbial community composition[5], in what regards dominance of main groups of organisms[6]. Furthermore, combined with the use of isotope (^{13}C or ^{14}C) labelled substrates, the lipid methods can also be used to identify the metabolically active part of the microbial community. This approach is well established in soil ecology and serves as a phenotypic, and thus complementary, tool to genotyping approaches for determining microbial diversity. Apart from taxonomic descriptions, the PLFA technique enables the determination of physiological changes within microbial consortia. For instance, the monoenic PLFA 16:1ω7c and 18:1ω7c are increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0 in Gram-negative bacteria in response to environmental stress[7].

Different methodologies are available for the determination of soil fatty acids. These methodologies present different levels of complexity when applied and provide different levels of resolution in the description of soil microbial communities. ISO/TS 29843-1 deals with the generally called “extended PLFA extraction method” while this document deals with the generally called “simple PLFA extraction method”[8],[9].

This document is accessible to most research and analytical laboratories involved in soil sciences. This methodology can be used for a wide range of soils. It provides a broad diversity measurement of a soil microbial community at the phenotypic level. It can be applied to biomass estimation and can be used to differentiate microbial communities among different soil samples (with the aid of an adapted statistical method). This method is especially adapted for detecting rapid changes in the soil microbial community structure. It can also be used to give a rough description of microbial groups present in soil samples (e.g. Gram-positive bacteria, actinomycetes, fungi[6]). Table 1 (adapted from Table 1 in Reference [8]), presents a comparison of the sensitivity of the “extended PLFA” versus “simple PLFA” techniques.

Table 1 — Comparison of the sensitivity of the “simple” and “extended” PLFA techniques in characterizing shifts in the composition of microbial communities

Property	PLFA (simple)	PLFA (extended)
Ability to differentiate between two communities (with the aid of multivariate statistical methods)	Yes	Yes
Applicability for biomass estimation	Yes	Yes
Ability to register all single components of an entire community structure (“fingerprint”)	No	Yes
Ability to register FAs other than EL-FAs	No	Yes
Estimation of number of FAs in soil samples	<50	200 to 400
Capacity to determine the linkage of the FAs to lipids in the molecule	Yes, EL	Yes, EL, NEL
Capacity to detect defined FAs in lower concentrations in the soil extract	No	Yes
Capacity to detect unusual FAs in the soil extract	No	Yes
Number of available signatures of FAs for defined organisms	Few	High numbers
Relationships of FAs widespread in the profile	High	Natura

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Property	PLFA (simple)	PLFA (extended)
Ability to identify the organisms causing the shift in microbial community	No	Basically yes
FA fatty acid EL ester-linked NEL non-ester–linked		

This method has been derived from the one first proposed in Reference [10]. This revised method has been widely used[11] and has also been discussed and compared to the extended PLFA extraction method in peer-reviewed articles[8],[9].

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TECHNICAL SPECIFICATION **ISO/TS 29843-2:2021**

Soil quality — Determination of soil microbial diversity —

Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method

1 Scope

This document specifies a simple method for the extraction of only phospholipid fatty acids (PLFA) from soils.

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 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

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

4 Symbols and abbreviated terms (except chemical products and reagents)

BAME	bacterial acid methyl ester(s)
FA	fatty acid
EL-FA	ester-linked fatty acid
NEL-FA	non-ester-linked fatty acid
FAME	fatty acid methyl ester(s)
PL-FAME	phospholipid fatty acid methyl ester(s)
w_w	mass fraction of water in the soil, in grams of water per gram of dry soil (g/g)
GC	gas chromatography
HPLC	high-performance liquid chromatography

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