TECHNICAL SPECIFICATION

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Soil quality — Determination of soil microbial diversity —

Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis

Qualité du sol — Détermination de la diversité microbienne du sol —

Partie 1: Méthode par analyse des acides gras phospholipidiques (PLFA) et par analyse des lipides éther phospholipidiques (PLEL)

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Foreword

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In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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ISO/TS 29843-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO/TS 29843 consists of the following parts, under the general title *Soil quality — Determination of soil microbial diversity*:

— *Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis*

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

Introduction

Phospholipids are essential components of membranes of all living cells, and their fatty acid (PLFA: phospholipid fatty acids) or ether-linked isoprenoid side chains (PLEL: phospholipid ether lipid) allow for taxonomic differentiation within complex microbial communities (References [5] and [7]). This approach is now well established in soil ecology and serves as a phenotypic and thus complementary tool to genotypic (molecular genetic) approaches for determining microbial diversity.

Different methodologies for determination of soil fatty acids are available. These methodologies present different levels of complexity when applied and provide different levels of resolution in the description of soil microbial communities.

The determination of total PLFA and PLEL provides a quantitative measure of the viable biomass of soil: microorganisms of all three domains of the biosphere (bacteria, fungi and archaebacteria). Viable microbes have an intact membrane, which contains phospholipids. Cellular enzymes hydrolyze and release the phosphate group within minutes or hours following cell death (Reference [6]).

Apart from taxonomic descriptions, the PLFA technique enables the determination of physiological changes within microbial consortia. For instance, the monoenic PLFA 16:1ω7c and 18:1 ω7c are increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0 in Gram-negative bacteria in response to environmental stress (Reference [2]).

Besides the method described in this part of ISO/TS 29843, other methods for the determination of PLFA are available (References [3] and [6]). With these methods, only bacterial and fungal PLFA can be estimated; the determination of hydroxy-substituted fatty acids (PLOH), non-ester-linked (NEL) fatty acids and PLEL is not possible.

Soil quality — Determination of soil microbial diversity —

Part 1:
Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis

1 Scope

This part of ISO/TS 29843 specifies an extended method for the extraction and determination of both phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL) from soils.

ISO/TS 29843-2 specifies a simple method for the extraction of only PLFA from soils.

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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Abbreviated terms

FAME fatty acid methyl ester(s)

(EL-)PLFA (ester-linked) phospholipid fatty acid(s)

PLEL phospholipid ether lipid(s)

SATFA saturated fatty acid(s)

MUFA mono-unsaturated fatty acid(s)

PUFA poly-unsaturated fatty acid(s)

PLOH hydroxy-substituted fatty acid(s)

NEL-PLFA non-ester-linked phospholipid fatty acid(s)

UNSFA unsubstituted fatty acid(s)

UNOH hydroxy-substituted fatty acid(s)

GC/MS gas chromatography/mass spectrometry

SCX strong cation exchange

HPLC high-performance liquid chromatography

4 Principle

Lipids are extracted using the Bligh and Dyer[9] extraction procedure. Lipid extracts are separated by liquid chromatography using a silica column (si-column). Phospholipids are transformed into fatty acid methyl esters (FAME) by mild alkaline hydrolysis and into phospholipid ether lipids (PLEL) by acid hydrolysis and methylation. Separation of FAME into saturated (SATFA), mono-unsaturated (MUFA), poly-unsaturated (PUFA), hydroxy-substituted (PLOH), non-ester-linked unsubstituted (NEL-UNSFA) and non-ester-linked hydroxy-substituted (NEL-UNOH) fatty acids is achieved on solid-phase extraction columns. The different FAME are measured using gas chromatography/mass spectrometry (GC/MS). A schematic overview of the procedures is given in Figure 1.

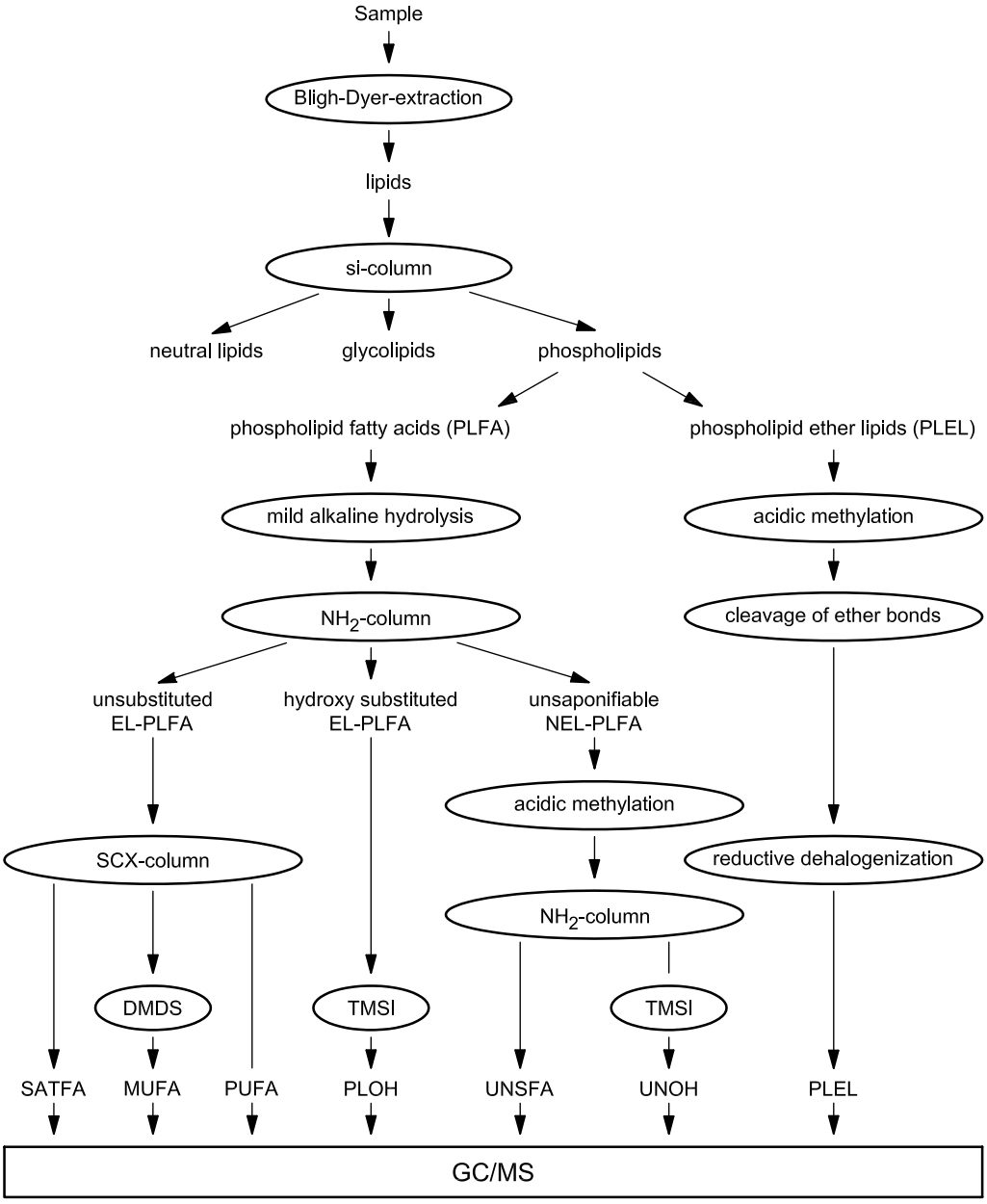

Figure 1 — Schematic overview of PLFA and PLEL analysis

5 Reagents and materials

5.1 Soil

Take soil samples and prepare them as specified in ISO 10381-6. If samples which have been sieved in the fresh state are not analysed immediately, they may be kept at –20 °C or stored in chloroform after lipid extraction (see 6.1).

5.2 Reagents

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

5.2.1 Acetone, C_3H_6O, residue analysis.

5.2.2 Acetonitrile, CH_3CN, for high-performance liquid chromatography (HPLC).

5.2.3 Bis(trimethylsilyl)trifluoroacetamide (BSTFA).

5.2.4 Celite 545[1)], particle size 0,02 mm to 0,10 mm.

5.2.5 Chloroform, $CHCl_3$.

5.2.6 Dichloromethane, CH_2Cl_2, for residue analysis.

5.2.7 Diethyl ether, $(C_2H_5)_2O$.

5.2.8 Dimethyl disulfide (DMDS), $(CH_3S)_2$.

5.2.9 Acetic acid, CH_3COOH.

5.2.10 Ethyl acetate, C_4H_8O.

5.2.11 Hexamethyldisilasane (HMDS).

5.2.12 Hexane, C_6H_{14}, for residue analysis.

5.2.13 Potassium hydroxide, KOH.

5.2.14 Methanol, CH_3OH, for residue analysis.

5.2.15 Sodium sulfate, Na_2SO_4.

5.2.16 Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$.

5.2.17 Aminopropyl-column [Chromabond[2)]**]** NH_2**-column**.

5.2.18 Pyridine, dried, maximum 0,01 % H_2O.

5.2.19 Hydrochloric acid, HCl.

5.2.20 Silver nitrate, $AgNO_3$.

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1) Celite 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) Chromabond is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.2.21 Toluene, C_7H_8, scintillation grade.

5.2.22 Trimethylchlorosilane (TMSI).

5.2.23 Potassium hydrogenphosphate, K_2HPO_4.

5.2.24 Nonadecanoic acid methyl ester, $C_{20}H_{40}O_2$.

5.2.25 Hydriodic acid, HI, 57 % stabilized with hydrophosphorous acid.

5.2.26 Isooctane, C_8H_{18}.

5.2.27 Iodine, I_2, 6 % in diethyl ether.

5.2.28 Sodium carbonate, Na_2CO_3.

5.2.29 Zinc powder, GR quality for analyses, particle size 45 µm.

5.3 Buffers and standards

5.3.1 Phosphate buffer, 0,05 mol/l, 17,42 g of K_2HPO_4 in 2 000 ml H_2O, adjusting with 4 mol/l HCl to pH 7,4.

5.3.2 Methanolic KOH solution, 0,2 mol/l , 0,11 g of KOH in 10 ml of methanol.

5.3.3 Acetic acid, 1 mol/l, 6,0 g of acetic acid (100 %) in 100 g of water.

5.3.4 Sodium carbonate solution, 0,1 mol/l, 5,3 g of Na_2CO_3 in 500 ml of water.

5.3.5 Sodium carbonate solution, 10 %, 40 g of $Na_2CO_3 \cdot 5H_2O$ in 360 g of water.

5.3.6 Sodium thiosulfate solution, 50 %, 272,5 g of $Na_2S_2O_3 \cdot 5H_2O$ in 200 g of water.

5.3.7 Standard ($C_{19:0}$ FAME), 25,0 mg nonadecanoic acid methyl ester in 25,0 ml of *i*-octane = stock solution => dilution 1:10 with *i*-octane [2,5 ml of stock solution (using a 5 ml pipette) adjusting to 25,0 ml with *i*-octane]; final concentration 32,05 nmol 100 μl^{-1}.

5.4 Apparatus

Usual laboratory equipment and the following.

5.4.1 SCX-column, strong-cation-exchange column, 0,5 g/3 ml, Bond Elut 1210-2040[3)].

5.4.2 SI-column, 2 g/12 ml, Mega Bond Elut 1225-6018[4)].

5.4.3 Gas chromatograph, with mass-selective detector equipped with a capillary column (length = 50 m, internal diameter = 0,2 mm, coated with a cross-linked 5 % phenylmethyl rubber phase with a film thickness of 0,3 µm).

3) Bond Elut 1210-2040 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) Mega Bond Elut 1225-6018 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6 Procedures

6.1 Lipid extraction (Bligh-Dyer-extraction)

About 10 g to 25 g of soil (dry weight) are put in a 500 ml volumetric flask; 125 ml of methanol (5.2.14), 62,5 ml chloroform (5.2.5), 0,05 mol/l phosphate buffer (pH 7,4; 50 ml minus the water content in soil, 5.3.1) are added. After shaking for 2 h, 62,5 ml of water and 62,5 ml of chloroform (5.2.5) are added. The mixture is kept for 24 h, then the water phase is removed and discarded. The organic phase and slurry are passed through a filtration funnel containing 2 cm of Celite 545 (5.2.4). The chloroform phase is transferred to a separating funnel, dried over anhydrous sodium sulfate (5.2.15) and reduced to a small volume (about 10 ml).

6.2 Separation of lipids by sI-column

Column size: 2 g/12 ml (reservoir volume, V).

Conditioning: 1V of chloroform (5.2.5).

Sample application: sample dissolved in chloroform (5.2.5); sample volume: < 12 ml.

Elution: 1V of chloroform (5.2.5) for neutral lipids, 1V of acetone (5.2.1) for glycolipids and 4V of methanol (5.2.14) for phospholipids. The last fraction is reduced to almost dryness.

The following protocol is divided into two parts: the analysis of the phospholipids, PLFA (6.3) and the ether lipids, PLEL (6.4).

6.3 PLFA analysis

6.3.1 Mild alkaline hydrolysis

During this step, ester bonds between the glycerol backbone and the fatty acid side chains are cleaved and fatty acid methyl esters (FAME) are formed from the released fatty acids.

The residue of phospholipids is dissolved in 1 ml of methanol (5.2.14):toluene (5.2.21) (1:1, volume fraction), then 5 ml of 0,2 mol/l KOH (5.2.13) in methanol (freshly prepared, 5.3.2) are added. This mixture is incubated for 15 min at 37 °C. Afterwards, the pH of this mixture is adjusted to approximately pH 6 with 1 mol/l acetic acid (5.3.3). 10 ml of chloroform (5.2.5) and 10 ml of water are added and transferred to a centrifugation tube. This tube is mixed for 1 min and centrifuged at 2 000 g for 10 min. After removing the chloroform phase (lower phase), the water phase is extracted once more with 5 ml of chloroform (5.2.5). The combined chloroform phases are dried over sodium sulfate (5.2.15) and reduced to a small volume.

6.3.2 NH_2 column: Separation of FAME from OH-substituted FAME (PLOH) and unsaponifiable lipids

FAME generated during step 6.3.1 are separated on the NH_2 column.

Column size: 0,5 g/3 ml.

Conditioning: 1V of hexane (5.2.12):dichloromethane (5.2.6) (3:1, volume fraction).

Sample application: sample dissolved in hexane (5.2.12):dichloromethane (5.2.6) (3:1, volume fraction), sample volume < 1,5 ml.

Elution: 1V of hexane (5.2.12):dichloromethane (5.2.6) (3:1, volume fraction) for unsubstituted FAME; 1V of dichloromethane (5.2.6):ethyl acetate (5.2.10) (9:1, volume fraction) for PLOH and 2V of 2 % acetic acid in methanol for unsaponifiable lipids.

6.3.3 SCX column: Separation of unsubstituted ester-linked PLFA (EL-PLFA)

Unsubstituted ester-linked PLFA obtained during step 6.3.2 are separated via a silver-impregnated SCX column.

Column size: 0,5 g/3 ml.

Conditioning: 0,1 g of silver nitrate (5.2.20) in 1,5 ml of acetonitrile (5.2.2):water (10:1, volume fraction), followed by 2V of acetonitrile (5.2.2), 2V of acetone (5.2.1) and 4V of dichloromethane (5.2.6).

Sample application: sample is dissolved in dichloromethane (5.2.6):hexane (5.2.12) (7:3, volume fraction); sample volume $<$ 3,0 ml.

Elution: 2V of dichloromethane (5.2.6):hexane (5.2.12) (7:3, volume fraction) to elute the SATFA; 2V of dichloromethane (5.2.6):acetone (5.2.1) (9:1, volume fraction) to obtain the MUFA and 4V of acetone (5.2.1):acetonitrile (5.2.2) (9:1, volume fraction) to elute the PUFA. All liquids should pass the column without pressure.

6.3.4 Acidic methylation of unsaponifiable lipids and separation into UNOH and UNSFA

NEL-PLFA are generated through the hydrolysis and methylation of the unsaponifiable lipid fraction obtained during step 6.3.2.

After solvent removal, the residue of the unsaponifiables is redissolved in 2 ml of methanol:chloroform:hydrochloric acid (37 %) (10:1:1, volume fraction) in a centrifugation tube, and is kept at 60 °C overnight. Then, 2 ml of 2 % sodium chloride are added. The extraction is done three times with 4 ml of hexane (5.2.12):toluene (5.2.21) (1:1, volume fraction). The sample is dried with sodium sulfate (5.2.15) (sodium sulfate is washed with hexane). The resulting NEL-PLFA are separated into UNOH and UNSFA using the procedure of step 6.3.2.

6.3.5 TMSI derivatization of PLOH and UNOH (see 5.2.22)

PLOH and UNOH obtained during steps 6.3.2 and 6.3.4, respectively, are derivatized prior to gas chromatography (GC) injection:

0,5 ml of a mixture of pyridine (5.2.18):BSTFA (5.2.3):hexamethyldisilasane (5.2.11):trimethylchlorosilane (5.2.22) (0,2:1:2:1, volume fraction) are added to the sample. This mixture is kept at 60 °C for 15 min, then evaporated by a stream of nitrogen.

6.3.6 DMDS derivatization of MUFA (see 5.2.8)

MUFA obtained during step 6.3.3 are derivatized prior to GC injection:

The sample is dissolved in 0,05 ml of hexane (5.2.12), 0,1 ml of DMDS (5.2.8), and three to five drops of iodine (6 % in diethyl ether, 5.2.27) are added. This mixture is kept at 60 °C for 72 h. The excess iodine is removed by addition of 1 ml of 5 % sodium thiosulfate (5.2.16) and the adduct is extracted three times with 1,5 ml of hexane (5.2.12). The hexane phases are combined, dried with sodium sulfate (5.2.15) and evaporated until nearly dry.

6.4 PLEL analysis

6.4.1 General

Aliquots of the phospholipid fraction equivalent to about 5 g of dry matter (organic soil) or 12,5 g of dry matter (mineral soil), respectively, are subjected to PLEL analysis.

6.4.2 Acidic methylation

This step is performed to cleave the polar head group to obtain ether core lipids.

After solvent removal, the aliquot of the phospholipid fraction is redissolved in 2 ml of methanol (5.2.14):chloroform (5.2.5):hydrochloric acid (37 %) (5.2.19) (10:1:1, volume fraction) and kept at 60 °C overnight. After cooling, 4 ml of water is added. The extraction of the ether lipids is carried out three times with 5 ml of hexane (5.2.12). The hexane phases are combined and dried with sodium sulfate (5.2.15) (sodium sulfate is washed three times with hexane).

6.4.3 Cleavage of etherbonds with hydroiodic acid (HI)

This step is performed to release the ether-linked hydrocarbons (as alkyl iodides) from ether lipids.

The sample with the ether core lipids is transferred in a 50 ml tube and dried using a nitrogen stream; 2,0 ml hydroiodic acid (5.2.25) is added (shaking for 20 s). The sample is incubated at 100 °C for 18 h. After cooling, 4 ml of water is added to stop the reaction. The extraction is done three times with 5 ml of hexane (5.2.12), the hexane phases are collected in a 100 ml separation funnel. The hexane extracts are washed with 4 ml of water (15 s shaking), 10 ml of sodium carbonate, 10 % (5.3.5) (30 s shaking) and 10 ml of sodium thiosulfate 50 % (5.3.6) (30 s shaking). After 15 min, the lower phase is discarded and the hexane phase is dried with sodium sulfate (5.2.15).

6.4.4 Reductive dehalogenization with zinc

300 mg of zinc powder (5.2.29) is added to the dried sample of alkyliodides (generated during step 6.4.3) in a centrifugation tube, 3 ml of acetic acid (100 %) (5.2.9) is added and the tube is shaken for 20 s. The sample is incubated at 100 °C for 18 h; after cooling, the neutralization is carried out with 5 ml of 0,1 mol/l sodium carbonate (5.3.4). The extraction is done three times with 7 ml of hexane (5.2.12) (centrifugation at 2 000g for 10 min) and the hexane phases are combined in a 100 ml separation funnel. Wash with 10 ml of 0,1 mol/l sodium carbonate (5.3.4) (15 s shaking/take into account the pressure balance), and twice with 8 ml of water (30 s shaking). After 15 min, the lower phase is discarded and the hexane phase is dried with sodium sulfate (5.2.15).

6.5 Measurement of PLFA/PLEL fractions

To the dried sample, 50 µl or 100 µl of ISTD (internal standard; nonadecanoic acid methyl ester, 100 ng/µl, 5.3.7) is added, and the liquid is transferred to a GC/MS vial; the sample is now ready for analysis via GC/MS. Samples are injected into a capillary column (50 m $\times$ 0,2 mm, 0,3 µm film thickness) coated with a cross-linked 5 % phenylmethyl rubber phase in the splitless mode with a 0,75 min venting time by an automatic sampler. Helium is used as the carrier gas at a flow rate of 1,0 ml·min^{-1}. For PLFA analyses, the temperature programme of the oven is begun at 70 °C (for 2 min) and increased to 160 °C at 40 °C min^{-1}, followed up to 280 °C at 3 °C min^{-1}, and held for 10 min (injector temperature 290 °C). For PLEL fractions, the initial temperature of 70 °C (for 2 min) is increased at a rate of 30 °C·min^{-1} to 130 °C, followed up to 320 °C at 4 °C·min^{-1} (held for 30 min).

7 Identification and calculation

Identification and quantification of individual components can be achieved by using chromatography software [e.g. HP ChemStation SOLVIT, Switzerland[5)]]. Alternatively, identification is based on comparison with spectra that were either obtained from standards or reported in the literature. The amounts of each FAME in soil extracts can be calculated using Equation (1):

$$c = \frac{R_f \times k \times m}{R_i \times V \times M} \times 1\,000 \quad (1)$$

where

c is the concentration of the compound of interest in soil, in nanomoles per gram (nmol g^{-1});

R_f, R_i are the mean responses of the compound of interest and the internal standard;

k is the factor for the compound of interest against the internal standard, in terms of response;

m is the mass of the internal standard injected, in nanograms (ng);

V is the volume of soil extract injected, in millilitres (ml);

M is the molar mass of the compound of interest, in grams per mole ($g \cdot mol^{-1}$).

Total microbial biomass can be estimated from the total PLFA content of soils. Individual FAME can also be expressed as log moles percent. Variations between patterns of different soils (polluted or unpolluted) or soils which have undergone different treatments (e.g. addition of chemicals, remediation) can be subjected to Principal Component Analysis (PCA).

5) HP ChemStation SOLVIT, Switzerland is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Bibliography

[1] ALEF, K. and NANNIPIERI, P. (1995) Methods in Applied Soil Microbiology and Biochemistry Chapter 9 Community Structure, pp. 428-462, Academic Press

[2] FINDLAY, R.H., TREXLER, M.B., GUCKERT, J.B. and WHITE, D.C. (1990) Laboratory study of disturbance in marine sediments: response of a microbial community. *Mar Ecol - Prog Ser*, **62**, pp. 121-133

[3] FROSTEGAARD, A., TUNLID, A. and BAATH, E. (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods*, **14**, pp. 151-163

[4] GATTINGER, A., RUSER, R., SCHLOTER, M. and MUNCH, J.C. (2002) Microbial community structure varies in different soil zones of a potato field. *Journal of Plant Nutrition and Soil Science*, **165**, pp. 421-428

[5] GATTINGER, A., GÜNTHNER, A., SCHLOTER, M. and MUNCH, J.C. (2003) Characterization of Archaea in soils by polar lipid analysis. *Acta Biotechnologica*, **23**, pp. 21-28

[6] WHITE, D.C., DAVIS, W.M., NICKELS, J.S., KING, J.D. and BOBBIE, R.J. (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, **40**, pp. 51-62

[7] ZELLES, L. and BAI, Q.Y. (1993) Fractionation of fatty acids derived from soil lipids by soil phase extraction and their quantitative analysis by GC-MS. *Soil. Biol. Biochem.*, **25**, pp. 130-134

[8] ZELLES, L. (1999) Fatty acid patterus of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils*, **29**, pp. 111-129

[9] BLIGH, E.G. and DYER, W.J. (1959) A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, pp. 911-917

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