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Gas chromatography – Optical fiber detector for assessment of fatty acids in urban soils

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ABSTRACT

Fatty acids have been used as biomarkers of the microbial community composition of soils and they are usually separated and quantified by gas-chromatography coupled to a flame ionization detector (GC-FID). The aim of this study was to develop, validate and apply a methodology based on gas chromatography coupled to optical fiber detection (GC-OF) for screening five fatty acids used as indicators of fungal and bacterial communities in urban soils. The performance of the GC-OF methodology (optical fiber detector at 1550 nm) was evaluated by comparison with the GC-FID methodology and it was found that they were comparable in terms of linear range, detection limit and analytical errors. Besides these similar analytical characteristics, the GC-OF is much cheaper than the GC-FID methodology. Different concentrations were determined for each fatty acid indicator which in turn varied significantly between the soil samples analyzed from Lisbon ornamental gardens. Additionally, the GC-OF showed a great potential as alternative for determination of eleven or more fatty acids in urban soils.

1. Introduction

In the last few years urban soil research efforts have been made in order to increase knowledge on the distribution of potentially toxic elements [1–6] and organic [7–9]. Fewer studies have been reported on the distribution of fatty acids in urban soils [10]. Fatty acids are useful biomarkers, and signatures for fingerprinting microbial communities as well as good indicators of bacterial and fungal biomass present in the soil [11]. Therefore, it is accepted that changes in the fatty acid profile would be a reflex of modifications in the microbial community caused by the presence of chemical contaminants and agricultural practices [12–14]. Although individual fatty acids cannot be used to represent specific species because individuals can have numerous fatty acids involved in their biochemistry, and the same fatty acids can occur in more than one species, it can be applied to detection of changes in the composition of the bacterial and/or fungal community as a whole [11]. Different assignations are attributed to different fatty acids for taking inferences about the microbial community (bacteria and fungi) profiles of soil. Thus, some authors [12,15–17] pointed out iC15:0, iC16:0, iC17:0, C17:0 as indicators of Gram-positive bacteria; cyC17:0 and C18:1n9t as indicators of Gram-negative bacteria and C18:1n9c, C18:2n6c and C18:3n6 as indicators of fungi. Based on these studies the fatty acids selected as soil microbial indicators for this work were heptadecanoic acid (C17:0) and elaidic acid (C18:1n9t) as indicators of bacteria, oleic acid (C18:1n9c), linoleic acid (C18:2n6c), and γ-linolenic acid (C18:3n6) as indicators of fungi. Usually, fatty acids are extracted directly from soil by saponification (e.g., NaOH or KOH in methanol), methylation (e.g., HCl in methanol or acetic acid in hexane), purification and extract (e.g., silica gel column and extraction diethyl ether) and finally separated and quantified either by gas chromatography coupled to flame injection detection (GC-FID) [11,18,19], or by gas chromatography coupled to mass spectrometry (GC-MS) [11,19–21].

The main objective of this research was to develop, validate, and apply a methodology based on gas chromatography coupled to optical fiber detection (GC-OF) for screening fatty acids as indicators of fungal and bacterial microbial communities in urban soils. To accomplish the objectives, five fatty acids were chosen and screened in urban soil from ornamental gardens:heptadecanoic acid (C17:0), elaidic acid (C18:1n9t), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and γ-linolenic acid (C18:3n6).

Optical fiber (OF) sensors and OF-based methodologies, have been proven to be of high sensitivity and of easy operation for several analytes [22–33]. The optical fiber detectors coupled to

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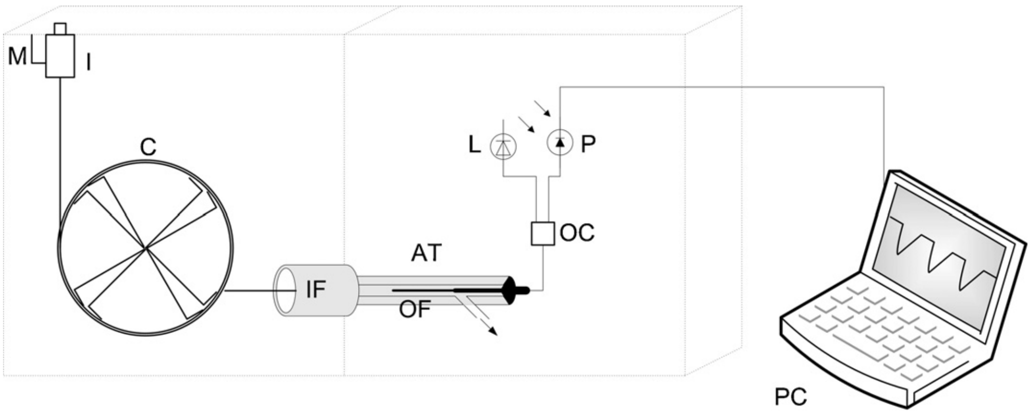

Fig. 1. Experimental apparatus used for GC-OF methodology (M – makeup gas, I – Injector, C – capillary column, IF – interface, AT – analytical tube, OF – optical fiber, OC – optical coupler, L – laser diode optical source, P – photodiode detector, PC – personal computer).

GC or HPLC showed analytical figures of merit such as detection limit, sensitivity, repeatability and linearity comparable with flame ionization detector [22,25] and electrochemical detector [34]. The OF-based sensors and methodologies also showed detection limits, sensitivity, repeatability and linearity comparable to GC-FID, GC-MS and HPLC-ED. The principal advantage of the use of OF as a detector, sensor or methodology relies on the fact that is less expensive than the other methodologies.

2. Materials and methods

2.1. Standard solutions

Standards solutions of fatty acids for calibration have been prepared by mixing appropriate amounts of heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, and γ-linolenic acid methyl ester (Sigma, Spain) in methylene chloride in concentrations of 5.00, 10.00, 40.00, 70.00, 100.00, 130.00 $\mu g\,L^{-1}$.

Ten different concentrations of standard mixtures (SM) of each fatty acid (FA) methyl ester in concentrations of 10.00, 30.00, 40.00, 50.00, 70.00, 80.00, 90.00, 100.00, 110.00, 120.00 $\mu g\,L^{-1}$ were appropriately prepared in order to compare the performance of the proposed methodology (GC-OF) with GC-FID based methodology.

2.2. GC-OF based methodology

Fig. 1 shows the experimental apparatus of GC-OF based methodology. The SP-2560 capillary column (C) (0.14 μm film × 0.18 mm ID × 75 m, Sigma) was connected to the injector unit (I) of the GC and to the analytical tube (AT) via interface (IF). The AT has an internal narrowed region of 0.4 cm of diameter and 6.5 cm long and inside it (AT) was the optical fiber (O). The optical fiber was previously uncladded and cleaved to a few millimeters extension of the optical path cord. The cleaved optical fiber section was dipped into a PDMS solution (by dip-coating technique) resulting in the sensitive component of the OF detection. The OF detection component of the GC-OF based methodology is constituted, besides the coated optical fiber and the analytical tube, by an laser diode optical source (L) and a photodiode detector (P). The laser diode optical source was set for a working wavelength 1550 nm to generate the interrogating signal and was connected to the optical coupler (OC). The OC was also connected to the photodiode detector (P) which measures the intensity of the modulated signal and is also connected to a laptop (PC) with home-made software. The optical component (OF) of the detector is constituted by a monomode optical fiber pigtail, core and cladding diameters of 9 and 125 μm, respectively, integrated into a directional 50:50 Y optical coupler (OC).

Standards of FA methyl ester, standard mixtures – SM, and samples (1 μL) were introduced by a gastight micro-syringe (Hamilton), in splitless mode at the injector (I) kept at 250 °C. The temperature program started at 168 °C, rising up to 220 °C with a program rate of 4 °C $\min^{-1}$. After separation the analytes reached the analytical tube which contains the coated optical fiber (OF) generating an analytical signal measured as changes in the reflected optical power. These changes in the reflected optical power are due to variations in the refractive index of optical fiber which depends on both coating film (PDMS) and analytes (FA methyl ester) properties and also its (coating and analytes) chemical interactions as previously discussed for volatile organic compounds by Silva et al. [22–34].

The working conditions of the OF-based detector were optimized, particularly in terms of the OF length (from 10 to 20 mm) and number of dips for sensitive film deposition.

2.3. GC-FID based methodology

In order to compare and validate the GC-OF methodology, a GC-FID based methodology was used. Standards of FA methyl ester, and SM (1 μL) were injected using a gastight micro-syringe (Hamilton) in splitless mode on a GC-FID (GowMac). The capillary column used was also a SP-2560 capillary column (0.14 μm film × 0.18 mm ID × 75 m, Sigma). The GC program temperature used was already mentioned in Section 2.2. The carrier gas was hydrogen with a constant flow of 40 cm s^{-1}.

2.4. Samples

A total of 12 samples of urban soil were collected from ornamental gardens, in the largest city of Portugal, Lisbon (38°46′52″N/9°8′9″W). Lisbon is the capital of Portugal with a municipal population of around 565 thousands inhabitants in 84.8 km^2, around 2.8 million inhabitants in the metropolitan area and around 3.34 million inhabitants in the broader agglomeration of the Lisbon metropolitan region. The metropolitan area of Lisbon is highly industrialized (oil refineries, chemical industry, textile industry, shipyards and steelworks) and is the largest corporate merger in the country.

The soils from Lisbon ornamental gardens were collected (0–10 cm depth) in summer. The samples were stored in aluminum foil inside opaque plastic bags, transported to the laboratory and frozen (−20 °C) until analysis.

2.5. Fatty acids extraction and analysis procedure

Fatty acids extraction and analysis was performed according to Silva et al. [10]. The soil samples (1.0 g) were weighted and introduced into a round-bottomed flask (100 mL), followed by the addition of 10.0 mL of potassium hydroxide (KOH, 0.2 M, Eka Chemicals AB). The mixture was placed in an oven (Binder) at 37 °C, for 1 h (agitated every 15 min), followed by addition of 1.0 mL of acetic acid (Riedel de Haën) and 5.0 mL of hexane (99%, Fluka). Then the mixture was homogenized for 1 min and kept in an ultrasonic bath (57 Hiney Ultra sonic) for 30 min. The organic phase (top layer) was filtered with anhydrous sodium sulfate (Sigma) and brought to dryness with a rotary evaporator (Laborato 4000, Heidolph). The residue was re-dissolved in 1.0 mL of dichloromethane/hexane (1:1) and then filtered in a silica gel column (Macherey-Nagel) pre-conditioned with 1.0 mL of dichloromethane/hexane (1:1) (Lab-scan) and 1.0 mL of diethyl ether (Panreac, Spain), in order to eliminate interferences. The fatty acids of interest were eluted from the column by adding twice 1.0 mL of diethyl ether. The sample eluates were then transferred to microreaction vessels and dried under nitrogen atmosphere. Finally, 200 μL of hexane was added to sample extracts prior to analysis. Samples were further diluted with hexane on ever was needed. Fatty acids were analyzed, as methyl esters, by the developed GC-OF methodology after its validation.

2.6. Quality assurance and quality control

In order to assure representativeness and reliability of the results obtained, QC/QA procedures included the procedure blanks, duplicates and recovery assays. Replicate analysis of the soil samples gave an uncertainty of <6% for fatty acids. Recovery assays were carried out to ensure effectiveness of the extraction procedure of fatty acids from soil and also evaluate the matrix effects: spiked soil were prepared adding each fatty acid in concentrations 5, 10 and 100 times higher than concentration in the soil samples, and recoveries ranged from 95 to 102%. For soil spiked samples dilution of samples was performed after extraction and before analysis using both methodologies.

Calibration models were built by injection of 1 μL of different concentrations of standard solutions. The concentration of each FA methyl ester was determined by direct interpolation in the standard curve within their linear dynamic range, and the detection limits were calculated using $y = y_B + 3s_B$, where s_B is the SD of the blank signal estimated as $s_{y/x}$, the residual SD taken from the calibration line, and y_B is the blank signal estimated from the intercept taken also from the calibration line [35].

3. Results and discussion

3.1. OF-detector optimization experiments

The results obtained during the optimization of the OF detector, regarding the length of the OF sensitive region are shown in Table 1. The OF detector constituted by a sensitive region (OF + sensitive film) of 15 mm long showed the most advantageous response profile, evaluated in terms of value of the analytical signal amplitude, i.e., optical power decreases (dB) resulting for the mean of five sequential FA determinations and % of error associated to the measure, in comparison to sensitive regions of 10 and 20 mm long. The decrease in the peak height/optical signal variation (dB) observed for a detector constituted by a sensitive region of 10 mm long could be explained taking into account that the reflected optical power also depends on the evanescent wave of the light on the sensitized fiber section, in set of other parameters of physical/optical and chemical nature, such as: optical components features, sensitive film and analyte properties, and chemical interaction analyte-light. Although the high analytical signal amplitude and sensitivity recorded for a detector constituted by a sensitive region of 20 mm long, this system showed a high error% associated to the measures. In addition, the insertion in the analytical tube of this sensitive component increases in complexity and imperfections on the coating surface in comparison to shorter components.

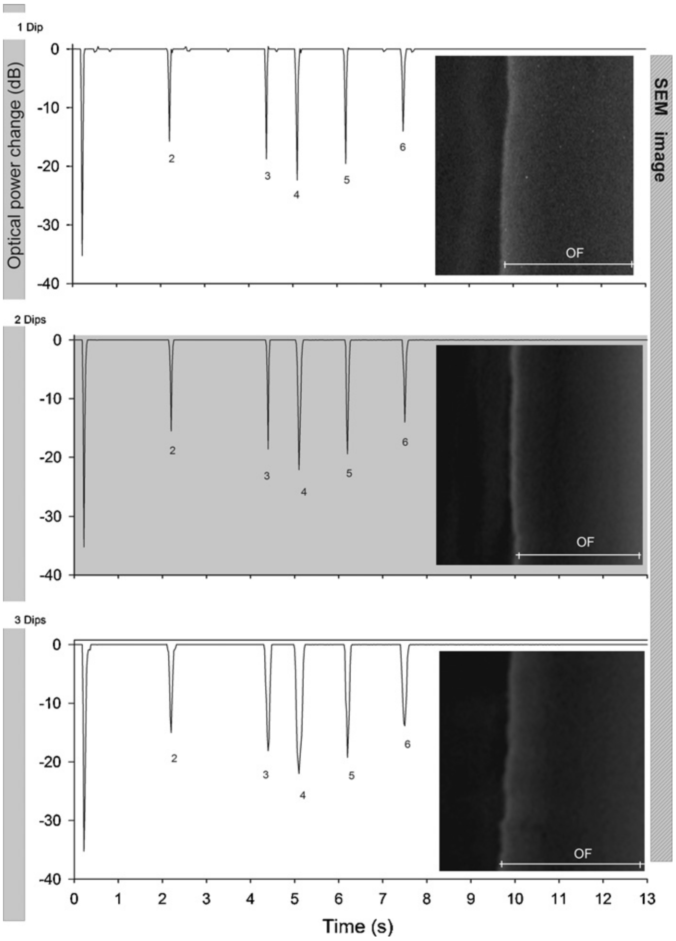

Fig. 2. Response profile and SEM images obtained by GC-OF method, during the optimization experiments regarding the length of the OF sensitive region, for a standard mixture 40.00 μg L^{-1} of heptadecanoic acid methyl ester (2), elaidic acid methyl ester (3), oleic acid methyl ester (4), linoleic acid methyl ester (5) γ-linolenic acid methyl ester (6) and solvent (hexane) (1).

Fig. 2 shows the analytical signal profile and Scanning Electron Microscopy (SEM) images obtained concerning the optimization of the number of dips for sensitive material deposition.

The use of a sensitive region constituted by an OF coated with a polymeric sensitive film performing 2 dips for deposition was based on the best ratio of analytical signal amplitude – recovering time – base line noise *vs.* optical signal stability, in comparison with systems using 1 and 3 dips for polymeric deposition. As initially foreseen, the results suggested that the quality of the OF detector response depends on: (a) quantity of the deposited material which establishes the film thickness, and (b) morphology of the film surface. This last parameter could be analyzed on basis of the SEM images obtained for the 3 tested optical system, that is: (a) the SEM image obtained for an OF covered using 1 dip for sensitive film deposition showed an irregular texture, which can promote light scattering phenomena from structural OF-sensitive surface inhomogeneities. In terms of analytical signal profile obtained for this OF system, a lower optical signal amplitude and high signal noise was observed; (b) the SEM image obtained for an OF covered using

Table 1
Analytical signal and associated statistics parameters obtained for the tested fatty acids using an OF detector with a sensitive region of 10, 15 and 20 mm long.

	Heptadecanoic acid methyl ester	Elaidic acid methyl ester	Oleic acid methyl ester	Linoleic acid methyl ester	γ-Linolenic acid methyl ester
10 mm					
Mean (dB)	13.36	16.34	19.42	18.36	13.30
SD (dB)	0.22	0.19	0.33	0.32	0.30
Error (%)	1.63	1.15	1.68	1.76	2.26
15 mm					
Mean (dB)	15.65	18.64	22.23	19.38	14.13
SD (dB)	0.12	0.05	0.17	0.13	0.08
Error (%)	0.77	0.25	0.74	0.65	0.54
20 mm					
Mean (dB)	16.35	19.50	23.43	20.12	15.43
SD (dB)	0.33	0.36	0.47	0.47	0.43
Error (%)	2.03	1.87	2.01	2.34	2.80

2 dips for sensitive film deposition showed a smooth and homogeneous coating surface; (c) the OF covered using 3 dips for deposition showed a lower degree of the texture homogeneity in comparison to the OF covered using 2 dips. The response prolife obtained for this system (using 3 dips for deposition of the polymeric film) was found to be also less advantageous due to an increase of recovering time.

3.2. GC-OF and GC-FID comparison

The total analytical time observed for the GC-OF based methodology (Fig. 3) was around 13 min and was the same as for the GC-FID methodology, since the same chromatographic components and operational conditions were used: namely temperature program and carrier gas flow rate. In optical fiber sensors based on polymeric film (PDMS in this case) as the sensitive component, the analytical signal generation and characteristics must be understood as an interaction between several physical and chemical factors. Firstly, the analytical signal depends on the wave guide, the polymeric cladding and the analyte proprieties. Secondly, it is important to take into account the molecular interactions that occur between the analyte molecules and the polymeric film with consequent changes in the intensity of the reflected light power [36,37]. When the analyte molecules contact with the polymeric film, the intensity of the reflected optical power will vary and the optical power changes are proportional to the amount of the analyte present. The different sensitivities (Fig. 3) obtained for the five compounds tested can be attributed to the different analyte properties, such as the boiling temperature, the molecular weight and the vapor pressure. In this case the sensitivity varies in the same way as the boiling temperature (γ-linolenic acid methyl ester < heptadecanoic acid methyl ester < elaidic acid methyl ester < linoleic acid methyl ester < oleic acid methyl ester).

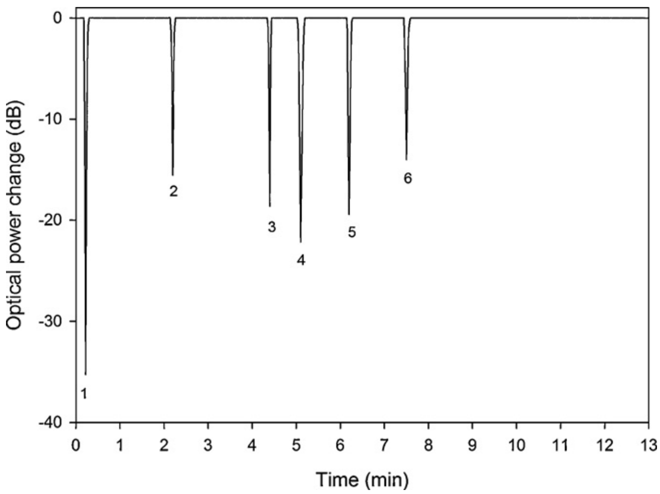

Fig. 3. Analytical signal obtained by GC-OF based methodology for a standard mixture 40.00 μg L^{-1} of heptadecanoic acid methyl ester (2), elaidic acid methyl ester (3), oleic acid methyl ester (4), linoleic acid methyl ester (5) γ-linolenic acid methyl ester (6) and solvent (hexane) (1).

The retention times and other analytical parameters are shown in Table 2. The detection limits, in μg L^{-1}, were in a range from 1.9 to 3.3, and from 1.9 to 3.6, for the GC-FID and GC-OF methodologies, respectively and were in the same order of magnitude for both methodologies. The linear range was 5–130 μg L^{-1} for both methodologies.

The OF methodology shows an increase in calibration sensitivities, measured as the slope of the calibration curve, according to the following order: γ-linolenic acid methyl ester < heptadecanoic acid methyl ester < elaidic acid methyl ester < linoleic acid methyl ester < oleic acid methyl ester. Such an increase in sensitivity of

Table 2
Analytical parameters obtained for heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester using GC-FID and GC-OF based methodologies.

	Methodology	Heptadecanoic acid methyl ester	Elaidic acid methyl ester	Oleic acid methyl ester	Linoleic acid methyl ester	γ-Linolenic acid methyl ester
Retention time (s)	GC-FID	2.22	4.60	5.02	6.17	7.50
	GC-OF	2.20	4.40	5.10	6.20	7.50
Linear range (μg L^{-1})	GC-FID	5–130	5–130	5–130	5–130	5–130
	GC-OF	5–130	5–130	5–130	5–130	5–130
Linear calibration	GC-FID	$y = 0.820x + 37.60$	$y = 0.835x + 40.59$	$y = 0.874x + 38.38$	$y = 0.839x + 43.36$	$y = 0.894x + 40.22$
	GC-OF	$y = 0.106x + 12.67$	$y = 0.107x + 12.60$	$y = 0.109x + 13.99$	$y = 0.108x + 12.56$	$y = 0.105x + 12.75$
Squared coefficient of correlation R^2	GC-FID	0.9991 ($p < 2.76 \times 10^{-7}$)	0.9997 ($p < 2.52 \times 10^{-6}$)	0.9992 ($p < 2.30 \times 10^{-7}$)	0.9997 ($p < 3.22 \times 10^{-8}$)	0.9991 ($p < 2.95 \times 10^{-7}$)
	GC-OF	0.9990 ($p < 3.90 \times 10^{-7}$)	0.9998 ($p < 7.01 \times 10^{-8}$)	0.9995 ($p < 1.02 \times 10^{-7}$)	0.9998 ($p < 3.394 \times 10^{-8}$)	0.9996 ($p < 2.89 \times 10^{-7}$)
Detection limit (μg L^{-1})	GC-FID	3.3	2.6	3.1	1.9	3.3
	GC-OF	3.6	2.3	2.6	1.9	3.3

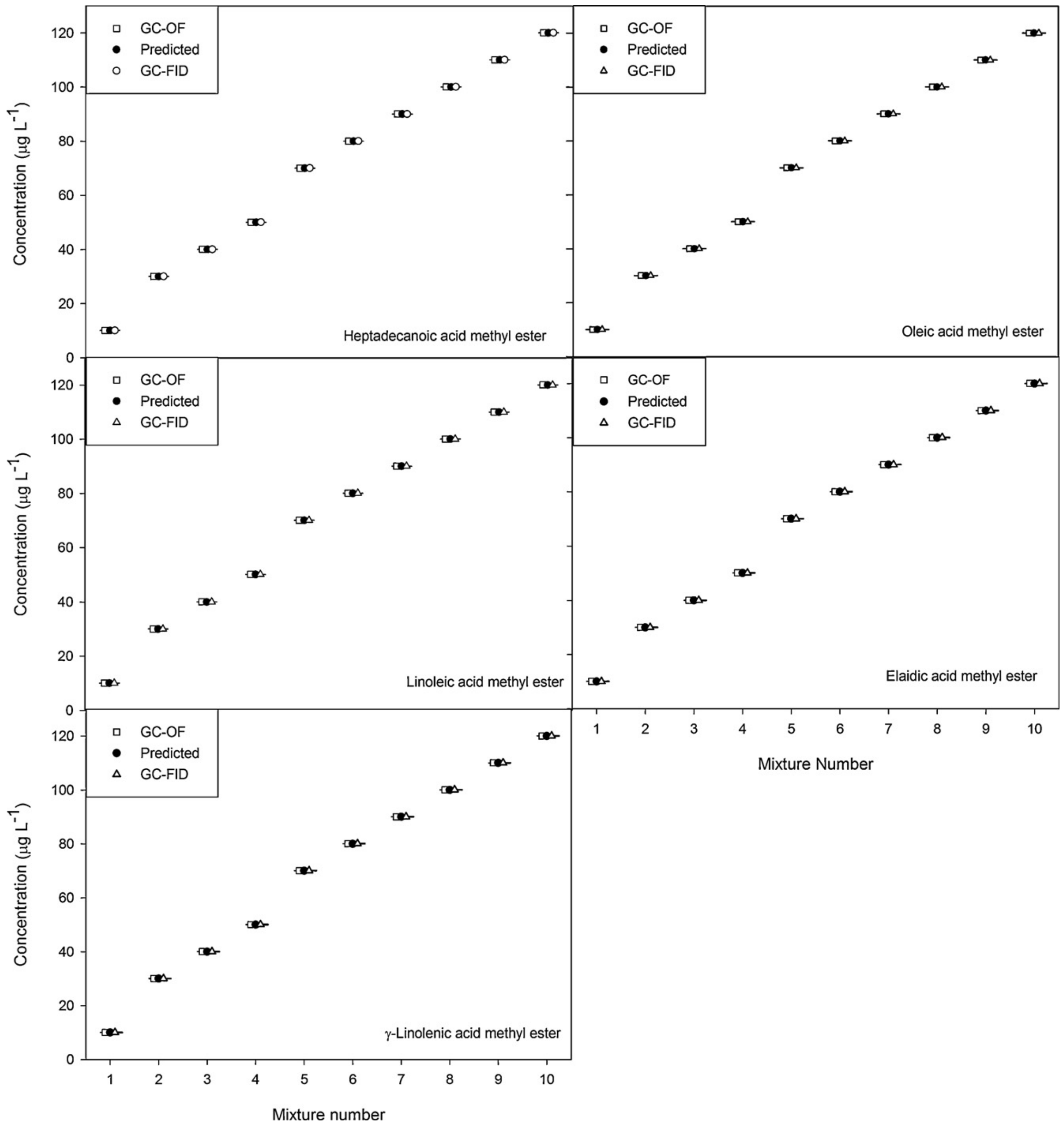

Fig. 4. Comparison of the results obtained for the ten SM (standard mixtures), (mean and standard deviation) of heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester using the GC-OF and GC-FID methodologies.

the analytical signal is in agreement with the boiling point of the components of the standard mixture as mentioned above.

The ten standard mixtures with different concentrations used to evaluate the performance of GC-OF methodology (Fig. 4) were subjected to an ANOVA [38] which allowed to observe that there are no significant differences between the results obtained by the two methods ($p = 0.885, 0.997, 0.981, 0.986$ and 0.975 for heptadecanoic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, elaidic acid methyl ester and γ-linolenic acid methyl ester, respectively). The results obtained in this study are reported in Table 3.

The analytical error, as evaluated by the coefficient of variation, was found to be less than 1% for the analysis of each FA methyl ester by GC-OF method; the maximum analytical error was found to be 0.51% for γ-linolenic acid methyl ester determination. Also for the analysis of heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester by GC-FID, the analytical error associated to the results obtained was found to be less than 1%.

The measurements of the five FA methyl esters performed by GC-OF method show an error level (taking as reference the expected value of concentration) ranging from 0.04% to 0.88%, and from 0.03% to 0.76% for the measurements performed by GC-FID method. Therefore, the accuracy of both methods can be considered in the same order of magnitude.

Table 3

Comparison of the results (mean ± coefficient of variation) obtained for heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester with GC-OF and GC–FID methods.

Standard number	Expected value	Heptadecanoic acid methyl ester		Elaidic acid methyl ester		Oleic acid methyl ester		Linoleic acid methyl ester		γ-Linolenic acid methyl ester	
		Mean ($\mu g\,L^{-1}$) ± [a] CV (%)	[b]Error (%)	Mean ± CV	Error (%)	Mean ± CV	Error (%)	Mean ± CV	Error (%)	Mean ± CV	Error (%)
GC-OF											
1	10.00	10.09 ± 0.47	0.86	10.09 ± 0.39	0.88	10.05 ± 0.19	0.48	10.07 ± 0.31	0.68	10.08 ± 0.51	0.80
2	30.00	30.07 ± 0.08	0.22	30.07 ± 0.16	0.25	30.04 ± 0.05	0.13	30.06 ± 0.12	0.19	30.08 ± 0.17	0.25
3	40.00	40.06 ± 0.05	0.15	40.06 ± 0.12	0.15	40.04 ± 0.06	0.10	40.06 ± 0.12	0.15	40.06 ± 0.11	0.15
4	50.00	50.05 ± 0.05	0.09	50.08 ± 0.09	0.16	50.04 ± 0.04	0.08	50.06 ± 0.06	0.11	50.08 ± 0.09	0.16
5	70.00	70.05 ± 0.03	0.07	70.06 ± 0.07	0.09	70.04 ± 0.04	0.06	70.05 ± 0.07	0.08	70.07 ± 0.07	0.10
6	80.00	80.04 ± 0.02	0.05	80.08 ± 0.05	0.10	80.05 ± 0.03	0.06	80.07 ± 0.03	0.09	80.07 ± 0.04	0.09
7	90.00	90.06 ± 0.03	0.06	90.08 ± 0.05	0.08	90.05 ± 0.03	0.06	90.07 ± 0.05	0.08	90.07 ± 0.05	0.08
8	100.00	100.04 ± 0.03	0.04	100.07 ± 0.04	0.07	100.04 ± 0.02	0.04	100.05 ± 0.04	0.05	100.07 ± 0.04	0.07
9	110.00	110.05 ± 0.02	0.04	110.06 ± 0.04	0.05	110.04 ± 0.02	0.04	110.06 ± 0.04	0.06	110.06 ± 0.04	0.05
10	120.00	120.05 ± 0.02	0.04	120.07 ± 0.03	0.06	120.06 ± 0.03	0.05	120.07 ± 0.03	0.06	120.07 ± 0.04	0.06
GC-FID											
1	10.00	10.06 ± 0.49	0.64	10.08 ± 0.49	0.76	10.04 ± 0.23	0.36	10.06 ± 0.52	0.56	10.07 ± 0.46	0.74
2	30.00	30.05 ± 0.10	0.16	30.05 ± 0.13	0.16	30.04 ± 0.08	0.13	30.05 ± 0.17	0.18	30.05 ± 0.16	0.17
3	40.00	40.05 ± 0.09	0.13	40.05 ± 0.12	0.13	40.04 ± 0.07	0.10	40.05 ± 0.11	0.13	40.06 ± 0.12	0.14
4	50.00	50.05 ± 0.05	0.10	50.06 ± 0.07	0.12	50.05 ± 0.05	0.10	50.06 ± 0.06	0.11	50.06 ± 0.08	0.12
5	70.00	70.04 ± 0.03	0.06	70.09 ± 0.05	0.13	70.05 ± 0.03	0.07	70.07 ± 0.05	0.10	70.08 ± 0.06	0.12
6	80.00	80.04 ± 0.03	0.05	80.09 ± 0.06	0.11	80.05 ± 0.03	0.07	80.07 ± 0.05	0.08	80.08 ± 0.06	0.11
7	90.00	90.05 ± 0.02	0.05	90.09 ± 0.05	0.10	90.05 ± 0.02	0.05	90.07 ± 0.04	0.07	90.09 ± 0.05	0.10
8	100.00	100.05 ± 0.03	0.05	100.09 ± 0.05	0.09	100.06 ± 0.03	0.06	100.08 ± 0.03	0.08	100.09 ± 0.04	0.09
9	110.00	110.04 ± 0.02	0.03	110.06 ± 0.04	0.05	110.04 ± 0.02	0.04	110.06 ± 0.04	0.05	110.06 ± 0.04	0.05
10	120.00	120.05 ± 0.03	0.04	120.04 ± 0.04	0.03	120.06 ± 0.02	0.05	120.05 ± 0.05	0.05	120.05 ± 0.04	0.04

[a] CV = (standard deviation/mean) × 100.

[b] Error = [(found value − expected value)/expected value] × 100.

Table 4
Concentration (μg kg^{-1}) of heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester in the soil from ornamental gardens from Lisbon.

Sample	Concentration (μg kg^{-1})				
	Heptadecanoic acid (C17:0)	Oleic acid (C18:1n9c)	Elaidic acid (C18:1n9t)	Linoleic acid (C18:2n6c)	γ-Linolenic acid (C18:3n6)
1	28,064 ± 11	34,568 ± 15	36,774 ± 17	30,136 ± 12	32,475 ± 11
2	24,064 ± 14	30,568 ± 19	32,774 ± 20	26,136 ± 13	28,468 ± 16
3	27,222 ± 17	33,531 ± 21	35,671 ± 14	29,232 ± 19	31,501 ± 23
4	16,240 ± 3	17,842 ± 5	18,372 ± 7	16,746 ± 9	17,329 ± 6
5	23,871 ± 16	30,323 ± 23	32,512 ± 25	25,927 ± 17	28,241 ± 19
6	24,826 ± 12	31,536 ± 17	33,812 ± 15	26,964 ± 19	29,370 ± 21
7	25,819 ± 15	32,798 ± 29	35,165 ± 31	28,043 ± 26	30,545 ± 24
8	26,852 ± 20	34,111 ± 18	36,571 ± 34	29,164 ± 17	31,766 ± 30
9	12,176 ± 8	15,415 ± 11	16,509 ± 13	13,207 ± 9	14,370 ± 16
10	28,194 ± 26	35,815 ± 35	38,400 ± 32	30,622 ± 22	33,355 ± 25
11	29,322 ± 18	37,248 ± 36	39,936 ± 38	31,847 ± 31	34,689 ± 39
12	19,597 ± 15	21,529 ± 19	22,169 ± 21	20,207 ± 24	20,911 ± 17

3.3. *GC-OF applications to urban soil samples*

After the development, comparison and validation against GC-FID methodology, the GC-OF methodology was applied to the analysis of heptadecanoic acid (C17:0), elaidic acid (C18:1n9t), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and γ-linolenic acid (C18:3n6) from urban soil samples from ornamental gardens from Lisbon (Table 4).

An indicator of the presence of microbial groups in urban soils, namely in the analyzed samples, can be obtained from the concentration of the bacterial indicators fatty acids (C17:0 for Gram-positive bacteria, and C18:1n9t for Gram-negative bacteria) and of the fungal indicators fatty acids (C18:1n9c, C18:2n6c, and C18:3n6) [12,15–17]. According to our results, there is a statistically significant difference ($p = 0.005$) between the concentration of each of the five screened fatty acid (Table 4) in the soil samples from Lisbon ornamental gardens. It can be observed in Table 4 that the fatty acids concentrations in all the twelve samples analyzed vary according to the following order: C18:1n9t > C18:1n9c > C18:3n6 > C18:2n6c > C17:0. Gram-negative bacteria, based on C18:1n9t concentrations, are present in higher quantity than Gram-positive bacteria according to C17:0 concentrations; this fact could probably be attributed to stress conditions, since is known that Gram-negative bacteria posses cyclo fatty acids in their membrane, and in the outer lipopolysacchride layer which are related to higher levels of survivability under stress conditions [12]. The observed differences between the 12 samples for the same fatty acid could be attributed to the fact that each sample is from an ornamental garden with different characteristics and microbial metabolism in soil is limited by the availability and types of organic substrates, and it is likely that ecosystems that differ on flora will have a different microbial growth [16].

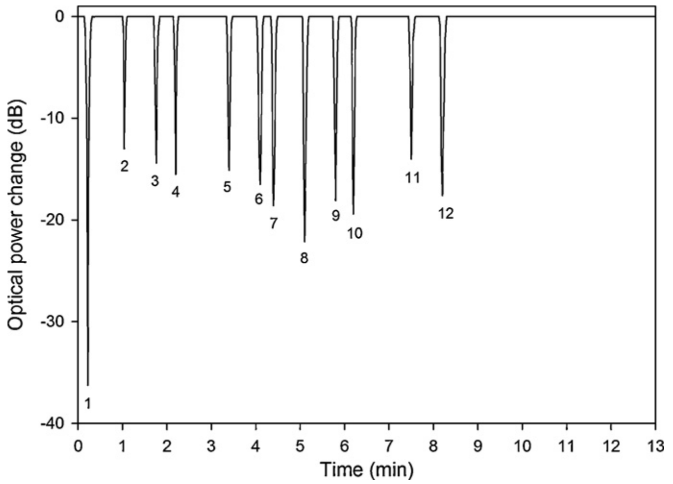

Fig. 5. Analytical signal obtained by GC-OF methodology for one of the samples of soil from ornamental gardens from Lisbon (1) solvent (hexane), (2) palmitic acid methyl ester, (3) palmitoleic acid methyl ester, (4) heptadecanoic acid methyl ester, (5) cis-10-heptadecanoic acid methyl ester, (6) stearic acid methyl ester, (7) elaidic acid methyl ester, (8) oleic acid methyl ester, (9) linolaidic acid methyl ester, (10) linoleic acid methyl ester, (11) γ-linolenic acid methyl ester, and (12) α-linolenic acid methyl ester.

Fig. 5 shows a chromatogram obtained by GC-OF methodology for one of the soil samples from ornamental gardens from Lisbon analyzed for heptadecanoic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and γ-linolenic acid methyl ester. In the chromatogram it can be observed eleven fatty acid methyl ester instead of the five used as indicators of microbial activity. This chromatogram shows that the proposed methodology is able of complete separation and detection of palmitic acid methyl ester, palmitoleic acid methyl ester, heptadecanoic acid methyl ester, cis-10-heptadecanoic acid methyl ester, stearic acid methyl ester, elaidic acid methyl ester, oleic acid methyl ester, linolaidic acid methyl ester, linoleic acid methyl ester, γ-linolenic acid methyl ester, and α-linolenic acid methyl ester. It can also be observed in Fig. 5 that these fatty acids (besides the five used as indicators) do not interfere in the analysis by GC-OF methodology. In summary, the obtained results (Fig. 5) allow to concluding that: (a) the GC-OF method shows an efficient peak separation and resolution, allowing selective analysis of the five fatty acid methyl ester under study, (b) the other substances detected were found not relevant in the target compounds analysis, since they produce a peak in a different analytical time, (c) the adjustment of the time window, through the optimization of chromatographic component of the analytical system, could be used for avoiding other potential interferences, and (d) the proposed method shows high potential for application to the analysis of other potential indicators of fungal and bacterial communities in urban soils.

4. Conclusions

The gas GC-OF detection methodology showed performance, detection limits (ranging from 1.9 to 3.6 μg L^{-1}) and linear range (5–130 μg L^{-1}) comparable to the GC-FID while avoiding the use of more expensive and sophisticated equipment.

The application of GC-OF to analyze different fatty acids in soil samples demonstrated its potential as alternative method to evaluate indicators of the microbial community in urban soils. Besides, the OF have potential for integration with GC in a commercial equipment.

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