



**HAL**  
open science

## **PLFAs of the microbial communities in composting mixtures of agro-industry sludge with different proportions of household waste**

Soumia Amir, Rajae Abouelwafa, Abdelilah Meddich, Salah Souabi, Peter Winterton, Georges Merlina, Jean-Claude Revel, Eric Pinelli, Mohamed Hafidi

► **To cite this version:**

Soumia Amir, Rajae Abouelwafa, Abdelilah Meddich, Salah Souabi, Peter Winterton, et al.. PLFAs of the microbial communities in composting mixtures of agro-industry sludge with different proportions of household waste. *International Biodeterioration and Biodegradation*, 2010, vol. 64, pp. 614-621. 10.1016/j.ibiod.2010.01.012 . hal-00793429

**HAL Id: hal-00793429**

**<https://hal.science/hal-00793429>**

Submitted on 22 Feb 2013

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in: <http://oatao.univ-toulouse.fr/>  
Eprints ID: 5818

**To link to this article:** DOI:10.1016/j.ibiod.2010.01.012  
<http://dx.doi.org/10.1016/j.ibiod.2010.01.012>

To cite this version: Amir, S. and Abouelwafa, Rajae and Meddich, Abdelilah and Souabi, Salah and Winterton, Peter and Merlina, Georges and Revel, Jean-Claude and Pinelli, Eric and Hafidi, M. *PLFAs of the microbial communities in composting mixtures of agro-industry sludge with different proportions of household waste*. (2010) *International Biodeterioration and Biodegradation*, vol. 64 (n°7). pp. 614-621. ISSN 0964-8305

Any correspondence concerning this service should be sent to the repository administrator: [staff-oatao@inp-toulouse.fr](mailto:staff-oatao@inp-toulouse.fr)

# PLFAs of the microbial communities in composting mixtures of agro-industry sludge with different proportions of household waste

Soumia Amir<sup>a</sup>, Rajae Abouelwafa<sup>b</sup>, Abdelilah Meddich<sup>b</sup>, Salah Souabi<sup>c</sup>, Peter Winterton<sup>d</sup>, Géorge Merlina<sup>e</sup>, Jean-Claude Revel<sup>e</sup>, Eric Pinelli<sup>e</sup>, Mohamed Hafidi<sup>b,\*</sup>

<sup>a</sup>Equipe Chimie Appliquée et Sciences de l'Environnement, Université Sultan Moulay Slimane, Beni Mellal, Morocco

<sup>b</sup>Laboratoire d'Ecologie et Environnement (Unité associée au CNRST), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390, Marrakech, Morocco

<sup>c</sup>Laboratoire Génie de l'Environnement, Faculté des Sciences et Techniques, Mohammedia, Morocco

<sup>d</sup>Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France

<sup>e</sup>ECOLAB, Ecole Nationale Supérieure Agronomique, Avenue de l'Agrobiopole, BP 32607, Auzeville-Tolosane, F 31326 Castanet-Tolosan Cedex, France

## ARTICLE INFO

### Keywords:

Compost  
PLFAs  
Oil industry  
Sludge  
Maturity indices

## ABSTRACT

Phospholipid fatty acids (PLFAs) were analysed at different time periods during composting of two waste mixtures rich in fats, M1 (22%) and M2 (39%), with the aim of monitoring changes in microbial community structure. The two mixtures consisted of a sludge sample collected from a vegetable oil refinery effluent treatment plant combined with household wastes. The PLFA profiles of both mixtures revealed that, at the start of the process, fungi and Gram-negative bacteria (G<sup>-</sup>) were more abundant in M2 than in M1. During the thermophilic phase, branched PLFA (i15:0, a15:0, i16:0, and i17:0) markers of Gram-positive bacteria (G<sup>+</sup>), became more abundant in M1, while G<sup>-</sup> bacteria were predominant in M2. The PLFA profiles in M1 representing non-specific, G<sup>+</sup> and G<sup>-</sup> bacteria as well as fungi decreased during the cooling phase (maturation) while an increase was recorded in M2, which was richer in fats. The Shannon–Weaver diversity index (I<sub>sh</sub>) showed a greater increase during M1 composting (from 0.69 to 1.05), mainly for G<sup>+</sup> bacteria and G<sup>-</sup> bacteria, than in M2 composting (from 0.79 to 0.84). Principal components and cluster analyses revealed a succession of different communities during composting, which varied from fungi and G<sup>-</sup> bacteria to G<sup>+</sup> and thermophilic and thermotolerant G<sup>-</sup> bacteria. The end of composting was characterized by a reduction of all these microbial entities, especially for M1, except actinomycetes, which are associated with compost stability.

## 1. Introduction

Composting is a process involving the decomposition and transformation of biodegradable organic waste under the action of various microbial populations occurring in damp aerobic environments. The initial phase is characterised by the activity and growth of mesophilic microorganisms leading to a rapid increase in temperature (Finstein and Morris 1975; De Bertoldi 1981; Miller 1993). Then, thermophilic microorganisms ensure the degradation process, the activity of non-heat-tolerant species being halted. The final phase, which includes a period of cooling and maturation, is characterised by the development of a new community of mesophiles. Maturity and stability are words expressing very different concepts: The maturity of compost refers to its degree of humification, and stability concerns the level of activity of the microbial

biomass (Iannotti et al. 1993). Analysis of phospholipid fatty acids (PLFAs) is an accurate method of examining the changes occurring in the microbial community during composting.

Analysis of PLFAs is a method of choice for many reasons. One is that most environmental microorganisms, although viable, cannot be cultured (Xu et al. 1982; McCarthy and Murray 1996; White et al. 1997). In addition, microorganisms contain phospholipids in their membranes that are not stored but have a relatively high turnover during metabolism (White et al. 1997). Consequently, the PLFAs present in a sample are representative of the viable microbial biomass contained in that sample (Vestal and White 1989). Moreover, some PLFAs can be considered to be taxonomic or physiological biomarkers for certain genera of microbes (White 1995; Zelles et al. 1995). Several studies, such as that of Herrmann and Shann (1997), have used PLFA profile variations to monitor changes occurring in the microbial community as composting progresses. They found that during the mesophilic phase, fatty acids (FAs) from eukaryotes and from mycetes were the most abundant. In the thermophilic phase, FAs from actinomycetes and

\* Corresponding author. Tel.: +212 5 24 43 76 65.  
E-mail address: hafidi@ucam.ac.ma (M. Hafidi).

Gram-negative (G-) bacteria were predominant. In the samples collected at the end of the process, they found biomarkers from mycetes and actinomycetes. Furthermore, when using very old samples for composting they found that the changes occurring in FA profiles during the process were similar. This suggests that PLFA analysis provides a reliable and powerful tool for evaluating the maturity of compost. However, it is not possible from current literature to get a clear picture of the microbial communities' variations during composting. These communities vary according to the composition of the initial mixture that is intended for composting and the environmental conditions during composting.

The major goal of the present study was to monitor the variations in microbial communities during composting of sludge samples collected at the outlet of a vegetable oil refinery wastewater treatment plant located at Ain Harouda in Morocco. This sludge tends to vary in quality and in quantity from day to day. The type of effluent depends on which oils are being processed (soybean, palm, sunflower, rapeseed) and the processes they undergo (soap making, production of distilled fatty acids, production of cake) (Bhah and Sidibe 2002; Soudi and Maftouh 2003; Battachi 2006; Lachhab and Herras 2006). There can also be lipid spills during the different stages of refining, which increase the level of fats in the sludge. On occasions, owing to the increase in production from the refinery – when a third production line is put into operation – the input of wastewater exceeds the treatment plant's capacity (1450 m<sup>3</sup>/day). In a study by Lachhab and Herras (2006), it was reported that the input to the treatment plant in May 2006 ranged from 350 m<sup>3</sup> day<sup>-1</sup> to 1680 m<sup>3</sup> day<sup>-1</sup>, and the level of fats in the effluent varied from 3054 to 6634 mg l<sup>-1</sup>.

In this work we studied the change in fats in the course of composting of two samples of the Ain Harouda sludge, initially containing different levels of lipids. In order to reduce the amount of lipids, both sludge samples were mixed with household wastes. The changes occurring in the PLFA profiles were studied to determine how the microbial community varied during the composting of both mixtures.

## 2. Materials and methods

### 2.1. Compost substrate

The substrate consisted of a mixture of sludge and household waste

- The sludge samples were taken from the vegetable oil refinery effluent treatment plant of Lesieur-Cristal Co. Ltd. (Ain Harouda, Morocco). They were produced after the liquid effluent had undergone three types of treatment: physical, chemical, and biological.
- The household waste was taken from the municipal dump of the town of Mohammedia (Morocco). It was sorted to remove non-biodegradable material.

The two sludge samples were collected at two different times during the effluent treatment. The proportion of organic carbon (43%) of the sample collected at the later step of the treatment, S2, was higher, and the nitrogen content (0.72%) and available phosphorus (885 ppm) were lower than the sludge sample S1, which was collected at an earlier treatment step (Table 1). Owing to these differences, two mixtures were prepared with different proportions of sludge and household waste:

- Mixture M1: 25 kg (dry weight) Lesieur sludge S1 (collected during an early step of the treatment) were mixed with 40 kg (dry weight) sorted household rubbish.

**Table 1**

Physical–chemical characteristics of the material to be composted (sludge samples S1 and S2, household waste (HW), and their mixtures M1 and M2).

	Mixture M1			Mixture M2		
	Sludge S1	HW	Mixture	Sludge S2	HW	Mixture
Moisture (%)	75.86	80.45	77.64	45.97	72.11	63.01
PH	5.16	4.82	4.96	5.24	5.85	5.70
Electrical conductivity (ms cm <sup>-1</sup> )	4.1	5.95	5.42	1.5	5.2	1.86
Total Kjeldhal nitrogen (% dry wt.)	1.15	1.54	1.5	0.72	3.46	1.3
Total organic carbon (%)	35.91	53.22	45.18	43.33	45.18	44.27
C/N	31.22	34.55	30.12	60.18	13.05	34.05
Ash (%)	39.74	10.18	21.39	25.58	16.66	24.70
Assimilable phosphorus (ppm)	6254	1858	4688	885	986	911
Total polyphenol (mg g <sup>-1</sup> W Wt.)	0.108	0.483	0.247	nd	nd	0.254

nd: not detected.

- Mixture M2: 77 kg (dry weight) Lesieur sludge S2 (collected 15 days after S1 sampling) were mixed with 28 kg (dry weight) household rubbish sorted to remove non-biodegradable items.

### 2.2. Compost experiment

The heaps were composted outside on a purpose-built composting slab for a period of five months. They were forked over every week to maintain aeration, and moistened regularly. Samples were taken after different times of composting (T0M1 and T0M2: initial mixtures M1 and M2; T2M1 and T2M2: after 2 months; and T5M1 and T5M2: after 5 months of composting of M1 and M2, respectively). Preliminary analyses of these samples have been reported previously (Abouelwafa et al. 2008a), and the rate of decomposition was determined from the following equation:

$$\text{Decomposition (\%)} = 100 \left( \frac{\text{Ash}_f - \text{Ash}_i}{\text{Ash}_f(100 - \text{Ash}_i)} \right) 100$$

where Ash<sub>i</sub> is the initial level of ash and Ash<sub>f</sub> the final level.

The humic substances were extracted using 0.1 M NaOH, humic acid was isolated by 3 M HCl and recovered in 0.1 M NaOH, and its level was determined by weighing a known volume after freeze-drying.

For the lipid and fatty acid analyses, the compost was sampled at different phases during composting and the samples were transferred to a plastic bag and stored at -20 °C until analysis. Immediately before sampling, the heaps were turned to mix the contents.

### 2.3. Lipid extraction and separation

The lipid extraction was carried out from 15 g of accurately weighed compost samples (three repetitions). The lipids were extracted with 360 ml of a chloroform/methanol mixture (2/1, v/v) (3 × 120 ml). After 24 h of maceration at 4 °C, the mixture was filtered through glass wool. The chloroform fraction containing the lipids was recovered following the addition of 60 ml of 0.73% NaCl, according to the method of Folch et al. (1956), and it was dehydrated with anhydrous sodium sulphate. The lipids were separated on silica columns. The neutral lipids, glycolipids, and phospholipids

were eluted by chloroform, acetone, and methanol, respectively (Frostegård and Bååth 1996). The phospholipids and neutral lipids content were estimated by weighing the two extracts after evaporation and drying. Both fractions were recovered in 5 ml of  $\text{CHCl}_3$ .

The phospholipid fraction, further dried under a stream of nitrogen gas, was taken up in 1 ml of heptane with C10:0 as the internal standard. The fatty acids were subjected to transesterification after alkaline methanolysis. The fatty acid methyl esters were analysed by gas chromatography (HP 5890 series II) coupled with mass spectrometry (HP 5971): column length 30 m, internal diameter 0.530 mm. Injection used the split mode, 1  $\mu\text{l}$  sample with 1  $\mu\text{l}$  hexane, carrier gas helium under 21 kPa pressure; the injector temperature was 260 °C, and that of the oven: from 140 °C (4 min) to 250 °C with 4 °C  $\text{min}^{-1}$ ; the detector temperature was 280 °C, and the ionisation energy was 70 eV. The methyl ester peaks obtained were compared with those of external standards corresponding to bacterial fatty acid methyl esters FAMES (26 known bacterial FAMES, supplied by Supelco, Bellefonte, PA).

#### 2.4. Fatty acid nomenclature

The fatty acid nomenclature used is as follows: total number of carbon atoms::number of double bonds, followed by the position w of the double bond from the methyl end of the molecule. The *cis* and *trans* configurations are indicated by c and t, respectively. The *anteiso* and *iso* branching are designated by the prefix a or i.

The PLFA profiles recorded in this investigation were classified into specific groups of organisms according to the literature (Hellman et al. 1997; Amir et al. 2008):

NM: Not only of microbial origin, they could be also in the vegetal waste (12: 0; 14: 0; 16: 0; 18: 0; 20: 0)

NSB: Non-specific bacteria exhibit the fatty acids patterns occurring in a large number of bacteria (13: 0; 15: 0; 17: 0; 19: 0).

Fatty acids patterns specific to microbial groups:

G+: Gram-positive bacteria (i-15: 0; a-15: 0; i-16: 0; i-17: 0);

G-: Gram-negative bacteria (2-OH12: 0; 16:1 $\omega$ 9c; cy17: 0; 18:1 $\omega$ 9t; cy19: 0);

F: Fungi (18:1 $\omega$ 9c; 18: 2 $\omega$ 6,9).

#### 2.5. Statistical treatment

Comparison of the averages was carried out by ANOVA test post-hoc Tukey. Principal components analyses (PCA) and cluster analyses were carried out between different PLFA profiles during composting.

### 3. Results and discussion

#### 3.1. The properties of composts

The microbiological activity during composting of mixtures M1 and M2 led to temperatures reaching, respectively, 62.6 and 65 °C (Fig. 1); each compost achieved pathogen kill temperatures of >55 °C and remained at these temperatures for several days (Abouelwafa et al. 2008a). The physicochemical characteristics of the final composts, after 5 months of composting, were, for M1 and M2, respectively, pH: 8.5 and 7.1; C/N: 10 and 16; proportion of decomposition: 78 and 55%;  $\text{NH}_4^+/\text{NO}_3^-$ : 0.78 and 1.02. Total polyphenol concentrations decreased during composting (Abouelwafa et al. 2008a), falling by 75% and 76% for M1 and M2, respectively. The levels of cellulose, hemicellulose, and lignin fell in M1 by 62, 63,

and 67% and in M2 by 39, 49, and 39% respectively. This was paralleled by a rise in the humic acid content, which reached 22 and 36  $\text{mg g}^{-1}$  in M1 and M2, respectively.

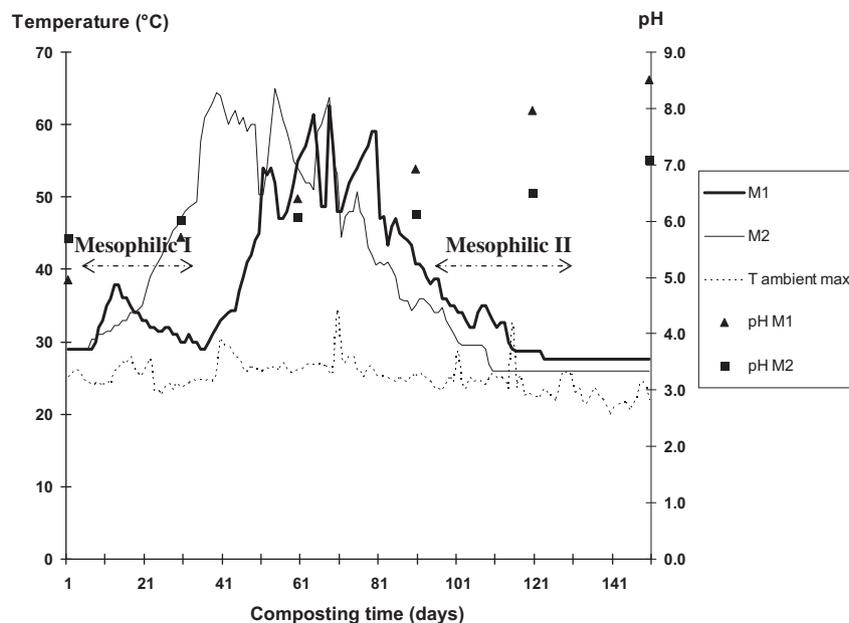
#### 3.2. Changes in total fats, phospholipids, and neutral lipids during composting

Lipid analysis indicates that sludge sample S2 contained a higher amount of fats (49%) than did S1 (31%) (Table 2); their mixtures with household waste paralleled these values. The lipid composition of the two mixtures in the initial sludge samples and at different phases during composting differed significantly. The respective percentages of neutral lipid comprised in total lipids (TL) varied from 27 and 29% before composting to 79 and 53% after composting for M1 and M2. In the case of phospholipids, maximal values did not exceed 10% of the total lipids except in the case of T5M1 (Table 2). As reported by Malosso et al. (2004), neutral lipids serve as energy storage in microbial organisms. However, the possibility that they originated from the refinery wastewater cannot be excluded.

During composting, TL degradation was extensive in both mixtures, reaching 81% and 72% for M1 and M2 respectively, although a non-negligible quantity of lipids (11% dw) remained in mixture M2. The degradation of neutral lipids reached 92% for M1 and 75% for M2 after 150 days of composting. By contrast, phospholipids fell slightly in compost M2 and increased in M1. The change in phospholipid levels can be well explained by the fact that the phospholipids originate mainly from the membranes of microorganisms which were abundant during the high-temperature phase of composting, as reported by Bai et al. (2000), Boulter et al. (2002), Barje et al. (2008), and Amir et al. (2008). Similar results were reported by El Hajjouji et al. (2008) during the aerobic treatment of olive mill wastewater (OMW), when PLFAs increased owing to the growth of a new population of microorganisms.

#### 3.3. Phospholipid fatty acid analyses

Table 3 shows the concentrations of the phospholipids and fatty acids detected in sludge S1 and S2 and in mixtures M1 and M2 before and after 2 months and 5 months of composting. In Table 3, the fatty acids are organized according to the groups of organisms in which they occurred (White 1995; Zelles et al. 1995). On the basis of PLFA profiles of the sludge samples, the microbial biomass of sludge S1 is richer than that of S2. The higher concentration of fats in the latter is probably hindering its development (Table 3). In fact, the concentrations of bacterial biomass (fungal and G- bacteria PLFAs) were much higher at the time of mixing S2 with household waste: TOM2 compared to TOM1 (Table 3). This is corroborated by the microbiological analysis reported in a previous study, which showed that TOM2 exhibited a higher microbial diversity with respect to total mesophilic flora, fungi, spore-forming bacilli, and actinomycetes compared to TOM1 (Abouelwafa et al. 2008b). In addition, PLFA analysis showed a higher level of PLFAs in TOM2 than in TOM1 (Table 3). This could be explained by the better conditions for the development of these microorganisms in M2 compared to M1 obtained by reducing the concentrations of fats through addition of more household waste, the presence of more readily available compounds for decomposition, and mesophilic temperature of M2 compost at the initial time (Fig. 1). As a consequence, the high microbial activity in M2 led to the emergence of an anaerobic state, as demonstrated by the presence of hydroxy fatty acids 2 OH-12:0 in TOM2 (Myers et al. 2001) (Table 3). In fact, the higher  $\text{NH}_4^+/\text{NO}_3^-$  ratio in M2 compared to M1 is further evidence that composting of M2 reaches an anaerobic state (Wang et al. 2008).



**Fig. 1.** Temperature and pH changes during composting of mixtures M1 and M2 consisting of mixtures of agro-industrial sludge samples and household wastes.

However, at the beginning of the thermophilic phase of composting, the amount of lipids associated with G+ and G- bacteria increased significantly ( $P < 0.01$ ) in M1 (T2M1) (Fig. 2), and those associated with fungi and NSB were reduced ( $P < 0.01$ ). A similar pattern occurred in M2 except that lipids associated with G- bacteria decreased (T2M2) (Fig. 2). Many authors have reported the use of increased *iso* and *anteiso* branched PLFAs (e.g., i-15:0; a-15:0; i-16; i-17) as indicators of the growth of G+ bacteria during the stabilisation phase of composting (Klamer and Bååth 1998; Carpenter-Boggs et al. 1998; Cahyani et al. 2002). The strong increase in 15- and 17-carbon chains is considered to be a good indication of the presence of thermophilic *Bacillus* sp [12]. According to these authors, a high proportion of branched *iso*-FAMES indicates the continuous and extensive survival of G+ bacteria in a strictly thermogenic phase of composting. In a recent study, Amir et al. (2008) examined the lipid variations during composting of sewage sludge and straw. They found that the branched fatty acids with *iso*- and *anteiso*-forms (i-15:0; a-15:0; i-16; i-17) increased mainly in the thermophilic phase, but decreased immediately afterwards. However, the decrease in M2 (T2M2) of G- bacteria PLFAs (mainly 16:1 and 18:1) was probably caused by a decrease in available carbon, which is evidenced by the observation that the C/N ratio and the carbon compounds (polyphenols, cellulose, hemicellulose, and lignin) decreased significantly during the thermophilic phase (Abouelwafa et al. 2008a).

**Table 2**

Proportion of total lipids, phospholipids, and neutral lipids in sludge S1 and S2 and in mixtures M1 and M2 after different times of composting.

Lipids (% dry wt)	Mixture or sludge	Sludge	Mixture		
			Mixture 1 day	Mixture 2 months	Mixture 5 months
Total lipids	(1)	30.6	21.7	7.7	4.1
	(2)	48.8	38.7	14.0	10.7
Phospholipids	(1)	1.9	0.1	0.7	1.2
	(2)	1.9	1.9	1.5	0.7
Neutral lipids	(1)	16.6	17.1	2.1	1.3
	(2)	14.1	20.4	5.1	5.3

All values are the means of three repetitions.

Zelles et al. (1992) reported that monounsaturated fatty acids 16:1 and 18:1 from G- bacteria are strongly related to high substrate availability.

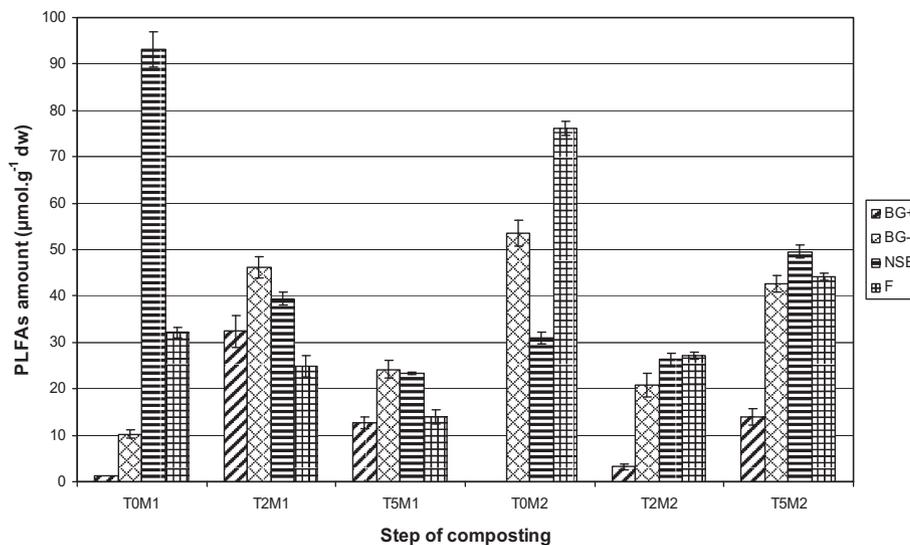
In the maturation phase, in M1, all microbial entities: G+ and G- bacteria and fungi decreased (T5M1), in contrast to M2 (T5M2), where all entities increased ( $P < 0.01$ ) (Fig. 2). This is an indication that at the end of the thermophilic phase, compost M1 was

**Table 3**

PLFA concentrations in sludge S1 and S2 and in mixtures M1 and M2 at time 0 (T0), during the hottest phase (T2: 2 months), and at the end (T5: 5 months) of composting.

PLFA ( $\mu\text{mol g}^{-1} \text{ d.w.}$ )	M1				M2			
	S1	T0M1	T2M1	T5M1	S2	T0M2	T2M2	T5M2
<i>NM</i>								
12:0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0
14:0	34.2	4.7	7.6	3.3	3.8	4.9	2.5	5.8
16:0	96.1	12.1	26.8	13.8	39.1	20.7	19.0	34.7
18:0	9.9	4.2	4.4	3.7	13.7	5.3	4.8	9.0
20:0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>NSB</i>								
13:0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
15:0	8.7	0.0	0.7	0.9	0.0	0.0	0.0	0.0
17:0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19:0	34.8	69.5	0.0	0.0	0.0	0.0	0.0	0.0
<i>BG+</i>								
i-15:0	20.0	0.0	12.7	5.1	0.0	0.0	1.9	5.9
a-15:0	37.3	1.4	13.1	4.2	0.0	0.0	1.3	5.4
i-16:0	0.0	0.0	5.2	2.3	0.0	0.0	0.0	2.8
i-17:0	0.0	0.0	1.4	1.2	1.8	0.0	0.0	0.0
<i>Fungi</i>								
18:1 $\omega$ 9c	60.9	20.1	17.6	10.2	48.8	46.4	23.5	38.8
18:2 $\omega$ 6,9	74.6	12.1	7.4	3.7	0.0	29.8	3.7	5.3
<i>BG-</i>								
2-OH12:0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0
16:1 $\omega$ 9c	20.5	0.0	13.2	5.3	0.0	4.1	3.2	2.0
cy17:0	0.0	0.0	0.9	1.5	0.0	0.0	0.0	0.0
18:1 $\omega$ 9t	127.8	10.3	30.8	13.5	9.9	22.4	16.0	35.2
cy19:0	0.0	0.0	1.4	3.8	0.0	0.0	1.5	5.4

NM: non-microbial lipids; NSB: non-specific bacteria; BG+: Gram+ Bacteria; BG-: Gram- Bacteria.



**Fig. 2.** Changes in bacterial population based on PLFA profiles during composting of mixtures M1 and M2. T0, T2, and T5 refer to time 0, 2 months, and 5 months of composting. BG-, G negative bacteria; BG+, G positive bacteria; NSB, non-specific bacteria; F, fungi.

stabilized, unlike M2, where the thermophilic microorganisms were replaced by an active mesophilic microbial population (Herrmann and Shann 1997).

Major conclusions can be drawn on the basis of the relative abundance of each group of microorganisms. The first is that bacteria were much more abundant than fungi in M1 than in M2; the bacteria/fungi ratio was of the order of 3.2–4.7 in M1 and about 1.0–2.4 in M2. This difference could, in all likelihood, be attributed to the high amounts of fatty acids in sludge S2, and therefore compost M2, that favour the presence of fungi. A second conclusion is that all bacteria (G+ plus G-)/fungi or G+/G- ratios increased and then decreased at the end of M1 composting, but in M2 these ratios continued to increase, which confirms that in M2, composting had not yet ended.

The Shannon-Weaver index (Ish) was used to calculate the degree of microbial diversity during composting. Low values of Ish correspond to low diversity, while a high index corresponds to a complex system. This ecological index was used by Alcaniz et al. (1983) and Ayuso et al. (1996) to examine the organisation of organic compounds in soil and humic acids.

The relative abundance,  $P_i$ , of each fatty acid is calculated from the ratio between the area of its corresponding peak to the sum of the areas of all the peaks considered in the chromatograms (Ayuso et al. 1996).

$$P_i = \frac{a_i}{\sum_1^n a_i}$$

The Ish index was calculated as follows:  $Ish = -\sum_1^n P_i \log_2 P_i$ ;  $0.5 < Ish < 4.5$ .

As seen in Table 4, the diversity index differed between sludge S1 and S2. The total microbial diversity  $Ish_{tot}$  increased more in compost M1 (from 0.69 to 1.05) than in compost M2 (from 0.79 to 0.84). The diversity index increased principally for the G+ and G- bacteria, whereas the diversity index for fungi did not increase.

The fatty acid profile variations during composting of mixture M1 were analyzed using the principal component analysis (Fig. 3a). The plots of the principal component scores are divided into three domains (I, II, and III). The first axis, PC1, explains 73.6 % of the variability between domain I of PLFA profiles (18:1 $\omega$ 9c; 18:2 $\omega$ 6,9; 19:0; 13:0) and domain II (16:0, 18:1 $\omega$ 9t; a15:0; i 15:0; 16:1 $\omega$ 9c; i 16:0; i 17:0). NSB and fungal PLFAs are clustered into domain I,

whereas PLFAs of G+ and G- bacteria, except for the G- molecules cy17:0 and cy19:0, are clustered into domain II. Thus, the high temperature contributes to the first principal components, separating thermo-sensitive species (PLFA profiles domain I) and thermophilic or thermotolerant species (PLFA profiles domain II). The PLFA profiles of domain II are presumed to express the index of stability of the material in the thermophilic phase of composting. The G- PLFAs (cy17:0; cy19:0) present with 15:0 in domain III can be distinguished from domain II by taking PC2 (26.4 %) into consideration. The molecules cy17:0, cy19:0, and 15:0 increased during composting from 0 to 1.5, 3.8, and 0.9  $\mu\text{mol g}^{-1}$  dry wt in T5M1, respectively. However, only cy19:0 increased from 0 to 5.4 in T5M2. Herrmann and Shann (1997) reported that cy17:0 and cy19:0 underwent a consistent increase as the compost aged. These authors suggested that high levels of these PLFAs would be associated with a decrease in metabolic activities as the readily degradable fraction of the compost is depleted. Therefore, the presence and level of phospholipids cy17:0 and cy19:0 have been suggested to provide a means of determining the age and maturity of a composted material. The PLFA 15:0 is generally associated with bacteria and can be found in small amounts in completely different bacterial groups (B  ath 2003).

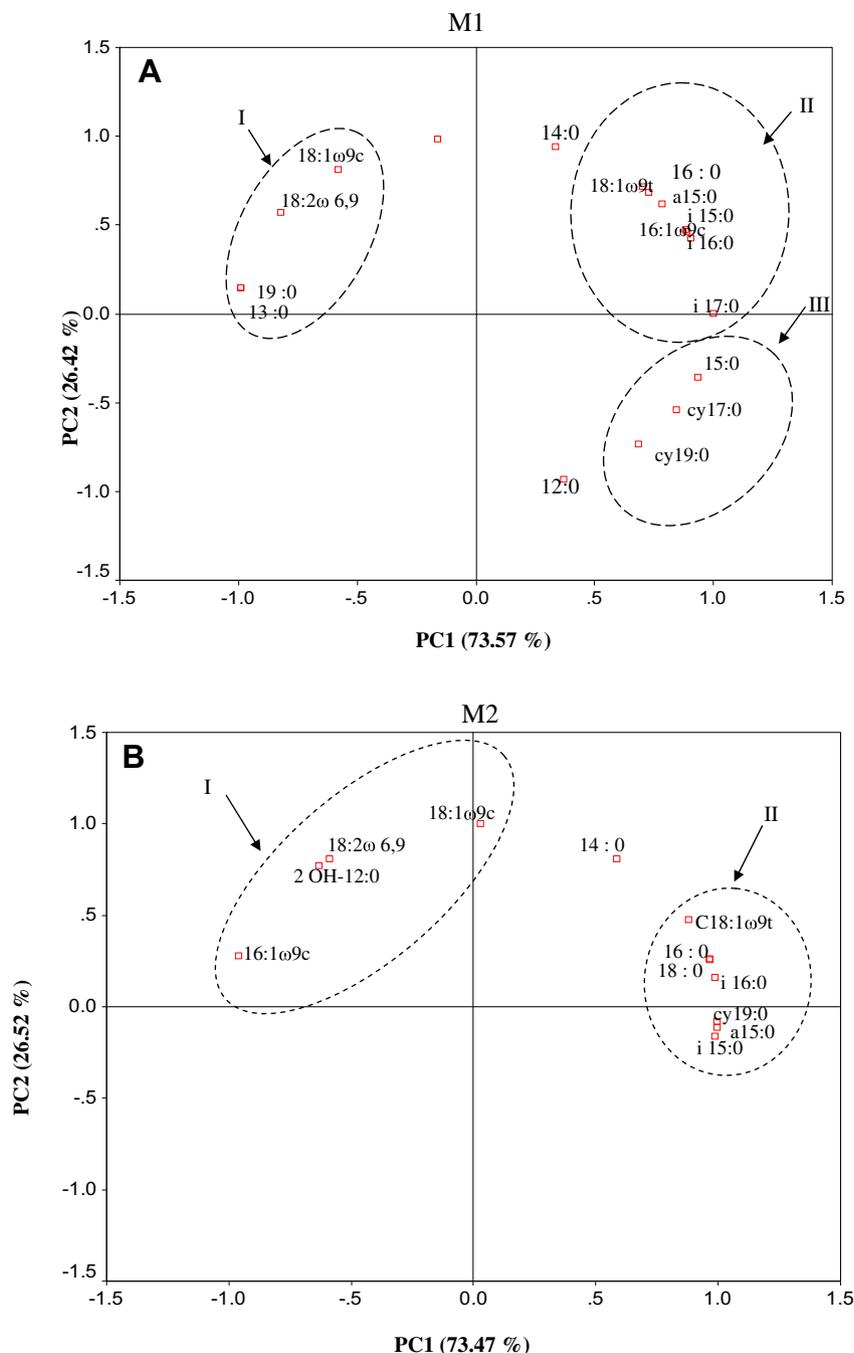
Similar observations were made from the PCA analysis for the PLFA profiles of compost M2, where the plot of the principal component scores was divided into two domains (I and II) (Fig. 3b). The first axis PC1 explains 73.6 % of the variability between PLFA profiles of domain I (fungal PLFAs: 18:1 $\omega$ 9c; 18:2 $\omega$ 6,9 and G- bacteria PLFAs: 2 OH-12:0; 16:1 $\omega$ 9c) and those of domain II (Gram+ bacteria: a15:0; i15:0; i16:0; Gram-: 18:1 $\omega$ 9t; cy19:0;

**Table 4**

The Shannon-Weaver index (Ish) calculated between all identified microorganisms (Ish tot) and within each microbial group during composting of mixtures M1 and M2 at time 0 (T0), 2 months (T2), and 5 months (T5).

	S1	T0M1	T2M1	T5M1	S2	T0M2	T2M2	T5M2
Ish tot	0.95	0.69	0.97	1.05	0.59	0.79	0.80	0.84
Ish NSB	0.63	0.38	0.39	0.52	0.34	0.38	0.33	0.35
Ish BG+	0.28	0.00	0.51	0.55	0.00	0.00	0.29	0.46
Ish Fungi	0.30	0.29	0.26	0.25	0.00	0.29	0.17	0.16
Ish BG-	0.17	0.00	0.35	0.49	0.00	0.39	0.30	0.25

NSB: non-specific bacteria; BG+: Gram+ Bacteria; BG-: Gram- bacteria.

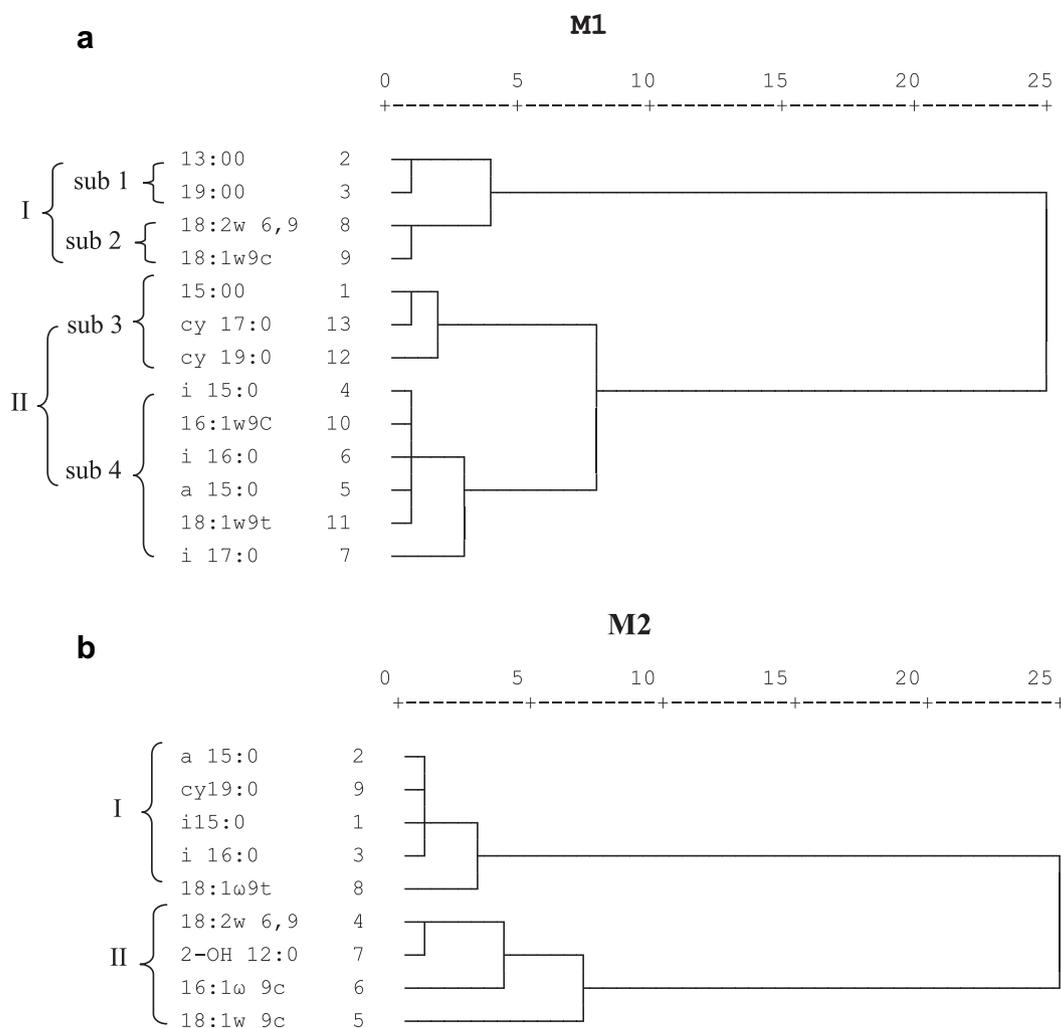


**Fig. 3.** Principal components analysis of PLFA profile variations during composting of (a) mixture M1, and (b) mixture M2.

NSB: 16:0, 18:0). Domain I appears to include the PLFAs of thermosensitive microorganisms in which the temperature is a selective factor for their development. In contrast, domain II gathers PLFA profiles of bacterial entities whose abundance increases in the first phase of composting, i.e., those specific to thermotolerant species or microorganisms contributing to the stabilisation of the material to be composted. PC2 separates the G<sup>-</sup> bacteria PLFA C18:1 $\omega$ 9t from G<sup>+</sup> PLFAs in domain II, as well as G<sup>-</sup> PLFA 16:1 $\omega$ 9c from fungal PLFAs in domain I.

The cluster analysis based on the PLFA profile variations during composting of mixture M1 shows two major clusters (Fig. 4a). Each one was divided into two sub-clusters, each corresponding to specific microbial groups: cluster I comprised NSB PLFAs (sub-

cluster 1) and fungal PLFAs (sub-cluster 2); cluster II comprised PLFAs profiles of G<sup>-</sup> bacteria (sub-cluster 3) and of G<sup>+</sup> bacteria (sub-cluster 4). Similar results were obtained for the analysis of mixture M2 composting (Fig. 4b); the PLFA profiles were divided into two large clusters, the first comprising the PLFA profiles of G<sup>+</sup> and G<sup>-</sup> bacteria, and the second, the fungal PLFAs. One exception is the presence of 2 OH-12:0 in the cluster assembling the fungal PLFAs. The cluster division clearly demonstrates that different groups of microorganisms proliferate in the compost and that the PLFA profiles detected during composting depend upon the conditions prevailing at each phase of treatment. In fact, it has been reported that, at the start of composting, eukaryotes, fungi, and some G<sup>-</sup> bacteria are less abundant in the compost biomass (Steger



**Fig. 4.** Cluster analysis of PLFA profiles during the composting of (a) mixture M1, and (b) mixture M2.

et al. 2003). The other G<sup>-</sup> bacteria are thermotolerant (Rhee et al. 2000), but G<sup>+</sup> bacteria represent the strict thermophilic bacteria that proliferate especially during the high-temperature phase.

Altogether, PCA and cluster analyses demonstrate the changes occurring in each group of microorganisms during the course of composting. The latter process is controlled by a succession of communities varying from fungi and G<sup>-</sup> to G<sup>+</sup> bacteria and other thermophilic and thermotolerant G<sup>-</sup> bacteria (Herrmann and Shann 1997; Klamer and Bååth 2004). The actinomycetes, considered by many authors to be an index of compost maturity, showed a continuous increase in the late steps of composting in M2, while in M1 they became stabilized from day 60 (Steger et al. 2007; Abouelwafa et al. 2008b).

From the PLFA changes that occurred in the course of composting, it appears that the succession of microbial communities was fairly similar in both composts, but differences in microbial composition were recorded depending on the initial chemical composition of the mixtures to be treated as well as the selective conditions and the degree of maturity achieved in each compost. PLFA analyses showing that mixture M1 reached a state of maturity during composting was corroborated by other analytical data based on physicochemical properties such as the spectroscopic analysis of humic substances (not shown). In contrast, in M2 the high fatty acid content hindered rapid stabilisation and maturation (Abouelwafa et al. 2008a).

This difference between the two mixtures was especially due to the composition of the initial mixture and the resulting temperature changes. Temperature showed a rapid increase in M2 from the start of composting, while in M1 a latency step and mesophilic phase was recorded before the thermophilic phase.

## References

- Abouelwafa, R., Amir, S., Souabi, S., Winterton, P., Ndira, V., Revel, J.-C., Hafidi, M., 2008a. The fulvic acid fraction as it changes in the mature phase of vegetable oil-mill sludge and domestic waste composting. *Bioresource Technology* 99, 6112–6118.
- Abouelwafa, R., Ait Baddi, G., Souabi, S., Winterton, P., Cegarra, J., Hafidi, M., 2008b. Aerobic biodegradation of sludge from the effluent of a vegetable oil processing plant mixed with household waste: physical-chemical, microbiological, and spectroscopic analysis. *Bioresource Technology* 99, 8571–8577.
- Alcaniz, J.M., Seres, A., Gassiot-Matas, M., 1983. Soil depth variations of humic composition studied by pyrolysis-gas chromatography (Py-GC). In: VIII International Symposium on Humus and Plant: Studies about Humus. Prague, vol. I, pp. 1–4.
- Amir, S., Merlina, G., Pinelli, E., Winterton, P., Revel, J.C., Hafidi, M., 2008. Microbial community dynamics during composting of sewage sludge and straw studied through phospholipid and neutral lipid analysis. *Journal Hazardous Materials* 159, 593–601.
- Ayuso, M., Hernandez, T., Garcia, C., Pascual, J.A., 1996. Biochemical and chemical-structural characterization of different organic materials used as manures. *Bioresource Technology* 57, 201–207.
- Bååth, E., 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of the soil fungi. *Microbial Ecology* 45, 373–383.

- Bai, Q., Gattinger, A., Zelles, L., 2000. Characterization of microbial consortia in paddy rice soil by phospholipid analysis. *Microbial Ecology* 39, 273–281.
- Barje, F., Amir, S., Winterton, P., Pinelli, E., Merlina, G., Cegarra, J., Revel, J.C., Hafidi, M., 2008. Phospholipid fatty acid analysis to monitor the co-composting process of olive oil mill wastes and organic household refuse. *Journal of Hazardous Materials* 154, 682–687.
- Battachi, K., 2006. Mémoire de fin d'étude de l'office de la formation professionnel et de la promotion de travail, p. 97.
- Bhah, A., Sidibe, B., 2002. Optimisation des rejets liquides. Rapport de stage de la 5ème année IAV Hassan II Rabat, Maroc, pp. 37–38.
- Boulter, J.I., Trevors, J.T., Boland, G.J., 2002. Microbial studies of compost, bacterial identification and their potential for turf grass pathogen suppression. *World Journal of Microbiology Biotechnology* 18, 661–671.
- Cahyani, V.R., Watanabe, A., Matsuya, K., Asakawa, S., Kimura, M., 2002. Succession of microbiota estimated by phospholipid fatty acid analysis and changes in organic constituents during the composting process of rice straw. *Soil Science and Plant Nutrition* 48, 735–743.
- Carpenter-Boggs, L., Kennedy, A.C., Reganold, J.P., 1998. Use of phospholipid fatty acids and carbon source utilization patterns to track microbial community succession in developing compost. *Applied and Environmental Microbiology* 64, 4062–4064.
- De Bertoldi, M., 1981. Pathogenic fungi associated with land application of sludge. W.H.O., Regional Office for Europe, W.R.C., Stenebager (G.B).
- El Hajjouji, H., Merlina, G., Pinelli, E., Winterton, P., Revel, J.C., Hafidi, M., 2008. <sup>13</sup>C NMR study of the effect of aerobic treatment of olive mill wastewater (OMW) on its lipid-free content. *Journal Hazardous Materials* 154, 927–932.
- Finstein, M.S., Morris, M.L., 1975. Microbiology of municipal solid waste composting. *Advances in Applied Microbiology* 19, 113–151.
- Folch, J., Lees, M., Stanley, S., 1956. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497–509.
- Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22, 59–65.
- Hellman, B., Zelles, L., Palojarvi, A., Bai, Q., 1997. Emission of climate-relevant trace gases and succession of microbial communities during open-windrow composting. *Applied and Environmental Microbiology*, 1011–1018.
- Herrmann, R.F., Shann, J.F., 1997. Microbial community changes during the composting of municipal solid waste. *Microbial Ecology* 33, 78–85.
- Iannotti, D.A., Pang, T., Toth, B.L., 1993. A quantitative respirometric method for monitoring compost stability. *Compost Science and Utilization* 1, 52–65.
- Klamer, M., Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. *FEMS Microbiology Ecology* 27, 19–20.
- Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6,9. *Soil Biology Biochemistry* 36, 57–65.
- Lachhab, L., Herras, M., 2006. Performance épuratoire de la station d'épuration des rejets liquides de Lesieur-Cristal, Projet de fin d'étude. Faculté des Sciences et Techniques- Mohammedia, Université Hassan II, Maroc, p. 100.
- Malosso, E., English, L., Hopkins, D.W., O'Donnell, A.G., 2004. Use of <sup>13</sup>C-labelled plant materials and ergosterol, PLFA and NLFA analyses to investigate organic matter decomposition in Antarctic soil. *Soil Biology Biochemistry* 36, 165–175.
- McCarthy, C.M., Murray, L., 1996. Viability and metabolic features of bacteria indigenous to a contaminated deep aquifer. *Microbial Ecology* 32, 305–321.
- Miller, F.C., 1993. Composting as a process based on the control of ecologically selective factors. In: Blaine, F., Metting, J. (Eds.), *Soil microbial ecology—applications in agricultural and environmental management*. Marcel Dekker, New York, pp. 515–539.
- Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Science Society of America Journal* 65, 359–367.
- Rhee, S.K., Lee, S.G., Hong, S.P., Choi, Y.H., Park, J.H., Kim, C.J., Sung, M.H., 2000. A novel microbial interaction, obligate commensalism between a new Gram-negative thermophile and a thermophilic *Bacillus* strain. *Biomedical Life Science* 4, 131–136.
- Soudi, K., Maftouh, M., 2003. Etude et suivi des traitements physico-chimiques des eaux résiduaires industrielles de Lesieur Cristal, Rapport de stage de fin d'étude de l'Institut Supérieur de Technologie Appliquée de Mohammedia (ISTA-M), p. 42.
- Steger, K., Jarvis, A., Smårs, S., Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. *Journal of Microbiological Methods* 55, 371–382.
- Steger, K., Jarvis, A., Vasara, T., Romantschuk, M., Sundh, I., 2007. Effects of differing temperature management on development of Actinobacteria populations during composting. *Research in Microbiology*, 1–8.
- Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. *Bioscience* 39, 535–541.
- Wang, S., Zhang, S., Peng, Ch, Akio, T., 2008. Intercross real-time control strategy in alternating activated sludge process for short-cut biological nitrogen removal treating domestic wastewater. *Journal of Environmental Sciences* 20, 957–963.
- White, D.C., 1995. Chemical ecology: possible linkage between macro- and microbial ecology. *Oikos* 74, 177–184.
- White, D.C., Pinkart, H.C., Ringelberg, A.B., 1997. Biomass measurements: biochemical approaches. In: Hurst, C.J., Knudson, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (Eds.), *Manual of environmental microbiology*. ASM Press, DC, pp. 91–101.
- Xu, H.S., Roberts, N., Singleton, F.L., Atwell, R.W., Grimes, D.J., Colwell, R.R., 1982. Survival and viability of non-culturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microbial Ecology* 8, 313–323.
- Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biology and Biochemistry* 24, 317–323.
- Zelles, L., Bai, Q.Y., Rackwitz, R., Chadwick, D., Besse, F., 1995. Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils. *Biology and Fertility of Soils* 19, 115–123.