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Wild Tropical Forest Soil Characteristics and Composition of Directly Extractable Soil Lipid Fraction

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Abstract: Soil characteristics of a wild tropical forest from Cote d'Ivoire and composition of directly extractable soil lipid fraction were investigated. This study was undertaken with the aim to establish soil characteristics, structures and origin of directly extractable lipids components as the first step in understanding diagenesis processes in soil of Cote d'Ivoire. Studies required the use of particle size fractionation and spectrometric (GC/MS) methods. Main characteristics of the soil are the following: neutral pH (6.7), absence of carbonates, sandy texture (88% of sand consisting of silica) and low total organic matter content (4% including 644 mg kg⁻¹ of extractable lipid) localized in fine fraction (90%). Directly extractable lipid fraction is composed by aliphatic series of alkanes, normal alcohols, fatty acids and triterpenes. The fatty acids, by their composition and the prominence of even carbon chains, could originate from higher plants and highlight a strong microbial activity. Long normal and odd carbon chains compose exclusively alkanes while long even carbon chains form alcohols. Such a distribution underlines the double origin (fungi and/or microbial) of these compounds. Few triterpenoids, found in directly extractable lipids, are formed exclusively by cholestene and derivatives. Such a structure indicates either the reductive character of this soil, or the triterpenes vegetal origin.

Key words: Soil characteristics, wild tropical forest, lipid composition

INTRODUCTION

Soil Organic Matters (SOM) play a key role in environment and have an effect on the soil properties by increasing the fertility and stability. According to Stevenson (1982) and Jambu *et al.* (1983), lipids play an important role in soil processes because they affect plants and soil microflora and influence soil physical properties. As a result, they are of prime importance for various soil features such as aggregate stability and water retention (Jambu *et al.*, 1978). The directly extractable lipids provide very significant data about the sources and diagenesis processes occurring in soils. They can be useful as biomarkers for the different sources of SOM (Jandl *et al.*, 2002). Moreover, the study of lipids is essential for the comprehension of the relationships between different parts of organic matter.

The GC/MS (Gas Chromatography/Mass Spectrometry), earlier shown to be a very sensitive

indicator of organic matter quality in compost or soils (Paré *et al.*, 1999; Dinel *et al.*, 1996; Dinel and Nolin, 2000), is particularly useful to provide data at the molecular level on the volatile organic matter of the directly extractable SOM. Consequently, we selected these techniques for the analysis of extractable lipids.

In literature studies of structure and characteristics of SOM are concentrated mainly on subsurface soil under agriculture, litter biodegradability in well-known forest and need to be extended to wilder types of soil. Diagenesis processes occurring in European, American and Asian soils are well known because of the important studies on SOM of these continents. Such studies on African and particularly on Ivorian soils have never been undertaken.

This study was undertaken with aim to establish soil characteristics, structures and origin of directly extractable lipids components as first step in understanding diagenesis processes in our country.

MATERIALS AND METHODS

This study was conducted from October 2005 to February 2006. Particle size fractionation was performed in Institut National Polytechnique Félix HOUPOUET-BOIGNY (Côte d'Ivoire). All the other analyses were conducted in Laboratoire Synthèse et Réactivité des substances Naturelles, UMR 6514 of Poitiers (France).

Geography: Oume is a city located in the center of Cote d'Ivoire at approximately 200 km to the North-West of Abidjan, at 6°22' northern altitude and 5°25' of western longitude. Around this city, government programmes protect some quantity of still existing wild forest. The climate of Oume and its region is of transitional tropical type characterized by a long dry season (from November to February) and a rainy season with two maxima in May or June and September or October.

Sampling: Soil samples were taken using a data (local instrument) at the depth ranging from 0 to 30 cm. The 12 samples of 2 kg each, collected on 1 ha area, were mixed, air dried during one week (~30°C) and a sample of 2 kg was formed for analyses.

Physicochemical analyses: Particle size fractionation is usually necessary for soil characteristics determination. For this analysis, soil aggregates were dispersed by ultrasound in a normal solution of sodium chloride using a Fischer Scientific Ultrasonic T420 apparatus during 1 min. The energy of the ultrasound was sufficiently effective for total dispersion and without significant redistribution of the organic matter as described by Schmidt *et al.* (1997). After dispersion, the sand fraction (>50 µm) was separated by wet sifting from 16 g of bulk soil and dried. The silt fraction (50-2 µm) and the clay fraction (<2 µm) were separated by sedimentation through thorough stirring in 100 mL of water and settling for 16 h (settling time calculated by applying Stoke's law for a particle density of 2.65 g cm⁻³). The suspension containing clay was removed and clay recovered by centrifugation (8000 t min⁻¹, 20 min). The process was repeated on the sediment until all clay was extracted.

Thermal analysis: For determination of total organic matter content, a soil sample was analyzed on a Universal TA instruments apparatus SDT Q 600. The oven temperature was raised from 30 to 900°C (5 min isothermally) at a rate of 5°C min⁻¹ in a synthetic air atmosphere (100 mL min⁻¹) and the DTA and TGA curves were recorded simultaneously.

Lipid extraction: A sample of whole soil (113 g) was extracted with a mixture of dichloro-méthane/méthanol (2/1 v/v) using an automatic high-pressure Accelerator Solvent Extractor (ASE 100, Dionex) in a cell from 100 mL. The oven temperature was 110°C, the pressure and the flush volume were respectively 100 bars and 60%. Two extractions of three cycles each were necessary for total lipids extraction.

GC/MS analysis: The extractable organic matter was separated in neutral, acid and polar component on a column treated with potassium hydroxide (KOH/SiO₂), using the process of McCarthy and Duthie (1962). Carboxylic acids were identified as methyl esters derivatives, after methylation of the acid and neutral fractions with TMS-diazomethane (0.2 M in hexane) as described by Hashimoto *et al.* (1981). Alcohols and sterols were transformed into corresponding acetates by acetylating the acid and neutral fractions in an excess of acetic anhydride using a catalytic amount of pyridine. After 15 min at 70°C and 2 h at room temperature, cold water was added and organic compounds were extracted with chloroform. GC/MS analyses were performed on a Hewlett-Packard 6890 gas chromatograph coupled with a ThermoFinnigan Trace GC-Automass.

The Hewlett-Packard 6890 gas chromatograph was equipped with a split/splitless injector, a FID detector and a 30 m×250 µm×0.25 µm fused silica capillary column containing 5% of methyl phenyl siloxane phase (BPX-5). The oven temperature was raised from 60 to 300°C (15 min isothermally) at a rate of 5°C min⁻¹.

The ThermoFinnigan Trace GC-Automass was equipped with a BPX5-MS column (30 m length, 0.25 mm i.d., 0.25 µm phase thickness). The heating rate was set to 5°C min⁻¹ from 60°C (1 min) to 300 °C (15 min isothermally).

The compounds were identified basing on their retention time, mass spectra and by comparison with literature data (Schnitzer and Schulten, 1995; Schnitzer and Schulten, 1989; Diné *et al.*, 1996).

The pH value was determined using a digital electronic pH-meter in a soil solution.

RESULTS AND DISCUSSION

Soil characteristics: According to particle size fractionation, sand, silt and clays form the soil of Oume in 88, 10 and 2%, respectively. Such particle size proportions highlight the sandy character of this soil and suppose a high degree of aeration and water infiltration.

However, soil did not contain carbonates and its pH value was neutral (6.70). Thermal analyses revealed 4% of total organic matter from whole soil. This total organic matter was investigated in relationship with particle size fractions. The most quantity of total organic matter was located in silt (58%) and clay (32%) and sand (10%) was lower than that generally observed in European forests soils (Amblès *et al.*, 1993, 1994).

These results are in conformity with those reported by Guggenberger *et al.* (1994) where organic matter enrichment in finest particle size fraction was commonly observed for sandy soil.

This content of total organic matter could reflect an especially high depletion in organic matter for Oume's soil caused by different reasons. In literature, precedent works reported by Dymess and Youngberg (1957); Hart (1961) showed that slash burning reduces the thickness surface humus layer and therefore soil organic matter. In Cote d'Ivoire the forest burning is usually undertaken for hunting. Accordingly we can notice that the low content of organic matter could be caused by this phenomenon. However, the neutral pH value and wet tropical climatic conditions characterized by a temperature varying between 27-35°C could support an intense biological activity.

Composition of the directly extractable lipids: The amount of directly extractable lipids was 644 mg kg⁻¹. After fractionation according to McCarthy and Duthie method, the neutral, acid and polar fractions represented, respectively 12, 40 and 48% of the total lipids fraction. The GC/MS analysis highlighted the presence of several compounds (Fig. 1) such as fatty acids, hydrocarbons, alcohols and triterpenoids. The distribution of the fatty acids was determined by the selective detection of their specific ion (m/z 74) and analyzes of it corresponding chromatograms. The FAME ranging from C₁₅ to C₃₄ showed a bimodal distribution with a maximum at C₂₄ and a submaximum at C₁₆. For both of them even carbon chains predominance was noted.

Compounds, with normal and ramified chains containing *iso* and *anteiso* isomers, characterize short distribution ranging from C₁₅ to C₁₈. Most abundant of them were C_{16:0}, C_{18:1} and C_{18:0} with contents of 9, 6 and 5%, respectively. The Palmitic acid (C_{16:0}) is ubiquitous in the living organisms including higher plants and bacteria (Padley *et al.*, 1992). Thus, its abundance in the lipid extracts does not bring any specific information. However, the ramified fatty acids (correspond chiefly to *iso* and *anteiso* compounds) are indicative of a bacterial contribution to the soil organic matter (Van Bergen *et al.*, 1997; Zelles, 1999).

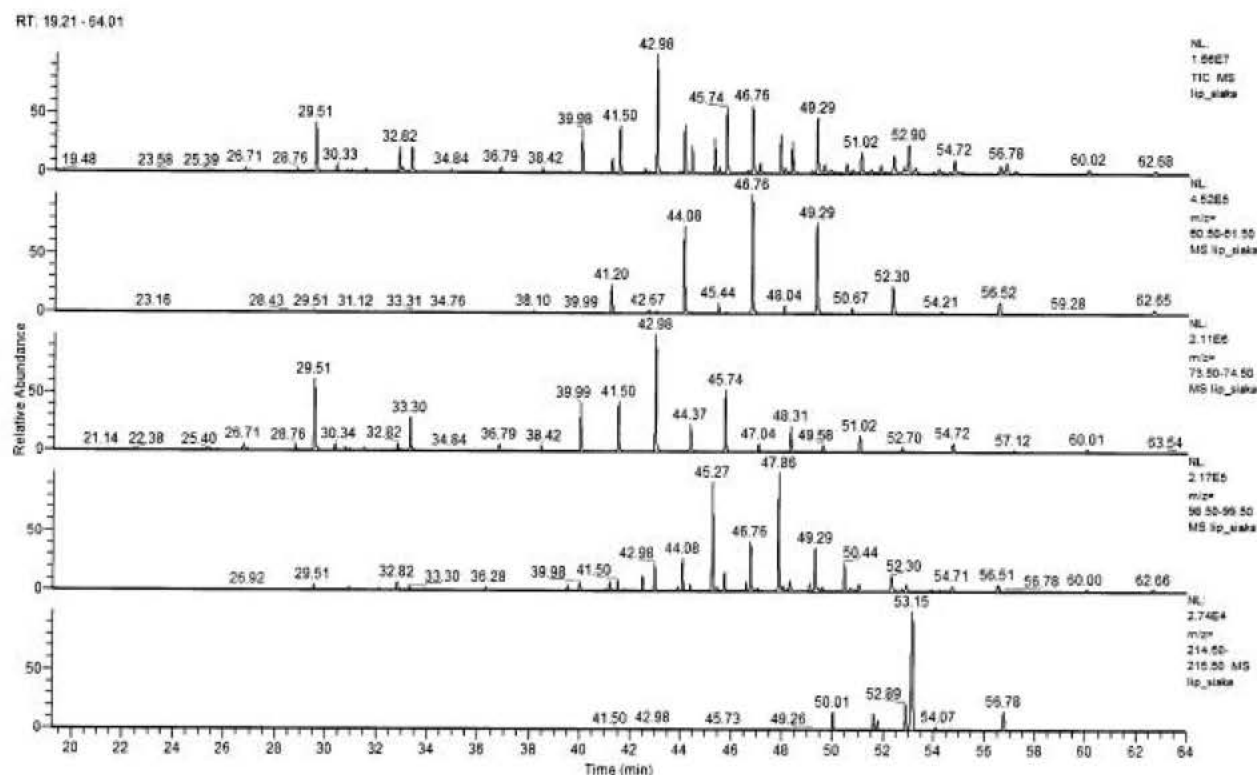


Fig. 1: Distribution of lipids compounds as revealed by chromatogram

Table 1: Triterpenes found in directly extractable soil lipid fraction

RT (min.)	MW	Formula	Name
50.01	370.0	C ₂₇ H ₄₆	Cholestene
51.79	394.0	C ₂₉ H ₄₆	Sigmasta-3,5,22-triene
52.90	396.0	C ₂₉ H ₄₈	Stigmasta-3,5-diene
53.15	398.0	C ₂₉ H ₅₀	Stigmastene
54.07	408.0	C ₃₀ H ₄₈	Ursa-2,12(13)-diene
56.78	410.0	C ₃₀ H ₅₀	Squalene

Table 2: Alkan-1-ols distribution in directly extractable lipid fraction

Acetate alcohol	Relative abundance (%)
C22	6.0
C24	19.8
C26	26.6
C28	25.6
C30	12.1
C32	6.1
C33	0.6
C34	3.1

Long distribution, in the range of C₂₀-C₃₄, presents compounds characterized by long, normal and saturated carbon chains. The most abundant components were C_{22:0}, C_{23:0}, C_{24:0}, C_{25:0}, C_{26:0}, C_{28:0} and C_{30:0} in content of 8, 8, 21, 5, 12, 6 and of 5%, respectively (Table 1).

According to the results reported by Kolattukudy *et al.* (1976); sKolattukudy (1980) these ester-bound acid moieties probably originate from higher plant components like wax esters and suberin derived components. In agreement with these results, the bimodal distribution FAME underlines a vegetal origin of a part of the fatty acids and a high microbial activity.

The alkan-1-ols, in the range of C₂₂-C₃₄, characterized by even chains carbon predominance (Table 2) were identified as their acetates derivatives.

The C₂₄, C₂₆, C₂₈ and C₃₀ compounds in contents of 20, 27, 26 and of 18% respectively were most abundant. According to (Amblès *et al.*, 1985 and Jambu *et al.*, 1983) normal alkan-1-ols are commonly observed in the lipids extracted from whole soils. However, normal alcohols with long carbon chains (above C₂₀) with even carbon numbered are typical components of the epicuticular waxes of higher plants with maxima generally found at C₂₂ and/or C₂₄ C₂₆ Kolattukudy (1980). Accordingly these alkanols probably originate from wax esters and suberin derived products.

The alkane's distribution, as reflected by selective detection of their specific ion m/z 57 in chromatogram, is illustrated by Fig. 1. This series of n-alkanes, ranging from C₂₂ to C₃₃ is characterized by an odd carbon distribution (Table 3) whose principal component is C₃₁ (41%).

The hydrocarbons with long carbon chain and odd distribution are the typical components of epicuticular waxes of higher plants (Tulloch, 1976; Van Bergen *et al.*, 1997). Accordingly they are

Table 3: Hydrocarbon distribution in directly extractable lipid fraction

Hydrocarbon	Relative abundance (%)
C22	5
C25	2
C27	5
C29	35
C31	41
C33	12

Table 4: FAME distribution in directly extractable lipid fraction

Fame	Relative abundance (%)
<i>i,a</i> C15	0.8
C15	0.4
<i>r</i> C16	0.6
C16	8.9
<i>i,a</i> C17	2.0
<i>r</i> C18:0	0.7
C18:1	5.6
C18:0	4.7
C20:0	1.0
C21:0	0.8
C22:0	7.8
C23:0	7.9
C24:0	20.6
C25:0	5.1
C26:0	11.8
C27:0	2.1
C28:0	5.7
C29:0	1.8
C30:0	5.1
C32:0	4.3
C33:0	0.7
C34:0	1.6

classically used as biomarkers for higher plant input (Wiesenberg *et al.*, 2004). Recent studies showed that fungi could produce long n-alkanes chains with odd carbons type C₂₃-C₃₃ (Bull *et al.*, 2000; Naafs *et al.*, 2004). Consequently the series of detected hydrocarbons could originate from the fungi and higher plants present in Oume's forest. Thus, the detection of only saturated n-alkanes could also highlight a reductive character of Oume's soil. The silica type of sand that composes soil could favourite it reductive character.

Few triterpenes derivatives were identified in the lipids fraction. The most abundant (Table 4) were the Cholestene, the Sigmasta-3,5,22-triene, the Stigmasta-3,5-diene, the Stigmastene, the Ursa-2,12(13)-diene and the squalene.

Except squalene, the triterpenes are characterized by the presence of an alkyl substituants at C₂₄ position of lateral carbon chain. They can also be regarded as reductive form of corresponding sterols. However, according to the studies undertaken by Patterson (1994) and Volkman (2005) the triterpenes from vegetal and algal origin are characterized by the presence of an alkyl substituants (methyl or ethyl) in C₂₄ position (lateral carbon chain). Consequently, the vegetative origin of these triterpenoid is more plausible.

CONCLUSIONS

Oume's soil is characterized by a sandy texture (80% of silicate) carbonates free and a neutral pH (6.7). The amount total organic matter (4%) is localized mainly in the fine fractions (silt and clays) of soil as usually reported in literature. These characteristics confer aird and permeable properties of the soil. The low content of total organic matter may be caused by the conjugation of three essential factors: the sandy nature of soil, forest burning practice occurring in region and high microbial activity due to tropical climate. A strong turnover aspect is not excluded.

The directly extractable lipids (644 mg kg⁻¹) are formed by neutral (12%), acid (40%) and polar (48%) components. The GC/MS analysis revealed the presence of homologous series of hydrocarbons, fatty acids, alkan-1-ols and triterpenoids. Bimodal distribution (short and long) characterized fatty acids. The predominance of long even carbon chains indicated the vegetative origin of these fatty acids while their *iso* and *anteiso* isomers highlighted high microbial activity. The structure of hydrocarbons and alkanols found in lipid fraction corroborated vegetal origin of the main component.

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