

PAPER • OPEN ACCESS

Lipid biomarkers in paleoreconstruction of lake sedimentogenesis

To cite this article: E Ts Pintaeva 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **320** 012016

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

Lipid biomarkers in paleoreconstruction of lake sedimentogenesis

E Ts Pintaeva¹

¹ Baikal Institute of Nature Management SB RAS, Ulan-Ude, 670047, Russia

E-mail: e-pintaeva@yandex.ru

Abstract. Lakes are important paleoenvironmental archives retaining abundant information due to their typical high sedimentation rates and susceptibility to environmental changes. Lipid biomarkers distributions in surface sediments were determined using gas chromatography (GC) and GC-mass-spectrometry in order to investigate the lipid biomarkers signature in sediments of four lakes of the Baikal region. They are linked to autochthonous and/or allochthonous sources, which can lead to distinct lipid molecular distributions. Lipid biomarkers of terrestrial origin (i.e. high-molecular weight n-alkanoic acids, n-alkanols, dicarboxylic acids, β -sitosterol) and aquatic source (i.e. short chain fatty acids, hydroxy fatty acids, etc.) were detected in all lakes in different ratios. The results of this study show the compatibility of different lipid biomarkers in assessing the origin of organic matter. Therefore, lipid molecular proxies in combination with molecular distribution of n-alkanes, dD or D/H ratios, palynological studies, etc. can be applied as paleoclimate and paleoenvironment proxies.

1. Introduction

Lakes are important paleoenvironmental archives of short-term processes at local to regional scale. They are systems susceptible to environmental changes (e.g. climate change, land uses, glacier retreatment, volcanic activity, biota, or human impact) that affect organic matter (OM) delivery and burial, thus imprinting the sedimentary record. Lakes receive abundant land-derived material that enhances lake fertility and aquatic production of OM, both producing high sedimentation rates that allow for retaining information from the water basement and catchment area. Lacustrine sediments constitute an integrative record of all local biogeochemical and geophysical processes [1] with a variety of indicators or proxies that can be used to reconstruct past climate and environmental conditions.

The recent climate changes dictate the need to study the changes of the Holocene paleoclimate as a relatively recent past. Despite the increasing of number and level of paleoclimatic studies, there is still a lack of reliable data on changes in the intercontinental climate. Small lakes, unlike large lakes, are less stable systems and more sensitive to climate fluctuations [2]. A large number of works are devoted to paleoreconstructions on bottom sediments of lakes of North-West Russia [3], southern Siberia [2,4-9]. Studies of small lakes to conduct paleoclimatic reconstructions are few and based mainly on data from palynological, diatom analyses, the distribution of macro- and microelements and mineral associations, whereas the use of lipid biomarkers is almost not covered.

During recent years, our knowledge about the late glacial and Holocene environmental evolution of the Lake Baikal region has significantly increased. Most of the results refer to lacustrine sediments.



Content from this work may be used under the terms of the [Creative Commons Attribution 3.0 licence](https://creativecommons.org/licenses/by/3.0/). Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

Most of the results refer to lacustrine sediments. Morley et al. [10] for instance, studying the diatoms in Lake Baikal sediments. Besides diatoms, pollen data also illustrate pronounced late glacial and Holocene environmental fluctuations in the Lake Baikal area [11-12].

Features of the structure and biosynthesis of fatty acids and lipids served as the basis for their wide application as biomarkers for the assessment of the origin and transformation of organic matter [13-14]. Furthermore, lipid biomarkers have been widely used to reconstruct paleoenvironments and changes in lacustrine systems [15-18]. In this study sediments of small lakes surrounding Lake Baikal have been used to trace biogeochemistry of organic matter.

2. Models and Methods

Four lakes located on the eastern coast of Lake Baikal are investigated (Figure 1). The lakes are located at a distance of no more than 3.5 km from the shore of Baikal and, nevertheless, have features in the macro- and microcomponent composition. Bormashevoe lake is mineral; total mineralization reaches 1,155 mg/l, it belongs to the sodium bicarbonate type. The bicarbonate content reaches 700 mg/l, and the sodium content - 292 mg/l. The lake is located on the isthmus of the Svyatoi Nos Peninsula, in 1.5 km from the coast of the Barguzinsky Bay and has a mineralization that is an order of magnitude greater than that of Lake Baikal. In the other three lakes (Dukhovoye, Dikoye, Kotokel) water belongs to the hydrocarbonate magnesium-calcium-sodium type - sodium prevails in the cationic composition, its relative content exceeds 40%, absolute sodium contents are low (9.8-22.5 mg/l), but with a low total mineralization, which is only 83-139 mg/l, dominance of this cation is observed. This fact is interesting because the water of region' rivers has a hydrocarbonate sodium-calcium composition, and the relative sodium content does not exceed 25%. It is known that the metamorphization of the chemical composition of water in the soda direction occurs under the influence of a stream of carbon dioxide, resulting in precipitation of calcium and accumulation of sodium in the solution. In addition, we know that nitrogen and methane thermal waters are also characterized by dominance of sodium in the cationic composition. It is likely that carbon dioxide or methane enters the lakes through faults, or hydrothermal discharge occurs. Lake Kotokel is of considerable size, biotic processes are intensively developed in its water area, a powerful horizon of sapropel deposits is formed, therefore the impact of faults is not clearly reflected in the trace element composition. In this lake, we did not find any anomalies in the microelement composition.

Lipids from sediments were extracted by the one-step extraction/methylation method [19]. An aliquot of sediment samples (~100 mg) were transferred to glass vials and 0.4 ml of methanol containing 1 M HCl was added. Vials were securely closed with Teflon-lined screw caps and placed in an oven for 1 h at 80°C for methanolysis. At this stage, fatty acids and aldehydes of the complex lipids of microorganisms and other cells of the sample are released in the form of methyl esters and dimethylacetals. These components are extracted with hexane (400 µl) for 5 min.

0.3 µg of deuterated methyl ester of 13:0 acid in hexane was added to the extract as an inner standard. Then the hexane was evaporated and the dry residue was treated with 20 µl of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) for 10 min at 80°C forming trimethylsilyl ethers of hydroxy acids and sterols. 80 µl of hexane was added to the reaction mixture and 1-2 µl of a solution was injected into the GC-MS system (Agilent 6890 gas chromatograph with MSD 5973N quadrupole mass-spectrometer). The GC-MS system was operated in the electron ionization mode (70 eV). A non-polar HP-5ms column (5% phenyl 95% dimethylpolysiloxane, 30m*0.25 mm*0.2µm) was used with helium as carrier gas.

For the identification of the component composition the total ion Scan mode was performed. The temperature of column was kept at 125°C for 0.5 min after injection and thereafter increased to 320°C at rate of 7°C/min. This temperature was maintained for 0.5 min.



Figure 1. Sampling map of bottom sediments of Dikoye, Kotokel, Dukhovoye and Bormashevoye lakes.

GC/MS quantification was performed using the external standard solution (GLC-68D from Nu-Chec-Prep; Elysian, Minnesota, USA), internal standard (deuterated methanol, m/z 90), their mass spectra (by considering the peak areas of key ions) and their relative retention time.

3. Results and Discussion

3.1. Total lipid classes

In total, 108 compounds were identified in lipid fraction of sediments. The major components of lipid fraction (see Table 1) were saturated fatty acids (SFA, 46.30% - 58.95% of total lipids, TL), followed by monounsaturated normal and branched fatty acids (MUFA, 12.30% - 29.41%), fatty alcohols (5.44% - 20.04%) and hydroxy fatty acids (0.20% - 11.70%). Also, aldehydes, dicarboxylic acids (DCA), cyclic acids (CycFA), sterols and alicyclic compounds are found in surface sediments.

3.2. Saturated fatty acids

Straight-chain fatty acids are often the most abundant lipids found in lacustrine sediments. The highest proportions of saturated fatty acids occur in Kotokel and Bormashevoye lakes sediments accounting for 58.95 and 56.13 % of total lipids, respectively. The distribution of these acids is bimodal, the first peak – hexadecanoic acid, the second - in the area of the long-chain FA. Within the short-chain fatty acids (SCFA), C16 (20.95 – 35.60 % of TLC) and C18 (4.57 – 11.08 %) (a biological marker of phytoplankton [13]) were most abundant, whereas C24 (up to 3.29 %), C22 (up to 2.66%) and C20 (up to 2.28%) had the high contributions among the long-chain fatty acids (LCFA), showing a strong even over odd predominance. Long-chain fatty acids (carbon number more than 20) in marine sediments are typically associated with terrestrial inputs of organic matter from higher plants [21]. Contribution of vascular plants to bottom sediments was the highest in Dukhovoye lake according to the higher level of terrestrial markers – LCFA.

The ratio Σ SCFAs / Σ LCFAs was applied to identify spatial and temporal variations in the contribution of marine vs. terrestrial sources for the saturated fatty acids. Predominance SCFA over LCFA was observed for all sediments (Table 1) and demonstrates a greater contribution of marine than

terrestrial OM to surface sediments, at that the highest ratio Σ SCFAs / Σ LCFAs is observed for Kotokel sediments, and the lowest value - Dukhovoye.

Further information can be gained from the carbon preference index (CPI), the ratio of even-over-odd numbered carboxylic compounds. CPI is used to reconstruct paleoclimate and paleoenvironment, including temperature, humidity [22] and dominant sources of related lipids [21, 23].

The reconstruction of paleoclimatic archives via CPI derives from the variation of diagenesis and degradation rates with climatic conditions. Under dry and cold climates, microbial diagenesis and degradation of organic material are reduced, corresponding to a high CPI value, while the accelerated microbial diagenesis and degradation of organic matter result in a low CPI value under a wet and warm climate [24, 25]. For instance, Zheng et al. [22] proposed a lower CPI value may result from the accelerated microbial degradation and changes of organic material linked to a warm and humid climate in the case of no geothermal heating.

Table 1. Relative amounts (as percentage of TL) of the lipid compounds and specific biomarkers/ratios in surface sediments of lakes.

Lipid compounds / biomarkers	Dukhovoye	Kotokel	Bormashevoye	Dikoye
Saturated (SFA)	44.95	58.95	56.13	44.30
Branched SFA	7.73	3.49	6.18	5.75
Σ SCFA ^a	31.84	56.84	48.15	38.19
Σ LCFA ^b	12.75	2.11	7.98	6.11
Σ SCFA / Σ LCFA	2.50	26.99	6.03	6.25
CPI _H ^c	12.77	0.11	2.90	1.98
Unsaturated	13.42	31.77	26.45	31.92
MUFA	12.30	29.02	25.18	29.41
16:1	7.01	11.45	12.21	9.17
18:1	4.98	16.75	11.66	12.69
PUFA	1.11	2.75	1.27	2.50
Aldehydes	1.30	0.27	0.00	0.49
Fatty alcohols	20.04	5.44	10.74	17.18
Hydroxy-FA	11.70	0.20	2.26	3.12
Cyc FA	0.45	0.00	0.00	0.00
Sterols	1.59	0.78	0.00	0.24
alicyclic	0.24	0.31	0.35	0.10
DCA	3.78	0.58	1.49	0.83

^a Short chain (SCFA < C20);

^b long chain (LCFA ≥ C20);

^c CPI_H - the carbon preference index for even- over odd-numbered high-molecular weight SFAs (CPI_H = (Σ C22–C30 + Σ C24–C32) even / (2 × Σ C23–C31) odd [20]).

It is proved that CPI reflects dominant sources of pertinent lipids, and thereby growth temperature and humidity. To begin with, Street et al. [26] suggested a strong odd-over-even predominance, which is recorded as a high CPI, amongst the more abundant chain length (nC21 to nC35) is an indicator of vascular plant dominated sources over the sediment sequence, and vice versa. The application of CPI as climate proxies may be perturbed by alien factors. Pollution is often considered to result in low CPI values of alkanes, e.g. vehicle exhaust [27], fuel or wood burning [28], or even marine sources [29].

Matsudo and Koyama [20] introduced a modification of the CPI for the application on FAs of high molecular weight (CPI_H, range: C22–C32) using the equation CPI_H = (Σ C22–C30 + Σ C24–C32)/2 × Σ C23–C31. Accordingly, the proportion of odd-numbered long-chain SFA (n-C23 to n-C31) is slightly increased in all lakes, except Dukhovoye, resulting in the lowest

observed CPI_H values of 0.11 – 2.90 (Table 1). The highest CPI_H values (12.77) observed in Dukhovoye lake is a result of high relative amounts of C22 and C24 FAs.

Terminally branched (iso-, anteiso-) FAs were detected in relative high amounts in the surface samples of Dukhovoye, Bormashevoye and Dikoye lakes. They are known to be abundant across multitude of bacteria, but less significant in algae, fungi as well as in terrestrial plants [30-32].

3.3. Unsaturated fatty acids

In highest relative amounts are found C16:1 and C18:1 MUFAs. These compounds are abundant in phytoplankton, since diatoms are enriched in C16:1, mainly C16:1d9, while other microalgae (e.g. dinoflagellates and prymnesiophytes) are enriched in C18:1 [33]. Thus, the greatest relative contribution from diatoms to the OM are in the sediments from Kotokel and Bormashevoye, as the highest relative amount of C16:1d9. In all sediments samples (except Dukhovoye lake), cis-vaccenic acid 18:1d11c was accompanied by 18:1d11t. This finding provides compelling evidence that bacterial cells can modify their membrane fluidity in response to stress [34]. In lakes Kotokel and Bormashevoye contribution of copepods to bottom sediments was significantly higher, than that in two other lakes, according to the levels of FA marker of copepods, 20:1d11 and 22:1d13 [35].

PUFAs are very low in relative amounts and the algal-derived PUFAs (20:5n-3 and 22:6n-3) are absent [36-38]. Low levels of PUFAs indicating that most of these labile fatty acids were effectively recycled during the whole settling and depositing process in lakes.

3.4. Hydroxy acids and fatty alcohols

Hydroxylated fatty acids have been found in all sediments, aliphatic long chain (C22-C27) α -monohydroxy fatty acids (2-hydroxy monocarboxylic acids) are more abundant. They are occurring in a wide range of organisms (i.e. in plants, animals and bacteria) and typically produced as intermediates in the α -oxidation of monocarboxylic fatty acids. In yeasts, α -hydroxy fatty acids are intermediates in fatty acid biosynthesis [39]. Hydroxy FA are of special significance in the determination of bacteria, because they are exclusively found in gram-negative bacterial biomass, as a part of the lipopolysaccharides in bacterial cells, and their amount is relatively constant. Thus, their detection in sediments is indicative of viable bacteria.

Long-chain hydroxy fatty acids are rarely reported in microalgae, although C22 to C26 saturated and monounsaturated α -hydroxy fatty acids have also been found as major lipid components of the cell wall of three marine chlorophytes and a series of saturated α - and β -hydroxy acids ranging from C24 to C30 with C28 predominating was detected in some freshwater eustigmatophytes [39].

Among β -hydroxy fatty acids, that commonly found in Gram-negative bacteria as amide-bound constituents of the lipid A component of the cell wall polysaccharide only hydroxy-C16 and hydroxy-C17 fatty acids in Dikoye lake sediments were found.

ω -hydroxy fatty acids are detected in all lake sediments, except Kotokel lake. C16 and C18 ω -hydroxy fatty acids are common constituents in plant cutin, while C16 – C22 ω -hydroxy fatty acids are found in suberin. The largest amount of hydroxy-FA were noted in the sediments of lake Dukhovoye (11.70 %), whereas in other lakes their quantity varied from 0.20 to 3.12 %. There is an assumption that hydroxy-FAs accumulated in significant amounts in stressful situations. This is more or less consistent with the CPI index, which shows a low degradation degree of OM in the Dukhovoye lake sediments.

At all sites, the fatty alcohols with even number of carbon atom range from C 22 to C26 are identified with the predominance in Dukhovoye (20.04%) and Dikoe (17.03%) lakes, suggesting that the alcohol fraction is of terrestrial origin. Volkman et al. [39] found the C22-alcohol to dominate in eustigmatophytes, phototrophic marine and freshwater microalgae, while Jaffe et al. [40] observed large amounts of this compound in epiphytes.

3.5. Aldehydes and Dicarboxylic acids

In the studied sediments were found simple linear, branched and mono-unsaturated aldehydes with a chain length of 14 to 18 carbon atoms. Aldehyde content is low and ranged from 0.27 % - 1.30 %. In natural waters, carbonyl compounds, which include aldehydes, may appear from algae, as a result of biochemical and photochemical oxidation of alcohols and organic acids, decomposition of organic substances such as lignin. The source of these compounds is also may be terrestrial plants, which form the series of aliphatic aldehydes and furan derivatives. Much of the aldehydes and ketones enter into natural waters as a result of human activities [41].

Only short chain C4, C9, C16 and C18 and long-chain C20-24 α , ω -dicarboxylic fatty acids are identified in all lake sediments, except Kotokel. Their largest amount is observed only in sediments of Dukhovoye lake. Shorter-chain dicarboxylic fatty acids such as azelaic acid (C9) occur as natural constituents of some plants and as degradation products from oxidative scission of the double bonds in unsaturated fatty acids. Azelaic and other short-chain dicarboxylic acids have been found in marine sediments and in aerosols [39]. C22-24 even-chain dicarboxylic fatty acids are often abundant in the plant biopolymer suberin [42]. Seagrasses also contain α , ω -dicarboxylic fatty acids, so most sediments containing higher plant organic matter can be expected to contain these long-chain dicarboxylic acids [39].

3.6. Sterols

Sterol composition of the lake sediments sampled appeared to be dominated by β -sitosterol (ranged from 0.65% to 2.48% of total lipids). Its largest amounts were founded in the Dukhovoye lake sediments. Cholesterol is very common in living organisms and predominate in Kotokel lake. In most cases, it is usually the major sterol encountered in environments with high productivity and, thus, high organic matter supply [43]. Cholesterol may derive from zooplankton such as ostracods or zoobenthos such as gastropods [44], however, it is also found in dinoflagellates, diatoms and some species of Prymnesiophyceae algae contain cholesterol as the major sterol [45].

3.7. Other compounds

Cyclopropane fatty acid with 17 carbon atoms was identified, which is quite common in both gram-positive and gram-negative organisms.

It should be noted the presence in all samples of dehydroabietic acid. It is a biomarker of coniferous residues in sediments and biomass burning.

4. Conclusion

This study illustrates the potential of lipid biomarkers as paleoclimate and paleoenvironment proxies. They are linked to autochthonous and/or allochthonous sources, which can lead to distinct lipid molecular distributions. The results of this study show the compatibility of different lipid biomarkers in assessing the origin of organic matter. Various fatty acid indices in combination with molecular distribution of n-alkanes, dD or D/H ratios, palynological studies, etc. can be applied to reconstruct the past precipitation, temperature and humidity.

Acknowledgments

The study was carried out within the framework of the state task of BINM SB RAS and partial financial support from RFBR, research project № 17-29-05085.

References

- [1] Cohen A S 2003 *Paleolimnology: The History and Evolution of Lake Systems* (Oxford University Press, New York) p 500
- [2] Danilenko I V, Solotchin P A and Solotchina E P 2018 *Izvestiya Rossiiskoi Akademii Nauk. Seriya Geograficheskaya* **4** 107-12
- [3] Subetto D A 2009 *Lake bottom sediments: paleolimnological studies* (HSPU Publishing House, St.Petersburg) 339 p

- [4] Kalugin I, Darin A, Rogozin D and Tretyakov G 2013 *Quaternary International* **290-1** 245-52
- [5] Vershinin K E and Rogozin D Y 2014 *Doklady biological sciences* **457 (1)** 248-51
- [6] Solotchina E P 2009 *Minerals; structure, properties, methods* **1** 58-60
- [7] Ptitsyn A B, Reshetova S A and Babich V V 2010 *Geogr. Nat. Res.* **2** 85-9
- [8] Bazarova V B, Grebennikova T A, Mokhova L M and Orlova L A 2011 *Russian Geology and Geophysics* **52 (3)** 333 - 42
- [9] Bezrukova E V, Abzaeva A A, Letunova P P, Kostrova S S, Tarasov P and Kulagina N V 2011 *Russian Geology and Geophysics* **52 (4)** 458-65
- [10] Morley D W, Leng M J, Mackay A W and Sloane HJ 2005 *Global and Planetary Change* **46** 221-33
- [11] Demske D, Heumann G, Granoszewski W, Nita M, Mamakowa K, Tarasov P E and Oberhansli H 2005 *Global and Planetary Change* **46** 255-79
- [12] Bezrukova E V, Tarasov P E, Solovieva N, Krivonogov S K and Riedel F 2010 *Palaeogeography, Palaeoclimatology, Palaeoecology* **296** 185-98
- [13] Hu J, Zhang H, Peng P 2006 *Estuar. Coast. Shelf Sci.* **66** 346-56
- [14] Holtvoeth J, Vogel H, Wagner B and Wolff GA 2010 *Biogeosciences* **7** 3473-89
- [15] Ishiwatari R, Yamamoto S, Uemura H 2005 *Org. geochem.* **36** 327-47
- [16] Arts M T, Brett M T and Kainz M J 2009 *Lipids in Aquatic Ecosystems* (New York: Springer Dordrecht Press) p 380
- [17] Ouyang X, Guo F and Bu H 2015 *Quat. Sci. Rev.* **123** 180–92
- [18] Carrizo D et al. 2019 *Sci. Total Environ* **672** 657-68
- [19] Osipov G A 1993 *Patent of Russia* 2086642, C12N 1/00, 1/20, C12Q 1/04 (Published 10 Aug. 1997, Bulletin No.22)
- [20] Matsudo H, Koyama T 1977 *Geochim. Cosmochim. Acta*, **41** 1825-34
- [21] Meyers P A 1997 *Organic Geochem.* **27** 213–50
- [22] Zheng Y, Zhou W, Meyers P A and Xie S 2007 *Org. Geochem.* **38** 1927-40
- [23] Horikawa K, Murayama M, Minagawa M, Kato Y and Sagawa T 2010 *Quat. Res.* **73** 573-82
- [24] Xie S, Nott C J, Avsejs L A, Maddy D, Chambers F and Evershed R P 2004 *Geochim. Cosmochim. Acta* **68** 2849-62
- [25] Zhou W, Xie S, Meyers P A and Zheng Y 2005 *Org. Geochem.* **36** 1272-84
- [26] Street J H, Anderson R S, Rosenbauer R J and Paytan A. 2013 *Quat. Res.* **79** 14-23
- [27] Simoneit B R T 1984 *Atmos. Environ.* **18** 51-67
- [28] Standley L J, Simoneit B R T 1987 *Environ. Sci. Technol.* **21** 163-9
- [29] Lichtfouse E, Derenne S, Mariotti A and Largeau C 1994 *Org. Geochem.* **22** 1023-7
- [30] Kaneda T 1991 *Microbiological Reviews* **55** 288–302
- [31] Gong C, Hollander D J 1997 *Organic Geochemistry* **26** 545–63
- [32] Dalsgaard J, John M St, Kattner G. et al. 2003 *Adv. Mar. Biol.* **46** 225–340
- [33] Volkman J K, Jeffery S W, Nichols P D et al. 1989 *J. Exp. Mar. Biol. Ecol.* **128** 219–40
- [34] Keweloh H and Heipieper H J 1996 *Lipids* **31** 129-37
- [35] Brett M T, Muller-Navarra D C and Persson J 2009 *Lipids in aquatic ecosystems* (New York: Springer Press) p 115-46
- [36] Zhukova N V and Aizdaicher N A 1995 *Phytochemistry* **39** 351–6
- [37] Zimmerman A R and Canuel E A 2001 *Est. Coast. Shelf Sci.* **53** 319–41
- [38] Volkman J K, Barrett S M, Blackburn S I et al. 1998 *Organic Geochemistry* **29** 1163–79
- [39] Volkman J K 2006 *The Handbook of environmental chemistry. Vol. 2: Reaction and processes.* (Berlin: Springer Press) pp 27-70
- [40] Jaffe R, Mead R, Hernandez M E et al. 2001 *Organic geochemistry* **32** 507-26
- [41] Vakulenko V F, Milyukin M V and Goncharuk V V 2006 *J. Appl. Chem.* **79 (3)** 397-403
- [42] Kolattukudy P E 1980 *The biochemistry of plants* (New York: Academic Press) pp 571-645

- [43] Gagosian R B, Volkman J K, Nigrelli G E 1983 *The use of sediment traps to determine sterol sources in coastal sediments of Peru. In: Bjoro M, Albrecht P, Cornford C, Groot K D, Eglinton G, Galimov E, Leythasuser D, Pelet R, Rulkoter J and Speers G (Ed.), Advances in Organic Geochemistry* (Chichester: Wiley) pp 369-79
- [44] Thiel V, Merz-Preiss M, Reitner J and Michaelis W 1997 *Biomarker studies on microbial carbonates: extractable lipids of a calcifying cyanobacterial mat* (Everglades: Springer-Verlag) **36** 163–72
- [45] Volkman J K 1986 *Org. Geochem.* **9** (2) 83-99