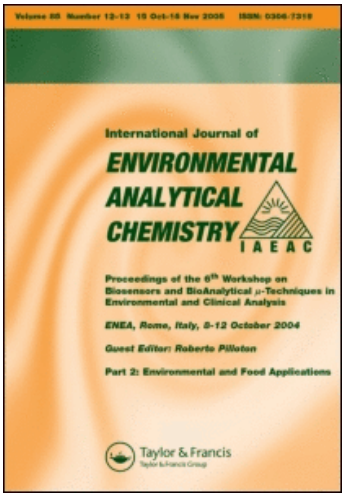

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Water-Soluble Lipids in *Carex* and *Sphagnum* Peats

Keijo Lehtonen[a]; Martti Ketola[b]; Kalevi Pihlaja[b]
[a] Department of Chemistry, and Department of Chemistry in Biomedicine, University of Turku, Turku, Finland [b] Department of Chemistry, University of Turku, Turku, Finland

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WATER-SOLUBLE LIPIDS IN *CAREX* AND *SPHAGNUM* PEATS

KEIJO LEHTONEN

Department of Chemistry, and Department of Chemistry in Biomedicine, University of Turku, SF-20500 Turku, Finland

MARTTI KETOLA and KALEVI PIHLAJA

Department of Chemistry, University of Turku, SF-20500 Turku, Finland

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Samples of *Carex* and *Sphagnum* peats of different degrees of humification were extracted with water at 20–25 °C for 20 h. Water-soluble lipids were isolated by dichloromethane extraction and analyzed by gas chromatography and mass spectrometry. The amount of lipidic matter (0.021–0.083% of dry peat, 0.54–2.70 mg/100 g of water, 4–5% of total dissolved organic matter) depended on the peat type and the degree of humification of the peat material. In *Sphagnum* peat the amount of total water-soluble lipids increased with increasing peat humification, while the reverse occurred in *Carex* peat. The concentration of dissolved organic carbon (DOC) compounds was found to be directly proportional with the concentration of the water-soluble lipids.

KEY WORDS: Peat lipids, water extracts, *Carex*, *Sphagnum*, DOC.

INTRODUCTION

Processed peat products for various purposes (energy production, chemical technology, agriculture, horticulture, etc.) have an increasing market in Finland. Draining of peatlands for productive use loads the surrounding bodies of water with dissolved material, causing eutrophication and problems in waterworks. Humic substances, especially aquatic fulvic acids, are the major organic constituents of the dissolved matter and they have been found to be mainly responsible for the environmental damage caused by peatland drainage.[1,2] The reaction of the disinfectant chlorine with humic compounds is probably the most significant factor in producing mutagenic activity in tap water.[3–5] The organic material dissolved in water contains some lipid-related compounds next to humic substances. This highly carbonaceous waxy matter has often been mistakenly incorporated in humic substances.

The aim of this research was to study the *Carex* (C) and *Sphagnum* (S) peat lipids soluble in water. The dichloromethane-extractable fractions in the water-soluble matter of C and S peats of different degrees of humification were isolated and analyzed for their extract content. One sample (C H3) was quantified both for its total lipid content and for its content of free and bound lipid monomers. The

other samples were qualitatively analyzed for their free lipid monomers. The efficiency of the water-extraction procedure was also investigated.

EXPERIMENTAL

Chemicals

Analytical-grade organic solvents and inorganic reagents were used as received from the suppliers (E. Merck AG, EKA). The model compounds included fatty acids and alcohols (C_{13}–C_{30}), α,ω-alkanedioic acids (C_8–C_{14}, C_{16}, C_{26}), ω-hydroxy acids (C_{10}, C_{12}, C_{16}, C_{26}), oleic acid, cholesterol, β-sitosterol, betulinol and betulinic acid and were obtained from Sigma Chemical Company, EGA-Chemie and Fluka AG except the C_{26}-α,ω-alkanedioic and C_{26}-ω-hydroxy acids, which were synthesized in our laboratory.[6] *n*-Heptadecanoic acid (Sigma, 99%) was used as internal standard. The derivatizing reagents BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide with 1% TMCS (trimethylchlorosilane)) (97%) and Diazald (N-methyl-N-nitroso-*p*-toluenesulphon-amide) (99%) were supplied by Fluka AG and EGA-Chemie, respectively.

Peat Samples

The peat materials were drilled from Norrbomuren fen (60° 30'N, 17° 3'E) in eastern Sweden in the summer of 1985. *Carex* (C) peat consisted of three samples with the degrees of humification[7] H3, H4 and H8, and *Sphagnum* (S) peat of two samples (H2 and H6). The botanical and technical characteristics of the peat materials as well as their lipid compositions are presented elsewhere.[8]

Extraction and Isolation Procedures

An outline for the isolation and analysis procedures is given in Figure 1. The peat materials were air-dried at ambient temperature and ground to pass a 1 mm sieve (drying at higher temperatures may change the inner structure of peat material, e.g. removal of physically bonded water and polymerization of lipidic matter). The moisture content of the samples varied from 16.1% to 50.0% by weight (triplicate determinations at 105 °C for 24 h). The peat samples (12.0–25.9 g) were mixed with distilled water (280–510 ml) to give dry peat/water ratios of about 1:30. Each peat–water mixture was stirred with a magnetic stirrer for 20 h at ambient temperature. Centrifugation of the mixture with 5500 r.p.m. for 1 h produced a greenish-brown translucent supernatant liquid (darker in the S peat samples, especially S H6), which was, after filtration (paper), extracted with dichloromethane (DCM, 3 × 100 ml). The DCM extracts, rich in water, were combined, 100 ml of DCM were added, and the mixture was shaken vigorously, whereafter the DCM phase was easy to separate. After drying over anhydrous sodium sulphate for 20 h, the DCM phase was evaporated to dryness under reduced pressure at 25 °C and weighed. This residue will hereafter be called water-soluble lipids.

The procedure applied to the C H3 peat sample was slightly different from the above. After centrifugation, a 1 ml sample of the water extract was stored for

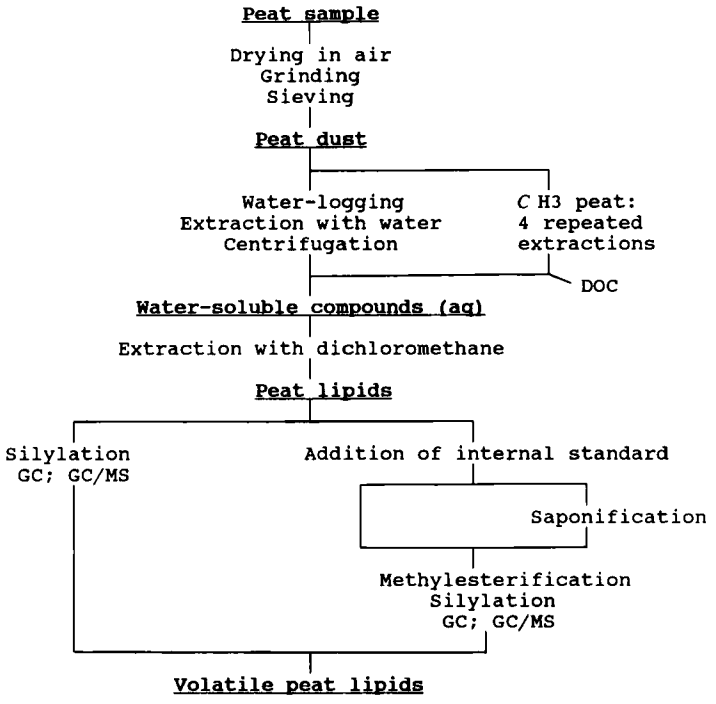

Figure 1 Sample pretreatment and analysis scheme for water-soluble peat lipids.

dissolved organic carbon (DOC) analysis. The extraction and the DOC sampling were repeated four times for the same C H3 peat sample (Figure 1).

Sample Pretreatment and Analysis

Each extract was qualitatively analyzed to determine its free lipid monomer composition. The first extract of the C H3 peat was quantified to determine its total lipids and free and bound lipid monomers. For quantification three different samples were used. One sample without internal standard was used to determine the natural abundance of *n*-heptadecanoic acid in peat. To the other two samples used for quantification, known amounts of *n*-heptadecanoic acid were added as internal standard. The first sample was used to determine the concentrations of the free lipid monomers. The second sample was refluxed with ethanolic alkali (0.4 M KOH in 90% EtOH) for 6 h to saponify the hydrolyzable ester bonds. Thereafter the mixture was acidified with 0.2 M sulphuric acid and extracted with diethyl ether (3 × 15 ml). The combined ether extracts were washed with distilled water until neutral and dried over anhydrous sodium sulphate for 20 h. After evaporating the solvent under medium vacuum at 25 °C, the free and released lipid monomers (as well as the unsaponifiable polymeric matter) remained in the residue.

The analyses were carried out by capillary gas chromatography (GC) and combined gas chromatography–mass spectrometry (GC–MS). For qualitative analysis the sample compounds were only trimethylsilylated with the BSTFA reagent (50 μl) in pyridine (20 μl) for 15 min at 80 °C. Quantification of the lipid monomers was based on the usual procedure using calibrated mass responses for the methyl esters of carboxylic acid derivatives and trimethylsilyl ethers of hydroxy compounds.[8] Therefore in the quantitative analysis the samples were at first methylesterified with fresh diazomethane for 3 h at 20–25 °C and then trimethylsilylated (Figure 1).[8]

Samples (1–2 μl) of the derivatized mixtures were injected into a Micromat HRGC 412 (Nordion, Finland) or Hewlett–Packard 5710A gas chromatograph equipped with a flame ionization detector (FID) and a wall-coated open tubular (WCOT) fused-silica capillary column (25 m, 0.20 or 0.32 mm i.d.) coated with a chemically bonded SE-54 stationary phase at a film thickness of 0.10 or 0.25 μm (Nordibond SE-54, Nordion). Hydrogen was used as the carrier gas in the Micromat at flow-rate of 0.7 ml/min (0.85 bar); with the H–P 5710A the carrier gas was nitrogen (2.0 ml/min, 1.4 bar). The injector and detector temperatures were maintained at 320 °C and 330 °C, respectively. The GC oven temperature was raised from 130 to 300 °C at a rate of 4 °C/min and maintained at 300 °C for 20 min. The splitting ratios were 25:1 and 15:1 in the Micromat and in the H–P 5710A, respectively.

For GC–MS analysis a double focussing VG Analytical 7070E mass spectrometer with a VG 11–250 data system and a Dani 3800 HRGC gas chromatograph was used under the conditions described earlier.[6] The GC separation of the compounds was achieved with a similar column and with the same temperature-programming as described above for GC.

Compounds were identified on the basis of their GC retention times and by comparing their mass spectra with those of standards or published data. Compound concentrations were obtained on the basis of electronically integrated GC peak areas relative to that of the internal standard (*n*-heptadecanoic acid) and the calibrated mass responses for the individual compounds.

DOC Measurements

Dissolved organic carbon concentrations were measured for the water extract samples of the CH3 peat after each extraction (4 DOC samples). Before measurement the samples were filtered through a 0.2-μm fluoropolymer membrane (ACRO LC 13, Gelman Sciences). DOC measurements were carried out with an Ionics Model 555 Carbon Analyzer.

RESULTS AND DISCUSSION

Lipid Content of the Water-extracts

The amount of water-soluble lipids varied from 0.021 % to 0.083 % (210–830 mg/

Table 1 Lipid contents of water extracts of *Carex* and *Sphagnum* peats of different degrees of humification

Lipids parameter	*Carex*			*Sphagnum*	
	C H3	*C H4*	*C H8*	*S H2*	*S H6*
Water-soluble lipids					
A, % of dry peat	0.049	0.027	0.029	0.021	0.083
B, mg/100 g of water	1.20	1.06	0.96	0.54	2.70
Total lipids[a]					
C, % of dry peat	9.8	6.5	11.2	2.4	8.1
Relative water-solubility					
100 A/C, %	0.50	0.42	0.26	0.88	1.03

[a]According to reference 8.

kg) in dry peat corresponding to 5.4–27.0 ppm or 0.54–2.70 mg/100 g of water (Table 1). Comparison of the concentrations with the corresponding values for palmitic (C_{16}) and stearic (C_{18}) acids, 0.72 and 0.29 mg/100 g of water at 20 °C,[9] respectively, indicates the solubility of the bulk of lipidic matter to be unexpectedly high. The total and relative water solubilities of the peat lipids decreased in the C peat and increased in the S peat with an increasing degree of humification (Table 1). The lipids of the humified S H6 peat were nearly three times as soluble as those of the C H8 peat, the relative water solubility of the former being almost four-fold that of the latter. In spite of the large differences in the water solubility, the lipid compositions of S and C peat samples were very similar. This result is unexpected because we have previously found that the lipids of these peat materials showed similar compositional and quantitative features when extracted with neutral organic solvents (DCM–acetone, 9:1, v/v).[8] The anomaly found in the water extracts could be caused by the difference in the association mode between humic and lipidic matter in both peat types, as was indicated by the much darker brown colour of the water extract of the S H6 peat compared with that of the C H8 peat.

Composition of the Water-soluble Lipids

Total matter. The composition of the water-soluble lipids of the C H3 peat is shown in Figure 2. The raw lipids included free lipid monomers (5%, A′ in Figure 2a), free other volatile materials (8%, B′) and non-volatile matter (87%, 100% A′–B′). During saponification the non-volatile matter (polymers) was partly (30%) hydrolyzed to lipidic monomer units. The unanalyzed wax esters in the other volatile materials of the raw lipids did, of course, also hydrolyze, but they increased the lipid monomer content only insignificantly. Actually, the total and relative amounts of the other volatile materials increased upon hydrolysis (Figure 2a). After saponification the lipid composition was the following: lipid monomers, 26%; other volatile materials, 14%; non-volatile polymeric matter, 60%. The amount of lipid monomers was thus increased about 5-fold during the saponification. The total lipid composition differs from that obtained by DCM–acetone (9:1,

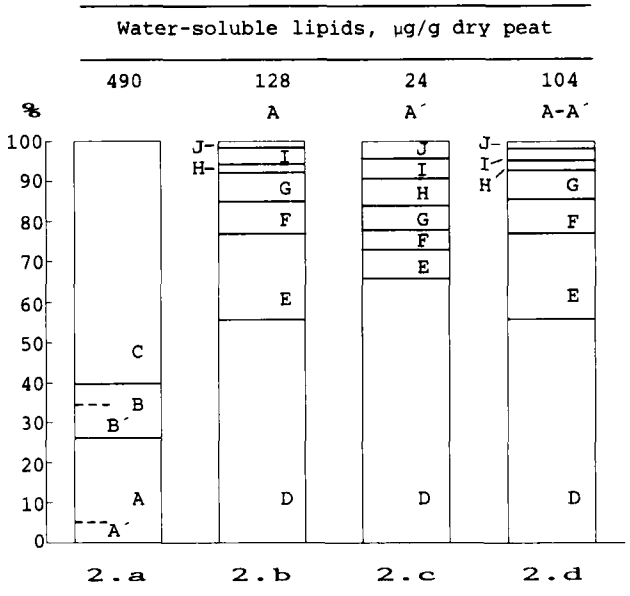

Figure 2 Composition of the water-soluble lipids of the C H3 peat: (a) total lipids; (b), (c) and (d) total, free and bound lipid monomers, respectively. A, lipid monomers (A', free lipid monomers); B, other volatile materials (B', free other volatile materials); C, non-volatile matter (polymers); D, *n*-fatty acids; E, ω-hydroxy acids; F, α,ω-alkanedioic acids; G, 1-alkanols; H, *n*-alkan-2-ones; I, sterols; J, triterpenoids.

v/v) extraction of the same C H3 peat:[8] lipid monomers, 35%; other volatiles, 22%; non-volatiles, 43%. Thus, the water extraction seems to be somewhat selective toward the non-volatile polymeric matter.

Monomeric lipid groups The total lipid monomers (128 µg/g dry peat), i.e. the lipid monomers after saponification (Figure 2b), consisted of normal fatty acids as the major group (58%), and ω-hydroxy acids (19%), α,ω-alkanedioic acids (8%), normal alcohols (7%), sterols (4%), triterpenoids (2%) and *n*-alkan-2-ones (2%) as the other monomer groups. This monomer composition differed from that obtained with DCM–acetone (9:1, v/v) extraction of the same C H3 peat:[8,10] *n*-fatty acids, 43%; ω-hydroxy acids, 43%; α,ω-alkanedioic acids, 2%; 1-alkanols, 7%; sterols, 4%; and *n*-alkan-2-ones, 1%. Consequently, the major differences existed in the composition of the acid fraction. In the water-extract the low ω-hydroxy acid content was counterbalanced by high α,ω-alkanedioic and normal fatty acid contents.

Free lipid monomers (24 µg/g dry peat) contained normal fatty acids in a high relative concentration (67%), the rest being composed of about equal amounts of the other monomer groups mentioned above (Figure 2c). This means that saponification released lipid monomers in high amounts (104 µg/g dry peat, Figure 2d), and with a high carboxyl content (>70% of the functional groups). This indicates that the hydrolyzable non-volatile matter contained mainly esterified

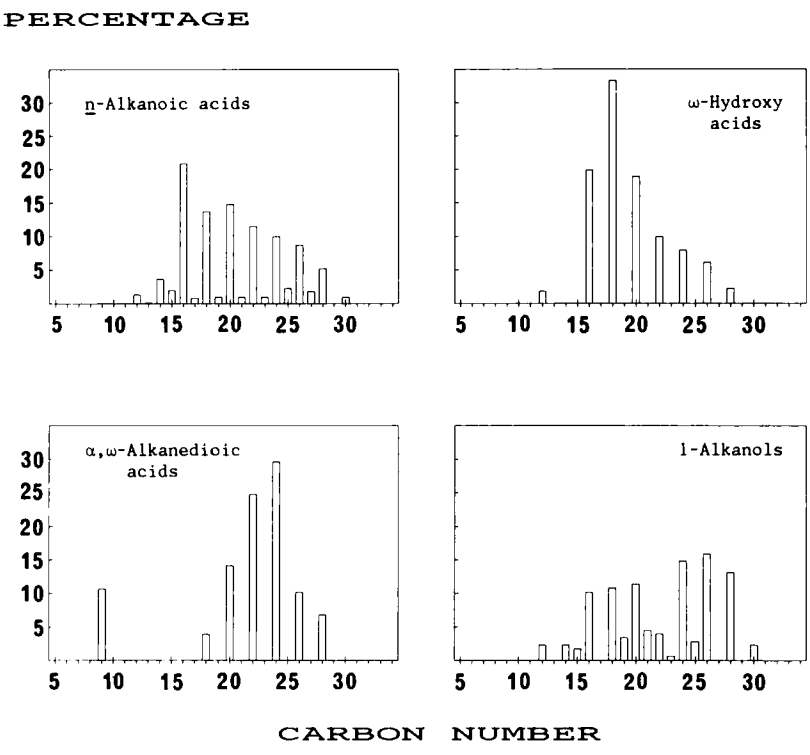

Figure 3 Individual compound distributions for total *n*-alkanoic, ω-hydroxy and α,ω-alkanedioic acids, and 1-alkanols.

hydroxyl groups. The relative amounts of ω-hydroxy and α,ω-alkanedioic acids of the acidic monomers increased during the saponification from 8 to 25% and from 7 to 10%, respectively, while that of normal fatty acids decreased from 85 to 65%.

Individual compounds The different lipid monomer groups were composed of the components usually found in peat extracts. This means long-chain fatty compounds in the range of C_{12}–C_{30}, with a predominance of the even-carbon homologues except the *n*-alkan-2-one fraction, which was composed of the odd C_{21}–C_{27} homologues. *n*-Fatty acids included *n*-alkanoic acids and two monoenoic *n*-fatty acids, namely palmitoleic (C_{16}) and oleic (C_{18}) acids. β-Sitosterol was the only remarkable sterol compound and betulin and betulinic acid were the most abundant triterpenoids in the extracts. The fatty compound distributions (Figure 3) differed, however, from those of the DCM–acetone extracts. In the total fractions of *n*-fatty acids, ω-hydroxy acids and 1-alkanols, the shorter-chain homologues ($C<22$) clearly predominated giving much higher proportions than in the case of the DCM–acetone (9:1, v/v) extract:[8] 63% (31% in DCM–acetone extract), 74% (25%) and 46% (21%), respectively. This kind of behaviour was not found for α,ω-alkanedioic acids. The difference can be partly explained by the

Table 2 Correlation between water-soluble *Carex* H3 peat lipids and DOC in four repeated extractions

Parameter	*Multiple water extractions of the C H3 peat*[a]			
	I	*II*	*III*	*IV*
Water-soluble lipids				
A, % of dry peat	0.049	0.045	0.054	0.036
B, mg/100 g of water	1.20	1.08	1.30	0.84
Dissolved organic carbon				
DOC, mg/100 g of water	13.9	11.7	13.1	9.1
Correlation factor[b]	0.93	1.00	1.07	1.00

[a]Extractions at 20–25 °C for 20 h except 96 h for IV.
[b]$(100B_i/DOC_i)/\{\sum 100B_i/DOC_i)/4\}$.

higher solubility of the more polar shorter-chain fatty compounds (or diacids) in the highly polar solvent, water, over the less polar longer-chain homologues and compound groups. The qualitative GC analysis showed that the relative compositions of the volatile free lipid monomers in the water-extracts of the various samples were quite similar.

Multiple Water Extractions: Lipids and DOC

Multiple water extractions of the C H3 peat sample resulted in an increasing amount of lipids (Table 2). The amounts of lipids obtained by the first three extraction steps were at the same concentration level (1.1–1.3 mg/100 g of water), whereas the fourth extraction gave a lower extraction yield. In the first three extractions the water solutions obviously were saturated by fatty material, or the slow acidic hydrolysis of ester bonds resulted in more lipids during the extraction steps, or these two factors both occurred. The DOC concentrations of the four water-extracts are shown in Table 2. The value of DOC was 12–14 mg/100 g of water (120–140 ppm) for the first three extracts, but only 9.1 mg/100 g of water for the fourth one. The correlation factors between the amounts of water-soluble lipids and the respective DOC concentrations were from 0.93 to 1.07 with a 3.5% relative standard deviation (Table 2). This result reveals that the higher DOC concentration correlates with the higher solubility of lipids in water. This probably means that the compounds which constitute DOC increase the solubility of lipids in water, possibly by means of hydrogen bonding. This obviously is the main factor responsible for the varying water-soluble lipid concentrations between and within *Carex* and *Sphagnum* peats.

Aquatic humic substances are known to account for 30–80% of DOC in natural waters,[2] and dissolved humic and fulvic acids enhance the water solubility of neutral organic pollutants and pesticides.[11] Aquatic fulvic acids (67%) and hydrophilic organic acids (20%) were shown to be the major DOC components in Thoreau's bog, an ombrotrophic floating-mat *Sphagnum* bog.[1] The DOC composition of Thoreau's bog can be supposed to be an example of the larger universal

peat bog system. Therefore we used the elemental analysis data of the major DOC components of Thoreau's bog[1] to estimate the total dissolved organic matter content in the multiple water-extracts of the C H3 peat. This estimation together with the lipid content found resulted in 20–30 mg of total dissolved organic matter in a 100 g portion of water. Dissolved organics contained 4–5% of water-soluble lipids.

CONCLUSIONS

Water-extracts of *Carex* (C) and *Sphagnum* (S) peats were found to contain varying amounts of lipidic matter (0.021–0.083% of dry peat, 0.54–2.70 mg/100 g of water, 4–5% of total dissolved organic matter). The lipid content was dependent on the peat type and the degree of humification of the peat material. The total and relative water-solubility of peat lipids increased in the S peat sample set with an increasing degree of humification, while the reverse was true for the C peat. In the most humified peat samples, the lipids of the S H6 peat were nearly three times as soluble as those of the C H8 peat, while the relative water-solubility of the former was about four-fold that of the latter. The DOC concentration obviously is one of the main factors controlling the water-solubility of the lipidic matter.

The primary water-soluble lipidic fraction of the C H3 peat included 87% of non-volatile polymeric matter from which 30% was hydrolyzed upon saponification. The other constituents were free lipid monomers (5%) and other volatile materials (8%). After saponification the lipids included 60% of polymers, 26% of lipid monomers and 14% of other volatile materials. The following constituents were found to exist in clearly higher proportions in the water extracts than in the organic solvent extracts: (i) the unsaponifiable polymers in total lipids (60%), (ii) the acidic lipid monomers in bound lipids (>70%) and (iii) the shorter-chain ($C<22$) homologues in various monomer groups (45–75%).

These results suggest that the dissolved non-volatile lipidic matter is composed of humic-like polymers with bound hydroxyl groups in their molecular structures. These hydroxyl groups in e.g. fulvic acids can be esterified by the carboxyl groups of various fatty acids.

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