Origin and formation pathways of kerogen-like organic matter in recent sediments off the Danube delta (northwestern Black Sea)

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Abstract

The chemical structure, source(s), and formation pathway(s) of kerogen-like organic matter (KL) were investigated in recent sediments from the northwestern Black Sea, off the Danube delta. Three sections from a sediment core collected at the mouth of the Sulina branch of the delta, under an oxic water column, were examined: \(S_0\) (0–0.5 cm bsf), \(S_{10}\) (10–13 cm bsf), and \(S_{20}\) (20–25 cm bsf). The bulk geochemical features of these sediments (total organic carbon, organic C/N atomic ratio, \(\delta^{13}\)C\textsubscript{org}) were determined. Thereafter, KL was isolated from the samples, as the insoluble residue obtained after HF/HCl treatment. KL chemical composition was investigated via spectroscopic (FTIR, solid state \(^{13}\)C and \(^{15}\)N NMR) and pyrolytic (Curie point pyrolysis–gas chromatography–mass spectrometry) methods, and the morphological features were examined by scanning and transmission electron microscopy. Similar morphological features and chemical composition were observed for the three KLS and they suggested that the selective preservation of land-plant derived material as well as of resistant aliphatic biomacromolecules (probably derived from cell walls of freshwater microalgae) was the main process involved in KL formation. Besides, some melanoidin-type macromolecules (formed via the degradation-recondensation of products mainly derived from proteinaceous material) and/or some encapsulated proteins also contributed to the KL chemical structure.

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Keywords: Kerogen-like organic matter; Recent sediments; Danube delta; Northwestern Black Sea; Preservation mechanisms; Selective preservation pathway; Degradation-recondensation

1. Introduction

A wide variety of organic and inorganic substances are transported from the continent to coastal marine environments via river discharge. The fate of riverine organic material in coastal areas largely depends on the physico–chemical partition between particulate and dissolved phases, and how this distribution is modified as freshwater mixes with saline water (Stumm and Morgan, 1970; Olsen et al., 1982). The rapid flocculation of terrestrially derived humates in seawater has been demonstrated (Narkis and Rebhun, 1975; Sholkovitz, 1976; Sholkovitz et al., 1978), and a large part of both riverborne particulate organic matter (POM) and flocculated colloids is expected to be exported from the water column to the sediment underlying estuarine systems.

The Danube delta is, after the Volga, the second largest delta in Europe, and 23rd in the world. It lies at the mouth of a river extending over a distance of 2860 km (exclusive of the delta itself) and drains an area of 805,300 km\(^2\). Within the delta, two major bifurcations...
give rise to three separate tributaries: the Chilia, Sulina and Saint Gheorghe branches. The Danube collects effluents of eight European countries, and accounts for 77% of the total freshwater discharge in the Romanian coastal shelf, which corresponds to a mean annual discharge of ca. 200 km$^3$. Increased anoxia in the north-western Black Sea indicates that anthropogenic activities in the drainage basin of the Danube raised the input of riverborne OM and the level of inorganic nutrients, and hence the production of autochthonous OM, beyond the natural assimilation capacity (Cociasu et al., 1996). The increase in nutrient and OM input from rivers, and from sources such as fertilisers from agriculture, and industrial and municipal wastewaters along the shoreline (especially phosphate), is the main cause for the increased eutrophication observed in the semi-enclosed Black Sea basin during the last decades (Mee, 1992). Indeed, due to a strong theromhaline stratification and the long residence time of water masses, the Black Sea ecosystem is highly sensitive to increased production of OM (Cociasu et al., 1996). In addition, the mainly wind-driven water circulation along the Romanian coast and the Coriolis force lead to a long residence time of the riverine waters on the Romanian shelf and the resulting negative effects on the coastal ecosystem have been observed (Mee, 1992). Distinct increases in phytoplankton cell densities, as well as the frequency of algal blooms, were reported. There was a more than double decrease of benthic biomass in the Romanian shelf area due to suboxia in the bottom water after the sedimentation of algal blooms (Cociasu et al., 1996).

The present study was designed to investigate the insoluble, HF/HCl resistant, OM isolated from three sections of a recent sediment core collected off the Sulina branch of the Danube delta. This organic fraction corresponds to the “kerogen” defined by Durand and Nicaise (1980) as the fraction of sedimentary OM isolated via organic solvent extraction and HF/HCl treatment, and is therefore referenced as “kerogen-like” material (KL) in this paper. KL is characterised by a macromolecular structure with a high resistance to bacterial, chemical, and diagenetic alterations. Only a few studies about KL have been reported so far, whilst such a fraction can account for a substantial part of total OM in marine sediments (e.g. Eglinton et al., 1994; Zegouagh et al., 1999). The aim of the present work is to derive information on the mechanism(s) and source(s) involved in KL formation and preservation in these Black Sea sediments. To this end, the morphological and chemical features of KLs were examined via a combination of electron microscopy observations and spectroscopic and pyrolytic methods, so as to deconvolute autochthonous versus allochthonous sources contributing to KLs in such a deltaic environment.

2. Experimental

2.1. Sediment samples

The sediment core was collected off the Sulina mouth of the Danube delta (N 45°08’; E 29°48’; water depth 20 m; salinity ca. 10; Fig. 1), under an oxic water column, during the EROS 21 (European River–Ocean Systems) cruise (9 April–3 May 1997). Three core sections were selected on the basis of their darker colour pointing to relatively high organic contents, wrapped in pre-combusted aluminium foil, frozen on board and kept frozen until analysis. The sections correspond to intervals 0–0.5 cm bsf (S$_0$ sample, oxic level), 10–13 cm bsf (S$_{10}$ sample), and 20–25 cm bsf (S$_{20}$ sample). The sediments were freeze dried and ground before further treatment.

2.2. Total organic carbon (TOC)

TOC contents were determined after decarbonatation by high temperature catalytic oxidation (HTCO) using a Shimadzu Solid Sample Module SSM 5000A coupled to a Shimadzu TOC 5000 analyser.

2.3. KL isolation

About 10 g of dry sediment were treated to isolate the “kerogen-like” fraction. Lipids were first removed by extraction with CH$_2$Cl$_2$/CH$_3$OH, 2/1, v/v (stirring overnight at room temperature). The bulk of the mineral constituents of the lipid-free samples was then eliminated via a classical HF/HCl treatment (Durand and Nicaise, 1980). The organic residue was submitted to extraction with CH$_2$Cl$_2$/CH$_3$OH, 2/1, v/v (stirring overnight at room temperature), and finally the insoluble organic material was recovered by centrifugation and dried under a nitrogen flow. It is important to note

Fig. 1. Map of the northwestern Black Sea, the Danube delta, and the sampling site.
that HF/HCl treatment, when applied to recent materials, results not only in the elimination of most minerals but also in the removal of some insoluble, macromolecular, organic constituents (e.g. labile protein- and polysaccharide-derived moieties) due to acid hydrolysis (Durand and Nicaise, 1980). However, some acid-labile organic constituents could be retained due to incorporation within the macromolecular network of the KLs.

2.4. Elemental analysis

Elemental composition of the three KLs obtained after the above treatments was determined by the “Service Central de Microanalyse”, CNRS, Vernaison.

2.5. Scanning (SEM) and transmission (TEM) electron microscopy

KL samples were fixed by 1% glutaraldehyde in cacodylate buffer (pH 7.4), post-fixed in 1% OsO4 and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and TEM observations were performed on a Philips 400 microscope.

For SEM, the fixed samples were dehydrated using the CO2 critical point technique and coated with gold, as previously described by Largeau et al. (1990), prior to observations on a Jeol 840 microscope.

2.6. Fourier transform infra red spectroscopy (FTIR)

FTIR spectra were recorded on a Bruker IFS 48 spectrometer using pellets prepared by grinding 1 wt.% of KL with KBr.

2.7. Solid-state $^{13}$C and $^{15}$N nuclear magnetic resonance spectroscopy ($^{13}$C and $^{15}$N NMR)

Solid-state $^{13}$C NMR spectra were obtained at 100.62 MHz for $^{13}$C on a Bruker MSL400 spectrometer with a recycle time of 5 s. High power decoupling, cross polarisation (contact time 1 ms) and magic angle spinning at 4.5 kHz, were used. The spectra were the results of ca. 8000 scans. Since all spectra recorded at 4.5 kHz are similar, only one sample (S20) was analysed at a higher spin frequency (15 kHz), on a Bruker MSL400 with the same parameters, to remove spinning side bands which make interpretation of some resonances difficult in the spectra at 4.5 kHz.

A Bruker DMX 400 operating at 40.56 MHz was used for acquiring the solid-state CP/MAS $^{15}$N NMR spectrum of one of the three samples (S20), applying a contact time of 1 ms and a recycle time of 150 ms. Ca. 2 million scans were accumulated at a magic angle spinning speed of 5.5 kHz. The chemical shift is referenced to the nitromethane scale (= 0 ppm) and was adjusted with $^{15}$N labeled glycine (−347.6 ppm).

2.8. Curie point pyrolysis—gas chromatography—mass spectrometry (CuPy–GC/MS)

KL aliquots (ca. 3 mg) were loaded in tubular ferromagnetic wires with a Curie temperature of 650°C and pyrolysis was performed by inductive heating of the sample-bearing wires to their Curie temperature in 0.15 s (10 s hold time). A Curie point high frequency generator was used to produce the magnetic field and the pyrolysis unit (Fisher 0316M) was directly coupled to GC–MS (a Hewlett Packard HP-5890 gas chromatograph and a Hewlett Packard HP-5889A mass spectrometer, electron energy 70 eV, ion source temperature 250°C, scanning from 40 to 650 a.m.u., 0.7 scan/s). Separation of the products was achieved by a 30 m fused silica capillary column coated with chemically-bound Restek RTX-5MS (0.25 mm i.d., film thickness 0.50 μm). Helium was used as carrier gas. The temperature of the GC oven was programmed from 50 to 300°C at a rate of 4°C/min, after a first stage at 50°C for 10 min to allow a better separation of the most volatile pyrolysis products. The wires were weighed after pyrolysis, and the weight loss calculated. Compounds were identified based on their mass spectra, GC retention times, comparisons with library mass spectra, and with published mass spectra (Danielson and Rogers, 1978; van de Meent et al., 1980a,b; van der Kaaden et al., 1983; Saiz-Jimenez and de Leeuw, 1984; Tsuge and Matsubara, 1985; Ralph and Hatfield, 1991).

3. Results and discussion

3.1. Bulk geochemical features of the untreated sediments

TOC contents, C/N atomic ratios and $\delta^{13}$C$_{org}$ values of the three sediment samples are reported in Table 1. TOC content varies between 1.34 and 1.61%. Previous studies on marine surface sediments exhibit TOC values around 0.1–1% in temperate, subtropical and equatorial climates (e.g. van Vleet et al., 1984; Kennicutt II et al., 1987; Bigot et al., 1989; Bouloubassi and Saliot, 1993), although higher values (1–5% range) were recorded for the Rhone delta (Bouloubassi et al., 1997), the Ebro delta (Grimalt and Albaige`s, 1990), the Mackenzie River delta (Yunker et al., 1993) and the Lena River delta (Zegouagh et al., 1996). Intermediate TOC contents are, therefore, observed in the present study.

The stable carbon isotopic composition ($\delta^{13}$C$_{org}$) of OM produced by photosynthetic organisms principally reflects the dynamics of carbon assimilation and the isotopic composition of the inorganic carbon source (e.g. Hayes, 1993). The difference in $\delta^{13}$C$_{org}$ between
OM produced by land plants and marine algae has successfully been used to trace the sources and distribution of OM in coastal ocean sediments (e.g. Prahl et al., 1994). Average values of $\delta^{13}C_{\text{org}}$ in coastal ocean sediments (e.g. Prahl et al., 1994) have been successfully used to trace the sources and distribution of OM produced by land plants and marine algae (e.g. Calvert and Fontugne, 1987; Fry et al., 1991; Arthur et al., 1994), whereas the terrestrial end-member has a $\delta^{13}C_{\text{org}}$ value of $-27\%$, on average (Arthur and Dean, 1998). In contrast to marine algae, freshwater algae have $\delta^{13}C_{\text{org}}$ values typically indistinguishable from those of OM from vascular land plants, i.e. between $-30$ and $-25\%$ (e.g. Benson et al., 1991; Calvert and Fontugne, 1987; Fry et al., 1991; Arthur et al., 1994), whereas the terrestrial end-member has a $\delta^{13}C_{\text{org}}$ value of $-27\%$, on average (Arthur and Dean, 1998). The stable carbon isotopic ratios of bulk OM in the three studied sediments, from $-26.3$ to $-25.9\%$, point to a strong contribution from land plants and/or freshwater algae. Indeed, surface particles collected near the rivermouth during the same period exhibited $\delta^{13}C_{\text{org}}$ values identical to those found in the present study (Saliot et al., in press).

Organic carbon to nitrogen atomic ratios (C/N) also have been widely used to distinguish between algal and land-plant sources of sedimentary OM (Premuzic et al., 1994; Ishiwatari and Uzaki, 1987; Meyer, 1994; Prahl et al., 1994; Silliman et al., 1996). Algae typically have atomic C/N ratios between 4 and 10, whereas vascular land plants have C/N ratios greater than 20 (Meyer, 1994). This difference arises from the protein richness of algal OM and is largely preserved in sedimentary OM (Jasper and Gagosian, 1990; Meyer, 1994). Riverine and estuarine sediments typically contain a larger contribution of OM derived from land plants and thus show elevated atomic C/N ratios, in the 13–35 range (Kennicutt II et al., 1987). Interestingly, our samples show relatively low C/N ratios with values in the 9–10 range, that characterise OM of mainly algal origin. Diagenetic modifications cannot explain these low values since early diagenesis rather results in an increase of C/N ratios due to the preferential loss of labile N-bearing components. Lowering of C/N ratios also has been observed in soils (e.g. Solins et al., 1984) and in ocean sediments (e.g. Muller, 1977), where it reflects the absorption of ammonia derived from OM decomposition, accompanied by remineralisation and release of carbon dioxide (Meyer, 1997; Boulobassi et al., 1998). In addition, the C/N ratios of OM in fine-sized sediments generally are lower than those of coarse sediments (Keil et al., 1994; Prahl et al., 1994). Fine sediment fractions contain a smaller proportion of intact land-plant debris than clay minerals. The latter have both large surface areas and negative surface charges and therefore efficiently adsorb ammonia. However, these artificially depressed C/N ratios ($<5$) are usually found in sediments having low OM concentrations (TOC < 0.3%) where inorganic N (nitrate, ammonium) largely contributes to the residual N (Muller, 1977). This is not the case here. Accordingly, in our samples, the low C/N values rather reflect the abundant presence of components derived from freshwater algae in the studied area (Ragueneau et al., in press; Saliot et al., in press), as well as the good preservation of protein-derived material, which could explain the unusually low C/N ratio associated to a low $\delta^{13}C_{\text{org}}$ value.

Consequently atomic C/N ratios and $\delta^{13}C_{\text{org}}$ values found in the sediment core indicate an admixture of terrigenous and freshwater materials as the main sources (Meyers, 1997).

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>TOC (wt.%)</th>
<th>C/N</th>
<th>$\delta^{13}C_{\text{org}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>0–0.5 cm bsf, oxic</td>
<td>1.34</td>
<td>8.7</td>
</tr>
<tr>
<td>S10</td>
<td>10–13 cm bsf</td>
<td>1.50</td>
<td>9.4</td>
</tr>
<tr>
<td>S20</td>
<td>20–25 cm bsf</td>
<td>1.61</td>
<td>9.6</td>
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### Table 2

<table>
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<tr>
<th>Sample</th>
<th>KL abundance (as wt.% of TOC in the raw sediments)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>S0</td>
</tr>
<tr>
<td>KL abundance</td>
<td>50.4</td>
</tr>
</tbody>
</table>

### Table 3

<table>
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<tr>
<th>Sample</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S_{tot}</th>
<th>Fe</th>
<th>Ash</th>
<th>H/C</th>
<th>N/C</th>
<th>$S_{org}/C^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>40.2</td>
<td>3.4</td>
<td>2.5</td>
<td>3.9</td>
<td>2.6</td>
<td>29.4</td>
<td>1.01</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>S10</td>
<td>36.7</td>
<td>3.3</td>
<td>2.7</td>
<td>6.2</td>
<td>3.5</td>
<td>24.3</td>
<td>1.08</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>S20</td>
<td>32.2</td>
<td>2.8</td>
<td>2.1</td>
<td>4.8</td>
<td>3.0</td>
<td>36.3</td>
<td>1.04</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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* $\delta^{13}C_{\text{org}}$ values typically have been reported for Black Sea suspended particles dominated by marine phytoplankton (Calvert and Fontugne, 1987; Fry et al., 1991; Arthur et al., 1994), whereas the terrestrial end-member has a $\delta^{13}C_{\text{org}}$ value of $-27\%$, on average (Arthur and Dean, 1998). In contrast to marine algae, freshwater algae have $\delta^{13}C_{\text{org}}$ values typically indistinguishable from those of OM from vascular land plants, i.e. between $-30$ and $-25\%$ (e.g. Benson et al., 1991; Calvert and Fontugne, 1987; Fry et al., 1991; Arthur et al., 1994), whereas the terrestrial end-member has a $\delta^{13}C_{\text{org}}$ value of $-27\%$, on average (Arthur and Dean, 1998). The stable carbon isotopic ratios of bulk OM in the three studied sediments, from $-26.3$ to $-25.9\%$, point to a strong contribution from land plants and/or freshwater algae. Indeed, surface particles collected near the rivermouth during the same period exhibited $\delta^{13}C_{\text{org}}$ values identical to those found in the present study (Saliot et al., in press).

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Consequently atomic C/N ratios and $\delta^{13}C_{\text{org}}$ values found in the sediment core indicate an admixture of terrigenous and freshwater materials as the main sources (Meyers, 1997).

### 3.2. Geochemical features and relative abundance of the three KLs

The amounts of organic carbon remaining after the HF/HCl treatment (as wt.% of the total organic carbon in the raw sediments), along with the elemental compositions of KLs in the three samples are reported in Tables 2 and 3, respectively.

As expected, the HF/HCl treatment resulted in the release of large amounts of organic carbon. However,
the resistant carbon retained after this treatment accounts for ca. 50 wt.% of the total initial carbon for the three samples (Table 2). The slightly more pronounced decrease observed for the surface sample S₀ is in agreement with the presence of intact (or slightly modified) biomacromolecules such as proteins and polysaccharides, which are acid labile. Indeed, the latter compounds are known to be partly retained in surface samples and to be rapidly degraded with depth in the upper layers of sediments (Hedges and Oades, 1997).

The organic sulphur to carbon atomic ratio is relatively low (Sₒᵣₒ/C ≈ 0.02), indicating a low contribution of organic sulphur compounds (OSC) in the three KLs. The hydrogen to carbon atomic ratio (H/C ≈ 1.04) indicates a substantial contribution of aromatic and/or unsaturated structures. Elemental analysis also revealed relatively high levels of nitrogen (ca. 2.5%) and iron (ca. 3%) in the three KLs. In a H/C ratio vs. N/C ratio diagram, the KL representative point falls close to OM in soils and terrestrial humic acids, thus pointing to a mainly continental origin.

X-ray analyses revealed that the residual inorganic fraction chiefly corresponds to fluorides, neo-formed during the HF/HCl treatment and not eliminated afterwards, which account for the substantial ash content of the KLs (ca. 30%).

3.3. KL morphological features

Observations of the three KLs by SEM (Fig. 2) showed that they are mainly composed of shapeless aggregates of various sizes ranging from ca. 8×10 up to ca. 30×40 μm (Fig. 2a and b). Most of these aggregates have a granular appearance (Fig. 2b and d), while a few of them exhibit a “gel-like” appearance (Fig. 2c). A number of pollen grains and/or land-plant spores (Fig. 2e and f), and of ligneous debris (Fig. 2g) were recognised, pointing to some contribution of land plants. A few framboids of pyrite, a mineral known to resist to HF/HCl attack, were also observed (Fig. 2h).

TEM observations (Fig. 3) revealed that the three KLs are largely composed of various types of lamellar structures with different thicknesses. The thinner ones (ca. 20–40 nm thick; Fig. 3a) represent a relatively minor fraction and are tightly accumulated into bundles. Similar lamellar structures, termed ultralaminae, have been recently observed by TEM in a number of kergenites from source rocks and oil shales (Largeau et al., 1990), so far considered as amorphous based on light microscopy observations. Ultralaminae have been shown to derive from the selective preservation of the thin outer walls of some microalgaes (Largeau et al., 1990; Derenne et al., 1991, 1992a,b; Gillaizeau et al., 1996), which are composed of highly resistant biomacromolecules termed algaenans (Tegelaar et al., 1989). Thicker structures (ca. 50–500 nm thick) resembling preserved cell walls of different shapes and thicknesses predominate in the three KLs (Fig. 3b–f). Based on their abundance these various lamellar structures likely correspond to the shapeless aggregates observed by SEM. Ligneous debris (Fig. 3g), pollen grains and/or land-plant spores (Fig. 3h), as well as a nanoscopically amorphous fraction (Fig. 3e) were also observed by TEM.

The combination of SEM and TEM observations thus allowed to conclude that the selective preservation of resistant cell walls (probably of microalgal origin) and of terrestrial material played an important role in the formation of the kerogen-like material of the three samples.

3.4. Spectroscopic studies

Because the three samples exhibit similar spectroscopic features, only the spectra of S₂₀ are discussed here in detail.

The FTIR spectrum of S₂₀ (Fig. 4) is characterised by an intense absorption centred at 3400 cm⁻¹ (wide band in the 3600–3000 cm⁻¹ range) corresponding to OH and/or NH groups. Carbonyl and/or carboxyl functions are responsible for the relatively weaker absorption at 1710 cm⁻¹. An intense band in the 1685–1585 cm⁻¹ range comprises two contributions: the stronger one, centred at 1655 cm⁻¹, corresponds to carbonyl groups in primary amide functions, while olefinic and aromatic unsaturations lead to a wide absorption occurring as a shoulder of the former band. The occurrence of aromatic groups is also suggested by absorptions at 790 and 750 cm⁻¹, although the bands in the aromatic C–H out-of-plane deformation region (900-700 cm⁻¹) are overlapped by relatively strong absorptions caused by residual minerals at wavelengths below 800 cm⁻¹. A weak absorption centred at 1540 cm⁻¹ is assigned to amide N–H bonds. Several absorptions are observed between 1000 and 1200 cm⁻¹ which can be due to C–O bonds (ethers and alcohols) and/or minerals. A wide band in the 1300–1200 cm⁻¹ range (maximum at 1230 cm⁻¹) is also noted. The latter may correspond to C–N bonds in aromatic amine functions. Relatively weak absorptions in the 3000–2800 cm⁻¹ range reflect the contribution of alkyl groups. The intensity ratio of the 1450 cm⁻¹ (CH₃ and CH₂ symmetric bending) and 1380 cm⁻¹ (CH₃ symmetric bending) absorptions shows that CH₃ groups are relatively abundant, pointing to a high degree of branching. A very weak absorption at 420 cm⁻¹ reflects the presence of a low amount of pyrite, in agreement with SEM observations.

The solid-state ¹³C NMR spectrum of S₂₀ KL (15 kHz; Fig. 5) is dominated by a peak centred at 30 ppm corresponding to aliphatic carbons without hetero-substituents (10–40 ppm range). This peak is relatively
wide indicating a substantial level of branching. The latter feature, which is in agreement with FTIR data, is also indicated by the occurrence of a shoulder at 15 ppm due to CH$_3$ groups. Several peaks reflect the occurrence of heteroelements in the KLs. The peak at 74 ppm corresponds to carbons bearing O or N atoms, including carbons from carbohydrates. The presence of the latter compounds is confirmed by a weak signal at 105 ppm due to anomic carbons (acetal groups). Carbons α to those occurring at 74 ppm are responsible for the peak at 55 ppm. However, the latter peak can also include carbons in methoxyl groups and/or carbons bearing the amino group in amino acids. The broad resonance centred at 130 ppm with a high relative intensity corresponds to aromatic and olefinic carbons. A weak signal at 150 ppm is assigned to phenols. A narrow peak centred at 175 ppm corresponds to ester and/or amide functions.

The solid-state $^{15}$N NMR spectrum of S$_{20}$ KL (Fig. 6) is dominated by a peak at −258 ppm assigned to amides. The shoulder between −200 and −230 ppm indicates the occurrence of both N-alkyl-substituted pyrroles: indoles or carbazoles (−200 ppm), and unsubstituted pyrroles (−230 ppm) (Derenne et al., 1993). A weak signal at −344 ppm may be due to amine functions.

The combination of FTIR and solid-state $^{13}$C and $^{15}$N NMR data showed that oxygenated and nitrogenous functions, together with aromatic and olefinic

![Fig. 2. Scanning electron microscopy of the three KLs.](image-url)
unsaturations, are predominant features for the three K\textls. These bulk chemical observations were completed by pyrolytic studies to derive further information about the molecular structure and origin(s) of K\textls.

3.5. Pyrolytic studies

CuPy-GC/MS of the K\textls isolated from samples S\textsubscript{0}, S\textsubscript{10}, and S\textsubscript{20} resulted in a substantial weight loss (ca. 20

![Fig. 3. Transmission electron microscopy of the three K\textls. (a) Thin lamellar structures similar to ultralaminae, (b) preserved cell walls packed together into bundles, (c) “organised” multicellular structures, (d) tangles of cell walls, isolated structures forming a closed (e) or an open (f) line, (g) ligneous debris, (h) pollen grain or land-plant spore. a, Amorphous.]
wt.%). The flash pyrolysates generated from the three KLs are highly complex and show similar compositions.

Their total ion current (TIC) traces (Fig. 7) are characterised by (i) an abundant presence of phenols and methoxyphenols, (ii) the presence of a complex mixture of alkylbenzenes, naphthalenes, indenes, indoles, pyrroles, pyridines and aromatic nitriles, with a low degree of alkylation (≤4 carbons) for the alkylated homologues of each family, (iii) a substantial contribution of normal alkanes and alk-1-enes, (iv) a virtual lack of sulphur compounds, (v) a low relative abundance of C_6–C_18 saturated fatty acids. The distributions of these different types of pyrolysis products are described in the following paragraphs and their origin and significance are discussed.

3.5.1. n-Alkanes/n-alk-1-enes

The mass chromatogram of m/z 57 (Fig. 8) shows an homologous series of n-alkanes ranging from C_9 to C_33 (maximum C_13), with no significant odd- or even-carbon-number dominance. A series of n-alk-1-enes with similar features was also observed. n-Alk-1-ene/n-alkane doublets are abundant in the pyrolysates of the highly aliphatic, resistant, biopolymers present in some higher plant tissues such as cutans (in cuticles), or suberans (in periderm tissues), as well as in tegmens (inner seed coats) (Nip et al., 1986a, b; van Bergen et al., 1993, 1994). Owing to the presence of such highly resistant components, these tissues and inner seed coats can retain their morphological features upon fossilisation. TEM observations on the three KLs revealed the abundant presence of relatively thick “lamellar” structures with different types of organisation (Fig. 3). A part of these structures might be related to some of the above resistant materials and correspond to selectively preserved structures derived from higher plants. In addition, SEM observations showed the presence of some plant spores and/or pollen grains (Fig. 2e and f). It is well documented that pollen and spore walls are composed of highly resistant materials, the so-called sporopollenins, which comprise aliphatic moieties and are also selectively preserved in the fossil record. Accordingly, a significant part of the n-alkanes and n-alk-1-enes observed in KL pyrolysates can be derived from landplant materials.

However, straight-chain hydrocarbons have also been described in the pyrolysates of lacustrine and marine materials (Fukushima and Ishiwatari, 1988; Fukushima et al., 1989). These hydrocarbons are likely derived from the algaenans which occur in the outer walls of numerous freshwater and marine microalgae (Largeau et al., 1984, 1986; Goth et al., 1988; Derenne et al., 1992b, 1995; de Leeuw et al., 1995; Gelin et al., 1999). The chemical structure of a number of algaenans was shown to be based on a network of long polymethyleneic chains (Largeau et al., 1986; Derenne et al., 1991, 1992 a,b;
Gelin et al., 1996, 1997), which generate large amounts of \( n \)-alkanes and \( n \)-alk-1-enes (up to C\(_{35}\)) upon pyrolysis. Moreover, owing to their extremely high resistance to chemical and microbial degradation (Tegelaar et al., 1989) such macromolecular constituents can survive almost unaffected during diagenesis. However, depending upon the thickness of the wall the initial morphology of the cell may be retained upon fossilisation, which leads to microfossils as in Torbanite (Largau et al., 1984, 1986) or in Messel Oil Shale (Goth et al., 1988), or may be partly altered as in ultralaminae (Derenne et al., 1991, 1992a). Such lamellar structures were observed by TEM in the KLs (Fig. 3) and they are probably derived from algaenan-composed thin outer walls of microalgae. However, the lack of predominance of the \( n \)-alk-1-ene/\( n \)-alkane doublets in the KL pyrolysates points to a moderate contribution of selectively preserved, resistant, aliphatic macromolecules. The latter are probably derived both from terrestrial plants and from freshwater microalgae (in agreement with the \( \delta^{13}C_{\text{org}} \) and C/N values).

3.5.2. Isoprenoid hydrocarbons

Prist-1-ene and trace amounts of prist-2-ene were identified in KL pyrolysates (Fig. 7 and 8). Several origins were previously put forward for these ubiquitous pyrolysis products. The phytol side chain of chlorophyll \( a \) was proposed as a precursor of pristene upon pyrolysis (Ishiwatari et al., 1991, 1993). However, Goossens et al. (1984) demonstrated that the pyrolysis of tocopheryl moieties produces pristene through an intramolecular rearrangement, in much higher yields than chlorophyll \( a \). Tocopherols are known to be relatively abundant in the chloroplasts of most photosynthetic microorganisms. Prist-1-ene and prist-2-ene in pyrolysates of fossil leaf material (Nip et al., 1986a; Logan et al., 1993) and tegmens of fossil water plants (van Bergen et al., 1994) were considered as being derived from tocopherol. The absence of phytadienes, which have been shown also to originate from chlorophyll-bound phytol (van de Meent et al., 1980a,b; Ishiwatari et al., 1991), suggests that chlorophyll \( a \) was probably not the precursor of pristenes in KL pyrolysates. Indeed the phytol chain is esterified to the cyclic tetrapyrrole moiety of chlorophyll \( a \), which renders it less stable than tocopheryl units upon hydrolysis during HF/HCl treatment.

3.5.3. Organic sulphur compounds (OSC)

The three KLs are characterised by a very low \( S_{\text{org}}/C \) atomic ratio and a virtual lack of organic sulphur compounds (OSC) in the three pyrolysates. Accordingly, the so-called “natural sulphurisation” pathway (Sinninghe...
Damsté et al., 1986, 1989; Schmid et al., 1987; Adam et al., 1993) only played a negligible role in their formation. The presence of pyrite (Fig. 2h) shows that some bacterial sulphate-reducing activity took place in the sediment. Nevertheless, this activity was probably low, and there was enough active iron to scavenge the reduced sulphur species.

3.5.4. Phenols

The KL pyrolysates are characterised by large amounts of phenolic compounds, including a large number of methoxyphenols (Fig. 7 and Table 4). Phenol and its alkylated homologues were identified by selective ion detection (m/z = 94 + 107 + 108 + 121 + 122; Fig. 9). This series is dominated by phenol, 3- and 4-methylphenols, and 4-ethylphenol, which also correspond to relatively important peaks in the TIC traces (Fig. 7). The abundance of the alkylated isomers decreases with the number of alkyl carbons, the amount of C_3+ homologues being quasi negligible.

Alkylphenols, associated to short-chain (C_1–C_3) methoxy-substituted alkylphenols are typical pyrolysis products of unaltered or weakly altered lignin (e.g. Martin et al., 1979; Obst, 1983; Saiz-Jimenez and de Leeuw, 1986a; Hatcher and Spiker, 1988), thus reflecting a terrigenous input. Catechols (1,2-benzenediols) are often assigned to demethylated units formed from microbial degradation of lignin (Hatcher et al., 1988; Stout et al., 1988). Therefore, the recognition of a wide range of lignin monomer units (Table 4) with intact methoxyl groups and the minor amount of catechols indicate that weakly altered lignins were significant precursors of the phenols in KL pyrolysates. This terrigenous contribution as evidenced by the pyrolytic study is consistent with SEM observations showing selectively preserved ligneous debris (Fig. 2g), as well as with the relatively 13Corg-depleted isotopic composition (ca. −26‰).

Lignin pyrolysis products are characterised by the occurrence (or predominance) of monomer units with very specific substitution patterns (e.g. Martin et al., 1979; Obst, 1983). Gymnosperms (non-flowering plants) and angiosperms (flowering plants) synthesise different types of lignin, which allows for the identification of the general vascular plant sources of sedimentary lignin-derived material (Hedges and Mann, 1979). The predominance of guaiacyl (vanillyl) over syringyl phenols (G vs. S) among KL pyrolysis products is consistent with the predominance of gymnosperms in the watershed vegetation of the Danube delta.

Alkylphenols with a similar distribution as in the present study have already been observed in pyrolysates of soil OM, marine sediments, and suspended and dissolved marine OM (Saiz-Jimenez and de Leeuw, 1986b;
Table 4
Phenolic compounds identified in KL pyrolysates

<table>
<thead>
<tr>
<th>Peak number&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Main mass fragments</th>
<th>Lignin parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94,66, Phenol</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>122, 121, 77, (4-Methoxytoluene)</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>108, 107, 79, 2-Methylphenol (o-cresol)</td>
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<tr>
<td>4</td>
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<td>5</td>
<td>107, 108, 79, 3-Methylphenol (m-cresol)</td>
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</tr>
<tr>
<td>6</td>
<td>109, 124, 81, Guaiacol</td>
<td>G</td>
</tr>
<tr>
<td>7</td>
<td>107, 122, 77, 2,6-Dimethylphenol</td>
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</tr>
<tr>
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<td>107, 122, 2,4-Dimethylphenol + 2,5-dimethylphenol</td>
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<td>11</td>
<td>110, 64, 1,2-Benzenediol (catechol) ?</td>
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<tr>
<td>12</td>
<td>123, 138, 95, 2-Methoxy-4-methylphenol (4-methylguaiaicol)</td>
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<td>120, 91, 4-Vinylphenol</td>
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<td>18</td>
<td>121, 136, 4-Ethyl-2-methylphenol ?</td>
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<td>34</td>
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<td>40</td>
<td>137, 152, 2-Methoxy-4-ethylphenol (4-ethylguaiaicol)</td>
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</table>

<sup>a</sup> See Figures.

Sicre et al., 1994; Peulvé et al., 1996a,b; van Heemst et al., 1993, 1997; Zegouagh et al., 1999). In some of these previous studies (Sicre et al., 1994; Peulvé et al., 1996a,b) the distribution is relatively invariant with depth, thus indicating that this series of alkylphenols is probably derived from complex resistant macromolecules, corresponding to melanoidin-type components. Melanoidins originate from random...
condensations of altered moieties derived from proteins and polysaccharides, and provide OM preservation via the so-called degradation-recondensation pathway. Melanoidins are characterised by relatively high oxygen contents, and their infra red spectra exhibit intense absorptions due to OH and C=O groups (Rubinsztain et al., 1984, 1986a,b). Their general IR features, as well as the solid-state $^{13}$C and $^{15}$N NMR spectra obtained for some synthetic, amino acid-rich, melanoidins (Ikan et al., 1986; Benzing-Purdie et al., 1983), show important similarities with those of the KLs. Van Heemst et al. (1997) also prepared synthetic melanoidins from mixtures of a tyrosine-containing protein and carbohydrate components, leading to similar distributions of alkylphenols upon pyrolysis as observed in the present study. These observations therefore point to melanoidin contribution to the three KLs. In addition, some contribution of proteinaceous material which would have survived diagenesis and HF/HCl hydrolysis cannot be excluded. Indeed, alkylphenols are also generated upon pyrolysis of tyrosine-containing proteins or derived materials (Tsuge and Matsubara, 1985), and as recently shown (Knicker and Hatcher, 1997; Zang et al., in press), proteins can be protected from diagenetic and chemical alterations via encapsulation in macromolecular organic substances. The presence of melanoidins and/or of such protected proteins is supported by the predominance of amides in the solid-state $^{15}$N NMR spectra of the three KLs (Fig. 6), as well as by their relatively low C/N atomic ratios.

The above observations suggest the significant occurrence of another type of precursor, in addition to lignins and lignin-derived components, for the alkylphenols of KL pyrolysates: tyrosine-derived moieties preserved from degradation by incorporation in melanoidin-type macromolecules and/or by encapsulation within the macromolecular network of the KLs.

3.5.5. Alkylbenzenes

Toluene, an ubiquitous pyrolysis product of sedimentary OM, represents a relatively important contribution to the TIC traces of the three KL pyrolysates (Fig. 7). Several isomers of alkylbenzenes with C$_2$- to C$_{16}$-chains were identified in mass chromatogram $m/z = 91 + 92 + 105 + 106 + 119 + 120$ (Fig. 10), but the relative abundance of the latter series is low and strongly decreases with the number of alkyl carbons. Some of these compounds (e.g. n-alkylbenzenes with a C$_{3}$- to C$_{16}$-chain, or n-alkyl-methylbenzenes with a C$_{6}$- to C$_{11}$-chain) are probably formed during pyrolysis by secondary reaction via cyclisation/aromatisation processes (Hartgers et al., 1994a). This is confirmed by the ortho-position observed for the latter series. Nevertheless, some isomers with a low alkyl carbon number (i.e. in the C$_{1}$-C$_{4}$ range) might be primary pyrolysis products formed by $\beta$-cleavage of substituted aromatic rings linked to the macromolecular structure via an alkyl chain (Hartgers et al., 1994b).

Toluene and C$_{2}$-alkylbenzenes can be produced upon pyrolysis of phenylalanine-containing proteins or...
protein derivatives (Tsuge and Matsubara, 1985; Stan-
kiewicz et al., 1998). The former source cannot be ruled 
out for the three KLs since, as already stressed, some 
proteins may have survived diagenesis and HF/HCl 
hydrolysis owing to encapsulation in the macro-
molecular structure of the KLs. Similar distributions of 
alkylbenzenes with C 1- to C 3-chains were recently 
observed in pyrolysates of particulate OM from the 
northwestern Mediterranean Sea (Peulvée et al., 1996a) 
and of refractory OM isolated from sediments of the 
northwestern African upwelling system (Zegouagh et 
al., 1999). In the above studies, these alkylbenzenes have 
been considered as originating from phenylalanine-con-
taining moieties derived from altered proteins, and 
incorporated within melanoidin-type structures. This 
suggests, as in the case of alkylphenols, that the degra-
dation-recondensation preservation mechanism also has 
played a role in the formation of the three KLs.

Alkylbenzenes, sometimes including long chain com-
ounds, and probably derived from algaenans, were 
detected as major pyrolysis products in a few previous 
studies on kerogens (e.g. Horsfield et al., 1992; Sin-
ninghe Damsté et al., 1993). Such compounds were also 
observed in the pyrolysis products of resistant bioma-
cromolecular material isolated from marine microalgae 
(Derenne et al., 1996; Kokinos et al., 1998). TEM 
observations of the three KLs showed the presence of 
numerous cell walls, probably containing resistant 
algaeans or algaenan-like, biopolymers, based on their 
excellent morphological preservation. Consequently, 
resistant macromolecular material from microalgal cell 
walls might be an additional source of some of the 
alkylbenzenes detected in the KL pyrolysates.

The presence of 1,2,3,4-tetra-methylbenzene (TMB) 
in the pyrolysates of various kerogens was ascribed to 
specific aromatic carotenoids of Chlorobiaceae (Hart-
gers et al., 1994b,c; Guthrie, 1996; Clegg et al., 1997). 
Chlorobiaceae are anaerobic, photosynthetic, green sul-
phur bacteria, and their presence is an indicator of 
anoxia in the photic zone. Since the water column is 
oxic in the zone where the studied sediment core was 
collected, such a bacterial origin can be ruled out. The 
occurrence of 1,2,3,4-TMB in pyrolysates of the resis-
tant material isolated from several marine algae (Hoefs 
et al., 1995) also suggests an algal origin, which would 
be consistent with TEM observations in the present 
case.

3.5.6. Other aromatic hydrocarbons

Naphthalenes and indenes were identified by selective 
ion detection at m/z = 128 + 142 + 156 + 170 (Fig. 11) 
and m/z = 115 + 116 + 129 + 130 (Fig. 12), respectively. 
Naphthalene and indene are the dominant compounds 
of both series, but their contribution to the KL pyr-
olyssates is minor. The distribution pattern of C 1- to C 3-
alkylated isomers shows decreasing relative abundance 
with the number of alkyl carbons. Such distributions of 
naphthalenes and indenes are comparable to those
observed in previous studies of marine, particulate and sedimentary, OM (Peulvé et al., 1996a; Zegouagh et al., 1999). However, the sources and precursors of both families are unknown at present.

3.5.7. Nitrogen-containing compounds

Pyrroles, indoles, pyridines, and aromatic nitriles were identified in KL pyrolysates. These series were identified in mass chromatograms $m/z = 103 + 117 +$
131 + 144 (alkylindoles and aromatic nitriles; Fig. 13), \( m/z = 67 + 81 + 95 \) (pyrroles), and \( m/z = 79 + 93 + 107 \) (pyridines). In each family the unsubstituted compound predominates, whereas C1 alkylated isomers are minor compounds and C2 homologues occur in negligible amounts.

Alkylated pyrroles can be derived from chlorophyll pigments. But, in the present case, such an origin cannot be considered since their distribution pattern differs from that reported for alkylpyrroles present in pyrolysates of porphyrin derivatives (Sinninghe Damsté et al., 1992).

All the above mentioned classes of N-containing compounds can be ascribed to pyrolysis products of proteins or protein-derived units. For instance, indoles and pyrroles are often associated with tryptophan and proline moieties, respectively (Danielson and Rogers, 1978; Tsuge and Matsubara, 1985). As already discussed, some proteins may have been protected via encapsulation in the three KLs. Similar distributions of alkylindoles and alkylpyrroles were also observed in previous studies of dissolved OM from Pacific Ocean, suspended OM from the Rhone delta, sediment trap material from the northwestern Mediterranean Sea, and marine sediments from the northwestern African upwelling system (van Heemst et al., 1993; Sicre et al., 1994; Peulvé et al., 1996a; Zegouagh et al., 1999) and considered to be related to melanoidin-type structures. Accordingly, as already concluded for alkylphenols and alkylbenzenes, these N-containing pyrolysis products may be related to melanoids comprising degraded proteinaceous material, and/or to proteins encapsulated in the most refractory part of the KL structure.

3.5.8. Polysaccharide pyrolysis products

Polysaccharides are ubiquitous compounds in the marine environment, occurring as constituents of cell walls, storage material, constituents of marine snow, and mucus (de Leeuw and Largeau, 1993).

Pyrolysis of polysaccharides and polysaccharide-derived components produces furans and furan-derivatives (Helleur et al., 1985a,b). Dimethylfuran, 2-furaldehyde, 5-methyl-2-furaldehyde and 2-furanmethanol were identified in the KL pyrolysates in very low amounts, 2-furaldehyde being the major furanic fragment. The presence of polysaccharidic moieties in the three KLs is confirmed by the signals at 74 and 105 ppm in their solid-state \(^{13}\)C NMR spectra. Furfurals and methylfurfurals have also been observed by Boon et al. (1986) in the pyrolysates of sedimentary OM from the estuary of the Rhine river, and cellulose was considered as a potential source for these pyrolysis products. Thermal degradation of cellulose results in a wide variety of products including furans and pyrans (van der Kaaden et al., 1983; Pouwels et al., 1989). These compounds are generally detected in minor amounts, whereas high yields of levoglucosan were obtained from different celluloses (20–60 wt.%) (Shafizadeh et al., 1979). Since (i)
most of these compounds, and in particular anhy-drosugars like levoglucosan, were not detected in KL pyrolysates, and (ii) the bulk of the polysaccharides that occurred in the sediments was most likely eliminated by the HF/HCl attack applied for eliminating the mineral matrix, the presence of furanic components in KL pyr-olysates can be attributed to degraded ligno-cellulosic complexes of higher plants and polysaccharide-derived moieties in melanoidin-type components.

3.5.9. Fatty acids

A minor series of straight-chain saturated fatty acids (C6-C18) was detected in KL pyrolysates (chromatogram of m/z = 60 + 73; Fig. 14). The C16 homologue is predominant and is observed in a significant relative abundance in the TIC traces of KL pyrolysates. This series is dominated by even-carbon-numbered homologues. Considering the moderate level of alteration of the acid moieties (indicated by this even predominance), it is likely that weakly altered saturated fatty acids were incorporated early within melanoidin-type structures, as previously observed for sediments from the north-western African upwelling system (Zegouagh et al., 1999). Indeed, Larter and Douglas (1980) reported on the participation of some lipids in the condensation reactions occurring during melanoidin formation. The absence of branched acids among KL pyrolysis products points to a negligible contribution from bacterial lipids in the condensation steps.

4. Conclusions

The main conclusions of this study, carried out via a combination of microscopic, spectroscopic, isotopic, and pyrolytic methods, on the KL isolated from three sections of a recent sediment core off the Danube delta, are summarised below:

(i) The kerogen-like material accounts for a sub-stantial fraction of total OM in the three samples (ca. 50%).

(ii) Similar morphological features and chemical composition are observed for the KL isolated from these sediments.

(iii) The main source of the KLs is an admixture of land-plant and freshwater microalgal material, whereas the contribution of bacterial material was low.

(iv) The selective preservation of terrigenous material (including ligneous debris and pollen and plant spores) as well as of resistant aliphatic bioma-cromolecules (probably derived from cell walls of microalgae) was the main process for KL for-mation.

(v) The KLs comprise substantial amounts of protei-naceous moieties, for which two preservation pathways can be considered: incorporation in melanoidin-type structures formed, via the degra-dation-recondensation pathway, from degraded
proteinaceous and polysaccharidic materials, and/or encapsulation of proteins within the macromolecular network of the three KLS.

(vi) The role of natural sulphurisation was negligible in KL formation.

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