

## FORMATION OF AN ALIPHATIC POLYMER DURING HEATING EXPERIMENTS WITH *LYCOPODIUM* SPORES

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The main components of *Lycopodium* spores is the biopolymer sporopollenin and significant amounts of ester-bound fatty acid material. The preservation of these components in the geosphere is currently the object of investigation (De Leeuw et al. 2006). To understand the chemical transformations that occur during the diagenesis of spores, solvent extracted and saponified examples from *Lycopodium* were heated under vacuum in sealed glass tubes at a series of temperatures (100-400 °C) for 48 hours. Hydrous pyrolysis of *Lycopodium* spores was also conducted in an N<sub>2</sub> atmosphere. Following heating, spores were solvent extracted with toluene and methanol (9:1) and allowed to dry before analysis by FT-IR. Spores were further characterised by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and thermochemolysis-GC-MS using tetramethylammonium hydroxide (TMAH) as the reagent.

With increasing temperature the FT-IR analyses show a decrease in oxygen containing functional groups, a decrease in overall aromaticity and an increase in the aliphatic component. The FT-IR spectra for the heated spores closely resemble that from fossil spores.

The Py-GC-MS chromatograms also clearly demonstrate that there is an increase in the aliphatic material in the pyrolysable component with increasing temperature. Figure 1 displays the Py-GC-MS total ion chromatogram from a sample of *Lycopodium* spores heated under vacuum at 300 °C for 48 hours. The main products released upon pyrolysis are a series of alkane/alkene doublets (C<sub>6</sub> to C<sub>17</sub>) along with benzene, toluene, ethylbenzene and xylenes (BTEXs).

FT-IR analyses reveal that the aliphatic component in the fresh *Lycopodium* spores is present as ester bound *n*-carboxylic acids (mostly *n*-C<sub>16:0</sub> and *n*-C<sub>18:1</sub>) and *n*-diacids (C<sub>8</sub> to C<sub>20</sub>). The distribution of the alkane/alkenes (i.e. predominance of *n*-C<sub>15</sub> and *n*-C<sub>17</sub>) is consistent with their source being the ester bound organic acids. It appears that this resistant aliphatic polymer has been formed from the hydrolysable component with sporopollenin, an observation that is consistent with the recent results and interpretations (e.g. Gupta et al.; *In press*).

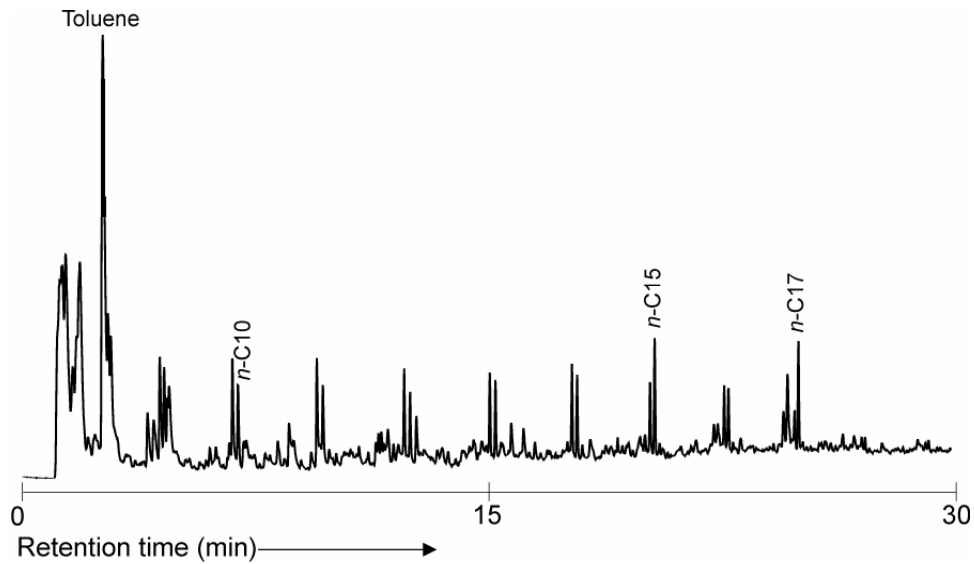


Figure 1. Py-GC-MS (at 610 °C) total ion current of *Lycopodium* after heating at 300 °C for 48 hours under vacuum.

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## DETECTION AND STRUCTURAL CHARACTERIZATION OF BLACK CARBON FROM NATURAL COMPLEX MATRICES

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Due to their well known geochemical and environmental implications, there is an increased interest in the study of the different forms of refractory organic matter (ROM) widely widespread in soils, water and sediments. Included in the refractory OM pool are kerogens, humic-like materials as well as the so-called “black carbon“ (BC). These materials might remain sequestered in soils and sediments (slow geological C cycle) from thousand to million of years, being considered as an important sink for atmospheric CO<sub>2</sub> (fast C cycle) and part of the “missing C” in the global C budget (Kuhlbusch, 1998).

Aiming to detect the occurrence of BC forms in natural complex matrices three set of samples including i) the reference samples recommended by an international interlaboratory research on Black Carbon (BC) (BC Ring-Trial), ii) a selection of fire affected soils and iii) a selection of marine sediments from the Southwest Atlantic coast of Spain (Gulf of Cadiz) were analysed. Bulk samples and isolated refractory forms were characterized by an array of analytical techniques including thermogravimetry (TG), TG coupled with differential scanning calorimetry (DSC), solid state <sup>13</sup>C CP-MAS nuclear magnetic resonance (NMR) spectroscopy and Pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC/MS).

Thermal analysis (TG, TG-DSC) provide mainly information about the proportions of labile, recalcitrant and refractory OM forms, whereas Py-GC/MS and <sup>13</sup>C NMR provide complementary windows of structural information on the bulk matrices and their OM fractions (López-Capel *et al.*, 2006; Quénéa *et al.*, 2005, 2006; Knicker *et al.*, 2006).

Good agreement was observed between recalcitrant C as determined by TG analysis and the aromatic content measured by <sup>13</sup>C NMR. Py-GC/MS showed an important presence of aromatic compounds in samples rich in BC like material. <sup>13</sup>C NMR revealed a loss of signal intensity in the alkyl C-O region of the spectra of the BC forms compared with demineralized sediment. Several samples from marine sources revealed a high contribution of aliphatic compounds to the refractory OM fraction suggesting the relevance of alkylic moieties in the stabilization of carbonous materials in this environment.

By using analytical pyrolysis (Py-GC/MS) it was possible to directly identify, with no pretreatment, specific changes in the product of burning/charring of different materials. Several specific compounds released after pyrolysis allows a clear identification of potentially BC-interference reference materials and, in BC rich samples, compounds that could be used as markers of pyrogenic events. Other markers were also identified that provide information about the origin of the OM.

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## RESOLVING UNRESOLVED COMPLEX MIXTURES: CHARACTERISTICS OF NATURAL ORGANIC MATTER DETERMINED BY ULTRAHIGH RESOLUTION MASS SPECTROMETRY

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Recent progress in the field of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) resulted in new and extensive insights in the molecular composition of natural organic matter (e.g. (Koch *et al.*, 2005; Kujawinski *et al.*, 2004; Stenson *et al.*, 2003)). In this work, we focus on the marine dissolved organic matter (DOM) which represents one of the largest active pools of organic carbon in the global carbon cycle. Its amount ( $700 \times 10^{15}$  g C) is comparable to that of carbon in atmospheric CO<sub>2</sub> ( $750 \times 10^{15}$  g C) and 30-times larger than all carbon in marine animals, plants, bacteria and marine organic particles combined ( $\sim 25 \times 10^{15}$  g C). Despite the importance of DOM, the proportion of deep-sea dissolved organic carbon (DOC) that can be characterized with conventional methods on the molecular level is marginal. The lack of molecular information prevents detailed characterization of the exact sources, availability to organisms, transformation and preservation mechanisms, binding to minerals and heavy metals and the role of DOC in the global carbon cycle.

Especially the enormous resolution power of  $>200000$  of modern FT-ICR-MS and the precise mass accuracy of up to 200 ppb allowed mass based separation of thousands of ions in the spectra and resulted in new insights into the molecular composition of marine DOM. We can assign thousands of molecular formulas which are suitable to distinguish between sources and degradation processes. Although every molecular formula can represent a multitude of chemical structures, FT-ICR-MS data can also provide structural information. The aromaticity index AI (Koch & Dittmar, 2006) is a conservative tool to identify aromatic or polyaromatic structures in a molecule and is solely based on the determination of the exact mass and the molecular formula. A new liquid-chromatography method allowed a polarity based separation of DOM molecules and to reduce the complexity of the FT-ICR-MS spectra. On the basis of this method, ions of different molecular elemental composition can be separated (Figure 1) and even dissolved nitrogen and sulphur compounds can be identified.

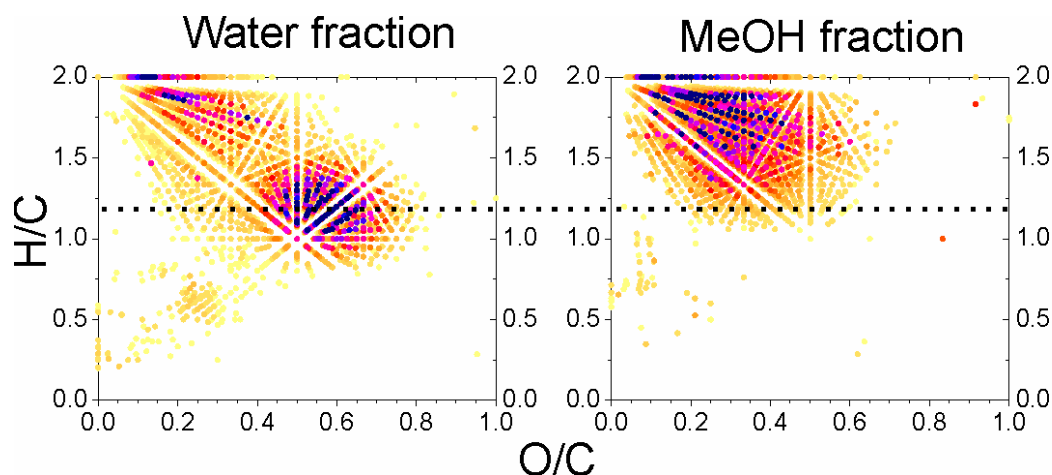


Figure 1. Molecular element ratios from an ESI-FT-ICR-MS analysis for a polar (left) and a less polar (right) chromatographic fraction of a marine DOM sample. Chromatographic separation and preparative enrichment was performed on an HPLC-system using an RP18-column and a H<sub>2</sub>O/methanol gradient. Decreasing polarity results in lower O/C and higher H/C ratios and shifts the ions to the upper left corner of the plot. The colours represent peak intensity.

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**NON-INVASIVE MOLECULAR CHARACTERIZATION OF KEROGEN AND ITS  
INSOLUBLE BIOPOLYMER PRECURSORS BY COMBINED NUCLEAR  
MAGNETIC RESONANCE AND FOURIER TRANSFORM MASS  
SPECTROMETRY**

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Molecular-level structural characterization of complex biopolymers (e.g., lignin, cutin, cutan) in plants and their fossilized counterparts is a key element to understanding the origin of fossil organic matter formation such as kerogen and coal. Molecular characterization of such geopolymers, however, is difficult due to their heterogeneity and insolubility in common aqueous or organic solvents. Accordingly, thermal or chemical degradative approaches have traditionally been utilized to examine fragments that presumably represent the original untreated structures. Here, we present two novel analytical approaches for changing tradition and gaining molecular-level insight into kerogen structure and that of its precursors in order to evaluate the various hypotheses for kerogen formation.

High Resolution Magic Angle Spinning (HRMAS) NMR is a recently developed solution NMR technique that allows for the single and multi-dimensional NMR analysis of solid polymers that become partially softened in a swelling solvent (i.e., DMSO-d<sub>6</sub>) leading to enhanced molecular mobility. As an example, Figure 1 shows a two-dimensional [<sup>1</sup>H-<sup>13</sup>C Heteronuclear Single Quantum Correlation (HSQC)] HRMAS spectrum of white oak lignin. Such a high resolution spectrum was not previously possible for solid lignin, and the observed cross-peaks are consistent with chemical moieties of lignin (see caption of Figure 1). One can also obtain other traditional solution multi-dimensional data, such as TOCSY, COSY, NOESY, etc. Thus, we can examine generally insoluble macromolecular structures in kerogen and its possible precursors to establish the nature and origin of the kerogen. Macromolecular structural elements in the precursors preserved in the kerogen would clearly indicate a selective preservation pathway for kerogen formation. Identification of unexpected structures would indicate that other structures have been incorporated by condensation reactions.

The other technique is ultrahigh resolution Fourier Transform – Ion Cyclotron Resonance - Mass Spectrometry (FT-ICR-MS) which requires solubility in a moderately polar solvent. To achieve this, kerogen and precursors are incubated with pyridine resulting in the

extraction of a portion of kerogen. Wu et al. (2003) employed such a method to examine coal extracts by FT-ICR-MS. The key to interpreting the results of these extracts as being representative of the insoluble macromolecular structure is establishing a correspondence between the extract and the solid kerogen structure. This is accomplished by multidimensional solution NMR studies of the extract, and comparing the spectra with those obtained by HRMAS. In our example for white oak lignin,  $^1\text{H}$ - $^{13}\text{C}$  HSQC solution-state data of the lignin extract produced the same crosspeaks observed in the HRMAS  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of the solid lignin sample (Figure 1). This observation confirms that the extract is *chemically representative* of the solid sample and that FT-ICR-MS analyses can therefore be used to molecularly represent the structural components of kerogen and precursors. The inset in Figure 1 shows a high resolution mass spectrum of white oak lignin in which detailed molecular formulas can be derived to describe the molecular components of lignin. We have examined a series of kerogens and precursor algal and terrestrial biopolymers to verify that selective preservation is an important process in kerogen formation but that molecular reorganization of the structures occurs due to maturation.

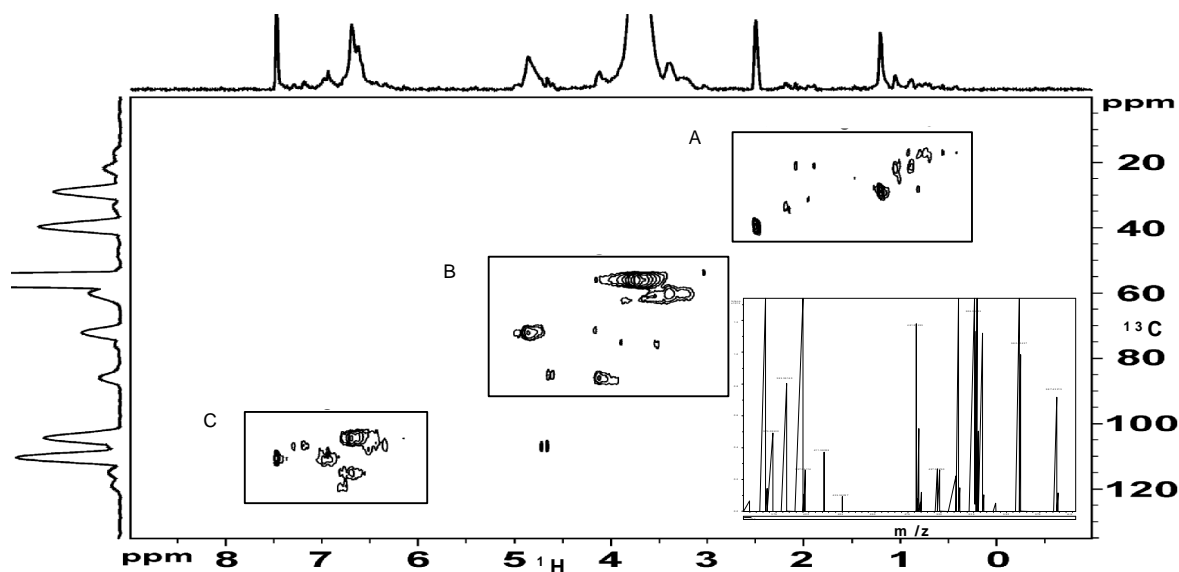


Figure 1.  $^1\text{H}$ - $^{13}\text{C}$  HSQC HRMAS spectrum of White Oak Lignin. Area A is the unsubstituted alkyl linkages, area B is the alkyl – O linkages, and area C is the lignin aromatic linkages. The inset is the high resolution mass spectrum of the lignin extract from  $m/z$  350 – 600.

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**NATURAL ORGANIC MATTER IN THE GLOBAL CARBON CYCLE:  
STRUCTURES AND ORGINS AS REVEALED BY MODERN NMR  
SPECTROSCOPY**

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Natural Organic Matter (NOM) in soils and water represent the largest reserves of carbon on earth. The global soil carbon pool is 3.3 times the size of the atmospheric pool and 4.5 times that of the biotic pool and it is considered that at least 50% of this soil carbon can be categorized as the chemically resistant component known as humic substances (HS). Conversely, dissolved organic matter (DOM) represents the largest pool of mobile carbon on the Earth and is a fundamental link between terrestrial and aquatic environments. This communication utilizes the latest technologies and novel 1-dimensional and multi-dimensional NMR spectroscopy to assess the composition and sources of NOM. As a culmination of years of NMR-based research, we attempt to define the major structural components in organic matter from soils and water, explain why the key structural components in the aquatic and terrestrial NOM are so different, and assess the key contributions from microbial biomass, which in many soil samples, is underestimated by orders of magnitude.

HS in soils have largely remained uncharacterized at the molecular level and have necessarily been defined operationally in terms of the methods used to extract or isolate them. Formation processes of HS have been debated for decades. It has traditionally been thought that extractable HS consist of novel categories of structures formed through varying biotransformation processes. Today, the predicted future and modelling of the soil carbon stock relies heavily on the temperature sensitivity of this carbon component. In this study, advanced Nuclear Magnetic Resonance (NMR) approaches were used to look at major components (proteins, carbohydrates, aliphatic biopolymers and lignin) that are known to be present in HS, and identify their fingerprints in humic mixtures. Theoretically, once all known components have been identified the remaining signals should be from materials with novel structures, themselves forming a distinct chemical category of humic materials. Surprisingly, nearly all of the NMR signals in traditional HS fractions could be assigned to intact and degraded biopolymers. We therefore suggest that the vast majority of operationally defined

humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products but not a distinct chemical category.

Furthermore, microbial contributions to soil organic biomass, appears in many cases to be significantly underestimated. Generally it is accepted that <5% of soils organic matter is comprised of microbial biomass (Alef 1993; Dalal 1998). However comparisons of cultured microbial biomass to soil organic matter indicate that in many cases  $\geq 50\%$  of the humic and humin (non extractable component) is actually derived from microbial cells. Much of this material that is highly enriched in proteinaceous material is likely present due to the release of microbial cell contents during lysis by the NaOH extraction, which is employed in the standard isolation procedure of humic substances. Much of this cellular material that in many cases contributes more than 50% by weight to the operationally defined "recalcitrant humic fraction", may be chemically labile and present inside living cells.

Similarly, aquatic DOM contains a range of interesting and novel structures very different from those derived from parent plant and microbial biopolymers in soils. Building on exceptional work by Hertkorn et al. (2006) and utilizing novel long range multiple bond correlations, we confirm that carboxyl-rich alicyclic molecules (CRAM), heteropolysaccharides (HPS) and open chain aliphatic polycarboxylic acids (OCAP) are the major components in aquatic DOM (Hertkorn et al. 2006). Using long range correlations the structure of the OCAP can be further refined and many of the carboxyl groups are found to be attached at methyl substituted carbons. 2-dimensional NMR identifies large concentrations of linear terpenoids present in Lake Ontario DOM that contain conjugated unsaturations with methylated branches analogous to those found in carotene structures. It appears these linear structures are the most likely the precursors of the OCAP material in the aquatic DOM whereas CRAM is likely derived from cyclic terpenoids. Implications of these findings in terms of the global carbon cycle will be considered and a range of novel NMR approaches designed for the study of extremely complex environmental mixtures will be introduced.

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**STRUCTURAL DOMAINS IN PEAT AS REVEALED BY PHYSICAL FRACTIONATION, SEQUENTIAL CHEMOLYSIS AND <sup>15</sup>N- AND <sup>13</sup>C-CPMAS NMR SPECTROSCOPIES**

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A quantitative assessment of the distribution patterns of different C and N forms in peat as seen by solid-state <sup>13</sup>C and <sup>15</sup>N NMR spectroscopies was carried out. A sapric peat (Buyo basement, Northern Spain) was subjected to a set of physical and chemical fractionations (sequential or parallel), as well as to degradation procedures to obtain the following three series of peat subfractions: **i**) particle-size fractions isolated by wet sieving (particle sizes > 1 mm, 1–0.5 mm, 0.5–0.25 mm, 0.25–0.15 mm, 0.15–0.10 mm, 0.10–0.04 mm, <0.04 mm), **ii**) peat residues after selective extraction with organic solvents and aqueous solutions removing either bitumen-like fractions (ethanol, butanol, dioxane) or humic substances (0.1M NaOH, 0.1M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>), and **iii**) peat residues remaining after mild degradation for preferential removal of esters (BF<sub>3</sub>-MeOH transesterification), breakdown of ether bonds in *O*-alkyl structures (IH-degradation), or preferential cleavage of carbohydrate, protein and other *O*-containing structures (acid and alkaline hydrolysis, or heating at 350 °C during successive periods). In particular the spectra from series **(i)** would inform on the role of particle size (in peats considered as index for decomposition degree) in the quantitative speciation of C and N forms, whereas series **(ii)** and **(iii)** would yield information on the occurrence of C and N structures in more or less environmentally recalcitrant structural domains (i.e., often considered to be related with resilience, diagenetic transformation and/or biodegradability) such are carbohydrate-derived, nonhydrolyzable alkyl and peptidic structures, residues, thermally-stable ‘cores’, etc.

The <sup>13</sup>C and to some extent <sup>15</sup>N NMR spectra of the size fractions, which was believed to reflect differences in peat decomposition stages, were very similar. This is interpreted as particle-size distribution being not necessarily surrogate indicator of maturity or diagenetic transformation in selectively-preserved organic matter pools. On the contrary, the fractions isolated or prepared by chemical and thermal treatments showed conspicuous changes as regards the spectrum of the whole peat material. In fact, the progressive thermal treatments removed most *O*-alkyl structures, which was also the case with the hydrolytic treatments, a

behaviour more pronounced after alkaline hydrolysis. Partial degradation with IH led to dramatic changes in the original peat spectrum (removal of the *O*-alkyl domain). In particular drastic chemical degradation methods confirm to us that not only aromatic C-forms, but also alkyl and protein-containing structures represent the recalcitrant core of the peat organic matter. On the other hand, the occurrence of comparatively labile aliphatic structures were recorded mainly in the spectra of the bituminous fractions extracted with ethanol, butanol and even with dioxane. The latter fraction did not show the expected lignin-like spectral profile, which could be interpreted as extensive rearrangement and condensation of residual plant macromolecules in peat.

The parallel use of  $^{15}\text{N}$  NMR spectra showed: **i)** the above-indicated very weak trend towards concentration of heterocyclic N-forms in the fractions lower than 100  $\mu\text{m}$  size; **ii)** removal of heterocyclic N forms as direct or indirect effect of transesterification treatments; **iii)** concentration of heterocyclic N in the humic acid fraction, extracted with a chelating reagent ( $\text{Na}_4\text{P}_2\text{O}_7$ ); **iv)** survival of amide N-forms after strong acid hydrolysis indicating that only some the amide N-forms in peat occur in hydrolyzable forms, the remainder being a conspicuous constituent of the stable domains seen in the 'protokerogen-like'  $^{13}\text{C}$  NMR profiles obtained after most degradation methods; **v)** significant increase the heterocyclic N moiety after thermal treatment. Quantitative values taking into account C and N balance in extractive fractions and degradation residues revealed that the latter fact was not only necessarily due to the removal of non-heterocyclic N forms but also to endothermic cyclisation reactions. The overall results suggest that distribution of C and N forms in peat structural domains depend more on the global impact of biogeochemical processes on the sedimentary organic matter (e.g., thermal impact, waterlogging, dehydration...) than on a multicompartiment dynamics associated to fractions of different maturity or residence time, which is not reflected in particle-size fractions, and is only fairly defined by patterns of nonpolar extractive fractions and the extent of the nonhydrolyzable aliphatic domain.

**CARBON-14 AGES OF PRODUCTS FROM RUTHENIUM TETROXIDE  
OXIDATION OF MACROMOLECULAR ORGANIC MATTER IN MARINE  
SEDIMENTS**

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Concurrently deposited organic carbon in aquatic sediments can derive from multiple sources that differ markedly in age. Autochthonous biomass, transported terrestrial biomass, and kerogen eroded from sedimentary rock can all contribute to the total organic carbon in the system. Deconvolving these various sources is of great importance not only in understanding an organic matter cycling within a specific environment, but in constraining global carbon cycling and budgets. In recent years, techniques have been developed to measure the carbon-14 ages of individual compounds (Eglinton *et al.*, 1996; Smittenberg *et al.*, 2002). Characterization of the ages of extractable source-specific biomarkers in a variety of environments has highlighted the complexity of sources and processes that contribute to organic matter content and composition (e.g., Eglinton *et al.*, 1997). Thus far, compound-specific carbon-14 measurements have primarily been applied to solvent extractable carbon pools, leaving a large percentage of the total organic carbon isotopically uncharacterized at the molecular level. In this study, we expand upon this approach by combining chemical degradation of the insoluble macromolecular organic matter preserved in a variety of aquatic sediments with carbon-14 age determination, in an attempt to further constrain the inputs and fate of organic carbon in these systems.

Ruthenium tetroxide (RuO<sub>4</sub>) is a relatively mild and selective oxidizing agent that allows the isolation of aliphatic and alicyclic compounds from kerogen or other macromolecules (Standen *et al.*, 1991). It selectively targets aromatic units, converts ether bonds into ester bonds, and alkenes into aldehydes and ketones (Boucher *et al.*, 1990). Thus, cross-linked aliphatic and alicyclic compounds are liberated from the macromolecular matrix and rendered amenable to characterization by traditional chromatographic and spectroscopic techniques for extractable molecules. This chemical degradation technique has been applied to study the composition of biopolymers (e.g., Blokker *et al.*, 1998; Blokker *et al.*, 2006; Tegelaar *et al.*, 1989) as well as fossil and modern kerogens (e.g., Li *et al.*, 2004; Standen *et al.*, 1991; Yoshioka & Ishiwatari, 2005). Additionally, the carbon-13 isotopic value of the resulting products has been shown to reflect the bulk isotopic composition of the original kerogen (Kuypers *et al.*, 2002). RuO<sub>4</sub> oxidation in combination with a determination

of the Carbon-14 ages are being determined for RuO<sub>4</sub> oxidation products from a range of marine surface sediment samples that have previously been characterized in terms of their molecular <sup>14</sup>C composition and differ widely in source input and depositional environment. In this way, assessments can be made concerning the origin and pre-depositional history of macromolecular components of sedimentary organic matter.

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## REFRACTORY MACROMOLECULE AND BIOMARKER ANALYSES OF CRETACEOUS WOODY FRAGMENTS IN CENTRAL HOKKAIDO, JAPAN

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Land plant biomarkers have been frequently found as free compounds in ancient sediments and plant fossils, and can be useful as chemotaxonomic marker. However, such free biomarkers account for only a small fraction in plant organic fossil. The most part of plant fossil consists of refractory macromolecule, which is non-extractable with organic solvent, acid, and alkali. Refractory macromolecule survives decay more readily than free biomarker during diagenesis. Major sources of refractory macromolecules are thought to be plant biomacromolecule that were selectively *in situ* preserved. Refractory macromolecular and biomarker analyses were carried out for woody fossil fragments in Cretaceous sandstones of Oyubari area, central Hokkaido, in order to evaluate chemical state of organic molecules within the fossil and to obtain chemotaxonomic information from refractory macromolecule.

Fine to medium sandstones were collected from the outcrops of the Okusakainosawa sandstone and mudstone member of the Cretaceous (Albian) Shuparogawa Formation (Yezo Group), Tengunosawa of Oyubari area. The Shuparogawa Formation abundantly contained woody and coaly fossil fragments (Takashima *et al.*, 2004). Several larger fragments of woody macro fossil in rock samples were individually picked out. Rock samples were crushed to a fine powder. From the powder sample, woody fossil fragments were obtained by density centrifugation method (Sawada, 2006). Free lipids were ultrasonically extracted from the woody fragments with dichloromethane and methanol. The lipid extract was separated by silica gel column to four fractions. The residue after extraction was saponified with 1M KOH in methanol to obtain bound lipids. These fractions were analyzed by GC/MS.

The isomer ratios of C<sub>29</sub> steranes (20S/(20S+20R)) and C<sub>32</sub> hopanes (22S/(22S+22R)) averaged to be 0.45 and 0.58, respectively. Thus, maturity levels of the woody fossil fragments were arrived at the stage of late diagenesis to early catagenesis. Gymnospermous biomarkers such as diterpenoids (retene, abietane and pimarane etc.) could be identified as free compounds in all samples. However, there were no angiospermous biomarkers such as triterpanes (e.g., oleanane).

Organic molecules bound in macromolecules constituting woody fragments (with ester bonds), obtained by saponification, were mainly composed of short-chain (C<sub>14</sub> - C<sub>18</sub>) fatty acids. Series of n-alkanols up to C<sub>20</sub> were also detected. From the distribution pattern,

these alkyl constituents might be originated from selectively-preserved refractory macromolecule such as cutin or suberin with ester bond. Meanwhile, other alkyl compounds (*e.g.* hydroxyl acid) that expected to be obtained as major hydrolysate from cutin or suberin within plant fossil fragments could not be detected. It suggests significant alteration or loss of these moieties during diagenesis. Nevertheless, even carbon-number predominance was observed in fatty acids and n-alkanols, which indicated that biological state may be preserved. Moreover, distribution patterns of fatty acids were found to be almost similar in all samples, while those of n-alkanol significantly varied.

Result of free biomarker analysis suggested all plant fragments were gymnosperms, which was concordant with palaeobotanical study in Hokkaido area (Nishida, 2005). Carbon number distribution patterns of fatty acids appears reasonable when compared with previous study for Cretaceous gymnosperm fossil (Almendros *et al.*, 1999). We suggest that can provide us chemotaxonomic information at the broad level of taxonomic groups, such as gymnosperm and angiosperm.

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## THE MOLECULAR COMPOSITION OF SPOROPOLLENIN FROM FOSSIL MEGASPORES AS REVEALED BY MICRO-FTIR AND PYROLYSIS-GC-MS

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Sporopollenin represents the naturally occurring macromolecules that constitute the chemically resistant component of the outer wall (exine) of spores and pollens. The actual chemistry of sporopollenin has been a topic of debate for years (Dutta 2006; de Leeuw et al., 2006 and references therein). Fossil sporopollenins are of particular interest as they make up the maceral sporinite, an important component of some coals. To investigate the chemical composition of fossil sporopollenin, megaspores of Lower Tertiary (*Azolla* sp.), Lower Cretaceous (*Dijkstraiporites helios*, *Paxillitriletes midas*, *Cabochoenicus carbunculus*) and Upper Carboniferous (*Tuberculatisporites* sp., *Laevigatisporites reinschii*, *Calamospora laevigata*, *Zonalessporites* sp.) age have been investigated. Following kerogen isolation, about 10-50 individual megaspore specimens have been handpicked, cleaned by dichloromethane to remove soluble organic matter, and analysed by micro-FTIR and Curie-point pyrolysis-GC-MS.

Both spectroscopic and pyrolytic investigations clearly indicate that fossil sporopollenin is composed of aliphatic and aromatic moieties. The micro-FTIR spectra of the walls of all spores are characterised by aliphatic CH<sub>x</sub> (3000-2800 and 1460-1450 cm<sup>-1</sup>) and CH<sub>3</sub> (1375 cm<sup>-1</sup>) absorptions, aromatic C=C (1560-1610 cm<sup>-1</sup>) and CH (700-900 cm<sup>-1</sup>) absorptions and various C=O group absorptions at 1740-1700 cm<sup>-1</sup>. Alkylbenzenes and alkylphenols are the major aromatic pyrolysates. A homologous series of *n*-alkene/*n*-alkane doublets were detected in all fossil sporopollenin. Oxygenated aromatic compounds like benzaldehyde, acetophenone and 4-vinylphenol were found in the pyrolysates of all megaspores of Lower Cretaceous and Lower Tertiary periods. However, they are absent or occur below detection limit in the pyrolysates of Upper Carboniferous megaspores. The present investigations show that the aliphatic building blocks became selectively abundant, and oxygenated aromatic compounds

selectively degraded with diagenesis and increasing thermal maturity and time of burial. 4-Vinylphenol is the pyrolytic decarboxylation product of *p*-coumaric acid which is believed to protect the spore from UV-B radiation (Blokker et al., 2006 and references therein). If oxygenated aromatic compounds are selectively degraded during burial and diagenesis,

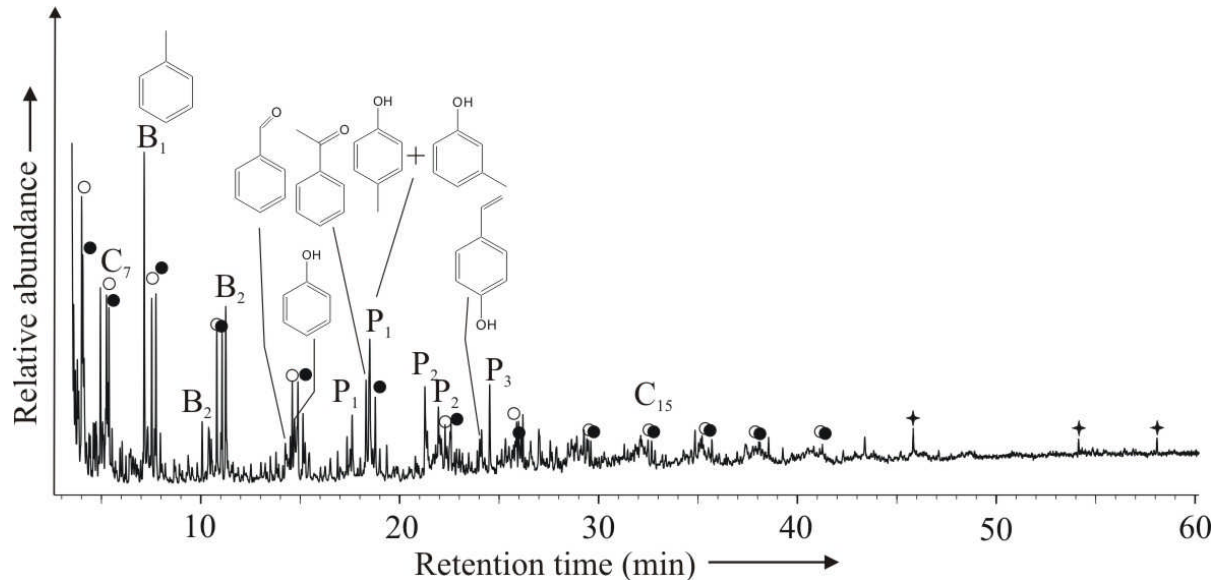


Figure 1. Total ion chromatogram resulting from the Curie point pyrolysis-GC-MS of Cretaceous megaspore *Dijkstraiporites helios*. Each doublet corresponds to an alkene (○) and alkane (●); selected C-numbers are indicated. B and P indicate alkylbenzenes and alkylphenols, respectively and their subscript indicate total number of methyl groups. + indicates contaminant.

then the presence of *p*-coumaric acid in spore wall holds little promise for assisting the reconstruction of past UV-B radiation. Within the realm of our present investigation, it is suggested that all megaspore walls had the same chemical composition prior to fossilization. The subsequent differences in chemical composition of fossil sporopollenin are likely due to the influence of different diagenetic processes and thermal history.

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## MOLECULAR MODELING AND EXPERIMENTAL STUDY ON SULFUR ISOTOPES CHANGES DURING SULFUR INCORPORATION INTO ORGANIC MODEL COMPOUNDS

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The reaction between sulfide (or polysulfides) and organic compounds has a significant role in the formation and preservation of sedimentary organic matter (SOM). Although the mechanisms for the formation of the sulfurized products were studied, not enough attention was given to the stable isotope ratio  $^{34}\text{S}/^{32}\text{S}$  ( $\delta^{34}\text{S}$ ) changes in these reactions.

The isotope fractionation during the reaction of inorganic reduced sulfur species and organic model compounds has recently been investigated (Amrani and Aizenshtat 2004). This study shows that the reaction of polysulfide anions with carbonyl compounds has a  $^{34}\text{S}$  enrichment of 4-5‰ at equilibrium. However, this study used polysulfides anions as nucleophiles assuming that the very complex polysulfide solution is isotopically homogenous and there is no  $^{34}\text{S}$  enrichment of the polysulfide as compare with the sulfide species in the same solution. A recent study challenged this idea showing that polysulfides are indeed  $^{34}\text{S}$  enriched by up to 6‰ as compare with the sulfide anions (Amrani et al., 2006). These finding may suggest that the observed  $^{34}\text{S}$  enrichment of the organic sulfur compounds is not due to  $^{34}\text{S}$  enrichment during the addition of sulfur into organic matter but rather selective addition of polysulfide anions into the aldehydes. This assumption is supported by the extremely higher reactivity of polysulfide in comparison with sulfide anions ((Loch et al., 2002).

In the present study, we combined theoretical calculations with experimental work in order to distinguish between these two mechanisms. We reacted aldehydes and haloalkanes with aqueous solutions of  $\text{CH}_3\text{SNa}$  or  $\text{HS}^-$  at pH=8-9 at 25°C. The reaction with  $\text{CH}_3\text{SNa}$  can not form polysulfides and therefore the isotopic effect can be attributed to the formation of C-S bond and measure its  $\delta^{34}\text{S}$  without the bias of polysulfide fractionation. The reaction with  $\text{H}_2\text{S}$  took place in an oxygen free environment to prevent oxidation of  $\text{H}_2\text{S}$  and formation of polysulfides. The results showed clearly that the incorporation of  $\text{CH}_3\text{SNa}$  results in  $^{34}\text{S}$  enrichment between 3.0 to 5.3‰. The main products from the reaction with the aldehydes were *gem* di-sulfides. The isotopic results are in a good agreement with thermodynamic calculations that put the upper limit for this enrichment at 5.5‰ for aldehydes. The reaction

with H<sub>2</sub>S (actually HS<sup>-</sup>) yielded the same general products but the isotopic enrichment was higher 5.5-7.8‰. Molecular modeling for this reaction put the upper limit for this isotopic enrichment at 9.3‰ for aldehydes. These results demonstrate an equilibrium isotope effect (Figure 1) as the reaction of aldehydes with sulfides is known to be reversible. Reaction with haloalkanes can demonstrate different mechanism controlled by kinetic isotope effect, as the results ranges between -3‰ to 0‰. Molecular modeling for these reactions involves the location of a transition state with high-level Density Functional Theory (DFT) calculation at the DFT/B3LYP/6-31G\*\* levels and are still in progress. Currently we study the isotope effect of other functionalities (such as conjugated double bonds etc.) in the incorporation of H<sub>2</sub>S under an anaerobic environment, from both molecular modeling and experimental approaches. In addition to the bulk measurement, organo-sulfur products will be isolated to individual compounds and measure for their δ<sup>34</sup>S value to give a better understanding of the mechanism pathway. The results of this study will help to interpret the isotopic differences that are often observed between OS and pyrite and may give insight into sedimentary organic sulfur composition and mechanism of formation as connected to their δ<sup>34</sup>S measured values.

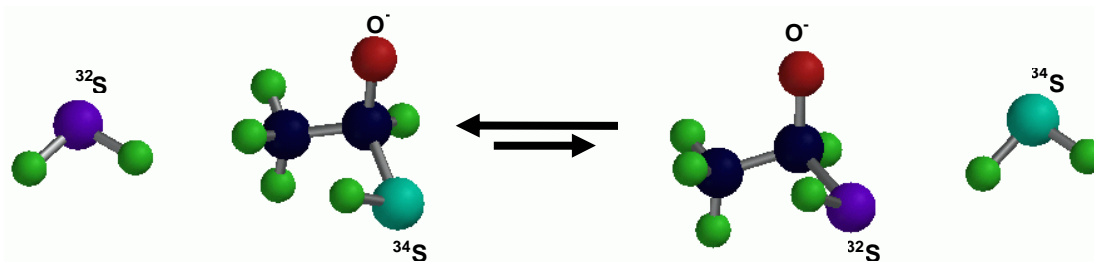


Figure 1. Isotopic equilibrium between <sup>32</sup>S and <sup>34</sup>S during reaction of H<sub>2</sub>S with aldehyde.

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**CHARACTERISATION OF ANTARCTIC FULVIC ACIDS BY A NOVEL AND SELECTIVE CHEMICAL REDUCTION – IMPLICATIONS FOR THE IDENTIFICATION OF PRECURSORS OF HUMIC SUBSTANCES**

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The complex supramolecular nature of fulvic acids (FA) can be attributed to the high abundance of carboxylic acids, phenols and hydroxyl functional groups present in their structures. The abundance of these polar functional groups also contributes to difficulties faced when analysing FA. Although various FA characterisation techniques have been reported (Abbt-Braun et al., 2004), no single technique or combinations of techniques have been completely successful in the structural elucidation of fulvic acid. The novel reduction technique used in this study enables the reduction of polycarboxylic acids to their corresponding methyl groups using *n*-butylsilane/tris (pentafluorophenyl) borane in a one-pot reaction (Nimmagadda and McRae, 2006a). The reduction of polycarboxylic acids to their hydrocarbon equivalents offers the potential for the transformation of supramolecular fulvic acid into simpler ‘backbone’ structures amenable to characterisation using GC and GC-MS. Similarly, aldehydes, ketones, primary, secondary and tertiary alcohols are reduced into their corresponding methyl functions (Nimmagadda and McRae, 2006b). Phenols and polyphenols are reduced to silylethers.

‘Backbone’ structures of standard fulvic acids such as Suwannee River fulvic acid, Elliot Soil fulvic acid and Waskish Peat fulvic acid were studied using the above reduction technique. The products identified from these standard fulvic substances were straight chain aliphatic compounds, methyl substituted cycloalkanes, methyl substituted aromatic hydrocarbons, reduced analogues of various phenolic and aromatic carboxylic acids, methoxy derived compounds and alkylated furans. The reduced compounds were still complex and correlation of input source to backbone structure still proved difficult.

In an attempt to improve the correlation of input source to backbone structure, samples of fulvic material isolated from fresh water and hypersaline lake systems found in Vestfold Hills, Antarctica. The Vestfold Hills is a 400 km<sup>2</sup> ice free region of the Antarctic continent. The lakes of the region are estimated to be 5000-8000 years old and are the result of the recent emergence of land above sea level (Pickard, 1986). These lake systems are considered pristine with well defined and relatively simple organic inputs and little or no anthropogenic

input (Pickard, 1985). The fulvic acids isolated from these lakes appear less oxidised, with precursor material being better preserved than their more equatorial counterparts. Interpretation of the structural information obtained from the reduced products of these Antarctic fulvic acids provides considerable insight into understanding the formation of fulvic acids from known organic inputs.

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## **STRUCTURAL HETEROGENEITY OF LIGNITE HUMIC ACIDS AS REVEALED BY ALIPHATIC LIPIDS RELEASED AFTER THERMOCHEMOLYSIS**

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Lignite and its alkali-soluble humic acids should not be viewed as some kind of low quality fuels only, but also as a valuable raw material for different chemical applications due to its naturally high content of functional groups which can, among other things, influence the bonding distribution of trace metals in the environment.

In this study we investigate and compare hydrocarbons and fatty acids from humic acids derived from the South-Moravian lignite. Humic acids were obtained following the IHSS procedure (Peuravuori *et al.*, 2006; Klučáková and Pekař, 2005). These compounds were released after solvent extraction (bitumens) or with a preparative thermochemolysis system with different alkylating agents such as TMAH and TEAAc to get structural informations and identification of minor products often hidden in flash pyrolysis (Grasset and Ambès, 1998). Tetraethyl ammonium acetate (TEAAc) is employed for the selective alkylation of free acids without transesterification of esterified ones (Grasset and Ambès, 2002). The products obtained after thermochemolysis, as well as bitumens, were separated and analyzed by capillary GC and GC-MS.

Analyzable compounds were mostly aliphatics. Aromatic units derived from lignous skeletons (known as one of the major constituent of lignite) were detected only as traces and then appear to be recalcitrant to thermochemolysis. Aliphatic compounds released after the solvent extraction of humic acids and after thermochemolysis are principally linear hydrocarbons and fatty acids.

The distribution patterns of fatty acids (C<sub>14</sub>-C<sub>32</sub>) present a strong even/odd carbon number predominance in the longer mode with a maxima at C<sub>28</sub> (Fig. 1) and attest to a plant origin. Linear fatty acids (methyl esters form) released after TMAH treatment present a greater proportion of the longer mode (originating from higher plants) in comparison with those of fatty acids from TEAAc (ethyl esters form) and of directly extractable one. The presence of FAMES as products from TEAAc treatment shows that some of FAMES released after the TMAH treatment were initially present as FAMES in the studied sample.

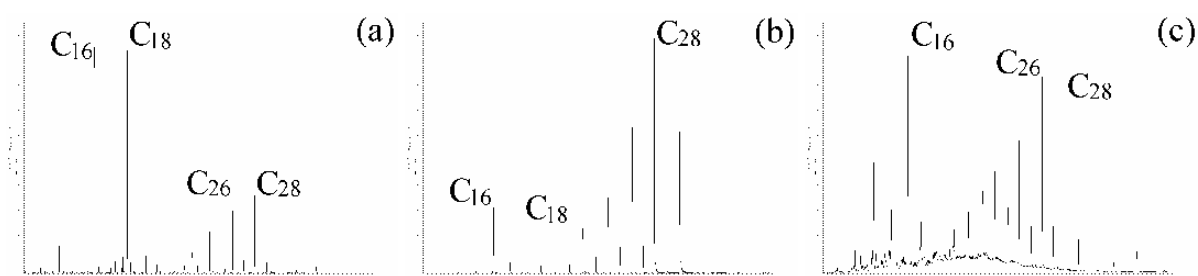


Figure 1. Distributions of fatty acids (as esters) obtained after solvent extraction (a), TMAH (b) and TEAAc (c) thermochemolysis.

Distributions of linear hydrocarbons obtained from the three procedures show sensible differences. The directly extractable hydrocarbons have a typical origin from higher plants with a monomodal distribution from  $C_{17}$  to  $C_{35}$  members with an odd/even carbon number predominance centered on the  $C_{29}$  component. The distribution of linear hydrocarbons obtained after thermochemolysis with TMAH were sensibly different with a slight even/odd carbon number predominance centered on the  $C_{24}$  and  $C_{26}$  components originating probably from a bacterial activity. The distribution of those obtained after thermochemolysis with TEAAc were centered on the  $C_{25}$  and  $C_{27}$  members without odd/even preference in the shorter mode ( $< C_{24}$ ).

TMAH and TEAAc thermochemolysis made it possible to discriminate between total and "free" fatty acids. Lipids trapped by macromolecular structures and free, directly-extractable, lipids have not the same origin. The differences observed between compounds released by thermochemolysis and by solvent extraction suggest that these moieties are not "intimately" mixed in humic acids, but forms juxtaposed blocks or "aggregates" of large size, which explains partly their heterogeneous nature.

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**ISOLATION AND CHEMICAL CHARACTERIZATION OF HIGH-MOLECULAR WEIGHT DISSOLVED ORGANIC MATTER (HMW-DOM) FROM FRESHWATER LAKES IN JAPAN, BY USE OF TANGENTIAL FLOW ULTRAFILTRATION.**

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Since Benner et al. (1992) succeeded in separation of high molecular weight dissolved organic matter (HMW-DOM) from a large volume of ocean water by use of tangential flow ultrafiltration technique (TFF), a great many works have been published on isolation and characterization of DOM in seawater and coastal waters (*e.g.*, Mannino and Harvey, 2000; Ogawa and Tanoue, 2002). However, only limited works have been done on freshwater regimes (Repeta et al., 2002). It led us to examine chemical characterization of HMW-DOM from freshwater lakes of different trophic status (eutrophic L.Suwa, mesotrophic L.Kizaki and L.Nakatsuna and oligotrophic L.Aoki).

Tens to hundreds mg of high molecular-weight dissolved organic matter (HMW-DOM) were isolated from 80-100 L of water samples from four freshwater lakes in Nagano Prefecture, Japan. Apparatus used was a Millipore Ltd. Pellicon Lab Cassette TFF equipped with a Millipore Ltd. PCAC and/or PLAC filter of nominal molecular cut-off of 1000 Dalton. Retention efficiency of TFF was assayed by filtrating aqueous solution (2 mg/L) of cyanocobalamine (MW=1355). It revealed that about 50 % of this size of molecules would be retained, when 100 L of sample water is condensed to 1 L (concentration factor = 100) (Yoshiyama et al., 2003). The DOM solution concentrated by TFF was further condensed by stirring ultrafiltration (Amicon YM1: MCO=1000 Dalton) and finally lyophilized.

The puffy powder accounted for 44-70% of the total dissolved organic carbon (DOC) in the sample waters. Chemical nature of the HMW-DOM was characterized by CN elemental, carbon and nitrogen stable isotopes, and lipid analyses.

Stable isotope ratios of carbon ( $\delta^{13}\text{C} = -29.3 \sim -23.9 \text{ ‰}$  against PDB) and nitrogen ( $\delta^{15}\text{N} = +0.5 \sim +8.6 \text{ ‰}$  against air) are heavier for HMW-DOM from a eutrophic lake (-24.1 ‰ and +8.6 ‰) and lighter for HMW-DOM from an oligotrophic lake (-29.3 ‰ and

+0.5 ‰). The data from two mesotrophic lakes (-27.4 ‰: +1.3 ‰ and -26.5 ‰: +4.1 ‰) fell between the two extremes. On the other hand, the C/N elemental ratio is low (11.1) for a eutrophic lake water and high for meso- to oligotrophic lake waters (12.6 to 17.4). Fig. 1 shows the correlation between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , where data dispersed on a straight line, suggesting a variable contribution of autochthonous microbial origin of organic matter to HMW-DOM according to the trophic status of the lake.

Fatty acids associated with HMW-DOM were compared with those of particulate matter (PM) and plankton collected in parallel. Fatty acids in HMW-DOM are mostly short-chain saturated and unsaturated homologues, being indicative of microbial origin. Relative proportion of branched bacterial fatty acids was also apparently large in HMW-DOM.

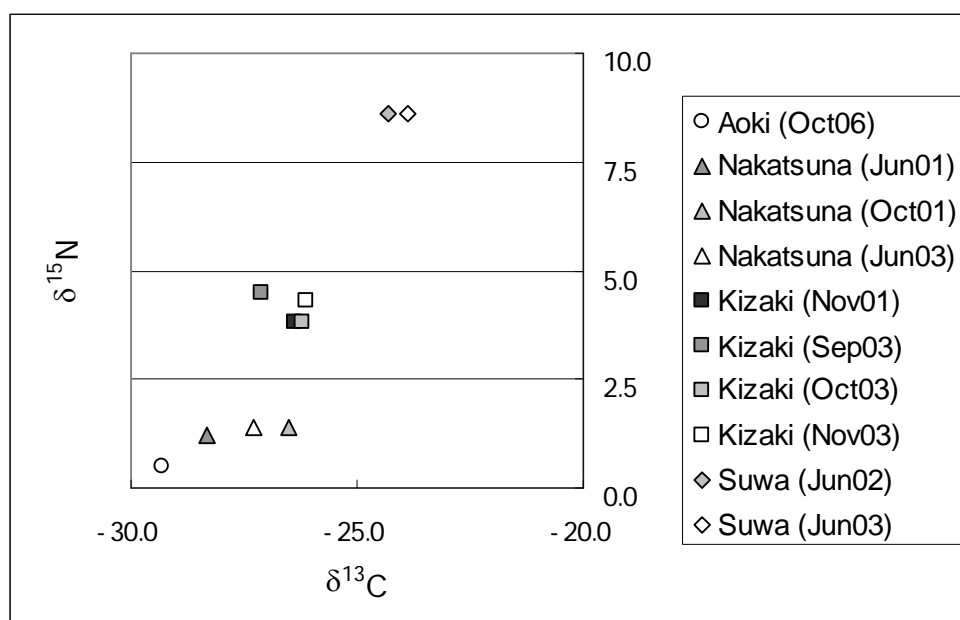


Figure 1. Correlation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of HMW-DOM separated from freshwater lakes.

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**ORGANIC GEOCHEMICAL CHARACTERIZATION OF HUMIC AND FULVIC ACIDS OF THERMAL WATERS IN DEEP AQUIFERS OF THE PANNONIAN BASIN (SE HUNGARY)**

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The presence and emerging of several organic solutes (e.g. humic acids, hydrocarbon gases, aromatic hydrocarbons, phenols and fatty acids) have been reported in thermal waters (TWs) from Pannonian Basin, their origin only is partly known (Kárpáti et al., 1999, Brukner-Wein, and Sajgó, 1990). In this study we concentrate on one group of the components: humic substances (HS) whose two fractions: fulvic and humic acids (FA and HA) usually are present in natural and ground waters, as parts of the dissolved organic matter (DOM). The aquatic FA and HA of TWs in deep aquifers may have many different origin: e.g. influx with recharge during sedimentation or subsidence; in situ generation in natural or in ground waters; dissolved from lignites where were generated in situ during subsidence. The FA and HA are chemically and physically very active components within their systems: they may physically and/or chemically bound to minerals, they act as detergent (solubilize hydrophobic compounds) and they are sensitive oxidation/mineralization, and their dissolution and precipitation processes occur continuously. The behavior of HS in aqueous solution is therefore of considerable interest, especially in view of the fact that it exists in many varieties, depending on age and origin influenced by other substances present in the environment, and likely on temperature that is in question in this study.

In this work after the isolation of humic substances from three TWs originating from the Makó area (SE Hungary), the FA and HA fractions have been characterized by different methods. Some general data for TWs are summarized in the following Table.

wells	depth [m]	temp. [°C]	pH	m-alk. <sup>1</sup> [mmol/L]	HA <sup>2</sup> [mg/L]	FA+HA <sup>3</sup> [mg/L]	TOC ppm	COD <sup>4</sup> [mg/L]
Makó Spa	993	46	8.90	14.90	6.4	2.89	8.5	8
Makó Hospital/2	1703	74	9.44	25.53	3.2	2.75	17.6	12
Agricult. use (Benkő Ltd.)	2300	92	9.14	44.56	23.2	5.53	257	47

<sup>1</sup>The methylorange alkalinity titrated potentiometrically; <sup>2</sup>measured spectrophotometrically (420nm) using Fluka standard (No: 53680); <sup>3</sup>after isolation measured gravimetrically; <sup>4</sup>Chemical Oxygen Demand

Hot groundwater samples (slightly yellow-greenish yellowish, tar-smelling brownish) were collected from three deep wells with 1332, 2432 and ~4109 mg/L total dissolved solids,

respectively, in February 2006. The temperature was measured on sampling sites, and the pH, alkalinity, UV-VIS absorption, FTIR, fluorescence and other chemical parameters were determined in the laboratory after cooling down. During isolations the methods for preparation of IHSS aquatic HAs and FAs were followed.

In earlier surveys (Varsányi and Bertalané, 1985 and Kárpáti et al., 1999) the HA contents in ~150 TWs were determined on the basis of photometrical analyses using Fluka or Aldrich standards (coming from modern depositional environments), but as it was suspected, this method provided only rough estimations of concentrations in case of TWs (see Table: HA and FA+HA results). It is also corroborated by that the IHSS preparation procedure of HA and FA from waters could not be applied in case of the hottest TW and in the appearance of the precipitates: flocculated (colder ones) lumpy/sticky (hottest) precipitate formed during separation.

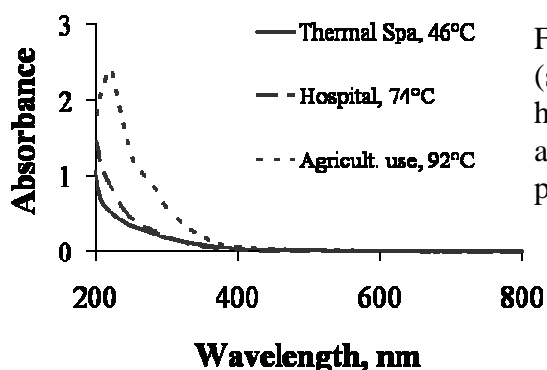


Figure 1. The larger the DOM contents (see COD and TOC values in Table), the higher the UV absorption. Increasing light absorption in the visible range indicates the presence of more and more HA.

The acidity of FA fractions is almost the same and greater than that of the HA samples both on basis of net proton surface and FTIR. On the basis of the previous 2 methods the amount of acidic groups in the HA fractions is significantly different in samples. The FTIR spectra of HA and FA of TWs are complex and the appearance and intensity of absorption bands (aromatic nuclei and carbonyl groups) are different and they also differ from the spectra of FA and HA extracted from coals or from natural waters, reflecting the elevated temperature and the effect of the complex physicochemical environment (facies) in the aquifers.

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**NEW DATA ON THE CHARACTERIZATION OF HUMIC SUBSTANCES  
EXTRACTED FROM PHOSPHATISED FAECAL “PELLETS” (TUNISIA)**

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Humic substances (HS) were extracted from faecal “pellets” - ovoid phosphatic grains 50 - 200 µm in size - collected from commercially mined phosphatic sediments, in the Gafsa Basin. The phosphatic constituents were characterized by C, H, O, N and S elemental analysis, Rock-Eval VI pyrolysis, Fourier-transformed infrared spectroscopy (FTIR) and solid state <sup>13</sup>C CPMAS NMR.

Humic (HA) and fulvic acids (FA) were isolated utilizing the standard method recommended by the International Humic Substances Society (IHSS). Their amounts were obtained by quantifying carbon in each fraction obtained at each step of the fractionation procedure. The total amount of HS carbon exceeds 75 % of the total organic carbon contained in the phosphatic grains, with a predominance of HA (about 70 %). These high contents of HS are well correlated with the total phosphorus content ( $P_2O_5 > 30 \%$ ) of the studied grains. Such high concentrations of HS in Eocene phosphatic sediments imply an excellent preservation of the organic matter within the pellets, both from a qualitative and quantitative point of view.

The elemental composition of HA and FA shows that both these fractions have rather low ash contents (7 % for HA; 13 % in FA) but distinctive elemental compositions. The C content increases from FA (55%) to HA (65 %), the H content being poorly variable between both fractions (ca.7 %). In contrast, the O content (13 % in HA and 29 % in FA) decreases with increasing C contents. Nitrogen and sulphur contents range from 2 to 4 % and 5 to 13 % for HA and FA, respectively. The atomic ratios O/C and H/C values of 0.15 and 1.29 % in HA and 0.40 and 1.55 % in FA, respectively, indicate that these humic compounds originated from marine aliphatic organic matter and only reached a low degree of thermal maturity.

The FTIR analysis of humic materials shows a striking predominance of aliphatic structures and O-containing functional groups over aromatic and condensed moieties. The spectra obtained for HAs show that they contain more aliphatic and aromatic structures than FAs, which are relatively richer in carboxylic groups and carbohydrates.

The structure of HAs and FAs was also investigated using solid state  $^{13}\text{C}$  CPMAS NMR (Fig. 1). The displayed spectrum shows a strong aliphatic carbon peak (0-50 ppm), aliphatic carbon bound to oxygenated functions (50-110 ppm), aromatic carbons (100-160 ppm) and, finally, carboxylic/carbonyl functions (160-220 ppm). The  $^{13}\text{C}$  NMR spectroscopic analysis is therefore consistent with elemental and IRTF data, all indicating abundant oxygen-bearing aliphatic structures.

The study of the HS using Rock-Eval VI pyrolysis confirms the marine planktonic origin of the organic matter located within phosphatic pellets as already stated by Belayouni and Trichet (1983). RE pyrolysis also provides HI, OI and Tmax values confirming the humic character of the organic matter contained in pellets, i.e. that of an immature material.

Thus, all the analytical data obtained on these HA and FA witness the surprisingly good preservation of this sedimentary organic matter, in Eocene series (i.e. 50 Ma old). This good preservation resulted from the conjunction of several processes, each participating to the protection of the HS within the pellets. This preservation might be due to a combination of several factors such as the early formation of pellet limiting the exchanges with the external medium and the incorporation of reduced S in the organic matter, thus increasing its stability.

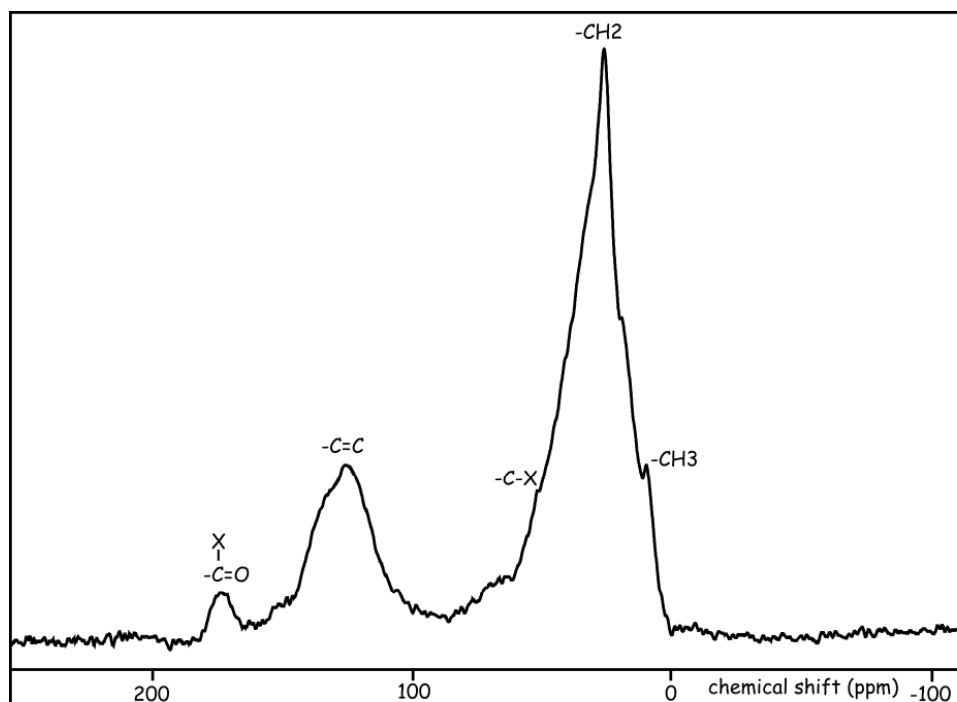


Figure 1.  $^{13}\text{C}$  solid state NMR spectrum of a HA extracted from phosphatic “pellets” collected from Gafsa Basin phosphatic sediments

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## COMPOSITIONAL CHARACTERISATION OF AQUATIC NOM BY MICRO-SCALE SEALED VESSEL (MSSV) PYROLYSIS

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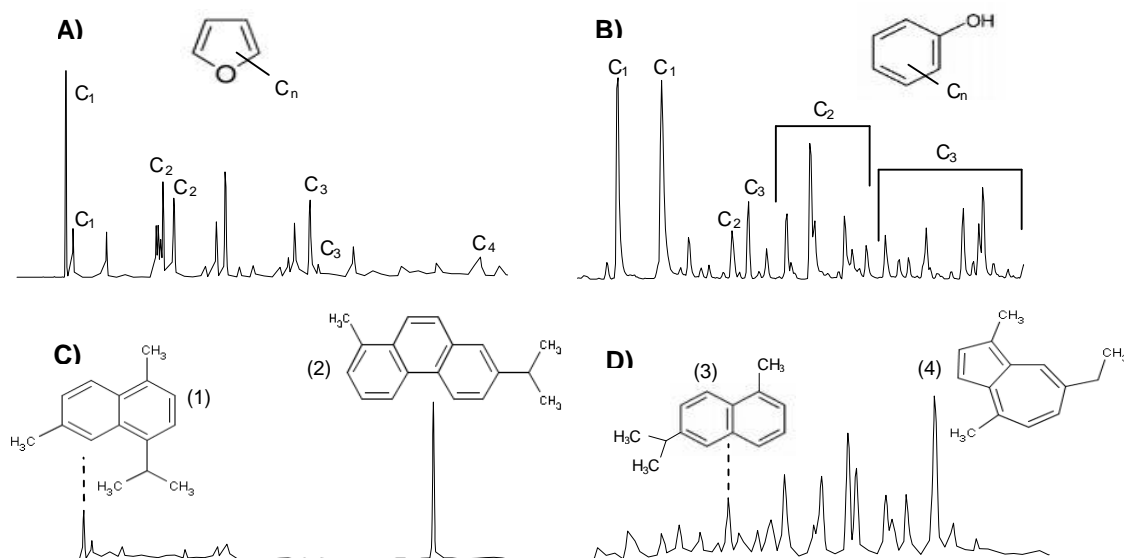
Analytical pyrolysis (e.g. flash or Curie-point) has proved useful for characterisation studies of naturally occurring OM, however, excessive degradation or high, biologically-inherited, structural polarity may limit GC (MS) detection of pyrolysis products. Closed-system micro-scale sealed vessel (MSSV) pyrolysis can overcome some of these issues. Although predominantly applied to kinetic studies of petroleum formation from sedimentary hydrocarbons (Horsfield *et al.*, 1989), the relatively mild thermal conditions of the MSSV experiment (i.e. 250-350°C/days Cf. >500°C and ballistic heating of flash pyrolysis) can facilitate both fragmentation and de-functionalisation of a wide variety of biochemicals. The recent MSSV detection of bacterial biomarkers in natural organic matter (NOM) of groundwater (Greenwood *et al.*, 2006) demonstrated the utility of this approach for yielding source diagnostic biomarkers from macro-molecularly bound precursors. Another recent study (Berwick *et al.*, 2006) similarly demonstrated the effectiveness of this approach for characterizing the nitrogen-containing pyrolysates of a number of aquatic NOM fractions.

This paper reports more holistically on the broad range of MSSV pyrolysates detected from a suite of NOM fractions isolated from various source and treated waters. An improved understanding of the structure and origins of NOM in potable sources will help understand practical NOM related issues such as disinfection by-product formation, fouling of membrane filters and microbial re-growth during distribution.

The hydrophobic (HPO), transphilic (TPI) and colloid NOM fractions yielded a broad range of pyrolysates, including many hetero-atomic products (i.e. oxygen- and sulphur- as well as the nitrogen-organics) of varied source diagnostic potential. The HPO fraction, for example, comprised alkylated furans indicative of polysaccharide precursors and higher plant derived products such as cadalene (1), retene (2), eudalene (3) and chamazulene (4). The colloid fraction yielded steranes indicative of eukaryotic input and many carbazole and indole products likely derived from alkaloid or bacterial structures.

Many more abundant MSSV products (e.g. alkylated phenols, benzenes and naphthalenes) are not diagnostic of specific structural precursors. A better understanding of

the biological origins and thermal generation of such products is sought by analysis of selected standards (e.g. polysaccharides, proteins, amino sugars and lignin) representing likely NOM structural precursors. Furthermore, the MSSV thermal profiles of pyrolysates from several samples have been investigated over the temperature range of 250°C - 350°C (at a constant 72hr). The product distributions are particularly scrutinised for evidence of additional molecular information to that provided by conventional pyrolysis and other characterisation methods (e.g.  $^{13}\text{C}$ -NMR, thermochemolysis) useful for establishing the structures and source inputs of recent organic materials like NOM.



**Figure 1.** Selected ion chromatograms from the MSSV pyrolysis GCMS of the HPO fraction of NOM from a Uruguay water source showing A) 81+82+95+96 Da - alkyl furans; B) 108+121+122+136 Da - alkyl phenols; C) 183+198+219 Da - miscellaneous aromatics; and D) 169+184 Da - miscellaneous aromatics.

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