ENVIRONMENTAL BIOGEOCHEMISTRY OF THE NORTHWEST ARM AND TRINITY BAY, NEWFOUNDLAND: NOVEL MOLECULAR AND CARBON ISOTOPIC APPROACHES

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Environmental biogeochemistry of the Northwest Arm and Trinity Bay, Newfoundland: novel molecular and carbon isotopic approaches

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A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

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July, 2001

St. John's Newfoundland

ABSTRACT

In this study, marine sedimentary organic matter of Trinity Bay and the Northwest Arm in spatial and temporal terms) was analyzed by pyrolysis and tetramethylammonium hydroxide chemolysis - techniques that degrade complex organic materials into moieties that are amenable to analysis. In addition, chemolysates were analyzed for their molecular isotopic signatures. The goal was to interpret the variation in the pyrolysate and chemolysate compositions in the context of sources and fates of organic matter and the influence of the surrounding communities on the distribution of terrestrially-derived material in the bay.

Pyrolysis products of organic matter present in near-shore sediments (Trinity Bay, Newfoundland) were characterized using GC/MS. The major products observed by "on-line" pyrolysis/GC/MS were nonadecene, phenol, methyl phenol, indole, and methylindole giving limited structural information about the sources of sedimentary organic matter. Spatial variation of the pyrolysates of the sediment were interpreted in the context of sources of organic matter, that were related to the historical economic activities of the study area.

A "batch-wise" TMAH thermochemolysis (*in-situ* methylation) technique was developed for the analysis of marine sediments. Standard phenolic aldehyde and acids and fatty acids were quantitatively methylated while triacylglycerol were converted to fatty acid methyl esters. Thermochemolysis of carbohydrates produced 1,2,4-trimethoxybenzene as an interesting

chemolysate marker. Polyunsaturated fatty acids could be analyzed using the thermochemolysis conditions employed.

TMAH thermochemolysis products of sediments and model compounds were characterized using GC/MS and GC/C/IRMS. Thermochemolysates of sediments included aromatic and aliphatic compounds determined as methyl esters and ethers. The dominant aliphatics are fatty acids that ranged from C_{14} to C_{28}, with a predominance of even carbon numbered species, and are either saturated, mono-unsaturated or branched. The aromatic compounds are phenols of which the most abundant is methylated vanillic acid, an important chemical marker in evaluating the terrestrial contribution to the organic carbon pool.

The spatial variation of the thermochemolysates indicates very high abundances of fatty acids and phenols in the near-shore sediments of the Northwest Arm rather than in the offshore sediment cores where fatty acids to phenol ratios were lowest for near-shore sediments. ^{210}Pb dates of sediments of two near-shore cores enabled calculation of annual preserved fluxes of organic carbon, total fatty acids and phenols. The fluxes of phenols were very high for one core, H1, and were related to saw-milling and logging activities of the community of Hickman's Harbour.

Compound-specific isotope analysis (CSIA) ($\delta^{13}C$) of thermochemolysates of standard fatty acids and phenols indicate that was was only minor isotopic fractionation ($0.4‰$ or better)

during high temperature methylation conditions. Also, there appears to be minor isotopic fractionation during base hydrolysis of ester bonds of a triacylglycerol. Theoretical mass balance calculations on phenols produced by TMAH thermochemolysis of a lignin dimer indicates that one of the phenols may have retained its isotopic signature after synthesis of the dimer. The $\delta^{13}C$ compositions of chemical markers were calculated after correction for the contribution of the methylating agent (TMAH) to the isotopic signature of individual the chemolysates. The isotopic composition of the phenolic markers, vanillin and vanillic acid show a strong similarity (depleted $\delta^{13}C$) to those same markers generated from adjacent terrestrial soil cover. Such supporting isotopic evidence, along with structural evidence that these TMAH products resemble guiacyl units of lignin, show that the TMAH thermochemolysates, methylated vanillin and vanillic acid are excellent biomarkers for terrestrially-derived carbon in a marine environment. In contrast, methylated coumaric acid shows a wide range in δ^{13} C values among locations, and with a more $\delta^{13}C$-enriched isotopic composition with a wide range (-28 to -19$^{o}/_{oo}$) originates from terrestrial and marine sources. Finally, the results of $\delta^{13}C$ values of fatty acids indicate that they are derived from multiple sources; $\delta^{13}C$ evidence also indicates that there are more contributors to the fatty acid pool in near-shore than in offshore sediments.

This thesis is dedicated to our good friend, **Allen Stark**, who passed away in 1996 before completing his MSc Project.

ACKNOWLEDGMENTS

I have accepted assistance from many individuals during the course of this study and I wish to extend my thanks to them. First, I thank Dr. Jun Abrajano for initiating this work and his support throughout this study. I extend sincere thanks to Dr. Robert Helleur and Dr. Christopher Periera for their comments, encouragement and advice on this thesis. I also thank Dr. Helleur and Dr. Periera for the timely rescue of this thesis and Ms. Linda Winsor for her day-to-day assistance in the laboratory and her words of wisdom and encouragement. Dr. Geoff Veinott has been a source of much-needed support in the final stages of this thesis and I thank him.

I am indebted to Darren Smith, Harold Whalen, Darren Jacobs, and Hannah Dayan for their extended assistance in computer related problems. I also acknowledge the assistance of the staff members of the Earth Sciences department, including Gerry Ford, Maureen Moore, Pat Browne, Gerry Sparkes, Pam King, Mike Tubrett, Liz Churchill, Maggy Piranian, Jeff Saunders, Allison Pye, Helen Gillespie and Rick Soper.

I appreciate the assistance of my academic peers including Dr. Vincent O'Malley, Ed Hudson, Yvette Favarro, Dr. Sue Budge and Dr. Terry Christopher. I would also like to thank my Information Technology colleagues, especially Derrick Roul, Duane Taylor, Carol Lidstone, Chris Jackson, Jeff Driscoll, Duane Carter and Catherine Bonnell. My

Information Technology instructors, especially Mr. Paul Gosse, have provided inspiration in many ways.

I extend thanks to the crews of the "Karl and Jackie", "Shamook" and "Nain Banker". I am also indebted to several members of Technical Services, including Jim Davis, Roy Crocker, Humphrey Dye, Doug Bolger, Don Filier, Ron O'Driscoll, Charlie Carter, Tom Perks and Jim Andrews.

In addition, I appreciate the financial support I received from the Eco-research Program. I am also very grateful to the School of Graduate Studies for granting me a much needed leave of absence.

I extend special thanks to my very close friends, Paul Blackwood, Ed Turpin, Allison Thorne, Pam and Vince Fulford, Dr. Mervin Marshall, Mrs. 'C' (Glenda) and Angela Clouter and Mary Elizabeth Archer. Finally, I extend special thanks to Judy and Alexander for their patience and understanding during this study.

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ABBREVIATIONS

CAD - *p*-Coumaric acid

CSIA - Compound-specific isotopic analysis

FAD - Ferrulic acid

FAME - Fatty acid methyl ester

GC/C/IRMS - Gas chromatography/Combustion/Ion ratio mass spectrometry

GC/MS - Gas chromatography/Mass spectrometry

ME - Methyl ester

OEP - Odd even predominance

OM - Organic matter

PUFA - Polyunsaturated fatty acid

SOM - Sedimentary organic matter

TOC - Total organic matter

TMAH - Tetramethylammonium hydroxide

TEAH - Tetraethylammonium hydroxide

VAD - Vanillic acid

VAL - Vanillin

PAD - *p*-hydroxybenzoic acid

SAD - Syringealdehyde

CHAPTER 1 - INTRODUCTION

1.1 OVERVIEW

Carbon combines covalently with up to four other atoms (mainly carbon, hydrogen, oxygen, nitrogen and sulphur) which in turn combines with the same atoms in a wide number of permutations. These combinations can produce an immense number of organic compounds of variable physical and chemical properties and environmental behaviours. The organic matter (OM) in terrestrial soils and marine sediments is quantitatively a small, but important fraction because the organic carbon content is linked to the biogeochemical cycling of many other elements, such as, nitrogen, sulphur and phosphorus. The cycling of OM influences the atmospheric abundances of carbon-containing greenhouse gases. Marine OM consists of a complex mixture of biologically derived compounds, including lignins, proteins, phenols, lipids, carbohydrates and humic compounds (Given, 1975; Henrichs, 1992). Humic compounds are widespread and comprise a major reservoir of organic carbon both in the soil, and in the oceans for the global carbon cycle (Aiken *et al.*, 1985); these compounds may constitute between 50 and 80% in modern sediments of the OM.

Although there has been significant advances in identification and analysis of organic compounds, there are many unresolved issues in the marine carbon cycle: for instance, over 50 % of the marine OM is unidentified at the molecular level. This study deals with the

analysis of the OM of the Northwest Arm and Trinity Bay area of Newfoundland using multiple geochemical tools. The organic geochemical data is interpreted in the context of sources and processes. In addition, these data are integrated with data from other studies to provide information about the sources of OM and processes leading to the formation of OM, which will be useful for subsequent studies.

1.1.0 Marine Carbon Cycle

The marine inorganic pools of carbon and biota are estimated at about 36860 and 3 Gt, respectively (Killops and Killops,1993) (Appendix1.1). Photosynthesis by phytoplankton accounts for about 95% of marine primary production and between 90 - 95% of the carbon that is produced by primary photosynthesis is consumed by heterotrophic activity, decomposed by microbial activity and oxidised by chemical processes (Rashid, 1985). This "recycled" OM is converted to new biomass, degraded particulate OM, regenerated nutrients and dissolved organic and inorganic matter; the remaining 5 - 10% settles as detritus (Wakeham and Lee, 1993).

The residual OM that eventually reaches the sediment/water interface is recycled by the detrital-feeding benthic community and oxidative alteration and destruction of OM continues after sedimentation (Mayer, 1993). The abundance of bacteria in sediments is highest at the sediment/water interface and these organisms consume a significant portion of the settling

OM. Sedimentary organic matter (SOM) at the sediment/water interface can be resuspended, resulting in re-exposure of OM to oxidation or further heterotrophic activity. In addition, bioturbation (or mixing that results from biological activity) also re-exposes OM to the sediment/water boundary. After burial below the bioturbation zone, SOM can be further altered by anaerobic bacteria. The end result is an accumulation of less reactive forms of OM. Generally, OM from aquatic sources are more easily degraded by bacteria; land-derived residues are relatively unreactive, possibly because of the nature of OM (being rich in lignins and tannins), but also because terrestrial OM generally have longer re-processing histories (Meyers and Ishiwitari, 1993). Reprocessing of terrestrial OM is influenced by its grain size and composition. Generally, terrestrial OM is delivered to the marine environment in larger particles than marine OM. Consequently, terrestrial OM has a lower surface area (compared to marine OM) that is exposed to oxidation agents.

Several factors affect the distribution of OM in marine sediments (Appendix 1.2). Primary productivity in the oceans results in OM being delivered to the sea floor. In coastal areas, primary productivity (by phytoplankton and marine algae) is high because the abundance of nutrients, and the TOC (total organic carbon) of sediments may vary between 3 and 5% (Rashid, 1985; Wakeham and Lee, 1993). Oxygen availability is also very important. Warm, oxygenated waters enhance primary productivity, as well as the growth of bacteria that decompose OM. In anoxic sediments, loss of organic carbon may vary from a few percent to about 40%; in oxic sediments this loss is usually as high as 80 - 90% (Rashid, 1985).

Seasonal variation in light intensity, temperature and nutrient availability result in a non-uniform rate of carbon production in seawater (Rashid, 1985).

Currents disturb, resuspend and redistribute sediments. This resuspension and extended residence time in the water column increase the exposure of SOM to bacterial degradation. Previous studies (e.g Henrichs, 1993) showed that there are positive correlations between the sedimentation rate and concentration of OM; high sedimentation can lead to rapid burial that appears to enhance preservation. Conversely, a slow sedimentation rate increases the duration of OM exposure to conditions of oxidation and degradation. In quiet bays where currents are not strong, the sedimentation rate is usually high and may be 500 cm per 1000 years (Duxbury and Duxbury, 1991). Hedges and Keil (1995) showed that the TOC generally increased with sedimentation rate; rapid deposition of sediment can protect SOM from overlying oxidising agents. TOC may also be related to the amount of dilution of sediments; i.e., low sedimentation rates and high fluxes of "preserved" OM can result in high TOC of sediments (Hedges and Keil,1995).

Geomorphological factors affect the distribution of OM. For example, sea floor depressions are areas in which sediments accumulate. Off-shore sediments usually have low carbon content (0.5%); in near-shore areas, the abundances of SOM are slightly higher (~3 to 5% or more) (Rashid, 1985). Sediments in coastal areas are richer in organic matter because of the high primary productivity resulting from the enrichment of nutrients derived from

drainage of terrestrially derived OM from the hinterlands (Hedges and Keil, 1995). Rainwater can leach organic soil, plant debris and inorganic constituents into dissolved and suspended phases (Millero, 1996). Several studies have indicated that flocculation and subsequent precipitation of OM in freshwater occurs when water from streams and rivers mixes with saline waters in estuaries. This apparent "salting out" of OM results in the restriction of allochthonous river-transported OM largely to coastal regions (Millero, 1996). Finally, atmospheric transport is another mechanism by which terrestrially derived carbon enters the marine environment (Hedges and Keil, 1995; Millero, 1996).

Research by several authors (Hedges and Keil, 1995; Mayer, 1994a and b) indicates that the surface area to volume of mineral particles available for adsorption of OM is a very important factor in its preservation; generally, the TOC increases with surface to volume ratio of sediment particles (Rashid, 1985). However, the specifics of OM/mineral interactions are poorly understood. Mayer (1994a and b) hypothesized that the burial of organic carbon is largely controlled by complex interactions of OM and mineral surfaces, and during burial terrestrial OM may be replaced by marine OM. OM in continental shelf sediment is shown to be primarily adsorbed on mineral surfaces (Mayer, 1994a). Correlations between mineral surfaces and TOC suggest an adsorptive control on the TOC stabilized in aluminosilicate sediments. Mayer (1994a and b) states that OM can be stabilized by its incorporation into mineral pores that are too small to allow entry and/or effective functioning of enzymes that degrade complex OM. Protection is achieved by restricting the entry of enzymes into pore

spaces that are occupied by OM and reducing the effectiveness of the enzymes by steric hindrance. OM is also protected by humus formation and selective preservation of refractory biochemicals. In addition, humus formation or condensation reactions may be pivotal in creating large molecules that become strongly attached to mineral surfaces by multi-point linkages.

1.1.1 Marine Organic Matter

A significant gap in understanding marine SOM diagenesis involves its compositional uncertainties (Henrichs, 1992). The OM of sediments can be divided into a chemically hydrolysable and a non-hydrolysable component (Chester, 1990). The hydrolysable component can be converted by acid or base hydrolysis into water-soluble substances that are amenable to characterization by various analytical procedures. The OM of rapidly accumulating coastal sediments consists of approximately 5% hydrolysable carbohydrates, 10 - 15% hydrolysable amino acids, less than 5% extractable lipids, less than 3% lignin and less than 1% volatile fatty acids (Henrichs, 1992) (Appendix 1.3). The non-hydrolysable component, accounting for over 70% of the TOC is poorly characterized and categorized as "humic-like" substances. Humic substances are defined as a class of relatively complex high-molecular weight, yellow to black coloured substances that are ubiquitous in sediments (Aiken *et al.*, 1985; Choudry, 1984; Stevenson, 1994). They constitute the largest pool of SOM (Aiken *et al.*, 1985). There are also major uncertainties associated with the pool of

marine humic substances because these compounds are not amenable to analysis.

Humic substances do not have well-defined chemical compositions. In addition, they are not comprised of easily identifiable monomeric units and should be considered as heterogeneous 'macromolecules' rather than natural 'polymers' (Tyson, 1995). Humic substances refer to three group of materials; fulvic acid, humic acid and humin. Humin is the insoluble residue which is produced by treatment of bulk humic materials with alkali (such as sodium hydroxide). Acidification of the alkaline extract precipitates humic acids, leaving fulvic acid in solution. These definitions originated from terrestrial soil science and are based on solubility characteristics. As a result, the definitions are purely operational. In marine sediments, between 120 and 150 individual constituents form an intricate network of humic material (Rashid, 1985). The organic precursors experience varying degrees of alteration with the resulting 3-D macromolecules showing very little resemblance to the original biomass components. The major components of humic substances include the following:

1. Proteins, amino acids and other related nitrogen containing compounds: The protein content of higher plants is generally low, between 1 and 10% (0.1 to 7 and 0.5 to 10% for coniferous and deciduous plants, respectively). Lower plants may have a nitrogen content of up to 40% (Rashid, 1985) while the protein content of marine bacteria varies between 40 to 70%.
2. Carbohydrates: are the most abundant constituents of terrestrial plants and may

constitute up to 70% of the organic carbon.

3. Lignin- and tannin-derived phenols and other aromatic compounds: are most abundant in higher land plants. Certain sea grasses have true lignin and are potential sources of lignin-derived phenols (Haddad and Martens, 1987) . However, aromaticity of marine humic materials is low as highly branched or cyclic aliphatic structures dominate (Hatcher *et al.*, 1981). The aromaticity of marine humic acids is estimated to be less than 15% (Hatcher *et al.*, 1981).
4. Lipids (fatty acids, fats and waxes) are found in both marine and terrestrial plants and are important structural components of marine humic compounds (Rashid, 1985). Aliphatic lipids comprise 24 and 22.2% of humic and fulvic acids, respectively (Rashid, 1985).
5. Other organic compounds resulting from microbial re-syntheses and chemical transformations. Phenols, polyphenols, quinones and other aromatics can react with amino acids, carbohydrates and other decomposition products during transformation to humic matter (Rashid, 1985).

The examination of the functional groups present in humic substances by infrared spectroscopy shows the presence of several oxygenated functional groups; these include -OH (from alcohols, phenols and acids), C=O (from aldehydes, ketones and carboxylic acids) and C-O (from alcohols, esters, ethers and carbohydrates) (Vandenbroucke *et al.*, 1985). C-H and N-H groups are also present. Carbon isotopic evidence suggests that marine humic

substances are primarily authochonous and plankton derived; humic acids from temperate marine sediments have $\delta^{13}C$ values between -22 and -17‰ (Vandenbroucke *et al.*, 1985). Humic substances are also characterized by a low degree of aromaticity and a predominance of aliphatic structures (Rashid, 1985; Tyson, 1995). However, in near-shore environments, many terrestrially derived phenolics enter the marine environment by a variety of processes. In addition, many microbes in the marine environment can synthesize phenols (such as 3,5-dihydroxy- and 2,3,5-trihydroxy phenol) that will ultimately be incorporated in marine humic substances (Rashid, 1985). It is expected that the isotopic signature of the phenols will reflect their sources.

Humic substances perform several important roles in the environment. The various functional groups of humic compounds (amine, carboxylate, etc.) facilitate formation of co-ordinate complexes between these sites and metal ions. As a result, humic substances are important agents for mobilization and transportation of metals. For example, lead and cadmium, which are highly toxic contaminants, have been immobilized through complexation with humic matter (Stevenson, 1994). Organic pollutants, such as pesticides and phthalates, also interact with humic substances that may act as a medium for promoting microbial degradation of many organic contaminants or form strong chemical bonds which will affect pollutant residues arising from the degradation of contaminants. In addition, primary productivity of coastal waters can be enhanced from the release of nutrients by humic substances, thereby stimulating the growth of phytoplankton and providing more food

for organisms that are higher in the food chain (Rashid, 1985).

All humic substances are variably interconnected (with respect to sources) through the medium of water, which is also the primary medium of transportation (Aiken *et al.*, 1985). Estuaries represent a transition zone from fresh water to a sea water environment, in which the humic substances consist of mixtures of terrestrial and marine end members (Mayer, 1985); also they are zones of intense humus formation (relative to the open ocean) (Mayer, 1985) .

In summary, the quality and quantity of OM in recent marine sediment depends on many factors. The distance away from the shoreline influences the type and amount of OM that reaches the marine sediments. The amount of terrestrially derived OM is influenced by human activities in coastal areas with significant contributions from vascular plants that are rich in lignin-derived phenols. The intensity of primary productivity influences the amount of marine-derived OM that reaches the sea floor and becomes incorporated into the pool of SOM. Environmental conditions will influence the degree of alteration of OM. The intensity of chemical degradation and the type of micro-organisms present in the water and sediment column are similarly dependent on water depth, which is indirectly related to position with respect to shoreline. Degradation through the water column determines the state of OM that is delivered to the sediment/water interface. Re-suspension and bioturbation are also important processes which further exposes OM to agents of degradation. Finally, the quality

and quantity of OM in marine sediments are closely related to interactions with detrital particles.

The initial stage of degradation of OM (applicable to this study) is diagenesis, during which, the OM loses nitrogen followed by loss of oxygen. Nitrogenous compounds, including proteins, appear to degrade faster than other classes of compounds (Killops and Killops, 1993). Carbohydrates are only slightly less readily degraded than proteins. Lipids are generally more resistant to degradation than nitrogenous compounds and carbohydrates. Lignins are the most resistant to microbial attack, although certain fungi and bacteria can decompose them. However, this order of stability may be altered by association of readily degradable compounds with resistant structures (Killops and Killops, 1993).

1.2 THE ECO-RESEARCH PROGRAM

The Eco-Research Program at Memorial University, commonly referred to as the "Green Plan" (*Sustainability in a Changing Cold-Ocean Coastal Environment*) was initiated in 1994 as a response to the Newfoundland fishery crisis. Earlier in 1992, a moratorium was imposed as a result of the depletion of the ground-fish stocks. In Newfoundland, the impact of the moratorium was staggering; at least 30,000 people were deprived of their normal source of income, resulting in a host of socio-economic problems (Ommer, 1998).

The overall objective of the Eco-Research Program was to establish the core components required to achieve sustainability for cold coastal communities in the future. It was deemed imperative to understand the elements that contributed to sustainability in the past, when groundfish was abundant, and identify how and when they have been disturbed. In this context, "sustainability" refers to people interacting in harmony with the natural systems of their environment in order to maintain health and integrity (Ommer, 1998).

The Eco-Research Program was a multi-disciplinary research endeavour that involved several teams of researchers in areas of social sciences, education, community health and the natural sciences which included a major study of the natural and anthropogenic influences on the carbon cycle in near- and offshore marine zones. The goal of the 'marine team' was to assess the health of the marine ecosystem in Trinity Bay and the extent of the changes (if any) of terrestrial and anthropogenic inputs in the present and recent past.

1.3 THESIS OBJECTIVES

A better understanding of the chemical composition and behaviour of OM of marine sediments will contribute to improvements in the carbon cycle model. It can be achieved by analysis of the SOM, in spatial and temporal terms in a cold coastal marine ecosystem. This study will contribute to the understanding of the geochemistry of sedimentary organic carbon of the Trinity Bay area and identify the factors that determine the quality of OM that is

preserved in the sediments. Specifically, the aims include the following:

1) To determine the chemical nature of SOM of the Northwest Arm and Trinity Bay study area. This involves degradation of the macromolecules into simpler marker units that can be detected and estimated. The signatures of specific markers, such as lignin-derived phenols, can then be utilized in tracing sources and processes.

2) To record the variations of the spatial and temporal profiles of "source" markers of the SOM in the study area and relate the distributions to sources and processes. Temporal profile studies are supported by ^{210}Pb dating of selected sediment cores.

3) To develop a method to isotopically characterize lignin-derived phenols and fatty acid markers. Fatty acids and phenols are major structural units of humic substances (Hatcher and Clifford, 1994) and other carbon pools in marine sediments. A critical undertaking involves the development of procedures which allow accurate and precise compound-specific isotopic analysis (CSIA) of marine sediment markers using gas chromatography-combustion-ion ratio mass spectrometry (GC/C/IRMS). The results are interpreted in the context of sources and diagenetic processes. Our study (Pulchan *et al.*, 1997) represents one of the few attempts at molecular level isotopic characterization of SOM.

4) To record the spatial and temporal variation of isotopic signatures of the SOM of markers and combine molecular signatures with their isotopic compositions in an attempt to interpret the historical carbon cycle of the study area.

1.4 ANALYTICAL APPROACH

Achieving the above goals would involve analysis of marine SOM, biomass tisssue, and local terrestrial soils by pyrolytically degrading and thermochemically fragmenting complex OM into small moieties that can be diagnostic of the parent OM. In addition, the carbon isotopic signature of markers were measured. Briefly, the analytical scheme is as follows:

1. **"On-line" Pyrolysis-gas chromatography/mass spectrometry** (GC/MS) (Chapter 2) - This is essentially an "on-line" thermal degradation process whereby macromolecules and other organic classes are fragmented (by the application of heat) into smaller molecules some of which are characteristic of the parent molecules. These daughter molecules are swept into an analytical GC column and identified by mass spectrometry.

2 **Tetramethylammounium hydroxide (TMAH) thermochemolysis-GC/MS** (Chapters 3 and 4) - Thermochemolysis in the presence of TMAH is a thermally assisted chemical degradation and methylation technique. The reaction temperatures are less severe than pyrolysis and information from this method complements that from pyrolysis. Results from TMAH thermochemolysis provide additional structural information because polar groups such as carboxylic acids and hydroxyl groups are methylated and not de-carboxylated or dehydrated as usually happens in direct pyrolysis. "Off-line" TMAH thermochemolysis products were dissolved in a solvent

and analyzed by GC/MS.

3. **Compound-specific isotopic analysis (CSIA) of TMAH chemolysates** (Chapters 5 and 6) - Model compounds were isotopically characterized by dual-inlet analysis. CSIA was performed on the chemolysates of model compounds added to an artificial sediment matrix in order to predict fractionation (if any) during derivatization and bond cleavage. This step is crucial for the interpretation of source identification of molecular markers of SOM. This was followed by CSIA of chemolysates of sediments and local soil samples using GC/C/IRMS.

Py-GC/MS and TMAH thermochemolysis are established techniques in organic geochemistry, and are also simple and inexpensive procedures that require minor sample preparation and small amounts of sample, thereby maximizing the information/effort ratio.

1.5 THE STUDY AREA

An important area of research includes the transfer of OM across interfaces where many important processes occur. The land/ocean and sediment/water interfaces are two important interfaces in the study area. The nature of sources, sinks and factors controlling rates of interaction between them are poorly understood at the above interfaces (Farrington, 1992).

Marine sediment samples were collected from the Northwest Arm and the main basin of

Trinity Bay, Newfoundland (Figure 1.1). The Northwest Arm is relatively shallow (50-80 m) and the water column well-oxygenated with bottom temperatures close to 0 °C throughout the year. Surface temperatures may reach 10 °C during the summer; winter temperatures are typically sub-zero (Budge and Parrish, 1998). The study area is highly productive because of a high supply of nutrients and oxygen. Marine production occurs in a strong seasonal cycle; Parrish (1998) measured highest concentrations of chlorophyll *a* (11.5 mg m^{-3}) during spring, with the maximum inputs during the month of May (1995 - 1997) in Trinity Bay. The study area also has a high preservation potential because the low water temperatures do not facilitate bacterial activity and reduce rate constants associated with degradation of OM (Budge and Parrish, 1998). Sediment trap data from Conception Bay, Newfoundland, that is adjacent to Trinity Bay, indicates deposition rates for particles in the water column 20 to 23 md^{-1} during the Newfoundland spring bloom (Parrish, 1998). In the open bay, sampling depth ranges from 302 to 335 m (stations ST10 and ST7). Assuming that settling rates in Conception Bay and Trinity Bays are similar, particles remain in the water column of this bay for approximately two weeks.

Marine OM is derived primarily from phytoplankton and macroalgal sources. The macro-algal flora include *Lithothamnion glaciale* (coraline algae), *Ptiloda serrata*, *Rhodomela confervoides* (laminaria), *Alaria esculenta* (Alaria), and *Zostera marina* (sea grass); the latter is abundant in low tidal areas. The isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) and amino acid ratios (aspartic/glutamic acid and tyrosine/phenylaniline) of OM from Trinity Bay cores

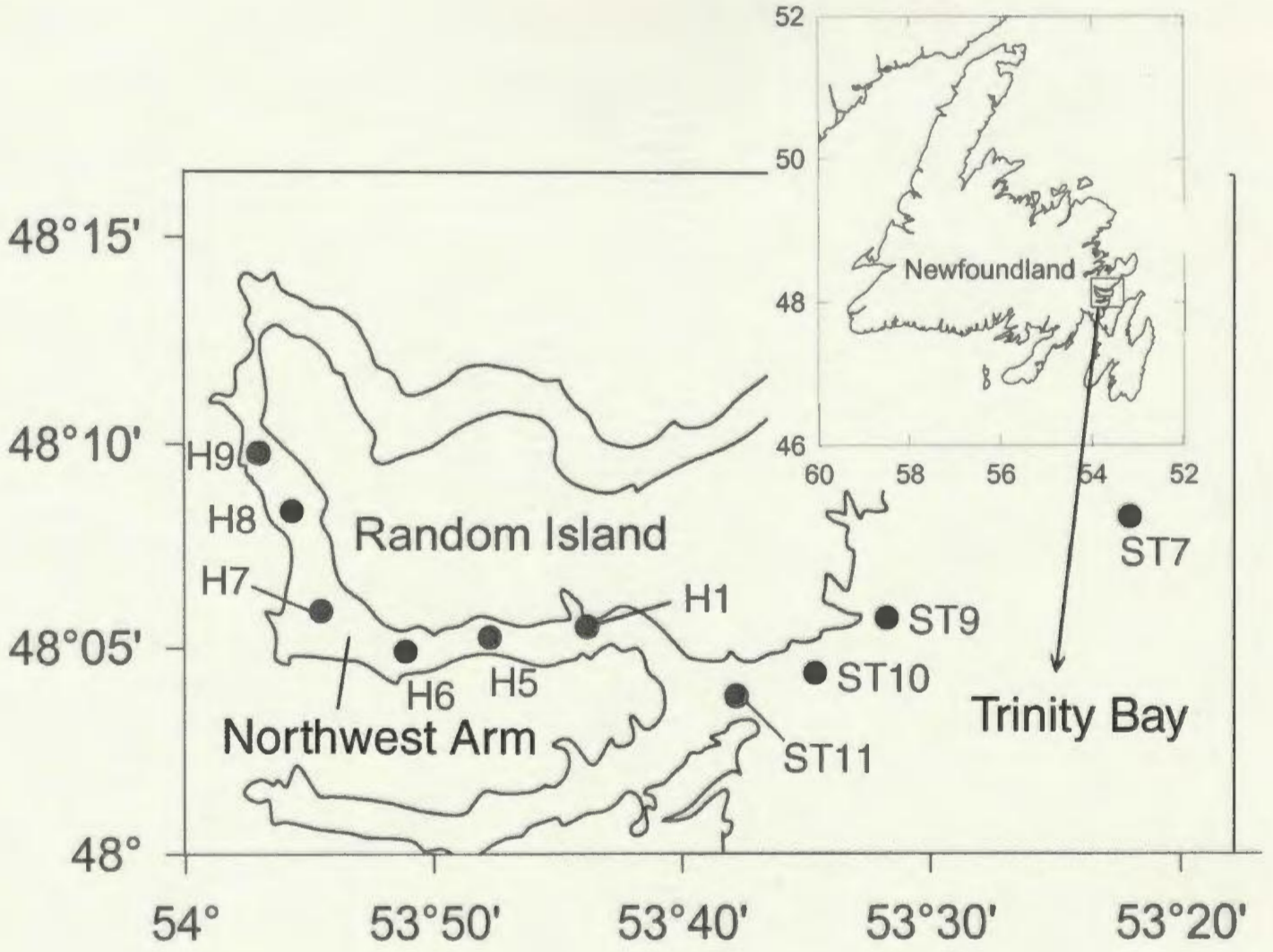


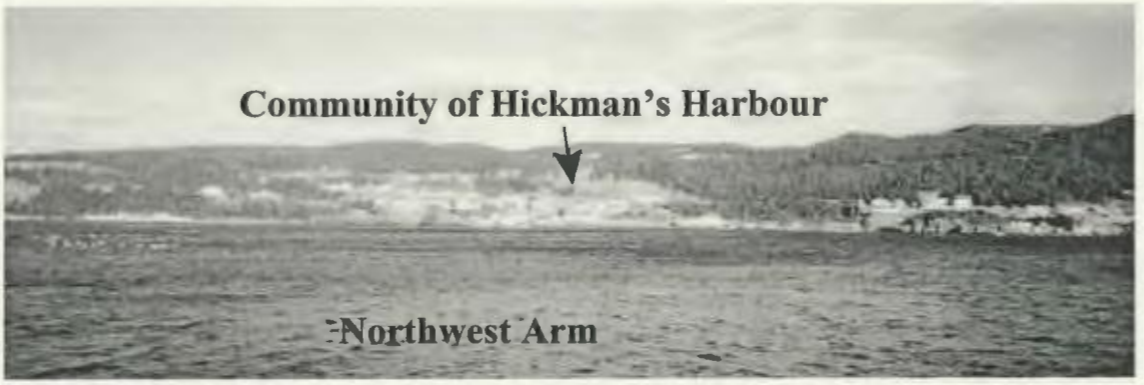
Figure 1.1 - The study area

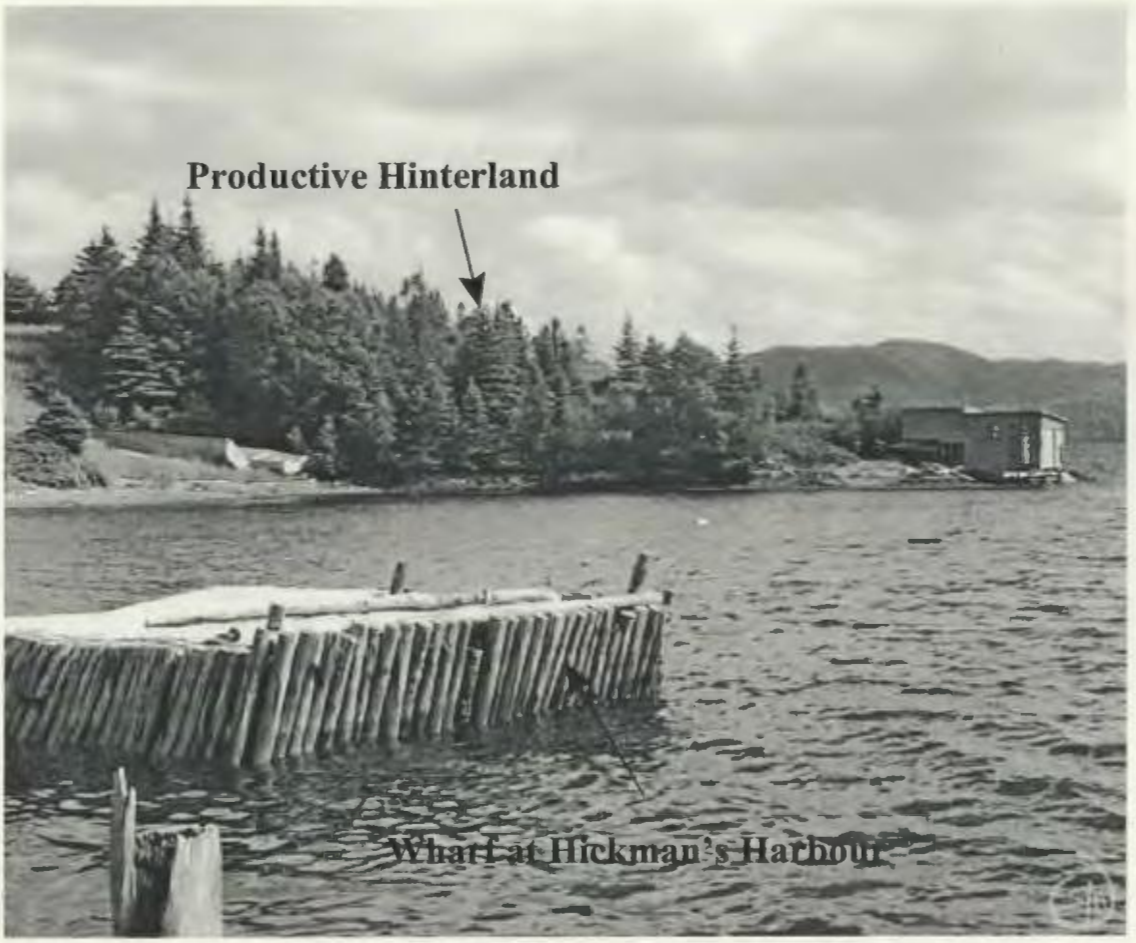
enabled Troke (1987) to infer a dominantly macrophytic algal source of OM for offshore locations in the area; the $\delta^{13}C_{TOC}$ values ranged from -21.8 to -20.7 $^{o}/_{oo}$. In addition, Troke (1987) concluded that microbial degradation of OM for two 68 cm long gravity cores was minimal. Other conclusions from that study included high productivity and high preservation potential for the Trinity Bay area which is likely the consequence of the sub-polar temperature regime of Trinity Bay. However, there is also an active benthic community in this region (at ST7, as indicated by bioturbation) which may facilitate microbial degradation and enhance mixing of different sediment horizons.

The Northwest Arm area receives much of its terrestrially derived inputs from the surrounding hinterland, including Random Island (Figures 1.1 and 1.2), through several small streams. The vegetation of the area is typical of a maritime boreal system and consists of sphagnum moss, spruce, birch, kalmia brush and grass. The area is also heavily and continuously logged and has few trees that are older than 80 years. A few small farms are scattered throughout the area and a large cattle ranch is situated close to Hickman's Harbour. The community is surrounded by forested hinterland that makes it suitable for timber extraction and saw-milling. Earlier this century, economic factors promoted these activities and by 1911 the Random Island area had 11 sawmills; all are now closed (Johnston, 1996). During this period, the value of sawn timber exceeded that of fishery products in the area.

Hickman's Harbour was probably discovered by Europeans around the middle of the 18th

Figure 1.2 - **Pictures of Random Island, Northwest Arm and Hickman's Harbour**





century. The Encyclopedia of Newfoundland indicates that the Harbour was surveyed by its namesake, Jonathan Hickman in 1770, when it was likely an area of Indian encampment. The Encyclopedia of Newfoundland also indicates that Hickman's Harbour was one of the last homes of the Beothuk Indians. This group of Indians became extinct in 1829 (Millenium WorldBook 2000).

1.6 WORK PLAN

1.6.1 Experimental

For Pyrolysis-GC/MS and tetramethylammoniun (TMAH) thermochemolysis, standard lignin model compounds, carbohydrates, tannins, phenolic acids and aldehydes, and fatty acids were examined in an artificial sediment matrix in order to identify useful markers for SOM studies and facilitate product/precursor relationships of chemolysates of SOM. Samples were also analyzed by tetraethylammonium hydroxide (TEAH) in order to determine the methyl groups that are added during thermochemolysis. The isotopic signature of the derivatizing agent (TMAH), model compounds and standards were determined by conventional mass spectrometry. The model compounds were then chemolyzed in a sediment matrix and the products analysed to predict bond and isotopic effects associated with the TMAH thermochemolysis process. The products and their yields were used as "in-house" standards for estimation of abundance of sediment markers (after calibration with authentic compounds and diagnostic kits) and also to monitor instrument performance (precision and

accuracy) during analyses by GC/MS and GC/C/IRMS.

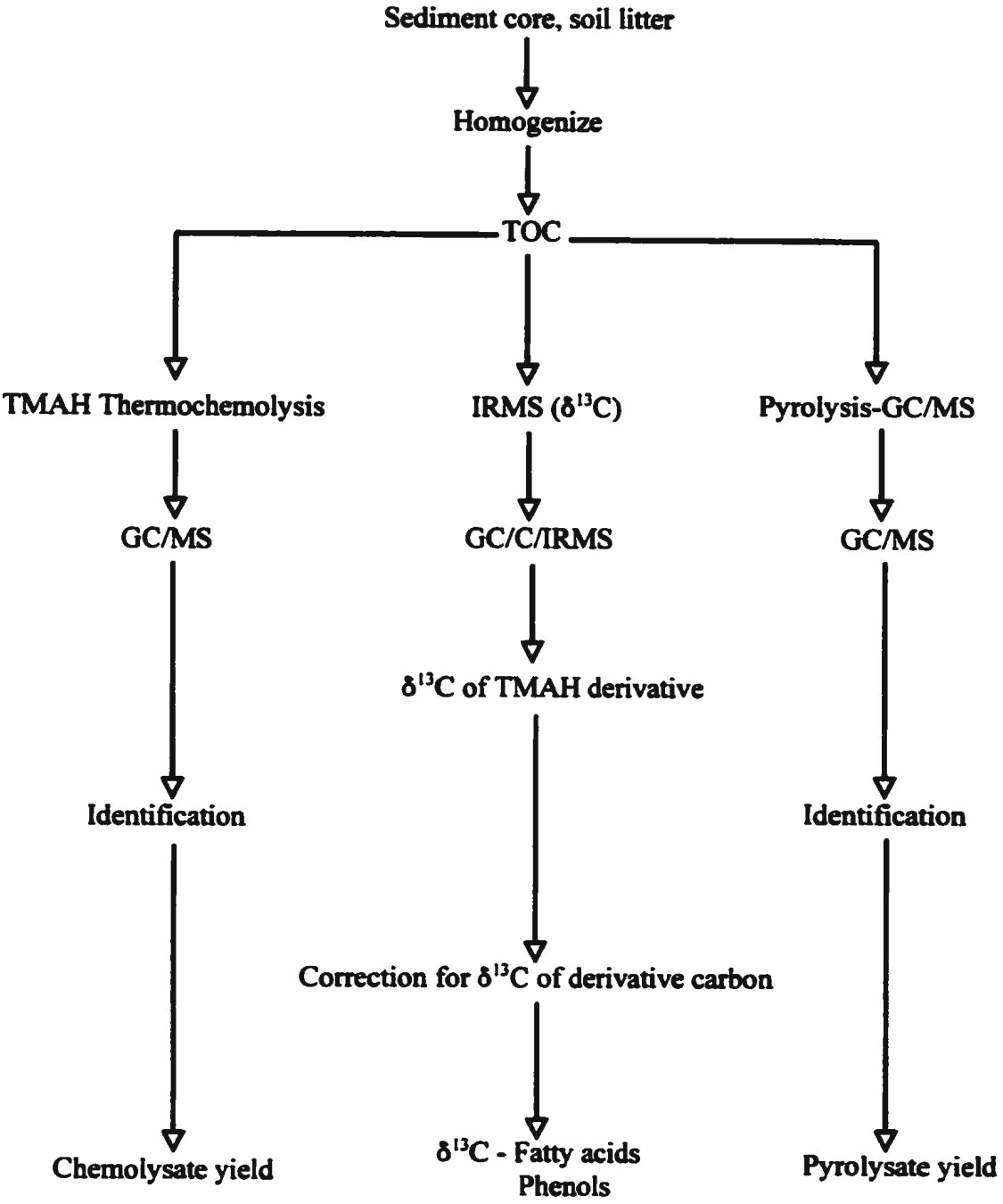
1.6.2 Sampling strategy

The marine sciences team of the Eco-Research Program at Memorial University of Newfoundland chose Trinity Bay as the study area. Northeast Arm and Trinity Bay are within the geographic proximity of the communities affected by the moratoria and they are easily accessible by boat. In addition, Trinity Bay had an abundance of cod during the months of May and June in the past (prior to the collapse of the fishery).

The sediment cores (H1, H9 and ST7), 6 inches in diameter and up to a foot long, and grab samples, were collected in order to obtain data for a reasonable transect of Northwest Arm (near-shore site) to the open bay (offshore site). Samples were taken using a box-corer and immediately put on ice and were later frozen for storage in order to prevent/minimize decomposition of SOM. Sample preparation involved drying individual sub-samples (between 10 - 20 g from 2 cm intervals) at room temperature in a fume hood followed by removal of any large stones and organisms (e.g. worms and clams). The dried clay-like sample was homogenized using a mortar and pestle and stored frozen until analysis. A small portion was acidified (30% HCl) in order to remove any carbonates, dried in a fume hood and stored for isotopic analysis ($\delta^{13}C$). Terrestrial soil samples (o to 6 cm deep) were collected, dried and ground to a fine powder using a clean spatula. The sample work-up

scheme is illustrated in Figure 1.3. In addition, sub-samples from cores ST7, H1 and H9 were dated using ^{210}Pb (see Schell, 1982). The results (ages of sediments and sedimentation rates) are given in Appendix 1.4.

Figure 1.3 - **Sample work-up scheme**



CHAPTER 2 - MOLECULAR CHARACTERIZATION OF MARINE SEDIMENTARY ORGANIC MATTER (SOM) BY DIRECT PYROLYSIS

2.1 REVIEW

Analytical pyrolysis involves the conversion of a sample (complex biomaterial) into a range of smaller molecules (pyrolysis fragments) by the rapid application of heat (Irwin, 1982). This is followed by chromatographic separation and chemical analysis of the pyrolysis fragments for the assessment of the original sample.

Pyrolysis, in conjunction with gas chromatography mass spectrometry (GC/MS), can be particularly useful in the analysis of complex, bio- and geopolymers that are not suitable for direct analysis by other analytical techniques. Modern pyrolyzers can be very easily interfaced to other analytical equipment such as GC or GC/MS systems to produce integrated systems. Capillary gas chromatography is desirable because of the ability to effectively separate a multitude of volatile pyrolysates usually observed from pyrolysis of complex biomass. This combination of pyrolysis-GC with mass spectrometric detection can be a very effective and efficient approach for molecular-level characterization of complex, non-volatile samples such as humic substances (Meuzelaar *et al.*, 1984).

Analytical pyrolysis has a list of compelling attributes. Samples that require minimal preparation can be rapidly analyzed thereby increasing sample throughput and reducing

analytical costs. In addition, because the amount of sample required is small (micro to milligram range), heterogeneity within collected samples can be studied. Analytical pyrolysis has numerous applications including characterization of organic compounds in forest humus, chemical characterization of wood and the study of environmental pollutants (Irwin, 1982).

An essential requirement of any analytical technique is the reproducibility with respect to reaction products and transfer of these products for separation, identification and quantification (Irwin, 1979). The development of the pyrolysis technique was not well coordinated and instruments and procedures were not standardized resulting in poor inter-laboratory correlation (Wampler and Levy, 1987). This was exemplified in the earlier inter-laboratory comparison of pyrolysis of identical compounds by Whitehouse *et al.* (1985) during which results ranged from encouraging to poor. This added to the perception that results from analytical pyrolysis can be difficult to reproduce. However, a high degree of precision can be achieved following optimization of the pyrolysis process and rigid control of the conditions. These include:

1. Temperature: Pyrolysis initiates primary-bond fission that may proceed by several temperature dependent and competing reactions. Hence, the product profile is usually very dependent on the heating characteristics of the pyrolyzer.
2. Temperature-time profile of the pyrolysis unit: A temperature rise in the order of milliseconds or better is desirable. This is essential because of the rapid degradation reactions that occur during pyrolysis and may result in completion of pyrolysis

before the equilibrium temperature is achieved. However, the average pyrolysis temperature must not be too high to avoid producing non-characteristic low molecular weight species.

3. Sample preparation: This may be the greatest and most easily remedied source of irreproducibility (Wampler and Levy, 1987). Small samples with large surface to volume ratios facilitate rapid and consistent thermal degradation (Wampler and Levy, 1987). Small sample size combined with rapid heating will minimize thermal gradients and secondary reactions within the sample. Homogeneity of sample and control of the size and shapes of solid samples are also of concern for reproducible results (Wampler and Levy, 1987). Finally, contamination may become important because very small samples are analyzed. Contaminant peaks are likely to be highly variable between analyses resulting in the perception that a sample is pyrolyzing in an erratic fashion. Hence, the cleanliness of all tools is absolutely essential.

4. Transfer of pyrolysates: Successful "on-line" analysis by pyrolysis-GC/MS requires efficient transfer of the pyrolysis products. Hence, the carrier gas velocity must be optimized for fast removal of the pyrolysates from the zone of pyrolysis to the GC (in order to prevent secondary reactions), without affecting the temperature-time profile.

5. Cold spots - These are most common between the pyrolysis zone and GC column. The transfer line should be kept sufficiently warm and insulated in order to prevent condensation of higher molecular weight pyrolysates that can result in partial transfer and poor reproducibility.

2.1.1 Analytical pyrolysis apparatus and methods

Three types of pyrolysis units can be identified depending upon the heating mechanism used. Heat can be applied using either a continuous source of energy (continuous mode heated micro-furnaces) by a pulsed source (pulse mode type - resistively and inductively heated filaments) or using ferro-magnetic Curie Point alloys.

"Off line" analytical pyrolysis can be performed with the pyroprobe mounted in a cooled glass condenser. Following pyrolysis, the condensed pyrolysates are retrieved from the inner walls of the condenser by washing with a suitable solvent and dried under a stream of nitrogen. "On line" analytical pyrolysis is carried out by coupling a pyrolyzer to another instrument (in this study - a GC/MS). "On-line" analytical analyses have the advantages of reducing the time required for each analysis and minimizing sample handling, thereby reducing the chances of contamination. However, although the "off-line" method is a little more time consuming and involves an additional step in sample handling, it has several noteworthy merits. The physical connection between the pyrolyzer and the GC/MS system of "on-line" analytical equipment introduces "dead space", which has been shown to negatively affect chromatographic separation. An improper connection will compromise the authenticity of analytical results by introducing cold spots, leaks, and memory effects of the pyrolysis system. Other advantages of the "off-line" method include the possibility of combining several pyrolysates for analyses that require large amounts of materials; addition of internal standards for quantification; and storage for re-analysis (by GC/MS and

GC/C/IRMS). A potential disadvantage of the "off-line" pyrolysis is possible alteration of sensitive degradation products and loss of volatile low molecular weight compounds. Faix *et al.* (1987) reported better reproducibility in the pyrolysis of lignins using the "off-line" method as opposed to the "on-line" method.

2.1.2 Pyrolysis of lignin, carbohydrates, amino acids and lipids

It has been demonstrated by Schulten *et al.* (1989) that thermal degradation products can characterize the main constituents of spruce needles such as carbohydrates and lignin. During pyrolysis, thermostable plant constituents and primary building blocks such as cellulose, hemicellulose and lignin are released from plant samples yielding characteristic products. The most common linkage in lignin is the alkyl-aryl ether with the α- and β-alkyl-aryl ethers forming 54 to 68% of the inter-unit linkages of lignin (Evans *et al.*, 1986). Bond energies dictate this type of linkage to be more thermally labile than carbon-carbon bonds and hence are important in determining the major primary pyrolysis reactions (and products) of depolymerization of lignin. The depolymerization products of lignin are readily identified by the recognition of the molecular ions in a suite of phenolic compounds (Klap, 1997; Faix *et al.*, 1990). The phenolic compounds include phenol, methylphenol, guaiacol, methylguaiacol, vinylphenol, vinylguaiacol and isoeugenol (Klap *et al.*, 1996).

An important step in the pyrolytic analysis of lignin-derived compounds is the identification and relative, inter-sample quantification of the numerous degradation products. This can be

achieved by comparing mass spectra of unknown products with those of model compounds as well as using mass spectra in reference libraries (Faix *et al.*, 1990). In addition, comparison of retention times and spectral characteristics of authentic compounds can supplement the identification schemes.

Polysaccharides can be deploymerized by transglycosylation or cycloreversion. During transglycosylation, only glycosidic bonds are cleaved. Cycloreversion occurs when one ring structure is cleaved to produce small molecular fragments (Klap, 1997). Thermal degradation of cellulose yields a variety of products including CO_2, methyl esters, acids, furans, pyrans and levoglucosan. Cellulose is abundant in undecomposed wood and can yield up to 51% levoglucosan (Kelly, 1992). Pyranones have also been identified and are found to be major pyrolysis products of amylose when pyrolyzed in a phosphate matrix (Van Der Kaaden and Haverkamp, 1983).

A number of studies (such as Sicre *et al.*, 1994; Ishiwatari *et al.*, 1993 and 1995) have identified nitrile products in recent biomass and they are believed to be from peptides, diketopiperazines, amino acids and aliphatic cyanides. The pyrroles and alkyl- and phenyl-nitriles may represent major product from nitrogeneous geopolymers (Ishiwatari *et al.* 1993 and 1995). Other possible sources of pyroles are chlorophyll and extensin, a highly resistant biopolymer that is rich in hydroxy-proline (Van Smeerdijk, 1987). Pyrolysis of tryptophan produces indole and methyl indole (Irwin, 1982). Methyl indole indicates the presence of proline, hydroxyproline and tryptophan. Tryptophan amino acid moieties are abundant in

algal proteins (Tsuge and Matsubara, 1985).

The pyrolysis products of lipids depend on pyrolysis and analysis conditions (Irwin, 1982). The principal pyrolysis products of a triacylglycerol lipid are characterized by three main homologous series, which are carboxylic acids, hydrocarbons and olefins. Pyrolysis of fatty acids esters, diacylglycerol (dipalmitin) and phospholipids (lecithin) is characterized by a homologous olefin and hydrocarbon series. During pyrolysis, higher molecular weight fatty acids become decarboxylated to produce hydrocarbons (Irwin, 1982).

2.1.3 Pyrolysis of marine-derived materials

Reports on pyrolysis of marine sediments are less abundant than those on pyrolysis on terrestrially derived sediment. The source of marine SOM is compositionally different from that of soil organic matter, and hence the profiles of pyrolysates are expected to be generally different. Soil organic matter tends to have high quantities of lignin-derived compounds. In the marine environment, the quantities of these compounds rapidly diminish with distance from shore. Sicre *et al.* (1994) in a study involving pyrolysis of riverine suspended material in the near-shore environment (Rhone delta, Northwestern Mediterranean Sea) found very low abundances of levoglucosan (from cellulose) and lignin-derived materials. The major products identified were derived from marine polysaccharides and lipids; the most abundant lipids were the fatty acids containing 14, 16 and 18 carbon atoms.

Ishiwatari *et al.* (1993 and 1995) investigated the compositional changes in OM in sinking particles in the Japan Trench using the pyrolysis-GC/MS technique. The biomass precursors of the pyrolysates were polysaccharide, protein and lipid in nature. The classes and abundance (in relative terms) of pyrolysates of the sinking particles were aliphatic hydrocarbons (2.7%), aromatic hydrocarbons (29.7%), aliphatic cyanides (0.8%), phenols (14.8%), furans (8.8%) and nitrogeneous compounds (43.2%). Aliphatic hydrocarbons (C_{10} to C_{27}) were assigned to lipid sources. The fatty acids and aliphatic cyanides (or fatty cyanides) were derived from fatty acid methyl ester (FAME) and aliphatic amides, respectively. Phenols (C_0 to C_3) have both lignin and protein-derived sources. Furans, furaldehyde and benzofurans were assigned to carbohydrate sources. Finally, several nitrogenous compounds were determined and assigned to sources that include protein, amino acids and related compounds. The nitrogenous pyrolysates were pyrolles, pyridines, benzene cyanides and indoles with average abundances (with respect to total N-compounds) of 41.8, 36.7, 9.5 and 12.1%, respectively.

Ishiwatari *et al.* (1995) computed ratios of classes of compounds that were used as parameters to test the degree of maturity of OM. The ratios include alkane, and alkene to indole abundances. Pyrolysates that are more abundant in nitrogen compounds and less abundant in aromatic compounds indicate the degree of freshness of OM of partculates in the water column. Fresh OM is relatively unaltered. Ishiwatari *et al.* (1995) established a relationship between grain size (of settling particles in the Japan Trench) and maturity of OM and determined that fresh OM was associated with large grain size particles (and vice versa).

Unfortunately there was no mention of analytical results performed on the sediment sample that was mentioned in the description of sampling procedure. This additional step would have provided insights into the compositional relationship between the TOC of particulates and sediment.

This Chapter deals with the investigation of the compositional changes in marine OM during early diagenesis as indicated by the signature of selected markers of sediment pyrolysates. To date, there have been few (if any) studies of this nature and these results will be a valuable complement to those of future analytical studies. The characterization of marine SOM, including the quantitatively important but poorly characterized non-hydrolysable fraction is central to the Py-GC/MS study.

2.2 METHODS

2.2.1 Samples

The samples analyzed were taken from cores in the Northwest Arm and Trinity Bay (Figure 1.1; Section 1.6.2). Cores H1, H9 and ST7 were box cores (30 cm long). Other samples were taken from grab samples. Cores H1 and H9 consisted of fine grained-dark mud. Core ST7 appeared to be coarser grained in texture and grayish in colour. The grab samples were also fine-grained dark mud.

2.2.2 "On-line" or Direct Pyrolysis-GC/MS

"On-line" or direct pyrolysis-GC/MS was adopted because of reduced sample handling and preparation and relative ease of analysis. It was carried out by attaching a pulsed (quartz tube) pyrolyzer (CDS Pyroprobe 120) to the injection port of a Hewlett Packard Model 5790A GC/MS equipped with a HP 5970A Workstation. The quartz boats were first flamed to remove any organic matter. The sediment samples were accurately weighed (5.0 ± 0.1 mg) and added to the centre of the boat and were then carefully inserted into the centre of the heating coil using clean tweezers. The probe was then screwed into position in the interface oven and the heated interface was maintained at 270°C.

Samples were pyrolyzed at 650°C (10 seconds) and swept into the GC by helium, at a total flow rate of 20 ml min^{-1} (column flow rate was 1.5 ml min^{-1}). Separation of the pyrolysates was carried out on a 30 m x 0.25mm I.D. fused silica capillary column of DB-1701 (1 μm film thickness), with the temperature held at 100°C for 2 min, and then increased at 5°C per minute to 260°C, which was held for 5 min. The electron impact ionisation voltage of the mass spectrometer was 70 eV and the mass range was 50 to 350 atomic mass units. Pyrolysis products were identified by comparisons with mass spectra in an NBS library and are listed as probable assignments as in Table 2.1a. Structural formulae of pyrolysates are shown in Table 2.1b.

Table 2.1a - **Compounds identified in pyrolysates of sediments**

Peak #*	Compound	Abbreviation*	Likely source◇
1	2-Methylpyrrole		Ch
2	2-Methylcyclopentanone	C2	Ps
3	3-Methylcyclopentanone	C3	Ps
4	6-Methyl-4-pyrimidione		Pr
5	Phenol	P1	Pr, Lg
6	2-Methoxyphenol		Lg
7	2-Methylphenol	P2	Ps, Pr, Lg
8	2-Pyridinecarbonitrile		Pr
9	4-Methylphenol	P3	Pr, Lg
10	2-Methoxy-3-methyl phenol		Lg
11	Benzeneacetonitrile	N1	Pr
12	2-Ethylphenol	P4	Lg
13	Butenedioic acid, monobutyl ester	L1	Lp
14	2,5-Pyrrolidinedione		Pr?
15	Benzenepropanenitrile	N2	Pr
16	4-Ethylenephenol	P5	Lg

17	2-methoxy-4-ethylenephenol		Lg
18	2-Methoxy-4(-2-propenyl)phenol		Lg
19	Benzeneacetonitrile, α-methylene		Pr?
20	1,2-Benzenediol		Ps, Pr, Lg
21	Indole	N4	Pr
22	3-Methyl-1,2-benzenediol		Ps, Lg
23	1,7,11-trimethyl-4-(methylethyl)cyclotetradecane		Lp
24	3-Methylindole	N5	Pr
25	Vanillin		Lg
26	Acetovanillone		Lg
27	9-Nonadecene	L2	Lp
28	Phenylacetaldehyde		Lg
29	5,6,7,8-tetrahydronapthaleneamine		Pr
30	Tridecanenitrile		Pr
31	2-Methylheptadecane		Lp
32	Levoglucosan	C4	Ps
33	4-(3-propanol)-2-methoxyphenol		Lg
34	7H-Furo[3,2-g][1]benzopyran-7-one	C1	Ps

35	3-Methylhexadecane		Lp
36	4-Propenal-2-methoxyphenol		Lg
37	Pentadecanenitrile	N3	Pr

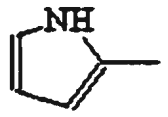
*Chromatographic peaks labelled in Figure 2.1

*Abbreviations used in tables and histograms

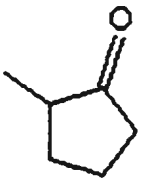
◆ Key: Ch = Chitin; Ps = Polysaccharide; Pr = Protein; Lg = Lignin; Lp = Lipid

Table 2.1b - **Structural formulae of pyrolysates**

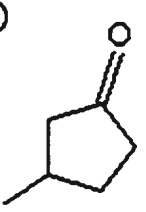
2-Methylpyrrole
(1)



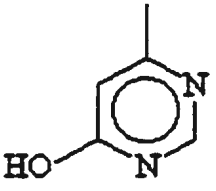
2-Methylcyclopentanone
(2)



3-Methylcyclopentanone
(3)

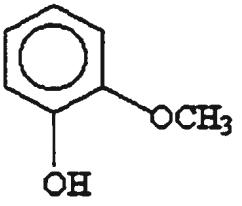


6-Methyl-4-pyrimidinone
(4)

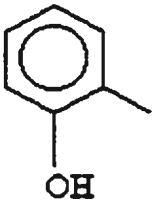


Phenol
(5)

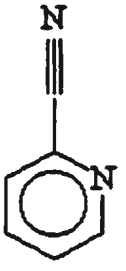
2-Methoxyphenol
(6)



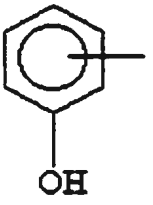
2-Methylphenol
(7)



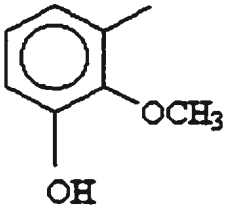
2-Pyridinecarbonitrile
(8)



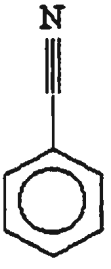
Methylphenol
(9)



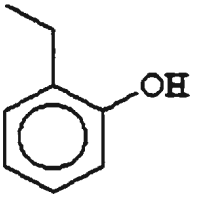
2-Methoxy-3-methylphenol
(10)



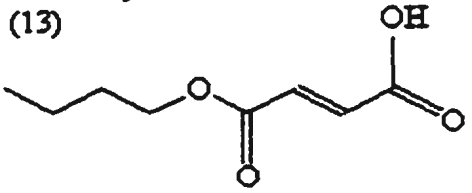
Benzeneacetonitrile
(11)



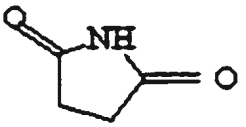
2-Ethylphenol
(12)



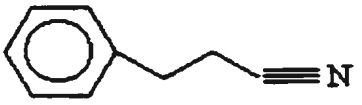
Butenedioic acid monobutyl ester
(13)



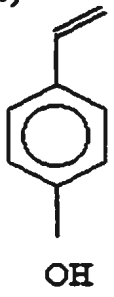
2,5- Pyrrolidinedione
(14)



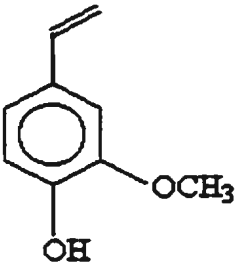
Benzenepropanenitrile
(15)



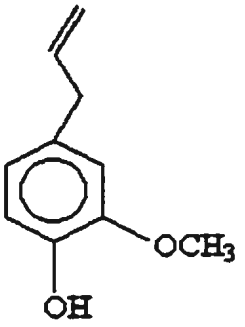
4-Ethylenephenol
(16)



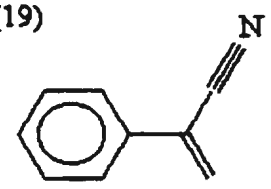
2-Methoxy-4-ethylenephenol
(17)



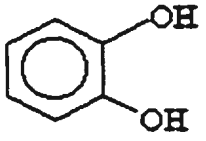
2-Methoxy-4-(2-propenyl)-phenol
(18)



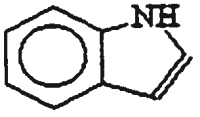
Benzeneacetonitrile, 4-methylene
(19)



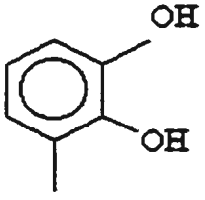
1,2- Benzenediol
(20)



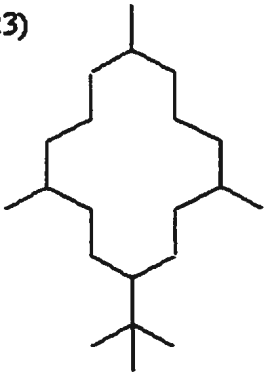
Indole
(21)



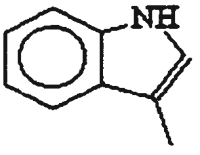
3-Methyl-1,2-benzenediol
(22)



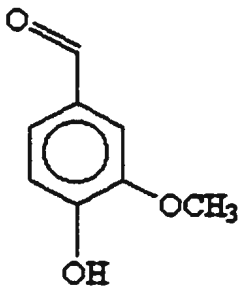
1,7,11-Trimethyl-4-(1-methylethyl)-cyclotetradecane
(23)



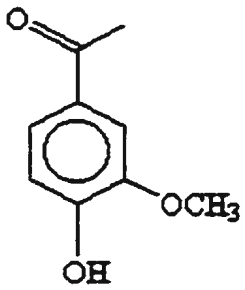
3-Methylindole
(24)



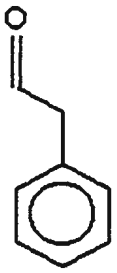
Vanillin
(25)



Acetovanillone
(26)

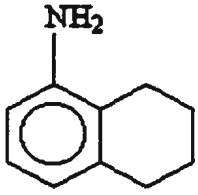


Phenylacetaldehyde
(28)



9-Nonadecene
(27)

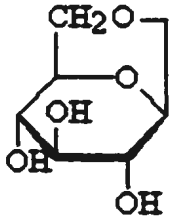
5,6,7,8-Tetrahydronapthaleneamine
(29)



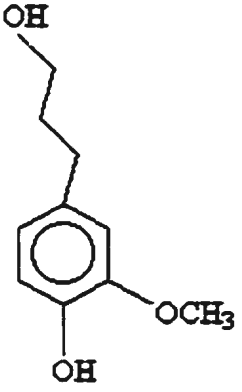
Tridecanenitrile
(30)

2-Methylheptadecane
(31)

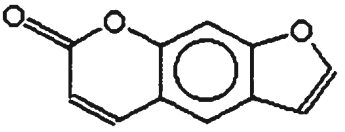
Levoglucosan
(32)



4-(3-Propanol)-2-methoxyphenol
(33)

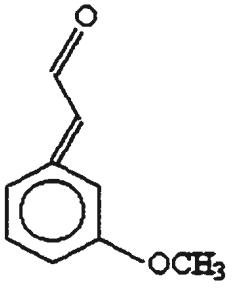


7H-Furo[3,2-g][1]-benzopyran-7-one
(34)



3-Methylhexadecane
(35)

4-Propenal-2-methoxyphenol
(36)

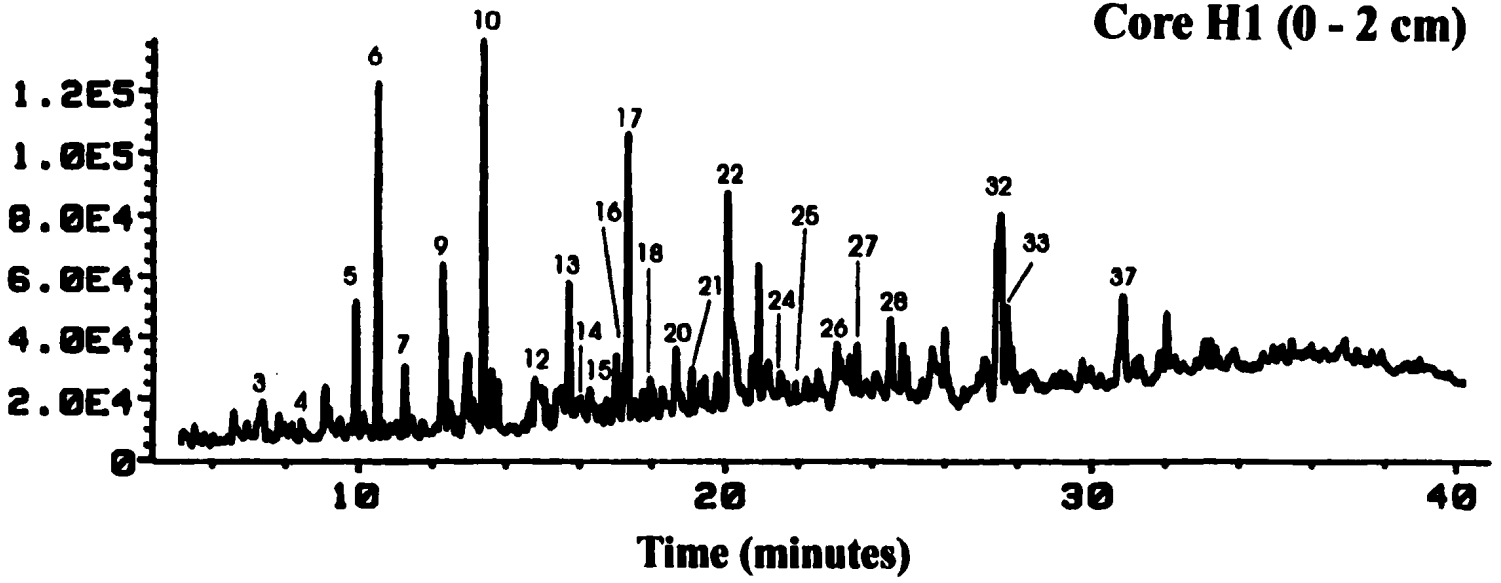


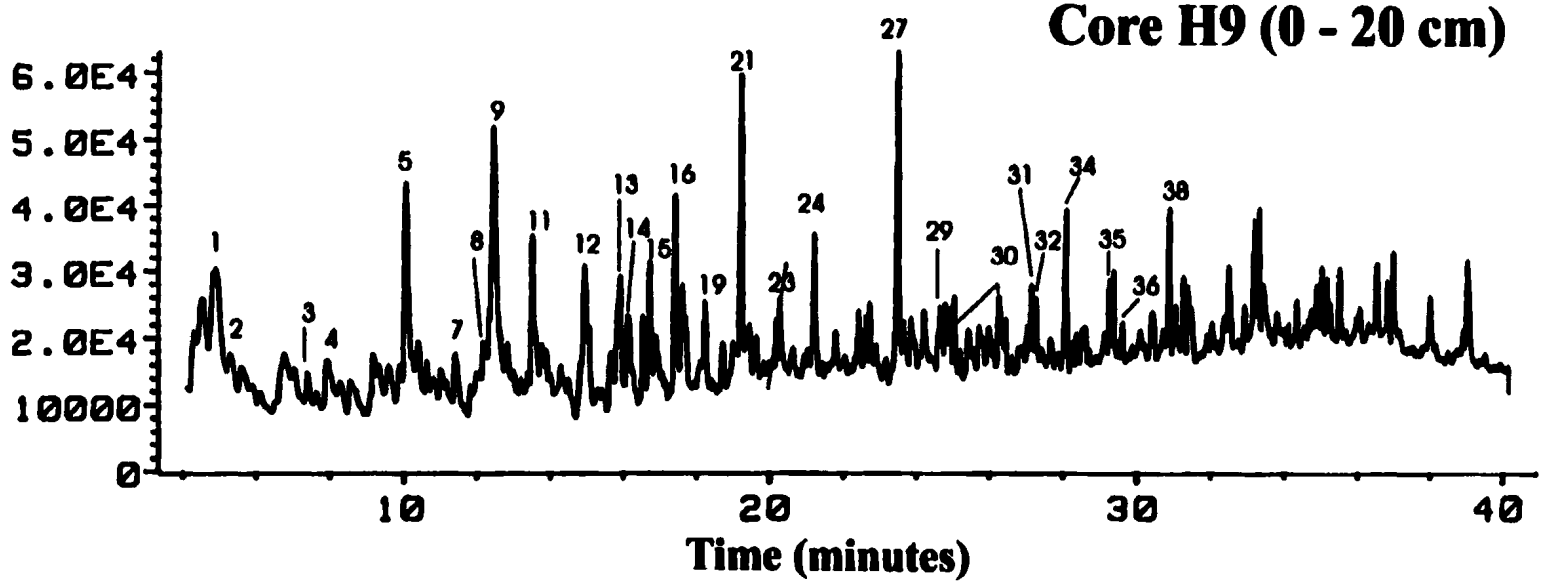
Pentadecanenitrile
(37)

Figure 2.1 - **Total ion chromatograms of sediment pyrolysates**

a. Core H1 (0-2 cm)
b. Core H9 (0-2 cm)

Peak numbers correspond to pyrolysates in Tables 2.1a and 2.1b.


Core H1 (0 - 2 cm)
1.2E5
1.0E5
8.0E4
6.0E4
4.0E4
2.0E4
0
10
20
30
40
Time (minutes)
3
4
5
6
7
9
10
12
13
14
15
16
17
18
20
21
22
24
25
26
27
28
32
33
37


Core H9 (0 - 20 cm)
6.0E4
5.0E4
4.0E4
3.0E4
2.0E4
10000
0
10
20
30
40
Time (minutes)
1
2
3
4
5
6
7
8
9
11
12
13
14
15
16
19
21
24
27
29
30
31
32
34
35
36
38

2.3 RESULTS AND DISCUSSION

2.3.1 Spatial variation of pyrolysates

The measurement of pyrolysate yields by analytical pyrolysis does not facilitate measurement of absolute concentrations of pyrolysate precursors; abundances of pyrolysates are expressed in units of peak area per mg of dry weight of sediment. Reproducibility of pyrolysis-GC/MS was determined by replicate analyses (n=5) for randomly chosen samples; the standard deviation of peak areas of individual compounds varied between 5.1 and 25.6%. These values result from instrument error (internal precision) and error resulting from sampling (external precision). This variation may be attributed to sample inhomogeneity since more than 90% of the sample is an inorganic matrix in which homogeneity may be difficult to achieve, and to the small sample pyrolyzed. When all phenols, N-compounds and carbohydrate pyrolysates are pooled independently together in each sample, the results in standard deviation are still acceptable. A sample of H1 (0-2 cm) was analyzed daily in order to correct for detector response if necessary.

Results of pyrolysis-GC/MS are discussed in the context of spatial and temporal variation. The former attempts to measure biogeochemical variations among surface sediment samples (0-2 cm of the cores). The latter deals with downcore trends and inter-variations of the core samples (ST7, H1 and H9). These three cores (30 cm long) were chosen because of their more detailed depositional histories (Appendix 1.3). The relative abundances of individual pyrolysates and the relative group abundances are given in Table 2.2. Also tabulated are

Table 2.2 - Spatial variation of sediment pyrolysate abundances of surface sediment

1. Class abundance of pyrolysates (peak area per mg of sediment x 1000 and normalized to TOC); n = 3, mean ± s.d.

2. Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis

Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16
Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37
Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34
Total lipids is the sum of peak numbers 13 and 27

Data plotted in Figure 2.2

Table 2.21 - **Class abundance of pyrolysates (peak area per mg of sediment x 1000 and normalized to TOC); n = 3, mean ± s.d.**

Station	H9	H8	H7	H6	H5
			Peak area per mg of sediment x 1000		
Phenols	91 ± 7	77 ± 6	50 ± 4	93 ± 7	56 ± 4
N-Compounds	56 ± 8	48 ± 7	28 ± 4	68 ± 10	30 ± 4
Carbohydrates	14 ± 2	11 ± 2	9.5 ± 1.5	15 ± 2	9 ± 1.5
Lipids	25 ± 3	27 ± 3	15 ± 2	30 ± 4	14 ± 2
			Peak area x 1000 per 100 mg of TOC		
Phenols	147 ± 11	202 ± 15	103 ± 7.6	152 ± 11	150 ± 11
N-Compounds	90 ± 13	125 ± 18	58 ± 8.5	111 ± 16	82 ± 12
Carbohydrates	23 ± 4	29 ± 5	19 ± 3	25 ± 4	25 ± 4
Lipids	40 ± 5	71 ± 8	31 ± 4	48 ± 6	37 ± 5
Phenol/N-compounds	1.6 ± 0.4	1.6 ± 0.4	1.8 ± 0.4	1.4 ± 0.3	1.8 ± 0.4
Phenol/Carbohydrates	6.5 ± 1.1	7 ± 1.7	5.3 ± 1.3	6.1 ± 1.3	5.9 ± 1.6
Phenol/Lipids	3.7 ± 0.8	2.8 ± 0.4	3.3 ± 1.0	3.1 ± 0.4	4.0 ± 2.3

Table 2.21 (continued)

	H1	ST11	ST10	ST9	ST7
			Peak area per mg of sediment x 1000		
Phenols	473 ± 35	80 ± 6	66 ± 5	47 ± 4	46 ± 3
N-Compounds	88 ± 13	78 ± 11	64 ± 9	40 ± 6	38 ± 6
Carbohydrates	128 ± 20	39 ± 6	36 ± 6	23 ± 4	24 ± 4
Lipids	479 ± 56	35 ± 4	41 ± 5	27 ± 3	25 ± 3
			Peak area x 1000 per 100 mg of TOC		
Phenol	364 ± 27	174 ± 13	221 ± 16	151 ± 11	100 ± 7
N-Compounds	68 ± 10	170 ± 25	214 ± 31	130 ± 19	84 ± 12
Carbohydrates	99 ± 15	85 ± 13	120 ± 19	74 ± 12	52 ± 8
Lipids	36 ± 44	76 ± 9	137 ± 16	88 ± 11	55 ± 7
Phenol/N-compounds	5.4 ± 1.2	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.3	1.2 ± 0.3
Phenol/Carbohydrates	3.7 ± 0.7	2.0 ± 0.7	1.8 ± 0.4	2.0 ± 0.5	1.9 ± 0.4
Phenol/Lipids	10 ± 2	2.3 ± 0.5	1.6 ± 0.3	1.7 ± 0.3	1.8 ± 0.2

Table 2.22 - **Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis**

Pyrolysate	H9	H8	H7	H6	H5	H1	ST11	ST10	ST9	ST7
Phenol	18	23	9.1	21	15	22	18	12	10	12
2-Methylphenol	5.6	6.7	5.0	7.6	4.2	4.6	4.2	7.4	5.0	5.2
Methylphenol	35	26	19	36	18	39	20	20	11	9.5
Ethyl phenol	18	11	11	16	10	2.7	27	16	14	14
4-Ethylenephenol	15	11	6.7	13	9.4	95	11	11	6.5	5.6
Other phenols	0.0	0.0	0.0	0.0	0.0	310	0.0	0.0	0.0	0.0
Benzeneacetonitrile	13	10	3.8	15	6.2	18	16	11	5.5	4.3
Benzenepropanenitrile	6.7	7.5	4.4	11	3.6	9.2	12	12	9.1	9.5
Pentadecanenitrile	7.7	6.4	3.6	8.3	4.2	38	12	8.4	5.7	5.7
1-H Indole	19	16	9.5	20	11	11	25	22	13	12
3-Methylindole	9.5	7.5	6.9	14	5.1	12	13	11	7.2	6.9
7-H Furobenzopyranone	8.8	8.0	6.1	9.4	4.7	19	1	9.0	5.7	6.8
2-Methylcyclopentanone	0.0	1.0	1.0	0.0	0.0	0.0	0.0	8.8	5.8	5.6
3-Methylcyclopentanone	5.3	2.1	2.5	5.8	4.7	1.9	13	18	12	12
Levoglucosan	0.0	0.0	0.0	0.0	0.0	107	13	0.0	0.0	0.0
Butenedioic acid, monobutyl ester	7.0	13	4.8	13	5.8	19	11	19	13	11
Nonadecene	18	14	18	17	8.0	29	24	22	15	14

compound group ratios, which will be compared to results obtained from TMAH thermochemolysis (Chapter 4).

The signatures of classes of pyrolysates for sediments of the Northwest Arm (H9, H8, H7, H6, H5, and H1) are different from those of Trinity Bay (ST11, ST10, ST9 and ST7) (Table 2.2). Generally, the histograms of abundances of pyrolysates indicate higher abundances of phenols for the cores of Northwest Arm than those of the offshore stations, although normalized (to TOC) abundances of phenols of offshore and near-shore sediments are comparable. The total phenols (in peak areas per mg sediment) correlates positively with the TOC (r^2=0.95, n=10). The histograms indicate higher abundances and normalized abundances of the other classes of pyrolysates (N-compounds, lipids and carbohydrates). Phenolic compounds are the dominant pyrolysis products of surface sediments, particularly for core H1, which is located in the close proximity of the community of Hickman's Harbour (Table 2.2; Figure 2.2). The high relative abundances of phenols in near-shore samples are the result of terrestrial sources of OM at the site. During the early 1900's, the community of Hickman's Harbour (site H1) was a saw milling and agricultural site and in 1911, there were fourteen sawmills. Later in 1921, there were thirty-seven sawmills in operation, all of which are closed today (Johnston, 1996). The dominant phenolic pyrolysis products of the surface sediments of core H1 are phenol (**5**), 2-methoxyphenol (**6**), methylphenol (**9**), 2-methoxy-3-methylphenol (**10**), 2-methoxy-4-ethenylphenol (**17**) and 3-methyl-1,2-benzenediol (**22**) (Figure 2.1[a]); many of these phenols are absent in the pyrolysates of other sediments. Molecular structural evidence indicates that sub-units of lignin are likely sources of the

Figure 2.2 - Spatial variation of pyrolysates of surface sediments

1. Total abundances of classes of pyrolysates (peak area per mg sediment); n = 3; mean ± s.d.
2. Abundances of individual pyrolysates normalized to TOC; n = 3; mean ± s.d.

Abbreviations for the individual pyrolysates are on the x axis and their peak numbers are in Table 2.1a.

Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16

Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37

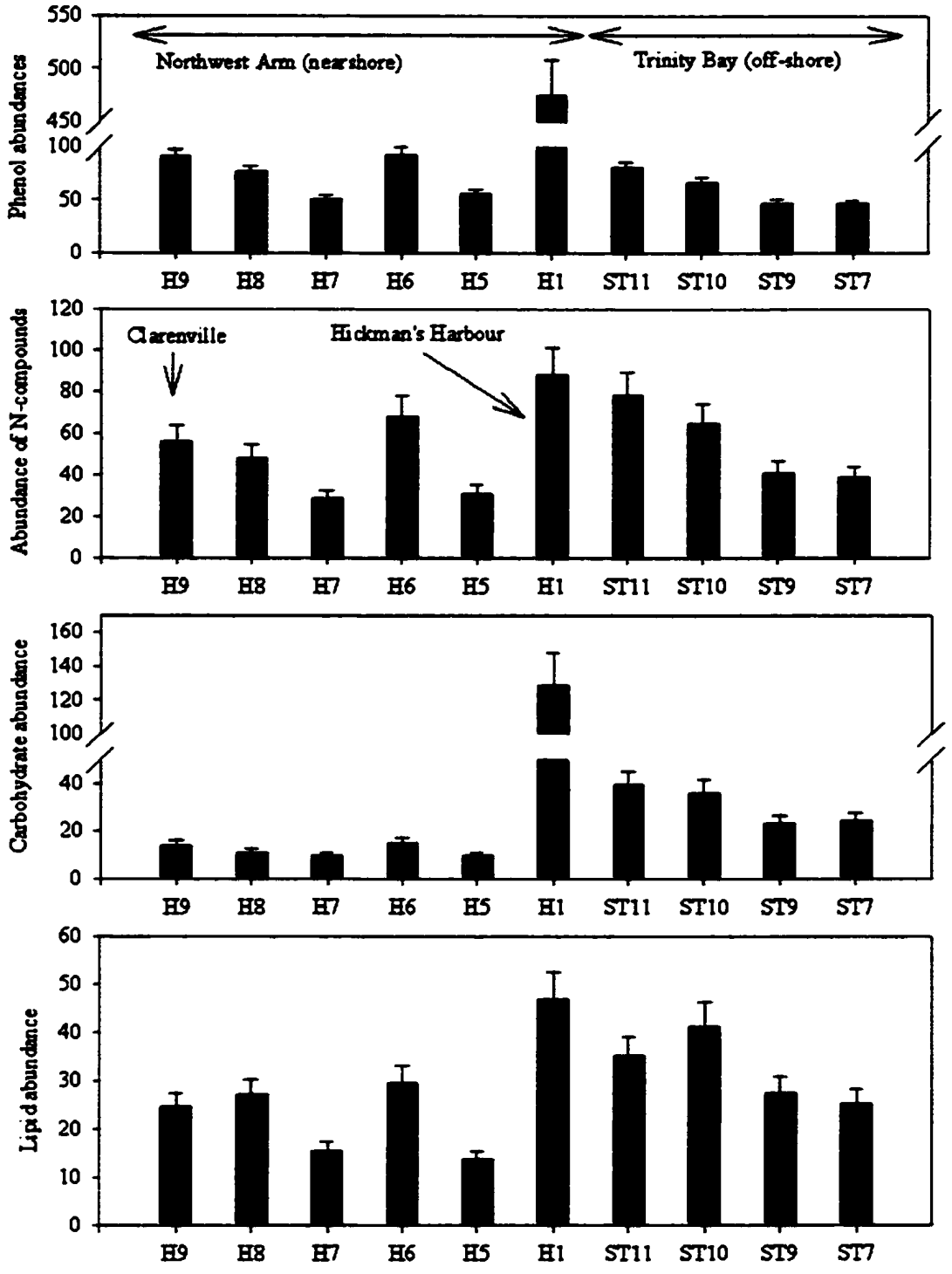
Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34

Total lipids is the sum of peak numbers 13 and 27

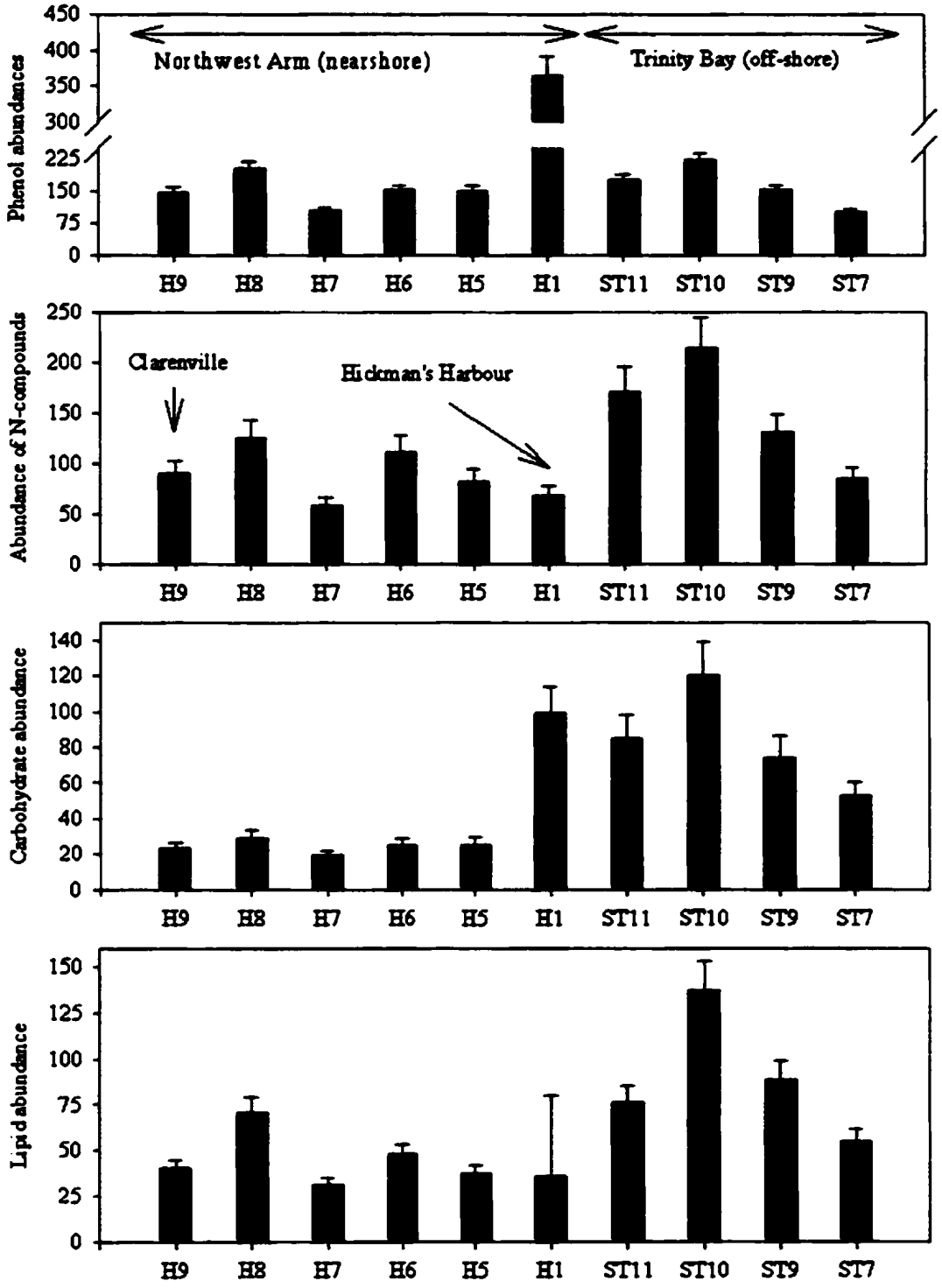
Data used for figures is in Table 2.2

Total abundances of classes of pyrolysates (peak area per mg sediment); n = 3; mean ± s.d.

Total abundances of classes of pyrolysates (peak area per mg sediment); n = 3; mean ± s.d.



Total abundances of classes of pyrolysates (peak area per mg sediment) normalized to TOC; n = 3; mean ± s.d.



phenols (Saiz-Jimenez and de Leeuw, 1986). The presence of methoxy phenol products has been interpreted as being pyrolysis products of lignin-derived materials (Ishiwatari *et al.*, 1993 and 1995).

Sicre *et al.* (1994) pyrolyzed riverine suspended matter and assigned phenol and methylphenol as products from lignin and protein, respectively. Similarly in this study, the more abundant phenol products (**5, 7, 9, 12** and **16**) in the pyrograms of all marine sediment samples can be assigned to lignin, marine humic and protein-derived sources (Tables 2.1) (Saiz-Jimenez, 1995). Phenol can also be a pyrolysis product of bacteria, algae and fungi (Irwin, 1982) - organisms that do not have lignin. Phenol, methyl- and ethylphenol can be produced by pyrolysis of tyrosine (Irwin, 1982).

Simple aromatic pyrolysates can have aliphatic sources. Alkylbenzenes, napthalenes and thiophenes have been related to the presence of aliphatic precursors in humic acids containing sulphur (Saiz-Jimenez, 1995). Aliphatic hydrocarbons in a six-membered cyclic system can undergo dehydrogenation to yield an aromatic product (Killops and Killops, 1993); minor amounts of phenol were detected during the pyrolysis of glucose (Kelly, 1992). In addition, alkylphenols were present in the pyrolysates of algal residues and were assumed to be derived from polyphenolic macromolecular sources (van Heemst *et al.*, 1996). Hence, the presence of simple phenolic compounds does not necessarily indicate that its original source was aromatic in nature or lignin-derived.

A wide variety of nitrogenous compounds were detected, including alkyl and phenyl-nitriles (**8, 11, 15** and **30**), indole (**21**) and methylindole (**24**) (Table 2.2; Figures 2.1and 2.2). A similar distribution of nitrogenous compounds was interpreted by Sicre *et al.* (1994) to have a freshly produced autochthonous source. Parrish (1998) measured chlorophyll concentrations (indicators of phytoplankton) in the water column of the study area and concluded that the Trinity Bay/Northwest Arm is highly productive, with Trinity Bay having higher chlorophyll concentrations. Hence, the settling particles at the offshore stations are expected to be richer in nitrogen because the nitrogen content of phytoplankton is high. It was found that both in the Trinity Bay and the Northwest Arm area, OM in the water column could be very efficiently stripped of the high-energy lipid component (Budge and Parrish, 1998; Parrish, 1998). Hence the fraction of the OM that reaches the sea floor and becomes incorporated as SOM would be depleted in lipids and likely richer in nitrogenous compounds; this is reflected in the higher abundances of OM that is enriched in nitrogen. Although the yields of N-containing compounds of Northwest Arm and the offshore stations are comparable, the offshore sediments have higher abundances of N-containing compounds (Figure 2.2). Higher amounts of N-containing compounds may also indicate greater quantitative importance of proteinaceous and related compounds at these locations (Tables 2.2) where the higher primary productivity by phytoplankton in the open bay is present, coupled with lower amounts of terrestrially-derived OM reaching the offshore.

Production of levoglucosan (**32**) was found to be high in the pyrograms of terrestrial soil samples, but it is present in only trace amounts in pyrolysates of all marine sediment

samples, except samples from core H1. This would indicate that polysaccharides such as cellulose, starch and amylose are degraded before incorporation into marine SOM. The presence of other carbohydrates in the sediments is also indicated by 7H-furobenzopyranone (34) and methyl-cyclopentanone (3) (Table 2.2). The abundances of carbohydrate pyrolysates (peak areas and normalized abundances) increase in sediments of offshore stations (Table 2.2). This observation may be a consequence of the relative decrease in abundance of phenols as inputs from terrestrial plant material become quantitatively less important further away from the land margin.

Most aliphatic hydrocarbons tend to yield similar mass fragmentograms, and only one hydrocarbon pyrolysate (nonadecene (27)) was identified based on GC retention time of selected hydrocarbon standards. Many of the peaks eluting after 30 minutes are aliphatic hydrocarbons (Figure 2.1). Hydrocarbons in the pyrolysates of marine particulates of the Japan Trench were assigned to lipid materials with long methylene chains (Ishiwatari *et al.*, 1993 and 1995). Pyrolysis induces decarboxylation of fatty acids to produce hydrocarbons, and the presence of fatty acid moieties in marine SOM of this study area is supported by TMAH thermochemolysis of sediments (Chapter 4). Results of TMAH thermochemolysis also indicated an increase in fatty acid abundance for the near-shore stations (Chapter 4; Table 4.2). The abundance (peak areas and normalized abundances) of nonadecene increased with distance offshore but this result may be a consequence of the decreasing abundances of phenols (also supported by TMAH thermochemolysis, Chapter 4). This pyrolysate (nonadecene) may also be related to the fatty acids because fatty acids are decarboxylated

during pyrolysis to produce hydrocarbons. However, hydrocarbon pyrolysates are also derived from other sources in SOM, such as other hydrocarbons.

SOM and mineral particles are intimately associated in natural environments (Hedges and Keil, 1995). Sediments from ST7 are relatively coarse grained (compared to other cores). Applying the grain size to maturity of OM relationship determined by Ishiwatari *et al.* (1993 and 1995), the OM of this core is probably not as mature (or degraded) as that of other cores. As seen in Table 2.2, the OM delivered at ST7 is richer in N-containing compounds as with the Japan Trench sediment; fresher OM is associated with high abundances of nitrogenous compounds.

Ratios of major classes of pyrolysates (phenols/N-compounds, phenols/carbohydrates and phenols/lipids) were determined in order to compare class signatures of the different sediments (Table 2.2). The phenol ratios are interpreted with the assumption that the major (but not only) sources of phenols are lignins (**5, 7, 9, 12 and 16**). This is based on results discussed in Chapter 3 in which yields of phenols from TMAH thermochemolysis of amino acid sources were very low. The abundances of terrestrially derived phenols (as determined by TMAH thermochemolysis correlates very strongly with abundances of phenols in the pyrolysates (r^2=0.98, n=10) (Table 2.2; Figure 2.2). There is also a strong correlation between abundances of pyrolysate and chemolysate phenols normalized to TOC (r^2=0.89, n=10). The ratio of phenols/N-containing compounds (Table 2.2) is generally higher in the Northwest Arm, and decreases in the offshore stations where contributions from N-

containing compounds (ultimately derived from phytoplankton and other marine producers) become more important. Again, this may be a consequence of the greater relative contribution of sources of N-containing compounds and/or reduced inputs from major sources of phenols such as terrestrial plants. Another potential contributor of sedimentary phenolic compounds is sea grass (*Zostera marina*) (Thayer *et al.*, 1978) which is abundant in the Northwest Arm. Simple and unicellular plants such as algae are lowest in the plant kingdom because they lack roots, leaves and other structures, but sea grasses such as *Zostera* are higher than algae and would have lignin; hence, they are potentially major contributors of lignin-derived material to the sediments.

Similarly the phenols/lipids ratios for Northwest Arm sediment are higher than those of Trinity Bay sediment. This is a consequence of higher phenol abundances in Northwest Arm sediment (Table 2.2; Figure 2.2). The ratios of phenols/carbohydrates are also higher for the Northwest Arm sediments because of higher abundances of phenols and lower abundances of carbohydrates in Northwest arm (Table 2.2; Figure 2.2). However, the differences in ratios between samples are within the percent error computed for these ratios.

2.3.2 Temporal variation of pyrolysates

The distribution of pyrolysis products of core H1 sediments indicates a pronounced loss of the phenols and carbohydrates (especially levoglucosan) with depth (Tables 2.31 and 2.32; Figure 2.31); this decrease in phenol abundance is the result of the loss of the "other

Table 2.3 - Temporal variation of sediment pyrolysates of core H1

1. Class abundance of pyrolysates (peak area per mg of sediment x 1000 and normalized to TOC); n = 3, mean ± s.d.

2. Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis

Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16
Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37
Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34
Total lipids is the sum of peak numbers 13 and 27

Data plotted in Figure 2.31

Table 2.31 - **Class abundance of pyrolysates (peak area per mg of sediment x 1000); n = 3, mean ± s.d.**

Core H1	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenols	474 ± 35	149 ± 11	120 ± 9	103 ± 8
N-compounds	88 ± 13	49 ± 7	56 ± 8	45 ± 7
Carbohydrates	128 ± 20	31 ± 12	21 ± 4	18 ± 3
Lipids	47 ± 6	45 ± 5	36 ± 4	26 ± 3
Phenols/N-compounds	5.4 ± 1.2	3.0 ± 0.7	2.2 ± 0.5	2.3 ± 0.5
Phenols/Carbohydrates	3.7 ± 0.6	4.8 ± 1.7	5.8 ± 1.5	5.6 ± 1.4
Phenols/Lipid	10 ± 2	3.3 ± 0.7	3.4 ± 0.7	4.0 ± 0.8

Table 2.32 - **Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis**

Core H1	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenol	22	40	34	30
2-Methylphenol	4.6	6.7	13	8.8
Methylphenol	39	45	50	38
Ethyl phenol	2.7	32	9.4	8.4
4-Ethylenephenol	95	26	14	18
Other phenols	310			
Benzeneacetonitrile	18	15	13	13
Benzenepropanenitrile	9.2	4.9	18	7.2
Pentadecanenitrile	38	4.5	7.9	4.6
1-H Indole	11	17	9.9	14
3-Methylindole	12	8.1	6.4	6.9
7-H Furobenzopyranone	19	6.3	5.4	6.1
3-Methylcyclopentanone	1.9	18	7.9	8.8
Levoglucosan	107	6.3	7.4	3.4
Butenedioic acid monobutyl ester	19	23	22	13
Nonadecene	29	22	14	2.6

Figure 2.31 - **Temporal variation of sediment pyrolysate classes of core H1. Abundances are in peak area (x 1000) per mg sediment.**

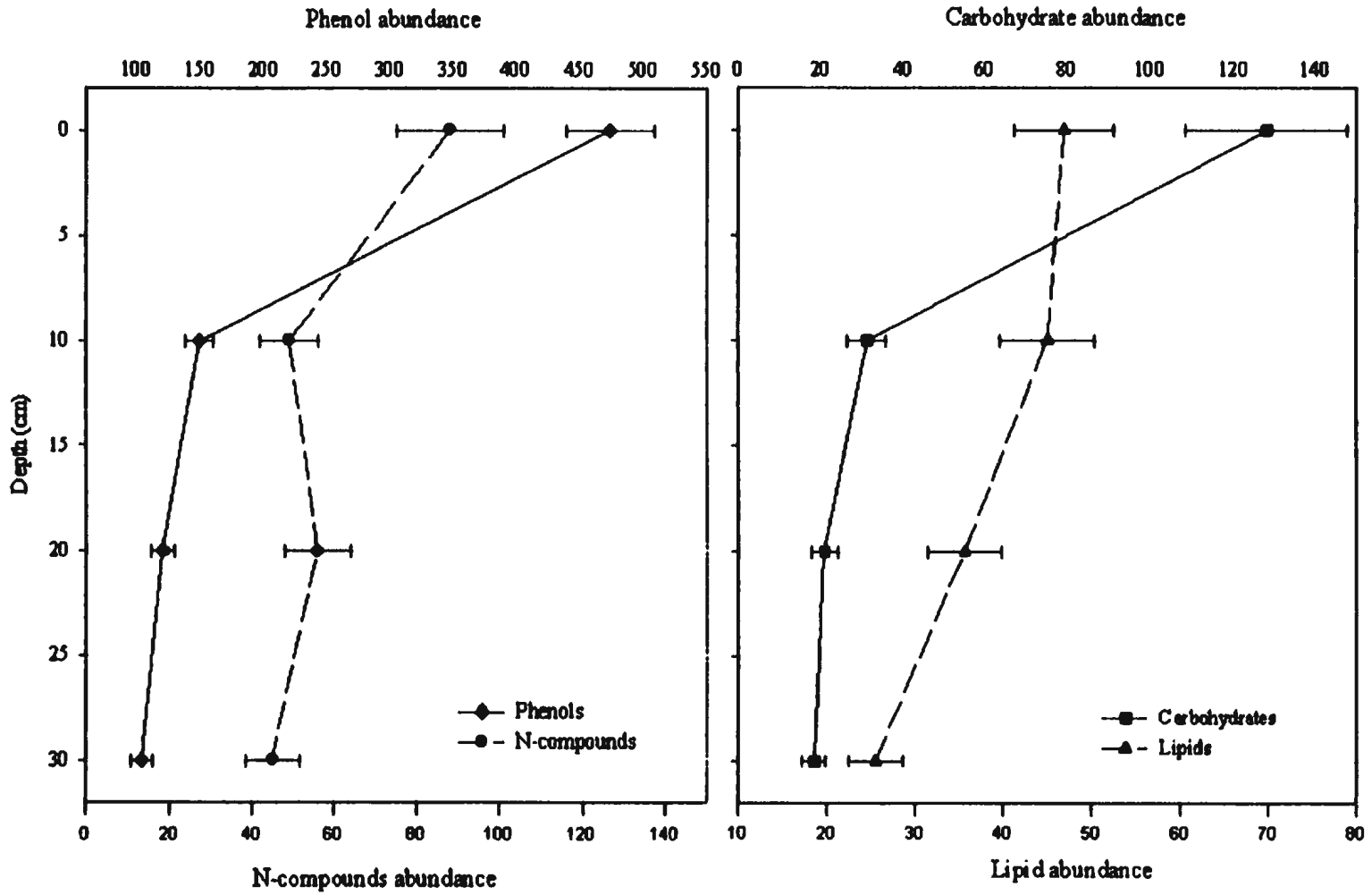
Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16

Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37

Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34

Total lipids is the sum of peak numbers 13 and 27

Data used for figures is in Table 2.3


Phenol abundance
100 150 200 250 300 350 400 450 500 550
Carbohydrate abundance
0 20 40 60 80 100 120 140
Depth (cm)
0 5 10 15 20 25 30
Phenols
N-compounds
Carbohydrates
Lipids
0 20 40 60 80 100 120 140
N-compounds abundance
10 20 30 40 50 60 70 80
Lipid abundance

phenols" (in Tables 2.2 and 2.3). The decrease below 10 cm in phenol and carbohydrate abundances is within the limits of their standard deviation. The nature of the "other phenols" makes this an interesting feature of core H1. The "other phenols" - 2-methoxyphenol (**6**), 2-methoxy-4-ethylenephenol (**7**), 2-methoxy-4-(2-propenyl) phenol (**18**), vanillin (**25**), acetovanillinone (**26**) and 4-propenal-2-methoxyphenol (**37**) - have methoxy side groups, and are unambiguous lignin markers. The disappearance of the lignin-derived phenols below the surface of core H1 indicates rapid alteration of lignin as particulate OM becomes incorporated into the SOM. The proportion of phenols in the pyrolysates and the phenols/N-containing compounds ratios are very high at the surface and decrease to relatively constant (but high compared to phenols/N-containing compounds ratios of cores H9 and ST7) values downcore (Table 2.3). The high values for the surface sediments may be reflective of inputs of OC from past logging, saw milling and agricultural activities; high phenol/N-compound ratios may also be reflective of the baseline signal that is determined by natural processes only. The main stream on Random Island discharges into Hickman's Harbour and terrestrially derived inputs from this source may account for the relatively high values in the lower half of the core. The high phenol/N-compound ratios in the lower half of core H1 may reflect the lifestyle of the Beothuk Indians, the pre-European settlers of Random Island, who likely camped at Hickman's Harbour. The Beothuks were not farmers, but used wood and wood products generously in their daily lives (Newfoundland Encyclopaedia). Hence, this entire core has received significant inputs from terrestrial plants. This will also explain the high phenol abundances found because terrestrial plants are rich in phenols and depleted in protein content (as low as 3%) (Rashid, 1985).

The N-compounds of core H1 decrease in abundance with sediment depth. However, the rate of utilization of nitrogenous compounds is at a lower rate than that of phenols in core H1; phenols and N-compound abundances decrease by 69 and 44%, respectively, from the surface layer to 5 cm (corresponding to 67 years). This is inconsistent with the reactivities of the two classes of compounds. Phenols are generally more stable (and less reactive) than nitrogenous compounds (Killops and Killops, 1993). Data from the pyrolysates of particulates of the Japan Trench indicate preferential removal of nitrogenous compounds with depth in the water column (Ishiwatari *et al.*, 1993 and 1995). In the Cape Lookout Bight (North Carolina) sediment, the hydrolysable amino acids/TOC ratio decreased by 30% after 2 years of sedimentation (corresponding to 20 cm depth of sediment) (Burdige and Martens, 1988). It is possible that for sediments of core H1, the high abundances of phenolics may have acted as a preservative (i.e. anti-bacterial) to the N-containing compounds thereby preventing post-burial microbial attack. Alternatively, N-containing compounds may become strongly incorporated in the humic fraction of the TOC through associations with other stable moieties.

There is a strong decrease in the yield of carbohydrates (especially levoglucosan) product below the surface of core H1; this resulted in the pronounced decrease in yield of total carbohydrates with depth. The phenol/carbohydrate ratios increase downcore (Table 2.3), indicating a strong decrease in the carbohydrate precursors with depth as a result of preferential removal of these compounds from the TOC pool. However, this trend, which is within the limits of the percent error of phenols/carbohydrates ratios, must be interpreted

with caution especially in the lower 20 cm of this core. Although both classes of compounds decrease with depth, the carbohydrates are removed faster than the phenols. This is the expected trend because phenols are more stable than carbohydrates (Killops and Killops, 1993). However, this is not consistent with the behaviour of OM in Dabob Bay (Washington) sediments, where the carbohydrate/TOC is constant in the upper 50 cm. The environment of Northwest Arm is different from that of Dabob Bay (Washington). For example, sediments of the latter are bioturbated, whereas there is no evidence of bioturbation in the sediments of core H1 (Chapter 1).

There are minor variations in the signature of the pyrolysates of core H9 (compared to core H1). This is partly related to the depositional histories of the two cores; sediments of core H1 accumulated more slowly than those of H9 and were exposed over longer periods to agents of degradation. Sedimentation rates for the upper 6-cm of cores H1 and H9 are 0.09 and 0.7 cm y^{-1} (average values). ^{210}Pb concentrations were too low below 6 cm of core H1 for estimation of dates using the ^{210}Pb isotope. The upper 26-cm of core H9 accumulated at an average rate of 0.4 cm y^{-1} (Appendix 1.4). All classes of pyrolysates (phenols, carbohydrates, N-compounds and lipids) decrease gradually in core H9 (Table 2.4; Figure 2.32). The yields of N-compounds also show a gradual decrease with depth.

Surface sediment of core H9 is characterized by a relatively low phenols/N-containing compounds ratio (1.6), compared to the rest of the core (between 1.8 and 2.0). This may be indicative of high amounts of N-containing compounds, thereby preserving mass balance

Table 2.4 - Temporal variation of sediment pyrolysates of core H9

1. Class abundance of pyrolysates (peak area per mg of sediment x 1000 and normalized to TOC); n = 3, mean ± s.d.

2. Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis

Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16
Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37
Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34
Total lipids is the sum of peak numbers 13 and 27

Data plotted in Figure 2.32

Table 2.41 - **Class abundance of pyrolysates (peak area per mg of sediment x 1000); n = 3, mean ± s.d.**

Core H9	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenols	91 ± 7	77 ± 6	80 ± 6	52 ± 4
N-compounds	56 ± 8	38 ± 5	40 ± 6	29 ± 4
Carbohydrates	14 ± 2	13 ± 2	13 ± 2	8.4 ± 1.7
Lipids	25 ± 3	24 ± 3	28 ± 3	15 ± 2
Phenols/N-compounds	1.6 ± 0.4	2.0 ± 0.5	2.0 ± 0.5	1.8 ± 0.4
Phenols/Carbohydrates	6.5 ± 1.1	5.8 ± 1.4	6.4 ± 1.5	6.1 ± 1.6
Phenols/Lipid	3.7 ± 0.7	3.1 ± 0.6	2.9 ± 0.6	3.5 ± 0.7

Table 2.42 - **Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis**

Core H9	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenol	18	15	20	13
2-Methylphenol	5.6	5.4	5.2	0.7
Methylphenol	35	29	26	17
Ethyl phenol	18	16	13	11
4-Ethylenephenol	15	12	16	9.2
Benzeneacetonitrile	13	8.3	10	7.5
Benzenepropanenitrile	6.7	5.4	6.0	3.4
Pentadecanenitrile	7.7	4.6	4.5	3.6
1-H Indole	19	13	13	10
3-Methylindole	9.5	6.8	7.1	4.8
7-H Furobenzopyranone	8.8	6.8	6.3	4.6
3-Methylcyclopentanone	5.3	6.3	6.3	3.8
Butenedioic acid, monobutyl ester	7.0	12	15	8.7
Nonadecene	18	12	13	6.2

Figure 2.32 - **Temporal variation of sediment pyrolysate classes of core H9. Abundances are in peak area (x 1000) per mg sediment.**

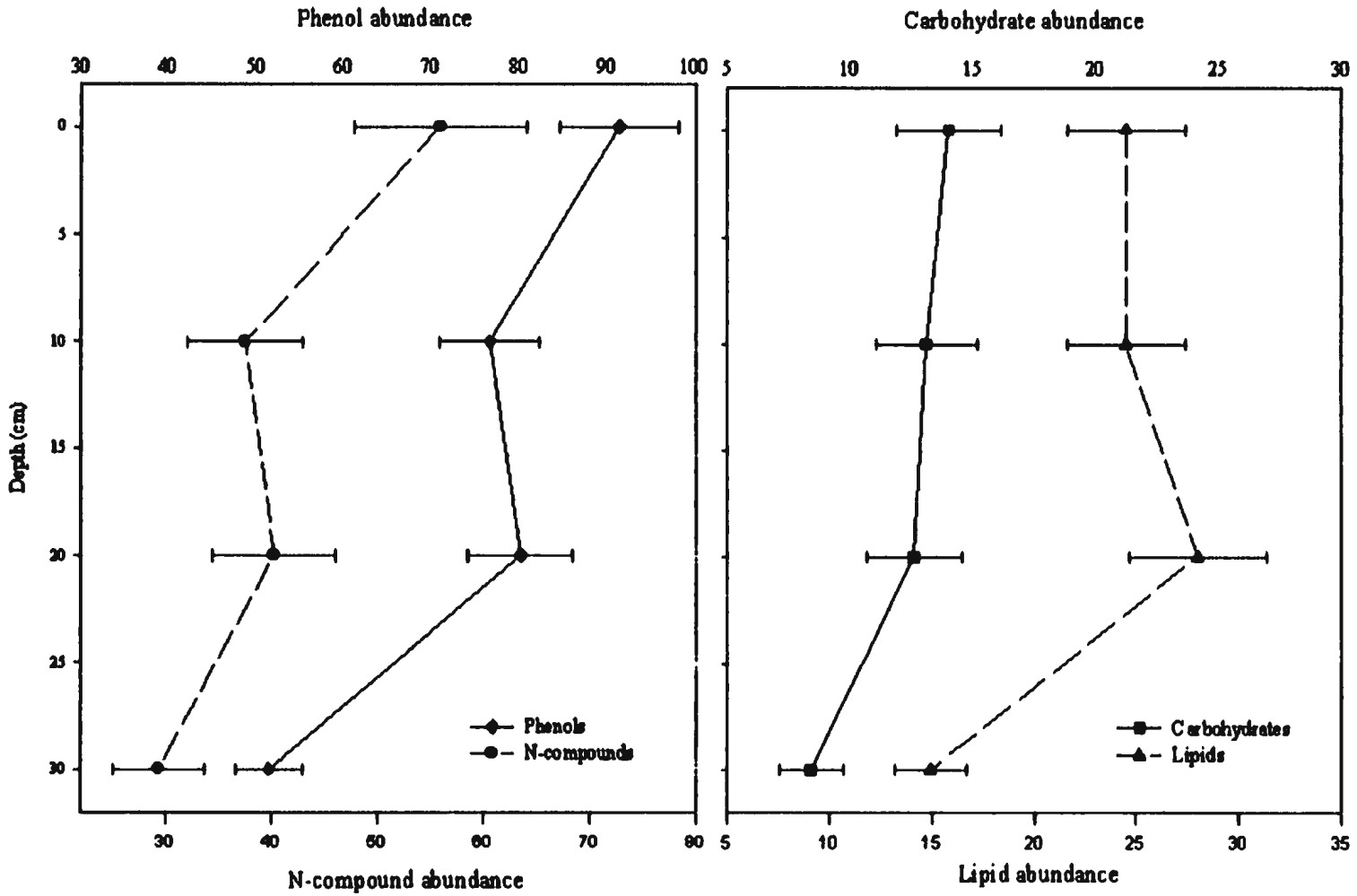
Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16

Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37

Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34

Total lipids is the sum of peak numbers 13 and 27

Data used for figures is in Table 2.4


Phenol abundance
Carbohydrate abundance
Depth (cm)
Phenols
N-compounds
Carbohydrates
Lipids
N-compound abundance
Lipid abundance

(discussed in Section 4.3.4) (Table 2.4). There is a negative correlation between organic content and phenol and fatty acid yields in the upper 5 to 10 cm of core H9 (Figure 4.52; Chapter 4). There must be an increase in the abundance of other classes of compounds, such as nitrogenous compounds, in order to preserve mass balance of total organic matter. Favaro (1998) measured elevated PAH concentrations (chrysene and benzo(a)anthracene) for core H9 and indicates that petroleum contamination is a major contributor of these contaminants. It is also likely that there are elevated levels of other contaminants. The low phenols/N-containing compounds ratios at the surface (1.6) may be the result of dumping of N-rich compounds in the Clarenville Harbour in the past 25 years as a result of various industrial activities. However, the downcore variation of phenols/N-compounds is within the limits of the percent error of phenols/carbohydrates ratios and must be interpreted with caution. Phenol/carbohydrates ratios are constant downcore indicating that carbohydrates are utilized at approximately the same rate as phenols.

The abundance of phenols of core ST7 sediment, lowest among all of the cores, is highest at the surface and decreases downcore (Table 2.5; Figure 2.33). The abundance of N-compounds and total carbohydrate follow the same trend (of decreasing abundance of phenols with sediment depth); hence, the phenol/N-compound ratios are relatively constant throughout core ST7. The surface sediment of ST7 is characterized by very high abundances of 1-H-indole, which decreases with depth indicating inputs of "fresh" OM that increases in maturity (downcore) as a result of early diagenesis and extensive bioturbation by the benthic community.

Table 2.5 - Depth variation of sediment pyrolysates of core ST7

1. Class abundance of pyrolysates (peak area per mg of sediment x 1000 and normalized to TOC); n = 3, mean ± s.d.

2. Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis

Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16
Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37
Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34
Total lipids is the sum of peak numbers 13 and 27

Data plotted in Figure 2.33

Table 2.51 - **Class abundance of pyrolysates (peak area per mg of sediment x 1000); n = 3, mean ± s.d.**

Core ST7	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenols	46 ± 3	22 ± 2	19 ± 1	13 ± 1
N-compounds	38 ± 6	21 ± 3	15 ± 2	10 ± 2
Carbohydrates	24 ± 4	11 ± 3	9.1 ± 1.6	4.2 ± 1.0
Lipids	25 ± 3	10 ± 1.3	7.6 ± 0.9	4.7 ± 0.6
Phenols/N-compounds	1.2 ± 0.3	1.0 ± 0.2	1.2 ± 0.3	1.3 ± 0.3
Phenols/Carbohydrates	1.9 ± 0.3	1.9 ± 0.5	2.1 ± 0.5	3.1 ± 0.9
Phenols/Lipid	1.8 ± 0.4	2.1 ± 0.4	2.4 ± 0.5	2.8 ± 0.5

Table 2.52 - **Individual pyrolysates abundance (peak area per mg sediment x 1000); single analysis**

Core ST7	0-2 cm	10-12 cm	18-20 cm	28-30 cm
Phenol	12	5.3	3.7	2.4
2-Methylphenol	5.2	2.3	1.9	0.9
Methylphenol	9.5	6.2	5.9	4.9
Ethyl phenol	14	5.3	5.0	3.1
4-Ethylenephenol	5.6	2.4	2.1	1.7
Benzeneacetonitrile	4.3	2.8	4.8	3.0
Benzenepropanenitrile	9.5	4.9	1.7	1.7
Pentadecanenitrile	5.7	3.0	1.9	1.0
1-H Indole	12	6.2	4.3	3.2
3-Methylindole	6.9	3.8	2.3	1.1
7-H Furobenzopyranone	6.8	1.8	2.5	1.3
2-Methylcyclopentanone	5.6	3.8	2.8	1.2
3-Methylcyclopentanone	12	5.7	3.8	1.8
Butenedioic acid, monobutyl ester	11	4.3	3.0	1.9
Nonadecene	14	6.1	4.6	2.8

Figure 2.33 - **Depth variation of sediment pyrolysate classes of core ST7. Abundances are in peak area (x 1000) per mg sediment.**

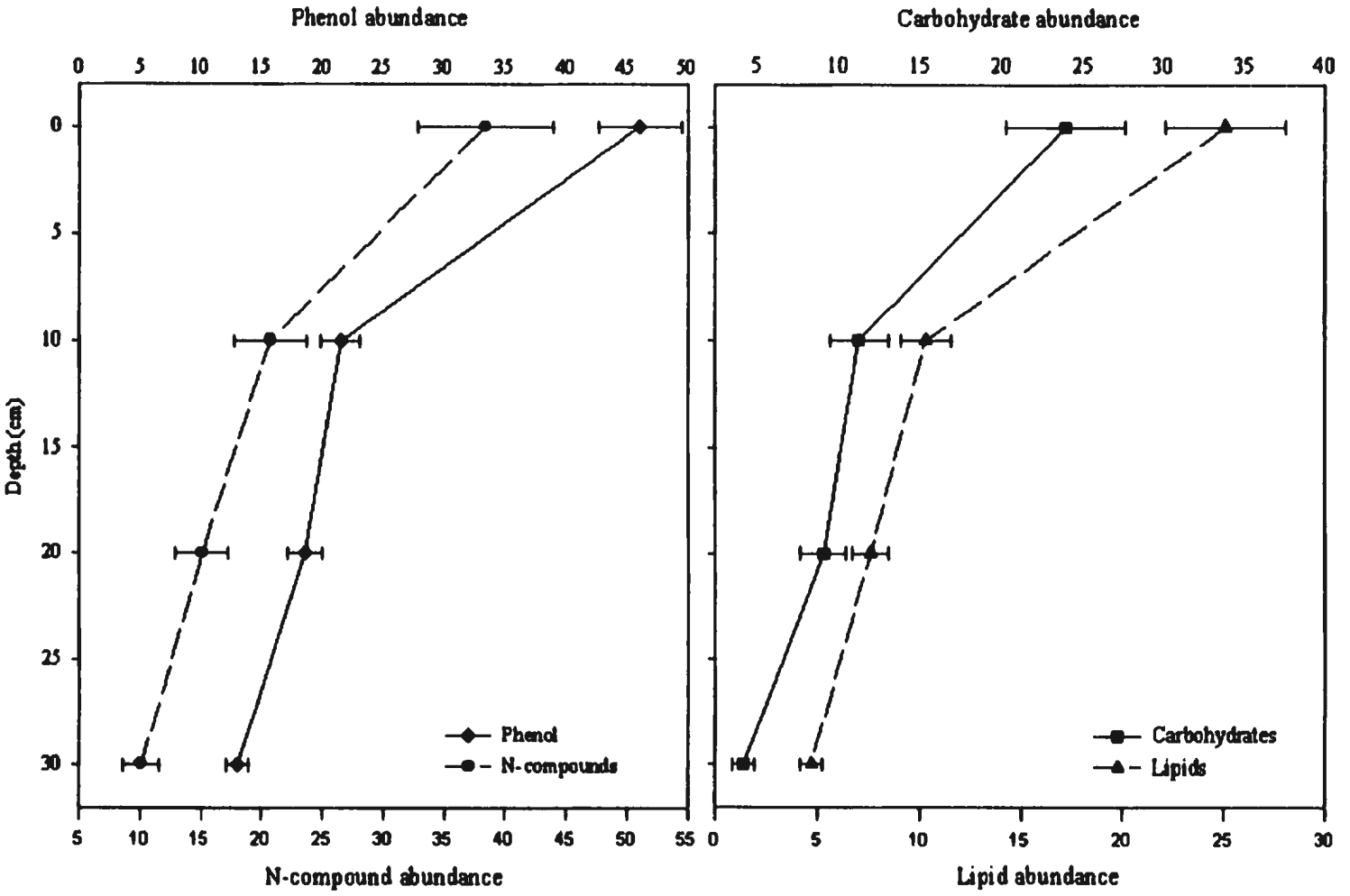
Total phenols is the sum of peak numbers 5, 7, 9, 12 and 16

Total N-Compounds is the sum of peak numbers 11, 15, 21, 24 and 37

Total carbohydrates is the sum of peak numbers 2, 3, 32 and 34

Total lipids is the sum of peak numbers 13 and 27

Data used for figures is in Table 2.5


Phenol abundance
Carbohydrate abundance
Depth (cm)
Phenol
N-compounds
Carbohydrates
Lipids
N-compound abundance
Lipid abundance

The phenols/carbohydrates ratios for core ST7 increase with depth, with the maximum value at the bottom of the core, indicating preferential degradation of carbohydrates and greater rate of utilization of carbohydrates than phenols. This is consistent with the reactivities of the two classes of compounds; carbohydrates are generally more reactive than phenols (Killops and Killops, 1993). Similarly the downcore increase in phenol/lipid ratio is interpreted in the context of lower reactivity of phenols in comparison to lipids (also consistent with their reactivities). However, both the downcore variations of phenols/carbohydrates and phenols/lipids ratios are within the limits of their percent error and must be interpreted with caution.

There are several differences between the signatures of the sediment pyrolysates of the study area and those of settling particles in the Japan Trench. Analysis of settling particles in the Japan Trench was carried out using a CDS pyroprobe (Model 100) connected to a Varian 3400 with a fused silica capillary column (DB-5). According to Ishiwatari *et al.* (1995), phenols accounted for a minor fraction of the total pyrolysates. On average, 13.8% of the total pyrolysates were phenols. Phenols were more abundant in sediment pyrolysates of Trinity Bay/Northwest Arm, varying between approximately 64 % (core H1, 0-2 cm) and 34% (core ST7, 10 cm) indicating preferential preservation of phenols (if the composition of organic matter of particulates in both areas are similar). The average percentage of nitrogenous compounds in the particulates from the Japan Trench is higher than that of sediment pyrolysates from the study area as expected from the reactivity of this class of compounds; nitrogenous compounds are very reactive and likely to be preferentially removed

from the SOM after sedimentation (Killops and Killops, 1993). The ratios of N-containing compounds/carbohydrates and N-containing compounds/alkanes of pyrolysates of settling matter in the Japan Trench generally decrease with depth in the water column. As particles descend in the water column of the Japan Trench, the organic content becomes more mature and preferentially becomes enriched in aromatic content and depleted in nitrogenous and/or oxygenated compounds. The differences in signatures of pyrolysates of particulates in the Japan Trench are different from those of sediments of Trinity Bay and Northwest Arm because the environments are different. It is likely that the quality of OM in particulates settling in the Japan Trench is different from that of sediments of Trinity Bay and Northwest Arm. Although the sampling device in the Japan Trench was set at 9200 m, it is likely that the OM in the water column of the Japan Trench is at an earlier stage of degradation than that of the sediments of Trinity Bay and Northwest Arm because it has not reached the sediment/water interface, a zone of intense biodegradation.

Although there were variations in the signatures of pyrolysates of the three cores (H1, H9 and ST7), temporal variations of pyrolysates in the study area are less pronounced than spatial variations. The most noticeable change is the strong decrease in phenol distribution with below the surface for core H1. An increase in the percentage nitrogenous compounds and a decrease in percentage carbohydrates accompany this. These changes reflect inputs from Hickman's Harbour. Downcore variations of signatures of different fractions of compounds were minor for core ST7. This is probably the result of bioturbation, which enhances homogenization. The minor variations indicate that OM that reaches the sediment is somewhat recalcitrant and illustrates its stability.

2.4 SUMMARY

1) The pyrolysates of sediments included a suite of phenolic, carbohydrate, and nitrogeneous organic compounds (Table 2.1; Figure 2.1). Phenols were structurally simple and lacked higher order side chains for all samples except for the surface of core H1. The carbohydrates in the pyrolysates included methylcyclopentanones, pyranones and levoglucosan. The N-containing compounds (acetonitriles and indole) of the pyrolysates reflect several classes of compounds including proteins, amino acids and chlorophyll.

2) The distribution of sediment pyrolysates from Trinity Bay is different from those of the Northwest Arm (Table 2.2; Figure 2.2). Sediment pyrolysates in the Northwest Arm have greater amounts of phenolic compounds. The offshore stations are characterized by higher abundances (peak areas and normalized abundances) of proteinaceous and related compounds.

3) Downcore variations of pyrolysates were most pronounced in core H1 (Table 2.3; Figure 2.31). The most noticeable changes occur just below the 0-2 cm depth, and are marked by a sharp decrease in abundance in phenols and carbohydrates. In addition, the unambiguous lignin pyrolysate markers (i.e. those with methoxy side chains) were absent below the surface of core H1. Variations in abundances of pyrolysates for cores H9 and ST7 were less pronounced (Tables 2.4 and 2.5; Figures

2.32 and 2.33).

4) Historical tree logging activities by pre-European and European settlers near Hickman's Harbour contributed to terrestrially derived OM being transported to site H1. As a result, pyrolysates of core H1 are abundant in phenols (Table 2.3; Figure 2.31). Natural processes such as drainage of the hinterland also contribute to high baseline abundances of phenols of core H1.

5) In Trinity Bay/Northwest Arm, spatial variation of the signatures of the sediment pyrolysates was more apparent than temporal variation. The most noticeable feature of the spatial variation of pyrolysates is the abundance and the signature of pyrolysates of core H1 pyrolysates. There are also apparent differences between the abundance and the signature of the Northwest Arm and Trinity Bay pyrolysates.

CHAPTER 3 - TMAH THERMOCHEMOLYSIS OF MODEL COMPOUNDS IN A SEDIMENT MATRIX

3.1 INTRODUCTION

Conventional flash pyrolysis of macromolecules releases many compounds with varying polarities. The more polar of these compounds are difficult to evaluate (Challinor, 1989; del Rio *et al.*, 1998; Garcetee-Lepecq *et al.*, 2000) and the less volatile of these compounds do not pass through standard chromatographic columns (Hatcher and Clifford, 1994; Challinor, 1995); these include compounds such as polyhydric phenols, benzene carboxylic acid and phenolic acids and long chain fatty acids. Derivatization of the polar moeities to produce compounds that can be more efficiently chromatographed is one analytical solution (Challinor, 1989; Filley *et al.*, 2000; Knapp, 1979). Also it has been demonstrated that pyrolysis can induce extensive decarboxylation (Saiz-Jimenez, 1995; Van der Heijden, 1994) and demethoxylation (Klap, 1997) which can lead to the underestimation of these functional compounds. Alkylation of hydroxyl and carboxyl groups is an applicable procedure that enhances preservation of these functional groups during thermochemolysis and chromatographic separation.

The most important class of organic compounds in recent sediments are the oxygenated biomolecules, and a suitable analytical approach which allows for analysis of these

compounds would be a useful tool. For example, the ratios of phenolic acids to phenolic aldehydes in degraded lignin can provide information on source of OM, as well as environmental conditions (Filley *et al.*, 2000; del Rio *et al.*, 1998; Hamilton and Hedges, 1988). A suitable technique is thermochemolysis with the use of tetramethylammonium hydroxide (TMAH) as an effective methylating agent (Challinor, 1989; Filley *et al.*, 1999; Filley *et al.*, 2000) (Appendix 3.1a).

This study thus examined the applicability of thermochemolysis with TMAH as a method of analyzing marine SOM. This technique involved thermochemolysis with TMAH at elevated temperatures, whereby organic components are hydrolyzed under basic conditions and the released acidic functional groups are methylated. As well, it is believed that other bonds are broken through pyrolytic process. As a relatively new technique, it has emerged as a suitable analytical procedure for lignin (Clifford *et al.*, 1995; Hatcher *et al.*, 1995), other polar group-containing compounds such as lipids (Hatcher and Clifford, 1994), humic materials (Martin *et al.*, 1995; del Rio *et al.*, 1998) and environmental samples (Hatcher *et al.*, 2000). It has been demonstrated that characteristic lignin monomers could be obtained using "on-line" pyrolysis/GC/MS at temperatures over 600 °C (Hatcher and Clifford, 1994; Martin *et al.*, 1995). McKinney *et al.* (1995) showed that thermochemolysis at much lower temperatures (300 °C) also produced the same lignin monomers. Hatcher *et al.* (1995) further demonstrated that this technique gave similar information to the more time consuming CuO oxidation method for lignin analysis. The reduced temperatures can facilitate the use of

thermochemolysis reaction with TMAH in sealed glass ampoules thus allowing for more controlled conditions, where internal standards can be added to provide quantitative measurement and where the products can be easily isolated for multiple or alternate analyses such as GC/C/ IRMS.

The distribution of phenol compounds allows for the distinction of the chemotaxonomic groups (angiosperm, gymnosperm, herbaceous tissues) and evaluation of the extent of degradation of lignins (del Rio *et al.*,1998; Filley *et al.*, 2000). This is achieved by comparison of lignin parameters. For example, the ratio of phenolic acid to corresponding phenolic aldehydes could provide information on the extent of lignin degradation (Filley *et al.*, 2000; Hedges *et al.*, 1988a, Hedges *et al.*, 1988b and Hedges *et al.*, 1988c). A potential problem with TMAH thermochemolysis of lignin is the occurrence of the Cannizzaro reaction (Appendix 3.1b) during which aldehyde moieties produce methylated products of the corresponding phenol acid (Tanczos *et al.*, 1997).

The methylation mechanism is shown in the Appendix 3.1a and is as follows:

1. The formation of tetramethylammonium salts of "free" acidic groups such as phenols and carboxylic acids.
2. A higher temperature base hydrolysis reaction of hydrolyzable functional groups of phenol ethers and carboxylic acid esters, and to some extent alkyl ethers to produce

tetramethylammonium salts (Appendix 3.1a, Eqs. 1a,1b).

3. Rapid thermal degradation of the quaternary salts to form methyl derivatives (ethers and esters) (Appendix 3.1a, Eqs. 1a,1b).

Hence, this reaction may also be referred to as thermally-assisted hydrolysis and methylation. Any excess TMAH undergoes thermal decomposition to produce triethylamine and methanol (Appendix 3.1a, Eq. 2). In short, the use of TMAH enhances bond cleavage of oxygenated linkages of macromolecules and assists in methylation of polar moieties containing hydroxyl groups, producing methyl esters of carboxylic acids and methyl ethers of hydroxy compounds (del Rio *et al.*, 1994; de Leeuw and Baas, 1993).

Before the products of TMAH thermochemolysates of marine sediments can be interpreted, it is necessary to proceed with analysis of useful model compounds in order to accurately establish bond cleavage and derivatization processes. The following issues need to be specifically investigated:

1) The behaviour of model compounds during TMAH thermochemolsis in the presence of a marine sedimentary matrix (surface core sediment ashed at 500°C to remove SOM). No previous work has been done to investigate the influence of inorganic matrices in thermochemolysis reactions.

2) Cannizzaro reaction: Tanczos *et al.* (1997) reported that the Cannizzaro reaction can occur during TMAH thermochemolysis of aldehydes (Appendix 3.1b). In the presence of concentrated alkalis, aldehydes containing no α-hydrogen undergo self oxidation and reduction reactions to yield a mixture of an alcohol and a salt of a carboxylic acid which is converted to the corresponding carboxylic acid under acidic conditions. Consequently, if the Cannizzaro reaction occurs during TMAH thermochemolysis of mixtures of phenolic acids and aldehydes (in a marine sediment matrix), the former will be overestimated and latter underestimated. This will distort any results of acid/aldehyde ratios of phenolic compounds, such as those reported by Hatcher *et al.* (1995). Hence, any TMAH-induced formation of phenolic acids from their respective aldehydes must be considered before acid/aldehyde ratios are interpreted.

3) The potential isomerization of conjugated biomolecules under the TMAH thermochemolysis conditions: TMAH thermochemolysis is known to induce isomerization of unsaturated compounds in studies of samples containing polysaccharides and fatty acids.

4) The efficiency of TMAH thermochemolysis conditions on bond cleavage of macromolecules: In humic materials, fatty acids are most likely linked to aromatic rings by ester bonds (Schnitzer and Neyroud, 1975). Phenols and fatty acids are major compounds of interest in this study. In lignin, the phenolic monomers are most commonly linked by ether bonds, which are thermally more stable than ester bonds.

5) The TMAH thermochemolysis behaviour of other classes of biomolecules on abundant naturally occurring compounds and material (other than phenols and fatty acids) normally present in marine sediments.

3.2 METHODS

3.2.1 Batch-wise TMAH thermochemolysis of model compounds

Individual fatty acid standards (Supelco Canada, Sigma-Aldrich), carbohydrates (Sigma, Aldrich), tricaprin (Supelco Canada) and phenolic standards (Sigma, Aldrich) were chosen for analysis. A lignin dimer kindly supplied by Dr. Phil Britt (Research Scientist), (Oak Ridge, Colorado), stearyl gallate and dihydroxydiphenyl ether (TCL America) were also chemolyzed. TMAH was purchased as its pentahydrate (Aldrich). Methanol (Aldrich) solutions (25% w/w) of TMAH were prepared fresh, as required.

The method employed is similar to that used by Hatcher *et al.* (1995). Approximately 0.5 mg of standard added to 50 mg of pre-combusted marine sediment in a pre-combusted (500^0 C, 60 minutes) 9 mm pyrex tube, to which 50 µl of 25% w/w methanolic TMAH (approx. 25 mg of reagent) was added. This ensures excess reagent for reaction.

The tubes were flame-sealed following evacuation of methanol and air on a high vacuum line. The samples were then placed in a sand bath maintained at 250 ± 5^0 C for 30 minutes.

The sand bath was contained in an enclosed furnace and a thermocouple monitored the temperature. After cooling, the tubes were opened and all internal surfaces were washed with spectrograde methylene chloride (1ml , three times). The solvent was then carefully removed under a stream of high purity nitrogen, and the products re-dissolved and dried again until there was no aroma of the trimethylamine by-product. The product residue was stored in the freezer until analysis.

The "in-house" standards (TMAH derivatives of phenolic aldehydes and fatty acids) were compared with commercially available standards (mixtures of fatty acids and phenols). This enabled estimation of recoveries. By using pre-combusted sediments in the reaction tube, the "in-house" standards and sediments were chemolyzed under similar conditions as the sediment samples. An aliquot of a commercial standard mixture was injected into the GC/MS. Peak areas obtained from a local standard mixture were compared to that of the commercial standard. Recoveries were then calculated as follows:

Percent recovery = [(Theoretical - Actual) * 100]/Theoretical

where Theoretical refers to the calculated peak area for a given amount of standard. Actual refers to the peak area obtained for the same amount of standard.

3.2.2 Identification of products by gas chromatography/ mass spectrometry

The GC/MS was a Varian Star 3400 gas chromatograph (equipped with an on- column

septum programmable injector (SPI) which was interfaced to a Varian Saturn 3 mass spectrometer (Ion Trap) detector. The capillary column was a J & W DB-5ms (0.25 mm i.d. x 30 m, 0.25 μ film thickness).The carrier gas (He) column pressure was set at 10 psi. The transfer line was heated at 280^0 C. The Ion Trap manifold temperature was set at 240^0 C. The mass range (40 - 500 m/z) was scanned at a rate of 500 ms under EI (70 eV) conditions. Dried samples were dissolved in 50 μl of methylene chloride and a 0.5 μl aliquot was injected on-column. The on-column injector was initially set at 80^0 C, then ramped to 280^0 C in 90 sec. The oven temperature program was as follows: initial temperature at 40^0 C (held for 2 min) ramped at 4^0/min to a final temperature of 280 ^{0}C (held for 20 min.). The temperature ramp for analysis of phenolic standards was 12^{0}C/min. Products listed in Table 3.1 were selected as useful markers and were authenticated by matching retention times and mass spectra of derivatized standards when they were commercially available. Other compounds were tentatively identified by matching with NBS mass spectral library and mass spectra published in the literature.

3.3 RESULTS AND DISCUSSION

3.3.1 Phenolic Compounds

The phenolic compounds of interest and their useful thermochemolysis product markers are listed in Table 3.1. The structures of these model compounds and their major thermochemolysis products are shown in Appendix 3.2. TMAH is a strong base (Knapp,

Table 3.1 - **TMAH thermochemolysis products of model compounds**

[1] Identified as a mixture of structural isomers

[2] Not the major product

[3] Chemical structures drawn in Appendix 3.2.

[4] Identification is tentative

*The fatty acids, sorbic acid, arachidonic acid and DHA are 2,4-hexadienoic acid, 5,8,11,14-eicosatetraenoic and 4,7,10,13,16,19-docosahexaenoic acids, respectively.

Model Compound[4]	Major Thermochemolysis Product marker
Phenolic compounds	
4-Hydroxybenzaldehyde	4-Methoxybenzaldehyde
3-Methoxy-4-hydroxybenzaldehyde	3, 4-Dimethoxybenzaldehyde
Syringealdehyde	3, 4, 5-Trimethoxybenzaldehyde
Benzoic acid	4-Methoxy benzoic acid, methyl ester
Vanillic acid	3, 4-Dimethoxy benzoic acid, methyl ester
Syringic acid	3, 4, 5-Trimethoxy benzoic acid, methyl ester
Ferrulic acid	3-(3,4-Dimethoxyphenyl)acrylic acid, methyl ester
Coumaric acid	3-(4-Methoxyphenyl)acrylic acid, methyl ester
Gallic acid	3, 4, 5-Trimethoxy benzoic acid, methyl ester
Fatty acids*	
Hexadecanoic acid	Hexadecanoic acid, methyl ester
16-Methyl-heptadecanoic acid	16-Methyl-heptadecanoic acid, methyl ester
Eicosanoic acid	Eicosanoic acid, methyl ester
Docosanoic acid	Docosanoic acid, methyl ester
Tetracosanoic acid	Tetracosanoic acid, methyl ester
Sorbic acid	Methyl esters of sorbic acid[1]
Arachidonic acid	Methyl esters of arachidonic acid[1]
DHA	Methyl esters of DHA[1]

Model Compound	Thermochemolysis Product marker
Carbohydrates	
Cellulose	1, 2, 4-Trimethoxybenzene
Glucose	1, 2, 4-Trimethoxybenzene[2]
Starch	1, 2, 4-Trimethoxybenzene[2]
Amino acids	
Tyrosine (TYR)	3-(4-Methoxyphenyl)acrylic acid, methyl ester[4]
	1-Ethenyl-4-methoxybenzene
Phenylalanine (PHE)	3-Phenyl-2-propanoic acid, methyl ester
	2-Methoxy-benzenepropanoic acid, methyl ester
	1-Methoxy-4-(1-propenyl)benzene[4]
Other Compounds	
Lignin-dimer[3]	1, 2-Dimethoxybenzene
	1-Ethenyl-4-methoxybenzene
Tricaprin	Decanoic acid, methyl ester
Stearyl gallate	1,2,3-Trimethoxybenzoic acid, methyl ester
	Octadecyl alcohol
4,4-Dihydroxydiphenyl ether	4,4-Dimethoxydiphenyl ether

1979). The classic reaction of TMAH reagent and a phenol is illustrated in Appendix 3.1a, Eq.1a. Derivatization of the phenolic aldehydes (syringealdehyde [SAL], vanillin [VAL], benzaldehyde [PAL]) resulted in conversion to their respective methyl ethers. No other products were observed. The phenolic acids (syringic acid [SAD], vanillic acid [VAD], benzoic acid [PAD], gallic acid [GAD], coumaric acid [CAD], and ferrulic acid [FAD]) produced methoxy phenolic methyl esters (Appendix 3.1a, Eq. 1b). Filley *et al.* (1999) also demonstrated that the aryl methoxy group of selected phenols (vanillic acid and eugenol) were stable during TMAH thermochemolysis and there was minimal (less than 3%) exchange with the methyl groups of TMAH.

There was no evidence of the occurrence of the Cannizzaro reaction which would result in partial oxidation of the phenolic aldehydes to the respective carboxylic acids (see Figure 3.1). The reactions of the aldehydes (4-hydroxybenzaldehyde, 3-methoxy-4-hydroxybenzaldehyde and syringealdehyde) to produce their respective methoxy derivatives were almost quantitative (when compared to calibration curves of products); 95, 96 and 98% conversion to 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde, respectively. The products have only small amounts of their respective benzoic acids (yield ratios of ~0.05, acid/aldehyde). Furthermore, the ion scans of the methoxy derivatives of the aldehydes indicate very low abundances of acid methyl esters. The conversions of the phenolic acids to the respective methyl esters were also quantitative (>98%) (Figure 3.2). Therefore, the acid/aldehyde ratios determined by this method should be representative of

Figure 3.1 - **Total ion chromatograms of thermochemolysates of phenolic aldehydes (1. Benzaldehyde, 2. Vanillin, 3. Syringealdehyde)**

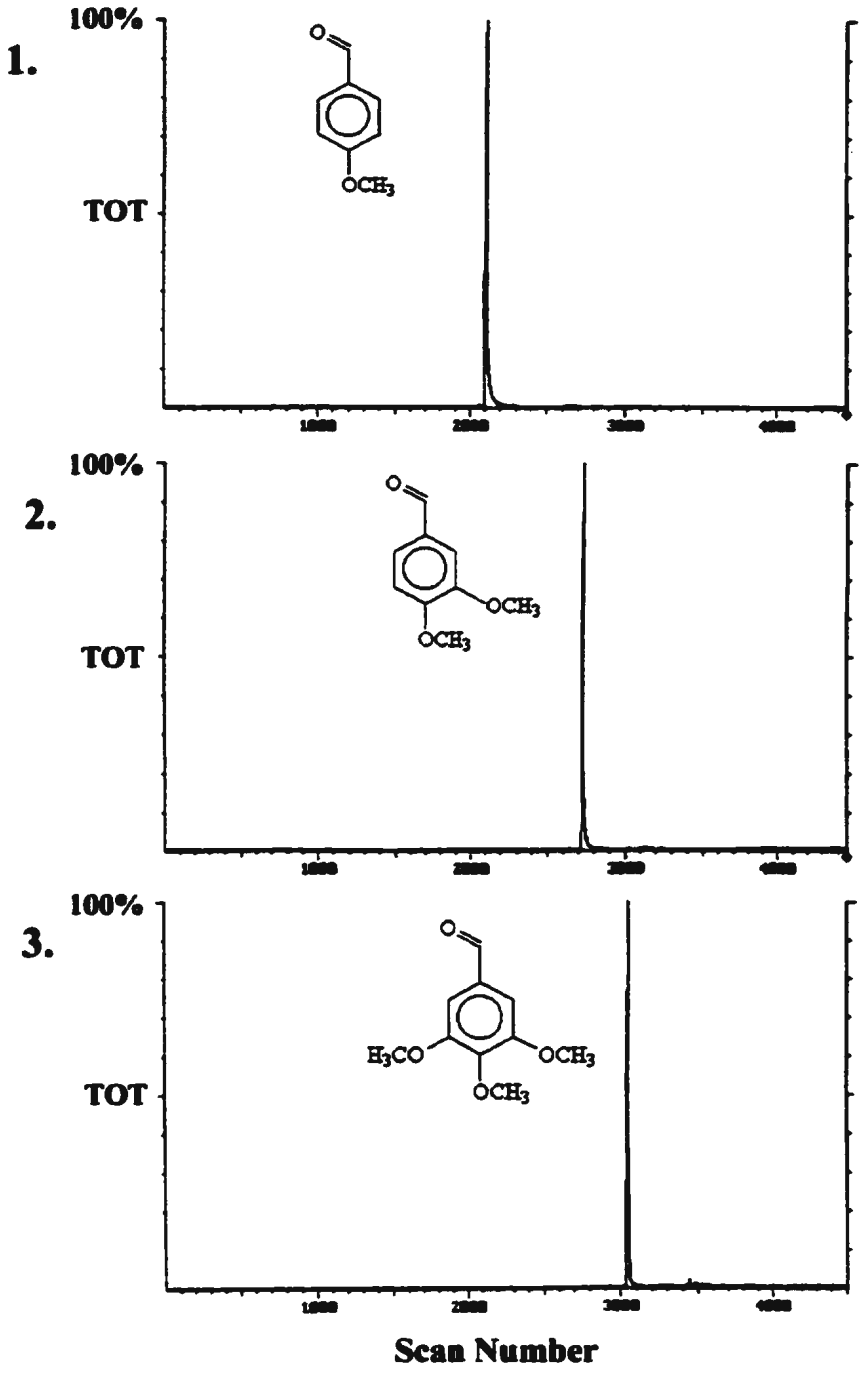
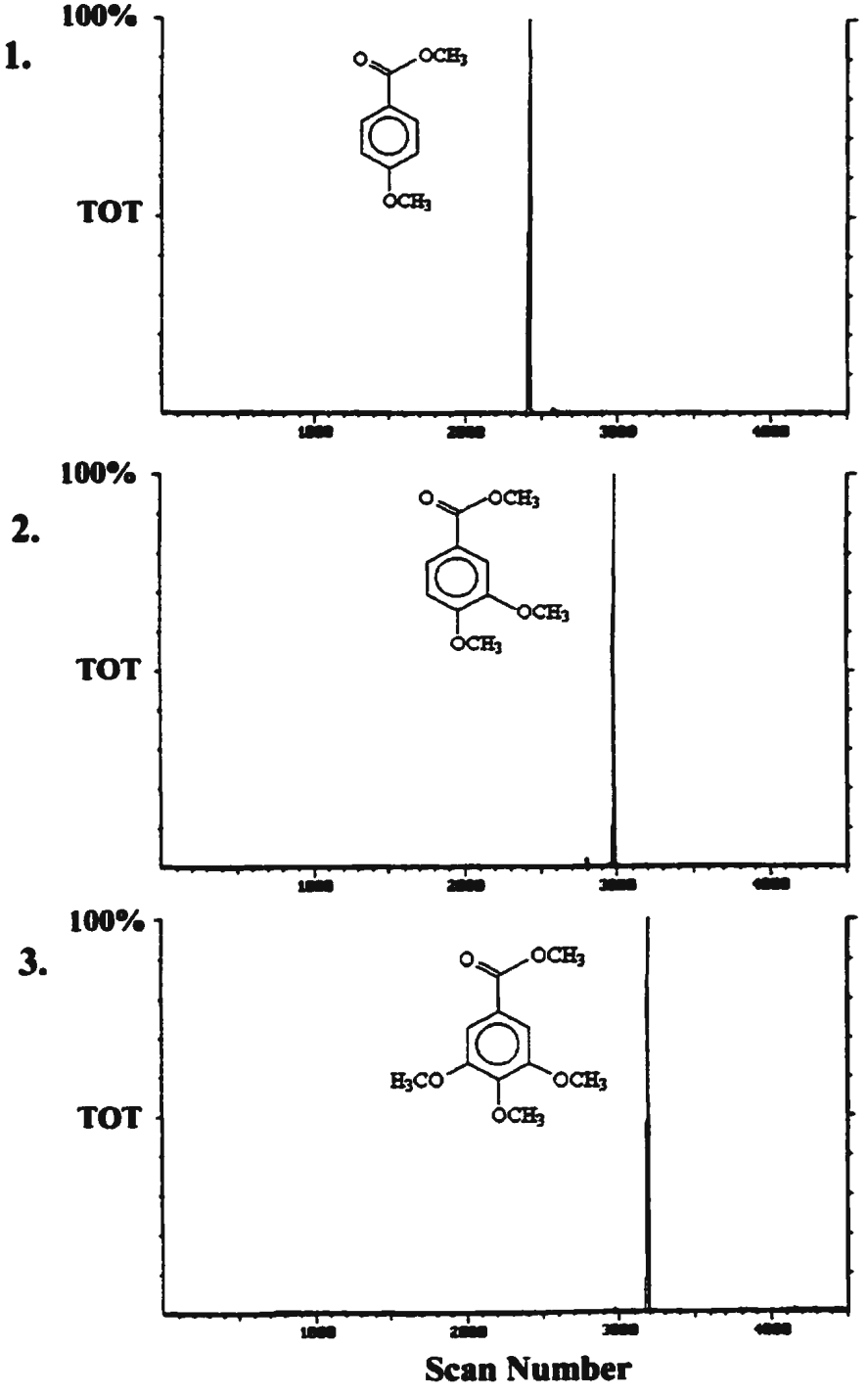


Figure 3.2 - **Total ion chromatograms of thermochemolysates of phenolic acids (1. Benzoic acid, 2. Vanillic acid, 3. Syringic acid)**



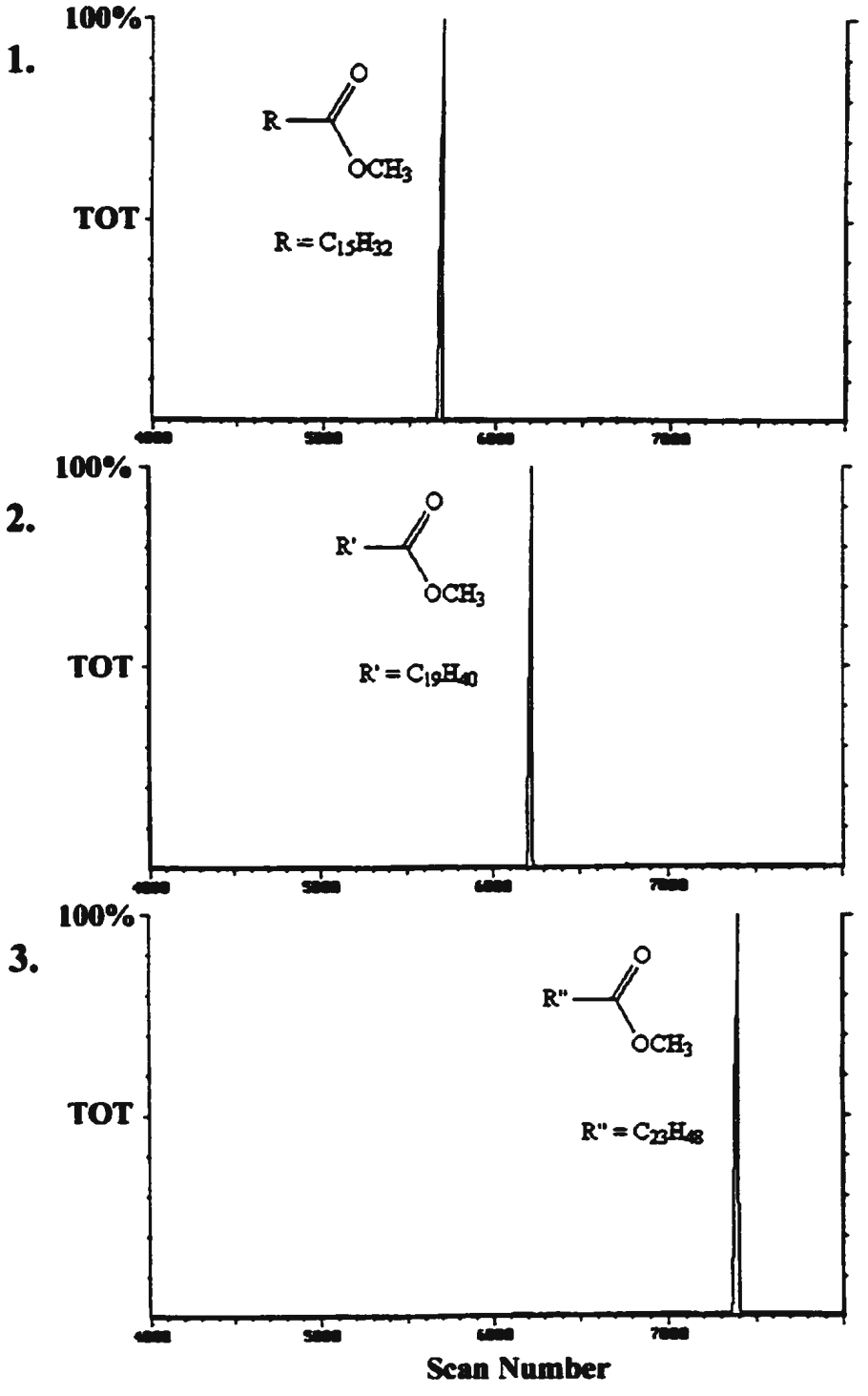
the abundances of these compounds in the free state.

3.3.2 Fatty acids

The names of fatty acid may be abbreviated by indicating the number of carbon atoms, and the number and position of double bonds. Hexadecanoic acid can be written as 16:0, indicating 16 carbon atoms (16) and no double bonds (:0). The shorthand C18:1Δ9 refers to 18 carbon chain length (18), one double bond (:1) at the 9 carbon position (Δ9). The prefix b indicates a branched carbon skeleton.

Alkaline TMAH solution can also act as a methylating agent for conversion of fatty acids into esters (Equation 1) (Knapp, 1979). Several fatty acids were thermochemolyzed (Table 3.1, Appendix 3.1a). The saturated fatty acids (C_{16}, C_{18}, C_{20}, C_{22}, C_{24}) were almost quantitatively recovered as their respective methyl esters after TMAH thermochemolysis (Figure 3.3). Challinor (2000) also reported rapid and quantitative conversion of fatty acids in oils and fats. Analysis of thermochemolysates of polyunsaturated fatty acids indicate varying degrees of isomerization of double bonds, and quite likely some degradation because they are unstable to the highly basic conditions of TMAH. The unsaturated fatty acids that were chemolyzed included sorbic acid ($C_{6:2}$), 5,8,11,14 eicosatetraenoic acid (arachidonic acid, $C_{20:4}$) and 4,7,10,13,16,19-docosahexaenoic acid ($C_{22:6}$). TMAH chemolysates of the unsaturated fatty acids consist of isomers; pronounced isomerization of the polyunsaturated

Figure 3.3 - **Total ion chromatograms of thermochemolysates of saturated fatty acids (1. Hexadecanoic acid, 2. Eicosanoic acid, 3. Tetracosanoic acid)**



fatty acids, 5,8,11,14 eicosatetraenoic acid and 4,7,10,13,16,19-docohexaenoic acid, was evident in their chromatograms (Figure 3.4). The TMAH chemolysis of the polyunsaturated fatty acids was very inefficient and yields of the fatty acid methyl esters were very low. As a result, the abundances of individual polyunsaturated fatty acids in sediments cannot be obtained using the conditions employed.

3.3.3 Triacylglycerol, lignin dimer, dihydroxydiphenyl ether, stearyl gallate

Tricaprin, a triacylglycerol, was thermochemolyzed to decanoic acid (C_{10}) methyl ester (Figure 3.5). Thermochemolysis of stearyl gallate (an ester consisting of gallic acid and a C_{18} unit) resulted in a quantitative conversion into methyl esters of the monomeric units (3,4,5-trimethoxybenzoic acid methyl ester and octadecyl alcohol) (Figure 3.5).

A model lignin dimer was also thermochemolyzed into its monomeric units (3,4-dimethoxybenzene and 1-ethenyl-4-methoxybenzene) (Figure 3.6). However, the major product is the methylated parent molecule. Similar results were obtained on thermochemolysis of dihydroxydiphenyl ether (Figure 3.6). Filley *et al.* (1999) proposed four mechanisms to explain TMAH chemolysis product distribution for a model lignin structure. Two of the mechanisms are observed in the chemolysis of the lignin dimer used in this study. There is a straightforward methylation of the phenolic group of the dimer to produce the methylated dimer. In addition, there is cleavage of the β-O-4 linkage to produce the

Figure 3.4 - **Total ion chromatograms of thermochemolysates of unsaturated fatty acids (1. Sorbic acid, 2. 5,8,11,14-Eicosatetraenoic acid, 3. 4,7,10,13,16,19-Docosahexaenoic acid)**

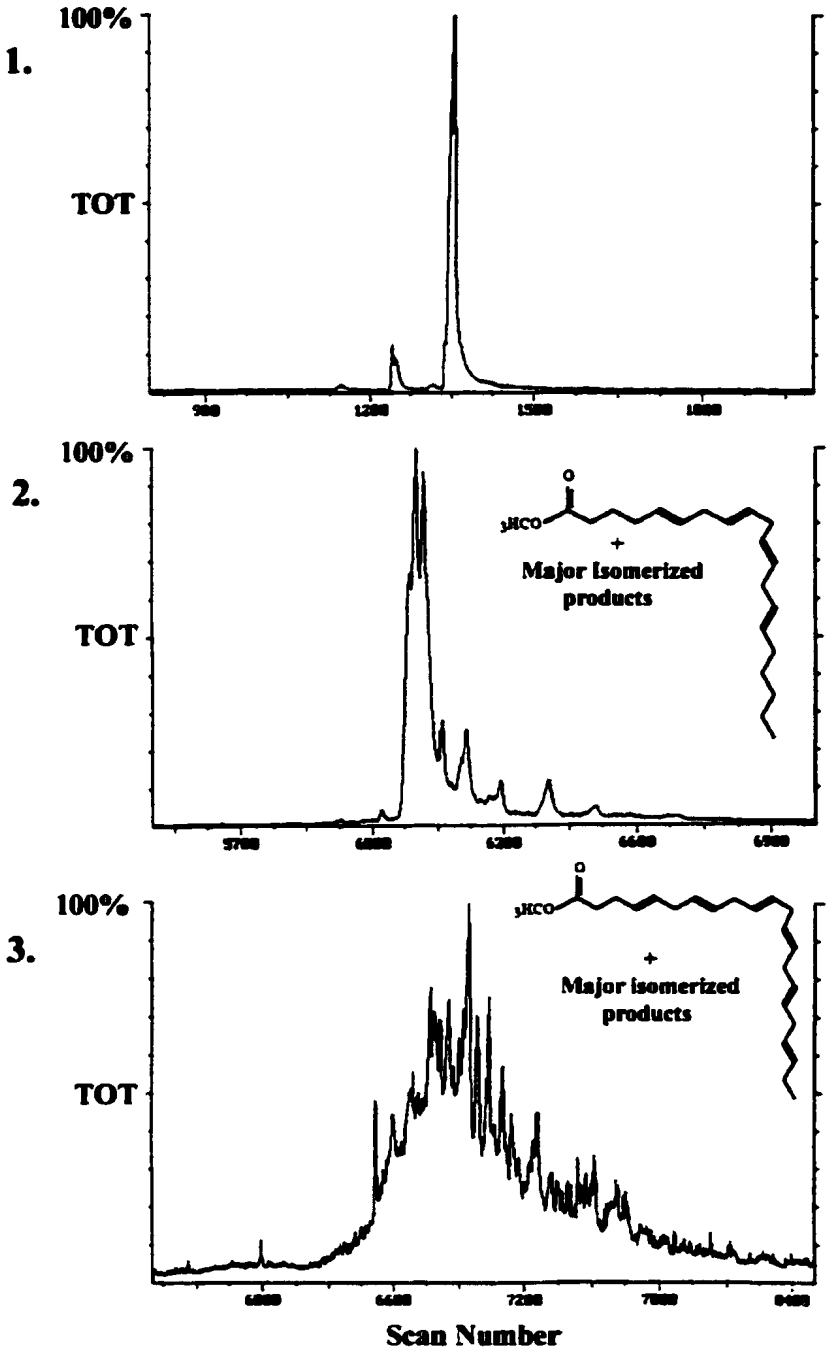


Figure 3.5 - Total ion chromatograms of thermochemolysates of (1) Tricaprin and (2) Stearyl gallate

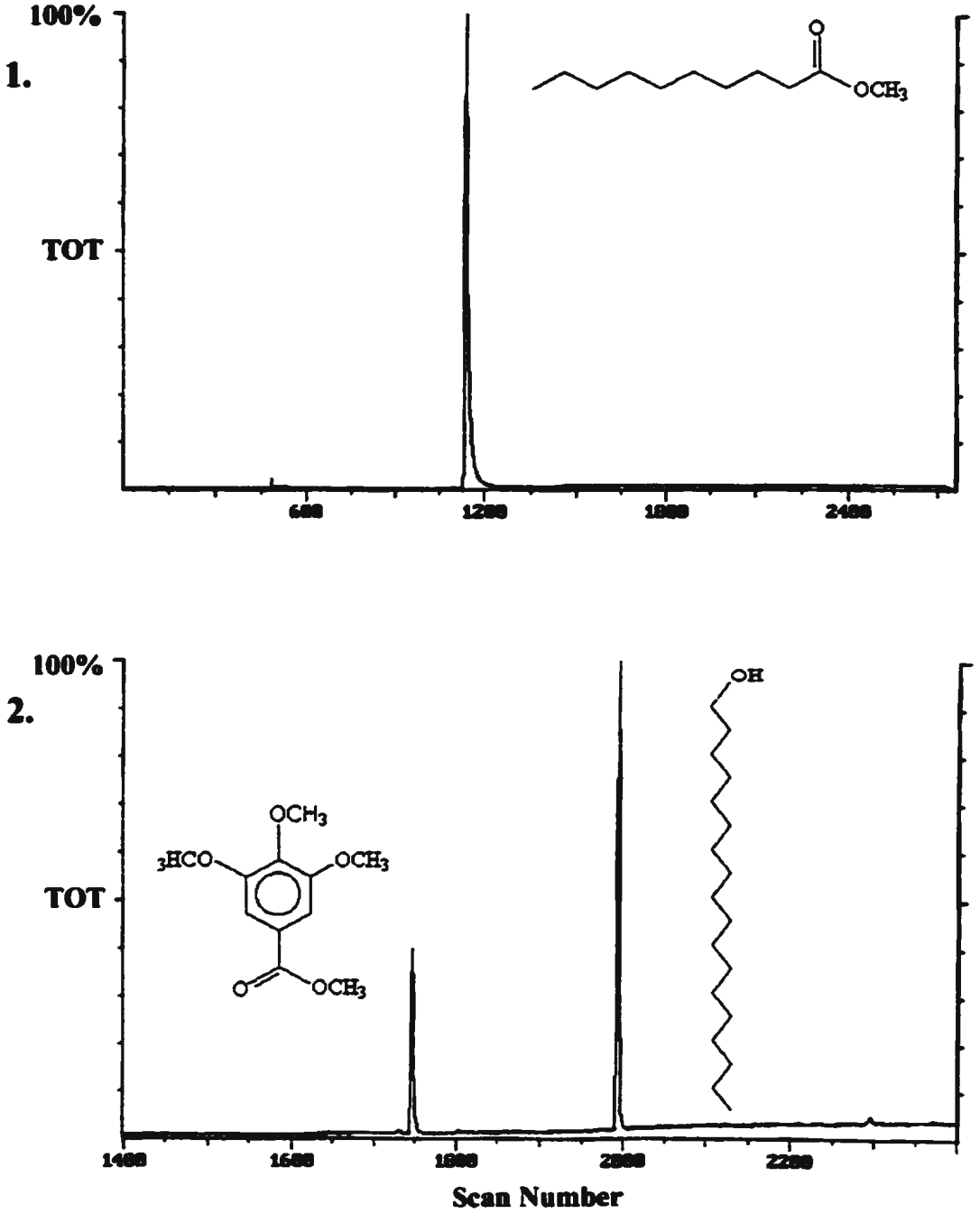
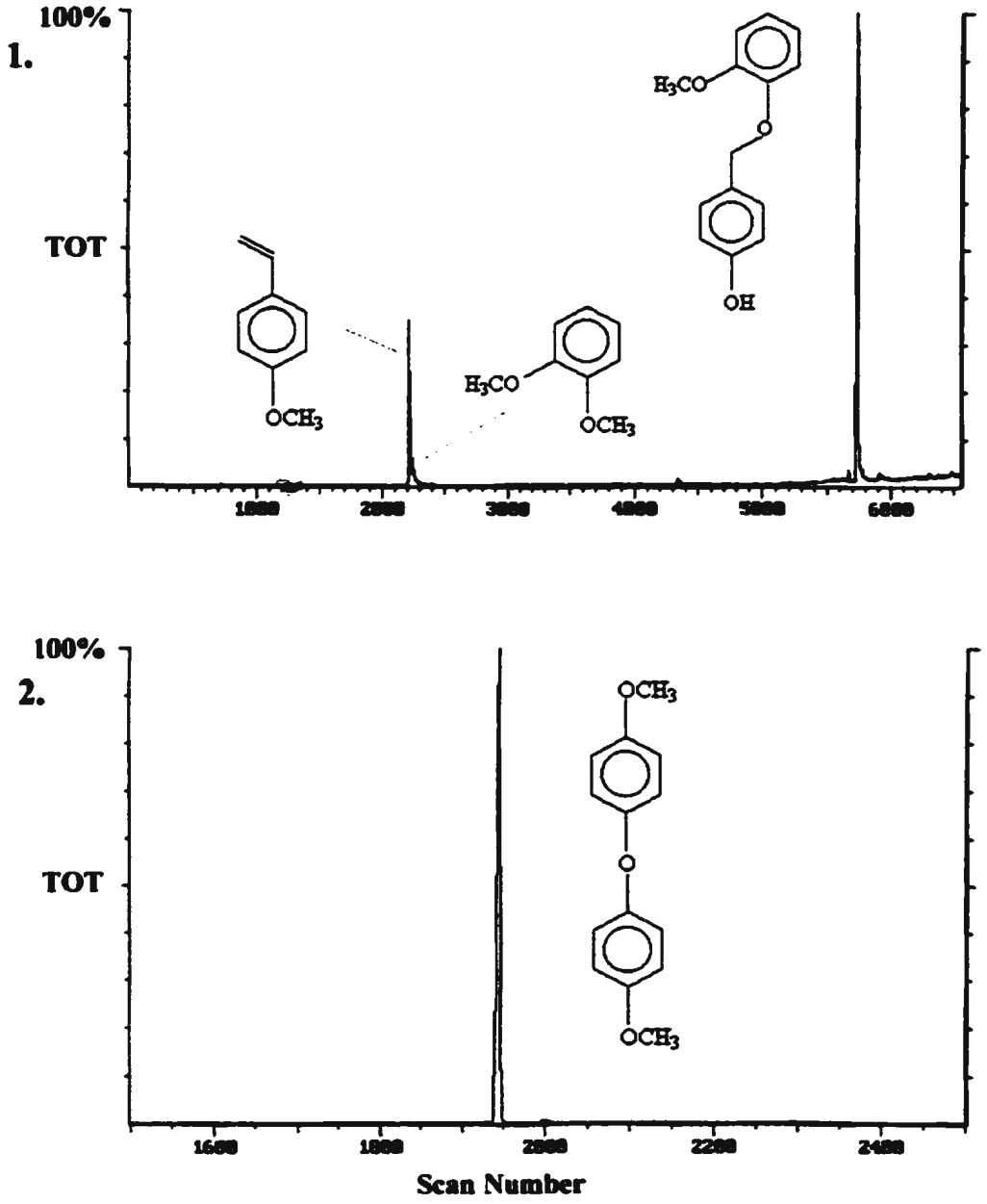


Figure 3.6 - **Total ion chromatograms of thermochemolysates of (1) Lignin dimer and (2) 4,4-Dihydroxydiphenyl ether**



monomeric units. The product distribution of TMAH thermochemolysis of the lignin dimer in this study are different from that of Filley *et al.* (1999) because the dimers are structurally different. TMAH effectively depolymerize β-O-4 linkages with adjacent hydroxyl groups, such as that used by Filley *et al.* (1999) and Filley *et al.* (2000). In addition, Filley *et al.* (1999) and Tanczos *et al.* (1997), proposed that the epoxide is the intermediate in the oxidation of 3, 4-dimethoxybenzaldehyde to 3, 4-dimethoxybenzoic acid methyl ester during TMAH thermochemolysis by a Cannizzaro-type reaction. However, this mechanism is only possible with lignin dimers that have hydroxyl groups adjacent to the β-O-4 linkages.

3.3.4 Carbohydrates

Preliminary work on the chemolysates of marine sediments revealed the presence of 1,2,4-trimethoxybenzene. Initially, this compound could be be attributed to aromatic precursors, possibly syringyl lignin types (lignin with monomeric units having two methoxy groups in the *meta* position). Model carbohydrates were chemolyzed, including cellulose, glucose, starch, pectin and gum. The major chemolysis product of cellulose is 1,2,4-trimethoxybenzene. Fabbri and Helleur (1999) obtained similar results from TMAH chemolysis (under alkaline conditions) of carbohydrates, and proposed that 1,2,4-trimethoxybenzene is formed by a "peeling mechanism". Although present in chemolysates of the other carbohydrates, 1,2,4-trimethoxybenzene is not the major product. Interestingly, phenols (3,4- and 2,6-dimethoxy) were also produced during TMAH thermochemolysis of

the carbohydrates; Fabbri and Helleur (1999) also identified these compounds as chemolysates of carbohydrates. In addition, del Rio *et al.* (1998) identified 1,2,4-trimethoxybenzene in the TMAH chemolysate of dissolved organic matter (DOM) from the North Pacific ocean.

The production of 1,2,4-trimethoxybenzene by TMAH thermochemolysis of carbohydrates is particularly interesting in that it demonstrates that aromatic compounds can be produced by thermochemolysis (TMAH) of non-aromatic compounds (Fabbri and Helleur, 1999). The yields of 1,2,4-trimethoxybenzene during chemolysis of cellulose, glucose, pectin and gum are 1.1%, 0.7%, 0.2% and 0.01%, respectively. TMAH thermochemolysis of marine sediments yielded high abundances of 1,2,4-trimethoxybenzene, as high as 0.32 mg per gram of sediment (core H1 - 20 cm depth), indicating possible existence of very large quantities of carbohydrate compounds in marine organic matter (discussed in Chapter 4). Unfortunately, the disparate yields 1,2,4-trimethoxybenzene from the TMAH thermochemolysis of different carbohydrates do not facilitate accurate quantification of carbohydrates in sediments. Hence, the methoxybenzenes were not used as markers in this study.

3.3.5 Amino acids

Selected amino acids were also chemolyzed, including tyrosine (TYR), phenyalanine (PHE),

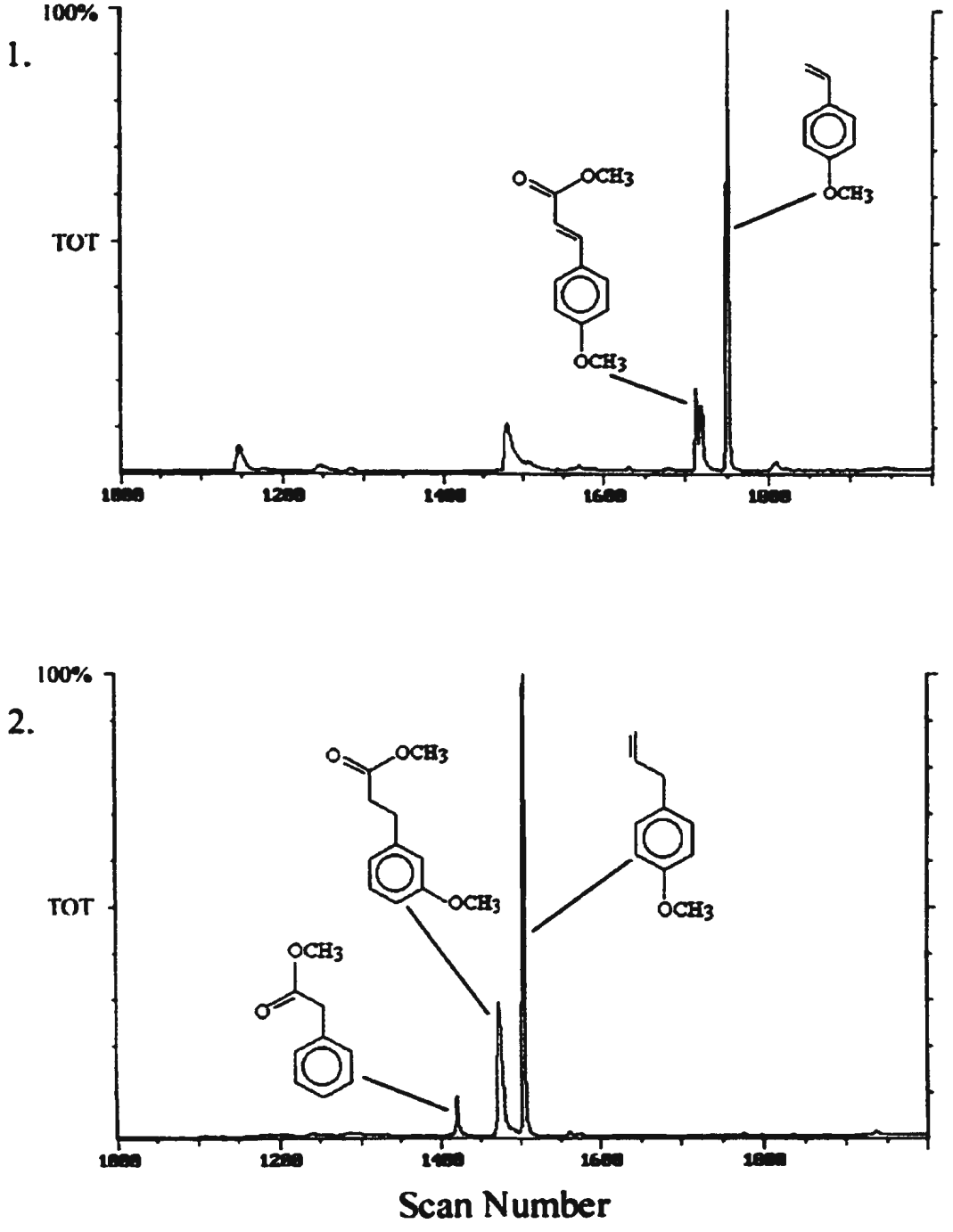
glycine (GLY), glutamic acid (GLU) and histidine (HIS). The aromatic amino acids yield a suite of phenolic products on TMAH thermochemolysis (Table 3.1; Figure 3.7). TMAH chemolysates of TYR were more abundant and different in structure from those of PHE. This may be explained by the additional hydroxy group in the para position in TYR. Of particular importance is the detection of 3-(4-methoxyphenyl) acrylic acid methyl ester. Initially, in this study, the precursor of this compound was assigned to coumaric acid (CAD). Knicker and Hatcher (1997) assigned proteins as a source for this compound in TMAH chemolysates of humic residues of algal sapropel from Mangrove Lake (Bermuda). Other TMAH chemolysates with structures similar to derivative lignin-derived phenols are 4-methoxybenzaldehyde, 4-methoxyethene and 1-(2-ethoxyphenyl)acetone.

3.4 SUMMARY

1 Phenolic aldehydes and acids are quantitatively converted to the respective ethers and methyl esters. Under the experimental conditions (marine sediment matrix, large excess TMAH, 250^{0}C) there was no evidence of unmanted Cannizzarro reaction (formation of respective acids) during derivatization of free phenolic aldehydes likely because the reaction conditions were not basic enough.

2 Saturated fatty acids are also quantitatively converted to methyl esters. TMAH thermochemolysis of a triacylglycerol proceeded as predicted by cleavage of the ester

Figure 3.7 - **Total ion chromatograms of thermochemolysates of amino acids (1. Tyrosine, 2. Phenylalanine)**



bonds to produce a fatty acid methyl ester and methanol in the sediment matrix.

3 TMAH chemolysates of polyunsaturated fatty acids consist predominantly of a mixture of structural isomers, thereby hindering the estimation of sedimentary unsaturated fatty acids.

4 TMAH thermochemolysis of carbohydrates produce 1,2,4-trimethoxybenzene, which is present in the chemolysates of naturally occurring SOM.

6. TMAH thermochemolysis of aromatic amino acids produce several phenolic compounds, some of which resemble methylated lignin-derived phenols, including 3-(4-methoxyphenyl) acrylic acid methyl ester.

CHAPTER 4 - MOLECULAR CHARACTERIZATION OF MARINE SEDIMENTARY ORGANIC MATTER BY TMAH THERMOCHEMOLYSIS

4.1 INTRODUCTION

Coastal marine sediments contain only small amounts (3 to 5%) of OM and, of this, an even smaller fraction is of terrestrial origin (Rashid, 1985; Killops and Killops, 1993). But in these regions, terrestrially derived OM plays a significant role in the carbon balance in the marine ecosystem. Both land drainage and atmospheric transport are important vehicles for terrestrial carbon input into the marine environment (See Chapter 1). One approach to study this terrestrial carbon flow is to examine marine sediments for terrestrially derived organic markers.

Lignin-derived products are ideal tracers of vascular plant material in sediments, particularly under anaerobic conditions (Hedges and Parker, 1976; Hedges *et al.*, 1988a; Hedges *et al.*, 1988b; Hedges, 1991; Manino and Harvey, 2000). This part of the study examines the suitability of thermochemolysis using TMAH as a method of structurally characterizing marine SOM and determining the contribution of terrestrially derived organic material. The hypothesis that chemolysates in TOC of marine sediments can be indicators of sources of OM to the TOC pool will also be tested.

4.1.1 TMAH Thermochemolysis of sedimentary organic matter

In Chapter 2, the results from molecular characterization of marine SOM by conventional pyrolysis/GC/MS show that in many cases, the pyrolysates were not unique enough to be suitable markers of terrestrial carbon input. For example, the benzene-containing pyrolysates, phenol, 2-methyl phenol, 4-ethyl phenol although identifiable in the pyrolysates of lignin standards, are not solely lignin pyrolysates; the pyrolysates of tyrosine (an aromatic amino acid) include phenol, cresol and ethylphenol (Irwin, 1982). Furthermore, fatty acids were not observed in the chromatograms (Irwin, 1982). Instead, hydrocarbons are readily produced as the result of decarboxylation. The method of TMAH thermochemolysis is adopted in this study because it has been established as a suitable technique in characterizing lignin (Challinor, 1995; Challinor, 2000; Filley *et al.*, 1999; Filley *et al.*, 2000; Hatcher, 1995; Hatcher *et al.*, 1995; McKinney *et al.*, 1995), marine dissolved organic matter (Manino and Harvey, 2000; van Heemst *et al.*, 2000) and soil humic materials (Clifford *et al.*, 1995; del Rio *et al.*, 1994; del Rio *et al.*, 1998; Fabbri *et al.*, 1996; Hatcher *et al.*, 1995; Martin *et al.*, 1994; Martin *et al.*, 1995). The chemical profiles obtained by TMAH thermochemolysis will complement those from conventional pyrolysis.

TMAH treatment of OM at elevated temperatures induces hydrolytic ester and ether bond cleavage and converts carboxylic and phenolic hydroxyl groups to their respective methyl derivatives, which are amenable to separation by gas chromatography (as discussed in

Chapter 1) (Lehtonen *et al.*, 2000). The technique of TMAH thermochemolysis is simple and routine, where sample preparation is minimal as described in Chapter 1. A big advantage of this method is the simultaneous determination of both fatty acid and phenolic moieties in complex OM (Hatcher and Clifford, 1994; Martin *et al.*, 1995; Martin *et al.*, 1994; Saiz-Jiminez, 1994). TMAH thermochemolysates identified in humic acids from a Haqlaquod (from Prince Edward Island, Canada) included fatty acids (C_6 to C_{25}), as well as several aliphatic diacids (Hatcher and Clifford, 1994) and aromatic products. Interestingly, fatty acids were more abundant than the phenolic chemolysates. TMAH thermochemolysis of humic acids from a podzol (soil located south of the tundra region, high in organics) yielded a suite of saturated fatty acids (C_{14} to C_{30}), diacids and monounsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$) (Saiz-Jiminez, 1995). A similar distribution of chemolysates was obtained by del Rio *et al.* (1998) on TMAH thermochemolysis of humic acids from Minnesota peat.

It must be noted that the observed yield of TMAH thermochemolysates comprises only a small portion of TOC. TMAH thermochemolysis cleaves ester and ether bonds and methylates fatty acids and phenols. In addition, the free forms of these compounds will also become methylated. As well, some classes of organics such as the lipid fraction, will have higher yields of product compared to more condensed structures such as the aromatic moieties in humic matter. To convey the results of TMAH thermochemolysis, abundances will be expressed as a fraction of the TOC. This was adopted by Del Rio *et al.* (1998) who analysed humic acids of Minnesota peat and expressed the amounts of chemolysates as a

percentage of TOC (mg of chemolysates per 100 mg of TOC). The amounts of individual lignin-derived phenols in the humic acids ranged from 0.04 to 1.28 mg per 100 mg of TOC. Vanillic acid methyl ester was the most abundant of the lignin-derived phenols. Fatty acid methyl esters (FAMEs) (C_{14} to C_{30}) were also readily identified in the peat sample having a strong predominance of even numbered carbon atoms. The amounts of FAMEs ranged from between 0.03 and 0.24 mg per 100 mg of TOC, the most abundant FAMEs being C_{16} to C_{20}. Only 4 - 5% of the TOC was accounted for by the thermochemolysis yield. However, what is important is the production of characteristic trace markers of organic classes in a reproducible fashion. Thus relative yields of chemolysates from different samples and yield ratios among organic classes will be used as indicators of the composition of SOM.

4.1.2 Lignin-derived compounds

Lignin is a generic term for complex irregular aromatic polymers unique to vascular plants. Lignins represent the second most abundant bio-polymer mineralized via the carbon cycle (Zeikus, 1980). These irregular three dimensional polymers are derived from an enzyme-initiated free radical polymerization of three phenyl propane moieties (C_6 - C_3 units) (Evans *et al.*, 1986; and de Leeuw and Largeau, 1993) (Appendix 4.1).

Lignins are classified into three main groups on the basis of their structural monomer units (Higuchi, 1980 and 1985; Kogel, 1986; Monties, 1989; Sjostrom, 1981) (Appendix 4.1).

Gymnosperm lignins (guaiacyl lignins) are dehydrogenation polymers of coniferyl alcohol that occur mostly in softwoods and contain a methoxy group in the para position (Appendix 4.1). Angiosperm lignins (guaiacyl-syringyl lignins) are mixed dehydrogenation polymers of coniferyl and sinapyl alcohols and are typical of hardwoods. Syringyl lignins are characterized by two *p*-methoxy groups. Grass lignins (guaiacyl-syringyl-*p*-hydroxyphenyl lignins) are composed of mixed dehydrogenation polymers of coniferyl, sinapyl and *p*-coumaryl alcohols (Appendix 4.1). *p*-Hydroxyphenyl lignins do not have methoxy groups. Furthermore, grass lignins also contain the cinnamyl alcohols. Therefore, compositional analysis of lignins can be used to characterize plant type (Saiz-Jimenez and De Leeuw, 1986; Goni and Hedges, 1990a and 1990b; Hedges, 1991).

Lignins are very resistant to microbial degradation because they are structurally compact, insoluble in most solvents and their inter-polymeric bonds are not directly hydrolysable (Zeikus, 1980). These properties render lignin a formidable substrate for microbial utilization. Nevertheless, lignins are metabolized by aerobic microbes such as white rot and brown rot fungi (Filley *et al.*, 2000; Rashid, 1985). The analytical decomposition of lignins by alkaline CuO oxidation results in the release of phenyl propane units, which in turn are broken down into simpler phenolic compounds such as C_6 - C_1 units. Some of the degradation products include vanillic, p-hydroxybenzoic, ferulic and syringic acids, vanillin, dehydroxyvanillin, coniferaldehyde, benzaldehyde, syringealdehyde and guaiacylglycerol (Appendix 4.2). Decomposition (hydrolysis and methylation) of lignin by TMAH yields a

suite of methylated phenolic compounds that include the above mentioned monomers, as well as C_6 - C_3 units with hydroxylated side chain (Filley *et al.*, 2000). The presence of hydroxylated (now methylated) side groups indicate that the TMAH procedure is milder than CuO oxidation. The hydroxylated C_6 - C_3 units are very similar to the structures of the monomeric precursors of lignin and are considered indicators of the β-O-4 linkages in lignins.

The abundances of the various lignin monomers have been manipulated to define several lignin parameters (Alberts *et al.*, 1991; Benner *et al.*, 1990a; Hedges *et al.*, 1985; Hedges and Welliky, 1989; Requejo *et al.*, 1991a and b). The relative proportions of phenolic monomers can be used as indicators of degradation (Goni *et al.*, 1993). Acid/aldehyde ratios (Ad/Al), a lignin parameter, can reflect environmental conditions and alteration of sedimentary lignin as a result of greater stability of the phenolic acids (Hedges *et al.*, 1988a; Hedges *et al.*, 1988b and Hedges *et al.*, 1988c). For example, an increase in the acid to aldehyde ratios can be used to infer increased diagenetic degradation. In laboratory controlled degradation of conifer wood, the $(Ad/Al)_{V\text{-}CuO}$ (the subscript V-CuO denotes acid to aldehyde ratios of vanillyl phenols [V] determined by CuO oxidation) increased from an initial value of 0.15 to a final value of 0.5 (Hedges *et al.*, 1988c). However, the $(Ad/Al)_{V\text{-}CuO}$ values also depend on the plant type and tissue. $(Ad/Al)_{V\text{-}CuO}$ values greater than 0.2 appear to be confined to non-woody vascular plant tissues. $(Ad/Al)_{V\text{-}CuO}$ ratios also depend on the type of micro-organism effecting oxidative degradation. White rot fungi increases the $(Ad/Al)_{V\text{-}CuO}$ values

while brown rotters caused less pronounced changes (Filley *et al.*, 2000; Hedges *et al.*, 1988c). Further, stability series that are based on reactivity have been established. One such series for hardwood lignins is as follows: vanillyl and p-hydroxyl lignins > syringyl lignins > cinnamyl lignins (Hedges *et al.*, 1985). The cinnamyl alcohols are susceptible to biodegradation (Hedges and Weliky, 1989; Benner *et al.*, 1990).

Lignin parameters can also be computed from TMAH thermochemolysis data (Hatcher *et al.*, 1995). However, the relative yields for the phenols are different from those of CuO oxidation, and consequently, the parameters have different numerical values. Hatcher *et al.*, (1995) determined a positive correlation between $(Ad/Al)_{v\text{-}CuO}$ and $(Ad/Al)_{v\text{-}TMAH}$ (the subscript V_{TMAH} denotes acid to aldehyde ratios of vanillyl phenols [V] determined by TMAH thermochemolysis). For example, $(Ad/Al)_{v\text{-}CuO}$ values increased from 0.39 to 0.93 for degraded Douglas fir. $(Ad/Al)_{v\text{-}TMAH}$ were 3 to 11.7 for corresponding samples of Douglas fir (Hatcher *et al.*, 1995). The $(Ad/Al)_{v\text{-}TMAH}$ ratios increase with degradation because of several factors. Filley *et al.* (2000) measured a significant increase and decrease in yields of methylated vanillic acid [3,4-dimethoxybenzoic acid methyl ester] and vanillin [3,4-dimethoxybenzaldehyde], respectively, for wood subjected to white and brown rot fungal inoculation. There are several sources of vanillic acid units in the chemolysates of degraded wood, including free and bound vanillic acid units, cleavage of a lignin subunit in an intermediate oxidation state and flavanoids (Filley *et al.*, 2000). Vanillin units in TMAH chemolysates of wood are most likely produced from decomposition of β-O-4 linkages with

adjacent hydroxyl groups to produce an epoxide intermediate that decomposes to produce the aldehyde (Filley *et al.*, 2000). Hence, the presence of methylated vanillin in chemolysates is contingent on the amount of β-O-4 linkages with adjacent hydroxyl groups; removal of these groups during lignin degradation results in lower abundances of methylated vanillin in the chemolysates (Filley *et al.*, 2000). Finally, del Rio *et al.* (1998) measured 1.28 and 0.15 mg (per 100 mg of TOC) of methylated vanillic acid and vanillin, respectively, in the humic acid fraction in Minnesota peat; this yields an $(Ad/Al)_{v\text{-}TMAH}$ ratio of 8.53. Lignin-derived compounds comprised 5.78 mg per 100 mg of TOC.

4.1.3 Fatty acids

Fatty acids are hydrocarbons that have a terminal carboxylic acid group (R-COOH) where R can be an aliphatic group (saturated or unsaturated). They are important components of most lipids and are typically of C_6 to C_{36} chain length. In the geochemical literature, long chain carboxylic acids are referred to as fatty acids. Nomenclature rules used in this study are defined earlier in Chapter 3.

The biological and chemical processes leading to the accumulation of *n*-fatty acids (straight-chain or normal saturated) within degraded OM are unclear (Litchfouse *et al.*, 1995a). These substances are derived from a variety of biological precursors (Colombo *et al.*, 1997a). Fatty acids in marine sediments (from C_{12} to C_{24}) are produced by both algae and higher plants.

The most common naturally occurring fatty acids are either saturated or unsaturated compounds of C_{16} or C_{18} chain length. The monounsaturated fatty acids are more useful as biomarkers than the saturated fatty acids (Napolitano, 2000). Among the unsaturated fatty acids, $C_{16:1\Delta9}$ can be indicative of phytoplankton source whereas $C_{18:1\Delta9}$ indicates zooplanktonic material (Wakeham *et al.*, 1984). High relative concentrations of *iso*- and *anteiso*-C_{15} and $C_{18:1\Delta11}$ are used as indicators of microbial alteration (bacterial activity) of OM matter and they represent *in situ* production of sedimentary lipids at the expense of the original OM (Bradshaw and Eglington, 1993; Napolitano, 2000; Rashid, 1985; Rezanka, 1989). Cranwell *et al.* (1987) observed that the highest concentrations of *iso*- and *anteiso* branched fatty acids were at the sediment/water interface for lacustrine sediments - the region of the most intense microbial activity. C_{24} and longer chain fatty acids were usually assigned to higher plant sources (Rashid, 1985). The presence of C_{24} was used as an indicator of allochthonous (terrestrial) source of OM in marine sediment (Wakeham *et al.*, 1984). Fatty acids of higher plant sources are usually C_{10} to C_{40} compounds, with greater abundances of fatty acids containing even number of carbon atoms (Matsumoto *et al.*, 1992; Rashid, 1985). Isotopic evidence indicate that the short chain fatty acids of TMAH thermochemolysates of dissolved organic matter of the Delaware Estuary, Delaware, are derived from terrestrial sources and are bound with lignin and other compounds within a macromolecular matrix (Manino and Harvey, 2000).

Environmental conditions can affect the biosynthetic production of lipid constituents of

organisms. This adaptive biochemistry creates the possibility for compounds indicative of climatic (and other environmental changes) to be preserved in the sedimentary record (Brassel, 1992). The abundances and degree of unsaturation of fatty acids are regulated by plants as a response to changes in environmental conditions, such as temperature (Kawamura and Ishiwatari, 1981). Hence, the degree of unsaturation can reflect temperature or climate (if preserved). Ratios of selected fatty acids ($C_{16:1}/C_{16}$, $C_{18:1}/C_{18}$ and C_{29}/C_{17}) were used as indicators of source and degree of preservation (Meyers and Ishiwatari, 1993). However, the survival of these compounds depends on their resilience to microbial and diagenetic alteration and the usefulness of these indicators can be limited by the ease by which the unsaturated acids are degraded and their lack of biological specificity (Kennicutt *et al.*, 1992).

Fatty acids abundances are highly variable in various environments as a result of different sources and environmental processes and conditions. Fatty acids in coals are comprised of C_{22} to C_{32} homologues that have a distinct even to odd predominance (Chaffee *et al.*, 1986). De Leeuw *et al.* (1995) identified several fatty acids in soxhlet extracts (KOH/methanol) of eel grass (*Zostera marina*). The fatty acids ranged from C_{14} to C_{28}. The most abundant fatty acids were n-C_{14}, n-C_{16}, n-C_{22}, n-C_{24} and n-C_{26}. Algal humic materials also contain long chain fatty acids and consist of C_6 to C_{24} (straight chain), and C_{12} to C_{19} (branched) fatty acids (Schnitzer and Khan, 1972). In addition, the relative distribution of long chain *n*-fatty acids enabled Gieren *et al.* (1999) to estimate the variability of the relative inputs of marine and

terriginous OM to sediments of the continental shelf off Costa Rica.

Haddad *et al.* (1991) analyzed near-shore marine sediments (Cape Lookout Bight, North Carolina) for free fatty acids and detected saturated fatty acids ranging from C_{12} to C_{34}, as well as unsaturated fatty acids in the C_{16} to C_{22} range. The surface samples were dominated by the medium chain range (C_{12} to C_{19}) with a predominance of straight chain compounds. The relative abundances of the long chain fatty acids (specifically C_{20} to C_{34}) increased with depth. The total free fatty acids off Cape Lookout Bight accounted for 2.2 mg per 100 mg of TOC. The total fatty acids comprise 0.93 mg per 100 mg of TOC between 25 and 30 cm depth. Humic acids from a black Chernozemic soil from central Alberta contained 98.2 mg of fatty acids per g of humic acid, of which 95 percent is considered tightly bound (Schnitzer and Neyroud, 1975). Del Rio *et al.* (1998) determined that the total fatty acids and alcohols of chemolysates of humic acids, from Minnesota peat, was 3.44 mg per 100 mg of TOC.

In mid latitude coastal areas, the production of fatty acids and other lipids show a pronounced seasonal effect. In this study area, Parrish (1998) determined that during the spring bloom in Trinity Bay, the production of fatty acids is very high and Budge and Parrish (1998) determined that all of the acyl lipid flux in the Trinity Bay area is of marine origin. However, up to 98 percent of this lipid, which includes fatty acids, is lost in the transition from 100 m in the water column to just below the water/sediment interface. In addition, the benthic and demersal communities are very efficient in recycling most of these nutrients.

This illustrates the focus of marine productivity; it is geared toward reproduction; terrestrial production is geared toward storage (Schlishinger, 1994). Temporal comparisons of fatty acids and organic contents of net-tow samples, sediment trap materials and sediment in the study area indicate a decrease in the TOC and lipids in the same order (Parrish, 1998; Budge and Parrish, 1998). The organic contents of net-tows, sediment trap materials and sediments were 57.14% and 46.16%, and between 3% and 13%, respectively (Parrish, 1998). Budge and Parrish (1998) also determined that the average chain lengths and double bonds of fatty acids decreased in the transition from net tow to sediments, and the results are summarized below.

Sample	Chain lengths	Double bonds	TOC (% organic carbon)
Net-tow	17.7 ± .20	2.25 ± .20	57.14
Sediment trap	17.2 ± 0.4	1.4 ± 0.4	46.16
Sediment	16.7 ± 0.1	0.6 ± 0.1	3 - 13

The fatty acids in sediments are shorter and more saturated than those of net-tow and sediment trap material; this may happen because the fatty acids are degraded in the sediment traps, resulting in shorter chain length and reduction in degree of unsaturation. The decrease in TOC in the sediment trap material is attributed to dilution of OM by inorganic materials. The transition between sediment trap and sediments yield the largest qualitative and quantitative difference in fatty acids. There is a significant decrease in lipid content (as a proportion of organic weight) from sediment trap to cores and below the surface of sediments, indicating that lipids are preferentially removed while the more refractory

materials are preserved.

4.2 METHODS

4.2.1 Batch-wise TMAH thermochemolysis

The method employed is similar to that used by Hatcher *et al.* (1995). Approximately 50 mg of marine sediment was placed in a 9 mm pre-combusted pyrex tube and 50 μl of 25 percent w/w aqueous TMAH (approx. 25 mg of reagent) was added to ensure excess reagent for reaction. An internal standard of 2-methoxybenzoic acid (25 ng μl^{-1} x 10 μl) in methanol was added to the sediment as a recovery standard. The rest of the procedure is similar to that described in Chapter 3. Compounds were identified by matching with an NBS mass spectra library and mass spectra published in the literature. Other products were authenticated by matching retention times and mass spectra of derivatized standards (where commercially available). Assignments are indicated in Table 4.1a. Structural formulae of the chemolysates is provided in Table 4.1b.

Great care was taken in quantifying the thermochemolysis yields of lignin and fatty acid markers in marine sediments. For sediment analysis, 2-methoxybenzoic acid was added (250 ng) to 50 mg of sediment as an internal recovery standard. For quantitative calculations, peak areas of selected ion monitoring (SIM) chromatograms (GC/MS) were measured. For aromatic products, the molecular ion was used; for fatty acid methyl esters, the sum of m/z

Table 4.1a - **List of TMAH chemolysates identified in marine sediments**

* Chromatographic peaks labeled in Figures 4.11, 4.12 and 4.13

** Lignin sources were based on the presence or absence of methoxy group in the parent precursor as determined by TEAH chemolysis (Section 4.33)

Peak*	Chemolysate	Comments **
1	Diethylhydrazone-2-butanone	?
2	2,6-dimethyl-3-formylthiacyclohex-3-one	?
3	1,2,3-Trimethoxybenzene	syringyl units (lignin)
4	3,4-dimethoxyphenol	incomplete methylation of peak # 6
5	3,4-dimethoxyphenyl ethane	guiacyl units (lignin)
6	1,2,4-trimethoxybenzene	precursor has two methoxyl groups (carbohydrate)
7	4-methoxybenzoic acid, methyl ester	*p*-hydroxyphenyl units (lignin from graminees)
8	1,3,5- trimethoxybenzene	1,3,5 -trihydroxybenzene (condensed tannin ?)
9	3,4,5-trimethoxytoluene	syringyl units (lignin)
10	4-methoxy-phenylacetic acid, methyl ester	*p*-hydroxyphenyl units (lignin from graminees)
11	3,4-dimethoxybenzaldehyde	guiacyl units (lignin)
12	3,4-dimethoxyphenyl ethene	guiacyl units (lignin)
13	dodecanoic acid, methyl ester	(lipid)
14	1-(3,4-dimethoxyphenyl) ethanone	guiacyl units (lignin)
15	3,4-dimethoxyphenyl acetone	"
16	3,4-dimethoxybenzoic acid, methyl ester	"

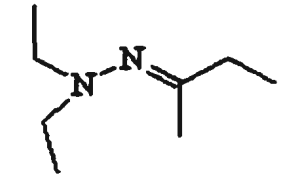
Peak	Compound	Comments
17	3,4-dimethoxyphenylacetic acid, methyl ester	guiacyl units (lignin)
18	nonanedioic acid, dimethyl ester	(lipid)
19	3-(4-methoxyphenyl) acrylic acid, methyl ester	*p*-coumaric acid (lignin)
20	12-methyl-tridecanoic acid, methyl ester	(lipid)
21	12-methyl-tetradecanoic acid, methyl ester	"
22	pentadecanoic acid, methyl ester	"
23	3-(3,4-dimethoxyphenyl)acrylic acid, methyl ester	ferulic acid (lignin)
24	9-hexadecenoic acid, methyl ester	(lipid)
25	hexadecanoic acid, methyl ester	"
26	heptadecanoic acid, methyl ester	"
27	9-octadecenoic acid, methyl ester	"
28	13-octadecenoic acid, methyl ester	"
29	16-methyl-heptadecanoic acid, methyl ester	"
30	hexadecanedioic acid, dimethyl ester	(lipid)
31	9,10-dimethoxy-hexadecanoic acid, methyl ester	"
32	eicosanoic acid, methyl ester	"

Peak	Compound	Comments
33	heneicosanoic, methyl ester	(lipid)
34	docosanoic acid, methyl ester	"
35	tricosacoic acid, methyl ester	"
36	eicosanedioic acid, dimethyl ester	"
37	tetracosanoic acid, methyl ester	"
38	pentacosanoic acid, methyl ester	"
39	docosanedioic acid, dimethyl ester	"
40	hexacosanoic acid, methyl ester	"

Table 4.1b - Structural formulae of chemolysates of marine sediment

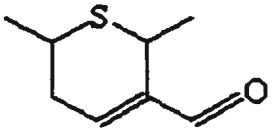
Diethylhydrazone-2-butanone

(1)



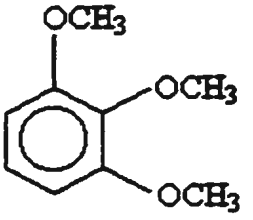
2,6-Dimethyl-3-formylthiacyclohex-3-one

(2)



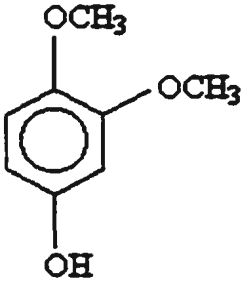
1,2,3-Trimethoxybenzene

(3)



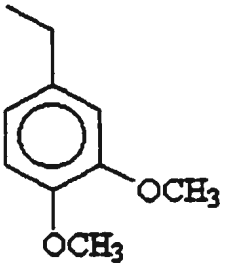
3,4-Dimethoxyphenol

(4)



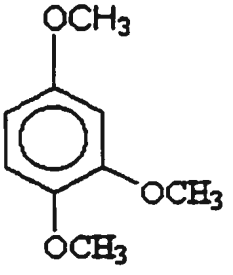
3,4-Dimethoxyphenyl ethane

(5)



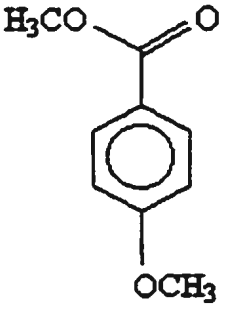
1,2,4-Trimethoxybenzene

(6)



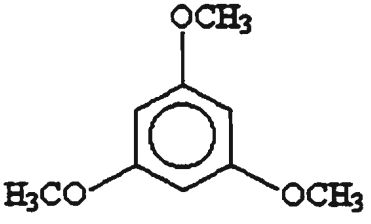
4-Methoxybenzoic acid, ME

(7)



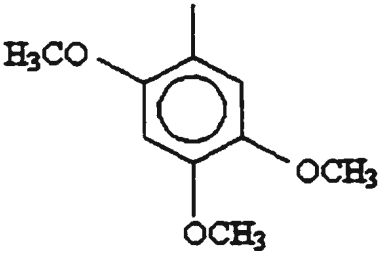
1,3,5-Trimethoxybenzene

(8)



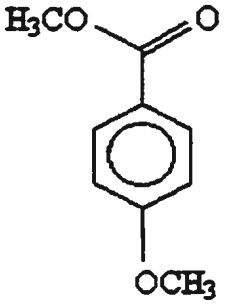
3,4,5-Trimethoxytoluene

(9)



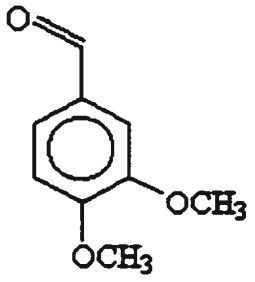
4-Methoxyphenylacetic acid, ME

(10)



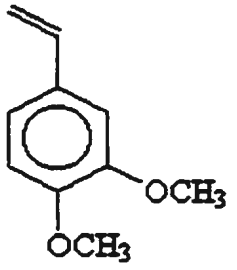
3,4-Dimethoxybenzaldehyde

(11)



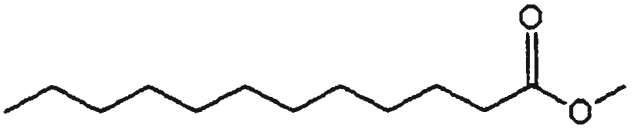
3,4-Dimethoxyphenyl ethene

(12)



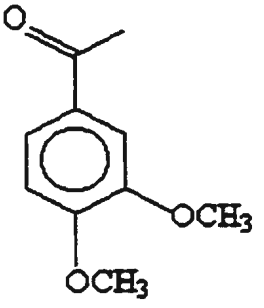
Dodecanoic acid, ME

(13)



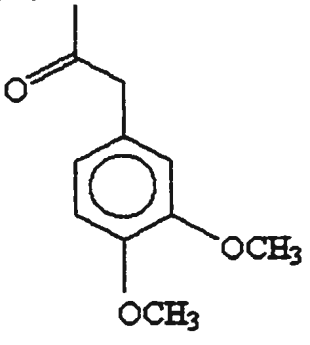
1-(3,4-Dimethoxyphenyl) ethanone

(14)



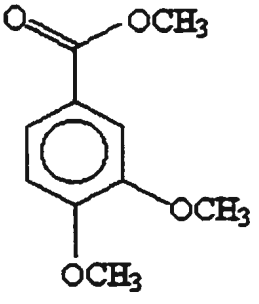
3,4-Dimethoxyphenyl acetone

(15)



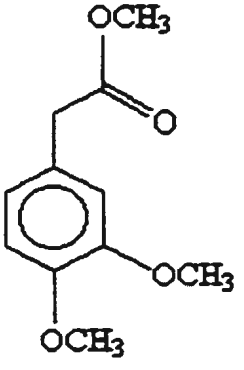
3,4-Dimethoxybenzoic acid, ME

(16)

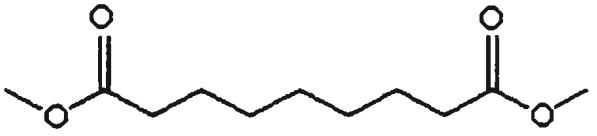


3,4-Dimethoxyphenylacetic acid, ME

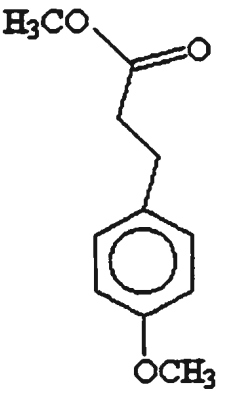
(17)



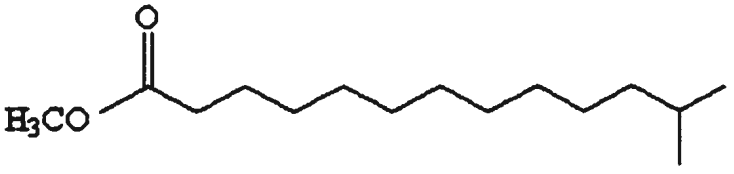
Nonanedioic acid
dimethyl ester



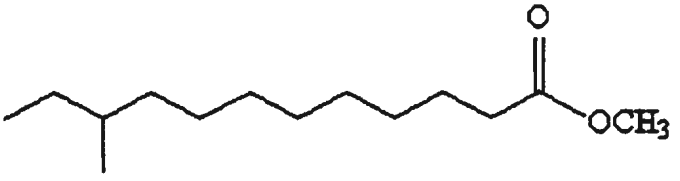
3-(4-Methoxyphenyl)
acrylic acid, ME



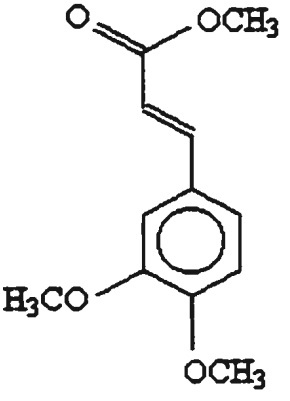
12-Methyl tridecanoic acid, ME



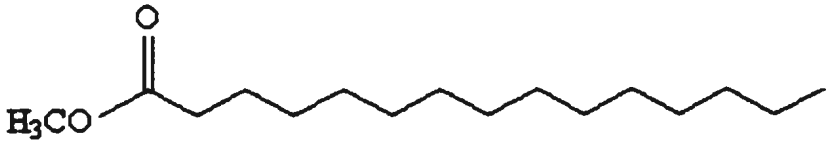
12-Methyl tetradecanoic acid, ME



3-(3,4-Dimethoxyphenyl)
acrylic acid, ME

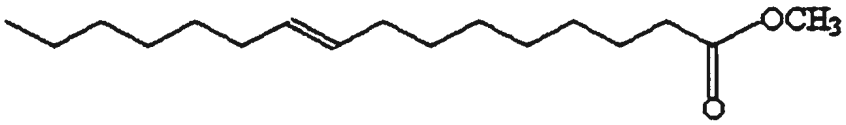


Pentadecanoic acid, ME



9-Hexadecenoic acid, ME

(24)

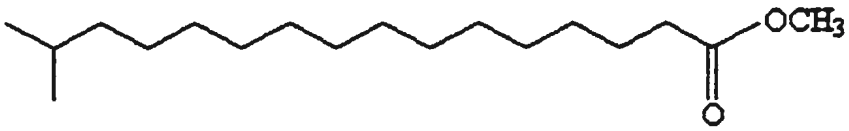


Hexadecanoic acid, ME

(25)

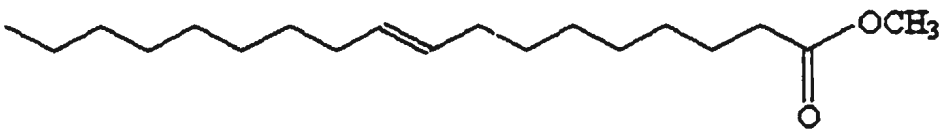
15-Methyl hexadecanoic acid, ME

(26)



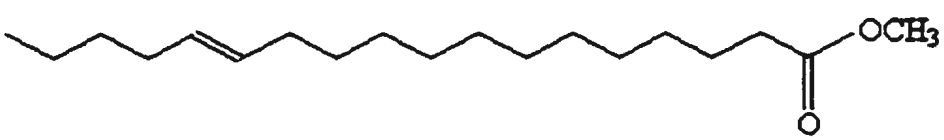
9-Octadecenoic acid, Me

(27)



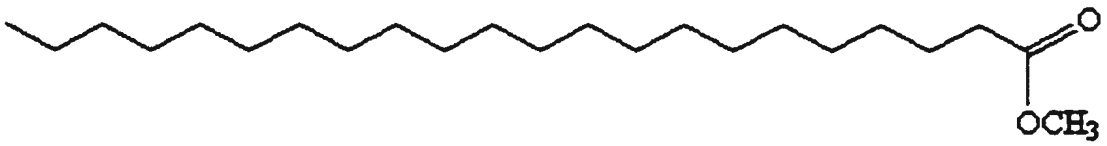
13-Octadecenoic acid, Me

(28)



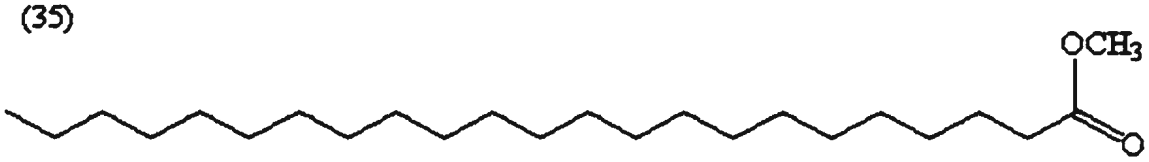
Docosanoic acid, ME

(34)



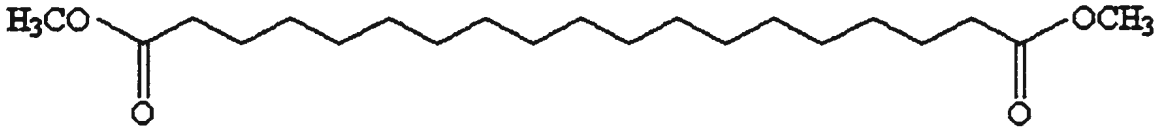
Tricosanoic acid, ME

(35)



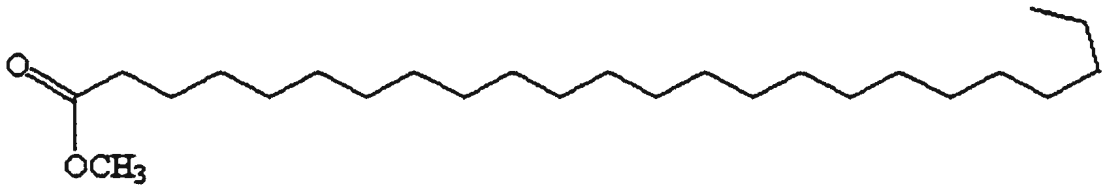
Eicosanedioic acid, dimethyl ester

(36)



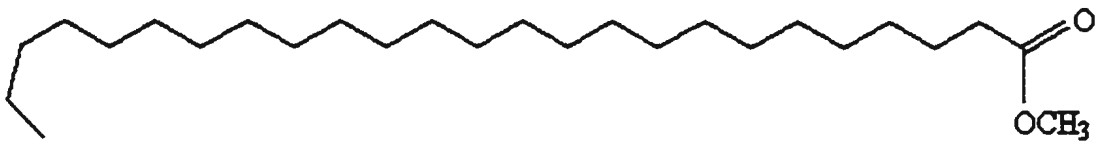
Tetracosanoic acid, ME

(37)



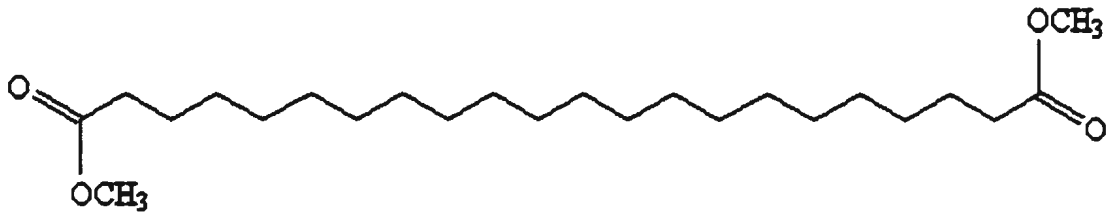
Pentacosanoic acid, ME

(38)



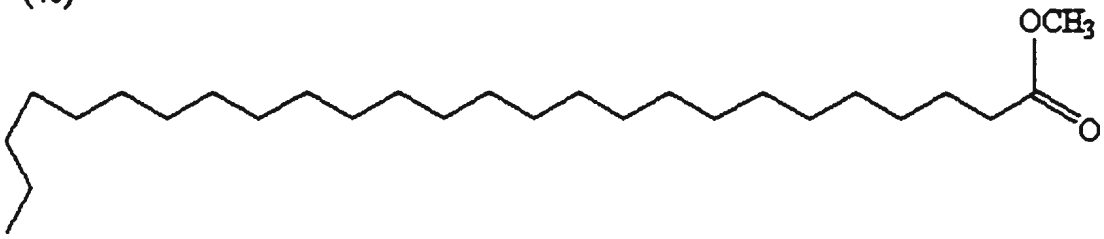
Docosanedioic acid, dimethyl ester

(39)



Hexacosanoic acid, ME

(40)



74 and 87 were chosen because they were the most abundant ions. Not all of the TMAH chemolysates identified in sediments were quantified. Only the standards that could be obtained in high purity were chosen as listed in the table of yield results. Yields were calculated from constructed calibration curves of standards (methylated if required). A variability of 8 to 28 was observed which results from instrument error (internal precision) and error resulting from sampling and sample preparation (external precision). Internal precision of the Varian Saturn 3 mass spectrometer (Ion Trap) detector (determined by multiple injections of sample H1, 0 - 2 cm) was between 5 and 10 percent.

4.2.2 Total Organic Carbon

Sediment samples were weighed and transferred into pre-combusted quartz tubes (9 mm, 500°C, 1 hour) to which excess cupric oxide was added. Following evacuation on a high vacuum line, the sample tubes were flame sealed and combusted (500°C, 1 hour). The tubes were then cracked on a high vacuum line and the purified CO_2 measured on a calibrated manometer. The purified CO_2 was collected and stored in 6 mm pyrex tubes for isotopic analysis (Chapter 6).

4.3 RESULTS AND DISCUSSION

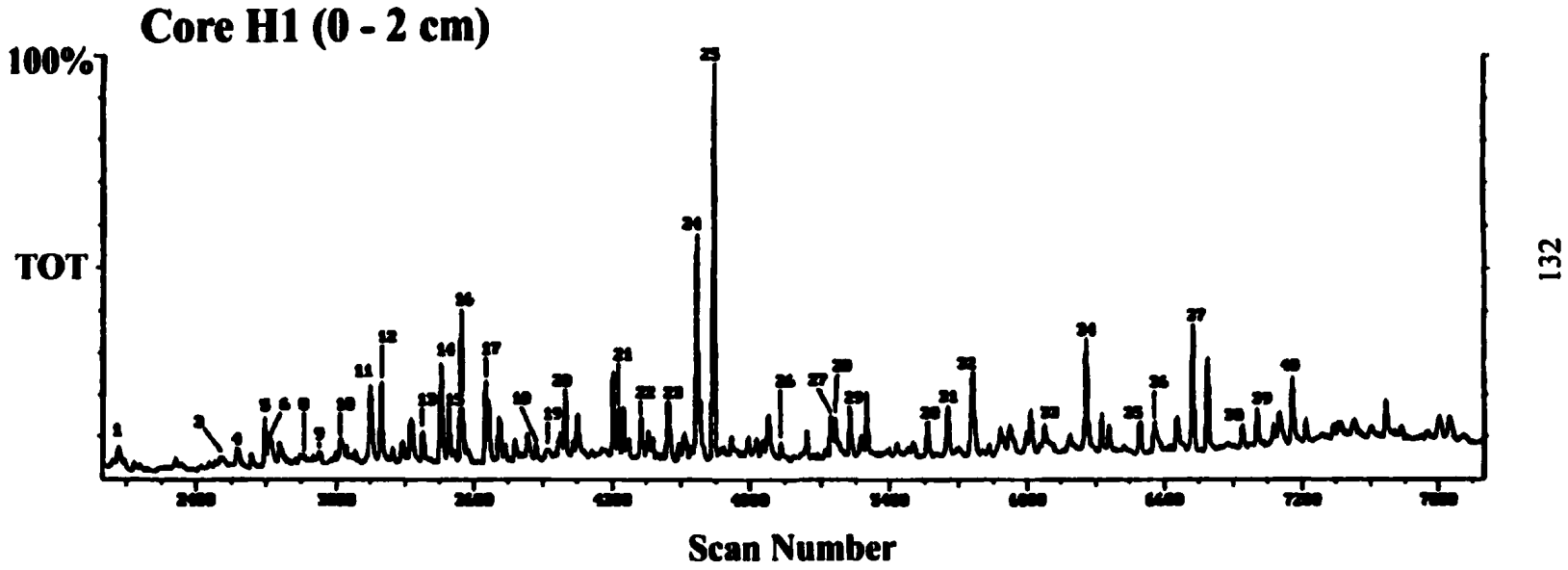
4.3.1 Distribution of compounds in sediments

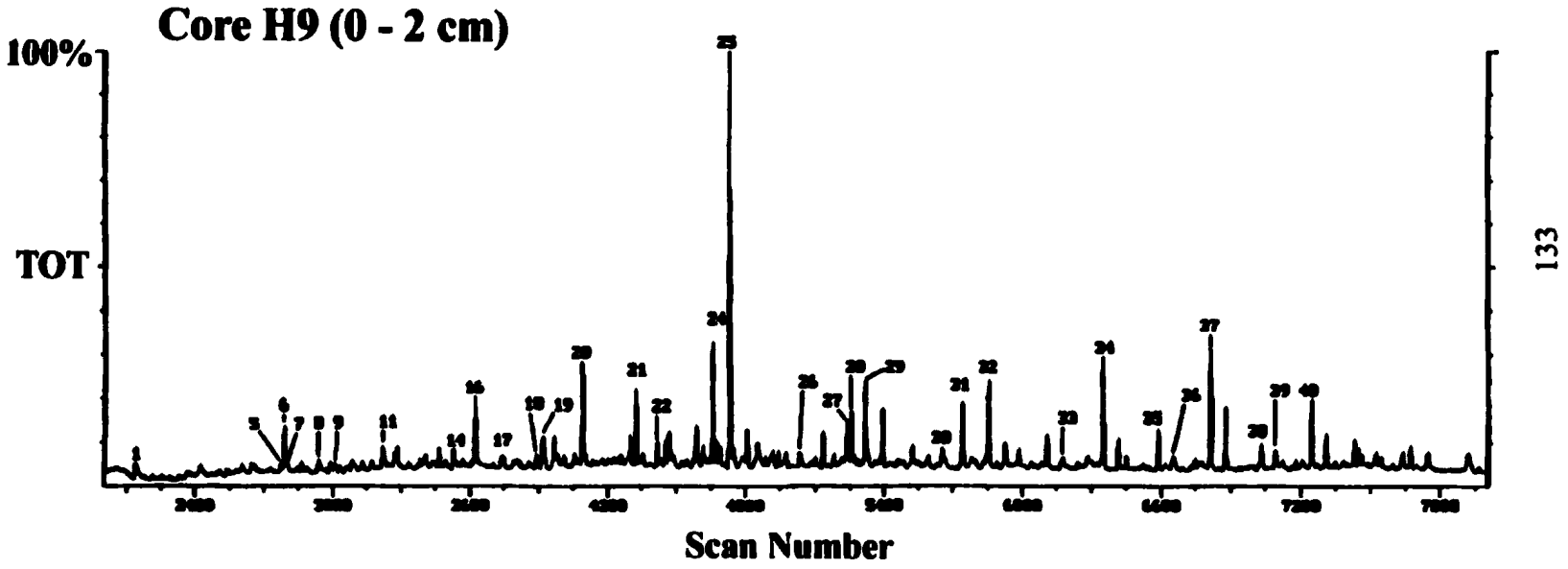
The chemical nature of marine sediment chemolysates is very different from that obtained from direct pyrolysis (Chapter 2) of corresponding samples. Products include a suite of methylated phenolic compounds and fatty acid methyl esters (Tables 4.1a and b). The relative abundances of these chemolysates vary among sample locations (Figure 4.1). The presence of certain products is similar to that obtained by Hatcher and Clifford (1994) and del Rio *et al.* (1998). The first half of the chromatograms contains the peaks of aromatic compounds (Figure 4.1). The second half consists of peaks that represent the more abundant aliphatic compounds, consisting mostly of fatty acid methyl esters. Almendros *et al.* (1998) analysed geological samples (organic material taken from a limestone quarry in Cerro de la Mesa, Madrid, Spain) by conventional pyrolysis-GC/MS and TMAH thermochemolysis. Fatty acid methyl esters were identified only by the latter analytical scheme. Conventional pyrolysis-GC/MS of samples released alkanes and alkenes instead of fatty acid methyl esters. Almendros *et al.* (1998) concluded that pyrolytic decarboxylation (during pyrolysis) resulted in conversion of fatty acids to hydrocarbons.

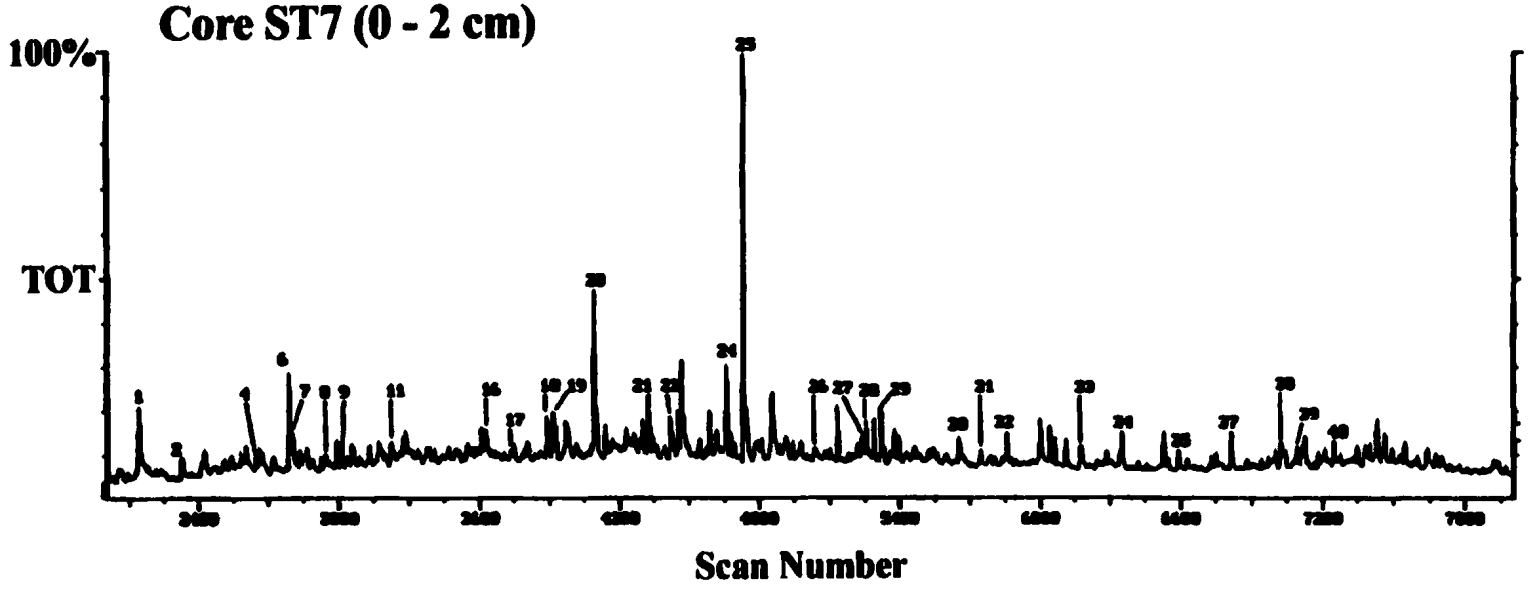
Figure 4.1 shows partial total ion chromatograms of the TMAH thermochemolysis products of three selected marine sediment samples (H1, H9 and ST7). No structurally unique chromatographic peaks were observed earlier than scan # 2,100 and those present were not

Figure 4.1 - **Total ion chromatograph of sediment chemolysates of cores H1, H9 and ST7 (0-2 cm)**

Peak numbers indicated correspond to chemolysates in Tables 4.1a and 4.1b.


Core H1 (0 - 2 cm)
100%
TOT
Scan Number


Core H9 (0 - 2 cm)
100%
TOT
133
Scan Number


Core ST7 (0 - 2 cm)
100%
TOT
Scan Number

identified. The chromatograms were dominated by fatty acid methyl esters. TMAH thermochemolysis seemed to effectively esterify fatty acids of triacylglycerol and other fatty acid-containing lipids to produce good yields of fatty acid methyl esters (Clifford *et al.*, 1995; McKinney *et al.*, 1995). Marine sediments usually contain substantial amounts of fatty acids particularly in productive marine ecosystems as the ones sampled in this study. It is estimated that fatty acids constitute a significant portion (up to 9 percent) of the TOC of marine sediments (Rashid, 1985). In this study, fatty acids ranged from C_{14} to C_{28} with a predominance of even carbon numbered species, possibly as a result of terrestrial plant inputs (Budge and Parrish, 1998). There are several types of fatty acids in marine sediments and those identified in this study include saturated, unsaturated and branched structures (Tables 4.1a and b). The branched fatty acids are b-C_{14}, b-C_{15} and b-C_{18} and they may indicate bacterial contributions to the TOC (Budge and Parrish, 1998).

In these samples, the saturated fatty acids are much more abundant than monounsaturated fatty acids, likely because there are fewer of the latter as a result of utilization and oxidative degradation. Colombo *et al.* (1997b) measured lower amounts of monounsaturated fatty acids in sediments than in the water column at the same sites in the Laurentian Trough. In Trinity Bay, there were unusually low levels of PUFAs (compared to literature values) in the sediments of the study area (Parrish, 1998). The spring bloom net-tow material of Trinity Bay contained elevated amounts of monounsaturated fatty acids (Budge and Parrish, 1998). Only 0.5% of all PUFAs in sediment trap materials are preserved in sediments of Trinity Bay

(Budge and Parrish, 1998). This high flux of PUFA and minor preservation in sediments suggests that these nutrients are very efficiently recycled or incorporated/transformed into refractory organic materials. The difference in amounts of PUFAs in Trinity Bay and the Laurentian Trough is the result of different environmental conditions and sources of OM.

The high reactivity of unsaturated fatty acids also contributes to their low abundances in sediments. Harvey and Boran (1985) experimented with unsaturated lipids in seawater, and postulated that marine humic materials in seawater are formed from unsaturated marine lipids. Unsaturated lipids can react spontaneously with oxygen (Harvey and Boran, 1985). These auto-oxidations are accelerated by light and auto-catalyzed by transitional elements. Essentially, the reaction of polyunsaturated lipids with oxygen is initiated by extraction of allylic hydrogen atoms to produce allylic radicals. The radicals may then react with another site of unsaturation to form a new C-C bond and proceed until aromatic rings are formed (Harvey and Boran, 1985). Hence, if humic compounds of seawater are synthesized by this pathway, some of the aromatic rings of marine humic materials may be derived from mainly planktonic and other marine sources.

Aliphatic dicarboxylic acid dimethyl esters (peaks **18** (C_3), **30** (C_{16}), **33** (C_{18}), **36** (C_{20}) and **39** (C_{22}) have been identified in the sediment chemolysates (Tables 4.1a and b). Recently, pyrolysis-field ionization mass spectrometric analysis of TMAH treated soils (Schulten and Sorge, 1995) revealed evidence of dicarboxylic acid residues up to C_{26}. They were also

identified after methylation/pyrolysis of fulvic acids (Martin *et al.*, 1994) and humic acids (Saiz-Jiminez, 1994). Dicarboxylic acids have been reported to be present in humic acids of peat (del Rio *et al.*, 1998). The potential of these dicarboxylic acid dimethyl esters as possible terrestrial markers remains to be fully evaluated.

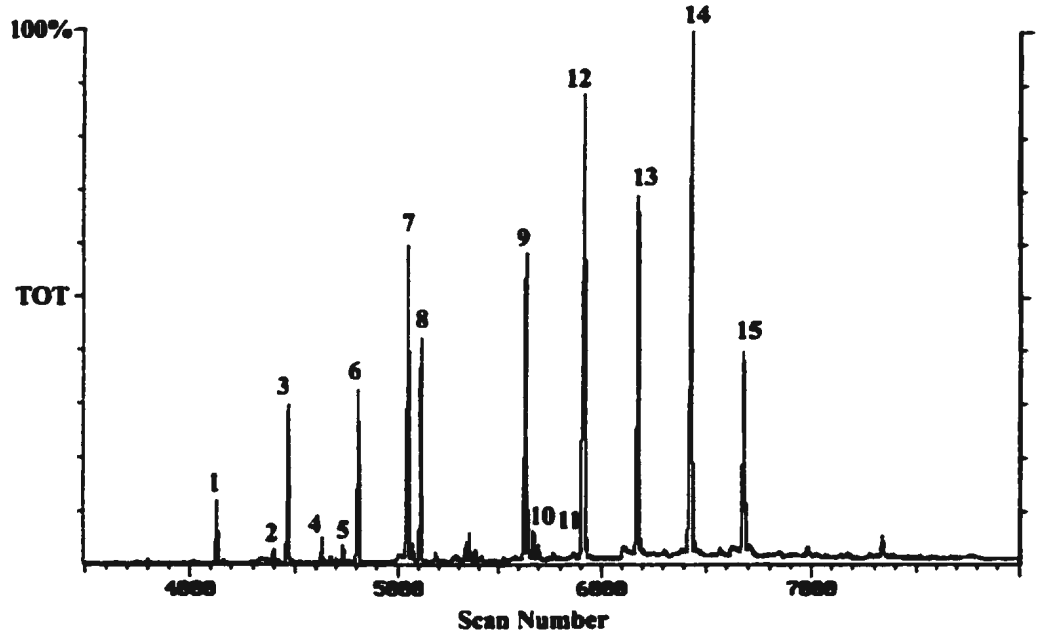
Interestingly, phenolic compounds were virtually absent in the chemolysates of net tow and sediment trap materials (discussed in Section 4.3.2). Hence, the amount of terrestrially derived OM in the water column is small. However, the preservation potential of the phenolic compounds is high and this results in subsequent concentration of these compounds in the sediments relative to other SOM. The methylated phenolic products are especially important in estimating the terrestrially derived OM in the study area. The most abundant are the 3,4-dimethoxybenzenes containing ethane (peak **5**), aldehyde (peak **11**), ethene (peak **12**), ethanone (peak **14**), acetone (peak **15**) side groups and the methylation of carboxylated side-chains yielding methyl esters (peaks **16**, **17** and **23**) (Figure 4.1). The most abundant 3,4- dimethoxybenzene products in all samples are 3,4-dimethoxybenzoic acid, ME (peak **16**) [methylated vanillic acid] and 3,4-dimethoxybenzaldehyde (peak **11**) [methgylated vanillin]. Less abundant are the methylated *p*-hydroxyphenyl products, 4-methoxybenzoic acid, ME (peak **7**), 3-(4-methoxyphenyl) acrylic acid, ME (peak **19**) [methylated coumaric acid] and 4-methoxy- phenylacetic acid, ME (peak **10**). Finally, the other class of phenolic compounds are the trimethoxybenzenes and trimethoxytoluene compounds. Syringyl lignin markers, indicating hardwood inputs, include 1,2,3-trimethoxybenzene (peak **3**) and 3,4,5-

trimethoxytoluene (peak 9). Of the remaining products, peak 8 (1,3,5-trimethoxybenzene) has been shown to be a good indicator of condensed tannins from terrestrial plants (Zhang, 1993) and 1,2,4-trimethoxybenzene (peak 6) has been determined to be a carbohydrate TMAH thermochemolysis product (Chapter 3) (Fabbri and Helleur, 1999).

4.3.2 Distribution of compounds in net-tow material and the macroalga, *Laminaria*

A small portion of the particulate OM in the water column settles to the water/sediment interface and becomes incorporated into the SOM. Therefore it is important to investigate the composition of the particulate OM that consists of planktonic material. The chemical nature of the chemolysates of the net-tow (horizontal tows, 20 μm mesh) material consisted mainly of fatty acid methyl esters (Figure 4.2). The monounsaturated fatty acids are more abundant than saturated fatty acids and include those of 16, 18, 19, 20 and 21 carbon atoms (Parrish, 1998). As noted in Chapter 3, PUFAs cannot be determined by TMAH thermochemolysis because of the strong basic conditions resulting in isomerization and oxidation. Ostrom and Macko (1992) identified macroalgae as a major producer of OM in Trinity Bay. The distribution of the chemolysates of Laminaria, a common macroalga that is listed as a major producer in Trinity Bay, shows a different distribution. The dominant compound is hexadecanoic acid, ME, followed by 9-octadecenoic acid, ME (Figure 4.3).

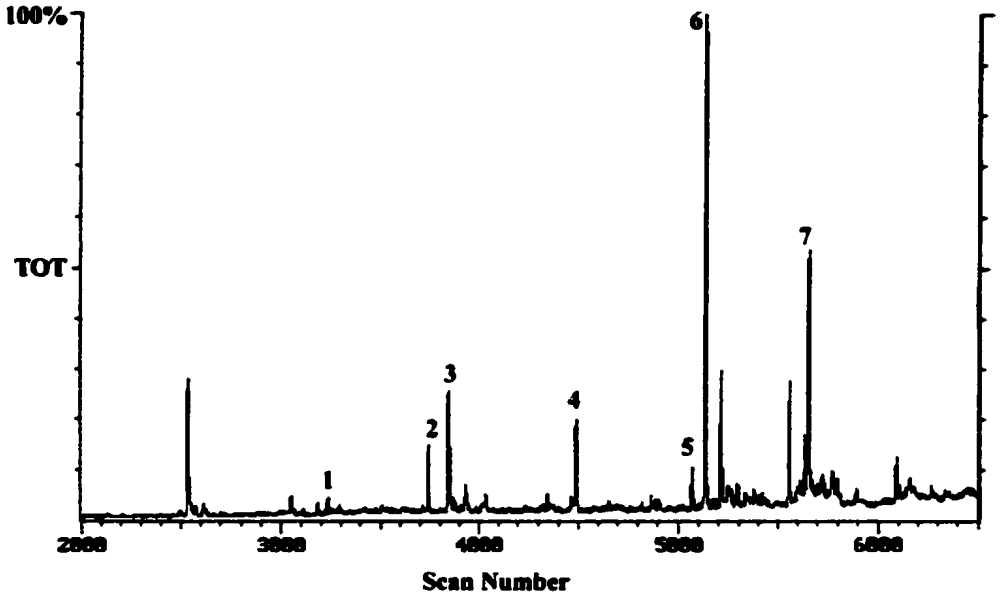
Figure 4.2 - **Total ion chromatogram of thermochemolysates of net-tow material**



Thermochemolysate key for the total ion chromatogram

1 - Pentadecanediol
2 - 2-Ethenyl-1-decanol
3 -12-Methyl-tridecanoic acid, methyl ester
4 - -12-Methyl-tetradecanoic acid, methyl ester
5 - Tetradecyne
6 - Pentadecanoic acid, methyl ester
7 - 9-Hexadecenoic acid, methyl ester
8 - Hexadecanoic acid, methyl ester
9 - 9-Octadecenoic acid, methyl ester
10 - 13-Octadecenoic acid, methyl ester
11 - Octadecanoic acid, methyl ester
12 - 9-Eicosenoic acid, methyl ester
13 - 11-Heneicosenoic acid, methyl ester
14 - 11-Docosenoic acid, methyl ester
15 - Tetracosenoic acid, methyl ester

Figure 4.3 - **Total ion chromatogram of thermochemolysates of the marine alga, laminaria**



Thermochemolysate key for the total ion chromatogram

1 - Phenol

2 - Myoinisitol

3 - Galictol

4 - Tetradecanoic acid, methyl ester

5 - 9-Hexadecenoic acid, methyl ester

6 - Hexadecanoic acid, methyl ester

7 - 9-Octadecenoic acid, methyl ester

4.3.3 Origin of the methylated products using tetraethylammonium hydroxide (TEAH) thermochemolysis

One of the drawbacks of using TMAH as the thermochemolysis reagent is that it does not allow for the determination of the presence of methoxy groups in the original structure of the compound. This drawback can be overcome by using ^{13}C-labelled TMAH (Filley *et al.*, 2000) or a different alkylating agent, such as TEAH; the latter was employed in this study. Some sediments were analyzed by TEAH thermochemolysis and the resulting ethylated derivatives were subsequently analyzed by GC/MS, and the presence of ethoxy groups in the products were then determined. From this information it is possible to assign the origin of these compounds to guaiacyl-based units (products that have at least one methoxy substituent related to its structure, Appendix 4.1) of lignin as noted in the comments column of Table 4.1a. It was found that all 3,4-dimethoxybenzene compounds produced by TMAH thermochemolysis listed in Table 4.1a had one original methoxy group. Furthermore, most of the 1,2,3-trimethoxybenzene products were found to contain two original methoxy groups thus indicating that these may be derived from syringyl units of lignin (Appendix 4.1). Finally, the *p*-hydroxyphenyl products were found not to contain methoxy groups and their origin may be *p*-hydroxyphenyl units of lignin, as well as the aromatic amino acids (Tyrosine and phenylalinine) (Chapter 3). Interestingly, a small amount of 1,2,4-trimethoxybenzene in the chemolysates of sediments had two methoxy groups in its original structure. Since 1,2,4-trimethoxybenzene has been found to be a major pyrolysate of carbohydrates (Chapter

3), this would indicate that there are other sources of methoxybenzenes in marine sediments.

Filley *et al.* (2000) investigated the effect of lignin degradation by brown and white rot fungi using ^{13}C TMAH to differentiate between added and original methoxy groups during thermochemolysis and found that white rot fungi were more efficient in effecting de-lignification of wood. They also found no significant exchange between methoxy groups derived from the lignin moieties those derived from the TMAH reagent. Although preparation of the ^{13}C reagent is time consuming, this derivatization scheme has an advantage in that the mass spectra of the compounds will indicate the number of methoxy groups derived from the reagent - each $O^{13}CH$ (methoxy) group will add 30 mass units (as opposed to 29 for unlabelled TMAH). An additional advantage of using ^{13}C TMAH is that retention times of compounds produced by this derivatization scheme are comparable to those produced by unlabelled TMAH.

4.3.4 Spatial variation of chemolysates in Trinity Bay sediments

Spatial variation in the chemical profile among sediment locations can be related to changes of the abundances of chemolysates. The variation in the abundances of the chemolysates at 0 - 2 cm core (surface) are discussed in the context of their precursors. Generally, the sediments from the Northwest Arm (H9, H8, H7, H6, H5, and H1) have higher abundances of lignin-derived phenols and fatty acids than the sediments of the offshore stations (ST11,

ST10, ST9 and ST7) that are located in Trinity Bay (Table 4.2; Figure 4.4). The total chemolysates correlates positively with the TOC (R^2=0.89, n=10). The most abundant phenols are methylated vanillic acid and coumaric acid (Table 4.2). Generally, the most abundant fatty acid chemolysates are hexadecanoic and octadecenoic acid methyl esters. Fatty acids can be produced from both marine and terrestrial plants, whereas lignin-derived phenols are produced by vascular plants and certain marine sea grasses.

TMAH chemolysates of core H1 sediments produced the highest yields of fatty acids and phenols (9.7 and 1.4 mg per g of dry sediment of fatty acids and phenols, respectively) (Table 4.2; Figure 4.4). The yields of chemolysate phenols correlates positively with those of pyrolysate phenol (R^2=0.99, n=10). Also, the chemolysates of core H1 sediments have several types of phenols, as opposed to chemolysates of other sediment samples. Core H1 sediments have several sources of phenols, including stream discharge into Hickman's Harbour. Vanillic acid methyl ester is the most abundant phenol chemolysate which has a yield of 614 μg per g of dry sediment in the chemolysates of core H1 sediments. The abundance of vanillic acid methyl ester ranged from 0.47 to 0.03 mg per 100 mg of TOC for cores H1 and ST7, respectively. These abundances are comparable to those obtained by del Rio *et al.* (1998). The yields of methylated vanillin and coumaric acid are much lower than that of vanillic acid and are 119 and 113 μg per g of dry sediment, respectively, for surface samples of core H1. Only six phenols are determined in the chemolysates of core H9. The most abundant are methylated vanillic acid, coumaric acid and vanillin (111, 60 and 20 μg

Table 4.2 - **Spatial variation of chemolysate abundances of surface sediment**

4.21. Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.

4.22. Individual chemolysate abundance (μg per g of sediment); single data analysis

Data plotted in Figure 4.4

Table 4.21- **Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.**

	H9	H8	H7	H6	H5
	mg per g of sediment				
Lignin-derived phenols	0.21 ± 0.03	0.18 ± 0.03	0.15 ± 0.02	0.29 ± 0.04	0.18 ± 0.04
Fatty acids	2.80 ± 0.34	2.35 ± 0.31	1.84 ± 0.27	3.29 ± 0.40	1.58 ± 0.22
Mono-unsaturated fatty acids	0.34 ± 0.05	0.55 ± 0.08	0.44 ± 0.07	1.00 ± 0.15	0.55 ± 0.08
Saturated fatty acids	2.46 ± 0.30	1.81 ± 0.22	1.39 ± 0.17	2.29 ± 0.27	1.03 ± 0.12
Branched fatty acids	0.48 ± 0.05	0.47 ± 0.05	0.33 ± 0.04	0.51 ± 0.06	0.29 ± 0.03
Medium chain fatty acid	1.70 ± 0.20	1.58 ± 0.19	1.22 ± 0.14	2.30 ± 0.28	1.21 ± 0.14
Long chain fatty acid	1.10 ± 0.14	0.77 ± 0.08	0.62 ± 0.13	0.99 ± 0.13	0.38 ± 0.05
	mg per 100 mg of TOC				
Lignin-derived phenols	0.34 ± 0.04	0.47 ± 0.10	0.31 ± 0.05	0.48 ± 0.06	0.49 ± 0.10
Fatty acids	4.52 ± 0.51	6.19 ± 0.81	3.75 ± 0.54	5.40 ± 0.75	4.28 ± 0.60
Mono-unsaturated fatty acids	0.56 ± 0.09	1.44 ± 0.21	0.91 ± 0.14	1.64 ± 0.24	1.49 ± 0.23
Saturated fatty acids	3.96 ± 0.48	4.75 ± 0.58	2.84 ± 0.34	3.75 ± 0.46	2.79 ± 0.34
Branched fatty acids	0.77 ± 0.09	1.23 ± 0.13	0.68 ± 0.08	0.84 ± 0.09	0.79 ± 0.09
Medium chain fatty acid	2.74 ± 0.32	4.15 ± 0.49	2.49 ± 0.30	3.77 ± 0.46	3.26 ± 0.04
Long chain fatty acid	1.78 ± 0.23	2.04 ± 0.26	1.26 ± 0.17	1.63 ± 0.21	1.03 ± 0.13
Long chain/Medium chain	0.64 ± 0.16	0.48 ± 0.11	0.51 ± 0.17	0.41 ± 0.11	0.31 ± 0.08
Fatty acids/Phenols	13.3 ± 3.6	13.1 ± 4.0	12.0 ± 3.5	11.2 ± 3.0	8.7 ± 2.8
VAD/CAD	1.9 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.3 ± 0.2	1.2 ± 0.3
TOC	6.2 ± 0.3	3.8 ± 0.3	4.9 ± 0.5	6.1 ± 0.4	3.7 ± 0.4

Table 4.21 (continued)

	H1	ST11	ST10	ST9	ST7
	mg per g of sediment				
Lignin-derived phenols	1.39 ± 0.16	0.11 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.02
Fatty acids	9.66 ± 1.62	3.64 ± 0.45	2.09 ± 0.32	1.18 ± 0.19	0.60 ± 0.12
Mono-unsaturated fatty acids	1.76 ± 0.26	1.15 ± 0.17	0.82 ± 0.12	0.43 ± 0.06	0.19 ± 0.03
Saturated fatty acids	7.90 ± 0.95	2.49 ± 0.30	1.27 ± 0.15	0.75 ± 0.09	0.47 ± 0.06
Branched fatty acids	1.09 ± 0.12	0.76 ± 0.08	0.43 ± 0.05	0.24 ± 0.03	0.15 ± 0.02
Medium chain fatty acid	5.65 ± 0.68	2.87 ± 0.35	1.74 ± 0.20	0.95 ± 0.12	0.54 ± 0.06
Long chain fatty acid	4.01 ± 0.52	0.77 ± 0.10	0.35 ± 0.05	0.23 ± 0.03	0.11 ± 0.01
	mg per 100 mg of TOC				
Lignin-derived phenols	1.07 ± 0.12	0.23 ± 0.05	0.27 ± 0.05	0.20 ± 0.03	0.14 ± 0.02
Fatty acids	7.43 ± 0.78	7.91 ± 0.99	6.98 ± 1.10	3.82 ± 0.61	1.43 ± 0.26
Mono-unsaturated fatty acids	1.35 ± 0.24	2.49 ± 0.38	2.74 ± 0.41	1.39 ± 0.21	0.41 ± 0.06
Saturated fatty acids	6.08 ± 0.73	5.42 ± 0.65	4.24 ± 0.50	2.43 ± 0.29	1.01 ± 0.12
Branched fatty acids	0.84 ± 0.09	1.65 ± 0.18	1.43 ± 0.16	0.77 ± 0.10	0.33 ± 0.03
Medium chain fatty acid	4.35 ± 0.52	6.24 ± 0.74	5.80 ± 0.69	3.07 ± 0.37	1.18 ± 0.14
Long chain fatty acid	3.08 ± 0.41	1.67 ± 0.22	1.18 ± 0.16	0.75 ± 0.10	0.25 ± 0.03
Long chain/Medium chain	0.71 ± 0.18	0.27 ± 0.07	0.20 ± 0.05	0.24 ± 0.06	0.20 ± 0.04
Fatty acids/Phenols	6.9 ± 2.0	34 ± 3	26 ± 4	19 ± 3	10.2 ± 4.2
VAD/CAD	5.4 ± 0.9	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
TOC	13.0 ± 0.8	4.6 ± 0.4	3.0 ± 0.3	3.1 ± 0.3	4.6 ± 0.4

Table 4.22 - **Individual chemolysate abundance (μg per g of sediment); single data analysis**

Aromatic products	H9	H8	H7	H6	H5	H1	ST11	ST10	ST9	ST7
4-Methoxybenzoic acid, ME	20	17	29	52	21	125				
1,2,4-Trimethoxybenzene	156	59	72	96	62	74	33	73	46	78
4-Methoxyphenylacetic acid, ME						111				
3,4-Dimethoxybenzaldehyde	20	10	8	8	24	119				
3,4-Dimethoxybenzoic acid, ME	111	96	75	122	75	614	33	18	13	15
3,4-Dimethoxybenzeneacetic acid, ME				17		263				
3-(4-Methoxyphenyl)acrylic acid, ME	60	56	42	94	62	113	75	63	48	51
3-(3,4-Dimethoxyphenyl)acrylic acid, ME						48				

Table 4.22 (continued)

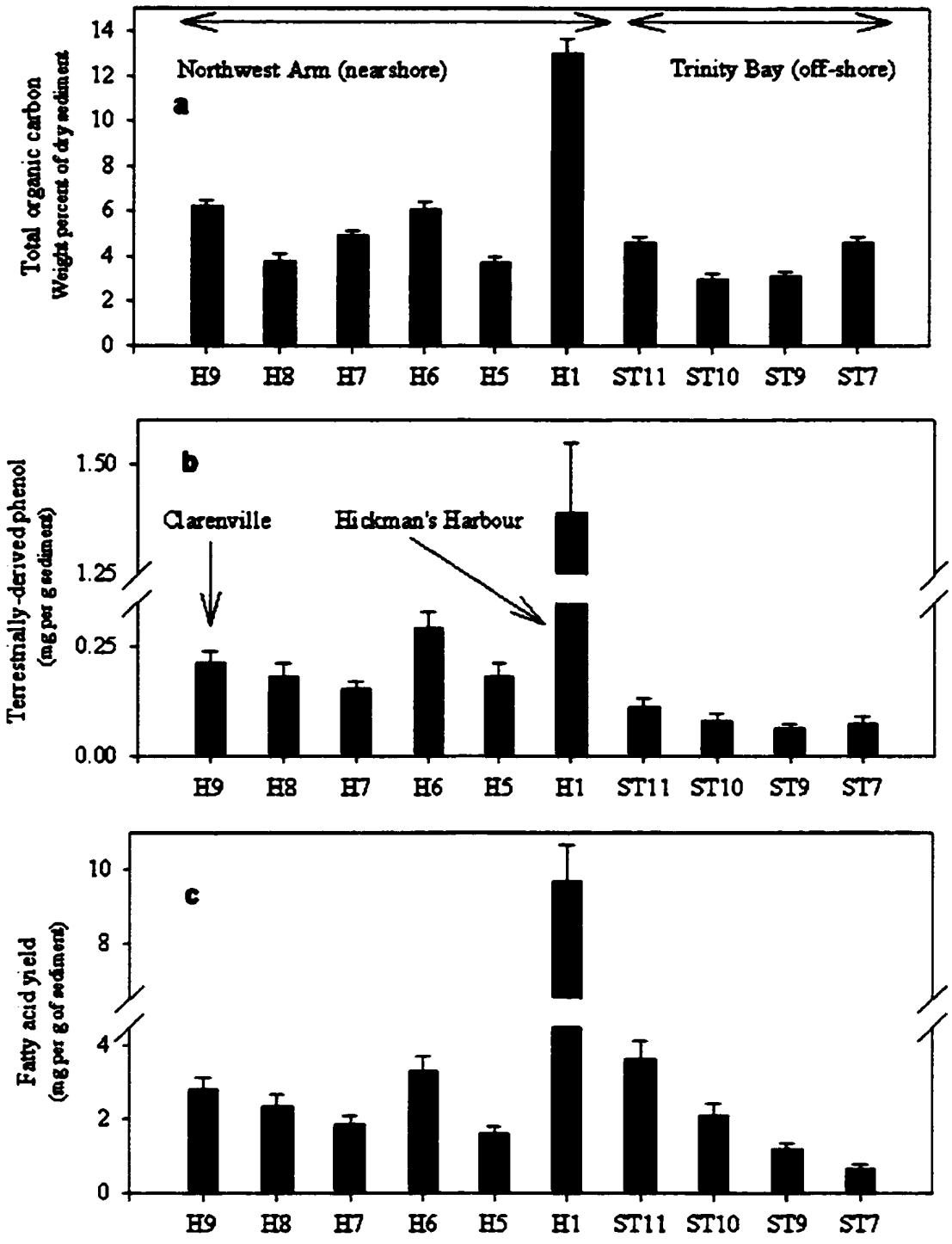
Fatty acid products	H9	H8	H7	H6	H5	H1	ST11	ST10	ST9	ST7
Dodecanoic acid, ME	32	26	26	26	25	124	13	23	15	23
12-Methyl-tridecanioc acid, ME	145	98	87	163	61	379	219	113	52	49
12-Methyl-tetradecanoic acid, ME	87	51	31	68	26	289	77	32	18	18
Pentadecanoic acid, ME	36	26	12	53	10	224	54	26	11	2
9-Hexadecenoic acid, ME	208	123	81	236	99	1371	259	204	61	48
Hexadecanoic acid, ME	808	511	405	706	327	2459	898	441	258	174
Heptadecanoic acid, ME	62	58	55	44	33	125	76	61	45	26
9-Octadecenoic acid	95	354	336	558	394	232	711	522	317	121
13-Octadecenoic acid	42	68	29	209	59	153	178	96	52	21
16-Methyl-heptadecanoic acid	184	261	159	238	170	297	386	223	123	62
Eicosanoic acid, ME	155	133	87	119	32	940	78	32	21	15
Heneicosanoic acid, ME	84	64	77	117	49	312	87	35	32	14
Docosanoic acid, ME	223	143	101	178	66	800	126	60	48	21
Tricosacoic acid, ME	55	99	68	130	44	234	67	33	1	9
Tetracosanoic acid, ME	280	183	149	225	82	867	155	86	59	28
Pentacosanoic acid, ME	66	1	12	60	24	126	68	16	15	4
Hexacosanoic acid, ME	175	108	95	130	57	443	102	48	45	24
Octacosanoic acid, ME	66	45	28	32	25	286	86	42	12	1

Figure 4.4 - **Spatial variation (for top of sediment) of chemolysate abundances**

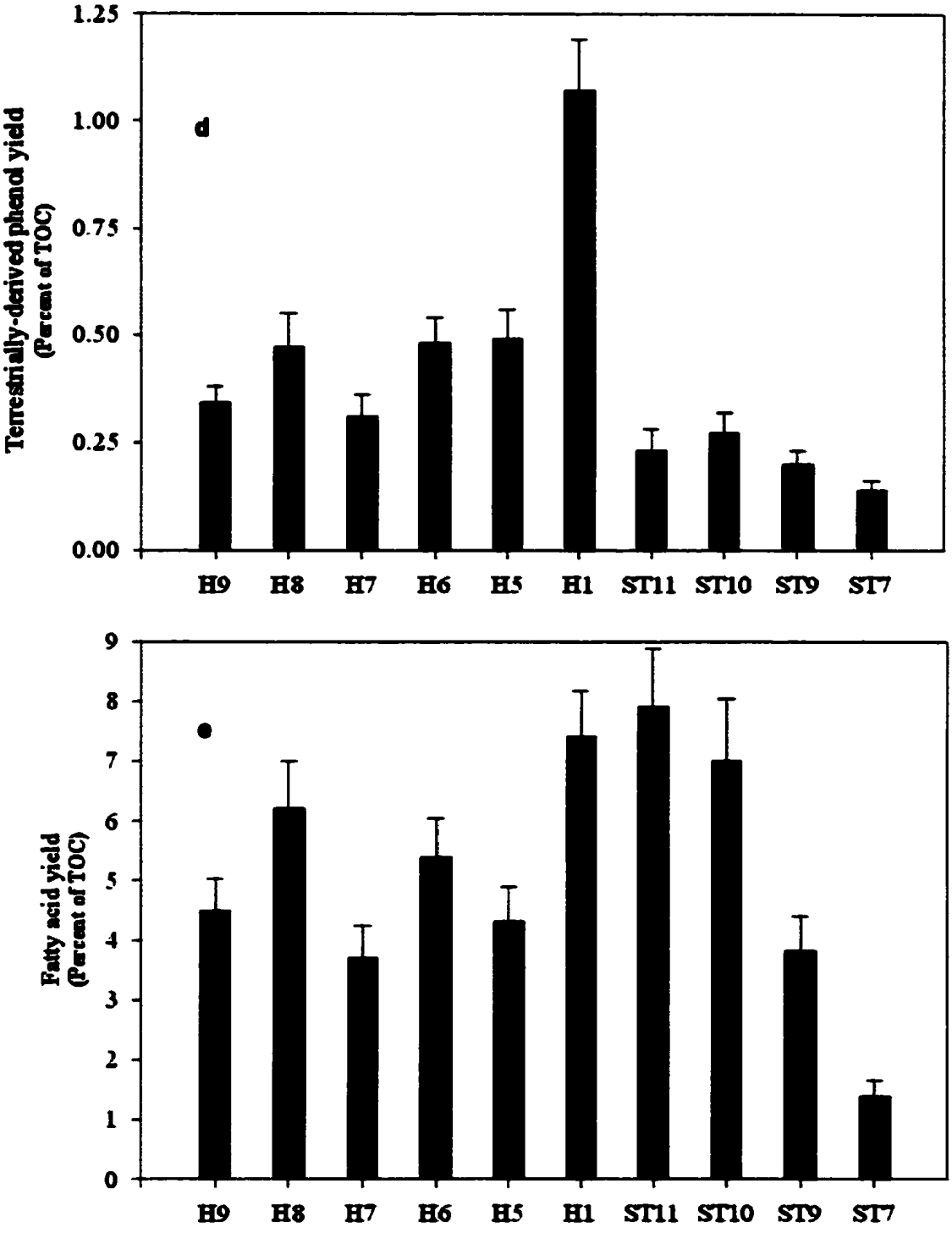
a) TOC (expressed as a percentage of dry weight of sediment or mg per 100 mg of dry sediment)
b) Yield of terrestrially-derived phenols (mg per g of sediment)
c) Yield of fatty acids (mg per g of sediment)
d) Yield of terrestrially-derived phenols normalized to TOC (mg per 100 mg of C)
e) Yield of fatty acids normalized to TOC (mg per 100 mg of C)

Data from Table 4.2

TOC, terrestrially derived phenol and fatty acid abundances



Total phenol and fatty acid sediment chemolysate abundances normalized to TOC



per g of dry sediment, respectively). Coumaric acid methyl ester becomes the quantitatively more important (i.e. increases as a percentage of total phenols) in offshore station sediments (Table 4.2). The lowest yields of phenols are seen in chemolysates of core ST7 and include 15 and 51 μg per g of dry sediment for methyl esters of vanillic and coumaric acids, respectively (Table 4.2; Figure 4.4). The higher yields of phenols in the Northwest Arm sediments is consistent with the observation of terrestrial inputs from natural processes and sawmilling/agricultural activities in the Random Island area. Northwest Arm also receives OM from sea grass (*Zostera marina*) located in low tidal areas. Sea grasses are also sources of lignin-derived phenols.

Terrestrial inputs have also resulted in differences in the composition of fatty acids in the Northwest Arm and Trinity Bay. The most abundant fatty acid chemolysate is hexadecanoic acid methyl ester which has a maximum yield of 174 and 2459 μg per g of dry sediment for cores ST7 and H1, respectively. There were longer chain fatty acids in the Northwest Arm affecting in the long/medium chain ratios. Long chain/medium chain fatty acids were highest in core H1 sediments (0.7 ± 0.18), most likely as a consequence of contributions from vascular plants; core ST7 sediments gave the lowest yields of fatty acids (0.6 mg per g of dry sediment) (Figure 4.4; Table 4.2). The lowest long/medium chain fatty acid ratio are for ST10, ST9 and ST7 sediment (Table 4.2), indicating reduced terriginous sources of OM.

The branched fatty acid methyl esters (12-methyl-tridecanioc acid, 12-methyl-tetradecanoic

acid, ME, 15-methyl-hexadecanoic acid, ME and 16-methyl-heptadecanoic acid, ME) comprise a minor fraction (less than 25 percent) of the fatty acids. The abundances of branched fatty acids are more variable in the Northwest Arm than in Trinity Bay samples. The abundances are highest for core H1 sediment (1.09 mg per g of dry sediment) and lowest for ST7 sediments (0.15 mg per g of dry sediment). However, the branched fatty acid fractions of the TOC are highest for core ST11 sediments and lowest for ST7 sediments, with values of 1.65 and 0.33 mg per 100 mg of TOC, respectively. The abundances of branched fatty acids correlate positively with TOC (R^2 = 0.83, n=10) implying that bacterial abundances correlate with the food supply.

1,2,4-Trimethoxybenzene is detected in the chemolysates of all the sediments, probably because of the presence of carbohydrates (Pulchan *et al.*, 1997; Fabbri and Helleur, 1999). The relative abundances of 1,2,4-trimethoxybenzene are highest in the chemolysates of sediment of offshore cores (as well as in core H1). However, it cannot be concluded that the relative carbohydrate abundances are higher in offshore sediments because of the variable yields of 1,2,4-trimethoxybenzene from TMAH thermochemolysis of different carbohydrate compounds (discussed in Chapter 3). On a carbon basis, abundances of 1,2,4-trimethoxybenzene vary between 0.6 and 2.5 μg per mg of TOC (for cores H1 and H9, respectively). Manino and Harvey (2000) measured 1.21 to 6.25 μg per mg of particulate organic carbon from the Delaware Bay.

Generally, the distribution of the chemolysates when expressed as fractions of the dry sediment are similar to their fractional composition of the TOC. The Northwest Arm cores have higher fractions of phenols compared to the offshore cores (Table 4.2). Core H1 sediments have the highest abundance of phenols. However, the distribution of fatty acids, as a fraction of the TOC, are different; ST11 has the highest fraction of fatty acids in its TOC (7.9 mg per 100 mg of TOC). In addition, all of the offshore stations have high percentages of fatty acids in their TOC with the exception of ST7 which is impoverished (in comparison with the other cores) in both phenols and fatty acids. Hence, in order to preserve mass balance, there must be greater quantities of other classes of organic compounds in sediments at ST7, such as N-compounds (as determined in Chapter 2). The passage of biopolymers and other OM through the digestive tracts of sediment-ingesting benthos may exert an important influence on OM transformations (Farrington, 1992). ST7 sediment are extensively bioturbated and homogenized which re-exposes OM to oxidising agents. In the absence of other factors, bioturbation will result in utilization of labile compounds such as N-compounds and fatty acids, and preservation of the more formidable substrates such as phenolic compounds. However, core ST7 sediments display enhanced preservation of the labile compounds. Knicker and Hatcher, 1997 reported enhanced preservation of N-compounds and suggested that it can be achieved through encapsulation in a network of refractory bioploymer.

Fatty acid methyl esters comprised the dominant class of compounds in the chemolysates of

all sediment samples of the Northwest Arm and Trinity Bay because marine production results in OM that is richer in fatty acids (and N-compounds). Although fatty acids are more labile than lignin-derived phenols, their survival in the sediment can be enhanced by associations with other stable compounds and interactions with mineral particles. The fatty acid/phenol ratios for surface samples varied between 6.9 to 34.0 (for cores H1 and ST7, respectively).

There are also differences in the distributions of the individual chemolysates (as a fraction of the TOC) and abundances of chemolysates in dry sediments. For example, the ratios of abundances of vanillic acid methyl ester for cores H1 and H5 are 614 to 75 μg per g of sediment (~8.2) and 0.47 and 0.2 mg per 100 mg of TOC (~2.4), respectively. Hence, normalization of abundances (in mg per g of sediment) of chemolysates to the TOC de-emphasizes the apparent quantitative importance of vanillic acid methyl ester for core H1.

4.3.5 Temporal/depth variation of chemolysates

Cores H1, H9 and ST7 have different sedimentation histories (Appendix 1.3). The sedimentation rate at H1 is much slower than that at H9. Extensive bioturbation at ST7 prevented dating of the sediments by ^{210}Pb. All three stations show a slight downcore decrease in the total organic carbon (Table 4.3, 4.4 and 4.5; Figures 4.51, 4.52 and 4.53) which is most likely the result of diagenetic degradation and/or changes in fluxes. The

Table 4.3 - **Temporal variation of chemolysates of core H1**

4.31. Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.

4.32. Individual chemolysate abundance (μg per g of sediment); single data analysis

Data plotted in Figure 4.51

Table 4.31. **Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.**

	0-2 cm	4-6 cm	8-10 cm	14-16 cm
	mg per g of sediment			
Lignin-derived phenols	1.39 ± 0.16	0.71 ± 0.07	0.59 ± 0.07	0.43 ± 0.05
Fatty acids	9.66 ± 1.62	8.23 ± 0.72	8.15 ± 0.82	7.94 ± 0.86
Mono-unsaturated fatty acids	1.76 ± 0.26	1.05 ± 0.25	0.99 ± 0.22	0.85 ± 0.20
Saturated fatty acids	7.90 ± 0.95	7.18 ± 0.63	7.16 ± 0.64	7.09 ± 0.63
Branched fatty acids	1.09 ± 0.12	1.42 ± 0.13	1.29 ± 0.12	1.14 ± 0.10
Medium chain fatty acids	5.65 ± 0.68	4.74 ± 0.57	4.43 ± 0.53	4.03 ± 0.48
Long chain fatty acids	4.01 ± 0.52	3.50 ± 0.46	3.72 ± 0.48	3.91 ± 0.51
	mg per 100 mg of TOC			
Lignin-derived phenols	1.07 ± 0.18	0.61 ± 0.06	0.54 ± 0.06	0.43 ± 0.05
Fatty acids	7.43 ± 0.78	7.04 ± 0.61	7.34 ± 0.73	7.03 ± 0.76
Mono-unsaturated fatty acids	1.35 ± 0.24	0.90 ± 0.28	0.89 ± 0.25	0.75 ± 0.21
Saturated fatty acids	6.08 ± 0.73	6.14 ± 0.54	6.45 ± 0.56	6.28 ± 0.70
Branched fatty acids	0.84 ± 0.09	1.22 ± 0.11	1.16 ± 0.12	1.00 ± 0.11
Medium chain fatty acids	4.36 ± 0.52	4.05 ± 0.49	4.00 ± 0.43	3.57 ± 0.49
Long chain fatty acids	3.08 ± 0.41	2.99 ± 0.44	3.35 ± 0.45	3.46 ± 0.57
Long chain/Medium chain	0.71± 0.18	0.74 ± 0.19	0.84 ± 0.21	0.97 ± 0.25
Fatty acids/Phenols	6.9 ± 2.0	7.4 ± 2.2	8.6 ± 3.1	10.1 ± 4.2
VAD/CAD	5.4 ± 0.3	2.2 ± 0.5	1.7 ± 0.3	1.5 ± 0.2
VAD/VAL	5.1 ± 0.2	7.4 ± 0.7	12.6 ± 0.9	14.9 ± 1.0
TOC	13.0 ± 0.8	11.7 ± 0.5	11.1 ± 0.05	11.3 ± 0.6

Table 4.31 (continued)

	18-20 cm	24-26 cm	28-30 cm
	mg per g of sediment		
Lignin-derived phenols	0.38 ± 0.05	0.32 ± 0.04	0.26 ± 0.04
Fatty acids	8.45 ± 0.73	8.17 ± 0.74	7.99 ± 0.76
Mono-unsaturated fatty acids	0.84 ± 0.19	0.84 ± 0.18	0.85 ± 0.19
Saturated fatty acids	7.62 ± 0.70	7.33 ± 0.68	7.13 ± 0.66
Branched fatty acids	1.18 ± 0.13	1.17 ± 0.11	1.18 ± 0.12
Medium chain fatty acids	4.10 ± 0.49	3.98 ± 0.48	3.89 ± 0.47
Long chain fatty acids	4.36 ± 0.57	4.19 ± 0.54	4.09 ± 0.53
	mg per 100 mg of TOC		
Lignin-derived phenols	0.38 ± 0.04	0.32 ± 0.04	0.23 ± 0.04
Fatty acids	7.83 ± 0.67	7.43 ± 0.67	7.13 ± 0.54
Mono-unsaturated fatty acids	0.78 ± 0.16	0.76 ± 0.17	0.76 ± 0.18
Saturated fatty acids	7.05 ± 0.65	6.67 ± 0.62	6.37 ± 0.59
Branched fatty acids	1.10 ± 0.10	1.07 ± 0.11	1.05 ± 0.11
Medium chain fatty acids	3.80 ± 0.46	3.62 ± 0.43	3.48 ± 0.45
Long chain fatty acids	4.03 ± 0.52	3.81 ± 0.50	3.65 ± 0.47
Long chain/Medium chain	1.06 ± 0.27	1.05 ± 0.23	1.05 ± 0.24
Fatty acids/Phenols	20.6 ± 4.9	22.9 ± 5.6	30.8 ± 7.9
VAD/CAD	1.7 ± 0.2	1.2 ± 0.2	1.4 ± 0.1
VAD/VAL	33.0 ± 1.4	27.0 ± 1.7	21.0 ± 1.9
TOC	10.8 ± 0.6	11.0 ± 0.4	11.2 ± 0.6

Table 4.32 - **Individual chemolysate abundance (μg per g of sediment); single data analysis**

Aromatic products	0-2	4-6	8-10	14-16	18-20	24-26	28-30
4-Methoxybenzoic acid, ME	125	134	71	12	20		
1,2,4-Trimethoxybenzene	74	164	267	188	324	322	261
4-Methoxyphenylacetic acid, ME	111	46	114	105	48	6	17
3,4-Dimethoxybenzaldehyde	119	32	16	11	6	6	6
3,4-Dimethoxybenzoic acid, ME	614	237	201	164	186	161	127
3,4-Dimethoxybenzeneacetic acid, ME	263	134	70	32	30	39	14
3-(4-Methoxyphenyl)acrylic acid, ME	113	106	115	107	109	134	90
3-(3,4-Dimethoxyphenyl)acrylic acid, ME	48	25	7	5	12	11	4

Table 4.32 (continued)

Fatty acid products	0-2	4-6	8-10	14-16	18-20	24-26	28-30
Dodecanoic acid, ME	124	103	147	105	155	147	145
12-Methyl-tridecanioc acid, ME	379	368	346	320	322	323	334
12-Methyl-tetradecanoic acid, ME	289	260	220	177	206	155	172
Pentadecanoic acid, ME	240	87	81	50	82	95	31
9-Hexadecenoic acid, ME	1371	134	104	83	75	64	65
Hexadecanoic acid, ME	2459	2068	1924	1891	1841	1726	1684
Heptadecanoic acid, ME	125	100	97	101	110	88	76
9-Octadecenoic acid, ME	232	791	768	669	672	698	691
13-Octadecenoic acid, ME	153	128	118	96	90	75	96
16-Methyl-heptadecanoic acid, ME	297	697	629	538	547	606	600
Eicosanoic acid, ME	940	520	616	374	407	385	439
Heneicosanoic acid, ME	312	419	186	212	213	299	260
Docosanoic acid, ME	800	766	621	697	590	545	596
Tricosacoic acid, ME	234	389	293	283	371	383	293
Tetracosanoic acid, ME	867	576	1000	993	1007	972	991
Pentacosanoic acid, ME	126	73	203	214	235	230	189
Hexacosanoic acid, ME	443	363	440	727	938	839	804
Octacosanoic acid, ME	286	393	358	412	595	538	521

Table 4.4 - **Temporal variation of chemolysates of core H9**

4.41. Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.

4.42. Individual chemolysate abundance (μg per g of sediment); single data analysis

Data plotted in Figure 4.52

Table 4.41 - **Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.**

	0-2 cm	4-6 cm	8-10 cm	14-16 cm
	mg per g of sediment			
Lignin-derived phenols	0.21 ± 0.03	0.23 ± 0.03	0.19 ± 0.03	0.21 ± 0.04
Fatty acids	2.80 ± 0.34	2.91 ± 0.33	3.44 ± 0.45	2.34 ± 0.23
Mono-unsaturated fatty acids	0.34 ± 0.05	0.37 ± 0.06	0.43 ± 0.06	0.25 ± 0.04
Saturated fatty acids	2.46 ± 0.30	2.55 ± 0.31	3.02 ± 0.36	2.09 ± 0.25
Branched fatty acids	0.48 ± 0.05	0.55 ± 0.06	0.58 ± 0.06	0.43 ± 0.05
Medium chain fatty acids	1.70 ± 0.20	1.81 ± 0.22	1.96 ± 0.24	1.36 ± 0.16
Long chain fatty acids	1.10 ± 0.14	1.10 ± 0.14	1.48 ± 0.19	0.98 ± 0.13
	mg per 100 mg of TOC			
Lignin-derived phenols	0.34 ± 0.04	0.40 ± 0.05	0.34 ± 0.05	0.36 ± 0.06
Fatty acids	4.50 ± 0.51	5.11 ± 0.59	6.26 ± 0.82	4.04 ± 0.49
Mono-unsaturated fatty acids	0.56 ± 0.09	0.65 ± 0.10	0.78 ± 0.12	0.44 ± 0.07
Total saturated fatty acids	3.96 ± 0.48	4.47 ± 0.54	5.49 ± 0.66	3.60 ± 0.43
Total Branched fatty acids	0.77 ± 0.09	0.97 ± 0.11	1.06 ± 0.12	0.75 ± 0.08
Medium chain fatty acid	2.74 ± 0.32	3.18 ± 0.38	3.57 ± 0.43	2.35 ± 0.28
Long chain fatty acid	1.78 ± 0.23	1.93 ± 0.25	2.69 ± 0.35	1.69 ± 0.22
Long chain/Medium chain	0.64 ± 0.16	0.61 ± 0.15	0.75 ± 0.19	0.72 ± 0.18
Fatty acids/Phenols	13.3 ± 3.6	12.8 ± 3.1	18.2 ± 4.8	11.3 ± 3.3
VAD/CAD	1.9 ± 0.3	2.7 ± 0.5	3.1 ± 0.5	1.7 ± 0.3
VAD/VAL	5.6 ± 1.2	11.1 ± 2.2		
TOC	6.2 ± 0.3	5.7 ± 0.6	5.5 ± 0.5	5.8 ± 0.6

Table 4.41 (continued)

	18-20 cm	24-26 cm	28-30 cm
	mg per g of sediment		
Lignin-derived phenols	0.20 ± 0.03	0.15 ±0.03	0.15 ± 0.03
Fatty acids	2.53 ± 0.31	2.85 ±0.34	2.35 ± 0.34
Mono-unsaturated fatty acids	0.23 ± 0.03	0.28 ±0.04	0.22 ± 0.03
Total saturated fatty acids	2.30 ± 0.28	2.57 ±0.31	2.13 ± 0.26
Total branched fatty acids	0.41 ± 0.05	0.51 ±0.06	0.40 ± 0.04
Medium chain fatty acids	1.42 ± 0.17	1.66 ±0.21	1.36 ± 0.16
Long chain fatty acids	1.11 ± 0.14	1.18 ±0.15	0.98 ± 0.13
	mg per 100 mg of TOC		
Lignin-derived phenols	0.40 ± 0.06	0.24 ± 0.04	0.25 ± 0.04
Fatty acids	5.06 ± 0.61	4.59 ± 0.55	3.98 ± 0.44
Mono-unsaturated fatty acids	0.46 ± 0.07	0.45 ± 0.07	0.37 ± 0.09
Saturated fatty acids	4.59 ± 0.55	4.15 ± 0.53	3.61 ± 0.45
Branched fatty acids	0.82 ± 0.09	0.82 ± 0.09	0.67 ± 0.12
Medium chain fatty acid	2.83 ± 0.34	2.68 ± 0.35	2.31 ± 0.28
Long chain fatty acid	2.22 ± 0.29	1.91 ± 0.25	1.67 ± 0.22
Long chain/Medium chain	0.78 ± 0.20	0.71 ± 0.18	0.72 ± 0.19
Fatty acids/Phenols	12.5 ± 3.5	19.2 ± 5.6	15.8 ± 5.1
VAD/CAD	1.8 ± 0.3	2.8 ± 0.5	2.2 ± 0.3
TOC	5.0 ± 0.4	6.2 ± 0.5	5.9 ± 0.4

Table 4.42 - **Individual chemolysate abundance (μg per g of sediment); single data analysis**

Aromatic products	0-2	4-6	8-10	14-16	18-20	24-26	28-30
4-Methoxybenzoic acid, ME	20						
1,2,4-Trimethoxybenzene	156	192	112	75	74	28	59
3,4-Dimethoxybenzaldehyde	20	14					
3,4-Dimethoxybenzoic acid, ME	111	156	143	130	129	109	103
3-(4-Methoxyphenyl)acrylic acid, ME	60	58	46	78	73	39	46

Table 4.42 (continued)

Fatty acid products	0-2	4-6	8-10	14-16	18-20	24-26	28-30
Dodecanoic acid, ME	32	17	30	18	10	20	20
12-Methyl-tridecanioc acid, ME	145	194	210	152	99	177	150
12-Methyl-tetradecanoic acid, ME	87	130	123	106	97	107	77
Pentadecanoic acid, ME	36	41	67	36	30	36	25
9-Hexadecenoic acid, ME	208	177	222	119	109	126	86
Hexadecanoic acid, ME	808	836	857	619	734	822	703
Heptadecanoic acid, ME	62	60	55	44	55	52	33
9-Octadecenoic acid, ME	95	98	117	66	63	88	84
13-Octadecenoic acid, ME	42	93	89	67	59	62	50
16-Methyl-heptadecanoic acid, ME	184	166	194	132	160	174	135
Eicosanoic acid, ME	155	179	244	131	183	170	140
Heneicosanoic acid, ME	84	79	80	69	76	83	59
Docosanoic acid, ME	223	223	282	193	229	230	197
Tricosacoic acid, ME	55	83	87	74	82	93	62
Tetracosanoic acid, ME	280	248	392	242	272	309	265
Pentacosanoic acid, ME	66	29	68	65	35	56	21
Hexacosanoic acid, ME	175	193	257	147	165	175	176
Octacosanoic acid, ME	66	67	71	61	69	69	65

Table 4.5 - **Depth variation of chemolysates of core ST7**

4.51. Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.

4.52. Individual chemolysate abundance (μg per g of sediment); single data analysis

Data plotted in Figure 4.53

Figure 4.51 - **Class yields of selected chemolysates (mg per g of sediment and normalized to TOC); n = 3, mean ± s.d.**

	0-2 cm	4-6 cm	8-10 cm	14-16 cm
	mg per g of sediment			
Lignin-derived phenols	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Fatty acids	0.60 ± 0.12	0.83 ± 0.11	0.75 ± 0.08	0.60 ± 0.05
Mono-unsaturated fatty acids	0.19 ± 0.03	0.26 ± 0.04	0.25 ± 0.04	0.18 ± 0.03
Saturated fatty acids	0.47 ± 0.06	0.56 ± 0.07	0.50 ± 0.06	0.42 ± 0.05
Branched fatty acids	0.15 ± 0.02	0.18 ± 0.02	0.16 ± 0.02	0.12 ± 0.01
Medium chain fatty acids	0.54 ± 0.06	0.69 ± 0.08	0.62 ± 0.07	0.47 ± 0.06
Long chain fatty acids	0.11 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.02
	mg per 100 mg of TOC			
Lignin-derived phenols	0.14 ± 0.02	0.18 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
Fatty acids	1.43 ± 0.26	2.36 ± 0.27	2.27 ± 0.24	1.87 ± 0.16
Mono-unsaturated fatty acids	0.41 ± 0.06	0.76 ± 0.11	0.74 ± 0.12	0.57 ± 0.09
Saturated fatty acids	1.01 ± 0.12	1.61 ± 0.19	1.52 ± 0.20	1.30 ± 0.16
Branched fatty acids	0.33 ± 0.03	0.50 ± 0.06	0.48 ± 0.05	0.39 ± 0.04
Medium chain fatty acids	1.18 ± 0.14	1.98 ± 0.24	1.87 ± 0.22	1.47 ± 0.18
Long chain fatty acids	0.25 ± 0.03	0.38 ± 0.05	0.39 ± 0.05	0.40 ± 0.05
Long chain/Medium chain	0.21 ± 0.04	0.19 ± 0.05	0.21 ± 0.06	0.27 ± 0.08
Fatty acids/Phenols	10.0 ± 4.2	13.1 ± 3.4	14.1 ± 4.1	12.0 ± 3.2
VAD/CAD	0.29 ± 0.10	0.15 ± 0.02	0.15 ± 0.03	0.14 ± 0.02
TOC	4.6 ± 0.4	3.5 ± 0.3	3.3 ± 0.3	3.2 ± 0.3

Table 4.51 (continued)

	18-20 cm	24-26 cm	28-30 cm
	mg per g of sediment		
Lignin-derived phenols	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Fatty acids	0.61 ± 0.06	0.64 ± 0.73	0.57 ± 0.06
Mono-unsaturated fatty acids	0.18 ± 0.08	0.22 ± 0.09	0.18 ± 0.08
Saturated fatty acids	0.43 ± 0.15	0.43 ± 0.14	0.39 ± 0.14
Branched fatty acids	0.15 ± 0.05	0.13 ± 0.04	0.13 ± 0.04
Medium chain fatty acids	0.49 ± 0.17	0.53 ± 0.17	0.47 ± 0.17
Long chain fatty acids	0.13 ± 0.05	0.11 ± 0.04	0.10 ± 0.04
	mg per 100 mg of TOC		
Lignin-derived phenols	0.09 ± 0.09	0.09 ± 0.01	0.05 ± 0.01
Fatty acids	1.76 ± 0.19	1.74 ± 0.20	1.68 ± 0.16
Mono-unsaturated fatty acids	0.52 ± 0.08	0.59 ± 0.09	0.54 ± 0.08
Saturated fatty acids	1.23 ± 0.15	1.15 ± 0.14	1.14 ± 0.18
Branched fatty acids	0.42 ± 0.05	0.35 ± 0.04	0.37 ± 0.04
Medium chain fatty acids	1.39 ± 0.17	1.43 ± 0.17	1.39 ± 0.17
Long chain fatty acids	0.37 ± 0.05	0.31 ± 0.04	0.29 ± 0.04
Long chain/Medium chain	0.3 ± 0.20	0.2 ± 0.15	0.2 ± 0.17
Fatty acids/Phenols	18.6 ± 6.3	19.5 ± 6.4	31.7 ± 7.4
VAD/CAD	0.50 ± 0.01	0.27 ± 0.01	0.50 ± 0.01
TOC	3.5 ± 0.3	3.7 ± 0.4	3.4 ± 0.3

Table 4.52 - **Individual chemolysate abundance (μg per g of sediment); single data analysis**

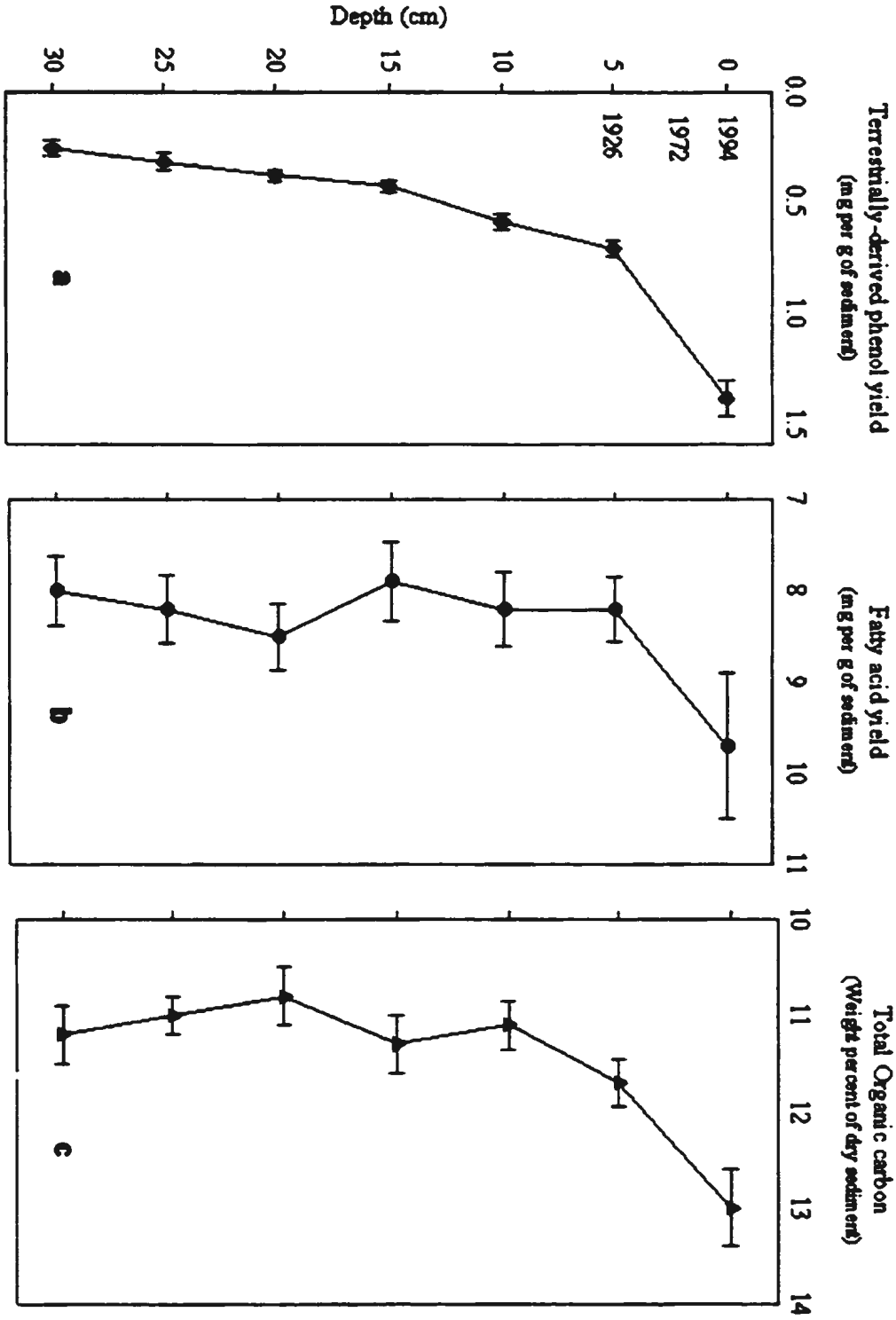
Aromatic products	0-2	4-6	8-10	14-16	18-20	24-26	28-30
1,2,4-Trimethoxybenzene	78	89	18	35	12	5	8
3,4-Dimethoxybenzoic acid, ME	15	8	7	6	11	7	6
3-(4-Methoxyphenyl)acrylic acid, ME	51	55	46	44	22	26	12
Fatty acid products							
Dodecanoic acid, ME	23	9	2				
12-Methyl-tridecanioc acid, ME	49	53	22	26	50	28	29
12-Methyl-tetradecanoic acid, ME	18	12	22	16	19	19	21
Pentadecanoic acid, ME	2	14	11	7	4	11	13
9-Hexadecenoic acid, ME	48	59	51	35	40	62	57
Hexadecanoic acid, ME	174	231	200	156	151	171	149
Heptadecanoic acid, ME	25	23	31	19	22	24	20
9-Octadecenoic acid, ME	121	163	160	124	119	124	101
13-Octadecenoic acid, ME	21	43	34	24	25	32	25
16-Methyl-heptadecanoic acid, ME	62	87	85	63	56	59	56
Eicosanoic acid, ME	15	17	22	15	16	12	15
Heneicosanoic acid, ME	14	22	12	11	15	12	8
Docosanoic acid, ME	21	18	30	31	25	20	20
Tricosacoic acid, ME	9	18	7	7	13	12	10
Tetracosanoic acid, ME	28	39	29	44	31	32	26
Pentacosanoic acid, ME	4	4	7	5	7	3	2
Hexacosanoic acid, ME	24	14	24	16	22	22	18

Figure 4.51 - **Temporal variation of sediment chemolysates of core H1**

a) Yield of terrestrially-derived phenols (mg per g of sediment)
b) Yield of fatty acids (mg per g of sediment)
c) TOC (expressed as a percentage of dry weight of sediment or mg per 100 mg of dry sediment)
d) Yield of terrestrially-derived phenols normalized to TOC (mg per100 mg of C)
e) Yield of fatty acids normalized to TOC (mg per100 mg of C)

Data from Table 4.3

Terrestrially derived phenol and fatty acid abundances and TOC of core H1



Total phenol and fatty acid sediment chemolysate abundances normalized to TOC

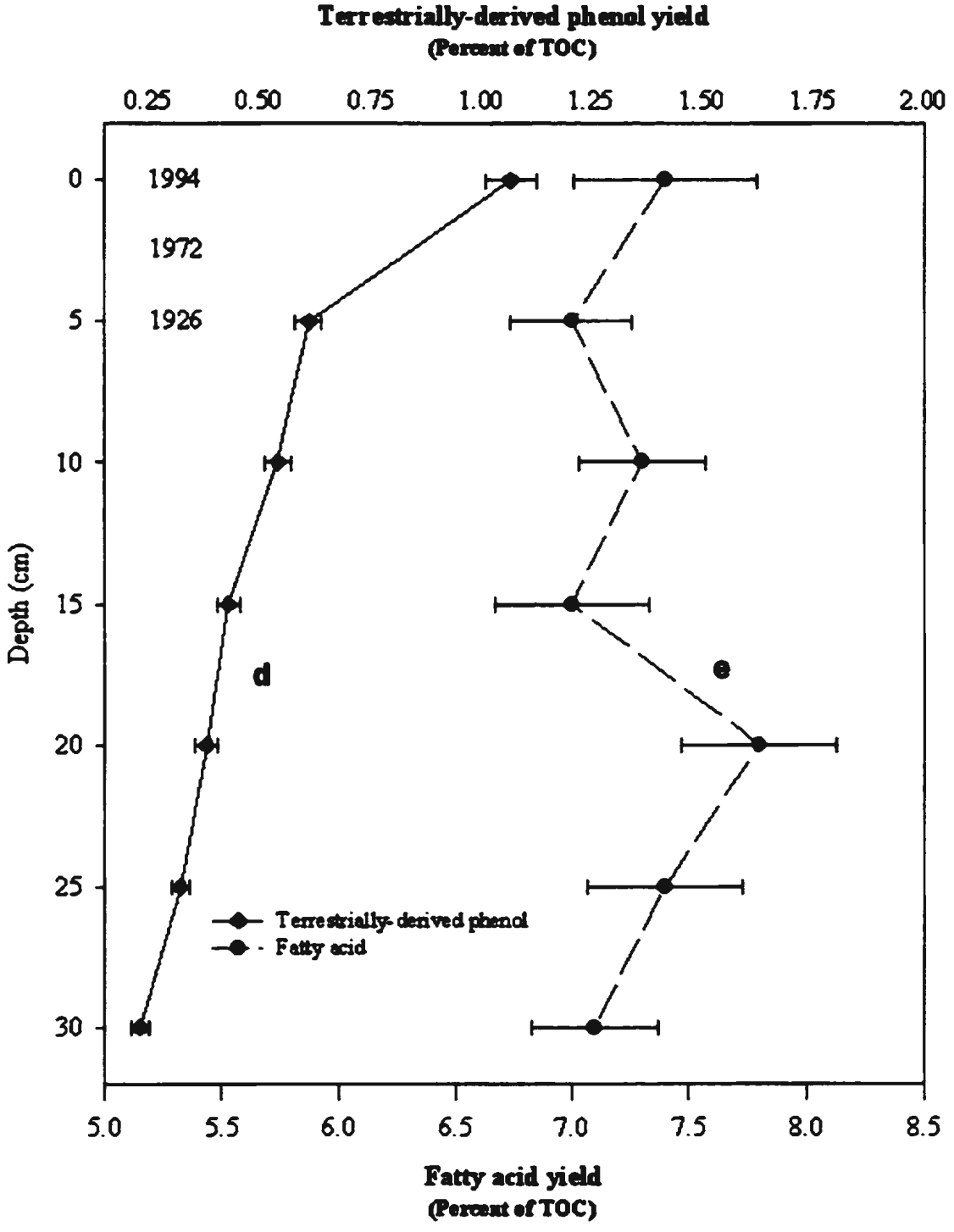
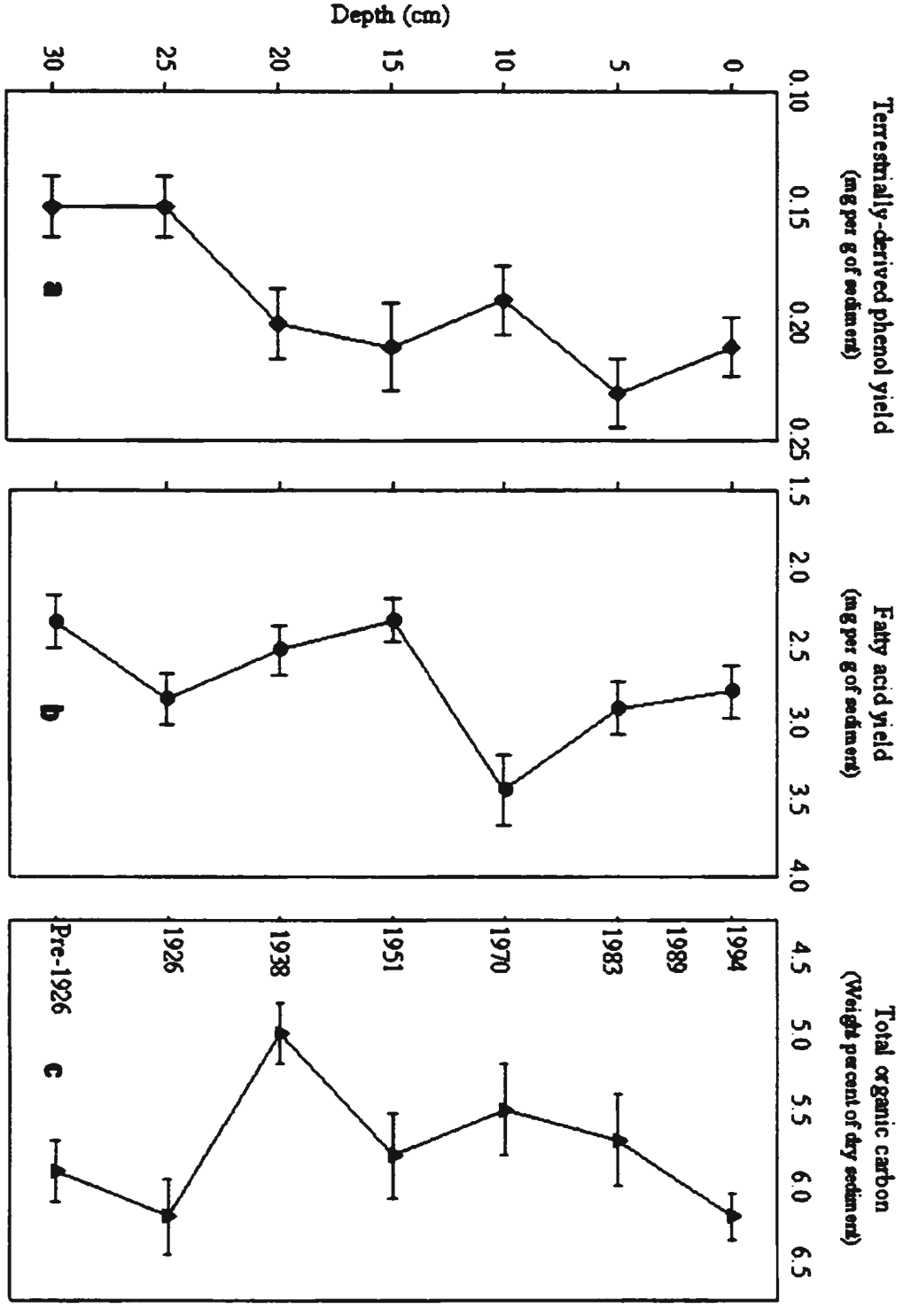


Figure 4.52 - **Temporal variation of sediment chemolysates of core H9**

a) Yield of terrestrially-derived phenols (mg per g of sediment)
b) Yield of fatty acids (mg per g of sediment)
c) TOC (expressed as a percentage of dry weight of sediment or mg per 100 mg of dry sediment)
d) Yield of terrestrially-derived phenols normalized to TOC (mg per100 mg of C)
e) Yield of fatty acids normalized to TOC (mg per100 mg of C)

Data from Table 4.4

Terrestrially derived phenol and fatty acid abundances and TOC of core H9



Total phenol and fatty acid sediment chemolysate abundances normalized to TOC

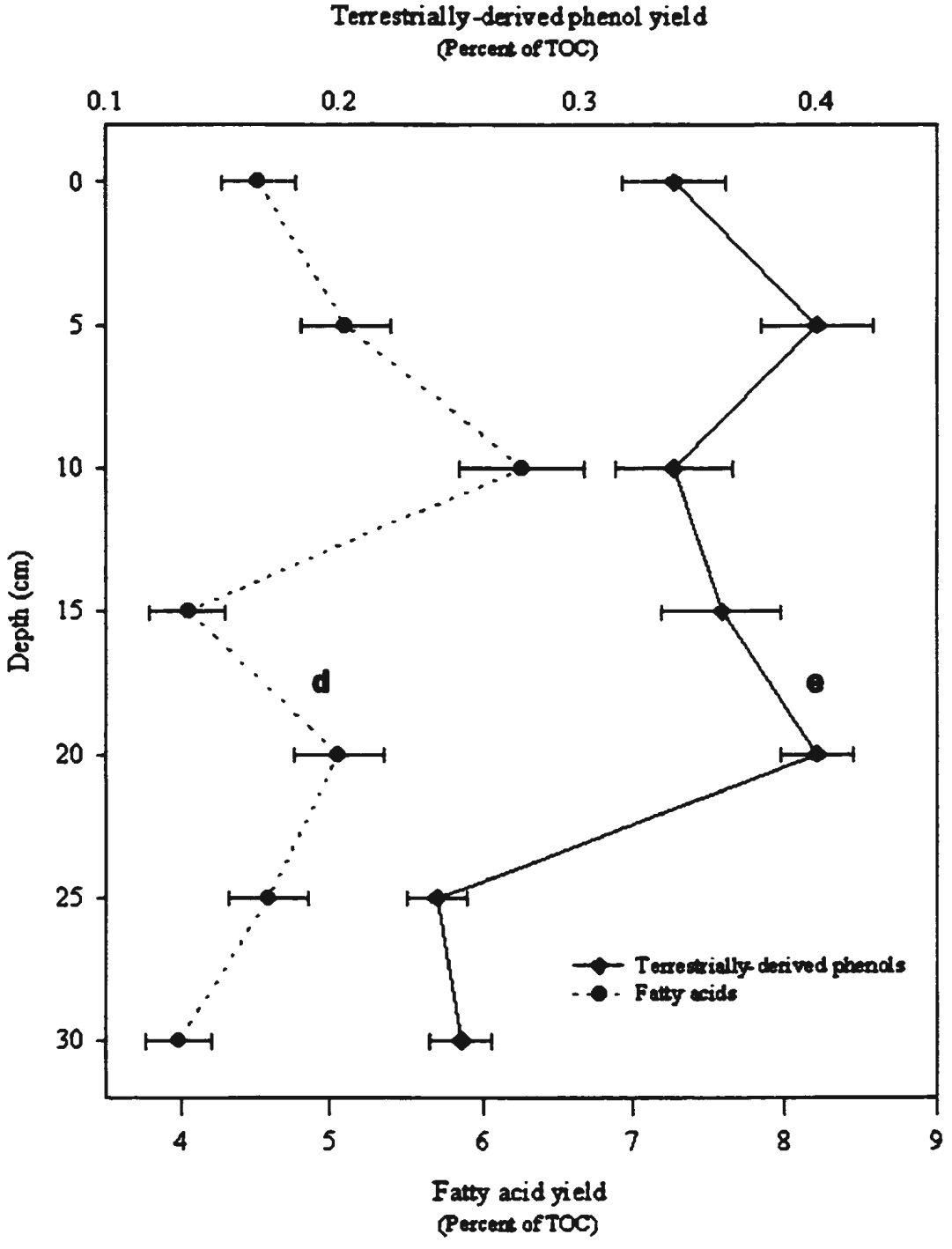
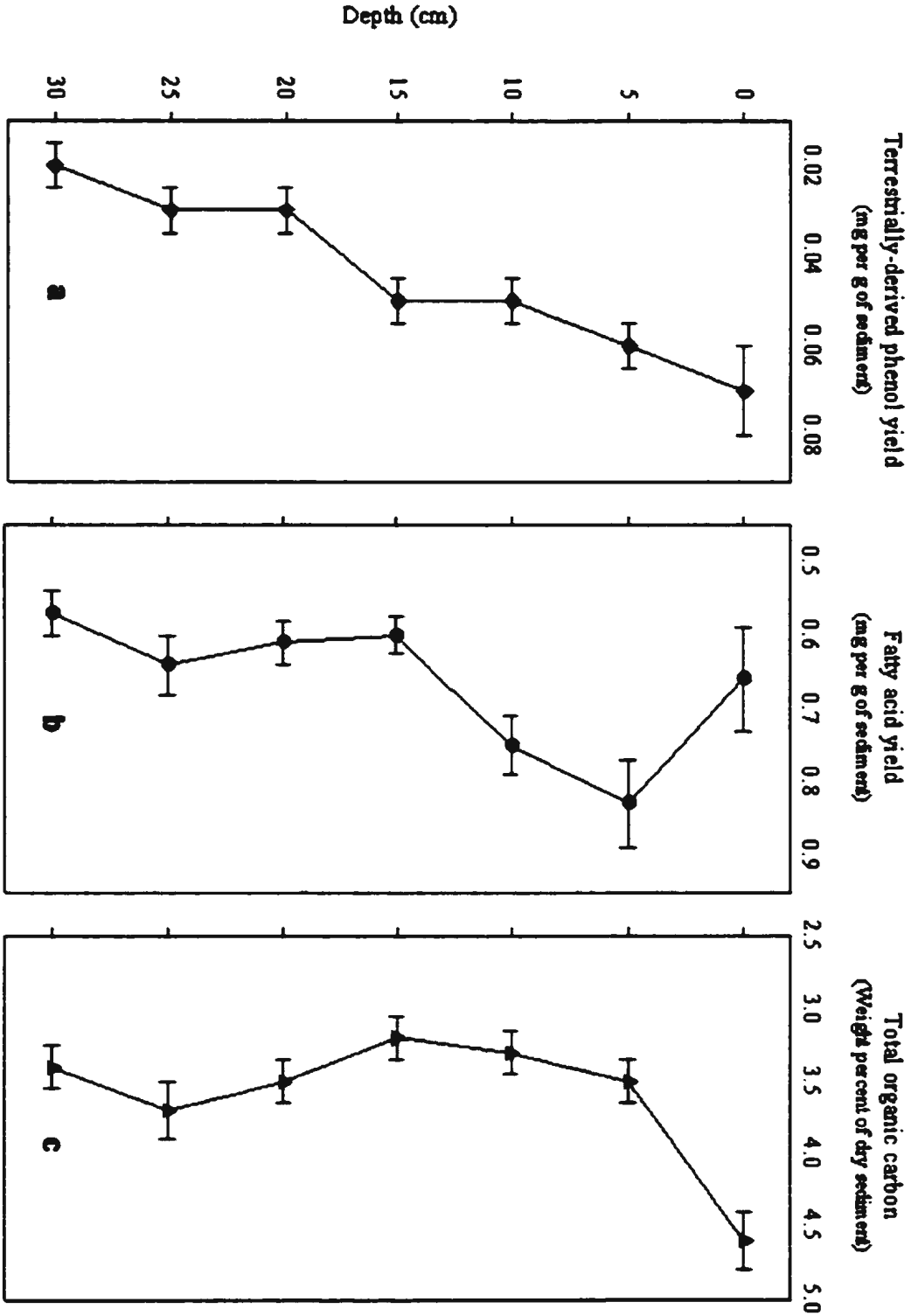


Figure 4.53 - **Temporal variation of sediment chemolysates of core ST7**

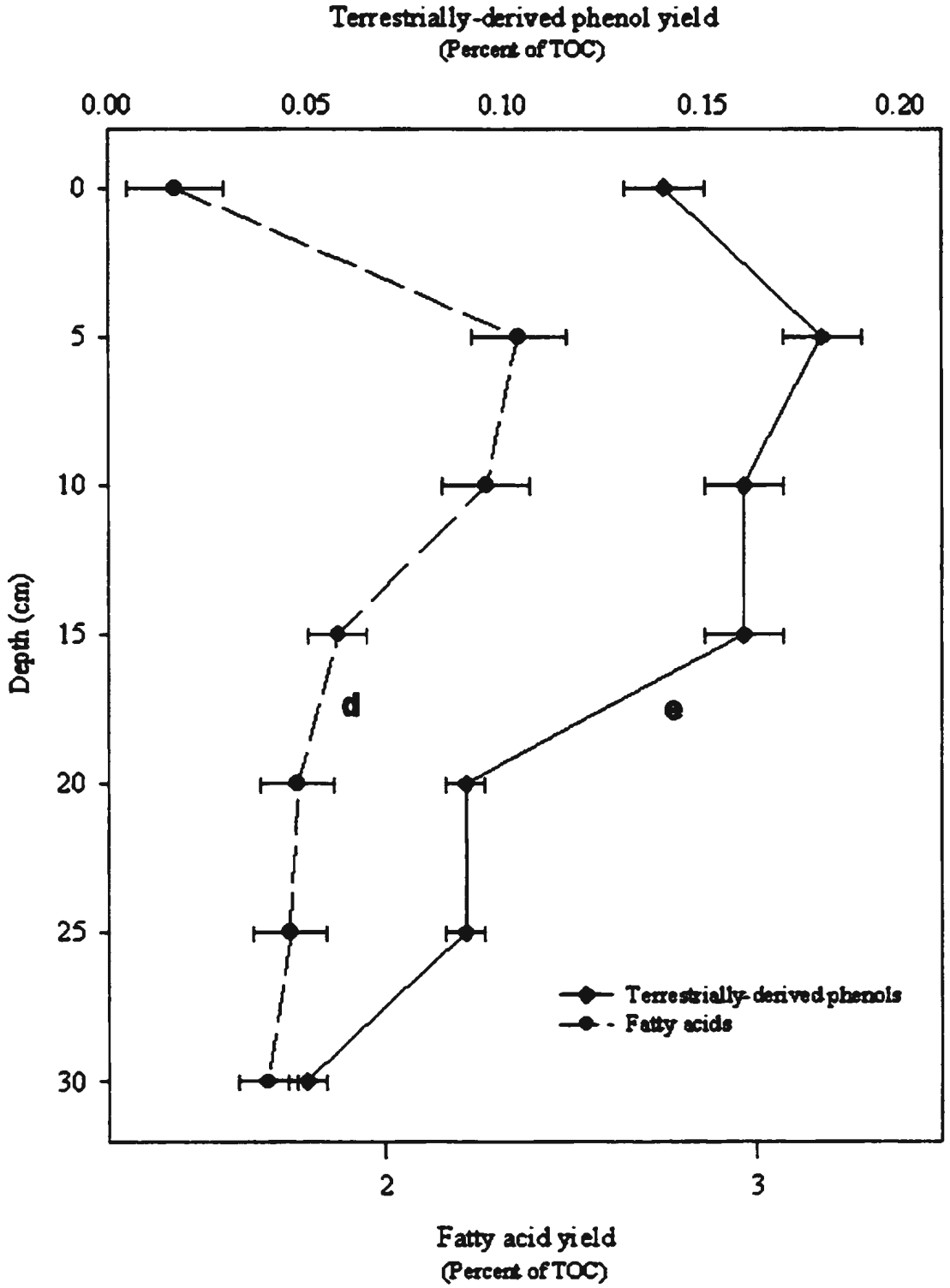
a) Yield of terrestrially-derived phenols (mg per g of sediment)
b) Yield of fatty acids (mg per g of sediment)
c) TOC (expressed as a percentage of dry weight of sediment or mg per 100 mg of dry sediment)
d) Yield of terrestrially-derived phenols normalized to TOC (mg per100 mg of C)
e) Yield of fatty acids normalized to TOC (mg per100 mg of C)

Data from Table 4.5

Terrestrially derived phenol and fatty acid abundances and TOC of core ST7



Total phenol and fatty acid sediment chemolysate abundances normalized to TOC



phenolic chemolysates of core H1 sediment decrease sharply downcore, but abundances are high throughout the core (Figure 4.51; Table 4.3). The yields of phenols in the chemolysates are 1.4 (in mg per g of sediment) of dry sediment (or 1.1 mg per 100 mg of TOC) at the surface and 0.26 mg per g of dry sediment (or 0.23 mg per 100 mg of TOC) at 30 cm. The profile of core H1 is interpreted in the context of increased anthropogenic activities following settlement of the Random Island area and surrounding communities; activities that result in the production and release of phenolic compounds that accumulate in the sediments. The activities of both the pre-European and European settlers of Hickman's Harbour have resulted in phenolic compounds being delivered to core H1 sediment. The high yields of phenols for the upper 5 cm of core H1 sediment is likely a consequence of sawmilling activities in the community of Hickman's Harbour in the early 1900's. The upper 5 cm of this core represents 66 years (1928 to 1994) of sedimentation, compared to 9 years for core H9 (1985 to 1994). (Figure 4.51) (Appendix 1.3). This time interval coincides with the settlement of Random Island by Europeans and industrial activities centred in the community of Hickman's Harbour.

There are other influences of the levels of phenols below 5 cm of core H1 sediment. The pre-European settlers of Hickman's Harbour were the native Indians. According to the Encyclopaedia of Newfoundland and Labrador, Hickman's Harbour was one of the last homes of the Beothuck Indians on the east coast of Canada (Rex Clarke); the last Beothuck died in 1829 (IBM World Book 2000). Their activities contributed to the high abundances

of phenols in the lower part of core H1. The Beothucks were hunters and did not practice agriculture. However, they made their homes using poles that were covered by birch bark. They were also nomadic in summer months, during which they built several homes (Marshall, 1985). They also used containers made of bark. Hence, the liberal use of wood and related materials by the Beothucks contributed to high amounts of lignin-derived phenols in sediments of core H1 via the main stream at Hickman's Harbour. In addition, the stream that drains the hinterland and enters Hickman's Harbour may be responsible for a high "baseline" signal of phenol abundance for core H1.

Fatty acid abundances show minor variation throughout core H1 after a sub-surface decrease. The yields of fatty acids in the chemolysates are 9.7 mg per g of dry sediment at the surface and 8.0 mg per g of dry sediment at 30 cm. Fatty acids, at both the top and 30 cm depth, comprise 7.4 mg per 100 mg of TOC in both instances. However, there are compositional changes in the fatty acid composition. The long chain to medium chain fatty acid ratio increases with depth (from 0.7 at the surface to 1.1 at 30 cm). This trend is consistent with observations of Haddad *et al.* (1991) and is related to the greater susceptibility of medium chain fatty acids and/or "packaging" of the long chain fatty acids by biochemical or pre-depositional diagenetic processes so that they become inaccessible to microbes in the sediments.

The abundances of unsaturated fatty acids decreased sharply below the surface and then

remained constant throughout the rest of the core. The reactivity of the unsaturated fatty acids may be responsible for the sharp loss in abundances of these compounds in the upper 5 cm of core H1 sediments. The steady values for the saturated to unsaturated chain fatty acid ratio below 5 cm of core H1 may be the result of the labile and reactive moieties becoming protected through various associations with mineral particles and stable organic structures. The increase in fatty acid to phenol ratios (for cores H1, H9 and ST7) with depth also exemplifies enhanced preservation of more reactive and labile sedimentary biochemicals (fatty acids).

The branched fatty acids are most abundant in core H1 sediments. The branched fatty acid fraction of the TOC of core H1 sediments also increase with sediment depth, although the fatty acid fraction of the TOC is relatively constant. Hence, bacterial activity may be responsible for removal of fatty acids from various sources accompanied by *in situ* production of the branched moieties. There are no significant trends in variation of abundances of branched fatty acids for cores H9 and ST7 sediments.

Although core H9 is also located in the Northwest Arm, its downcore profile (in the abundances and the signatures of chemolysates) is different from that of core H1. Yields of phenols increase from 0.21 mg per g of dry sediment (or 0.34 mg per 100 mg of TOC) at the surface to 0.23 mg per g of dry sediment at 5 cm (or 0.4 mg per 100 mg of TOC) (Figure 4.52). Below 5 cm, yields of phenols for the upper 20 cm are relatively constant, followed

by a decrease at 25 (dated 1922 by ^{210}Pb geochronology) and 30 cm. At 25 and 30 cm of core H9, the abundances of phenols are lowest; 0.15 mg per g of dry sediment and comprising approximately 0.25 mg per 100 mg of TOC. Variation of abundances of chemolysates in sediment correlate with variation in its fractional composition of the TOC. The decrease in phenols at the surface is interpreted in the context of reduced inputs of phenolics after intensification of wood cutting, ship building and sawmilling activities on Random Island and nearby communities, such as Clarenville, in the early 1900's. The highest yield of fatty acids occurs at 10 cm depth, dated 1970 (^{210}Pb dating) and may be related to industrial activities at Clarenville Harbour or events such as dumping of waste products from the various industries. In addition, the abundances of branched fatty acids are highest at 10 cm depth, indicating increased production from bacteria.

Microbes preferentially degrade shorter fatty acids resulting in preservation of longer chain analogs (Rashid, 1985). The long/medium chain fatty acid ratios in cores H9 and ST7 are lower than that of H1 and are generally constant downcore (Tables 4.3, 4.4 and 4.5). The increase in long/medium chain fatty acid ratios with depth in core H1 is a result of a longer sedimentation period during which microbial alteration occurred (Appendix 1.3). The long/medium chain fatty acid ratio increases from 0.7 at the surface to 1.1 at 30 cm. The long/medium chain fatty acid ratios throughout core H9 is also relatively high (between .8 and 1). However, the shorter interval of sedimentation which core H9 represents, is insufficient for noticeable preferential preservation of long chain fatty acids. Core ST7

sediments receive smaller amounts terrestrially-derived OM (which contains long chain fatty acids) than sediments of cores H1 and H9, resulting in low long/medium chain fatty acid ratios (between 0.3 and 0.5 throughout the core). Bioturbation at the site of core ST7 is important in homogenizing sediments and producing relatively constant long/medium chain fatty acid ratios throughout the core.

Fatty acids decrease from the surface to 10 cm of core H9 while the TOC tend to increase within this interval. The phenol abundances tend to increase at 5 cm followed by a decrease at 10 cm (Figure 4.52). However, the downcore (10 cm) trend of the total phenols may be de-emphasized because it approaches the limit of error of analysis. To preserve mass balance of total OM, there must be an increase in the abundance of other classes of compounds. This becomes clearer when the abundances of chemolysates are expressed as a fraction of the TOC. The phenols and fatty acids comprise a smaller fraction of the TOC at the surface and 5 cm depth. Hence, there must be an increase in the abundances of other classes of organic compounds in order to preserve mass balance. Elevated concentrations petrogenic alkanes and PAH's were measured for this section, suggesting increased inputs from anthropogenic activities (Favarro, 1997). There were several industries in the town of Clarenville that experienced rapid growth from the early 1900's. Industrial products include emulsified asphalt (also known as colas), cresote and tar. In addition, there had been shipbuilding (on a small scale) in Clarenville. These industrial activities may have contributed to anthropogenic inputs of OM to core H9. The ratios of phenols to N-compounds of

pyrolysates (Chapter 2) at the sediment surface and 30 cm are low indicating the possibility of high abundances of nitrogenous organic compounds such as proteins and amino acids (Chapter 2). Furthermore, high yields of 1,2,4-trimethoxybenzene (156 - 192 μg per g of dry sediment) were measured for the upper 10 cm of core H9, possibly indicating high abundances of carbohydrates (Table 4.4).

In the upper 5 cm of core ST7, the total fatty acid abundances increase with depth. The phenol abundance also tend to follow this trend (in the upper 5 cm) which is accompanied by a decrease in TOC with depth (Figure 4.53). The fatty acids comprise 1.4 mg per 100 mg of TOC at the surface and increases to 2.4 mg per 100 mg of TOC at 5 cm. This is accompanied by a corresponding increase in phenols from to 0.14 to 0.18 mg per 100 mg of TOC. Hence, there must be an increase in other classes of compounds in order to preserve mass balance. Sediments of core ST7 have the highest yields of petrogenic alkanes and PAH's compared to the sediment of other offshore cores (Favarro, 1997). Also, relatively high yields of 1,2,4-trimethoxybenzene were measured in the upper half of core ST7 indicative of high abundances of carbohydrates.

Below 5 cm, the phenol fraction of the TOC decreases with depth for core ST7 sediments. Generally, the fatty acid/phenol ratio increases with depth which becomes pronounced below 20 cm (Table 4.5; Figure 4.53). This supports the contention of enhanced preservation of OM through associations with resistant materials. Interactions with mineral particles may be less

important because sediments of core ST7 consisted of coarse grained mud - the interactions with mineral particles become important when grain size is small (Mayer, 1994a).

The abundances of methylated vanillin decreases downcore in both cores H1 and H9 (Tables 4.3 and 4.4), consistent with the observation of preferential degradation of aldehydes (Hedges *et al.*, 1988a; Hedges and Weliky, 1989). Methylated vanillin is not detected in the chemolysates of most of the samples. Methylated vanillin is present in the upper 10 cm of core H9 sediments (Table 4.4). The absence of methylated vanillin indicates that β-O-4 bonds with adjacent hydroxy (-OH) groups are absent and are readily degraded in sediments. Absence of methylated vanillin also indicates that it is not present in the free form or bound to fatty acids by ester bonds.

Generally, the abundance of 1,2,4-trimethoxybenzene increases with depth for core H1 and decreases with depth for cores ST7 and H9 (Tables 4.3 to 4.5). The abundances of 1,2,4-trimethoxybenzene at the surface and 30 cm for core ST7 are 78 and 8 μg per g of dry sediment. Abundances of trimethoxybenzene for core H9 are 156 and 59 μg per g of dry sediment for surface and 30 cm sediments, respectively. The yields of 1,2,4-trimethoxybenzene increase with depth for core H1, reaching a maximum abundance of 324 μg per g of dry sediment at 20 cm. The downcore increase in abundances of 1,2,4-trimethoxybenzene may be indicative of an increase in abundances in some classes of carbohydrate compounds with depth, thereby preserving mass balance in light of the loss of

phenols and fatty acids with depth and relative constant TOC values for core H1. However, it should be noted that an increase in yield of 1,2,4-trimethoxybenzene does not necessarily increase in total carbohydrates because of variable yields of 1,2,4-trimethoxybenzene from different carbohydrate compounds.

4.3.6 Comparison of annual "fluxes"

Sediments with higher TOC do not necessarily have higher fluxes of OM. This is apparent when the OM of cores H1 and H9 are normalized on a per year basis. The upper 2 cm of sediment for cores H1 (TOC=13%) and H9 (TOC=6.1%), represents 16 and 3 years of sedimentation, respectively (Figures 4.51 and 4.52) (Appendix 1.3). The annually preserved flux of TOC contributed to the upper 2 cm of sediments at these sites can thus be calculated:

Preserved flux of organics for core H1 = 0.8 percent OM per year.

Preserved flux of organics for core H9 = 2.0 percent OM per year.

The results indicate that the amount of OM that is preserved in the sediments at H9 exceeds that at H1. The higher sedimentation rate at H9 simply results in greater degree of dilution of OM.

It should, of course be noted that what is really measured is the amount of OM preserved on an annual basis. Therefore, the estimate of relative "flux" assumes a similar degree of preservation at both sites (H9 and H1). This assumption is supported by very similar

$(Ad/Al)_{V\text{-}TMAH}$ at both sites (5.5 and 5.1 for cores H9 and H1, respectively) (Table 4.2). As indicated in Chapter 1, $(Ad/Al)_{V\text{-}TMAH}$ ratio is a sensitive indicator of diagenetic alteration of sedimentary lignin (del Rio *et al.*, 1998; Hedges and Weliky, 1989; Meyers and Ishiwatari, 1993).

Similar calculations indicate that the quality of OM with respect to TMAH chemolysate yields of phenols and fatty acids preserved in the sediment at these sites is also different. TMAH yields of phenols for cores H1 and H9 are 1.4 and 0.21 mg per gram of sediment. Fatty acid yields for cores H1 and H9 are 9.7 and 3.1 mg per gram of sediment. Therefore

Preserved flux of phenols for core H1 = 0.09 mg per g of sediment per year.

Preserved flux of phenols for core H9 = 0.07 mg per g of sediment per year.

Preserved flux of fatty acids for core H1 = 0.62 mg per g of sediment per year.

Preserved flux of fatty acids for core H9 = 1.03 mg per g of sediment per year.

The higher flux of phenols (mg per g of sediment) in H1 is consistent with the observation of extensive logging, sawmilling and agricultural activities in the area of the community of Hickman's Harbour for the past century. Core H9 is located in the vicinity of Clarenville Harbour. Larger amounts of fatty acids are preserved at the site of core H9 because of multiple sources including inputs from marine and terrestrial productivity and industrial activities of Clarenville.

4.4 SUMMARY

1. The technique of batch-wise TMAH thermochemolysis is a rapid and straight forward method of identifying chemical markers of terrestrial origin in the marine environment. OM may have acidic and phenolic functional groups that require derivatization for GC analysis. The method of TMAH thermochemolysis is effective and suitable for this purpose.

2. The presence of carbohydrates is indicated by the presence of 1,2,4-trimethoxybenzene. However, the disparate yields of 1,2,4-trimethoxybenzene from various carbohydrates does not facilitate quantitative estimation of total carbohydrate contribution. Further work on TMAH thermochemolysis of carbohydrate compositional analysis of SOM will be performed.

3. Fatty acids/phenol ratios are generally higher for offshore cores. These ratios are lowest for core H1 sediments because of multiple source of phenols, including logging and sawmilling in the community of Hickman's Harbour.

4. Chemolysate yields constitute a minor portion of the TOC. Fatty acids are up to 7.4 mg per 100 mg of TOC, while phenols are approximately 1.4 mg per 100 mg of TOC. These values are for core H1 which has the highest chemolysate yields of all

cores.

5. The most abundant phenol in sediment of Northwest Arm is vanillic acid methyl ester. In sediment of the offshore sites, coumaric acid methyl ester becomes quantitatively more important than vanillic acid methyl ester.. This is not in line with the stability series established by Hedges *et al.* (1985) and indicates preferential preservation by mechanisms such as mineral associations.

6. The signature of the chemolysates in the sedimentary record is interpreted in context of the historical activities of the study area. Chemolysates of core H1 and H9 reflect the activities of the community of Hickman's Harbour and Clarenville over the past century.

7. Pre-settlement signatures of fatty acids and phenols in the SOM indicate high abundances for core H1. This may be a consequence of inputs by natural processes such as stream discharge into the harbour.

8. Generally, the sediments of Trinity Bay contain lesser amounts of OM (TOC), fatty acids and terrestrially derived phenols than sediment of Northwest Arm. Hence, there is an important terrestrial carbon input near-shore.

9. The TOC, fatty acids and phenols of sediments do not necessarily reflect the amounts of these materials preserved at these sites as determined by calculating the preserved annual fluxes. For example, although sediments of core H1 (located next to Hickman's Harbour) gave the highest yields of fatty acids in the study area, larger quantities of fatty acids are preserved at the site of core H9 on an annual basis. The OM of core H9 were diluted as a result of higher sedimentation rate at this site.

CHAPTER 5 - COMPOUND SPECIFIC ISOTOPIC ANALYSIS ($\delta^{13}C$) OF CHEMOLYSATES OF MODEL COMPOUNDS IN A SEDIMENT MATRIX

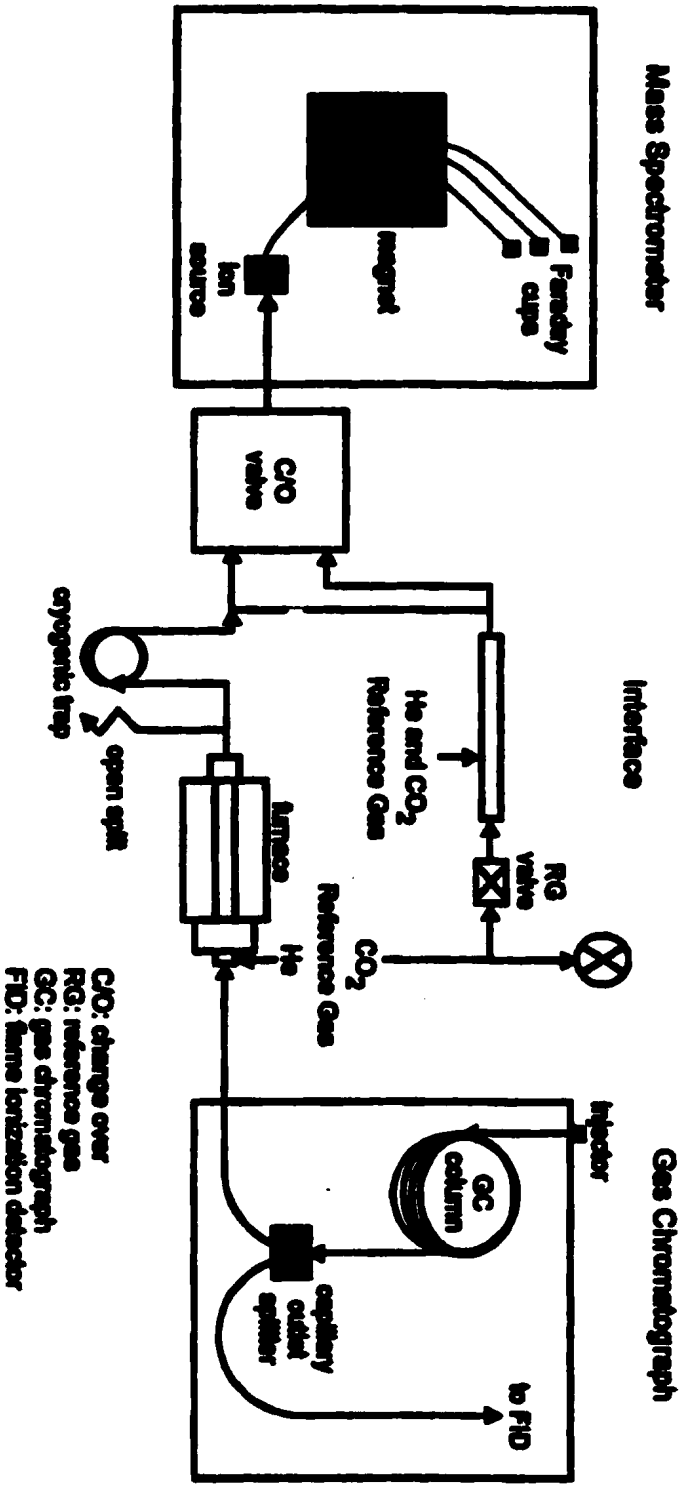
5.1 INTRODUCTION

Carbon has two stable isotopes, ^{12}C and ^{13}C that have abundances of 98.9 and 1.1%, respectively (Boutton, 1991a; Hoefs, 1997). The isotopic signatures of biochemical compounds of an organism can be preserved after death. In addition, changes in isotopic composition of geochemicals (biochemicals in the sedimentary record) may yield valuable insights into post-depositional processes affecting OM. Delineation of sources and diagenetic history of OM is enhanced by more detailed chemical/isotopic characterization of the OM. The $\delta^{13}C_{TOC}$ (or bulk carbon isotopic composition) of the sediments and biomass are composite values that depend on the relative amounts and isotopic composition of multiple components. In the case of sediments, the $\delta^{13}C_{TOC}$ may be influenced by post-depositional alteration, given the different reactivities of compounds with different isotopic compositions. Many studies have been done to isolate and isotopically characterize the different organic fractions. For example, Benner *et al.* (1987) established that the lignin-derived fraction of biomass and sediments was isotopically depleted relative to the $\delta^{13}C_{TOC}$. Macko *et al.* (1990 , 1991) isolated the carbohydrate content of peat samples; this fraction was hydrolyzed and individual sugars were chromatographically separated and combusted for isotopic analysis ($\delta^{13}C$) by conventional (or dual inlet) mass spectrometry.

On-line determination of isotopic composition for individual compounds by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) enabled measurement of molecular level isotopic composition of complex mixtures. A GC/C/IRMS system consists of a GC attached to a stable isotope mass spectrometer via a combustion furnace (Figure 5.1). The GC/C/IRMS technique combines the separation of individual compounds by gas chromatography, quantitative combustion of eluting compounds to CO_2 in the combustion furnace, and sequential real time isotopic analysis of the CO_2 by IRMS. A cryogenic trap is incorporated into existing instrumental designs because water and potentially other gases are produced at the combustion interface. These additional gases could compromise the actual ratios that are measured and have detrimental effects on critical components of the mass spectrometer; they are collected in the cryogenic trap and removed when the GC/C/IRMS system is not in data acquisition mode.

In some cases, it may be necessary to convert the compounds of interest into volatile derivatives in order to facilitate chromatographic separation. For instance, fatty acids are converted to fatty acid methyl esters (FAMEs) prior to GC/C/IRMS analysis. This necessitates estimation and correction for the isotopic signal of the added carbon atom/s of the derivatizing agent. Abrajano *et al.* (1994) investigated the kinetic isotope effect during fatty acid esterification and developed a simple mixing equation to correct for the contribution of the methylating agent (BF_3-methanol). The stoichiometric mass balance equation is as follows:

Figure 5.1 - **Schematic of GC/C/IRMS system**



$$\delta^{13}C_{FAME} = [X]\delta^{13}C_{FA} + [1\text{-}X]\delta^{13}C_{Methanol} \quad \text{.....(1)}$$

This enables computation of the isotopic composition of fatty acids ($\delta^{13}C_{FA}$) from the isotopic composition of the FAME ($\delta^{13}C_{FAME}$ measured by GC/C/IRMS) and isotopic composition of the methylating agent ($\delta^{13}C_{Methanol}$ measured by conventional mass spectrometry). In equation 1, X and 1 - X represents the fractional contribution of carbon atoms from the fatty acid and methanol, respectively, to the FAME. Different correction schemes have been developed for the analysis of other classes of derivatized compounds such as amino acids (e.g., Silfer *et al.*, 1991; Silfer *et al.*, 1994). Another method of correcting for added carbon is to derivatize several model compounds of known isotopic compositions into volatile compounds and analyze them by GC/C/IRMS. The isotopic composition of the derivatizing agent is then computed as a function of the difference between the measured isotopic compositions of the derivatives and that of the model compounds (Goni and Eglington, 1994). Silfer et al. (1994) analyzed derivatized amino acids (by GC/C/IRMS); the corrected $\delta^{13}C_{TOC}$ of the individual amino acids was computed subtracting the fractionation due to bond cleavage (specific for each amino acid) and performing mass balance.

Several factors limit the precision (internal) and accuracy of a GC/C/IRMS system. The amount of the individual compounds in the mixture of the sample injected will determine the size of the ion beams (Abrajano *et al.*, 1994). Concentrations of compounds that yield

quantities of CO_2 that will produce ion beams that are within the linear operating range of the mass spectrometer are desirable. It is also imperative that the degree of chromatographic separation/extent of co-elution is minimized so that peaks of one compound do not "interfere" with another (Abrajano *et al.*, 1994; Merrit *et al.*, 1994; Meir-Augenstein, 1999). Relative isotopic composition can be sensitive to the background concentrations of CO_2 as a result of background column bleed (Abrajano *et al.*, 1994; Ricci *et al.*, 1994; Meir-Augenstein, 1999). Post-acquisition reworking of data can incorporate background subtraction.

Pulchan *et al.* (1997) first reported CSIA data ($\delta^{13}C$) on TMAH chemolysates of marine sediments. One of the goals of this study was to analyze the isotopic composition of individual TMAH thermochemolysis products of sediments (phenols and fatty acids) using GC/C/IRMS (Chapter 1). It is therefore important to investigate whether isotopic fractionation occurred during thermally assisted TMAH thermochemolysis before the $\delta^{13}C$ of these products are used as proxies of the isotopic signature of their precursors. Specifically, it is necessary to investigate potential isotopic fractionation effects (if any) during bond cleavage, and subsequent derivatization of standards added to a real sediment matrix (surface core ashed at 500°C). In this study, the ester bonds are of particular interest because it is likely that in the uncharacterized fraction of the TOC, fatty acids are attached to aromatic rings by ester bonds (Schnitzer and Neyroud, 1975) and/or incorporated in lipid molecules such as triglycerides. Potential ether bond cleavage is also important because of

the presence of this bond type in the TOC of marine sediments and lignin-derived compounds. In addition, because addition of the derivative carbon atoms (from TMAH) results in analytes (esters of phenols and fatty acids) with isotopic compositions that are altered from the original compounds, it is imperative to understand how to derive the carbon isotope composition of the free phenolic and fatty acids from the measured carbon isotopic composition of the methylated products.

5.2 METHODS

5.2.1 Bulk isotopic analysis by conventional mass spectrometry

The $\delta^{13}C$ values of standards and TMAH were determined in triplicate using a dual inlet isotope ratio mass spectrometer (VG Optima). A 5 mg sample was placed in a previously combusted (500°C, 60 minutes) quartz tube to which excess ashed CuO (850 °C, 60 minutes) was added. The tube was evacuated, flame sealed and combusted in an 850 °C furnace for one hour. The CO_2 produced from combustion was cryogenically purified and trapped, and analyzed on the isotope mass spectrometer. The $^{13}C/^{12}C$ ratio of the organic carbon in the samples were reported relative to PDB standard in the per mil ($^{o}/_{oo}$) notation according to:

$$\delta^{13}C_s = 1000\,[R_s/R_r - 1] \quad \text{.....(2)}$$

where R represents the ratio $^{13}C/^{12}C$ and the subscripts *s* and *r* refer to sample and reference,

respectively.

5.2.2 CSIA by GC/C/IRMS analysis

Molecular level isotopic characterization (CSIA) of the TMAH thermochemolysis products (from standards mixed in a sediment matrix) were performed using a VG Isochrom system equipped with a Hewlett- Packard 5890 gas chromatograph (Figure 5.1). The gas chromatographic conditions were similar to those of GC/MS analysis except that samples (1 μl) were injected via a split/splitless injection port (heated at 280 ^{0}C) in splitless mode for 2 min. The individual compounds eluting from the column were combusted to CO_2 and H_2O in a furnace at 800 °C with CuO. The H_2O was then cryogenically collected in a water trap at -100 °C before the gas stream enters the mass spectrometer. For all GC/C/IRMS analyses, standardization was accomplished by comparing integrated $^{13}C/^{12}C$ ratios for each compound peak with similar ratios from pulses of reference CO_2 gas introduced before and after the sample chromatographic window. The accuracy of this procedure was tested daily by repeated analyses of a mixture of alkane standards (VG Instruments) and TMAH-derivatized standard mixture of phenolic aldehydes and acids and fatty acids as listed in Table 5.1.

During CSIA measurements of $\delta^{13}C$ values by GC-C-IRMS, m/z masses of 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{18}O^{16}O$) are determined as the individual components

Table 5.1 - $\delta^{13}C$ ($^{0}/_{00}$) **values of model compounds analyzed by conventional mass spectrometry and GC/C/IRMS** (details are in Pulchan *et al.*, 1997)

Legend for columns are as follows:

[1] $\delta^{13}C$ values of underivatized compounds obtained by dual inlet analysis

[2] Expected $\delta^{13}C$ values of derivatized compounds that are calculated using equation (3), Chapter 5

[3] $\delta^{13}C$ values of derivatized compounds obtained by GC/C/IRMS analysis

[4] Difference between [3] and [2]

$\delta^{13}C$ of TMAH = $-44.6^{0}/_{00}$

Compound	[1]$\delta^{13}C$	[2]Predicted δ^{13}	[3]Actual δ^{13}	[4] Δ
PHENOLS				
3-Methoxybenzaldehyde	-26.63	-28.6	-28.4	-0.2
Vanillic acid	-28.50	-31.7	-32.0	+0.3
Coumaric acid	-25.90	-29.3	-29.7	+0.4
Syringic acid	-40.24	-41.0	-40.6	-0.4
FATTY ACIDS				
Hexadecanoic acid	-26.64	-27.7	-27.9	+0.2
16-Methyl-heptadecanoic acid	-15.77	-17.3	-17.4	+0.1
Eicosanoic acid	-25.14	-26.1	-25.9	-0.2
Docosanoic acid	-27.78	-28.5	-28.7	+0.2
Tetracosanoic acid	-28.16	-28.8	-28.7	-0.1

Compound	[1]$\delta^{13}C$	[2]Predicted $\delta^{13}C$	[3]Actual $\delta^{13}C$	[4] Δ
Decanoic acid [5]	-30.48			
Decanoic acid [6]		-31.8	-31.5	-0.3
Decanoic acid [7]			-31.4	-0.4
Lignin Dimer	-31.63			
1,2-Dimethoxybenzene	-25.83			
1-Ethenyl-4-methoxybenzene	-36.7 [8]	-40.0	-37.6	-0.4

[5] Free acid after saponification of lipid and isolation by solvent extraction
[6] TMAH methylated decanoic acid from 0.5 mg standard
[7] TMAH methylated decanoic acid from 1.0 mg standard added to 25 mg of pre-combusted sediment

[8] Calculated (see Appendix 5.2)

elute from the chromatographic column and are combusted to CO_2. The data output from each analysis includes a mass 44 chromatographic trace, in which individual compounds eluting from the capillary column are represented as peaks whose intensities are a function of carbon concentration in the sample. This trace is broadly analogous to that of a GC/MS total ion chromatogram. In addition, a mass 45 to 44 ratio trace for each peak is simultaneously produced and displayed as a function of ($^{13}CO_2/^{12}CO_2$) owing to vapour-pressure isotope effects and the interaction with the liquid phase of the column, ^{13}C-containing molecules elute earlier than those containing ^{12}C. Thus, each peak is manifested in the m/z 45/44 ratio trace as an inflection. The software separately integrates ion currents of m/z 44, 45 and 46 and after correction for instrument offsets, background and contributions due to ^{17}O and ^{18}O, reports the $\delta^{13}C$ of each integrated peak after comparison with injections of a calibrated CO_2 standard gas. The chromatograms of all samples and standards prepared using TMAH required manual integration and background correction.

5.2.3 Analysis of triacylglycerol standard

The decanoic acid content of tricaprin was isolated by saponification of the lipid using ethanolic/10% KOH/reflux conditions followed by acidification (pH 2) of the aqueous layer and extraction of decanoic acid using ether. The ether layer was repeatedly washed with water to remove trace amounts of glycerol (the purity of decanoic acid was confirmed by GC/MS analysis). The $\delta^{13}C$ of this lipid-derived decanoic acid was measured by

conventional isotope ratio mass spectrometry. Tricaprin was also subjected to TMAH thermochemolysis and the product decanoic acid methyl ester analyzed by GC/C/IRMS. Another test, involving the spiking of a methanolic solution of tricaprin (1.0 mg) to 25 mg of ashed (550°C, 60 minutes) marine sediment, followed by TMAH-treatment.

5.3 RESULTS AND DISCUSSION

5.31 Derivatization using TMAH

The formation of methyl ether and ME (methyl ester) groups from free phenolic and carboxylic acids groups involves the addition of a methyl group from the TMAH reagent. If the methylating carbon from TMAH is isotopically identical to the parent TMAH carbons, a mass balance relationship of the form:

$$\delta^{13}C_{\text{product}} = [x]\delta^{13}C_{\text{parent}} + [1\text{-}x]\,\delta^{13}C_{\text{TMAH}} \quad \text{.....(3)}$$

can be used to estimate the carbon isotope composition of the underivatized chemical markers ($\delta^{13}C_{\text{parent}}$) from the known isotopic composition of TMAH ($\delta^{13}C_{\text{TMAH}} = -44.6\,^{0}/_{00}$) and the measured isotopic composition of the product ($\delta^{13}C_{\text{product}}$). In equation (3), x is the fractional carbon contribution of the underivatized compound to the methylated product. For example, x has the value of 16/17 and 8/10 for the methylation of hexadecanoic acid and vanillic acid, respectively (Appendix 5.1).

In this study, efforts were undertaken to determine the original (i.e., corrected for derivative carbon) isotopic composition of a selected number of representative phenolic and fatty acid TMAH methylated products likely to be present marine sediments and soil samples. Phenolic aldehydes (vanillin and syringealdehyde) phenolic acids (vanillic and coumaric acids) and saturated fatty acids (C_{16}, C_{18}, C_{20}, C_{22} and C_{24}) were TMAH chemolyzed in a sediment matrix as described previously in Chapter 3. Table 5.1 compares the $\delta^{13}C$ values predicted by equation (1) to those measured by GC/C/IRMS and conventional IRMS for a series of phenolic and fatty acid standards. The predicted and measured values are within the analytical precision of the instrument (0.4 $^{0}/_{00}$) and the calculated isotopic difference can be positive or negative. To be consistent with a kinetic isotopic effect at methyl carbon during derivatization, the measured $\delta^{13}C$ values should have been consistently lower than the predicted values. It is concluded that only minor isotopic fractionation (within the limits of the analytical precision of the instrument or internal precision) was found under the experimental conditions. In addition, derivatization of the phenolic acids involved addition of two carbon atoms, as opposed to the addition of one carbon atom for phenolic aldehydes and aliphatic fatty acids. However, systematic fractionation effects dependent on the number of substitutions were not observed.

5.32 Bond Cleavage

Experiments were performed to investigate the isotopic effects (if any) of bond cleavage during thermochemolysis of the tricaprin (by TMAH) and a lignin-derived dimer, compounds containing ester and ether chemical bonds, respectively. Decanoic acid (C_{10}) was obtained by saponification/base hydrolysis of the tricaprin (a triacylglycerol) standard. The purified decanoic acid was then combusted to produce CO_2 that was analyzed for $\delta^{13}C$ by conventional mass spectrometry. Decanoic acid was then analyzed by GC/C/IRMS as a FAME product of TMAH. The results in Table 5.1 also indicate comparable isotopic compositions of decanoic acid either from isolation via saponification or by TMAH thermochemolysis (corrected for derivatized carbon). The results support the supposition that cleavage of the ester bond by basic TMAH conditions resulted in minor isotopic fractionation (within limits of the internal precision of the instrument).

The ether bond of a lignin-type dimer (Figure 3.6), of known $\delta^{13}C$ composition, was cleaved by TMAH thermochemolysis and the chemolysates analyzed by GC/C/IRMS. The $\delta^{13}C$ composition of one of the two reactants (1,2-dimethoxybenzene) (Appendix 5.2) of the dimer was measured by conventional mass spectrometry. This enabled calculation of the $\delta^{13}C$ composition of the second reactant, 1-ethenyl-4-hydroxybenzene (without the methyl group that is taken off in the bond formation). The calculated $\delta^{13}C$ composition of 1-ethenyl-4-hydroxybenzene was comparable to the $\delta^{13}C$ composition (obtained by GC/C/IRMS) of its

methylated analog (and back-corrected for the methyl addition during TMAH thermochemolysis) (Table 5.1). Details of the calculations for the isotopic composition of the dimer and its precursors are in Appendix 5.2. The theoretical mass balance calculations indicate that one of the phenols might have retained its isotopic signature after synthesis of the dimer. It should be noted that the results of isotopic fractionation during chemolysis of the lignin dimer may be considered preliminary and warrants further investigation because of very poor yields during TMAH thermochemolysis of the dimer and poor chromatographic separation of the derivatized monomers during GC/C/IRMS.

The absence of significant fractionation during pyrolysis was attributed to isotopic effects associated with bond energies (Goni and Eglington, 1994, discussed below). It is possible that the same explanation might apply for TMAH thermochemolysis of ester bonds of tricaprin and ether bonds of the dimer; isotope effects may be minimized if the activation energies are greatly exceeded during thermochemolysis at 250 °C.

Previous studies also support the above results. Goni and Eglington (1994) investigated isotopic fractionation effects of pyrolytic cleavage of ether bonds of lignin. The $\delta^{13}C$ of the lignin-derived phenols (corrected for derivative carbon) are comparable to that of the $\delta^{13}C$ of the lignins, and lead to the conclusion that the pyrolysis products retained the isotopic signature of the parent polymer and that the $\delta^{13}C$ of the phenols produced by pyrolysis may be used to estimate the average isotopic composition of lignin macromolecules in

geochemical samples. Goni and Eglington (1994) provided evidence "that thermal dissociation of lignin macromolecules does not appear to produce any major isotopic fractionations". In addition, Eglington (1994) pyrolyzed polyethylene and analyzed the pyrolysis products (various hydrocarbons) by GC/C/IRMS. The $\delta^{13}C$ compositions of the individual pyrolysates are similar to the bulk $\delta^{13}C$ of the polyethylene and lead to the conclusion that specific pyrolysis products can be representative "isotopic markers of the parent macromolecule". Eglington (1994) also determined that the $\delta^{13}C$ compositions of pyrolysates of kerogens, which consist only of hydrocarbons, are representative of the bulk $\delta^{13}C$ composition of the kerogen i.e. there appears to be minor isotopic fractionation as a result of pyrolytic production of hydrocarbons.

5.4 SUMMARY

1 The technique of batch-wise TMAH thermochemolysis is a quick and simple method of analyzing organic compounds that have acidic and phenolic functional groups by GC/C/IRMS.

2 Correction of the isotopic compositions of TMAH chemolysates of phenolic aldehydes and acids and fatty acids follow a simple mixing relationship described by Equation 3.

3 Analysis of decanoic acid, methyl ester produced by TMAH thermochemolysis of tricaprin indicates minor isotopic fractionation as a result of bond cleavage.

4 Theoretical mass balance calculations on the isotopic $\delta^{13}C$ of a lignin dimer and its phenolic monomers indicate that one of the monomers might have retained its isotopic composition after synthesis of the dimer. However, further investigation is warranted on isotopic fractionations associated with bond cleavage (and synthesis).

CHAPTER 6 - COMPOUND SPECIFIC ISOTOPIC ANALYSIS ($\delta^{13}C$) OF CHEMOLYSATES OF MARINE SEDIMENTARY ORGANIC MATTER

6.1 INTRODUCTION

Unravelling the origins of SOM can be a formidable challenge due to its diverse and somewhat complex nature. The use of flash pyrolysis or thermochemolysis in combination with GC/MS is one way of providing useful structural information on biological markers of terrestrial flora. However, the potential may not be not fully realized because the carbon-skeleton structures of the products are not always sufficiently diagnostic to resolve individual sources. A strategy to derive additional information on biological sources for SOM is to use stable carbon isotopes (Fry and Sherr, 1984; Gearing, 1988; Macko *et al.*, 1993; Gleixner *et al.*, 1999). Organisms discriminate against ^{13}C during biosynthetic reactions. The degree of discrimination depends on the biosynthetic pathway of fixation (O'Leary, 1988; Troughton, 1979). For example, photosynthetic organisms, which often represent the base of the food chain in most ecosystems, fix CO_2 to synthesize OM with $^{13}C/^{12}C$ ratios that are substantially lower than atmospheric CO_2. Typical average $\delta^{13}C$ values for C_3 photosynthesis, which incorporate CO_2 through a three carbon organic intermediate, include $-27^{o}/_{oo}$ for terrestrial plants and $-21^{o}/_{oo}$ for marine plants (Boutton, 1991b). These characteristic isotopic signatures may be preserved after death and decomposition and thus can be utilized to distinguish and quantify the biological sources of OM to sedimentary environments (Boutton, 1991a and b;

Dienes, 1980; Fry and Sherr, 1984; Gearing, 1988; Tiezen and Boutton, 1989; Schulten and Gleixner, 1999; Gleixner and Schmidt, 1998).

It has been well established that the isotopic signatures of various plant biochemicals are different (Galimov, 1985). The $\delta^{13}C$ of sedimentary lipids, including fatty acids can differ from the $\delta^{13}C_{TOC}$ (Van der Meer *et al.*, 1998; Duan *et al.*, 1997). Revill *et al.* (1999) measured $\delta^{13}C_{Fatty\ Acids}$ depletion of 2.3 to 4.1‰ from the $\delta^{13}C_{TOC}$ for *Emiliania huxleyi* (a phytoplankton). Collister *et al.* (1994) determined that the total lipid extracts for plants were depleted in ^{13}C relative to bulk tissue by 3.0 to 7.3‰. Benner *et al.* (1987) established that the lignin derived fraction was isotopically depleted relative to the $\delta^{13}C_{TOC}$ by 2 to 6‰. In order to conserve mass balance, other biochemicals have to be isotopically enriched in ^{13}C, and these include amino acids and carbohydrates. It has also been demonstrated that reduced C-H-bonded positions are systematically depleted in ^{13}C and oxidised C-O-bonded positions are enriched in ^{13}C within organic molecules (Hoefs, 1997). Current emphasis is now directed at the isotopic compositions of individual compounds (CSIA). This will most likely give definitive insights into sources and processes affecting SOM.

During the past few years, several classes of compounds comprising SOM were analyzed by GC/C/IRMS, including alkanes (Bakel *et al.*, 1994; Freedman *et al.*, 1988), polycyclic aromatic hydrocarbons (O'Malley *et al.*, 1994), amino acids (Silfer *et al.*, 1991 and 1994) and fatty acids (Abrajano *et al.*, 1993 and 1994; Collister *et al.*, 1994; Litchfouse *et al.*,

1995a, 1995b and 1995c). Alkanes are perhaps the most commonly isotopically characterized class of compounds. Bakel *et al.* (1994) analyzed alkanes from samples of POM and Holocene marine sediment by GC/C/IRMS; they determined that the $\delta^{13}C$ of the n-alkanes of from the samples ranged between -26.0 and -33.0‰. However, several alkanes from the Holocene sediments that Bakel *et al.* (1994) analyzed, are isotopically depleted in comparison to those of POM. Bakel *et al.* (1994) also determined that the $\delta^{13}C$ of individual n-alkanes of POM from low productivity periods are isotopically enriched by about 1‰ over their counterparts from high productivity periods. High productivity results in a shortage of cellular CO_2, which reduces the availability and discriminatory utilization of ^{12}C. Hence, the biochemicals that are synthesized by primary producers during high productivity periods are isotopically heavier than those of low productivity periods.

Collister *et al.* (1994) measured $\delta^{13}C$ of individual n-alkanes of C_3, C_4 and CAM plants to be -30.7‰ (C_{23}) to -38.4‰ (C_{33}), -18.0‰ (C_{26}) to -25.8‰ (C_{33}), and -23.0‰ (C_{21}) to -29.2‰ (C_{34}), respectively. They also demonstrated a saw-tooth pattern of isotopic composition verses carbon number, in which the even carbon number alkanes were enriched in ^{13}C compared to odd carbon number alkanes. Ishiwitari *et al.* (1994) demonstrated a similar saw-tooth pattern for n-alkanes of Holocene sediments of Tokyo Harbour that became pronounced with depth. The saw-tooth distribution of isotopes was a result of multiple sources of hydrocarbons with different molecular and isotopic signatures in Tokyo Harbour including polluted oils and indiginous plant materials (Ishiwatari *et al.*, 1994). The

polluted oils have low OEP or mostly even carbon number, and are relatively enriched in ^{13}C. The indiginous plant materials that produce hydrocarbons in the Tokyo Harbour sediments are charcaterized by higher OEP and relatively depleted $\delta^{13}C$ values for n-alkanes and n-alkanes. A saw-tooth pattern ($\delta^{13}C$ verses carbon number) was also observed for soil fatty acids by Litchfouse *et al.* (1995a and 1995b) and Litchfouse and Collister, (1992). Fatty acids having even number carbon atoms were also isotopically heavier (Appendix 6.1).

Goñi and Eglinton (1994) analyzed kerogens and kerogen precursors by "off-line" pyrolysis in combination with GC/C/IRMS in which the phenolic pyrolysis products were isolated and subsequently analyzed as their trimethylsilyl derivative. They established that the isotopic composition of the pyrolysis products are representative of the parent macromolecule. For phenolic products, it was found that thermal dissociation of lignin macromolecules does not appear to produce any major isotopic fractionation. The $\delta^{13}C$ compositions of phenols for *Poplar* lignin and *Ginkgo* wood (with $\delta^{13}C_{TOC}$ values of -27.4 and -25.8‰, respectively) ranged between -33.3 to -25.6‰ and -31.7 to -23.8‰, respectively. Most of the phenols were depleted in the heavy isotope relative to the bulk composition.

Goñi and Eglinton (1996) also demonstrated that there are no major isotopic fractionation effects associated with oxidative hydrolysis of lignin polymers during CuO oxidation. In the study, they also measured the $\delta^{13}C$ of CuO oxidation products from purified lignins and determined that overall, the $\delta^{13}C$ compositions of major lignin products are within 1‰ of

the $\delta^{13}C_{Lignin}$. They also measured variations in individual phenols of each lignin. The acids (vanillic and syringic acids) were 1 to 3‰ more negative (isotopically lighter) than the aldehyde and ketone counterparts. The cinnamyl alcohols (coumaric and ferrulic acids) were consistently heavier than the rest of the phenols, and in some cases, heavier than the corresponding $\delta^{13}C_{lignin}$.

The technique of pyrolysis in conjunction with GC/C/IRMS is relatively new. To date, few molecular-level isotopic studies have reported on the isotopic characterization of pyrolysates (and none on TMAH thermochemolysis products). Early attempts to isotopically characterize individual pyrolysates were reported by Eglington (1994) and Goni and Eglington (1996). More recently, CSIA of pyrolysates were performed by Schulten and Gleixner (1999), Kracht and Gleixner (2000) and Gleixner *et al.* (1999); these studies clearly demonstrate the potential of the combination of structural and isotopic characterization of OM in investigating product/precursor relationships for biogeochemical processes. CSIA on chemolysates of TOC of sediments is a very efficient and cost effective method of obtaining molecular level isotopic data on fatty acids and phenols. This data is also be a valuable supplement to that obtained on molecular characterization (Chapter 4) and, in conjunction, will provide valuable information on the sources and fates of OM to the sediments of Northwest Arm and Trinity Bay.

6.2 METHODS

Purified CO_2 that was used to estimate the TOC of sediments (acidified with 30% HCl in order to remove carbonates), was analyzed for $\delta^{13}C_{TOC}$ by a mass spectrometer (VG Optima) in dual inlet mode of operation. The chemolysates that were previously analyzed by GC/MS were stored frozen and later analyzed by GC/C/IRMS for CSIA measurements as described in Chapter 5. The scheme used for computing the isotopic compositions of the underivatized parent compounds have been also previously described. The $^{13}C/^{12}C$ ratios are reported relative to PDB standard in per mil ($^{o}/_{oo}$) notation according to:

$$\delta^{13}C_s = 1000\,[R_s/R_r - 1] \quad \text{.....(2)}$$

where R represents the ratio $^{13}C/^{12}C$ and the subscripts *s* and *r* refer to chemolysates sample and reference, respectively.

6.3 RESULTS AND DISCUSSION

6.31 General observations

It was found that the $\delta^{13}C_{TOC}$ values of Northwest Arm sediment are isotopically lighter than those of Trinity Bay, with core H1 having the most depleted value (-24.3$^{o}/_{oo}$). Northwest Arm receives more terrestrially-derived sediment (isotopically lighter) than Trinity Bay

(Appendix 6.2). In order to observe the $\delta^{13}C$ variability within a sample (internal precision), duplicate analyses of selected core samples were performed; two duplicate analyses in a spatial variation study (H5 and ST11) and five duplicate analyses among temporal core studies. The variability of isotopic compositions for duplicate analyses of TMAH sediment chemolysates are higher than those observed for model compounds (chemolyzed in a sediment matrix), as high as 1.3‰ in some cases. This may be a consequence of a "size" effect of chemolysate yield of compounds observed. Generally, reproducibility was much better for yields with chromatographic peaks of ion intensities that are within the linear range of the isotope ratio mass spectrometer, that is between 5 and 8 E-9 A ion intensity for the major beam (m/z = 44). Smaller peak yields are more strongly affected by background corrections and instrument noise. The chromatograms of the sediment chemolysates are much more complex than those of standard chemolysates and this necessitates greater corrections.

6.3.2 Spatial variation

The $\delta^{13}C$ composition of 1,2,4-trimethoxybenzene showed the most depleted values ranging from -34.3 (ST7) to -41.3‰ (H5) (Table 6.1). These are uncorrected values because of the uncertainty in estimating the contribution of carbon atoms from the carbohydrate precursor/s (Chapters 3 and 4). Assuming that 3-methoxy groups are derived from TMAH and the remaining six carbon atoms in each molecule are from the parent carbohydrate (see "peeling"

Table 6.1 - **$\delta^{13}C$ of sediment chemolysates as determined by GC/C/IRMS**

Duplicate analyses (of same sample) are reported as mean values (n=2) and range. Standard deviation results from internal precision (machine error and error due to variations in amount of sample injected). All other data are single analyses. The $\delta^{13}C$ of 1,2,4-trimethoxybenzene are uncorrected and each is a composite value that is influenced by the $\delta^{13}C$ of its precursor/s and reactant TMAH. The $\delta^{13}C$ of all other chemolysates were determined as methyl esters and ethers and their $\delta^{13}C$ are corrected for the methoxy groups derived from TMAH using Equation (3) (Chapter 5).

Data plotted in Figures 6.1 - 6.5.

$\delta^{13}C$ of chemolysates of surface sediment of Northwest Arm cores (Figure 6.4)

Chemolysate	H8	H7	H6	H5
1,2,4-Trimethoxybenzene	-38.3	-40.1	-40.3	-41.3 ± 1.1
Vanillin	-30.8			
Vanillic acid	-33.0	-32.4	-33.4	-33.9 ± 0.7
Coumaric acid	-23.7	-23.9	-23.4	-23.9 ± 1.2
12-Methyl-tridecanioc acid	-29.6	-28.2	-28.3	-30.2 ± 1.0
Pentadecanoic acid	-26	-26.2	-26.6	-29.1 ± 0.9
Hexadecanoic acid	-27.1	-26.9	-27.6	-28.8 ± 0.4
16-Methyl-hexadecanoic acid	-28.5	-26.2	-27.7	-29.5 ± 0.7
Eicosanoic acid	-26.3	-27.8	-27.2	-30.4 ± 0.6
Heneicosanoic acid	-33.6	-31.7	-32.4	-33.7 ± 1.2
Docosanoic acid	-28.4	-30.2	-27.1	-26.8 ± 0.8
Tricosacoic acid	-32.5	-32.8	-32.0	-32.7 ± 1.3
Tetracosanoic acid	-26.6	-28.1	-27.2	-26.9 ± 1.0

$\delta^{13}C$ of chemolysates of surface sediment of Trinity Bay cores (Figure 6.5)

Chemolysate	ST9	ST10	ST11
1,2,4-Trimethoxybenzene	-36.2	-35.6	-36.2 ± 1.2
Vanillin	-30.3		-30.3 ± 0.6
Vanillic acid	-33.0	-32.0	-33.0 ± 0.7
Coumaric acid	-25.6	-24.6	-25.6 ± 0.6
12-Methyl-tridecanioc acid	-29.1	-28.3	-29.1 ± 0.5
Pentadecanoic acid	-28.7	-28.0	-28.7 ± 0.6
Hexadecanoic acid	-28.1	-27.8	-28.1 ± 0.4
16-Methyl-hexadecanoic acid	-28.1	-27.5	-28.1 ± 0.5
Eicosanoic acid	-30.1	-28.7	-30.1 ± 0.5
Heneicosanoic acid	-31.1	-29.4	-31.1 ± 1.1
Docosanoic acid	-27.2	-27.6	-26.7 ± 0.6
Tricosacoic acid	-28.1	-28.3	-27.5 ± 1.2
Tetracosanoic acid	-26.1	-26.2	-24.6 ± 1.1

$\delta^{13}C$ of sediment chemolysates of core H1 (Figure 6.1)

Chemolysate	0-2 cm	8-10 cm	18-20 cm	28-30 cm
1,2,4-Trimethoxybenzene	-40.6 ± 1.1	-38.2	-40.3 ± 0.9	-38.1
Vanillin	-32.5 ± 0.5			
Vanillic acid	-34.7 ± 0.5	-31.4	-32.7 ± 0.6	-31.7
Coumaric acid	-19.7 ± 0.5	-19.6	-19.2 ± 0.7	-21.9
12-Methyl-tridecanioc acid	-29.7 ± 0.7	-28.0	-29.8 ± 0.7	-28.2
Pentadecanoic acid	-29.8 ± 0.4	-24.7	-25.6 ± 0.7	-23.9
Hexadecanoic acid	-25.3 ± 0.3	-22.4	-24.1 ± 0.5	-23.4
16-Methyl-hexadecanoic acid	-26.8 ± 0.6	-25.6	-23.2 ± 0.4	-25.7
Eicosanoic acid	-28.6 ± 0.5	-23.9	-23.8 ± 0.8	-24.0
Heneicosanoic acid	-32.1 ± 0.5	-29.5	-30.9 ± 0.9	-30.0
Docosanoic acid	-27.9 ± 0.8	-23.5	-23.0 ± 0.8	-23.2
Tricosacoic acid	-31.3 ± 1.1	-29.9	-28.5 ± 1.2	-28.0
Tetracosanoic acid	-25.4 ± 0.9	-21.2	-21.3 ± 1.1	-20.0

$\delta^{13}C$ of sediment chemolysates of core H9 (Figure 6.2)

Chemolysate	0-2 cm	8-10 cm	18-20 cm	28-30 cm
1,2,4-Trimethoxybenzene	-38.8 ± 1.0	-36.7	-36.6 ± 1.2	-34.7
Vanillin	-33.1 ± 1.0			
Vanillic acid	-34.4 ± 0.6	-33.2	-34.2 ± 0.8	-32.4
Coumaric acid	-21.1 ± 1.2	-22.5	-20.1 ± 1.1	-20.0
12-Methyl-tridecanioc acid	-28.3 ± 0.6	-28.9	-28.7 ± 0.9	-27.1
Pentadecanoic acid	-27.5 ± 0.7	-28.0	-28.8 ± 0.7	-27.0
Hexadecanoic acid	-25.4 ± 0.5	-24.4	-24.3 ± 0.3	-24.1
16-Methyl-hexadecanoic acid	-24.5 ± 0.4	-22.6	-23.2 ± 0.4	-22.0
Eicosanoic acid	-25.0 ± 0.5	-22.6	-23.3 ± 0.6	-23.4
Heneicosanoic acid	-32.7 ± 1.0	-31.5	-32.4 ± 0.9	-30.1
Docosanoic acid	-24.9 ± 0.4	-21.4	-22.4 ± 0.5	-21.9
Tricosacoic acid	-31.5 ± 1.4	-29.8	-26.9 ± 1.2	-27.3
Tetracosanoic acid	-25.2 ± 0.8	-20.9	-20.6 ± 0.9	-20.3

$\delta^{13}C$ of sediment chemolysates of core ST7 (Figure 6.3)

Chemolysate	0-2 cm	8-10 cm	18-20 cm	28-30 cm
1,2,4-Trimethoxybenzene	-34.3 ± 1.3			
Vanillic acid	-32.9 ± 0.7		-33.1	
Coumaric acid	-27.9 ± 0.4	-24.5	-25.5	-24.3
12-Methyl-tridecanioc acid	-31.0 ± 0.9	-28.4	-27.6	-27.0
Pentadecanoic acid	-31.7 ± 0.3	-27.6	-26.1	-26.4
Hexadecanoic acid	-28.3 ± 0.5	-28.2	-27.9	-27.9
16-Methyl-hexadecanoic acid	-28.7 ± 0.4	-29.4	-28.0	-28.5
Eicosanoic acid	-28.9 ± 0.6	-27.9		-28.4
Heneicosanoic acid	-27.0 ± 0.5			-25.8
Docosanoic acid	-29.8 ± 0.5			-27.7
Tricosacoic acid	-31.9 ± 0.7			-29.7
Tetracosanoic acid, ME	-27.5 ± 1.3			-26.7

$\delta^{13}C$ of chemolysate of miscellaneous samples

Chemolysate	TOPSOIL	SOIL - 5 cm
1,2,4-Trimethoxybenzene	-38.9	-38.3
Vanillic acid	-32.5	-34.1
Coumaric acid	-26.6	-31.6
12-Methyl-tridecanioc acid	-34.7	-34.2
Pentadecanoic acid	-31.7	-35.5
Hexadecanoic acid	-33.3	-32.1
16-Methyl-hexadecanoic acid	-28.5	-29.2
Eicosanoic acid	-30.6	-30.2
Heneicosanoic acid	-33.2	-34.9
Docosanoic acid	-30.4	-30.4
Tricosacoic acid	-34.6	-34.4
Tetracosanoic acid	-30.9	

mechanism proposed by Fabbri and Helleur, 1999), the $\delta^{13}C$ compositions of its precursors would fall between -34 and -39‰. These "corrected" $\delta^{13}C$ values do not reflect $\delta^{13}C$ compositions of carbohydrates of marine plankton origin (pectins -16.7‰, hemicellulose -18.7‰ and cellulose -22.4‰) and terrestrial plants (hemicellulose -24.3‰ and cellulose -23.8‰ for *Pinus elliotii*) (Appendix 6.1). It is possible more than three carbon atoms of 1,2,4-trimethoxybenzene are derived from TMAH whereby mass balance calculations would yield heavier $\delta^{13}C$ compositions of precursors of 1,2,4-trimethoxybenzene - $\delta^{13}C$ values that are closer to those of marine carbohydrates. Nevertheless, $\delta^{13}C$ composition of 1,2,4-trimethoxybenzene indicate that precursors of this compound in the offshore locations are isotopically enriched in ^{13}C than those of the near-shore cores. Interestingly, the difference in $\delta^{13}C$ compositions of 1,2,4-trimethoxybenzene of offshore and near-shore cores (approximately 5‰) approaches the difference in $\delta^{13}C$ compositions of marine and terrestrial carbohydrates. In addition, it should be noted that the $\delta^{13}C$ composition of 1,2,4-trimethoxybenzene is a composite number because it is derived from the $\delta^{13}C$ compositions of several carbohydrate precursors.

Generally, the $\delta^{13}C$ compositions of the phenols are isotopically lighter than the fatty acids, with the exception of $\delta^{13}C$ values of coumaric acid units (Table 6.1 and Figures 6.1 - 6.5). The $\delta^{13}C$ values for vanillic acid and its aldehyde vary between -34.7 to -32.0‰ and -33.1 to -30.3‰, respectively. Generally, the $\delta^{13}C$ composition of vanillin units is slightly heavier than that of vanillic acid units. This trend is consistent with $\delta^{13}C$ values for these phenols in

Figures 6.1 to 6.5 - **Molecular isotopic signature ($\delta^{13}C$) of chemolysates of sediment cores**

Abbreviations for chemolysates on the x axis are as follows:

Lignin-derived phenols
VAL - 3,4-Dimethoxybenzaldehyde
VAD - 3,4-Dimethoxybenzoic acid
CAD - 3-(4-Methoxyphenyl)acrylic acid

Fatty acids
C14 - 12-Methyl-tridecanioc acid
C15 - Pentadecanoic acid
C16 - Hexadecanoic acid
C18 - 16-Methyl-heptadecanoic acid
C20 - Eicosanoic acid
C21 - Heneicosanoic acid
C22 - Docosanoic acid
C23 - Tricosacoic acid
C24 - Tetracosanoic acid

Figure 6.1 - **Molecular isotopic signature ($\delta^{13}C$) of sediment chemolysates of core H1**
Data used for figures is in Table 6.1

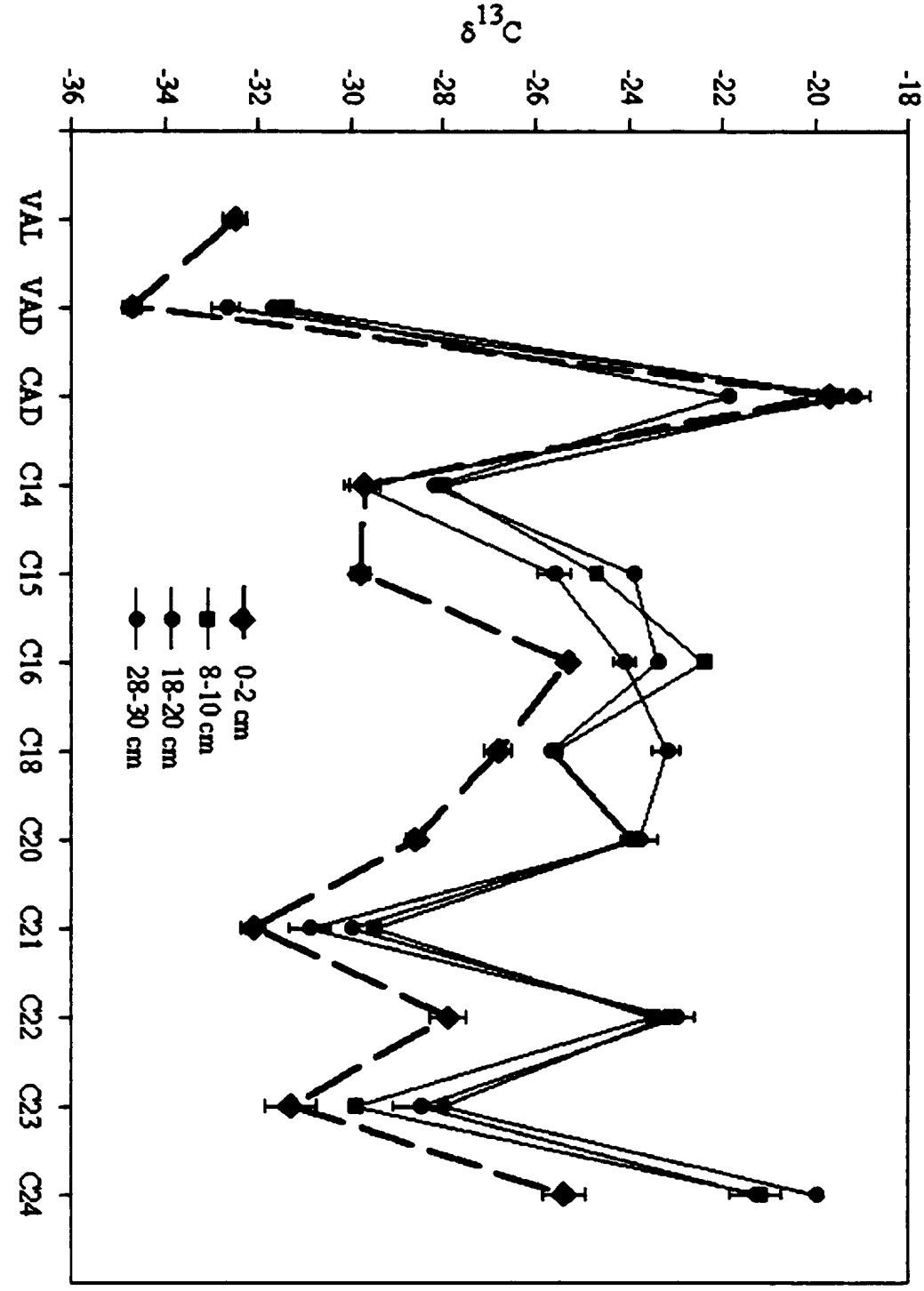


Figure 6.2 - **Molecular isotopic signature ($\delta^{13}C$) of sediment chemolysates of core H9.** Data used for figures is in Table 6.1

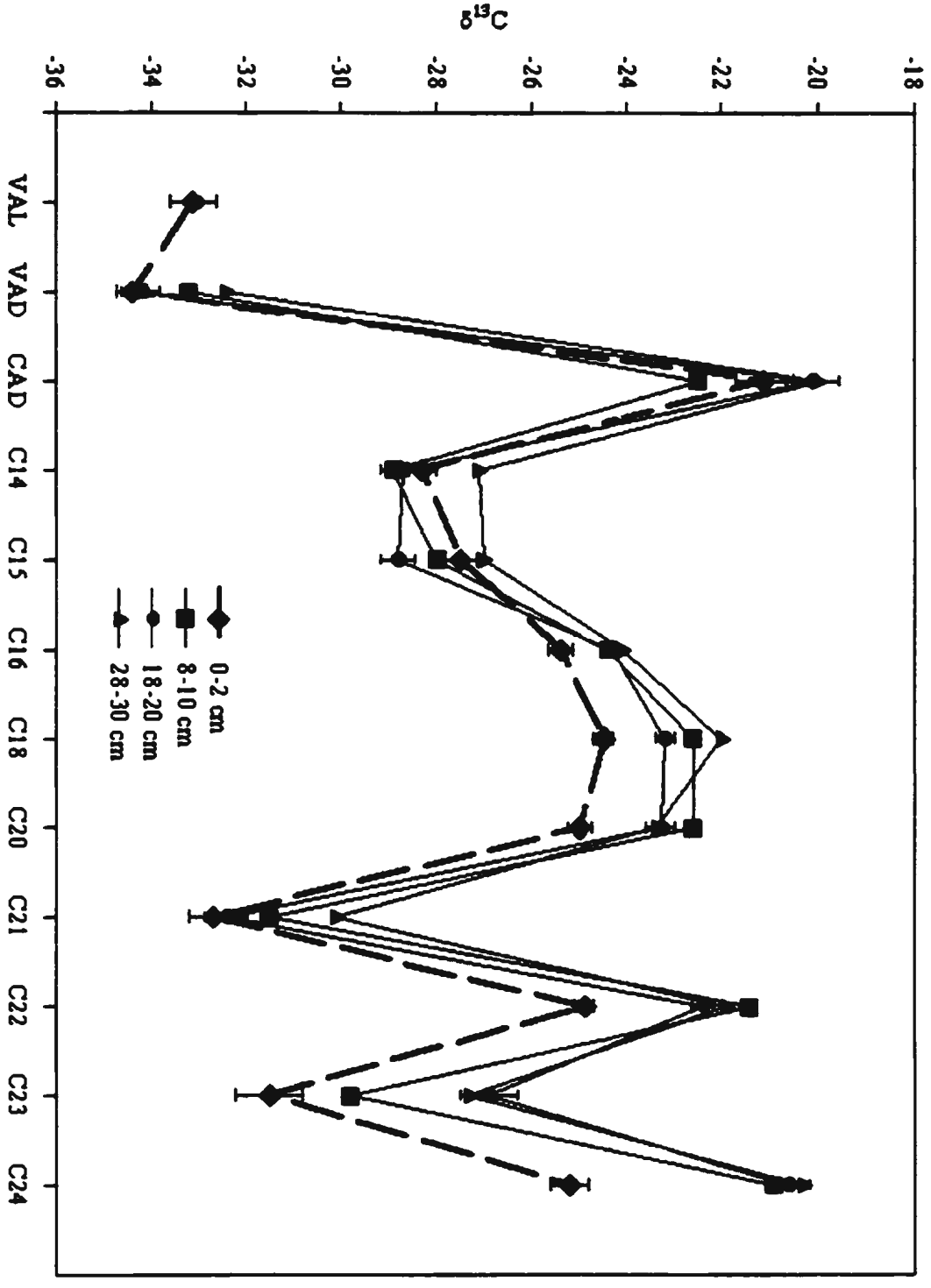


Figure 6.3 - Molecular isotopic signature ($\delta^{13}C$) of sediment chemolysates of core ST7
Data used for figures is in Table 6.1

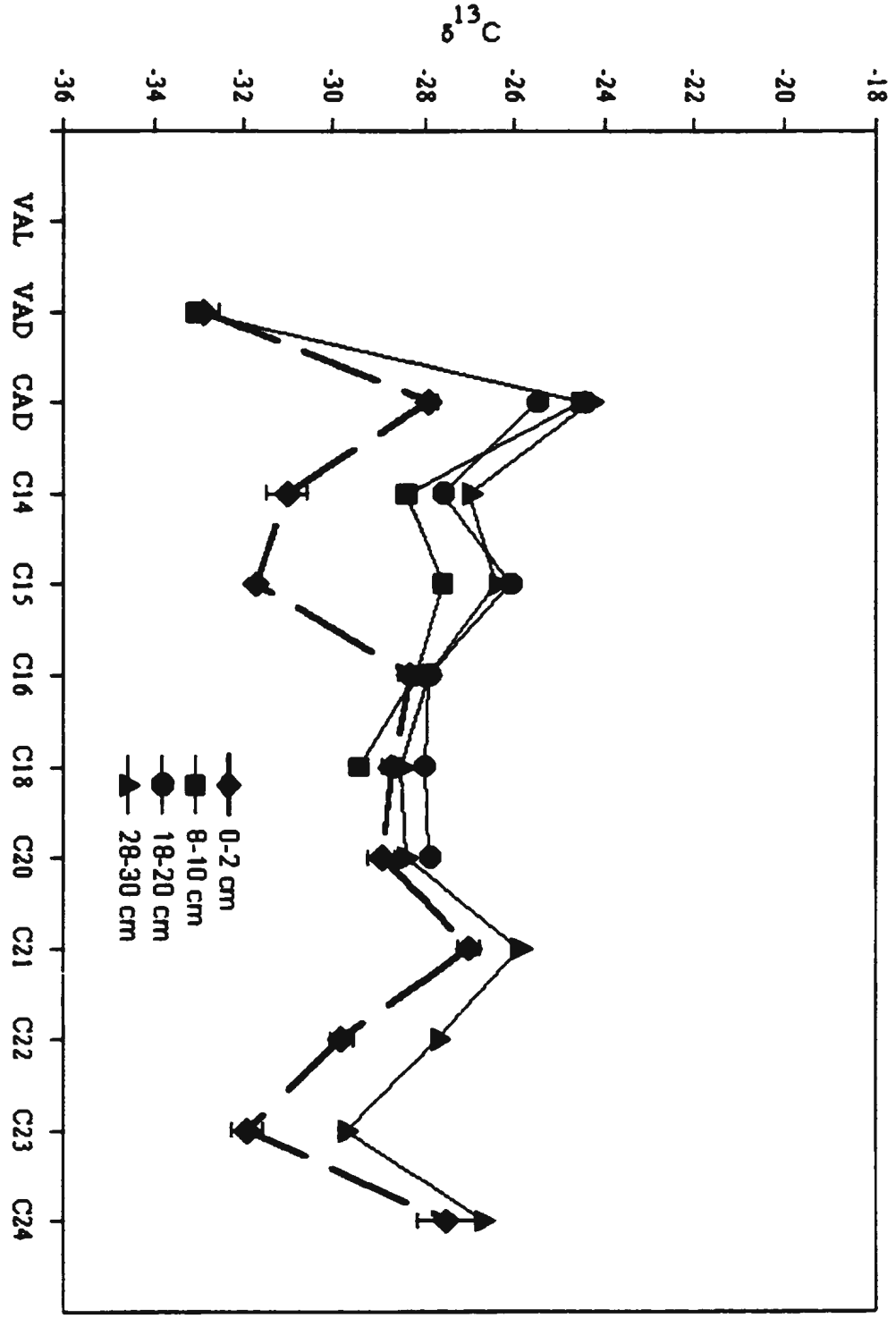


Figure 6.4 - **Molecular isotopic signature ($\delta^{13}C$) of sediment chemolysates of Northwest Arm (surface sediments).** Data used for figures is in Table 6.1

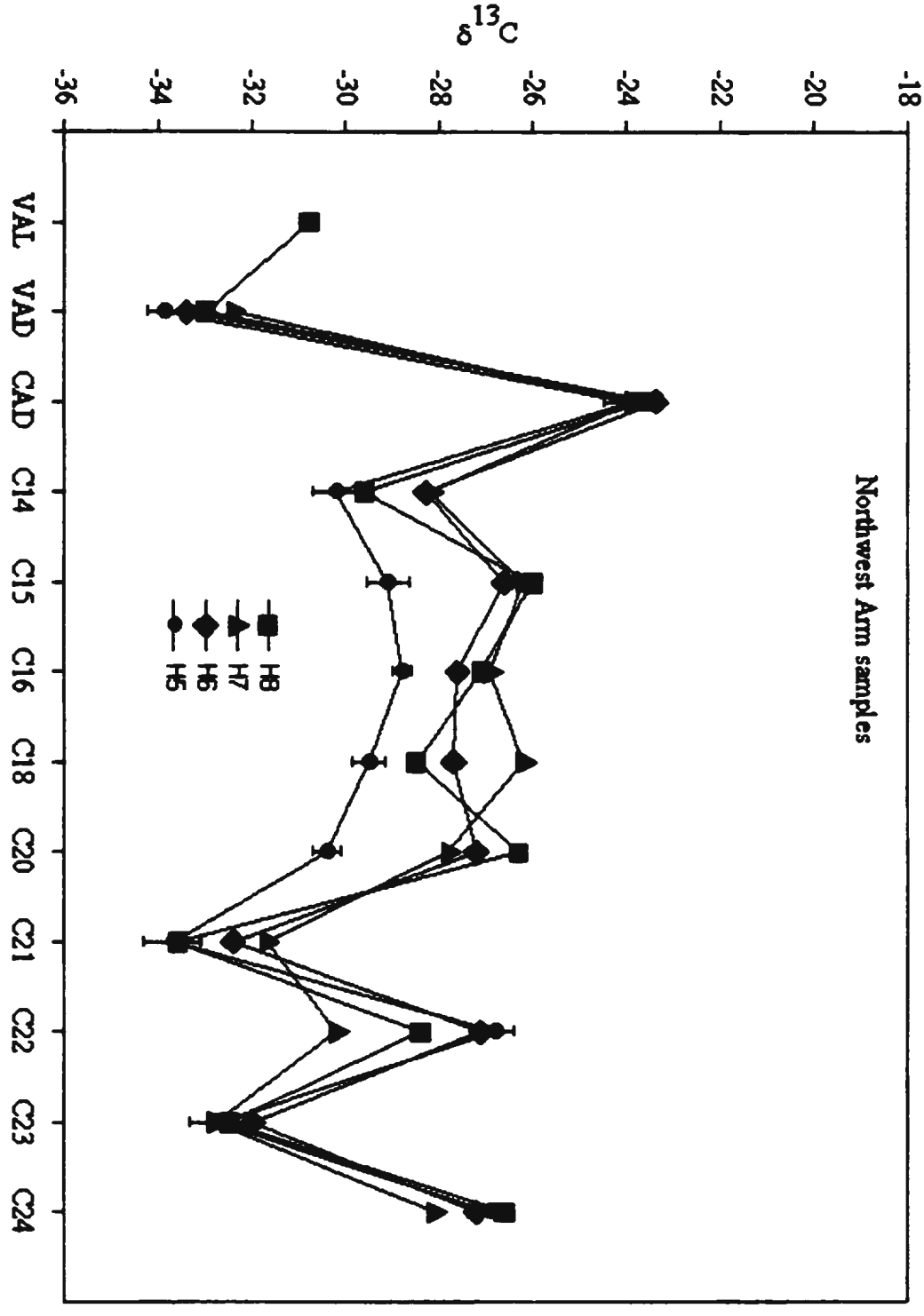
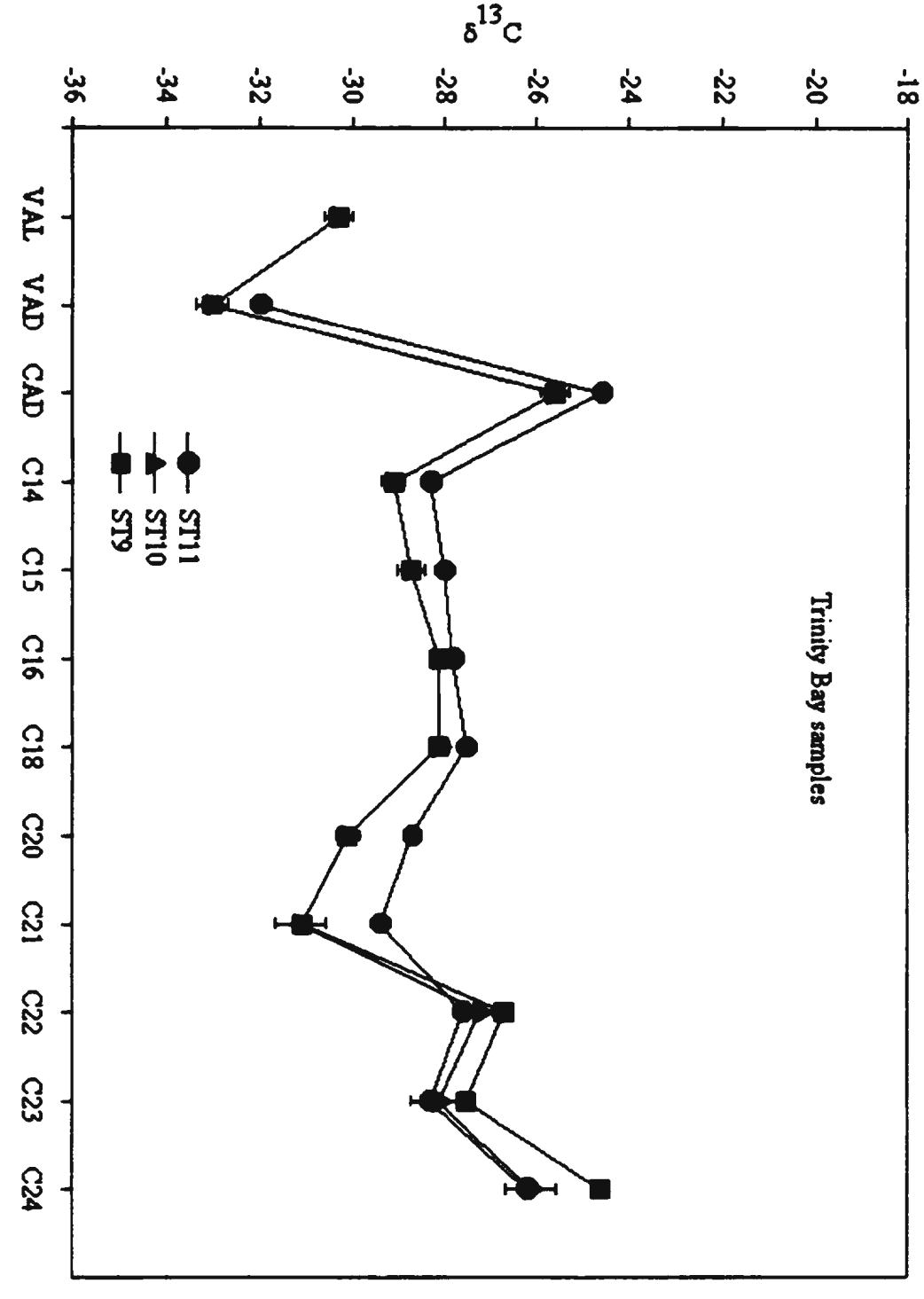


Figure 6.5 - Molecular isotopic signature ($\delta^{13}C$) of sediment chemolysates of Trinity Bay (surface sediments). Data used for figures is in Table 6.1



various lignins and SOM as determined by Goni and Eglington (1996).

The $\delta^{13}C$ values of coumaric acid units varies between-24.7 and -19.2°/$_{oo}$ from cores in the Northwest Arm, and -23.4 to -27.9°/$_{oo}$ from cores that are located offshore. The isotopic composition of terrestrial plants of the C_3 type range between -32 to -22°/$_{oo}$ (Galimov, 1985). Benner *et al.* (1987) analyzed the lignin fraction of plants and recorded up to 6°/$_{oo}$ depletions from $\delta^{13}C$ of the whole plants. Hence, the relatively light $\delta^{13}C$ isotopic values for the vanillin and vanillic acid are consistent with typical $\delta^{13}C$ values of C_3 plants (Appendix 6.1). In addition, the $\delta^{13}C$ compositions of these phenols are very similar to the $\delta^{13}C$ compositions of terrestrial soil samples, reinforcing the assumption that the lignin-derived phenols, vanillin and vanillic acid units, are good markers for terrestrial plant inputs into the marine environment (Table 6.1).

Isotopic evidence presented in Table 6.1 challenges the molecular evidence that coumaric acid derived from thermochemolysis of marine sediment is a marker for terrestrial plant lignin only. The $\delta^{13}C$ compositions of coumaric acid observed in core ST7 (and other offshore cores) are isotopically depleted and surprisingly similar to bulk $\delta^{13}C$ compositions of typical terrestrial C3 plants. In contrast, the $\delta^{13}C$ compositions of coumaric acid isolated from the near-shore sediments of the Northwest Arm are enriched in ^{13}C, having $\delta^{13}C$ compositions between -23.9°/$_{oo}$ and -19.2°/$_{oo}$. This observation is contrary to the expectation that the cores closer to the land margin should have a lignin-derived phenol that has an

isotopically enriched (in ^{13}C) signature which is not typical of C_3 plant biochemicals.

The $\delta^{13}C$ composition of coumaric acid can be interpreted in the context of differences in its sources. The $\delta^{13}C$ composition of coumaric acid is isotopically heavier than the $\delta^{13}C$ composition of other lignin-derived phenols because coumaric acid (and ferrulic acid) undergo fewer enzymatically-mediated steps prior to incorporation into lignin polymer (Goni and Eglington, 1996) (Appendix 6.1). Goni and Eglington (1996) measured a 9.6‰ difference in $\delta^{13}C$ composition between coumaric and vanillic acids for pine leaves. However, this explanation cannot adequately account for a difference of 15 and 13.3‰ between the $\delta^{13}C$ composition of coumaric and vanillic acids for cores H1 and H9, respectively. Coumaric acid is abundant in lignin from non-woody plants (Klap *et al.*, 1996; Martin *et al.*, 1995). The Northwest Arm is characterized by an abundance of sea grass (*Zostera marina*). These plants have true lignin as opposed to "psuedo-lignin" of lower marine plants that are described by Degens *et al.*, (1968). Haddad and Martens (1987) measured very high cinnamyl to vanillyl phenol ratio (C/V=3.47) of *Zostera marina* compared to a C/V ratio of 0 to 0.09 for gymnosperm wood (Meyers and Ishiwatari, 1993). The abundance of sea grasses in the study area and the high cinnamyl/vanillyl ratios seem to indicate that sea grasses are a source of the coumaric acid moieties present in the Northwest Arm. The $\delta^{13}C$ signature of *Zostera* ranges between -13 and -10‰ (a C_4-like composition) (Thayer *et al.*, 1978) and consequently, coumaric acid moieties from this source will create an isotopically heavy $\delta^{13}C_{\text{coumaric acid}}$ signal. There are other potential

sources of coumaric acid, such as sphagnum moss and terrestrial plants that inhabit the hinterland. However, $\delta^{13}C_{TOC}$ of these terrestrial plants (-25.8, -25.7 and -36.6‰ for kalmia, rush and sphagnum, respectively) indicate that they are probably not major sources coumaric acid in the Northwest Arm.

Amino acids and proteins are other possible sources of methylated coumaric acid in the chemolysates of the Northwest Arm sediments. Amino acids and proteins are isotopically heavy (Galimov, 1985), therefore, coumaric acid from amino acid sources are expected to be isotopically enriched in ^{13}C. Macko *et al.* (1987) determined isotopically enriched compositions for tyrosine and phenylalinine in blue-green algae and bacteria. However, the TMAH thermochemolysis yield of methylated coumaric acid from phenylalinine is minor (approximately 0.7 percent of total yield). Troke (1987) measured the concentration of amino acids in sediments of Trinity Bay and recorded the abundance of total (hydrolysable and free) tyrosine to be as high as 7.1 μg/g of sediment. Assuming that the abundance of amino acids in core ST7 are comparable to the abundances measured by Troke (1987), the expected yield of methylated coumaric acid from this source (tyrosine) is 0.14 μg/g of sediment (based on a 1 percent yield of methylated coumaric acid from tyrosine calculated in Chapter 3). Hence, the contribution of coumaric acid from amino acid to the total concentration is very small and will have a very proportionate impact on the overall $\delta^{13}C$ composition measured for coumaric acid units in light of mass balance considerations. However, coumaric acid may be derived from other amino acid mixtures and proteins as suggested by Manino and Harvey

(2000).

Another potential source of isotopically heavy coumaric acid in marine environments is phytoplankton; *p*-hydroxy phenols can be synthesized by phytoplankton (Requejo *et al.*, 1991b). Galimov (1985) describes the *p*-hydroxy phenols as a "lignin fraction" of phytoplankton and and these aromatic metabolites have isotopic signatures that are enriched in ^{13}C (approximately -17.3‰, Appendix 6.1).

The $\delta^{13}C$ isotopic signatures of fatty acids of surface cores and grab samples range between -33.7 and -25.1‰. Even-chain fatty acids were slightly more enriched in ^{13}C than the odd-chain counterparts resulting in a saw-tooth pattern (Figures 6.1 and 6.2). Litchfouse and Collister (1992) analyzed lacustrine sediments and reported even-chain fatty acids being isotopically heavier than the odd-numbered fatty acids from bacterial production (Appendix 6.1). This pattern was also obtained from isotopic data on individual alkanes and was attributed to the alkanes being derived from more than one source (Ishiwatari *et al.*, 1994). Each source may have different isotopic composition and odd-even predominance (OEP, the carbon number distribution in straight chain lipids). Hence, the contribution to the isotopic composition of each fatty acid compound becomes uneven, resulting in the saw-tooth pattern of isotopic composition verses carbon number. However, Uhle *et al.* (1997) has shown that intermolecular $\delta^{13}C$ variation within a single organism. Inter-compound isotopic variations throughout the Northwest Arm samples are generally larger than those of Trinity Bay

samples, which supports the suggestion that OM of the offshore cores may be derived from fewer sources than that of the Northwest Arm cores. This estuarine environment receives abundant runoff from several small streams that deliver a variety of terrestrially derived OM from the productive hinterland. In addition, macrophytic algae, phytoplankton and sea grasses are also potential contributors to the OM of the sediment of the Northwest Arm. It is expected that the contribution of OM from the macroalgae and sea grasses are less for the offshore stations which are located farther away from the land margin. The $\delta^{13}C$ record of the sedimentary fatty acids is further complicated by the fact that there can be isotopic variation in individual fatty acids produced by the same organism; Revill *et al.* (1999) measured different values (which were depleted by up to 3.8‰. from the $\delta^{13}C_{TOC}$) for individual fatty acids of *Emiliania huxleyi* in batch cultures.

Columbo *et al.* (1997a) estimates that most of the degradation of OM occurs between the top 150 m in the water column and between 0 to 3 cm in the sediment of the Laurentian Trough. Parrish (1998) calculates that particulate matter at ST7 and ST9 settles from 100 m in the water column to the water/sediment interface in 6 to 11 days. The Northwest Arm is shallow (50 to 80 m). This results in a shorter residence time of OM in the water column in the Arm (i.e. OM that reaches the water/sediment interface in the Northwest Arm is fresher that OM that reaches the water/sediment interface in Trinity Bay). Hence, preferential degradation of less resistant sources is minimized and OM from a wider variety of sources are likely to reach the sediment/water interface in the Northwest Arm. At ST7, inputs of OM from

terrestrial sources is expected to be lower than that in the Northwest Arm because ST7 is located farther offshore than the other cores. The depth of the water column (310 m) at this station (ST7) results in greater the residence time of OM in the water column compared to that of the Northwest Arm. The longer water column residence time and re-working of sediments by marine benthos at ST7 precipitates the preferential preservation of the more resistant, terrestrially-derived, OM. Hence, chemolysates of ST7 sediments should have isotopic signatures that are strongly influenced by terrestrially-derived OM sources. In addition, there is smaller isotopic variation of the phenols and fatty acids of sediments of core ST7 and other offshore stations compared to those of sites of the Northwest Arm) (Table 6.1; Figures 6.1 and 6.2). This indicates that there may be fewer sources of fatty acids and phenols for the offshore stations. In addition, the high degree of bioturbation throughout core ST7 is the result of a very active benthic and demersal communities that efficiently recycle the marine derived lipids, including PUFA's and other fatty acids (Budge and Parrish, 1998; Parrish, 1998). Furthermore, the chemolysates of ST7 has the highest percentages of branched fatty acids - indicators of bacterial activity. The $\delta^{13}C$ compositions of the branched fatty acids in all of the samples are typical of C_3 plants and bacteria.

Ostrom and Macko (1992) performed bulk isotopic analyses on seston, macroalgal (*Laminaria Solidungula*) and phytoplankton samples in the Trinity Bay area. The $\delta^{13}C$ values of the macroalgae were in the range between -20 and -12‰. The $\delta^{13}C$ of phytoplankton end member was -24.6‰ (Appendix 6.1). They also measured the $\delta^{13}C$ of seston at stations RS6

and TB6 to be -23.6 and -25.7‰, respectively. Stations RS6 and TB6 are located in Southwest Arm and in Trinity Bay, respectively.

6.3.3 Temporal/depth variation

There is minor variation (less than 1‰) in the $\delta^{13}C_{TOC}$ compositions of cores ST7, H1 and H9, similar to that observed by Troke (1987) (Appendix 6.2). The $\delta^{13}C$ compositions of the phenols showed minor downcore variations (compared to the fatty acids) (Table 6.1; Figures 6.3 to 6.5). Downcore variations in $\delta^{13}C$ compositions of coumaric acid for cores H1, H9 and ST7 ranged between -21.9 and -19.2‰, -24.7 and -20.0‰, and -28.3 and -23.0‰, respectively. The $\delta^{13}C$ compositions of vanillic acid and vanillin units (for cores H1, H9 and ST7) were much more depleted and have values ranging between -34.7 to -32.0 and -33.1 to -32.5‰, respectively. The most depleted $\delta^{13}C$ isotopic values are those obtained from the surface sediments in all of the sites. The most pronounced enrichments in ^{13}C of fatty acids and phenols are observed in the transition from the water/sediment interface to subsurface sediments (Table 6.1; Figures 6.3 to 6.5). Generally, the subsurface $\delta^{13}C$ compositions of fatty acids and phenols are relatively constant. Downcore enrichments of some compounds may be as high as 7.5‰ (e.g., $\delta^{13}C$ values of fatty acid C_{15} of core H1). Enrichments in ^{13}C have been associated with the synthesis of marine humic materials in the marine environment. Harvey and Boran (1985) simulated the synthesis of humic-type compounds using trilinolein, an unsaturated triglyceride, with $\delta^{13}C$ composition of -28‰. The isotopic

composition of the synthesized fulvic acids was -19.7‰ and represents an enrichment of 8.3‰ in $\delta^{13}C$. Hence, large carbon isotope fractionations are associated with these sediment-mediated reactions and is a plausible explanation for the downcore enrichments of the saturated fatty acids. Kracht and Gleixner (1998) measured 1.9‰ enrichment (by pyrolysis-GC/C/IRMS of peat) in ^{13}C of anhydropyranose. This enrichment was thought to be the result of a trophic level effect.

In order to preserve mass balance considerations in light of downcore enrichments in ^{13}C of TMAH thermochemolysates (fatty acids and phenols of cores H1, H9 and ST7) and minor variation of the $\delta^{13}C_{TOC}$, other biogeochemicals of SOM must become isotopically depleted in ^{13}C. It is expected that other classes of abundant organic compounds, such as carbohydrates and amino acids will become isotopically depleted. Kracht and Gleixner (2000) analyzed peat samples (up to 10 cm deep and with relatively constant $\delta^{13}C_{TOC}$) by pyrolysis-GC/C/IRMS; certain pyrolysates (e.g. phenol, toluene) became depleted with depth and others (e.g. isopropylphenol, 4-ethyl-2-methoxyphenol) were enriched.

It is interesting to note that the $\delta^{13}C$ composition of coumaric acid units for 5 cm deep terrestrial soil sample showed a 5‰ depletion in $\delta^{13}C$ (compared with its isotopic composition from the topsoil). This is interpreted in the context of changes in sources as a result of natural progression of the hinterland or changes in agricultural activities in the community of Hickman's Harbour. Microbial alteration does not adequately explain the

enrichment in the $\delta^{13}C$ of coumaric acid. Coumaric acid is at the bottom of the stability series established by Hedges and Weliky (1989) and hence susceptible to microbial degradation. However ^{13}C-^{12}C bonds are stronger than ^{12}C-^{12}C bonds and microbes will preferentially degrade the latter, leaving a substrate which is enriched in ^{13}C.

The most striking downcore variation (through H1 and H9) is an enrichment in $\delta^{13}C$ of the fatty acids (C_{16-24}), especially those with even-numbered carbon atoms . This is interesting in that the fatty acids with long chain even-numbered carbon atoms are considered markers for terrestrial plant inputs. This observation is in contrast to a much lesser downcore $\delta^{13}C$ isotopic variations throughout core ST7 (Table 6.1; Figure 6.5). The $\delta^{13}C$ compositions of the short-chain fatty acids (C_{14} and C_{15}) become enriched in ^{13}C with increasing depth, whereas $\delta^{13}C$ compositions of long-chain fatty acids remain relatively constant. It is possible that the long-chain fatty acids are preserved through associations with the mineral particles of sediment of ST7. Alternatively, the isotopically heavy short-chain fatty acids could be produced by rupture of ^{13}C-^{12}C or ^{12}C-^{12}C bonds of long-chain fatty acids; in both instances, the short-chain fatty acid that is produced will have fewer isotopically lighter C-H bonded carbon atoms. Enrichments may become more pronounced the ^{13}C-^{12}C bond ruptures and the ^{13}C portion becomes incorporated in the short chain fatty acid. Bacteria are responsible for input of the short chain fatty acids and the variation of their $\delta^{13}C$ compositions with depth are likely the result of microbial-mediated reactions. Pronounced bioturbation of ST7 sediments (discussed earlier in Section 4.3.4) enhances mixing of sediment horizons, which

results in less isotopic variation with depth. Overall, for the three cores (H1, H9 and ST7), most of the compounds show enrichment with burial, followed by relatively constant isotopic compositions below the zone of active re-working (more so for cores H1 and H9). Hence, if the $\delta^{13}C$ composition of these compounds are taken as source markers, then the isotopic effects must also be considered because the $\delta^{13}C$ compositions of plant biochemicals can become altered during diagenesis (Nguyen *et al.*, 1999).

The $\delta^{13}C$ enrichment with depth can also be interpreted in the context of changes in productivity. Over periods of high productivity in the marine environment, dissolved CO_2 becomes depleted and marine plants utilize the isotopically heavier bicarbonate ion during photosynthesis. Hence, it is possible that productivity could have been higher in the past, especially in light of downcore enrichments in all of the cores.

Finally, there are differences in enrichment of $^{\delta 13}C$ of the chemolysates when comparing the sediment profiles of cores H1 and H9 (Figures 6.3 and 6.4); the enrichments are greater for core H1. This can be explained by the age of the sediment core section (Appendix 1.3). The entire profile of core H9 represents less than 100 years of sedimentation. An approximate equivalent to this is in the upper 4-6 cm of core H1. Hence, sediments at equal depths of both cores are at different stages of humification. The bottom sediments of core H1 are at a more advanced stage of humification and this results in greater depletion of isotopic values.

6.4 SUMMARY

1. Using carbohydrate standards, the $\delta^{13}C$ values of 1,2,4-trimethoxybenzene do not reflect the $\delta^{13}C$ of its carbohydrate precursors.

2. The $\delta^{13}C$ signatures of lignin-derived phenols found in marine sediments (except coumaric acid) are extremely depleted values and reflect the isotopic signature of terrestrial sources. Generally, fatty acids are isotopically heavier.

3. Molecular isotopic characterization of chemolysate products indicates that there are more sources of OM for sediments of near-shore cores of the Northwest Arm than for sediments of offshore areas. In addition, the $\delta^{13}C_{TOC}$ of the Northwest Arm sediments reflect greater inputs from terrestrially-derived sources (compared to Trinity Bay sediments).

4. The Northwest Arm sediments are characterized by isotopically heavy $\delta^{13}C$ signatures for coumaric acid (compared to $\delta^{13}C$ of typical lignin-derived phenols).The isotopically heavier $\delta^{13}C$ for coumaric acid sediment may be a result of larger multiple inputs of OM, including the sea-grass *(Zostera marina)*. Interestingly, isotopic signatures indicate that terrestrially-derived OM may be a major source of coumaric acid in the offshore core sediments.

5. Fatty acids and phenols generally become enriched in ^{13}C below the sediment/water interface. These $\delta^{13}C$ enrichments are consistent with enrichments in $\delta^{13}C$ observed during synthesis of marine humic materials by Harvey and Boran (1985). However, minor variation in $\delta^{13}C_{TOC}$ with depth is observed. Hence, in order to preserve mass balance, other classes of organic compounds in the SOM are expected to become isotopically lighter or the isotopically light compounds increase in abundance with depth of sediment.

6. The isotopic profiles of chemolysate markers of core H1 show greater enrichments in $\delta^{13}C$ than those of core H9 because core H1 is the result of a longer period of sedimentation than core H9. As a result, sediments of core H1 are exposed for a longer time interval to the processes that produce the $\delta^{13}C$ enrichments with depth.

CHAPTER 7 - SUMMARY AND CONCLUSIONS

7.1 SUMMARY

7.1.1 Novel Analytical Approaches

The SOM of marine sediment is a complex mixture of organic compounds. The bulk of the SOM is the non-hydrolysable component that is composed of "humic-like" substances and is not totally characterized. According to Ishiwatari (1992), only a small percentage (less than 30%) of marine OM has been identified as common components of living organisms. Harvey and Boran (1985) proposed that marine humic compounds consist essentially of macromolecules comprising amino acid-carbohydrate condensation products and fatty acids attached through ester linkages, and minor amounts of other organic compounds present in the environment. Neyroud and Schnitzer (1975) also proposed that fatty acids are attached to phenolic units of humic materials by ester bonds. The hydrolysable carbohydrates, amino and fatty acids, phenols, sterols and hydrocarbons are components of the free fraction. It is essential to analyse SOM of marine sediment in order to establish relationships between compositional units and sources, as well as diagenetic alterations.

The analytical strategy was to systematically degrade SOM into characteristic fragments that can be more easily analyzed; direct pyrolysis and TMAH thermochemolysis are well-established analytical methods that can achieve this. The techniques of "on-line" pyrolysis-

GC/MS and "off-line" TMAH chemolysis/GC/MS proved to be inexpensive and very useful complementary methods of characterizing SOM of the study area (Trinity Bay and the Northwest Arm, Newfoundland). An additional advantage of "off-line" TMAH chemolysis is that the chemolysates can be isolated and analyzed by other techniques such as GC/C/IRMS.

The $\delta^{13}C$ signature in TMAH chemolysates of sediments can be interpreted only if there is no significant isotopic fractionation (or a predictable fractionation) during bond cleavage and subsequent derivatization or synthesis. Several model compounds which are known to be present in SOM chemolysates were tested by reaction with TMAH and isotopically analyzed by GC/C/IRMS. It appears that there was minor isotopic fractionation after correcting for the contribution of the added derivative carbons (of methylation). In addition, $\delta^{13}C$ compositions of C_{10} fatty acid (isolated from tricaprin after saponification) were comparable with that of C_{10} fatty acid produced by TMAH chemolysis of tricaprin (corrected for the methylation). It also appears that there was minor isotopic fractionation as a result of bond cleavage (in this case - ester bonds) , although additional work on isotopic fractionation during bond cleavage is warranted. Theoretical mass balance calculations on phenols produced by TMAH thermochemolysis of a lignin dimer indicates that one of the phenols may have retained its isotopic signature after synthesis of the dimer. However, these results are preliminary and more work is warranted in this area. Others (e.g. Eglington, 1994; Goni and Eglington, 1994; Goni and Eglington, 1996) have demonstrated that pyrolytic cleavage of

various chemical bonds, including ether bonds, do not result in significant isotopic fractionation.

7.1.2 Comparison and integration of data sets - source implications of SOM

Direct pyrolysis of sediments of the study area yield a suite of compounds that include simple phenol, alkylphenols, and typical pyrolysates of carbohydrates, N-compounds (pyrroles, nitriles and indoles) and several lipid products. Generally, the yield of phenol pyrolysates from near-shore Northwest Arm sediment is higher than that of offshore Trinity Bay sediment. Normalized abundances of N-compounds (to methylphenol) are higher for Trinity Bay sediment. In addition the yield of carbohydrate pyrolysates of offshore Trinity Bay cores is higher than that of Northwest Arm cores (with the exception of core H1 which has a major input of OM from logging operations).

Simple phenols in pyrolysates have multiple sources including lignins and certain amino acids units. Phytoplankton are also capable of synthesizing p-hydroxyphenols that can be precursors of the simple phenols. The N-containing pyrolysates are derived from several sources, including amino acids, proteins and chlorophylls. Chlorophyll, extensin, and collagen are possible sources of pyrrole. The abundances of pyrolysate N-compounds (to methylphenol) are also interpreted in terms of the inputs of OM. Generally, pyrolysate distribution indicate that sediments of Trinity Bay (offshore sites) have higher proportion of N-compounds than sediments of Northwest Arm (near-shore sites). Higher abundances of

N-compounds in the offshore sediments result from major inputs of marine production and may indicate that the OM delivered to these sites are relatively "fresh" because the nitrogen content decreases with alteration of OM. Terrestrial polysaccharides, such as cellulose, pyrolyse into simple products such as levoglucosan. Levoglucosan, present only in the pyrolysate of surface sediment of core H1 and ST11, is a major pyrolysis product of wood. The lack of levoglucosan below the surface of these cores indicates that cellulose is rapidly transformed as it becomes consumed by microbial activity.

TMAH chemolysates of SOM consist mainly of methylated fatty acids and phenolic compounds. Generally, the abundances of phenol and fatty acid markers are higher for Northwest Arm (near-shore) samples than those of Trinity Bay(offshore). TMAH chemolysis and conventional pyrolysis have yielded complementary data sets for the same sample. The chemical information they supply are different because the conditions during the thermolysis steps are not the same. Although the phenols derived from direct pyrolysis and TMAH chemolysates are structurally different, the strong positive correlation of total pyrolysate and chemolysate phenol abundances and their normalized abundances (to TOC) (R^2=0.99 and 0.89, n=10, respectively) for surface sediment samples, suggest that their sources are related. There are also strong downcore correlations of abundances of pyrolysate and chemolysate phenols (R^2=0.99, 0.92 and 0.92, n=4, for cores H1, H9 and ST7, respectively). Lipid pyrolysates (i.e. hydrocarbons) correlate reasonably well with abundances of fatty acid methyl esters generated by TMAH chemolysis (R^2=0.7, n=10, for surface samples) and are most likely derived from the fatty acid moieties present in sediments.

Results of the distribution of pyrolysates, TMAH chemolysates and compound-specific isotopic analysis of chemolysate phenols and fatty acids together have provided valuable insights into sources and processes affecting the OM of sediments. The composition of marine SOM materials reflects the activities and processes that deliver OM to the sediments. Spatial variation of TMAH chemolysates and pyrolysates reflect recent (up to the 16 years before 1994) activities that influence the input of OM to the sediments of Northwest Arm and Trinity Bay. Lignin-derived phenols are more abundant in Northwest Arm, and in particular, close to Hickman's Harbour. As well, the yield of vanillic acid units from TMAH chemolysates is about fivefold greater for sediments of Northwest Arm than for Trinity Bay, demonstrating a strong influence of OM derived from terrestrial sources and, likely the prevalent utilization of wood on Random Island. Sediment from core H1, which is located close to Hickman's Harbour, have the highest abundances of pyrolysate and TMAH chemolysate phenols. The economy of Hickman's Harbour used to be driven mainly by logging and sawmilling activities that resulted in large amounts of terrestrially-derived organic matter being delivered to the harbour via the local stream. The abundance of terrestrially- derived phenols decreases, and contribution OM from marine production to the TOC increases with distance from the land margin. Higher normalized abundances of N-compounds in the sediments of the offshore cores reflect increased quantitative importance of marine-derived OM that are generally richer in N content than terrestrially-derived OM. This supports the observations of Parrish (1998) that phytoplankton are more abundant in the water column in Trinity Bay than in the Northwest Arm.

Compound-specific isotopic analysis confirmed that methylated phenols of TMAH chemolysates (with the exception of coumaric acid units) are derived from terrestrial vascular plants. The $\delta^{13}C$ data shows that the vanillic units (vanillin and vanillic acid) are derived from terrestrial lignin materials and coumaric acid units is likely derived from both terrestrial plants and sea grass. Hence, $\delta^{13}C$ composition of coumaric acid units suggests that assumptions that all lignin-like phenols in marine environments are not simply terrestrial bio-markers (Hedges and Mann, 1979; Hedges and Parker, 1976; Hedges *et al.*, 1988a and b). Methylated coumaric acid, vanillin and vanillic acid are present in the chemolysates of both marine sediment and terrestrial soil samples. However, the $\delta^{13}C$ compositions of these markers from marine sediment indicate different sources. $\delta^{13}C$ compositions of vanillin, coumaric and vanillic acid moieties of SOM in Northwest Arm and Trinity Bay vary between -35.7 to -30.7‰, -33.1 to -30.3‰ and -27.9 to -19.7‰, respectively. The $\delta^{13}C$ values for coumaric and vanillic acid units of TMAH chemolysates of soil samples are isotopically depleted and close to C_3 terrestrial sources (as expected). In the sediments, especially those of Northwest Arm, the $\delta^{13}C$ signatures indicates a C_3 terrestrial source for vanillic acid. The isotopic composition of coumaric units may be interpreted in the context of multiple sources - sea grasses as well as terrestrial sources. In Northwest Arm, *Zostera marina* is abundant and can be a major contributor to the coumaric acid pool. In summary, structural information in conjunction with isotopic data indicate that vanillic acid and vanillin are derived from terrestrially-derived sources. Coumaric acid units are derived from terrestrial sources and sea grasses (*Zostera marina*) in Northwest Arm; $\delta^{13}C$ evidence indicates that coumaric acid is derived mainly from terrestrial sources at the offshore stations (ST7, ST9,

ST10 and ST11).

Fatty acids identified in TMAH chemolysates can also have multiple sources. In Northwest Arm, there is a strong terrestrial influence on input of OM, whereas primary productivity by phytoplankton becomes more important away from the shore in Trinity Bay. Relative abundances of fatty acids (compared to abundances of phenols) increased in sediments of the offshore cores; as a result the chemolysate fatty acid/phenol ratios of offshore cores are higher than corresponding ratios for near-shore cores. The $\delta^{13}C$ signatures of fatty acids provide supplemental evidence of the sources of fatty acids in the chemolysates; highly variable $\delta^{13}C$ values for fatty acids (-33.7 to -24.5‰) of Northwest Arm indicate multiple sources of OM (marine plankton, terrestrial plants, sea grass and anthropogenic activities such as wood cutting). The $\delta^{13}C$ compositions of fatty acids of the offshore stations in Trinity Bay are less variable (-31.9 to -24.6‰) and indicate fewer sources of OM.

7.1.3 Organic matter transport and accumulation in marine sediment

OM from the hinterland is delivered to Northwest Arm (and to a lesser extent ,Trinity Bay) by numerous pathways. A substantial portion of OM may also be delivered to the water column by aeolian transport. The terrestrially- and marine-derived OM will degrade as it sinks through the water column. The main stream on Random drains the major portion of the hinterland and empties into Hickman's Harbour. Core H1 is located near to the community of Hickman's Harbour. This core is unique because of its high TOC and its organic signature

- high abundances of terrestrially-derived phenols (this study), lipids (Parrish, 1998), aliphatic hydrocarbons and PAH's (Favaro, 1997) and sterols (Hudson et al., 2000). As a result, the harbour can also be a source of terrestrially-derived OM for the rest of Northwest Arm and Trinity Bay because sediment can be transported by ocean and tidal currents. The widespread occurrence of terrestrial markers and contaminants in marine sediment implies a propensity for land-derived contaminants to become easily dispersed in the system.

Lesser amounts of terrestrially-derived OM are transported to the offshore locations after having been exposed to various oxidation agents that affect biochemical changes; as a result, the reactive organic components are preferentially removed and OM that become incorporated into the sediment are more chemically resistant. Interestingly, the $\delta^{13}C$ of coumaric acid units of the offshore cores is typical of average terrestrial C_3 plants, compared to isotopically heavier $\delta^{13}C$ compositions for Northwest Arm sediment. This indicates that coumaric acid of the OM of offshore sediments is derived mainly from terrestrial land plants whereas coumaric acid of the OM of Northwest Arm sediments is probably derived from terrestrial land plants and sea grass. Coumaric acid from sea grass in Northwest Arm is most likely degraded en route to the offshore stations.

The isotopic signatures for fatty acids of the deeper offshore stations, such as ST7, which are closer to those derived from terrestrial plants, may be explained by preferential preservation of terrestrial acids that are arranged such that they are inaccessible to microbial utilization. Haddad *et al.* (1991) explained the preferential survival OM in Cape Lookout Bight (North

Carolina) in terms of its reactivity, which is controlled by the chemistry of the compound and the microbial accessibility of its pre-diagenetic matrix. Fatty acids are more labile than phenols. Hence, it was expected that the chemolysate fatty acid/phenol ratios will decrease with depth of sediment. However, the opposite effect is observed - downcore increases in fatty acid/phenol ratios are observed in all three cores; the reactivity of OM is determined by many factors such as OM/mineral interactions which can result in preferential preservation of more reactive classes of organic compounds. Changes in sources of organic matter can also contribute to these changes.

^{210}Pb measurement facilitated calculations of preserved fluxes (i.e. the amount of material that is preserved on an annual basis, assuming similar environmental conditions) of organic matter, fatty acids and phenols for cores H1 and H9. The ^{210}Pb dating results revealed different sedimentary environments for the three stations (H1, H9 and ST7) with respect to fluxes and benthic processes. Although the TOC of sediments of core H1 is higher than those of core H9, the preserved flux of OM is lower than that of core H9. The OM of sediments of core H9 becomes "diluted" with the inorganic sediment matrix as a consequence of a higher sedimentation rate (Appendix 1.4). Preserved fluxes also indicate that the quality of OM delivered to the locations of cores H1 and H9 are different. OM delivered to core H1 sediments contains more phenols and less fatty acids than that delivered to core H9 sediments. ^{210}Pb dating was difficult for core ST7 because of extensive bioturbation and is not reported.

7.1.4 Quality of organic matter and geochemical implications

This is the only study that dealt with TMAH thermochemolysis of marine sediments, which precludes direct comparisons with other studies (of TMAH thermochemolysis of marine sediments). Manino and Harvey (2000) compared the yields of fatty acids from TMAH thermochemolysis and solvent extraction procedures on particulate and dissolved OM and determined that the yields of the former can be much higher than those of the latter (by as much as 25 x, estimated from the figures). However, when thermochemolysis yields are normalized to TOC, reasonable comparisons can be made between data sets of this study and others (that involved TMAH thermochemolysis) (Appendix 7.1). The normalized abundances of lignin-derived phenols of Minnesota peat indicate higher yields of individual and total phenols and lower yields of individual and total fatty acids compared to the marine sediments of the study area (Appendix 7.1). The highest abundance of lignin phenols and fatty acids are from core sediments (1.07 and 7.43 mg per 100 mg of OC). The total abundances of phenols and fatty acids (and alcohols) of Minnesota peat are 5.78 and 3.44 mg per 100 mg of OC. Although the levels of abundances of the thermochemolysates are comparable, the differences can be explained in terms of sources; the OM Minnesota peat is derived from terrestrial materials that are rich in lignin-derived phenols, whereas the marine sediments (in the study area) are derived from lipid-rich marine OM and terrestrially-derived materials. Similarly, normalized lignin-derived phenol abundances are higher and individual fatty acid (C_{24} and C_{26}) abundances are lower for particulate organic matter from the Delaware Estuary when compared to those of marine sediments of the study area.

The mechanism proposed by Harvey and Boran (1985) wherein marine humic acids are formed, is not be the only mechanism by which humic materials of marine sediment are generated. The distribution of compounds identified by TMAH chemolysis and pyrolysis of marine sediment does not refute other pathways, such as the sugar amine condensation. This study supports the structures for humic compounds that were proposed by Schnitzer and Neyroud (1975) in which fatty acids can be attached to phenols via ester bonds. However, the yields of fatty acids and phenols account for a maximum of about 8.5 mg per 100 mg of TOC, and hence, there are other structures in the marine non-hydrolysable portion of the TOC that are quantitatively important.

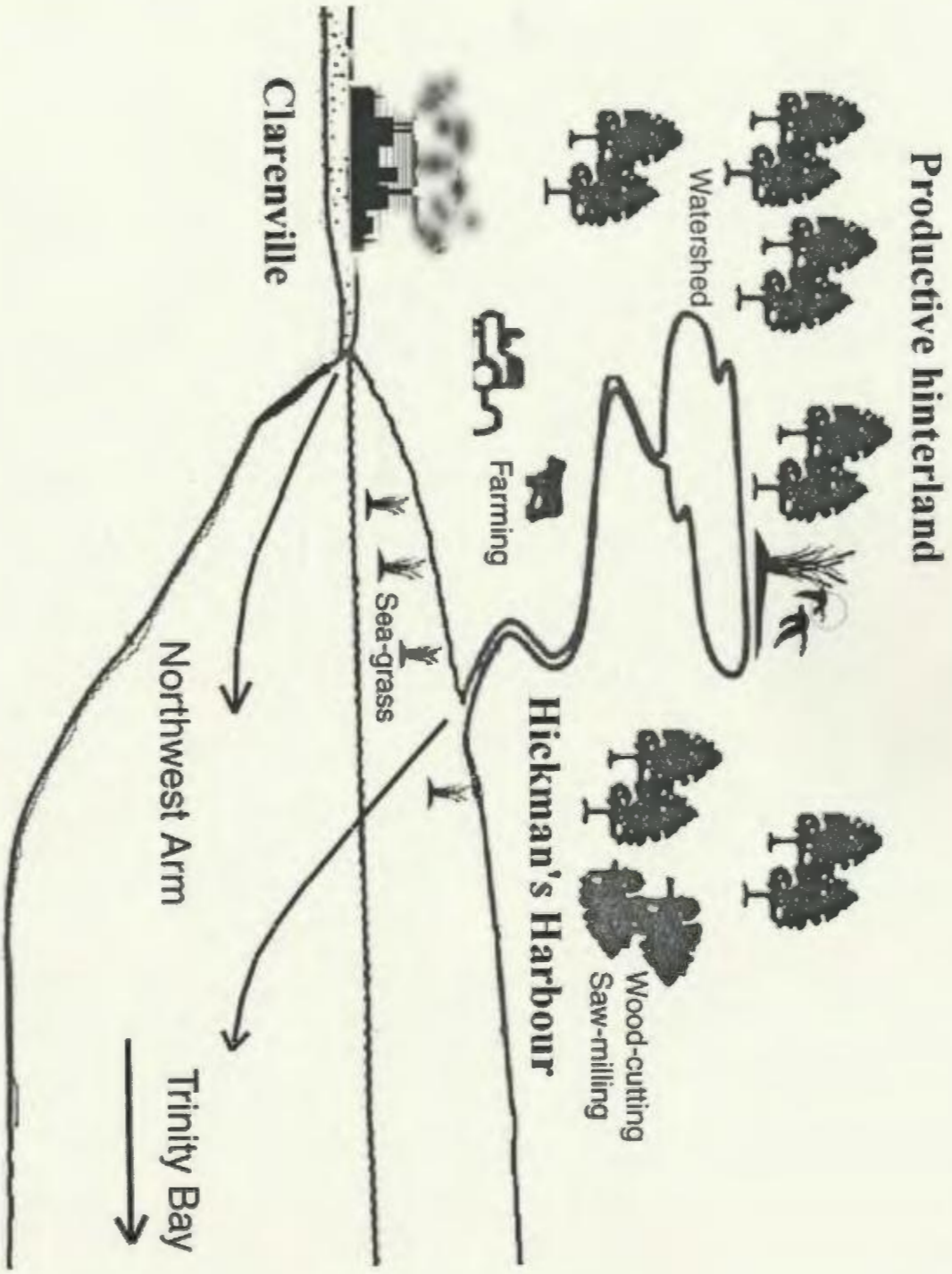
Substantial enrichments in $\delta^{13}C$ in the chemolysate fatty acids and phenols are observed during SOM humification of cores H1, H9 and ST7. The humification reactions that produce these $\delta^{13}C$ enrichments are probably similar to those proposed by Harvey and Boran (1985) because of the similarity in isotopic effects (an enrichment of 8.3‰). Finally, the magnitude of the $\delta^{13}C$ enrichments appears to be dependent on the humification stage. Generally, $\delta^{13}C$ enrichments (with depth) for core H1 are larger than those for H9 and ST7 (Figures 6.1 and 6.2). Average $\delta^{13}C$ enrichments, for each compound, for 30 cm of sedimentation for cores H1, H9 and ST7 are 3.6, 2.4 and 2.0‰, respectively. Core H1 represents a longer period of sedimentation than core H9 (Appendix 1.3) and hence OM at this site were exposed to agents of oxidation and degradation for a longer time period that OM at corresponding depth at the location of core H9. As a result, the magnitude of $\delta^{13}C$ enrichment is more pronounced for core H1 than for core H9. In light of isotopic mass balance considerations, other classes of

organic compounds have to become isotopically depleted in ^{13}C or the fraction of isotopically depleted (in ^{13}C) compounds increase in abundance. Although core ST7 is bioturbated, its downcore $\delta^{13}C$ profile also shows an enrichment that is less pronounced than those of cores H9 and H1.Bioturbation may be responsible for reducing the magnitude of enrichments.

7.1.5 Interdisciplinary Studies

One of the main objectives of the marine team of the Eco-Research Program was to assess the past and present "environmental health" (with respect to past climate, productivity, concentration of pollutants, etc.) of the study area. The scientific teams concluded that the environment (Bonavista headland and surrounding area) is generally clean (Ommer, 1998). Lacustrine core data indicated that there were no major perturbations in climate patterns in the past century. Spatial and temporal profiles for phenols, fatty acids and hydrocarbon (from Favaro, 1997) for cores H9 and H1 suggest that the level of land use has only weakly impacted the pattern of marine biogenic productivity in the study area. Finally, variations in lipids in the water column and sediment indicate efficient recycling of these compounds in the past few hundreds of years (Budge and Parrish, 1998; Parrish, 1998). The scientific conclusion is a common observation from "traditional ecological knowledge" - the disappearance of the groundfish off the coast of Newfoundland is a result of highly efficient and technologically sophisticated fishing techniques coupled with poor management of the industry.

Figure 7.1 - **Schematic of flow of terrestrially-derived carbon cycling in the study area**



Parrish (1998) measured concentrations of lipids in sediment samples from Trinity Bay and Northwest Arm; Favaro (1997) determined aliphatic hydrocarbons and PAH concentrations. There were significant correlations between the concentrations of organic compounds determined by Parrish (1998), Favaro (1997) and yields of chemolysates in this study. Spatial distribution of total lipids and hydrocarbon (from Parrish, 1998) correlates strongly with fatty acid chemolysates (R^2=0.94 in each instance). There was also a strong correlation between the spatial distribution of PAH concentration and phenol chemolysate (R^2=0.94, n=10). Hudson *et al.*, (2001) analyzed SOM of Trinity Bay and Northwest Arm (H1, H9 and ST7) for sterols as indicators of sewage contamination; the total free sterols (μg per g of OM) for the three sites (H1, H9 and ST7, respectively) are 410 ± 101, 405 ± 17 and 263 ± 46. In addition, the three sites contained high proportions of C_{28} and C_{29} sterols, which suggests significant terrestrial plant input. A schematic is developed that shows the flow of terrestrially-derived OM (as shown in Figure 7.1), with Hickman's Harbour being a major sink for terrestrially-derived OM and anthropogenic pollutants such as PAHs and sterols. Hickman's Harbour can also be a source for organic materials to other marine sediment locations by re-suspension processes.

There are also positive temporal/depth correlations. For example, the abundances of terrestrially-derived phenols correlate strongly with concentrations of PAH's for core H1 (R^2=0.80). The abundances of terrestrially-derived phenols correlate positively, although weaker, with downcore concentrations of PAH's for cores H9 and ST7. It is possible that the post sedimentary processes impose different types of alterations on the abundances of these

compounds which changes the strong surface correlations. Collectively, these markers-total lipids (Parrish, 1998), total aliphatic hydrocarbons and PAH's (from Favaro, 1998), phenols and fatty acids - indicate a significant influence of terrestrial plant inputs, specifically leaf and wood in Hickman's Harbour.

7.1.6 Emerging Issues

It has shown that during direct pyrolytic analysis, fatty acids are decarboxylated to yield a suite of hydrocarbons. An emerging issue that is related to these compounds is the verification of a relationship (or lack of) between fatty acid methyl esters present in TMAH chemolysates and these hydrocarbons present in pyrolysates. Verification can be achieved by using model compounds (such as tricaprin) and subjecting them to both direct pyrolysis and TMAH chemolysis, followed by isotopic analysis of the individual compounds. This is based on the premise that no significant isotopic fractionation occur during pyrolytic decarboxylation of fatty acids or during TMAH chemolysis. Modern pyrolyzers are easily interfaced to gas chromatographs (GC); connecting a pyrolyzer to the GC of the GC/C/IRMS system has been a routine procedure. Another emerging issue is comparison of molecular level isotopic characterization of "on line" TMAH chemolysates with those of the "off-line" procedure. Sample handling during "on-line" pyrolysis/GC/C/IRMS is minimized. In addition, the volatiles that are lost during "off-line" analyses will also be isotopically characterized. "On-line" analysis may also be useful to investigate temperature effects on cleavage of ether bonds. Only partial cleavage was achieved under experimental conditions

in this study and the use of pyrex break seals as combustion vessels above 300°C is limited. Higher temperatures can be used during the on-line procedure.

Similarly, by utilizing pyrolysis or TMAH chemolysis in conjunction with GC/C/IRMS, it is possible to determine whether the higher order phenols of the TMAH chemolysates are the source of alkylphenols during pyrolysis. Multiple sources of alkylphenols and alkylbenzenes have been reported. Biomacromolecules other than lignin have generated alkylphenols. Van Heemst *et al.* (1996, 1999) identified both alkylphenols and alkylbenzenes in pyrolysates of extracts of various algae and proposed that alkylphenols in pyrolyastes of DOM may be derived from algal sources. Results of this study indicate that the pyrolysate alkyl phenols and alkylbenzenes and the chemolysate lignin-derived phenols are related because of their positive correlation of their abundances (R^2=0.97 , n=10, for grab samples; R^2 varied between 0.98, 0.92 and 0.92, n=10, for downcore variations in cores H1, H9 and ST7, respectively). Comparison of the $\delta^{13}C$ compositions of phenolic pyrolysates and chemolysates will provide additional information on whether the compounds are related; this can be achieved by "online" pyrolysis-GC/C/IRMS.

TMAH chemolysis of model compounds were useful for interpreting the molecular and compound-specific isotopic characterization of SOM afforded by TMAH chemolysis. Phenolic acids and aldehydes and fatty acids were quantitatively converted to methylated derivatives. In the case of the free aldehydes, there is no evidence of Canizzaro reaction (during which aldehyde moieties produce methylated products of the corresponding phenol

acid) in the presence of excess TMAH. Ester bonds are easily broken during TMAH chemolysis. This is particularly significant because Schnitzer and Neyroud (1975) provided evidence that fatty acids are bound to phenols (in humic substances) via ester bonds and it is likely that these bond types are cleaved to produce a significant portion of the TMAH chemolysates (fatty acids and phenols). Further studies using pyrolysis/TMAH thermochemolysis in conjunction with GC/C/IRMS on specific processes of huminification, such as the *in-vitrio* biomass degradation will provide more insights into product/precursor relationships during this biogeochemical phenomenon.

Phenol ether bonds of model compounds that do not have hydroxyl side groups are more resistant to cleavage by TMAH chemolysis. In this study, partial cleavage of such a bond in a lignin dimer was obtained by TMAH chemolysis under experimental conditions. The distribution of TMAH chemolysis products of the lignin dimer (and the lack of compounds that can be produced from epoxide intermediates) can be explained by the mechanism proposed by Filley *et al.* (1999). An emerging issue related to this problem is investigation of temperature effects on the extent of bond cleavage of the above-mentioned lignin dimer which can be investigated by "on-line" TMAH thermochemolysis; the "off-line" procedure is performed in sealed glass tubes that are unsuitable for TMAH thermochemolysis at pyrolytic temperatures. Although it was demonstrated by Hatcher et al., (1994) that the TMAH technique yielded similar products at pyrolytic (600°C) and sub-pyrolytic temperatures (250-300°C), Lehtonen et al., (2000) reports greater numbers of TMAH thermochemolysates at 600°C.

7.1.7 Future applications of the analytical approach

This study is one of the first attempts at performing CSIA on phenols and fatty acids of marine SOM. The technique of TMAH chemolysis in conjunction with GC/C/IRMS has been shown to be appropriate for the analysis of OM that contains these compounds and markers. Fatty acids, fatty alcohols and lignin-derived phenols comprise 9.22 mg per 100mg of TOC of humic compounds of Minnesota peat (del Rio *et al.*, 1998). In this study, the fatty acids and phenols of TMAH chemolysates constitute between 1.6 to 8.5 mg per 100 mg of TOC. Hence, TMAH chemolysis/GC/C/IRMS can be useful to characterize these compounds in environments such as wetlands and contaminated sites (e.g. harbours, dumps, etc.) where the objective is to fingerprint specific compounds for source apportionment. The technique of TMAH chemolysis is also applicable to analysis of biomass tissues. It may be of particular use in cases where the environmental stress impacts various biosynthetic pathways/enzyme activity to the extent of changing the synthesis of plant biochemical and the associated kinetic isotopic effects. For example, several mutations have been identified that affect the lignin biosynthetic pathway, affecting the quantity of specific biochemicals that will be synthesized (Ralph *et. al.*, 1997). TMAH chemolysis/GC/C/IRMS is a simple and inexpensive method of monitoring these changes.

TMAH chemolysis/GC/C/IRMS is also potentially useful for monitoring the progress of contaminated sites that are undergoing remediation, such as waste-water deposits that have been contaminated by pyrolysis water deposits. This technique can also be used to evaluate

aerobic bacteria as a cleansing potential in bio-remediation for contaminated sites providing that the compounds that are affected are amenable to TMAH chemolysis. An emerging issue for bio-remediation studies involves measuring the magnitude and direction of isotopic changes in a given time period for specific biochemicals. This can be done under laboratory as well as field conditions.

Another application of TMAH chemolysis is the rapid detection and analysis of aerosols as a response to the potential for biological terrorism (Vorhees *et al.*, 1999). A new instrument has been designed at Oak Ridge National Laboratory (Colorado) which has the capability of to facilitate the analysis of an atmospheric aerosol sample. In this procedure, TMAH heated to above 200 °C hydrolyzes and methylates biochemicals within organisms in the aerosol samples. The subsequent analysis by GC/MS produces profiles of fatty acid methyl esters, methylated DNA bases, methylated thermal products and other compounds with acidic sites, that are characteristic of various microbes. There is also the potential for analysis of sedimentary protein analysis by TMAH chemolysis.

7.2 CONCLUSIONS

- TMAH chemolysis, and to a lesser extent, direct pyrolysis have produced markers that are excellent tracers of sources of SOM of marine sediments. SOM of Trinity Bay sediments (offshore) were more depleted in terrestrially-derived phenols than SOM of Northwest Arm (near-shore). Fatty acids and N-compounds are

quantitatively more important in the SOM of Trinity Bay.

- The variations of the spatial and temporal profiles of constituents of the SOM in the study area are clearly related to the distributions to sources and processes. Recent land-based activities have influenced the spatial distribution (in terms of quality and quantity) of markers in the Trinity Bay and Northwest Arm area, particularly in Hickman's Harbour and Clarenville. The SOM in Hickman's Harbour is also strongly influenced by discharge of the main stream on Random Island. Temporal variations of the pyrolysate and chemolysate markers may reflect the historical activities of the European and pre-European settlers of Random Island and nearby communities.

- Pyrolytic methods have been shown to be excellent for isotopic characterization of constituents of complex organic matter. It is demonstrated that TMAH derivatization of acidic and phenolic functionalities in sediment matrix is predictable and involves minor $\delta^{13}C$ fractionation. In addition, the $\delta^{13}C$ of monomers produced during bond cleavage (ether and ester) by TMAH chemolysis appear to be predictable. However, these results are preliminary and more work is warranted to resolve $\delta^{13}C$ fractionation during bond cleavage

- Isotopic information, in conjunction with molecular structural information of phenols provides strong evidence of sources of these compounds; this "dual tag" evidence

indicates that phenols are generally terrestrially-derived, but coumaric acid in the SOM of Northwest Arm appears to be derived from both terrestrial sources and sea grass (*Zostera Marina*). The $\delta^{13}C$ of the fatty acids indicate multiple sources (such as C_3 plants and bacteria).

- Finally, there are pronounced enrichments in the $\delta^{13}C$ compositions of both fatty acids and phenols with depth of sediment. Greater enrichments in $\delta^{13}C$ are observed for core H1 than for core H9 because the former represents a longer period of sedimentation.

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Appendix 1.1 - **Elements of the carbon cycle**

The cycle can be represented as four main reservoirs of carbon that are interconnected by pathways of exchange. The reservoirs are the atmosphere, terrestrial biosphere (usually includes freshwater systems), oceans, and sediments (includes fossil fuels). The carbon exchanges between reservoirs occur because of various chemical, physical, geological, and biological processes.

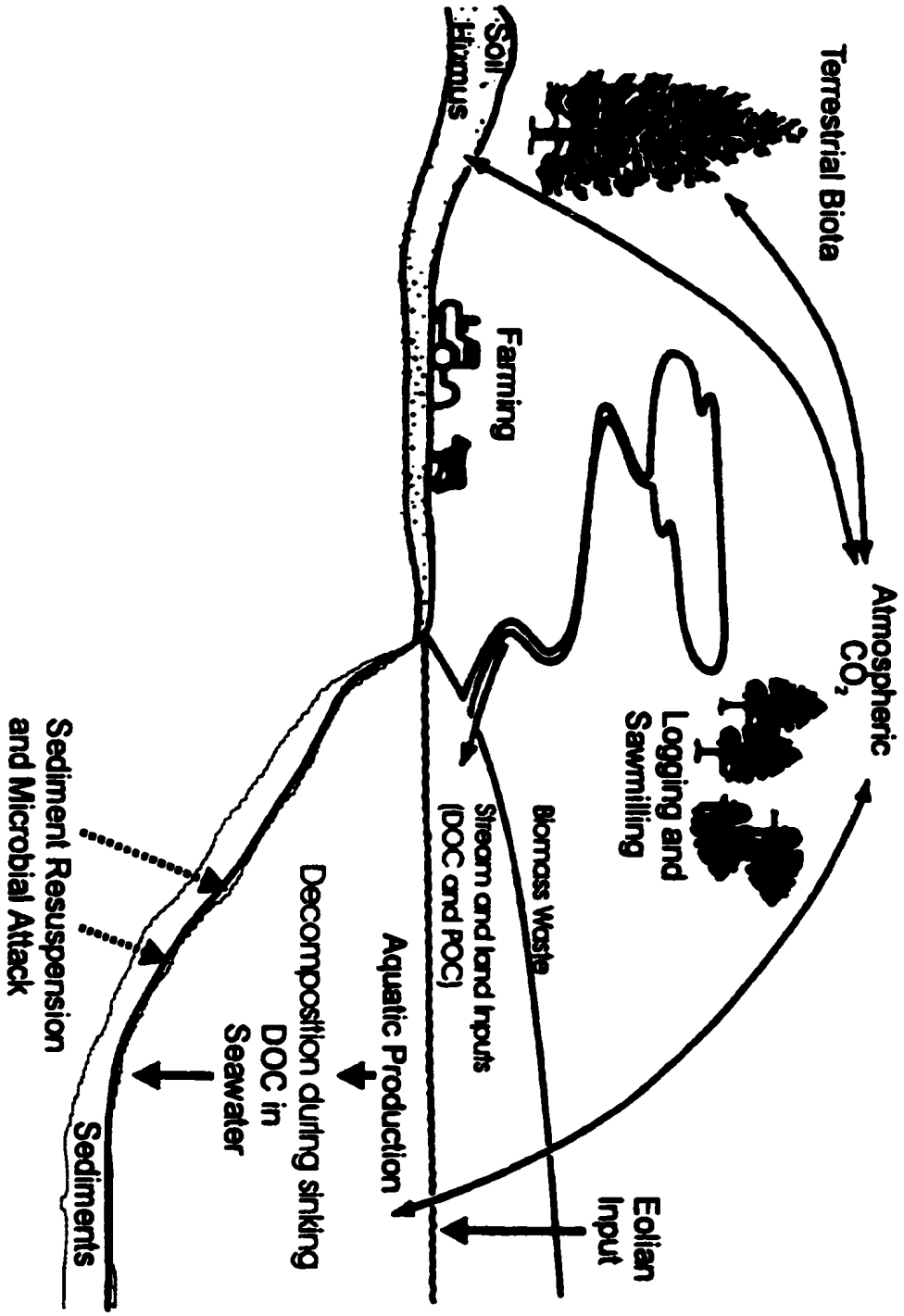
Estimated size of the major pools of carbon in the World Carbon Budget (in units of Gt - 10^9 t or 10^{15} g) (from Killops and Killops, 1993)

ATMOSPHERE	740
LAND	
Biota	550
Soil + Peat	1600
OCEANS	
Biota	3
Dissolved Organic Carbon	1140
Dissolved Inorganic carbon	36860
FOSSIL FUELS	5000

It is estimated that between 1 and 2 billion metric tons of carbon per year are *"missing"* (i.e. has not been observed) from the global carbon budget. The location of between 15 and 30 percent of the carbon released into the atmosphere each year from fossil fuel burning cannot be accurately constrained. Globally, humans annually release about 7 billion tons of carbon. Of that amount, 3 billion tons remain in the atmosphere, 2 billion tons are absorbed into the ocean. It is assumed that the remainder is absorbed by land vegetation, but the specifics about location and quantities have remained elusive.

Atmospheric increase = Emissions from fossil fuels + Net emissions from changes in land use - Oceanic uptake - *Missing carbon sink*

Appendix 1.2 - **Carbon cycling scheme in the study area**



Appendix 1.3 - **Composition of sedimentary organic matter in marine sediment of various locations (as percentage of TOC)**

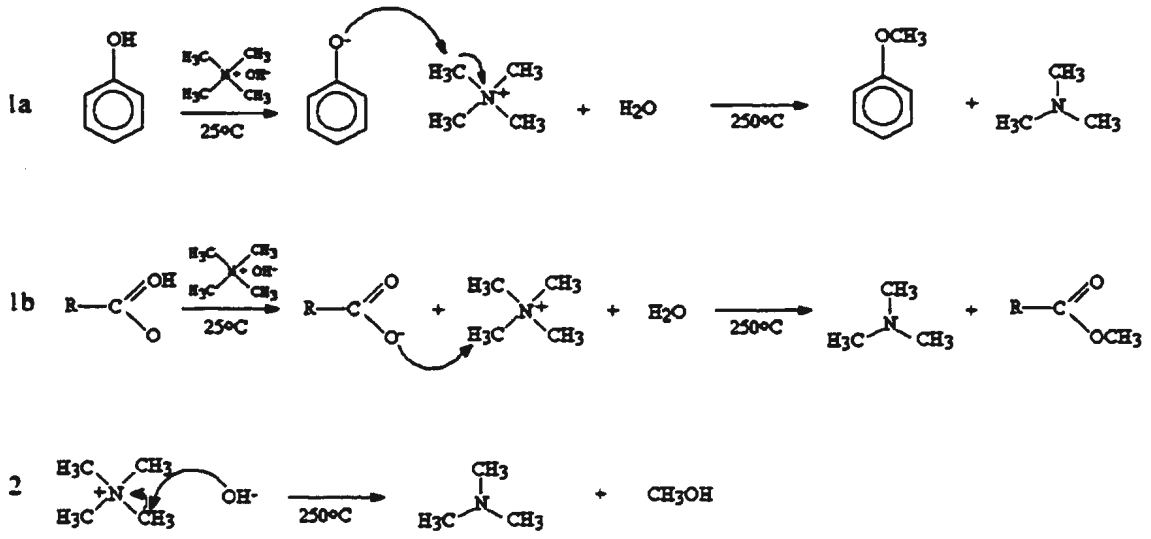
Site	Carbohydrate	Amino Acids	Lipid	Lignin
Cape Lookout Bight, N.C.	7[1]	11-15[2]	2.8[3]	0.7[4]
Dabob Bay	6[5]	<40[5]	2.3[5]	
Buzzards Bay		12-15[6]		

1 - Haddad, 1989, total hydrolysable carbohydrates
2 - Burridge and Martens, 1988, total hydrolysable amino acids
[3] - Haddad et al., 1991. The lipid fraction is the total unbound fatty acids ($1.1 mg g^{-1}$) in the upper 10 cm of the sediment.
[4] - Haddad and Martens, 1987.
[5] - Hedges et al., 1988 a and b
[6] - Henrichs and Farrington, 1987, total hydrolysable amino acids.

Appendix 1.4 - ^{210}Pb dates and sedimentation rates for cores H1 and H9

Sample site	Depth	Age (Year)	Sedimentation Rate (g/m^2/yr)
H1	0-2	1994	44.38
H1	2-4	1978	23.63
H1	4-6	1928	18.69
H9	0-2	1994	138.72
H9	2-4	1991	120.69
H9	4-6	1985	99.45
H9	6-8	1979	99.83
H9	8-10	1972	108.95
H9	10-12	1966	99.03
H9	12-14	1959	110.71
H9	14-16	1953	105.63
H9	16-18	1947	92.06
H9	18-20	1940	128.15
H9	20-22	1935	113.18
H9	22-24	1929	97.82
H9	24-26	1922	90.73
H9	26-28	Pre-1922	N/A

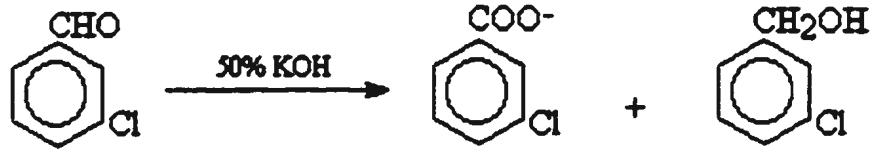
Appendix 3.1a - **Methylation by TMAH reagent**



In the first reaction of Equation 1a and 1b, tetramethylammonium salts of "free" acidic groups (such as phenols and carboxylic acids) are formed. The second reaction of Equations 1a and 1b is a higher temperature base hydrolysis reaction of hydrolyzable functional groups of phenol ethers and carboxylic acid esters, and to some extent alkyl ethers to produce tetramethylammonium salts (Appendix 3.1a, Eqs. 1a,1b). This is followed by rapid thermal degradation of the quaternary salts to form methyl derivatives (ethers and esters). In addition, under the reaction conditions, excess TMAH breaks down to produce a tertiary amine and methanol (Equation 2).

Appendix 3.1b - **The Cannizzaro reaction**

In the presence of concentrated alkalis, aldehydes that do not contain α-hydrogens undergo self oxidation-and-reduction to yield a mixture of an alcohol and a salt of a carboxylic acid. which is converted to the corresponding carboxylic acid under acidic conditions. An example of the Cannizzaro reaction is shown below.



1 2 3

Compounds in the equation are as follows:

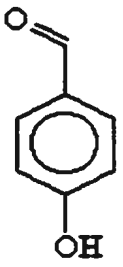
1 - 3-Chlorobenzaldehyde

2 - 3-Chlorobenzoate ion

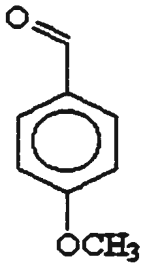
3 - 3-Chlorobenzyl alcohol

Appendix 3.2 - Structural formulae of model compounds and their TMAH thermochemolysis products

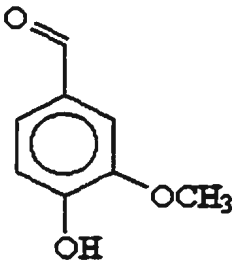
4-Hydroxybenzaldehyde
(PAL)



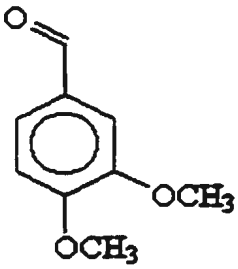
4-Methoxybenzaldehyde



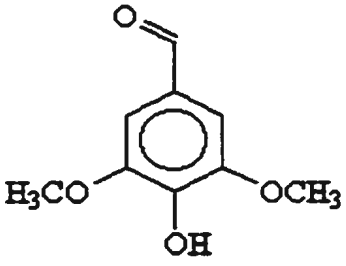
Vanillin
(VAL)



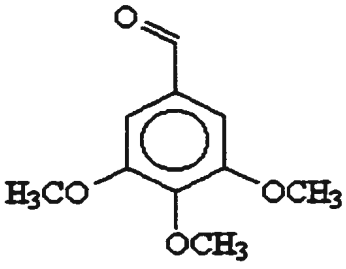
3,4-Dimethoxybenzaldehyde



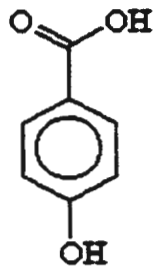
Syringealdehyde
(SAL)



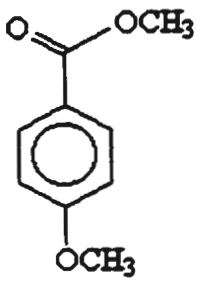
3,4,5-Trimethoxybenzaldehyde



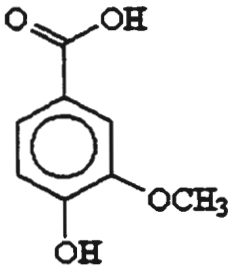
4-Hydroxybenzoic acid
(PAD)



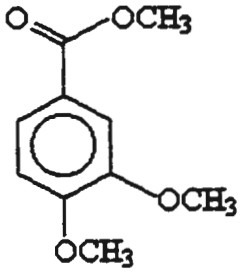
4-Methoxybenzoic acid, ME



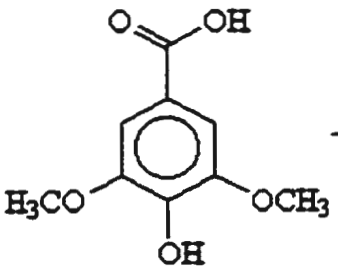
Vanillic acid
(VAD)



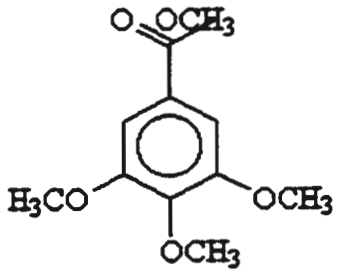
3,4-Dimethoxybenzoic acid, ME



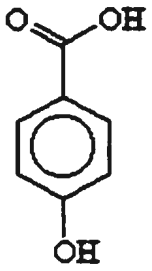
Syringic acid
(SAD)



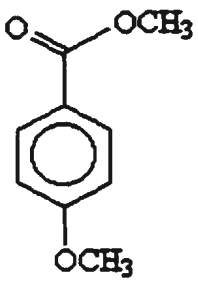
3,4,5-Trimethoxybenzoic acid, ME



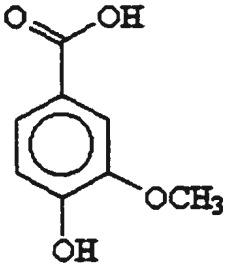
4-Hydroxybenzoic acid
(PAD)



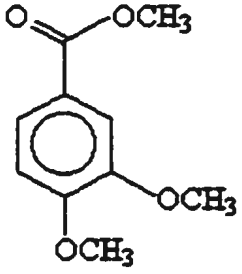
4-Methoxybenzoic acid, ME



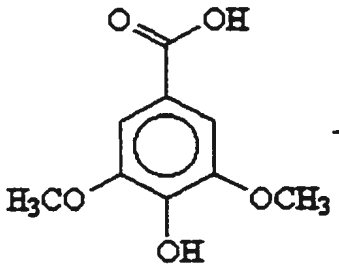
Vanillic acid
(VAD)



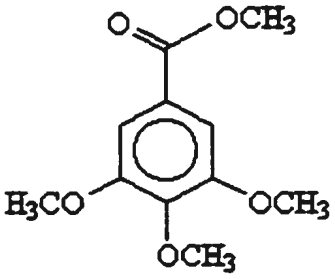
3,4-Dimethoxybenzoic acid, ME



Syringic acid
(SAD)

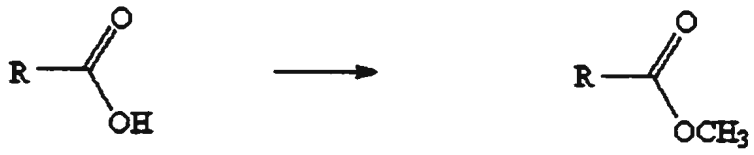


3,4,5-Trimethoxybenzoic acid, ME



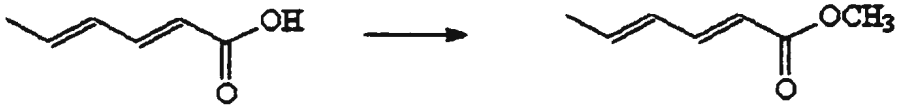
Saturated fatty acids
(C_{16}, C_{18}, C_{20}, C_{22}, C_{24})

Fatty acid ME

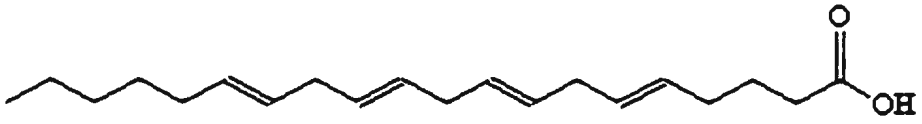


Sorbic acid

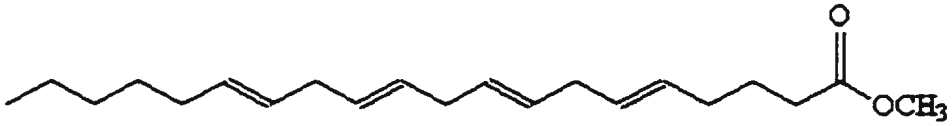
2,4- Hexadienoic acid, ME



5,8,11,14- Eicosapentaenoic acid



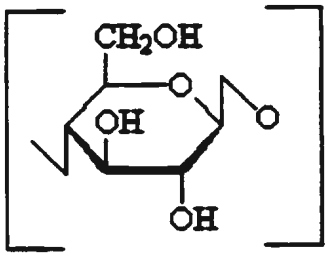
5,8,11,14- Eicosapentaenoic acid , ME



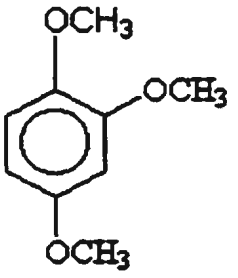
+

major isomerized products

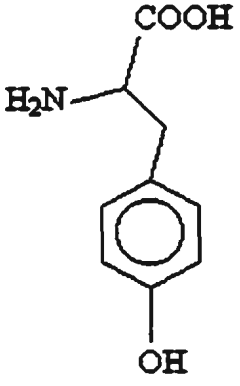
Cellulose, glucose, starch, pectin



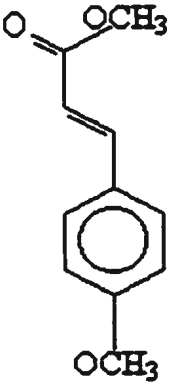
1,2,4-Trimethoxybenzene



Tyrosine
(TYR)

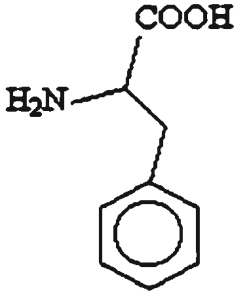


3-(4-Methoxyphenyl)
acrylic acid, ME

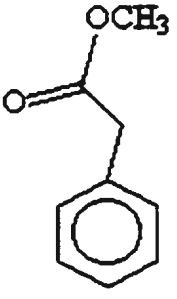


+ Others

Phenylalanine
(PHE)



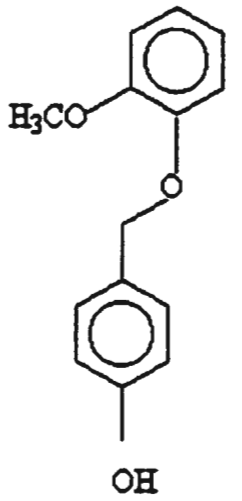
3-Phenyl-2-propanoic acid, ME



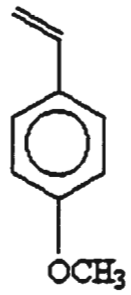
+ Others

Lignin dimer

4-[2-(2'-Methoxyphenoxy)ethyl] phenol

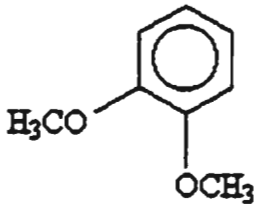


1-Ethenyl-4-methoxybenzene

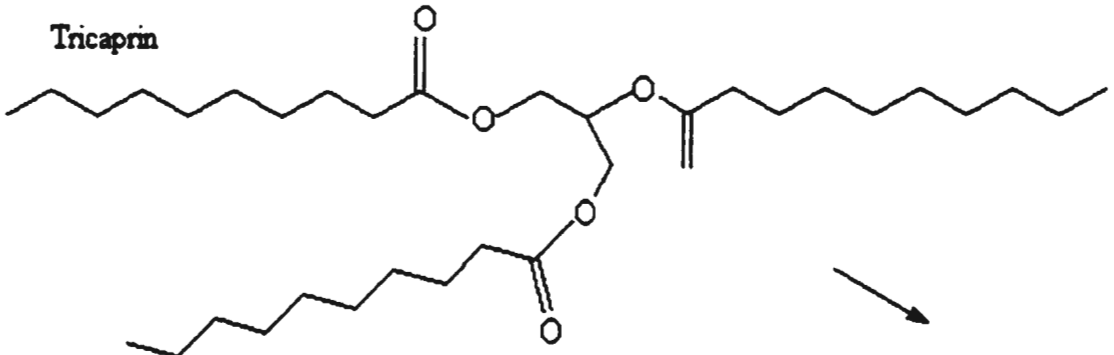


+

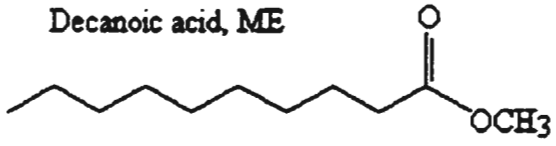
1,2-Dimethoxybenzene



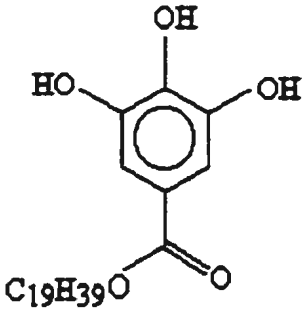
Tricaprin



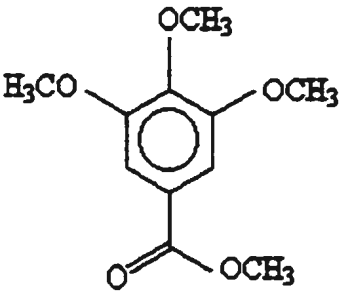
Decanoic acid, ME



Stearyl gallate



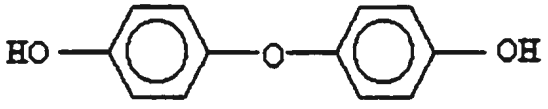
1,2,3-Trimethoxybenzoic acid, ME



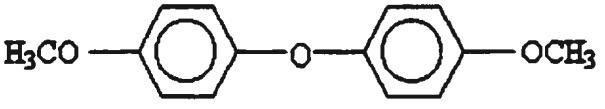
+

Octadecyl alcohol

4,4-Dihydroxydiphenyl ether

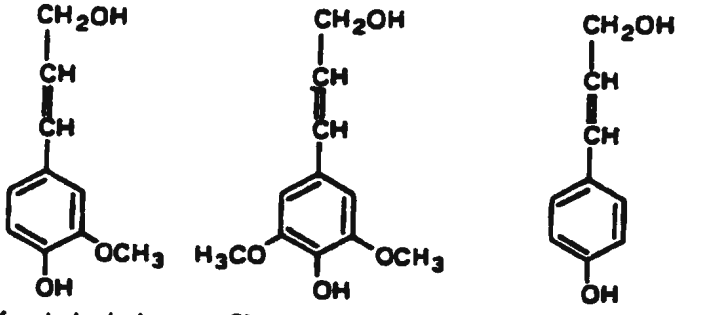


4,4-Dimethoxydiphenyl ether



Appendix 4.1 - **Structural Formulae of lignin alcohols and distribution of lignin types**

Monomers of lignin



	Coniferyl alcohol	Sinapyl alcohol	*p*-Coumaryl alcohol
Conifers	+	0	0
Deciduous trees	+	+	0
Gramineae	+	+	+

After Flaig et. al., 1975

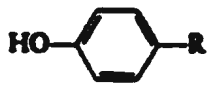
Lignin oxidation product distribution in different vascular plant tissues. The amounts are expressed as weight percent of vascular plant organic carbon.

	Syringyl	Cinnamyl	Vanillyl
Non-vascular woody plants	0	0	0
Non-woody angiosperms	1-3	0.4 - 3.1	0.6 - 3
Woody angiosperms	7-18	0	2.7 - 8
Non-woody gymnosperms	0	0.8 - 1.2	1.9 - 2.1
Woody gymnosperms	0	0	4 - 13

After Hedges and Mann, 1979.

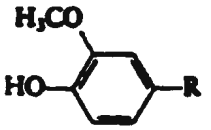
Appendix 4.2 - **Structural formulae of lignin-derived monomers determined by CuO oxidation (after Killops and Killops, 1993)**

p-Hydroxyl group



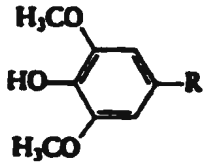
R = —C(=O)—H	p-Hydroxybenzaldehyde
R = —C(=O)—CH_3	p-Hydroxyacetophenone
R = —C(=O)—OH	p-Hydroxybenzoic acid

Vanillyl group



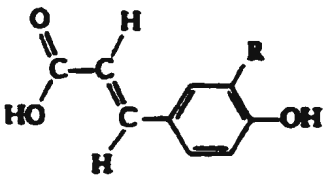
R = —C(=O)—H	Vanillin
R = —C(=O)—CH_3	Acetovanillone
R = —C(=O)—OH	Vanillic acid

Syringyl group



R = —C(=O)—H	Syringaldehyde
R = —C(=O)—CH_3	Acetosyringone
R = —C(=O)—OH	Syringic acid

Cinnamyl group



R = —H	p-Coumaric acid
R = —OCH_3	Ferulic acid

Appendix 5.1 - Sample calculation for estimating the $\delta^{13}C$ composition of sediment chemolysates (using equation 2 of Chapter 5)

A molecule of VAD has 8 carbon atoms. TMAH thermochemolysis results in addition of methyl groups (2 carbon atoms) from TMAH at the hydroxy and carboxyl groups (according to 1A) to produce 3,4-dimethoxybenzoic acid methyl ester. The derivatized product has 10 carbon atoms. The $\delta^{13}C$ of VAD and TMAH (measured by conventional mass spectrometry) are -28.5 and -44.6‰. The equation below is used to compute the predicted isotopic value of the derivatized product.

$$\delta^{13}C_{product} = [x]\delta^{13}C_{parent} + [1-x]\,\delta^{13}C_{TMAH}$$

In the equation, x is the fractional carbon contribution of the underivatized compound to the methylated product and has a value of 0.8.

$$\delta^{13}C_{product} = [.8 \times -28.50]‰ + [.2 \times -44.6]‰$$

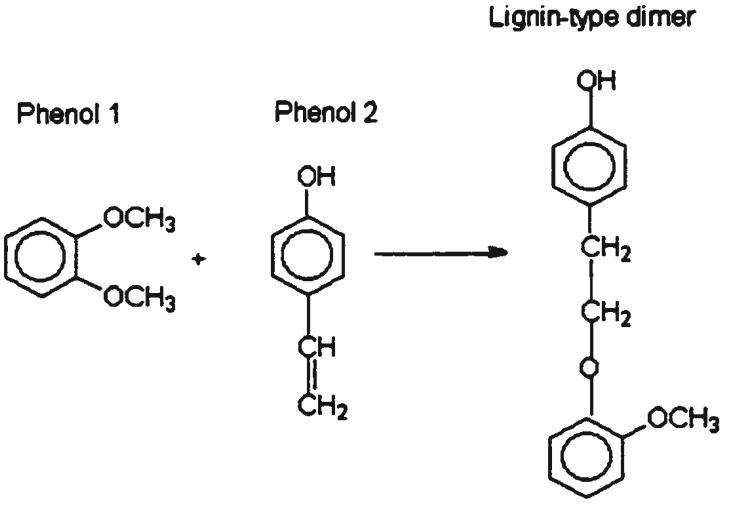
$$= -22.8‰ + [-8.92‰]$$

$$= -31.72‰$$

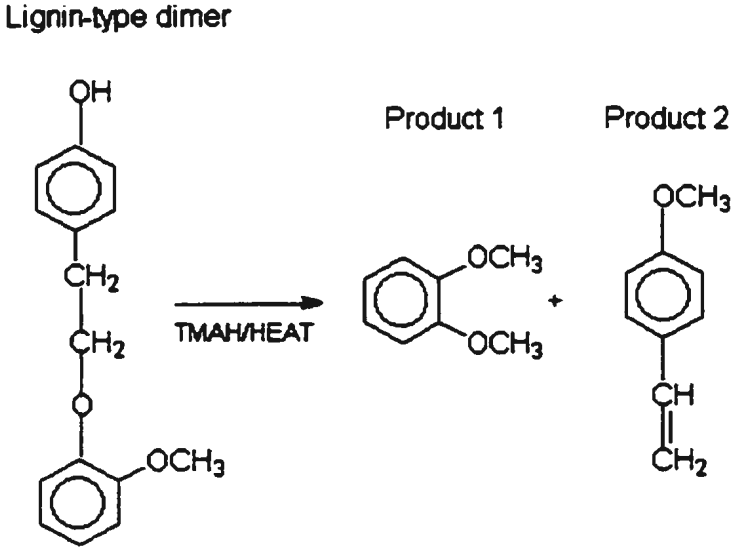
The purpose of CSIA on thermochemolysates is to determine the isotopic composition of the starting material ($\delta^{13}C_{parent}$). This is easily accomplished by re-arranging the equation and applying measured values of $\delta^{13}C_{product}$, $\delta^{13}C_{TMAH}$, and known value of x.

Appendix 5.2 - Synthesis and TMAH chemolysis of lignin dimer and isotopic compositions of reactants and products

Synthesis (performed by Dr. Phil Britt, Oak Ridge, Tennessee)



TMAH Chemolysis



Isotopic compositions

The isotopic compositions ($\delta^{13}C$) are as follows:

Dimer = -31.5 $^{0}/_{00}$ (measured by dual inlet mass spectrometry)

Phenol 1 (1,2-Dimethoxybenzene) = -25.8 $^{0}/_{00}$ (measured by dual inlet mass spectrometry)

Product 2 (Ethenylbenzene) = -38.8 $^{0}/_{00}$ (measured by GC/C/IRMS)

The dimer has 15 carbon atoms, of which 7 are derived from phenol 1. Therefore the remaining 8 carbon atoms are derived form phenol 2 (of unknown isotopic composition). The isotopic composition of phenol 2 can be computed as follows:

$$\delta^{13}C_{\text{Product 2}} = [(\delta^{13}C_{\text{Dimer}} \times 15) - (\delta^{13}C_{\text{Phenol 1}} \times 7)]/8$$

$$= [(31.63 \times 15) - (-25.83 \times 7)]/8$$

$$= [474.45 - 180.81]/8$$

$$= -36.7\ ^{0}/_{00}$$

Derivatization of phenol 2 produces product 2, which has one of nine carbon atoms from TMAH ($\delta^{13}C_{\text{TMAH}}$ = -44.6 $^{0}/_{00}$). The isotopic composition of product 2 is calculated as follows:

$$\delta^{13}C_{\text{Product 2}} = [(-37.58 \times 8) + (-44.6)]/9$$

$$= -37.6\ ^{0}/_{00}$$

The $\delta^{13}C_{\text{Product 2}}$, measured by GC/C/IRMS is -38.0 $^{0}/_{00}$. The difference between the expected and measured $\delta^{13}C_{\text{Product 2}}$ is within the analytical precision of the GC/C/IRMS system.

Appendix 6.1 - $\delta^{13}C$ composition of selected samples and composition of selected samples and compounds

Sample/Comments	$\delta^{13}C$ (‰)	Source
Seston (White Bay, Newfoundland)	-25.7 to -24.4	Ostrom and Macko, 1992
Phytoplankton "end members" (White Bay, Newfoundland)	-24.6	Ostrom and Macko, 1992
Plankton		
Bulk tissue	-20.2	Degens et. al., 1968
Lipid (Alcohol extract)	-21.1	
Lipid (CH_3Cl extract)	-29.8	
Pectins	-16.7	
Hemicellulose	-18.7	
Cellulose	-22.4	
Total sugars	-19.2	
Amino acids	-17.3	
Freshwater seston (White Bay, Newfoundland)	-25.5 to -24.9	Ostrom and Macko, 1992
Laminaria solidungula (White Bay, Newfoundland)	-20.3	Ostrom and Macko, 1992
Laminaria saccharina		
Bulk tissue	-18.4	Galimov, 1985
Lipid	-25.1	
Lupinus letus		
Bulk tissue	-27.6	Galimov, 1985
Lipid	-33.0	
Macroalgae	-20 to -12	Dienes, 1980
Sediment grabs (White Bay, Newfoundland)	-21.9 to -21.4	Ostrom and Macko, 1992

Sample/Comments	$\delta^{13}C$ (‰)	Source
POC (deposited) (Skan Bay)	-19.1 ± 0.2	Alperin et. al., 1992
POC (preserved) (Skan Bay)	-20.6 ± 0.1	Alperin et. al., 1992
Phytoplankton (Skan Bay)	-21.5 ± 0.1	Alperin et. al., 1992
Kelp	-16.4 ± 0.4	Alperin et. al., 1992
Sargassum		
Whole tissue	-16.1 ± 1.3	Kennicutt et. al., 1992
Lipid	-26.1 ± 0.09	
Zostera marina (live)	-10.2 ± 0.75	Thayer et. al., 1978
Zostera marina (dead)	-10.6 ± 0.75	
POC (from *Zostera* bed)	-19.4 ± 0.29	
Plankton		
Lignin*	-17.3	Degens et. al., 1968
Spartina alterniflora (C4)		
Bulk tissue	-13.2 to -12.6	Benner et. al., 1987
Hemicellulose	-11.6 to -11.2	
Cellulose	-12.3 to -11.1	
Lignin	-17.9 to -17.0	
Cynodon dactylon		
Bulk tissue	-18.7	Benner et. al., 1987
Hemicellulose	-14.5	
Cellulose	-18.2	
Lignin	-25.4	

Sample/Comments	$\delta^{13}C$ (‰)	Source
Pinus elliotii		
Bulk tissue	-25.3	Benner et. al., 1987
Hemicellulose	-24.3	
Cellulose	-23.8	
Lignin	-27.9	
Kalmia	-25.8	This study
Rush	–25.7	This study
Sphagnum	-36.6	This study
PAL		
Reed	-29.5	Galimov, 1985
VAL		
Reed	-29.9	Galimov, 1985
Wheat	-30.2	
SAL		
Reed	-30.6	Galimov, 1985
Wheat	-31.3	
OCH3 group of VAL		
Reed	-32.7	Galimov, 1985
Wheat	-32.4	
VAL		
Pine needles	-31.4	Goni and Eglington, 1996
Oak leaves	-35.0	
Spartina plant	-15.9	
Crab Creek sediment	-22.7	
VAD		
Pine needles	-34.5	Goni and Eglington, 1996
Oak leaves	-38.2	
Spartina plant	-19.0	
Crab Creek sediment	-22.9	

Sample/Comments	$\delta^{13}C$ (‰)	Source
CAD		
Pine needles	-24.9	Goni and Eglington, 1996
Oak leaves	-30.2	
Spartina plant	-13.2	
Crab Creek sediment	-21.1	
Fatty acids		
Blue-green algae	-31.9	Galimov, 1985
Krill	-28.1	
Lupine	-32.7	
Fatty acids (soils)		
C_{14}	-28.4	Litchfouse, 1995a
C_{16}	-25.0	
C_{18}	-24.8	
C_{20}	-29.5	
C_{21}	-29.9	
C_{22}	-30.7	
C_{23}	-31.5	
C_{24}	-30.0	
C_{25}	-32.9	
C_{26}	-32.5	
C_{27}	-34.5	
C_{28}	-33.9	

* The "lignin fraction" refers to aromatic metabolites, chiefly hydroxybenzoic acids and can be regarded as arbitrary analogs of lignin in higher plants (Galimov, 1985).

Abbreviations are as follows:

PAL - Benzaldehyde
VAL - Vanillin
SAL -Syringealdehyde
VAD - Vanillic acid
CAD - Coumaric acid
OCH_3 - methoxy group

Appendix 6.2 - **$\delta^{13}C$ of TOC of the Northwest Arm and Trinity Bay cores (carbonate free)**

Spatial variation

H9	-22.9
H8	-23.2
H7	-22.3
H6	-22.7
H5	-23.4
H1	-24.3
ST11	-22.6
ST10	-21.6
ST9	-20.9
ST7	-21.7

Temporal variation

Depth	H9	H1	ST7
0	-22.9	-24.5	-21.7
10	-23.1	-23.6	-21.6
20	-22.7	-23.4	-21.4
30	-22.8	-23.9	-20.8

Appendix 7.1 - **Comparison of TOC data sets**

Comparison of abundances (mg per 100 mg of OC) of selected chemolysates of the study area with those of Minnesota peat

Sample	H9	H7	H1	ST10	ST7	Peat[1]
Chemolysate						
PAD	0.03	0.06	0.10	0.00	0.00	0.6
VAL	0.03	0.02	0.09	0.00	0.00	0.15
VAD	0.18	0.15	0.47	0.06	0.03	1.28
CAD	0.10	0.09	0.09	0.21	0.11	0.2
C16	1.30	0.83	1.89	1.47	0.38	0.19
C20	0.25	0.18	0.72	0.11	0.03	0.14
C24	0.45	0.30	0.67	0.29	0.06	0.13
C28	0.11	0.06	0.22	0.14	0.00	0.06
Totals						
Phenols	0.34	0.31	1.07	0.27	0.14	5.78
Fatty Acids	4.52	3.75	7.43	6.98	1.43	3.44[2]

[1] Humic acid of Minnesota peat (Del Rio *et al.*, 1998)
[2] Sum of fatty acids and fatty accohols

Comparison of range of abundances (mg per g of OC) of selected chemolysates of the study area with those of particulate organic matter (POM) of the Delaware Estuary

Chemolysate	This study	Delaware Estuary[3]
1,2,4-Trimethoxybenzene	0.6[a] to 2.5[b]	1.21 to 6.25
Lignin-derived phenols[c]	0.14[d] to1.07[a]	0.15 to1.23
Tetracosanoic acid (C_{24})	0.6[d] to 2.15[a]	0.15 to 0.55[e]
Hexacosanoic acid (C_{26})	0.6[d] to 1.36[a]	0.05 to 0.3

[a], [b], [d] indicates surface abundances of core H1, H9 and ST7
[c] indicates the sum of the p-hydroxy, syringyl and vanillyl lignin phenols
[e] estimated form Figures
[3] POM of the Delaware Estuary (Manino and Harvey, 2000)