Measurement of lignin and organic carbon in Lake Mead sediment

Ekhlas Lisa Nemr
University of Nevada, Las Vegas

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MEASUREMENT OF LIGNIN AND ORGANIC CARBON
IN LAKE MEAD SEDIMENT

by

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Bachelor of Science
University of Alexandria, Egypt
1969

A thesis submitted in partial fulfillment
of requirements for the

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Measurement of Organic Carbon and Lignin in Lake Mead Sediments

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ABSTRACT

Measurement of Lignin and Organic Carbon in Lake Mead Sediments

by

Ekhas Lisa Nemr

Dr. Spencer M. Steinberg, Examination Committee Chair
Professor of Chemistry
University of Nevada, Las Vegas

Lignin distribution in sediment is a useful tool for tracing the transport of land derived organic matter in an aquatic environment. Tetramethylammonium hydroxide (TMAH) thermochemolysis at sub-pyrolysis temperatures of 300 °C followed by GC-MS analysis showed a great promise in being able to semi quantitatively assess the nature of organic carbon in sediments. TMAH chemopyrolysis produces methylated syringyl (S), vanillyl (V), and cinnamyl (C) phenols from lignin, these methylated phenols are an indirect measure of lignin in sediment. The concentration of lignin phenols is indicative of the contribution of terrestrial runoff to lake sediments because lignin is exclusively a product of land plants. The S/V ratio is a powerful tool to trace the terrestrial origin of organic matter in sediment.

Two chemolysis methods (on-line and off-line) were examined and compared. Organic carbon and lignin in several cores from Lake Mead were investigated. The results from sediment cores indicate significant variations in the sources of sedimentary materials. The concentration of organic carbon and the composition and amount of lignin provides an interpretable record of historical changes.
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CHAPTER 1

INTRODUCTION

1.1 The Macromolecule Lignin

Lignin is one of the most abundant natural polymers in vascular plants. This biopolymer occurs as a coating on cellulose and gives plants structural integrity. Lignin is produced almost exclusively in land environments (Table 1.1).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>60%</td>
</tr>
<tr>
<td>Sugars, Starches</td>
<td>5%</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>5-30%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>15-60%</td>
</tr>
<tr>
<td>Organic acids (amino, nucleic)</td>
<td>5%</td>
</tr>
<tr>
<td>Protein</td>
<td>2-15%</td>
</tr>
<tr>
<td><strong>Lignin</strong></td>
<td><strong>5-30%</strong></td>
</tr>
<tr>
<td>Fats, Waxes</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table 1.1: The major kinds of organic materials in plants (Biology classes, Oregon University, 2002)

Most plant material detritus is rapidly mineralized in sediment by bacteria. Lignin is very complex and is only slowly metabolized. Lignin persists in sediment for long
periods and is thought to be major contribution to humic acids. When lignin is completely mineralized, the result is CO$_2$ + H$_2$O. A multitude of enzymes are needed for lignin degradation. The initial reactions are mediated by extra cellular lignin and manganese peroxidases, primarily produced by white-rot fungi. In nature, lignin is rarely broken down completely; instead, various lignin fragments that remain, and may be slowly converted to lower molecular weight fragments over time. This results in humus (humic acid, humin, and fulvic acid) formation, which is: dark-colored soil organic matter, about 50 % carbon, 5 % nitrogen, and 0.5 % phosphorus, and colloidal. Humus is negatively charged due to the carboxylate and phenolate contents. Humus consists of different components that enumerated in table 1.2.

<table>
<thead>
<tr>
<th></th>
<th>Humin</th>
<th>Humic acid</th>
<th>Fulvic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td>large</td>
<td>medium</td>
<td>small</td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td>dark</td>
<td>medium</td>
<td>light</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>insoluble</td>
<td>soluble at high pH</td>
<td>soluble</td>
</tr>
</tbody>
</table>

Table 1.2: Humus components according to size, color, and solubility

The common characteristics of humus components are their high molecular weights and their backbone structure consisting of characteristic molecular substructures. Lignin as an example is a cross-linked polymer of phenyl propane subunits.
1.2 History of Lignin

Investigations of lignin started in 1838 when Anselme Payen recognized the composite nature of wood (McCarthy, 2000). He found that treatment of wood with nitric acid then an alkaline solution yielded a major insoluble fibrous residue that he called "cellulose", and dissolved incrusting material (carbon-rich substance) which embedded the cellulose in the wood. The term "lignin" was introduced by Schulze in 1865 (McCarthy, 2000). Kalson, Freudenberg, Nimz, Adler, Hibbert, Kratzl, Tischeno, Nakano and others conducted pioneering classical organic chemistry, research that revealed much fundamental knowledge about the chemistry structure of lignin (McCarthy, 2000). In 1907 Kalson demonstrated lignin to be high molecular substance, he observed that the removal of carbohydrates from wood by hydrolysis with strong sulfuric acid yields a brown-colored lignin product as insoluble residue (Adler, 1977). During the period of 1926-1932 numbers of structural hypotheses were published by Freudenberg Durr (Adler, 1977). Figure 1.1 shows one of these proposals. According to that proposal, the monomer is converted by C-C condensation into a polymer and the latter reacts further by loss of water to give the cyclic phenyl ether structure.
During the period 1970-1980, factual evidence of the level of natural organic carbon carried by terrestrial streams has been slowly gathering, but increases in the understanding of chemical characteristics of the complex natural products have been modest (Christman, 1981).

Throughout the following decades until present, many researchers concentrated on improving laboratory methods to detect and quantify lignin. The methods include permanganate oxidation and nitrobenzene oxidation, CuO oxidation, and recently thermochemolysis.

Lignin phenols can be classified as syringyl, vanillyl, or cinnamyl phenols. All vascular plants contain vanillyl phenols in their lignin structure. Angiosperms also contain syringyl phenols, while gymnosperms lack syringyl phenols. The ratio syringyl/vanillyl (S/V) in lignin samples provides information on the type of plants that
produced the lignin. S/V ratio can be measured by degrading the macromolecules into low-molecular-mass compounds that can be analyzed by GC/MS. When lignin degrades, a cocktail of phenolic compounds is produced (Figure 1.2), (Hyötyläinen et al., 1997).

Syringyl phenols:

- Syringyldehyde
- Acetosyringone
- Syringic acid

Vanillyl phenols:

- Vanillin
- Acetovanillone
- Vanillic acid

Cinnamyl phenols:

- p-coumaric acid
- Ferulic acid

Figure 1.2 the main products of lignin degradation
Information at the molecular level can be achieved by degrading the macromolecules into low-molecular-mass compounds that can be analyzed by gas chromatography mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC), (Hatcher et al., 2003).

1.3 Use of Lignin

Lignin is a product recovered from paper-pulp sulfite liquor; it is a complex material, C_{41}H_{32}O_{6} with four hydroxyl and four methoxyl groups per formula unit depending on origin (Angiosperm or Gymnosperm). Lignin melts at 250 to 275 °C and can be chemically modified and used as stabilizer against biodegradation for bio-degradable polymers and as stabilizer for thermal oxidation of rubbers, lignin also used in plastics, corrosion inhibitors adhesives coatings and compressed wood products (Wilson, Newsgroups, 1997).

1.4 Pyrolysis Gas Chromatography Mass Spectrometry (PY/GC/MS)

Relatively new results show that characteristic lignin monomers could be obtained by using pyrolysis/GC/MS at temperature ~ 600 °C (J. Challinor et al., 1991). The high temperature decomposes the original polymer into a mixture of products. Some of the products retain information about the parent structures. The chromatogram of pyrolysis products is called pyrogram and pyrolysis products that correlate with polymer structure often referred to as chemical markers. Coupling pyrolysis to high-resolution capillary GC separation (Py-GC), followed by mass spectrometry (Py/GC/MS), has proven to be a
powerful tool for characterization and identification of wide variety of polymeric materials.

Several advantages recommend the use of pyrolysis GC for polymer analysis: minimal sample preparation is usually required, and only a small amount of sample is used. Chemolysis procedures involve a chemical reaction between the polymer sample and a suitable reagent. McKinney et al., 1997 reported that thermochemolysis in combination with tetramethylammonium hydroxide (TMAH) at much lower temperature (350 °C) showed great promise in being able to semi quantitatively assess the amount and levels of degradation of lignin.

It has been proposed that the chemolysis of lignin by TMAH is thermally assisted chemolytic reaction (de Leew and Bass, 1993; Clifford et al., 1995; McKinney et al., 1995). Based upon work with natural lignin and model lignin dimers, Hatcher and Mirand (1995) concluded that hydrolysis of the β-O-4 ether bond, considered to be the principal structure linkage in lignin (Adler, 1977), results in the formation of carbocation at the β carbon of the propyl side chain of lignin monomer. Subsequent elimination reactions account for the wide but highly reproducible, distribution of chemolytic products. Filley et al., (1999) reported that the hot alkaline conditions of TMAH thermochemolysis suggest that chemolysis mechanism is more akin to that proposed for the degradation of alkyl-aryl ether bonds during alkali (soda) pulping (e.g. aqueous NaOH, pH 13-14, 155-175 °C) (Gierer 1970). Gierer demonstrated that alkali pulping cleaves the β-alkyl-aryl ether bonds in lignin via the formation of an epoxide intermediate with an intermolecular nucleophilic displacement of the β-phenoxide by either the α or γ alkoxide formed from the alcohol in strong base. Subsequent nucleophilic attack by
hydroxide cleaves the epoxide ring and produces a trihydroxy propane structure. Figure 1.3 shows the mechanism proposed by Gierer (1970) and supported by Filley (1999), for the cleavage of β-O-4 linkage in lignin during alkali (soda) pulping of lignin. The reaction shows the β-O-4 ether bond cleaved by an intermolecular epoxide formation.

![Chemical structure](image)

**Figure 1.3** Mechanism proposed by Gierer (1970), supported by Filley (1999) for the cleavage of β-O-4 linkage in lignin

1.5 Lignin in Lake Mead Sediment

Lake Mead is the largest reservoir on the Colorado River, it was created by Hoover Dam in 1934. Lake Mead covers approximately 593 square kilometers. More than 20 million people depend on Lake Mead and the lower part of Colorado River for domestic and agriculture water (LaBounty et al., 1997). Urban runoff, wastewater, and suspended solids (including terrestrial plants) enter the Las Vegas bay and Boulder basin of Lake Mead via the Las Vegas Wash (19 km). The Las Vegas Wash episodically develops rapid
flow carrying suspended sediment into the lake. These episodic events are potentially recorded in the sedimentary record.

Several reports have indicated that Lake Mead receives a significant variety of pollutants (Steinberg, 2002). Pollutants such as (NO$_3^-$) and (PO$_4^{3-}$) are nutrients that promote algal and phytoplankton growth. Other pollutants such may be endocrine disruptors that interfere with the reproductive cycle of fish and birds (Giesy, 1998). Some of the pollutants could be a result of human activities such as fishing and boating in the Lake.

Lake Mead sediments have been investigated for lignin and organic carbon. In the present study, several cores were taken by vibracore (VC) or gravitycore (GC) methods from Boulder Basin region of Lake Mead (Figure 1.1)
The occurrence of lignin phenols in sediment chemo pyrolysates indicate the contribution of terrestrial runoff to Lake Mead sediments from Las Vegas valley via Las Vegas Wash. Core VC1, which was taken near to the mouth of Las Vegas Wash shows dramatic fluctuation in organic carbon and lignin. On the other hand core GC26 (~12 kilometers) far from the mouth of Las Vegas Wash lower concentrations of OC and no lignin (Steinberg, 2002). One explanation for this phenomenon is that turbidity flows have episodically transported coarse material long distance into the lake (Zybala 2002). The presence of lignin in the sedimentary core is indicated by the occurrence of 165 m/e and 195 m/e mass fragments at the retention times corresponding to the methylated lignin.
phenols. The results from 2 cores indicate significant variations in the sources of sedimentary materials over the last 70 years.

Lignin peaks in core VC1 may record periodic changes in erosion patterns in the drainage basin. The composition and amount of lignin provides an interpretable record of historical changes in lake sediments.

The method chemopyrolysis involves heating organic materials in the presence of TMAH. This procedure results in the decomposition of organic matter to simpler products and methylation of these decomposition products so that they are amenable to gas chromatography mass spectrometry (GC/MS).

The objectives of this thesis are:

- To quantify organic carbon (OC) and lignin in sediment cores from Lake Mead.
- To evaluate two chemopyrolysis methods for determining lignin and to compare results from these methods in several cores.
- To supply data, useful for understanding of sediment dynamics.
CHAPTER 2

LITERATURE REVIEW OF LIGNIN DEGRADATION METHODS

In recent decades, several procedures have been developed for analysis of lignin and organic matter. However a lot of studies still use the traditional procedures such as CuO, nitrobenzene oxidation with the steps of extraction, purification, and derivatization prior to gas chromatography analysis. Recently rapid methods based upon thermochemolysis or chemopyrolysis have evolved. These rapid methods result in an ability to process more samples with results similar to conventional methods and create new research possibilities (quantitative/semi quantitative analysis of lignin and organic matter in waters, plants and sediments).

2.1 Permanganate Oxidation

A procedure based on permanganate oxidation was presented by Reuter et al., (1981). A sample of aquatic humus (0.5 g) was dissolved in minimum amount of water. To this solution, aqueous potassium permanganate (KMnO₄) solution (4% 125 ml) was added, and the mixture was heated slowly. The reaction mixture was refluxed for 8 hours. Excess permanganate was then destroyed with methanol and the mixture was filtered. The black residue (MnO₂) was washed with dilute NaOH, the filtrate acidified to pH 2 and continuously extracted for 4 hours each with ethylacetate (300 ml) four times. The
organic solvent was separated, dried (Na$_2$SO$_4$) and evaporated to leave a residue which was methylated with diazomethane in ether.

Freudenberg et al., (1936-1938) heated spruce lignin (spruce wood) with 70% aqueous potassium hydroxide in order to bring about hydrolytic cleavage of ether linkages and subsequently protected the phenolic groups liberated by methylation. Potassium permanganate (KMnO$_4$) oxidation of the methylated product at pH 6-7 gave veratric acid in a yield of about 8% of the lignin and minor amount of isohemipinic and dehydro-deveratric acids as shown in figure 2.1.

![Figure 2.1: KMnO$_4$ oxidation scheme](image_url)

2.2 Nitrobenzene Oxidation

Nitrobenzene oxidation of humic substances (from sediments and waters) was performed by Pempkowiak et al., (1981). The samples (50-100 mg) were transferred to glass reaction vials, 1 ml of nitrobenzene and 5 ml of 2 $M$ NaOH solution were added. The vials were placed in a constant-temperature block and held for four hours at 170 °C. The contents of each vial were washed into a separatory funnel (100 ml) using an equal volumes of 2 $M$ NaOH, the solids were suspended, centrifuged, and the supernatant was added to the content of the separatory funnel. The solution was extracted twice with
methylene chloride (2 x 5 ml) and CH$_2$Cl$_2$ layer discarded. The aqueous layer was acidified to pH 1 with 5 ml of 6 M HCl and the phenolic products were extracted into methylene chloride (2 x 5 ml). The CH$_2$Cl$_2$ extract was dried over CaSO$_4$, then filtered and washed (10 ml CH$_2$Cl$_2$) in Buchner funnel with a filter. The filtrate was reduced in volume to 500 μl under N$_2$. An aliquot (2 μl) of this solution was injected into the gas chromatograph.

### 2.3 CuO Oxidation

The basic procedure used in many studies were reported by Hedges et al, 1979-1984. The degradation is carried out in Teflon vessel at temperature 170° C under nitrogen atmosphere for 3 hours (2 g sediment, or 30 mg wood, 1.0 g CuO, 0.1 Fe(NH$_4$)$_2$ (SO$_4$)$_6$·(H$_2$O), and NaOH (2M)). The content then centrifuged and washed with distilled water for three times, acidified with HCl and extracted for three times with ether. The sample is concentrated by evaporation, derivatised by silation, and then analyzed. This method is used in many studies. Steinberg et al., 1984, reported the results from analysis of the products of the oxidation of lignin by CuO in different samples (wood, peat, and marine sediment) using reverse phase high-performance liquid chromatography (HPLC). The authors reported that a comparison of their results with Hedges et al., 1984, GC analysis. The differences in the proportions of the aldehydes, ketones, and acids could be due to the differences in the oxidation conditions. The recovery experiments reported in the study demonstrate the stability of phenolic compounds (under the conditions used). The procedure for oxidation of lignin with cupric oxide was applied successfully to a variety of sample types.
Hetherington. et al. (1997) reported a developed simplified procedure for characterization of lignin by CuO oxidation which saves approximately 40% of sample preparation time and double the number of samples which can be processed with the same period of time. The authors performed the method described by Kögel and Bochter (1985) with modifications by Guggenberger and Zech (1994). The authors investigated four different materials, two plant litters, (sweet chestnut from mixed woodland, heather from moorland). The two soil samples were from the mineral (A1) horizon beneath sweet chestnut and surface organic matter (Ao) from heather moorland. The analysis results show that lignin signatures of the chestnut and heather litter were dominated by vanillyl and syringyl derivatives as in typical of angiosperms (Sarkanen and Hergert, 1971; Campbell and Sederoff, 1996). The soils have signatures similar to those of parent plant materials with dominant concentrations of syringyl and vanillyl moieties. The quantity of each derivative recovered from soil samples showed no significant effect of using the original method of CuO oxidation and the modified (rapid) method. The rapid procedure produces comparable results with the original procedures.

2.4 Pyrolysis/GC/MS

Gas chromatography (GC) is very important method in the analysis of organic compounds. A wide variety of columns and detectors are available for use with GC, giving the method the ability to separate a wide variety of compounds.

Pyrolysis at high temperature ~ 600 °C decomposes the original polymer into a mixture of products. Some of the products retain information about the parent structures. The chromatogram of pyrolysis products is called pyrogram and pyrolysis products that
correlate with polymer structure often referred to as chemical markers. Coupling pyrolysis to high-resolution capillary GC separation (Py-GC), followed by mass spectrometry (Py/GC/MS), has proven to be a powerful tool for characterization and identification of wide variety of polymeric materials.

Several advantages recommend the use of pyrolysis GC for polymer analysis: minimal sample preparation is usually required, and only a small amount of sample is used.

Struczynski et al., 1997 investigated the use of pyrolysis GC/MS for assessing changes in soil organic matter quality. The authors reported that Py/GC/MS technique hold promise as a rapid procedure for detecting changes in the organic matter composition in the soils, which contrasts with the often laborious chemical methods used commonly for analyzing organic matter. To evaluate Py/GC/MS for this use, the authors characterized changes in the organic matter composition of bed ash-amended soils by both chemical and pyrolytic analysis. The chemical analysis of the soil samples had shown substantial degradation and loss of organic matter as a result of the ash amendments, and the pyrolytic analysis reflected these losses of soil organic matter by decrease in the total peak areas within the total ion chromatogram. Information on the changes in composition of soil organic matter was reflected by changes peak area for individual pyrolytic products, and the identity of more than 40 pyrolytic products was determined from mass spectral data. The authors concluded that the good agreement between classical chemical analysis and pyrolytic analysis indicates that Py/GC/MS can be use to monitor changes in the organic matter composition of soils.

Pyrolysis, in combination with TMAH was first introduced by Robb and Westbrook
(1963). The method has been termed thermally assisted hydrolysis and methylation (THM), (Challinor 1989). Because the method combines thermal decomposing chemical by decomposition and methylation, it has also been referred to as chemopyrolysis. THM-GC was successfully applied to the analysis of glycerol-bound fatty acids in fats, and oils as well as in several kinds of microorganism. For example, Dworzanski et al., 1991, converted fatty acids to the corresponding methyl esters in the hot injection port of a gas chromatograph. Classes of compounds investigated with this technique include synthetic polymers, fats, oils, coals, humic acids, microorganisms (Haken et al., 1996) and different macromolecules containing polar functionalities such as carboxylic acids and phenolic compounds. A review of the chemistry and application of the type of methods was published by Kossa et al., (1979).

Pyrolysis with in situ methylation has become a powerful technique for structural analysis of macromolecules. The application of TMAH as methylating reagent has been extended to synthetic polymers since Challinor (1989). He described in situ derivatization of polyesters under flash heating conditions using an external pyrolyzer. The technique was applied to several biopolymers (Del Rio et al., 1996), humic substances (Martin, 1994), or whole soil organic matter (Schulte et al., 1995).

Asperger et al., (1999) presented a study of advances in the analysis of natural waxes (mainly high molecular weight), using thermally assisted hydrolysis and methylation (THM) in combination with GC-MS, in the presence of TMAH. The mechanism of THM method is based on the alkaline character of the TMAH promoting the hydrolysis of waxy esters and the formation of quaternary ammonium salts of the carboxylates, which are thermally converted into the corresponding methyl derivatives (de Leeuw and Bass,
1893). Chemopyrolysis resulted in a chromatogram showing very specific signal patterns for each of the material investigated. Mass spectrometric detection of the methyl derivatives enabled the identification of more than ninety compounds present in the wax samples. The investigations show that THM-GC-MS is able to overcome many problems that arise from conventional Py-GC analysis of waxes, where thermal fragmentation can lead to non-specific pyrolysis products. Therefore, THM-GC-MS is an advantageous technique for comprehensive analysis of natural waxes. The authors indicated that there are no significant differences in the methylation yields within a temperature range between 350 °C and 600 °C. The conclusion of the Asperger and other study is that THM products reflects the very complex wax composition in a much more detailed form compared to the results obtained from other analytical techniques and can therefore, be considered valuable tool for advanced wax analysis.

2.5 TMAH-Thermochemolysis-GC-MS

Chemolysis procedures involve a chemical reaction between the polymer sample and a suitable reagent. McKinney et al, 1997 reported that thermochemolysis in combination with TMAH at much lower temperature (350 °C) showed great promise in being able to semi quantitatively assess the amount and levels of degradation of lignin. The TMAH thermochemolysis technique was performed by McKinney et al., (1996) to study the aliphatic biopolymer cutan, (which is derived from the leaf cuticles of Agare americana). The cutan biopolymer yielded fatty acid methyl esters (FAMES) of varying carbon length and a benzenecarboxylic acid methyl ester that point to the possibility of a chemical structure containing functionalized benzene rings. This type of structure is absent from
the current structural model of cutan. Under conventional flash pyrolysis conditions, the dominant products were alkanes, alkenes, and \( \alpha \)-\( \omega \)-alkadienes. Py-GC-MS data (without using TMAH) does not show any contribution from these oxygenated benzene structures (Tegelaar et al., 1989). This may be due to a number of possibilities. (a) The TMAH thermochemolysis procedure is more likely to produce oxygenated types of structures, (b) The relative contribution of these oxygenated functionalized rings is small (8%) in comparison to the aliphatic contribution so they are not seen by conventional pyrolysis or (c) Conventional flash pyrolysis when applied to functionalized alkyl chains, is more likely to produce alkane/alkene moieties. In piecing together the analytical data collected in this study, the authors have devised a new structural model for the cutan polymer, which take into account Py-GC-MS, \(^{13}\)C NMR, and TMAH thermochemolysis results.

Pulchan et al., (1997) studied the characterization of TMAH thermochemolysis products of near-shore marine sediments using gas chromatography/combustion/isotop ratio mass spectrometry (GC/C/IRMS) in order to examine the usefulness of TMAH thermochemolysis products in identifying organic markers of terrestrial-derived sources in marine sediments. Thermochemolysis conditions (excess TMAH, 250 °C, sealed glass tube, 30 min) were used on sediments and standards. The authors reported the first attempt to: (a) characterize organic matter in marine sediments using TMAH thermochemolysis and (b), analyze the isotopic composition of TMAH thermochemolysis products. The use of TMAH as a thermochemolysis reagent has one drawback in that it does not allow for the determination of presence of methoxy groups in the original structure of the compound. del Rio et al., 1998, presented a study of the structural characterization of bio- and geo-molecules using off-line TMAH thermochemolysis.
technique. The authors examined a set of macromolecules originating from gymnosperm and angiosperm woods, natural polyesters such as cutin, dissolved organic matter in natural and oceanic waters, and humic substances. TMAH thermochemolysis was performed in sealed glass ampoule; the procedure consists mainly of a thermally assisted chemolysis and subsequent methylation. Methylation of carboxylic and hydroxyl groups renders the chemolytic products more amenable to GC separation. TMAH thermochemolysis induces cleavages of the β-O-4 ether bond in lignin. This is significant because it shows the potential to characterize lignin-derived compounds similar to the CuO oxidation and the same ratios of lignin-derived phenols can be determined. Unlike pyrolytic methods, TMAH avoids decarboxylation of benzcarboxylic acids, and produced more products, with intact or any partially altered side chains, therefore, giving a better insight into molecular structure of bio-geo-polymers.

Guignard et al., (2000), presented a study of the structural characterization of humic substances from an acidic peat, using TMAH thermochemolysis techniques. Humic acid and humin were investigated, this technique yielded various ranges of hydrocarbons, fatty acid methyl ester and αω-dicarboxylic acid methyl esters. In both cases, pyrolysates contain high amount of polar macromolecular components and aromatic products. The presence of TMAH avoids decarboxylation of aromatic acids and as consequence prevents the loss of important structural information. Many of the pyrolysis products also become more volatile and more easily analyzed by GC-MS upon methylation. Aromatic compounds represent the major portion of identifiable compounds in thermochemolysis products of both humin and humic acids. The authors reported that infrared and NMR spectroscopy indicate that structural and functional similarities exist between humin and
humic acids but important differences can also be found in their lipid constituents.

Chefetz et al., 2000 employed TMAH thermochemolysis to study the chemical structure of soil organic matter sampled from a soil plot in which corn (Zea mays L.) was farmed continuously for 15 years. The chromatograms exhibited peaks related to compounds derived from lignin and fatty acid methyl esters (FAMEs). With depth, the ratio of syringyl to vanillyl compounds (S/V) decreased, suggesting a preferential degradation of the syringyl by microorganisms. The decrease of S/V ratio and the relative increase of aromatic and carboxylic C-containing groups exhibited in the $^{13}$C-NMR spectra support the theory that side-chain oxidation of lignin structures is one of the major humification processes occurring in soil. Fatty acids methyl esters of varying C-chain length (C$_7$ to C$_{27}$) were identified in the soil chromatogram. The TMAH thermochemolysis indicated the presence of a relatively large fraction of long-chain fatty acids that seem to be highly resistant to biological degradation. The authors concluded that the changes identified in the chemical components provide clues as to the nature of the humification processes in the soil profile and also yield information on the nature of sources of soil organic matter.

We conclude from the above studies, that the mechanism of thermally assisted TMAH reaction of acid includes at least two steps. Saponification yielding the TMA salts of the original ester constituents, and subsequently, the conversion of the saponification products to the corresponding methyl esters and methyl ethers, respectively, by the nucleophilic attack of phenolic and carboxylic anions to one of the methyl groups of TMA cation (De Leeuw et al., 1993). Therefore, the method has been termed thermally assisted hydrolysis and methylation (Challinor et al., 1995). THM-GC technique was
successfully applied to the analysis of glycerol-bound fatty acids in fats and oils as well as several kinds of microorganism. de Leeuw and Bass, 1993, and Martin et al., 1994 reported that the reaction involved in TMAH pyrolysis scheme is one of chemolysis, rather than pyrolysis by showing that the technique is equally as effective at temperatures below conventional pyrolysis conditions of 300-310 °C (Hatcher and Clifford, 1994). These authors have shown that pyrolysis of humic acids in the presence of TMAH, in comparison to Py-GC-MS of the same samples, leads to the identification of a larger quantity of structurally significant compounds. Thus, polar functionalities, which were poorly chromatographed under Py-GC-MS conditions, are better resolved and identified using TMAH. Pulcan et al., 1997 reported that the thermochemolysis reactions with TMAH can be conducted in a sealed glass tube (instead of pyrolysis system), thus allowing for more controlled conditions. Internal standards can be added to provide quantitative measurements and where the products can be easily isolated for alternate analysis.

2.6 Lignin as a Biomarker

Lignin is used as a biomarker to study various kinds of sediment deposition process. Historical trends in seagrass abundance (Thalassia testudium) in dated sediment cores from Florida Bay, Bob Allen key and Pass key were studied, using lignin phenols as a proxy (William et al., 2002). The total lignin phenol contents of sediment from Florida Bay at Pass key and Bob Allen key sites ranged from 0.2 to 3 μg/mg OC. The higher lignin phenol concentrations at Pass key reflect the influence of mangrove-derived lignin transported from the nearby coastal zone mangrove forest. Down-core variability in total
lignin content was relatively high at Pass key probably reflecting variability in the flux of mangrove–derived lignin from the coastal zone. Values of S/V ratios ranged from 0.1 to 0.6 indicating that the sediments at the sites contained a mixture of lignin derived from seagrass and mangroves. Values closer to 0.1 reflected a greater dominance by seagrass. Values closer to 0.6 reflected a greater dominance by mangroves. S/V values began to rise from the mid 1940’s to early 1970’s suggesting a moderate die-off in seagrass abundance. After the early 1970’s S/V decreased indicating an increase in seagrass abundance over this most recent period. In summary seagrass has very low levels of syringyl, phenols (S/V at 0.02) vs. mangrove (S/V > 0.5). Lignin phenols in sediment, provides a record of historical changes in seagrass abundance. Recent seagrass die-off may result from anthropogenic change to the ecosystem.

Fisher et al., 2003, presented an interesting study of the sediments from Gormire Lake (Yorkshire, UK). The site was chosen for the study because of the fact that its catchment has undergone periodic land use change and deforestation during the Holocene period. The authors considered whether the distributions of molecular markers in lake sediments reflect the sources of OM to the sediment and whether molecular parameters can act as a proxy for change in catchment vegetation. The TMAH thermochemolysis technique was used for characterization of lignin in dated sediments (14C labeled) from different locations, and depths (Table 2.1).
Table 2.1: Location and depth (cm) of dated samples from Gormire Lake (Fisher et al., 2003)

<table>
<thead>
<tr>
<th>Samples location</th>
<th>Depth in cm</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPZ4 (zone 4)</td>
<td>17 cm</td>
<td>1200-1970</td>
</tr>
<tr>
<td>LPZ3 (zone 3)</td>
<td>91 cm</td>
<td>800-1200</td>
</tr>
<tr>
<td>LPZ2 (zone 2)</td>
<td>137 cm</td>
<td>600 BC-800 AD</td>
</tr>
<tr>
<td>LPZ1 (zone 1)</td>
<td>261 cm</td>
<td>1100-600 BC</td>
</tr>
</tbody>
</table>

The yield of lignin products relative to TOC varied between zones. The highest levels in zone 2 reflect the significant change in nature of the material deposited in lake sediment from woody to grass dominated which is contemporaneous with deforestation ~600 BC.

The authors concluded from the study that the chemolysis of lignin confirms the significant change in the nature of material deposited in the lake. Bulk sedimentary OM composition (lignin) reflects changing catchment vegetation. Organic geochemistry of Gormire reflects its' changing environment over the past 3000 years.
CHAPTER 3

EXPERIMENTAL

3.1 Samples Collection

Sediment cores were collected from Las Vegas Bay and Boulder Basin of Lake Mead using a vibrating corer system and gravity corer system shown in Figures 3.1 and 3.2.

Figure 3.1: A: Coring devices (Vibrocore), B: Schematic diagram of Electric Vibrocore
Core liners were aluminum for vibracores (VC) and poly carbonate for gravity cores (GC). Core liners are marked in cm intervals so as to provide a length measurement of the core section. After retrieval, cores were cut to sections, capped, length measured, labeled, logged, and stored in a cooler until returned to a central cold storage location. Initial processing consisted of splitting the cores vertically with circular saw and then photographing. Detailed descriptions of core appearances, textures and sediment characteristics, with depth, as well as a notation of any unusual properties (i.e. wood chips) were entered in the samples log. Split cores were wrapped in plastic (to prevent desiccation/drying), and returned to cold storage. For chemical analyses, samples were taken every other 1.0 cm or every 2.0 cm starting at the top of the core. Samples were collected using a metal spatula cleaned between each
sampling with ethanol. The samples were placed in sterile glass containers, labeled and stored until performing chemical analyses. Coring and core description were performed by investigators from UNLV Geosciences and USGS (David C. Twichell; Robyn A. Howley; Andrew D. Hanson). The core descriptions entered by T. Johnson and J. Zybala. Figure 3.3 presents the description of VC1; the corresponding photograph is shown in 3.3 B.
**LEGEND**

<table>
<thead>
<tr>
<th>Sandstone</th>
<th>Claystone</th>
<th>Clayey sand</th>
<th>Siltstone</th>
<th>Silty claystone</th>
<th>Silt/sandstone</th>
<th>Silt/sandy siltstone</th>
</tr>
</thead>
</table>

**SHADES**

- Light grey
- Medium grey
- Dark grey
- Black
- White

**CONTACTS**

- Contact plane

**GRAIN SIZE**

- Fine sand
- Medium sand
- coarse sand
- gravel
- cobbles
- boulders

Data logged: June 17, 2020
Logged by: Hickson, Ho (entered by T. Johnson)
Datum elevation: 0.00 m
Remarks: SE of LV bay marina

**Figure 3.3: VC1, A: Core description, B: Section of corresponding photograph**

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3.2 Instrumentation

GC-MS analyses of chemolysis products were performed using Varian Saturn III (Figure 3.3) GC/MS system. The Varian 3400 Gas Chromatograph was fitted with a splitless injector and a fused high temperature silica column (Supelco EC-5). The GC was connected to a Varian Saturn 3 Ion-Trap mass-spectrometry through a heated transfer oven. Helium was used as a carrier gas (10 PSI). The GC oven was programmed initially at 40 °C, 10 °C min⁻¹ to 250 °C, 20 °C min⁻¹ to 280 °C, and hold at 280 °C for 15 min. Pyrolysis isothermal products were identified by comparison of their mass spectra with standard compounds and by comparing collected mass spectra with the Natural Institute Standard Technology (NIST 2002) mass spectral library.

For the on-line analysis, samples were pyrolyzed with a CDS Pyroprobe 2000. Samples (10-20 mg) are packed into a quartz tube. The sample is held open on place with quarts wool. The tube is placed into a coiled platinum (Pt) wire. An electric current is then passed through the platinum wire surrounding the tube, which is rapidly heated to 350 °C. Helium carrier sweeps the decomposition products into the GC column through the injector.

An Exeter CE 440 (CHN analyzer) was used for total carbon analysis using procedure prescribed by manufacturer (Model 440 CHN/O/S Elemental Analyzer Manual). Total carbon is measured by elevated temperature combustion (900 °C) in oxygen. Elemental carbon is determined using the same instrument and method after pre-combustion of the samples at 500 °C in a muffle furnace.
3.3 Reagents and Reference Materials

Alkali lignin was used as a standard for lignin analysis by GC/MS. The calibration standard for carbon analysis was acetanilide. Alkali lignin and acetanilide were purchased from Aldrich Chemical Company. Tetramethylammonium hydroxide (TMAH 25% in H₂O) was obtained from J. T. Baker. A 25% mixture of TMAH in methanol from Aldrich Chemical Co. was used for on-line analysis. Dichloromethane (DCM) was obtained from EMD Chemical Inc. DCM was used to extract the methylated products from the samples (off-line technique). Kaolinite clay was obtained from Ward's Natural Science Establishment, Inc.

3.4 Total and Organic Carbon Analysis

Samples were weighed in labeled glass test tubes (approximately, 100.0 mg each), saturated with 200.0 μl distilled water. Hydrochloric acid (25.0 μl of 12 M) is added to remove inorganic carbon. The acidified samples were dried overnight in an oven at 80 °C (Figure 3.4).
Figure 3.4: Drying the sediment samples in the oven overnight at 80 °C

Samples were homogenized by grinding and stirring with a stainless steel spatula and 15.0-20.0 mg of the dried samples were weighed into tin capsules. Tin capsules were purchased from Exeter. The tin capsules then were crimp sealed and inserted in the numbered holes on the sample carousel (Figure 3.5), and analyzed. Instrument calibration was performed by combusting 1.0-2.0 mg of acetanilide. Calibration samples were run every 5 samples.
Figure 3.5: A: Exeter CE 440 (CHN analyzer)
B: Sample carousel

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After performing total carbon analysis, the remainders of the samples were preheated at 500 °C overnight in ambient air. This treatment destroys organic carbon leaving behind only elemental carbon (i.e. charcoal) and the percentage of organic carbon in the samples is calculated as the difference between the percentage of total carbon and percentage of elemental carbon.

3.5 TMAH Chemolysis-GC/MS

TMAH Chemolysis involves heating organic materials in the presence of TMAH. This procedure results the decomposition of organic matter to simpler products and methylation of some of these decomposition products (carboxylic acids and phenols) so that they are amenable to gas chromatography mass spectrometry (GC/MS). The products were identified by comparison of mass spectra with standard compounds and by comparison to National Institute of Standard Technology (NIST 2002) mass spectral library.

3.5.1 Off-Line TMAH Chemolysis

The sample is weighed (approximately 100.0 mg), into a glass ampoule (obtained from Wheaton Co.) and saturated with 25 % TMAH in H₂O (obtained from J. J. Baker Co.). The inside walls of the ampoule are rinsed with 100 µl DI water to concentrate the sample and TMAH at the bottom of the ampoule. The sample is vacuum dried over NaOH pellets in a vacuum dessicator and sealed under vacuum (< 0.1 µ). The ampoule is wrapped in a piece of aluminum foil, and heated at 250° C for 30 minutes. After the sample is cooled to room temperature, the ampoule is scored and the contents are
extracted with 3 ml dichloromethane (DCM). The DCM is concentrated to near dryness under a gentle stream of nitrogen. The residue is dissolved in 50.0 μl of DCM and 2.0 μl is injected in the GC/MS with no further treatment. Figure 3.6 shows a schematic diagram of the procedure of sample treatment.

Figure 3.6: Schematic diagram of the procedure of sample treatment.
3.5.2 On-Line TMAH-Chemolysis

Cotton gloves are worn during sample handling, to avoid contamination. An open quartz tube (2.0 x 0.2 cm) is heated, using the pyrolysis probe at 1000 °C for 10 sec. (cleaning step). One end of the quartz tube is packed with glass wool at one end and the tube is tarred (weighed). The sediment sample (~ 20.0 mg) is loaded into the quartz tube and reweighed, the other end of tube is packed with glass wool from the other side. The sample is saturated with 10 µl of 25% TMAH (in methanol). Analysis are performed in a Pyroprobe 2000 (CDS Analytical) interfaced to 3400 Varian Saturn III ion trap Mass Spectrometer (MS) for detection and quantitation. Current passed through the platinum wire surrounding the tube. First the sample is dried for 10 seconds at 80 °C and then ballistically heated to 350 °C for 3 minutes (some runs were performed at 500 °C for 20 sec. and the results were similar). Figure 3.7 shows, A: Pyrolysis interface resistively-heated pyrolyzer, and B: the pyroprobe with the platinum wire.
Figure 3.7: A: Pyrolysis interface resistively-heated pyrolyzer and B: The pyroprobe with the platinum wire

Helium carrier into the GC column sweeps the decomposition of the products (Figure 3.8)

Figure 3.8: Schematic diagram of system pyrolysis

Figure 3.9 shows A, pyrolysis device attached to GC/MS and B, using the pyroprobe to inject the sample
Figure 3.9: A: Pyrolysis device attached to GC/MS, and B: Using the pyroprobe to inject the sample
3.6 Instrument Calibration

Mass calibration for GC/MS is performed using Fluoro Carbon 43. The calibration procedure was designed by the manufacturer. Standard response curves for lignin are constructed (linear) using reference material (RM) prepared by dispersing 1-4 mg of alkali lignin in 100 mg of kaolinite clay (Figure 3.10). Standards are run every 5 samples. For CHN analyzer, series of blanks and standards (acetanilide) are run, then blank and standard are run every five samples. Figure 3.10 shows the calibration curve off-line (165 m/e).

Slope $y = 406313$, correlation coefficient $(r) = 0.9873$.

The intersection of the curve at $x=1$ denotes the threshold before a response is registered by the instrument.

![Calibration curve off-line (165 m/e)](image)

**Figure 3.10:** Calibration curve for different concentrations of alkali lignin in kaolinite clay
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Carbon Analysis

Detailed carbon analyses were conducted on four sediment cores. The results are shown in figures 4.1–4.4. The total carbon is predominantly organic carbon. The concentration of elemental carbon in VC1 was higher than in other cores. The elemental carbon may reflect material transported from the Las Vegas Wash such as charcoal or asphalt particles.

Figure 4.1: Percentage of A: Total carbon, B: Organic carbon, and C: Elemental carbon down core VC1

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Concentrations of OC on the graphs reflect the amount of organic materials deposited to the lake sediments during different periods. The great amount of OC may be related to the changes of the lake levels through time or climatic or seasonal changes.
Changes in the levels of OC in the sediment layers could record the influence of climatic or seasonal variations on primary productivity or redistribution of large amounts of previously deposited material as a result of turbidity flows. Molecular changes in organic carbon can indicate the variations in the sources of sedimentary materials.

4.2 TMAH Chemolysis Results

Gas chromatography / mass spectrometry (GC/MS) provides specific structural information about the compounds that comprise natural organic matter.

Off-line chemolysis analysis of VC9 and VC16 (Figures 4.5, 4.6) were conducted to study the lignin signal (the sum of integrated peak areas of vanillyl and syringyl, aldehyde, ketone, and acid on the chromatogram) and for general characterization of organic matter. Lignin phenols can be identified by characteristic ions. The occurrence of
165 (m/e) ion at the appropriate retention time indicates fully methylated vanillyl phenols (produced from gymnosperm). Syringyl phenols are indicated by the occurrence of 195 (m/e) ions (produced from angiosperm). Vanillyl phenols predominate in all cores indicating that most of the plants materials deposited in lake sediments are derived from gymnosperm such as conifers or pines. Insufficient syringyl phenols were present for estimation of S/V ratio.

Figure 4.5: VC9, A: Percentage OC, B: TIC of OC, and C: TIC of lignin phenols (165 m/e)
Analyses of VC9 and VC16 show variation in organic carbon and lignin products with depths, indicating variation of the material deposited into the lake sediment. The presence of lignin indicates the contributions of terrestrial vascular plants. The higher concentrations of lignin phenols from VC9 at depth (32 cm) and from VC16 at (14 cm) are not highly correlated with the higher concentrations of organic carbon in the core indicating that organic matter is determined by other processes such as primary productivity and lignin deposition is a result of episodic turbidity currents or runoff. At depth 71-73 cm (VC16), lignin products were at the lowest abundance, while OC showed broad local maxima.

There is some similarity between the two cores. VC9 (45-65 cm) and VC16 (24-42 cm), these depths have the lowest concentrations of organic matter.
The estimation of lignin concentration in sediment samples was made by using the calibration curve developed by analysis of commercial alkali lignin dispersed in kaolinite clay (Chapter 3, figure 3.10) and its line equation:

\[
y = 406313 x - 464002 \quad \text{and} \quad r = 0.9873
\]

\(y\): the lignin signal (165 m/e), \(x\): lignin concentration, \(r\): correlation coefficient

All samples concentrations from each core are calculated, the average of concentration in cores reported on table 4.1, along with the average of organic carbon of same core. The semi quantitative analysis results of sediment VC1, VC9, VC16, and GC26 (Table 4.1) indicate the presence of lignin in all cores investigated. TOC concentrations ranged from 26.2 to 29.9 mg/g sediment except for GC26, which is far from the mouth of Las Vegas Wash (as mentioned before). The average of lignin phenols concentration was approximately 4.0 – 4.8 % of average organic carbon at the same core.

<table>
<thead>
<tr>
<th>Core</th>
<th>TOC (mg/g)</th>
<th>Lignin phenols (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg. Max Min</td>
<td>Avg. Max Min</td>
</tr>
<tr>
<td>VC1</td>
<td>29.9 61.2 1.0</td>
<td>1.21 1.36 0</td>
</tr>
<tr>
<td>VC9</td>
<td>29.7 61.8 1.0</td>
<td>1.23 1.51 0</td>
</tr>
<tr>
<td>VC16</td>
<td>26.2 58.3 2.5</td>
<td>1.25 1.44 0</td>
</tr>
<tr>
<td>GC26</td>
<td>7.7 23.1 0.3</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Table 4.1: Concentrations of TOC and lignin phenols in sediment cores

4.3 Comparison between On-Line and Off-Line Techniques
Chemopyrolysis involves heating organic materials in the presence of TMAH. This procedure results in decomposition of organic matter to simpler products and the methylation of these decomposition products so that they are amendable to gas GC/MS. The method can performed online using a gas chromatography pyrolysis interface (CDS analytical), or offline using a sealed glass ampoule.

Analysis of using on-line and off-line techniques (Figure 4.7 A and B) show that both methods give similar results. Total ion chromatogram (TIC) of both methods show results are similar. Our experience with lignin is that off-line method provided lower detection limits and greater consistency in analysis.

![Total integrated peak areas off-line (VC9)](image)

![Total integrated peak areas on-line (VC9)](image)

Figure 4.7: Comparison between total integrated areas (VC9), A: on-line and B: off-line techniques

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Differences between the two techniques stem from differences in temperature and decomposition time. Using on-line technique (350 °C for 3 min. or 500 °C for 20 sec.), decomposition and methylation are likely time limited. The efficiency of transfer of phenolic byproducts to the vapor phase during on-line hydrolysis may also limit the quantitative accuracy of the method. Figures 4.8 and 4.9 illustrate the lower detection limit for off-line method. The chromatograms (vanillin, acetovanillone, and vanillic acid) obtained from off-line and on-line techniques (same sediment samples at depths 27-28 cm and 63-64 cm) are compared. Lignin phenols were readily apparent with off-line but could not be recognized with the on-line.
Figure 4.8: The chromatograms obtained from A: off-line and B: on-line techniques, depth (27-28 cm)
Figure 4.9: The chromatograms obtained from A: off-line, and B: on-line techniques, depth (63-64 cm)
The results from off-line and on-line were plotted together as x and y. Figure 4.10 shows that the degree of confidence between the two techniques is reasonably high ($r = 0.7275$).

![Total integrated peak areas (VC9) off-line vs on-line](image)

**Figure 4.10: Conclusion of integrated signals from off-line and on-line methods**

This comparison between the two methods chromatograms leads us to believe that on-line pyrolysis could be used as a rapid screening method to estimate the lignin concentration in sediment samples before performing the quantitative analysis with other methods.
4.4 Lignin Biogeochemistry

One of the goals in this research is to investigate the relationship between the concentration of organic carbon and lignin and the sedimentary history in Lake Mead. For example, we wanted to see if the concentration of organic carbon and lignin is correlated to some events such as the 1975 flood event that increased headcutting, and contributed greatly to drainage and deterioration of the wetland vegetation (http://climchange.cr.usgs.gov).

Sediment cores show inter-bedded sand and clay. The sandy beds mostly are graded and are consistent with transport by turbidity flows. Sands beds in cores as much as 8 km from the mouth of Las Vegas Wash indicate that turbidity flows do transport coarse material long distances into the lake from urban runoff via Las Vegas wash (Zybala, 2003).

$^{137}$Cs variations in cores indicate that the above ground nuclear tests of the late 1950’s and early 1960’s are recorded in the cores (Rudin, 2002, personal communication). $^{137}$Cs results indicate that sedimentation has increased during recent years. Figure 4.11 shows the concentration of $^{137}$Cs, lignin phenols and organic carbon in cores VC1, VC9, and VC16. This figure prepared by adding our results of OC and lignin phenols on core description with $^{137}$Cs, which were performed by Rudin, Zybala, Hansen, and Twichell (personal communications).

Studying the concentrations of organic carbon and lignin in cores VC1, VC9, and VC16 with the cores description indicate that the higher concentrations of organic carbon and lignin phenols appear in the clay layers. The lowest concentrations of organic carbon and lignin phenols appear in the sand layers.
Figure 4.11: $^{137}$Cs, lignin phenols, and organic carbon in cores VC1, VC9, and VC16

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4.5 Fatty Acids in Lake Mead Sediment

TMAH thermochemolysis analysis (Off-line) of sediment samples (VC9) resulting in releasing fatty acids as methyl esters shown in figures 4.12 B, 4.13 B - 4.14 B for depths, 29, 65, 87 cm. Table 4.2 represents identity of the numbered main peaks on the corresponded chromatograms. The distribution of fatty acids methyl esters were quite similar, with a strong even carbon number predominance. Figure 4.2 shows that the concentration of fatty acids is getting lower by depth, and few of them do not exist at lower depth, probably because of bacteria use. Fatty acids in Lake Mead sediment will be studied in detail, and presented in a subsequent thesis.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Molecular mass</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>214</td>
<td>Dodecanoic acid methyl ester (lauric acid)</td>
</tr>
<tr>
<td>2</td>
<td>242</td>
<td>Tetradecanoic acid methyl ester (myristic acid)</td>
</tr>
<tr>
<td>3</td>
<td>270</td>
<td>Hexadecanoic acid methyl ester (palmitic acid)</td>
</tr>
<tr>
<td>4</td>
<td>298</td>
<td>Octadecanoic acid methyl ester (stearic acid)</td>
</tr>
<tr>
<td>5</td>
<td>326</td>
<td>Eicosanoic acid methyl ester (arachidic acid)</td>
</tr>
<tr>
<td>6</td>
<td>354</td>
<td>Docosanoic acid methyl ester (behenic acid)</td>
</tr>
<tr>
<td>7</td>
<td>382</td>
<td>Tetracosanoic acid methyl ester (lignoceric acid)</td>
</tr>
</tbody>
</table>

Table 4.2: The identified peaks of fatty acids methyl esters on the chromatograms obtained from sediment samples, VC9 depths, 29, 65, and 87 cm

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Figure 4.12: Thermochemolysis chromatograms of sediment, VC9 (depth 29-30 cm),
A: Total ionic chromatogram (TIC), B: Distribution of fatty acids (m/z 74)

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Figure 4.13: Thermochemolysis chromatograms of sediment, VC9 (depth 65-66 cm), A: Total ionic chromatogram (TIC), B: Distribution of fatty acids (m/z 74)

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Figure 4.14: Thermochemolysis chromatograms of sediment, VC9 (depth 87-88 cm), A: Total ionic chromatogram (TIC), B: Distribution of fatty acids (m/z 74)
CHAPTER 5

CONCLUSIONS

Distribution and concentration of organic carbon and lignin within the sediment layers in all cores indicate the contribution of organic materials and terrestrial vascular plants runoff to lake sediment.

Changes in the levels of organic carbon in the sediment layers could record the influence of climatic or seasonal variations on primary productivity or historical events such as the 1975 flood event or large amounts of previously deposited material as a result of turbidity flows.

Studying the concentrations of organic carbon and lignin in cores VC1, VC9, and VC16 with the cores description indicate that the higher concentrations of organic carbon and lignin phenols appear in the clay layers. The lowest concentrations of organic carbon and lignin phenols appear in the sand layers.

The comparison between thermochemolysis on-line and off-line techniques lead us to believe that on-line pyrolysis could be used as a rapid screening method to estimate the lignin concentration in sediment samples before performing the quantitative analysis with other methods. Both methods give similar results. Total ionic chromatogram (TIC) of both methods show results are similar. Our experience with lignin phenols is that off-line method provided lower detection limits and greater consistency in analysis.
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