

Characterization of sedimentary humic matter by alkaline hydrolysis

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Abstract—Humic matter fractions from modern sediments of Lake Huron and Lake Michigan have been compared. Large yields of saccharinic acids from alkaline hydrolysis suggest that these fractions contain large portions of carbohydrate materials. Evidence for contributions of aquatic lipid (C-16 fatty acids) and of lignin (phenolic acids) to these sediments is also present in the hydrolysis products. Qualitative differences among fulvic acid, humic acid and humin from the same lake are minor, suggesting common (or similar) organic sources for these fractions. The lability of sedimentary humic matter to alkaline hydrolysis is inversely related to its degree of exposure to oxidative weathering. Lability may also be related to diagenetic state as fulvic acids generally yield greater quantities of hydrolysis components than humic acids which in turn yield more than humin.

INTRODUCTION

Humic matter comprises the bulk of organic matter in natural systems. Although much research effort has gone into the study of soil humic matter, comparatively few studies have focused on sedimentary humic materials. Among these, elemental and spectroscopic characteristics of the humic materials isolated from freshwater sediments have been reported (Otsuki and Hanya, 1967; Ishiwatari, 1970, 1973; Kemp, 1973; Kemp and Mudrochova, 1973). In general, these studies have shown that sedimentary humic matter is composed of material of both aquatic and terrestrial origin. Recent studies of humic matter of various origin by NMR spectroscopy have shown varying proportions of aliphatic and aromatic structures (Stuermer and Payne, 1976; Hatcher *et al.*, 1980a,b; Dereppe *et al.*, 1980; Hatcher, *et al.*, 1981a,b; Wershaw *et al.*, 1981).

Chemical degradation has been used to characterize soil humic matter (Schnitzer and Khan, 1972) and has also been applied to freshwater studies. Ishiwatari (1975) oxidized humic acid from Lake Haruna sediment with alkaline KMnO_4 and released a variety of aliphatic and aromatic acids. Kemp and Mudrochova (1973) studied amino acids released by acid hydrolysis of fulvic and humic acids from a Lake Ontario sediment sample. Alkaline hydrolysis has been used successfully on soil humic matter by Jakab *et al.* (1963), Neyroud and Schnitzer (1975) and Tsutsuki and Kuwatsuka (1979).

Here we report chemical characteristics of humic matter from modern sediments of Lakes Huron and

Michigan. The focus of this paper is on products released by alkaline hydrolysis of humic matter fractions. These are compared to hydrolysis products from a number of locally derived materials which are potential sources of, or precursors to, the sedimentary humic matter.

EXPERIMENTAL

Sediment samples

The sediment samples analyzed were obtained with a 6.7 cm I.D. Benthos[®] gravity corer. Physical disturbance during the coring operation was minimized by using a butterfly type valve and leaving the penetration end open, without a core catcher. Cores were extruded vertically and sectioned within one hour after collection, frozen immediately and stored at -20°C in glass containers until analysis. One core, designated SLH75-18, was taken in the Goderich Basin of southern Lake Huron ($44^\circ00'\text{N}$, $81^\circ59'\text{W}$; water depth 63 m) in September, 1975. The other core designated SLM77-26, came from southeastern Lake Michigan ($42^\circ20'\text{N}$, $86^\circ50'\text{W}$; water depth 118 m) and was collected in July, 1977.

The Lake Huron site is characterized by a present-day sedimentation rate of 0.11 ± 0.01 cm/yr. This was determined by ^{210}Pb geochronology measured on a companion core by Robbins *et al.* (1977) at their station 18. Vertical mixing caused mostly by bioturbation extends to a depth of 3 cm at this location (Robbins *et al.*, 1977; Krezoski *et al.*, 1978). The present day sedimentation rate at the Lake Michigan site is 0.10 cm/yr (Edgington and Robbins, 1975, their station 26). This was determined from ^{137}Cs data as was the vertical mixing depth of 2 cm.

Fractionation of humic matter and chemical analyses were done on 1 cm sections for the upper parts of both cores. For the purposes of this report, data for the top 4 cm of each core were combined and weighed according to the proportion that the dry sediment weight of each 1 cm section is to the total dry weight of the 0-4 cm interval.

These combined data therefore represent material deposited during about the last 50 years at both sites.

Ancillary samples

The diatom *Asterionella formosa* and the green alga *Chlamydomonas* sp. were cultured on sterilized "WC medium" (Guillard, 1975). The bacterium *Escherichia coli* was grown on Difco Nutrient Broth. After sufficient growth had taken place, the cultures were centrifuged for 2 hr at 4000 g, washed twice with sterile 0.85% NaCl to remove traces of media and freeze-dried.

A sample of white spruce wood (*Picea glauca*) from a forest buried by glacial outwash 10,000 years ago (Hughes and Merry, 1978) was freeze-dried, ground and homogenized. This sample is designated "Pleistocene spruce". For comparison, a piece of freshly cut white spruce was prepared in the same way and designated "modern spruce" (Meyers *et al.*, 1980b). A piece of celery (*Apium graveolens*) was used as a source of crude cellulose for comparative purposes.

A topsoil sample was obtained from an eroding bank on the eastern shore of southern Lake Michigan (42°26.1'N., 86°15.8'W.). A sample of glacial till was taken from a lower sedimentary layer on this same bluff. Further details on these soil samples are discussed by Bourbonniere (1979), where they are named LMS-4 and LMS-1, respectively. The soils were freeze-dried, ground and homogenized before use.

Glaciolacustrine clay was sampled from the lower part of a core taken from another site in southern Lake Huron (43°50'N., 82°00'W.) (Meyers *et al.*, 1980a). This material underlies much of the post-glacial mud in Lake Huron and outcrops in several areas. Thomas *et al.* (1973) interpret glaciolacustrine clays as deep-water deposits of main and post-Algonquin glacial lakes, and as such were deposited about 10,000–11,000 years before the present. These authors also suggest that the extremely fine grain size, deficiency in organic carbon, and high quartz content of this clay is consistent with a rock flour origin. The profile of inorganic carbon at this location indicates a high sedimentation rate consistent with periglacial conditions (Iwasiuk *et al.*, 1977).

Fractionation of organic matter

The scheme in Fig. 1 constitutes the operational definitions of fulvic acid (FA), humic acid (HA) and humin (HU) as used most often by soil scientists (Schnitzer and Khan, 1972). In this study no attempt was made to isolate the HU fraction from the mineral phase, and this is thus the non-extractable or residual organic matter. FA is that fraction of the alkaline extract which remains soluble at low pH. Note that according to these operational definitions, the sum of the fractions constitutes the total organic matter.

FA is soluble in methanol (Griffith and Schnitzer, 1975), and this property is utilized in separating it (89–90% recovery) from the large quantities of NaCl which result from concentration of the FA solution. The FA's isolated in this way from these sediments are yellow to light brown fine powders which contain large quantities of ash, probably Si and Al hydroxides. HA appears as a brown colloid in low pH solution. It flocculates after standing several hours and is removed by centrifugation (4000 g). The HA precipitate is purified by repeated dissolution and precipitation, thus removing fine clay mineral contaminants. Purified and freeze-dried HA's from these sediments are dark brown to black granular solids with low ash contents.

Elemental analyses

Carbon and nitrogen data reported here were determined on a Hewlett-Packard Model 185-B CHN Analyser using cystine (N.B.S.) as a calibration standard. Total organic carbon (TOC) was determined on sediment samples treated with 1N HCl to remove carbonates. Standard deviations of atomic N/C ratios ranged from 3 to 7% of the sample means for humic matter fractions.

Alkaline hydrolysis

The apparent selectivity towards breaking C–O bonds and relative inefficiency for breaking C–C bonds were reasons for selecting this degradation method. The successful use of alkaline hydrolysis by Jakab *et al.* (1963) and Neyroud and Schnitzer (1975) on soil humic matter prompted our adaptation of their procedures to a micro-scale level. FA, HA and HU fractions were hydrolyzed for the SLH75-18 and SLM77-26 sediment samples. The ancillary samples were hydrolyzed as whole, unfractionated samples.

Samples to be hydrolyzed were placed in (10 ml) stainless steel reaction tubes. After addition of internal standard, (dodecanoic or heptadecanoic acid), nitrogen-purged 5N NaOH was added and the tube sealed. Reaction tubes were placed in a steel pressure vessel half-filled with water. The hydrolysis proceeded for 12 hr at 170°C.

After cooling, the basic solution in the tubes was acidified with 6N HCl, and the hydrolysis products were extracted from the acid solution with ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum at 30°C.

Gas-liquid chromatography

The dry extracts were silylated at 125–130°C for 45 min with 100 µl of BSFTA (Pierce Chemical Co.) prior to analysis on a Hewlett-Packard Model 5710 gas chromatograph coupled with an HP Model 3380A Integrating Recorder. The primary column used was 3 m × 1/8 in. (3.1 mm) o.d. stainless steel packed with 10% SP2250 (50% methyl, 50% phenyl silicone, Supelco Inc.) on 100/120 mesh Supelcoport temperature-programmed from 110 to 300°C at 4°C/min with a final hold at 300°C.

Chromatographic peaks were tentatively identified by comparing their retention indices on SP2250 to those of authentic standards. TMS esters of *n*-alkanoic acids (C5:0–C24:0) were used as a reference series for calculating retention indices after Kovats (1965). Standards and selected samples were also analyzed on a packed 10% SP2100 (100% methyl silicone) column programmed from 80 to 270°C at 4°C/min and a 30 m × 0.25 mm i.d. glass capillary column coated with SP2250 programmed from 75 to 250°C at 4°C/min. Further confirmation was achieved by capillary GC–MS of selected samples on a Finnigan 1015 Quadrupole system. A 21 m × 0.32 mm i.d. glass capillary coated with SE52 was used in the GC–MS work. It should be noted that the packed columns used in this study produced essentially the same chromatograms, with these samples, as were produced by the capillary columns.

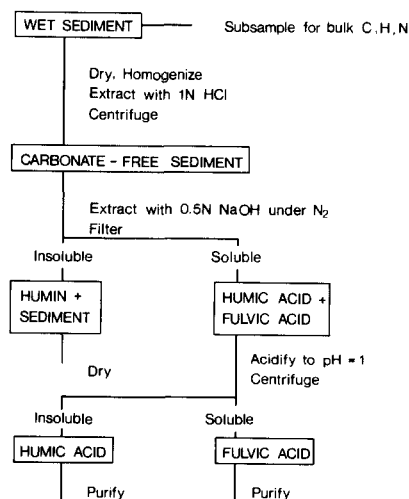


Fig. 1. Humic matter extraction and fractionation scheme.

Quantification was achieved by comparing peak areas to those of internal standards on packed column SP2250 chromatograms. Differences in FID response factor relative to that of the internal standard were determined on quantitative mixtures of authentic standards. Corrections reflecting these differences were applied to all data.

All organic solvents used were ACS reagent grade redistilled in glass. Any solvent used as a reagent in sample workup was certified free of contaminants by GLC. All water used was deionized, glass-distilled and glass-distilled a second time from alkaline KMnO_4 solution. All reagents were ACS reagent grade. Concentrated stock solutions were extracted with GLC certified dichloromethane and diluted to working concentrations with KMnO_4 -distilled water. Solid reagents were washed with appropriate GLC certified organic solvents and oven-dried.

RESULTS AND DISCUSSION

Character of bulk organic matter

Sediments from both Lake Huron and Lake Michigan contain *c.* 2.45% organic carbon (Table 1), which is typical of surficial sediments in the Goderich Basin of Lake Huron (Thomas *et al.*, 1973) and in southeastern Lake Michigan (Shimp *et al.*, 1971). The major part of the organic matter in these sediments is composed of the non-extractable HU fraction. FA is a lesser, and HA a minor proportion of the total organic matter (Table 1).

Atomic N/C ratios of sedimentary humic matter and of ancillary samples (Table 2) suggest some differences in origin among sedimentary humic matter fractions. Values for the HU fraction of both sediments are nearer to those for topsoil, glaciolacustrine clay, celery and values reported for soil HU. Organic matter in the HU fraction of these sediments may be derived largely from eroded terrestrial material, and may contain insoluble plant tissue fragments which are low in nitrogen, e.g., white spruce, Table 2. Sedimentary HU may also contain organic matter of aquatic origin which has lost nitrogen prior to sedimentation.

Both sedimentary HA fractions and FA from SLM77-26 show N/C ratios like those of phytoplankton and are much higher than those typical of soil FA and HA (Table 2). The ratio for FA from SLH75-18 is even higher and is more like that of zooplankton and bacteria. A number of authors (Otsuki and Hanya, 1967; Ishiwatari, 1970; Nissenbaum and Kaplan, 1972; Kemp, 1973; among others) have suggested that sedimentary FA and HA are derived primarily from aquatic sources. Our N/C results agree with this suggestion.

Composition of alkaline hydrolyzates

Hydrolyzates of the humic matter fractions and the ancillary samples yielded 30–50 GLC peaks. Thirteen

Table 1. Organic carbon distribution in sediment samples

	SLH75-18	SLM77-26
TOC (% dry sediment)	2.46	2.44
OC distribution (% of TOC)		
Fulvic acid	24.4	31.4
Humic acid	1.3	1.8
Humin	74.3	66.8

Table 2. Atomic N/C ratios of sedimentary humic matter and related materials^a

SLH75-18:	Fulvic acid	0.209
	Humic acid	0.111
	Humin	0.087
SLM77-26:	Fulvic acid	0.137
	Humic acid	0.132
	Humin	0.073
Soil humic matter ^b :	Fulvic acid	0.012–0.056
	Humic acid	0.024–0.062
	Humin	0.071–0.078
Topsoil, Lake Michigan shore		0.063
Till, Lake Michigan shore		— ^f
Glaciolacustrine clay, Lake Huron		0.073
White spruce, modern		0.022
White spruce, Pleistocene		0.049
Celery (<i>Apium graveolens</i>)		0.062
Phytoplankton:	<i>A. formosa</i>	0.116
	<i>Chlamydomonas</i> sp.	0.133
	<i>Scenedesmus</i> sp. ^c	0.152
	Marine diatoms ^d	0.115
	Copepods, Lake Ontario ^e	0.197
Zooplankton:	Copepods, Marine	0.187
	<i>E. coli</i>	0.244
Bacteria:	Soil bacteria ^d	0.209

^aThis work unless otherwise noted.

^bSchnitzer and Khan (1972).

^cOtsuki and Hanya (1967).

^dCited by Bordovskiy (1965)

^eKemp (1973).

^fNo organic N found.

Table 3. Selected components isolated by alkaline hydrolysis^a

Key ^b	Retention index ^c	Tentative Identification
1	572	Lactic acid
2	604	Oxalic acid
3	629	2-hydroxybutanoic acid
4	884	Succinic acid
5	908	Maleic/fumaric acids
6	1031	3,4-dihydroxybutanoic acid
7	1127	2-hydroxybenzoic acid
X	1158	Compound X
8	1229	4-hydroxybenzoic acid
9	1270	2,5-dihydroxy-3-pentenoic acid
10	1409	Vanillic acid
11	1600	<i>n</i> -C16 fatty acids
12	1768	Ferulic acid
13	1800	<i>n</i> -C18 fatty acids

^aFor complete information on identification criteria, refer to Bourbonniere (1979).

^bFor reference to figures.

^cRelative to *n*-alkanoic acid TMS esters.

of these peaks (Table 3) were chosen for closer study on the basis of: (1) their frequency of occurrence in the samples; (2) the amount of information available for their identification; (3) their generally high relative abundance in the hydrolyzates.

Aliphatic hydroxy acids. The four aliphatic hydroxy acids identified in the hydrolyzates of the humic matter fractions and of the ancillary samples are lactic acid, 2-hydroxybutanoic acid, 3,4-dihydroxybutanoic acid and 2,5-dihydroxy-3-pentenoic acid. These acids belong to a group of so-called saccharinic acids (Sowden, 1957), and are found frequently in liquors from the kraft paper process, which is an aqueous alkaline degradation of wood chips (Malinen and Sjoström, 1975; Lowendahl *et al.*, 1976).

Wood chemists have studied extensively the alkaline degradation of cellulose and related materials. Lowendahl *et al.* (1976) isolated lactic, 2-hydroxybutanoic and 3,4-dihydroxybutanoic acids in a 40:3:1 mass ratio from pine kraft black liquor among 25 hydroxy acids. Samuelson and Stolpe (1973) noted that lactic acid is the major component isolated when the process is carried out in the absence of oxygen. These results are significant because in the present study alkaline hydrolysis is accomplished in the absence of oxygen, and lactic acid is commonly a major component of hydrolysates. Isolation of 2,5-dihydroxy-3-pentenoic acid from alkaline oxidations of carbohydrates has not been reported. However 3,4-unsaturated compounds are postulated as intermediates in these reactions (Sowden, 1957).

Lactic and 2-hydroxybutanoic acids also may arise from microbial sources. Lactic acid is a common end product of glucose fermentation in many microorganisms, and lactic acid bacteria live in association with plants and decomposing plant tissues (Stanier *et al.*, 1970). Nuti *et al.* (1975) reported appreciable concentrations of 2-hydroxybutanoic acid in several genera of soil microfungi.

An unidentified compound (X) is a major component of the hydrolysates of the topsoil and glaciolacustrine clay samples GC-MS data suggest that this

component is an unsaturated hydroxy acid. Arguments to support this are similar to those used to identify 2,5-dihydroxy-3-pentenoic acid (Bourbonniere, 1979, Appendix C).

Aliphatic dicarboxylic acids. The three aliphatic dicarboxylic acids identified in the alkaline hydrolyzates were oxalic acid, succinic acid, and maleic/fumaric acids.

Oxalic and succinic acids were found among the alkaline oxidation products of cellobiose (Lowendahl *et al.*, 1975) and of pine chips (Lowendahl *et al.*, 1976), usually at concentrations an order of magnitude lower than those of aliphatic hydroxy acids.

Oxalic acid constitutes a large portion of the dry weight of certain leaves, and is typically produced by fungi (Stevenson, 1967; Briggs *et al.*, 1975). Wood-destroying fungi of the genera *Formitopsis* and *Coriolum* produce a considerable quantity of this acid (Milova, 1973). The fungus *Aspergillus niger* has been shown to produce oxalic acid from citric acid by a pathway involving the tricarboxylic acid cycle (Muller, 1975; Muller and Frosch, 1975).

Succinic acid is commonly found in all types of plants including the fungi. It is an intermediate in the tricarboxylic acid cycle (Stevenson, 1967; Stanier *et al.*, 1970), and is one of the products of fermentation of glucose by a number of bacteria which are referred to as "mixed-acid fermenters" (Stanier *et al.*, 1970). Maleic and fumaric acids are not resolvable by the chromatographic system used in this study. Fumaric acid is an intermediate in the tricarboxylic acid cycle (Stanier *et al.*, 1970).

Aromatic acids. The four aromatic acids isolated and identified in alkaline hydrolyzates are 2-hydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, and ferulic acid. Vanillic, 4-hydroxybenzoic and ferulic acids are typical of the degradation products related to the lignin of vascular plants. Numerous studies have focused on the incorporation of lignin-derived materials into humic matter (cf. Schnitzer and Kahn, 1972).

4-Hydroxybenzoic acid can be derived from the degradation of *p*-coumaric acid or related compounds by microbial processes or by chemical oxidation. It is indicative of the lignin of lower vascular plants (monocotyledons) because it does not occur in appreciable abundance in the lignins of conifers or hardwoods (Brown, 1964; Schubert, 1945; Flaig *et al.*, 1975).

Vanillic acid is a more reliable indicator of vascular plant lignin. Precursors to vanillic acid are the only type that occur in conifer lignin; they are also found in appreciable quantities in hardwood lignin, as well as in monocotyledon lignin (Schubert, 1965; Flaig *et al.*, 1975; Hedges and Parker, 1976). Thus vanillic acid is a general lignin indicator, and is not an indicator of a specific type of lignin.

Syringic acid was not detected in the hydrolyzates from this study; precursors so it are found only in hardwood and monocotyledon lignin (Schubert,

1965; Flaig *et al.*, 1975; Hedges and Parker, 1976). Its absence may indicate that these lignins are not significant sources in these sediments, or simply that syringic acid is generally a minor constituent of lignin relative to vanillic acid.

Ferulic acid is widespread in the plant kingdom, and can be considered as a non-specific indicator of plant sources to the sediments (Robinson, 1963). It is a derivative of coniferyl alcohol, a common lignin component, and can be converted to vanillic acid by β -oxidation (Towers, 1964; Schubert, 1965). Hedges and Parker (1976) found ferulic acid in the alkaline cupric oxide oxidation products of angiosperms.

The remaining aromatic acid, 2-hydroxybenzoic acid, is not a lignin phenol. It has been found among the products synthesized by the fungus *Hendersonula toruloida* growing on glucose (Martin *et al.*, 1972). The tissues of higher plants are capable of *ortho*-hydroxylation of benzoic acid to form 2-hydroxybenzoic acid. The benzoic acid may in turn be produced by β -oxidation of cinnamic acid (Towers, 1964). The bark of members of the genera *Salix* (willow) and *Populus* (poplar) contain salicin (Baumann and Pigman, 1957; Robinson, 1963), which may form 2-hydroxybenzoic acid upon oxidation and hydrolysis.

Benzenepolycarboxylic acids are detectable by the chromatographic methods employed in this study. None were found in any of the hydrolyzates. These acids are thought to result from the degradation of condensed aromatic structures. Evidently alkaline hydrolysis as used here is ineffective in breaking C-C bonds. This result confirms those of Neyroud and Schnitzer (1975) on soil FA and HA.

Longer-chain aliphatic (fatty) acids. In most of the hydrolyzates only two components were identified as fatty acids. These were the C-16 and C-18 fatty acids. With the chromatographic system used for quantitative analysis of all samples in this study, resolution of saturated and unsaturated fatty acids was not possible. Thus the C-16 fatty acid peak on the chromatograms most likely represents a combination of palmitic acid and palmitoleic acid. Stearic acid, oleic acid and possibly other unsaturated C-18 acids are similarly combined in one chromatographic peak with packed SP2250 columns.

Qualitative comparisons

Compositional differences among the hydrolyzates of the sediment humic matter fractionations and the ancillary samples are best illustrated by relative abundance diagrams. All components separated by GLC are plotted relative to the most abundant component (the "base peak") in each chromatogram which is set to 100 units. These diagrams can be considered to be reconstructed gas chromatograms. Unlike the actual chromatograms, relative abundance diagrams take response factors into consideration. Thus, relative abundance diagrams are more realistic representa-

tions of the composition of a hydrolyzate than are the original chromatograms.

Interpretation of relative abundance diagrams, however, must be done with caution. These diagrams do *not* give absolute quantitative data which can be compared on *different* samples. However, quantitative differences *within* a sample and qualitative differences *between* samples are shown well by these diagrams.

Ancillary samples. The ancillary samples serve to illustrate the distribution of hydrolysis components from potential source materials to the sediments. They are described first to provide a framework for the understanding of the hydrolysis data from the humic matter isolated from sediment samples.

Relative abundance diagrams for the alkaline hydrolyzates of the modern and Pleistocene spruce samples (Fig. 2) show lactic acid as the base peak in both cases. The Pleistocene spruce contains higher proportions of oxalic, vanillic and ferulic acids. In the modern spruce hydrolyzate C-16 and C-18 fatty acids are higher in relative abundance.

Higher proportions of lignin components (vanillic and ferulic acids) in the Pleistocene spruce relative to the modern spruce indicate a selective preservation of lignin with respect to polysaccharides. This observation is similar to that of Hatcher *et al.* (1981b) who compared NMR spectra from this same Pleistocene spruce sample to those from modern spruce wood and its lignin preparations. The lack of fatty acids in the Pleistocene spruce hydrolyzate suggests their labile nature as noted by Meyers *et al.* (1980) in extracts of these wood samples.

Lactic, oxalic and succinic acids are the dominant components of the celery hydrolyzate (Fig. 3A). Other hydroxy acids expected from cellulose degradation are also found but in lesser proportions. The dextrose hydrolyzate (Fig. 3B) is dominated by

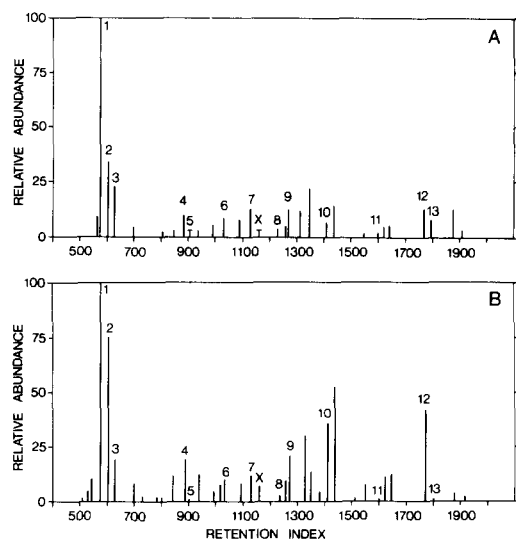


Fig. 2. Relative abundance of alkaline hydrolysis products. (A) Modern spruce; (B) Pleistocene spruce.

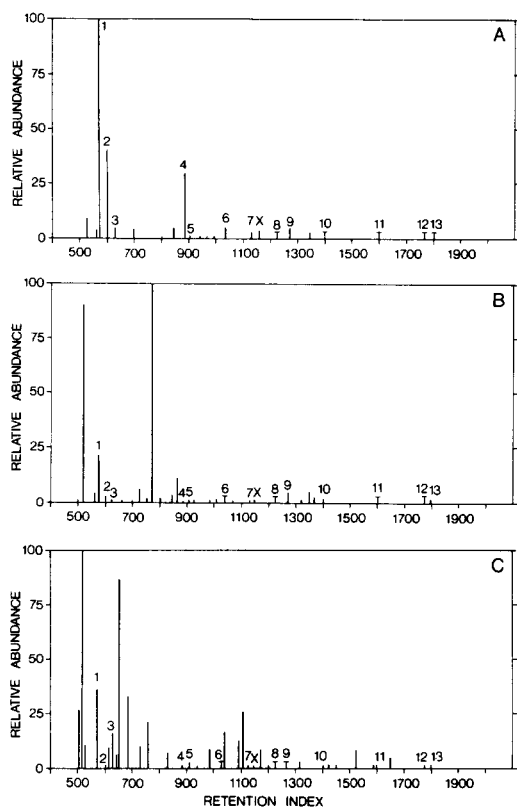


Fig. 3. Relative abundance of alkaline hydrolysis products. (A) Celery; (B) Dextrose; (C) *Escherichia coli*.

two unidentified components with retention indices of 522 and 771. Lesser proportions of lactic, oxalic and a few other hydroxy acids were isolated from the dextrose hydrolyzate. Differences in the composition of the celery and dextrose hydrolyzates may reflect the polymeric character of cellulose and the lack of such character in D-glucose. In cellulose the D-glucose monomers are joined by 1→4, β-D-glycosidic linkages. Oxidation of such 4-O-substituted D-glucose leads to products different from those of unsubstituted D-glucose (Sowden, 1957). The relative proportions of hydroxy and dicarboxylic acids in the celery hydrolyzate (Fig. 3A) resemble those of the modern spruce hydrolyzate (Fig. 2A) which is also expected to be dominated by cellulose degradation products.

Two unidentified components at retention indices of 516 and 653 dominate the hydrolysis products of *E. coli* (Fig. 3C). The component at 516 may be the same as a dominant component in the dextrose hydrolyzate. The only identified components in appreciable abundance in the *E. coli* hydrolyzate are lactic acid and 2-hydroxybutanoic acid. Degradation of carbohydrates could explain the origins of both of these components.

Products isolated from alkaline hydrolyzates of the freshwater diatom *A. formosa* and of the freshwater green alga *Chlamydomonas* sp. are represented by

relative abundance diagrams in Fig. 4. These hydrolyzates differ from each other, and they both differ significantly from the spruce, celery and dextrose samples.

Alkaline hydrolysis of *A. formosa* yields C-16 fatty acids as the base peak. Oxalic, lactic and succinic acids are also present in high relative abundances. The hydroxy acids 2-hydroxybutanoic, 3,4-dihydroxybutanoic and 2,5-dihydroxy-3-pentenoic show appreciable abundances as do 2-hydroxybenzoic acid, and C-18 fatty acids (Fig. 4A). High relative abundance of the C-16 fatty acids in the *A. formosa* hydrolyzate suggests their possible use as indicators of diatomaceous sources to the organic matter in sediments, but may be limited to young sediments (Meyers *et al.*, 1980a).

Alkaline hydrolysis of *Chlamydomonas* sp. yields a number of unidentified components with retention indices between 500 and 800. Several of these components are high in relative abundance (Fig. 4B). Rogers (1965) reported that carbohydrates in algae from two Minnesota lakes consist mostly of cellulose and hemicellulose. However, these algae also contain appreciable quantities of sugars which are not derived from cellulose. Glucose made up only about one-fifth of the carbohydrates isolated from marine phytoplankton, indicating a lower content of cellulose and hemicellulose (Degens and Mopper, 1975). It is possible that the unidentified components in *Chlamydomonas* sp. hydrolyzate are hydroxy acids and/or dicarboxylic acids which are degradation products of carbohydrates other than cellulose. A similar argument can be made for the high relative abundances of 2,5-dihydroxy-3-pentenoic acid and compound X in this hydrolyzate.

Oxalic acid, succinic acid and 2,5-dihydroxy-3-pentenoic acid are the dominant components in the hydrolyzate from the glacio-lacustrine clay and till

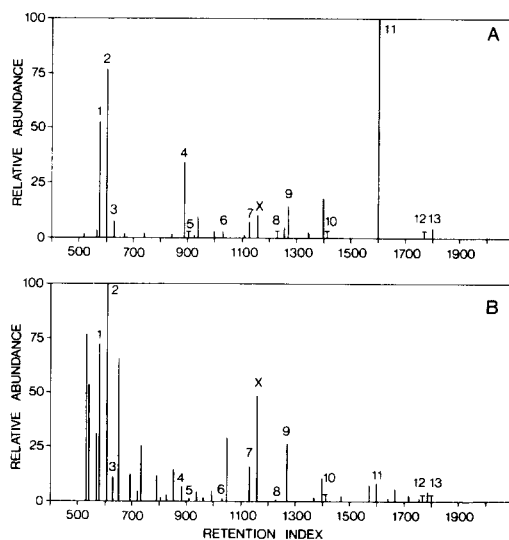


Fig. 4. Relative abundance of alkaline hydrolysis products. (A) *Asterionella formosa*; (B) *Chlamydomonas* sp.

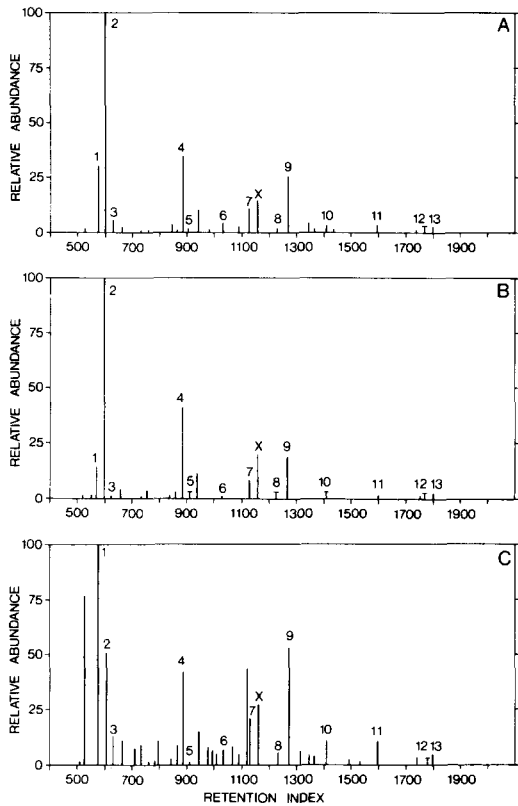


Fig. 5. Relative abundance of alkaline hydrolysis products. (A) Glaciolacustrine clay; (B) Till; (C) Topsoil.

samples (Figs 5A and 5B). Lactic acid, 2-hydroxybenzoic acid and compound X are also important components in these hydrolyzates. These samples may be considered as representative of the contribution of "reworked" detrital carbon to the sediments. Compositionally, the hydrolyzates are relatively simple. This may be related to the low organic carbon content of the samples. High proportions of succinic and 2,5-dihydroxy-3-pentenoic acids relative to that of lactic acid suggest that they come from some source other than cellulose. The topsoil sample from the Lake Michigan bluff yielded a wide variety of hydrolysis products (Fig. 5C). Components representative of a number of sources have been isolated in appreciable abundance.

Sediment samples. Distribution of products released by alkaline hydrolysis of all three humic matter fractions isolated from the SLH75-18 sediment are very similar (Fig. 6). Lactic, oxalic, 2-hydroxybutanoic and succinic acids are the predominant products, suggesting that cellulose materials (cf Fig. 3A) are by far the dominant precursors to or components of fulvic acid (FA), humic acid (HA) and humin (HU) found in this Lake Huron sediment.

Humic matter fractions from the SLM77-26 sediment yield hydrolysis products as shown in Fig. 7. They are distributed somewhat differently than those from the SLH75-18 sediment. Differences in distribu-

tion also occur among hydrolyzates from the FA, HA and HU fractions isolated from this Lake Michigan sediment. Cellulose-derived materials are indeed major components of these hydrolyzates but other precursors are also important. High relative abundances of 2,5-dihydroxy-3-pentenoic acid and compound X are found in the hydrolyzates of all three fractions. As discussed previously, these may indicate a significant contribution of non-cellulosic carbohydrates to the sedimentary humic matter. The original source of such material could be algal (cf. Fig. 4B), but may also be terrigenous as the topsoil hydrolyzate also contained these compounds in significant proportions (Fig. 5C). C-16 fatty acids show a much larger relative abundance in the HA and HU fractions of the Lake Michigan sediment compared to those from Lake Huron, suggesting that diatoms (cf. Fig. 4A) contribute a relatively greater proportion of the hydrolyzable organic matter in these Lake Michigan fractions.

In a qualitative sense, humic matter fractions from the Lake Michigan sediment are more complex than those from the Lake Huron sediment. We interpret this to mean that the hydrolyzable organic matter from the Lake Michigan sediment originates from a wider variety of sources than that from the Lake Huron sediment. The proportion of cellulose precursors in the Lake Huron sedimentary humic matter is

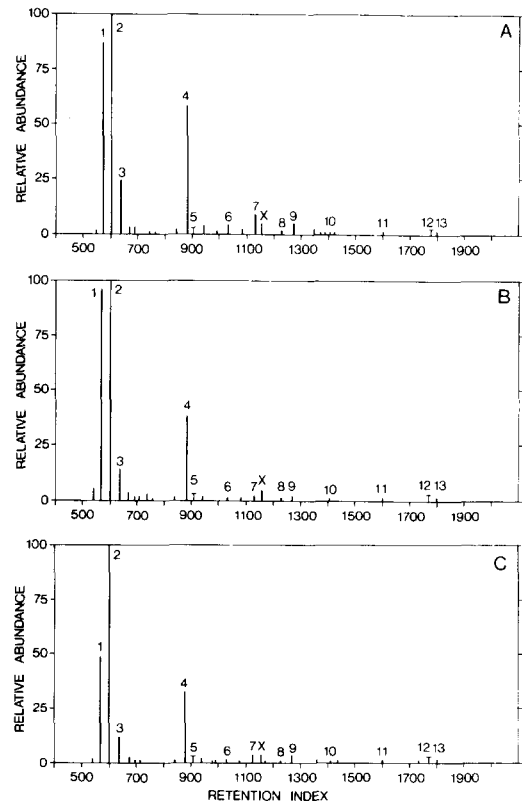


Fig. 6. Relative abundance of alkaline hydrolysis products from humic matter fractions of SLH75-18 sediment. (A) Fulvic acid; (B) Humic acid; (C) Humin.

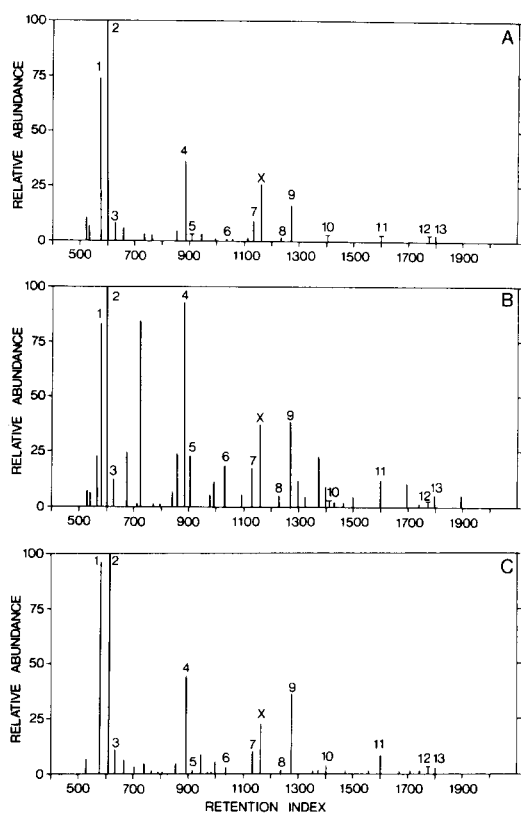


Fig. 7. Relative abundance of alkaline hydrolysis products from humic matter fractions of SLM77-26 sediment. (A) Fulvic acid; (B) Humic acid; (C) Humin.

greater relative to other sources. Humic matter from the Lake Michigan sediment probably contains degraded cellulose, suggesting that a reworked detrital component is important.

Along these lines it should be noted that products which should be of lignin origin (4-hydroxybenzoic, vanillic and ferrulic acids) are only minor components of all humic matter hydrolyzates from both sediments. It could be that lignin is not a significant precursor to these sedimentary humic materials, or that it is bound in a non-hydrolyzable state. The second possibility seems unlikely since all FA and HA hydrolyzates were clear solutions (HU hydrolyzates contained mineral precipitates) and lignin precursors were found in three ancillary samples, spruce and topsoil, as expected. These results agree in general with those of Meyers and Takeuchi (1979); Meyers *et al.* (1980a) and Leenheer (1981), who determined that lipids in sediments from nearby sites in both of these lakes are derived from both aquatic and terrestrial sources.

QUANTITATIVE CONSIDERATIONS

The previous discussion dealt with qualitative aspects of the distribution of products released by alkaline hydrolysis. The major organic source materi-

als which contribute to the humic matter in these sediments may be either aquatic or terrestrial in origin. Carbohydrate derivatives and fatty acids found in the hydrolyzates suggest both sources. Lignin derivatives imply terrestrial higher plant contribution to the humic matter in these sediments. This section deals more quantitatively with hydrolysis products representative of these classes and sources.

Four of the hydrolysis products were chosen for quantitative discussion on the basis of their frequency of occurrence and uniqueness as an indicator of source. To represent polysaccharides and other carbohydrates, we chose 3,4-dihydroxybutanoic acid. This compound is found in kraft liquors and unlike some of the other aliphatic hydroxy acids and dicarboxylic acids, it is not reported to have any other biochemical source. The C-16 fatty acids were chosen to represent aquatic organic sources. C-16 fatty acids are not unique to aquatic organisms but their relative abundance is secondary to that of the C-18 acids in land plants as exemplified by the modern spruce hydrolyzate (Fig. 2A) and as discussed in Robinson (1963). Furthermore, the C-16 fatty acids peak is the "base peak" in the hydrolyzate from the diatom *A. formosa* (Fig. 4A). Diatoms dominate the phytoplankton in these lakes (Stoermer and Kopczynska, 1967; Stoermer and Yang, 1968). Two aromatic acids representing lignin sources, 4-hydroxybenzoic acid and vanillic acid, were discussed in detail previously. Yields of these compounds in the alkaline hydrolyzates are expressed in Table 4 on an equal carbon basis along with data from other workers for comparison.

Comparison of sediment and ancillary samples

Comparison of the yields of all four components for corresponding humic matter fractions from the SLH75-18 sediment and that from SLM77-26 suggests that the humic matter in the Lake Huron sediment is more labile to alkaline hydrolysis than that from the Lake Michigan sediment. The SLH75-18 hydrolyzates contained 3-37 times greater quantities of these components than did the SLM77-26 hydrolyzates. This observation probably reflects differences in the source of sediments. Modern sediments in southeastern Lake Michigan make up the Waukegan Member of the Lake Michigan formation (Lineback *et al.*, 1980), and have as major sources fluvial contributions from rivers and shoreline erosion of glacial tills (Lineback and Gross, 1972). These modern sediments contain reworked detrital organic matter as indicated by radiocarbon ages of about 2000 yr (King *et al.*, 1976; Rea *et al.*, 1980). Detrital organic matter would be more refractory to alkaline hydrolysis.

In contrast, sediment in eastern Lake Huron contains a significant component from erosion of the Silurian dolomite of the Bruce Peninsula region of Ontario as well as a glacial till component (Thomas *et al.*, 1973). The Silurian component is relatively low

in organic matter. Thus the Lake Huron sediment contains a higher relative proportion of newly synthesized organic matter of aquatic origin which would be more labile to alkaline hydrolysis. Simple ratios of C-16 fatty acids to 4-hydroxybenzoic acid are higher for all fractions (especially HA and HU) of the Lake Huron sediment, showing that aquatic organic sources are indeed proportionately more important.

However, it is not only the aquatic source material that is more labile in Lake Huron sediments. The previous sedimentological arguments suggest that, as a whole, the SLM77-26 organic matter experienced long periods of subaerial exposure prior to deposition on the lake bottom. Thus the lability of the SLH75-18 humic matter suggests shorter periods of subaerial exposure. High yields of all four components in Table 4 for the Lake Huron organic matter and comparisons with the ancillary samples bear this out.

High yields of these hydrolysis products were obtained from the FA of the Prince Edward Island (PEI) podzol by Neyroud and Schnitzer (1974, 1975), who reported this soil to be poorly drained. Under poor drainage conditions one would expect preservation of labile organic matter to be greater compared to a drier soil (such as the Alberta chernozem) but possibly less than that of a subaqueous sediment. The FA and HA data (considered together) for these

sediment and soil samples are consistent with this ranking of increasing degree of exposure to oxidative weathering: SLH75-18 < PEI podzol < SLM77-26 < Alberta chernozem.

Hydrolysis yields from the Lake Michigan topsoil and till samples (Table 4) indicate that they could be sources of a portion of the SLM77-26 humic matter. The topsoil yielded amounts of hydrolysis products generally in the same range as the Lake Michigan sediment, whereas the till gave very low yields consistent with its age and degree of subaerial exposure.

Glaciolacustrine clay hydrolysis yields must be explained with reference to its environment of deposition as detailed earlier, rather than simply considering its age. The presence of carbohydrates represented by the 3,4-dihydroxybutanoic acid yield can be explained by preservation due to rapid burial and isolation from oxidizing environments (unlike the Lake Michigan till). A similar argument can explain why the yields of the other three products are higher than those of the till. The difference between the glaciolacustrine clay and modern Lake Huron sediment is explained by lower aquatic productivity under periglacial conditions, little terrestrial productivity, and degradation over 10,000–11,000 yr.

Yield data from the remaining ancillary samples (Table 4) cannot be compared directly to the sediment and soil samples since they represent potential

Table 4. Yields of various components by alkaline hydrolysis and other oxidation methods^a (values are expressed as $\mu\text{g}/\text{mg}$ C in sample)

		3,4-dihydroxybutanoic acid	n-C16 fatty acids	4-hydroxybenzoic acid	Vanillic acid
<i>Alkaline hydrolysis</i>					
SLH75-18:	Fulvic acid	360	68.8	157	81.6
	Humic acid	322	127	99.8	149
	Humin	10.6	48.3	8.1	39.3
SLM77-26:	Fulvic acid	9.7	3.2	9.5	Nil
	Humic acid	18.8	11.2	30.7	8.8
	Humin	3.4	1.9	2.2	Nil
Podzol, P.E.I.:	Fulvic acid ^b	NR	82.5	27.3	62.4
Chernozem, Alta:	Humic acid ^b	NR	114	8.4	17.1
Podzol, Japan:	Humic acid ^c	NR	NR	4.2	1.0
Topsoil, Lake Michigan shore		5.2	8.2	4.3	8.4
Till, Lake Michigan shore		1.0	2.1	Nil	Nil
Glaciolacustrine clay, Lake Huron		10.5	8.0	4.4	8.1
White spruce, modern		8.3	2.3	3.7	6.6
White spruce, Pleistocene		7.2	Nil	1.8	24.8
Celery (<i>Apium graveolens</i>)		39.4	4.2	2.3	Nil
Phytoplankton: (<i>A. formosa</i>)		5.4	163	1.2	Nil
(<i>Chlamydomonas</i> sp.)		Nil	19.5	Nil	Nil
<i>Alkaline CuO oxidation</i>					
Podzol, P.E.I.:	Fulvic acid ^d	ND	56.0	15.9	17.3
Chernozem, Alta:	Humic acid ^d	ND	28.7	9.6	17.2
Terrebonne Bay, LA:	Sediment (No. 2) ^e	ND	NR	2.6	3.1
	Sediment (No. 4) ^e	ND	NR	1.5	2.0
Continental Shelf sediment, off LA ^f		ND	NR	0.2	0.2
<i>Alkaline KMnO₄ oxidation</i>					
Spruce forest soil,	Fulvic acid ^f	ND	ND	11.8	23.9
Norway:	Humic acid ^f	ND	ND	9.1	19.4
	Humin ^f	ND	ND	7.3	17.5

NR—Not reported, presence not excluded by analytical scheme.

ND—not determined, analytical conditions precluded determination.

^aThis work unless otherwise noted.

^bNeyroud and Schnitzer (1975).

^cTsutsuki and Kuwatsuka (1979).

^dNeyroud and Schnitzer (1974).

^eHedges and Parker (1976).

^fOgner (1973).

sources undiluted by mineral matter. Nevertheless, a few observations can be made which aid in understanding the levels of hydrolysis components obtainable from the various end numbers. The modern and Pleistocene spruce samples contained 45 and 49% TOC, respectively (Bourbonniere, 1979). Yields of 3,4-dihydroxybutanoic acid are similar for both woods, suggesting about the same lability of the cellulose in each sample. However, the yield of vanillic acid is almost four times greater for the Pleistocene spruce suggesting a selective preservation of lignin. An alternative explanation could be that the particular spruce tree contained a greater proportion of lignified tissue when it was alive 10,000 yr ago. Fatty acids are very labile and more than 90% degraded over the 10,000 yr period of burial (Meyers *et al.*, 1980b). This is shown again by the present alkaline hydrolysis results.

High yields of 3,4-dihydroxybutanoic acid from celery are to be expected as it is almost pure cellulose. The much higher yields from the SLH75-18 FA and HA fractions compared to celery might indicate that the sedimentary polysaccharides are partially degraded and possibly more labile to alkaline hydrolysis. Yields of C-16 fatty acids from the phytoplankton are high enough to consider these organisms as potentially significant contributors to sedimentary fatty acids. Low yields of the other components confirms that phytoplankton are not significant sources of cellulose or lignin indicators.

Utility of alkaline hydrolysis

The quantitative data in Table 4 suggests that alkaline hydrolysis as used in the present study and by Neyroud and Schnitzer (1975) is superior to the degradation schemes that cause more oxidation. Hydrolysis in the absence of oxygen evidently preserves aliphatic structures such as fatty acids. Carbohydrate moieties are broken down into smaller aliphatic components which are representative of the original structure. In contrast, carbohydrates are easily degraded to CO₂ and H₂O by oxidative schemes. For example, comparison of the alkaline hydrolysis results of Neyroud and Schnitzer (1975) with results of their alkaline CuO oxidations on the same FA and HA samples (Neyroud and Schnitzer, 1974) shows significantly higher yields by non-oxidative hydrolysis than by oxidative procedures. Aromatic components appear to be less affected by the oxidative process, confirming the utility of this procedure for specific purposes such as the phenolic aldehyde study of Hedges and Parker (1976) in their transect of Gulf of Mexico sediments.

SUMMARY AND CONCLUSIONS

Degradation by alkaline hydrolysis in the absence of oxygen preserved aliphatic and carbohydrate-related components of sediment humic matter fractions and of ancillary samples in this study. This is an

advantage over other degradation methods which oxidize these components and compares well with the non-destructive NMR studies of Hatcher *et al.*, (1980a, b, 1981 a, b). Alkaline hydrolysis has the added advantage that components may be isolated which yield information about specific organic source materials. Fatty acids of algal origin, saccharinic acids from cellulose degradation, and lignin derivatives can be determined by analysis of the hydrolyzates.

Humic matter fractions from these Lake Huron and Lake Michigan sediments contain high relative proportions of carbohydrate moieties. Evidence is also found for aquatic lipid and terrestrial lignin contributions to the organic matter of these sediments. Qualitative differences among the fulvic acid, humic acid and humin fractions from the same lake are minor, suggesting commonality of organic source materials for these fractions. Since alkaline hydrolysis in the absence of oxygen is ineffective in breaking C-C bonds, the existence of condensed aromatic structures in the sedimentary humic matter cannot be investigated by this procedure.

Yields of hydrolysis components are related to the lability of samples. Fulvic acids are generally more labile to hydrolysis than humic acids, which are in turn more labile than humin, suggesting that yields are indicative of diagenetic stages. This in turn can be related to a sample's history of exposure to oxidative weathering and, in the case of a sediment, its environment of deposition. For instance, glaciolacustrine clay yielded significant quantities of saccharinic acids suggesting that even carbohydrates can be preserved if rapidly buried under high sedimentation rate conditions.

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