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GEOCHEMICAL STUDIES OF FATTY ACIDS AND THEIR RELATED COMPOUNDS IN RECENT SEDIMENTS

Kimitaka KAWAMURA

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現世堆積物中の脂肪酸と関連化合物の地球化学的研究

GEOCHEMICAL STUDIES OF FATTY ACIDS AND THEIR RELATED

COMPOUNDS IN RECENT SEDIMENTS

by

Kimitaka KAWAMURA

Department of Chemistry, Faculty of Science
Tokyo Metropolitan University
Fukazawa 2-1-1, Setagaya-ku
Tokyo, Japan

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ABSTRACT

We studied fatty acids and their related compounds (hydroxy acids,
dicarboxylic acids, hydrocarbons and alcohols) in a 200 m sediment
core taken from Lake Biwa to clarify their post-depositional changes
and also changes in their input to the sediment in the past. We also
conducted thermal alteration experiments to interpret the observation
results from a view point of diagenesis.

It was found that lower M.W. fatty acids (C_{12}-C_{19}) in unbound and
bound fractions decreased drastically in the upper 20 m sediment layers.
We concluded that such a phenomenon was caused by α-, β- and ω-oxidative
degradation of those acids. Higher M.W. (C_{20}-C_{30}) fatty acids, ω-hydroxy
acids and α,ω-dicarboxylic acids showed vertical fluctuations with some
peaks. These fluctuations were considered to be involved with allo-
chthonous contribution of organic matter to sediments based on the
thermal alteration experiment.

β-Hydroxy acids, which are constituents of bacterial cell wall,
were found to be mostly present in the residual fractions which are
separated by harsher saponification of the pre-extracted sediments.
This suggests that bacterial activity is associated with the formation
of geopolymers such as kerogen and humic compounds in sediments.

A considerable amount of polyunsaturated fatty acid ($C_{18:2}$) was
detected in the core sediment of Lake Biwa (0-20 m). The $C_{18:2}/C_{18:0}$
ratio was proposed as a possible indicator of paleoclimate. The times
of 200, 1000-4000, 15,000 and 20,000 yr BP were suggested to have been
cooler or colder than other ages. This hypothesis seemed to be supported
by other evidence from pollen analyses and oxygen isotopic analyses.

Thermal alteration experiment (65-112°C) showed that the concentra-
tion of fatty acids firstly decreased and then increased with increasing
temperature and time. We concluded that these acids were firstly
trapped in organic and/or inorganic matrices at milder conditions and
then released at higher temperature or in longer time.

A considerable amount of β-hydroxy acids were released upon heating

at 154°C for 1 day. Their concentration became 3 times larger than the initial one (unheated). It is probable that these acids are mainly present in geopolymers such as kerogen and humic compounds and they are released by breakdown of their bondings with geopolymers upon heating.

Thermal stability of organic compounds in the heated sediment samples (68°C-325°C, 1 day) were in the order: unsaturated fatty acids $<$ β-hydroxy acids $<$ ω-hydroxy acids $<$ α,ω-dicarboxylic acids $<$ saturated fatty acids. Saturated fatty acids were stable at severe thermal condition (325°C) where n-alkanes were abundantly generated.

ACKNOWLEDGEMENTS

CONTENTS

GENERAL INTRODUCTION

1. Historical view of the previous studies on fatty acids and related compounds in sediments

Fatty acids

The studies on fatty acids in sediments seem to have been conducted from three streams of the interests and the needs.

Since fatty acids have been considered to be a possible precursor of petroleum hydrocarbons (Cooper and Bray,1963), their distributions in ancient sediments have been widely observed together with paraffinic hydrocarbons (e.g. Kvenvolden, 1968; Burlingame et al., 1969; Douglas et al., 1968). These observations showed that odd carbon numbered fatty acids are abundantly present together with even carbon numbered fatty acids in old sediments. On the other hand, fatty acids in organisms and recent sediments show even carbon numbered predominance. So, it has been considered that their characteristic distributions in ancient sediments was caused by the diagenesis and considerable amounts of fatty acids were decarboxylated and n-alkanes were produced during diagenesis. These ideas have been a driving force of the observational researches of sedimentary fatty acids. However, Van Hoeven et al. (1969) showed that the even/odd predominance of n-fatty acids can be preserved throughout geological time, that is, even carbon numbered fatty acids are predominant in several ancient sediments and oils.

In recent years, surface or young sediments have been subjected to fatty acid analyses by many workers (e.g. Brooks et al., 1973, 1976, 1977; Johns and Calder , 1973; Boon et al., 1975; Ishiwatari and Hanya, 1975; Matsuda and Koyama, 1977; Barnes and Barnes, 1978) in order to elucidate an early diagenesis of organic matter in sediments. The fatty acids in sediments have been considered to be a possible indicator to evaluate diagenesis. These studies showed that saturated fatty acids in surface recent sediments are characterized by the even numbered predominance and a bimodal distribution with peaks at C_{16} and C_{22} to C_{28} acids. In addition, the lower carbon numbered fatty acids such as palmitic acid (C_{16}) decrease with

depth in the surface sediments. This phenomenon has been explained
by selective microbial degradation of these acids (Matsuda and Koyama,
1977). However, degradation process and degradation products have
not been clearly presented.

The presence of unsaturated fatty acids has been also reported
in recent sediments. Mono-unsaturated fatty acids decreased more
rapidly compared to saturated ones in the surface sediments (Farrington
and Quinn, 1977; Matsuda and Koyama, 1977). This fact indicates that
mono-unsaturated fatty acids are more reactive than saturated ones
in sediments and suggests that these compounds may play a role in
a diagenetic process of organic matter.

Polyunsaturated fatty acids, which are major components of
aquatic organisms, have been rarely reported in sediments (Poltz,
1972; Volkman and Johns, 1977). This suggests that these acids are
labile compounds in water column and sediments, and also indicates
that a portion of these acids can be preserved in suitable conditions
(Cranwell, 1978) such as highly primary productivities, shallow water
column, anoxic conditions, etc.

Since the distribution of fatty acids in organisms depends on
the species and environments, some fatty acids in sediments are valid
as a possible indicator to estimate the biological and environmental
conditions. The studies on these fatty acids have been also conducted
with a view of an application of organic geochemistry to paleoenviron-
mental or paleolimnological studies.

Sedimentary normal fatty acids, in general, show bimodal distri-
butions in the lower molecular weight region (e.g. C_{14}, C_{16}, C_{18}) and
higher molecular weight region (e.g. C_{20}-C_{30}). The former acids are
considered to be mainly derived from autochthonous organisms such as
phytoplankton and bacteria. The latters are considered to be of allo-
chthonous origin (higher plant wax esters). Therefore, the lower/higher
distribution of fatty acids has been proposed as an indicator to
estimate a relative contribution of autochthonous/allochthonous organic
matter to sediments (Brooks et al., 1976; Cranwell, 1974; Ishiwatari
and Hanya, 1975). For example, Ishiwatari et al. (1980) studied the

organic compounds in a 1.2 m long sediment core of Lake Haruna and elucidated the lake history, i.e. changes in primary production in the past, based on the characteristic distribution of organic compounds including fatty acids.

Branched iso/anteiso fatty acids in sediments have been discussed to evaluate the activity of bacteria, which contain these acids abundantly (Kaneda, 1967). Cranwell (1978) reported that the concentration of branched fatty acids are more abundant in a bound fraction than in an unbound fraction, and considered that fatty acids in bound form are more related to bacterial activity than those of unbound form.

In those studies, fatty acids are used as a chemical fossil. Finding, proposal and application of new chemical fossils are important problems in the field of the organic geochemistry since such studies may contribute to interdiciplinary fields of science. However, it is needed to study the origin and the behavior of fatty acids in the sediments (their reaction and preservation) before use of these compounds as chemical fossils.

Hydroxy acids and α,ω-dicarboxylic acids

Hydroxy acids have been reported to be present in sediments. Eglinton et al. (1968) have detected α-, β- and ω-hydroxy acids in 5000 years old lacustrine sediments. They considered that α- and β-hydroxy acids are derived from fatty acids by α- and β-oxidation and that ω-hydroxy acids were derived from terrestrial plant cutin. Cardoso et al. (1977) proposed the use of cutin acids in the recognition of higher plant contribution to recent sediment and suggested that β-hydroxy acids are probably derived in the main form from the original microbial biomass in the sediments. Boon et al. (1977) detected α-, β-, ω- and ω-1 hydroxy acids in Walvis Bay diatomaceous ooze. They Considered that ω- and ω-1 hydroxy acids are produced by an aerobic micro-organism capable of oxygenation on very long chain fatty acids in the water column and that β-hydroxy acids probably reflect the microbial activity in the sediments. Cranwell (1977) detected ω-hydroxy

acids in Cam Loch sediments and considered that they are of terrestrial origin. However, at present time, little is known about the distribution of hydroxy acids in the sediments. In particular, vertical distribution of those acids has been rarely reported.

α,ω-Dicarboxylic acids have been reported in the ancient sediments (Douglas et al., 1968, 1971; Haug et al., 1967; Shimoneit and Burlingame, 1973) and recent sediments (Eglinton et al., 1968; Johns and Onder, 1975; Ishiwatari and Hanya, 1975; Cranwell, 1977; Ishiwatari et al., 1980). These acids have been considered to be derived by the oxidation of ω-hydroxy acids (Eglinton et al., 1968), by the oxidation of n-saturated fatty acids (Ishiwatari and Hanya,1975) and from the contribution of mangroves and sedimentary organisms (Johns and Onder, 1975). However, little is known about the origin and distribution of α,ω-dicarboxylic acids in the sediments.

Since hydroxy acids and dicarboxylic acids are not found in algae, which are major source of sedimentary organic matter and they have similar carbon skeletons to fatty acids, it is of interest to study the vertical distribution of these acids in sediment core, compared with fatty acid distribution. These studies would provide evidence to interpret a diagenesis of fatty acids, and might provide information about the paleolimnological evidence (Cardoso et al., 1977). However, the studies on their verical distribution have been scarcely conducted.

The problems to be solved

Although many works on fatty acids and related compounds in sediments have been reported, there seems to be an important problem to be solved, i.e. to decipher both origin and diagenetic history of these compounds in sediments. Such a problem is also important from a view point that these compounds are valuable as chemical fossils (Eglinton and Calvin, 1967). However, adequate studies have not been conducted as described below.

Lacustrine sediments contain a few percent of organic matter. The organic matter is originated from aquatic organisms as well as

terrestrial organic sources. After deposition, these organic compounds
suffer diagenetic changes. Therefore, the qualitative and quantitative
distributions of organic compounds in sediments are determined by
two factors: primary by their input to sediment, and secondly by their
post-depositional change (diagenesis). Those two factors are very
important when we study the organic compounds including fatty acids
in sediments.

So far, many observational results on organic compounds in sedi-
ments have been discussed on assumptions as follows. The change in
the input of organics to sediment in the past is not taken into account
when diagenetic processes are discussed, whereas diagenetic processes
are ignored when the change in the input of organic compounds to
sediments in the past is discussed. However, those assumptions are
unreasonable, because two factors are simultaneously operative in the
sediments. In fact, it is very difficult to differentiate between two
factors in the vertical distribution of organic compounds observed in
a sediment core. However, there seems to exist some possible ways to
conquer the difficulty.

Detailed observation of organic compounds in a long sediment core
would provide the way to interpret the vertical distribution. A unimodal
direction in the vertical distribution of a compound may be strong
evidence to show or suggest that the distribution is caused by dia-
genesis. The peaks which are observed and are cleary distinguishable
from the general trend in the vertical distribution of an organic
compound would be caused by the change in the input of the compound in
the past. Furthermore, the analyses of the related compounds would
give us a valuable information on both diagenesis and origin of organic
compounds.

Apart from the observation studies, laboratory simulation experi-
ment is a useful methodology for the study of diagenesis of organic
matter in sediments (Eglinton, 1972) and also for the studies on the
existence forms of organic compounds in sediments, which would
seriously related to their geochemical behavior in sediments.

The fatty acids so far studied are concerned with recent (ca. several thousand yrs old) and ancient sediments (including Precambrian age). The description on fatty acids in a long sediment core with wide time range has been rarely reported. Therefore, it is attractive to extract the informations of the diagenesis of fatty acids and the changes in the input of these acids to sediments, based on the observation of the vertical distribution of those compounds in a long sediment core.

Fatty acids in sediments may exist in different chemical states, such as free, ester, and trapped or incorporated forms to organic and/or inorganic matrices. However, those chemical states have not been elucidated, because of difficulties of the studies. Since fatty acids in aquatic environment are subjected to chemical and biological attack after the death of organisms, their chemical states might be very complicated in sediments. So far, organic solvent extraction has been used as a popular separation procedure of fatty acids from sediments. Recently, a considerable amount of fatty acids has been obtained by saponification of pre-extracted sediment sample (Farrington and Quinn, 1971). The fatty acids which are separated by solvent extraction have been defined as "unbound fatty acids" and those that are separated on saponification of pre-extracted sediment have been defined as "bound fatty acids". Bound fatty acids have been found to comprise 30-70 % of the total (unbound + bound) fatty acids in recent sediments (Farrington and Quinn, 1973, 1977; Cranwell, 1974; Ishiwatari et al., 1980). The chemical state of bound fatty acids may be involved with the ester linkage with humic materials and other bondings with organic and inorganic matrices (Farrington and Quinn, 1977), although existing forms of bound fatty acids are not clearly elucidated.

Furthermore, other forms of fatty acids have been suggested to exist in sediments by thermal alteration experiments (Baedecker et al., 1977; Harrison, 1978), in which a considerable amount of extractable fatty acids was obtained by heating the sediment and pre-extracted sediment. They considered that fatty acids released on heating might

have been incorporated in kerogen and/or humic materials and sediment surface. The author also conducted a laboratory experiment on diagenesis of fatty acids and concluded that major part of the fatty acids released on heating came from bound fatty acids and the remaining part came from the residual form (see Chapter 7). It is very important to study main forms of fatty acids present in sediments when their diagenesis are discussed, because fatty acids may probably change their existence forms during diagenesis. We must bear in mind that the term "diagenesis" contain two concepts: (1) the change in the organic molecules which is concerned with the change of the functional groups and the skeleton,and (2) the changes in the existence forms of orgnaic molecules.

2. Purpoese of the present study

It is a fascinating problem to elucidate the factors which control the distribution of orgnaic matter in sediments: changes in their supply and diagenesis. The present author initiated the study with the following purposes.

The first is to describe the vertical distributions of fatty acids and related compounds (hydroxy acids, dicarboxylic acids, alcohols, hydrocarbons, etc), which exist in the main forms in a 200 m long sediment core of Lake Biwa.

The second is to interprete their distributions in terms of the changes in the input of organic compounds to sediments in the past and their post-depositional changes (diagenesis). The general criteria to differentiate diagenesis from the changes in the input are (1) whether or not an unimodal direction in the vertical distribution of orgnaic compounds is present, (2) whether or not the distribution pattern of other related compounds correlates with that of fatty acids, (3) whether or not the diagenetic potential of organic compounds, suggested from thermal alteration experiments of sediments, is high.

The third is to propose possible indicators for the estimation of paleoenvironments and to discuss paleoenvironmental conditions by using

the organic compounds.

This thesis consists of three parts. Part I deals with the observational results of fatty acids and the related compounds in the 200 m sediment core of Lake Biwa. Their vertical distributions are explained in terms of diagenesis and the changes in their input to the sediment in the past. Part II deals with the experimental studies of fatty acids and their related compounds in recent sediments. Their behaviors in the heated sediment samples are explained in terms of changes in their existing forms and chemical reaction. Part III deals with general discussion on diagenesis based on agreement (or disagreement) between observation (Part I) and experiment (Part I) and with future problems.

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PART I

OBSERVATIONAL STUDY OF A 200 M CORE TAKEN FROM LAKE BIWA

CHAPTER 1

SAMPLES AND ANALYTICAL PROCEDURE

1.1. Description of Lake Biwa and sampling

The author has considered that the sediment cores of Lake Biwa
seem to be most appropriate for the observational study on diagenesis
of organic matter, since the lake is very large and the lake conditions
such as water depth, drainage basin, and lake water conditions may
have been relatively stationary throughout long ages, consequently
the sediment layers are seemingly homogeneous.

Lake Biwa is the largest freshwater lake in Japan and one of the
most ancient lakes in the world. The lake is oligotrophic and its
altitude, area, volumn, maximum water depth and age are 85 m, 674.4 km^2,
27.8 km^3, 102.1 m and ca. 5 M yr old, respectively. Under the bottom
of the lake, very thick sediment layers (ca. 2 km) are thought to be
present. The lake is located in Omi Basin in the central part of Japan.
Figure 1 shows locality, geological and vegetational maps of Lake Biwa.
Alluvial and terrace deposits, several formations (Kobiwako group),
and basement rocks lie around the lake. In the terrace near the shore,
paddy and farm are developed and both needle-leaved and broad-leaved
forests are developed in the mountain area. Lake water is input through
approximately a hundred inflow rivers, whose drainage area is about
3800 km^2, and the outlet is to Osaka Bay through the Seta River.

Two sediment cores (5 and 200 m long) were taken by Professor
S. Horie and coworkers near the center of Lake Biwa (Location No. Ie-1,
water depth 70m). The 5 m core sample was taken in October 1973 using
a boring corer (Horie et al., 1974; Yokoyama and Horie, 1974). After
collection, the samples were cut horizontally at 5 cm intervals and
stored at 4°C for the 5 m core and at -20°C for the 200 m core.

a   b   c
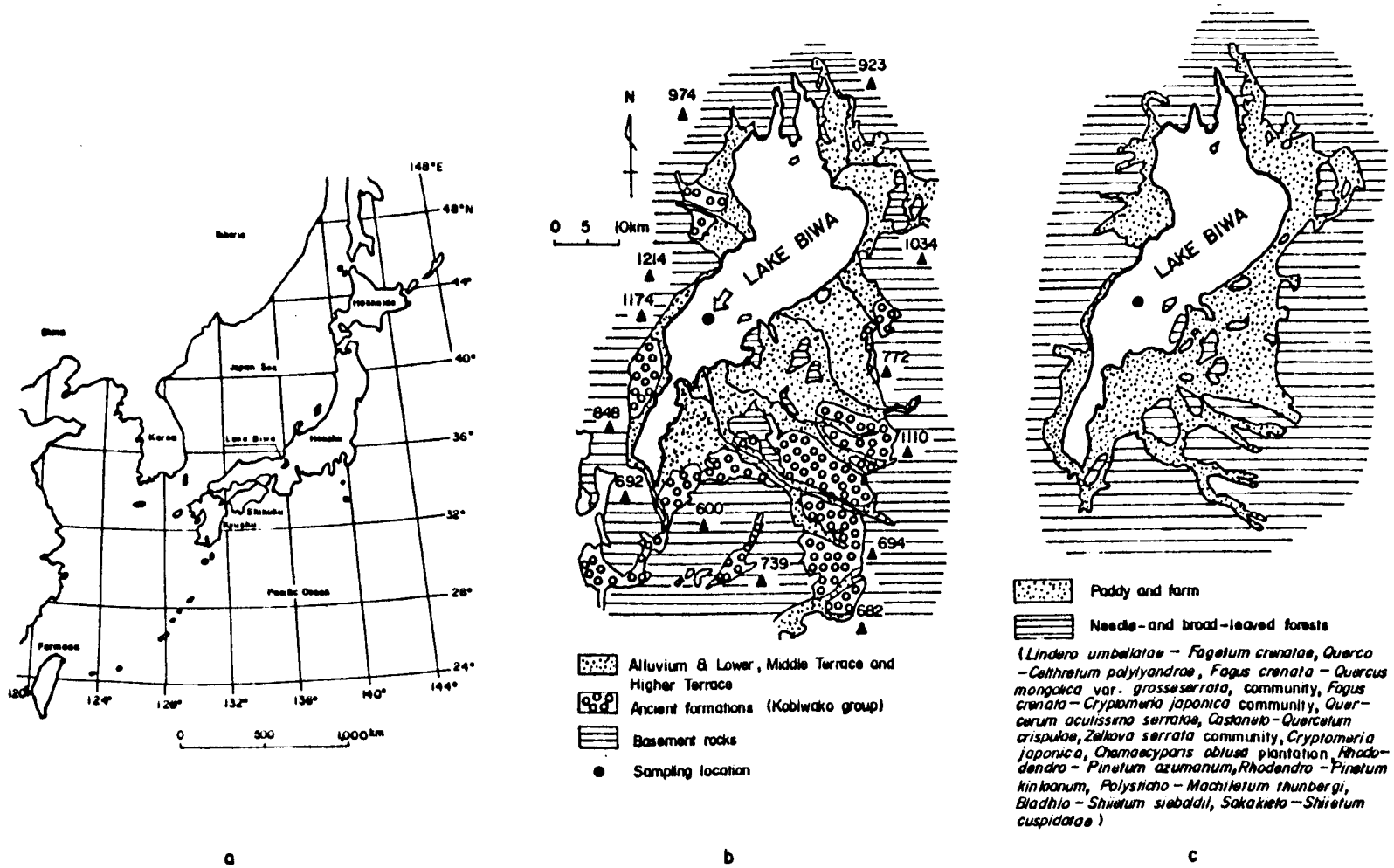
Fig. 1. Locality (a), geological (b) and vegetational (c) maps of Lake Biwa. b: modified from IKEBE and YOKOYAMA (1976); c: modified from FUJI (1976b).

The 200 m core has been studied by many workers from different view points, i.e. physics, geology, paleontology, palynology, biology, chemistry, etc. The papers on the studies can be found in books entitled Paleolimnology of Lake Biwa and the Japanese Pleistocene (ed. S. Horie, 1973-1980), Vol. 1-7.

1.2. Analytical procedures of fatty acids, dicarboxylic acids, hydroxy acids, hydrocarbons and alcohols in sediments

In the present study, the author used three separation procedures of fatty acids and related compounds in sediments, as follows.
(1) organic solvent extraction, which separates the free and ester type of the compounds (Eglinton, 1969).
(2) saponification procedure of pre-extracted sediments, which separates the organic compounds combined to organic and inorganic matrices in sediments.
(3) harsher saponification procedure, which releases the organic compounds trapped or incorporated into the organic or inorganic matrices in sediments.

The third method has not been applied to sediments, yet, although Schnitzer and Neyround (1975) have used this procedure and obtained a considerable amount of fatty acids and hydrocarbons from humic and fulvic acids.

Fig. 2 shows a separation procedure of unbound, bound and residual fractions from sediments. Fig. 3 shows a fractionation procedure of organics into individual compound groups for gas chromatographic analyses.

1.2.1. Separation of unbound, bound and residual fractions from sediments

Unbound fraction : The wet sediment samples (5-10 g) were extracted three times with 80 ml benzene/methanol (6:4) by using a homogenizer (Nihonseiki AM-1). The extracts were separated by filtration (GF/C), concentrated and then saponified with 5 ml 0.5 N KOH/methanol for 2 hours. These extracted lipids were named as "unbound fraction" and the fatty acids in the fraction were called "unbound fatty acids" or "fatty acids in unbound form".

```
                              ┌─────────────┐
                              │Wet sediment │
                              └─────────────┘
                                 │ extraction
                                 │ benzene/methanol (6:4)
           ┌─────────────────────┴──────────────────────┐
       ┌─────────┐                                  ┌─────────┐
       │ residue │                                  │ extract │
       └─────────┘                                  └─────────┘
          │ saponification                     (unbound fraction)
          │ 0.5 N KOH/MeOH
     ┌────┴────────────────────────────┐
 ┌─────────┐                      ┌─────────┐
 │ residue │                      │ extract │
 └─────────┘                      └─────────┘
    │                          (bound fraction)
    │ harsher saponification
    │ 2 N KOH soln.
    │ 180°C, 3 hrs.
  ┌──────┴───────────────┐
┌─────────┐         ┌──────────┐
│ residue │         │ solution │
└─────────┘         └──────────┘
(discard)         (residual fraction)
```

Fig. 2 Separation procedure of unbound, bound and residual
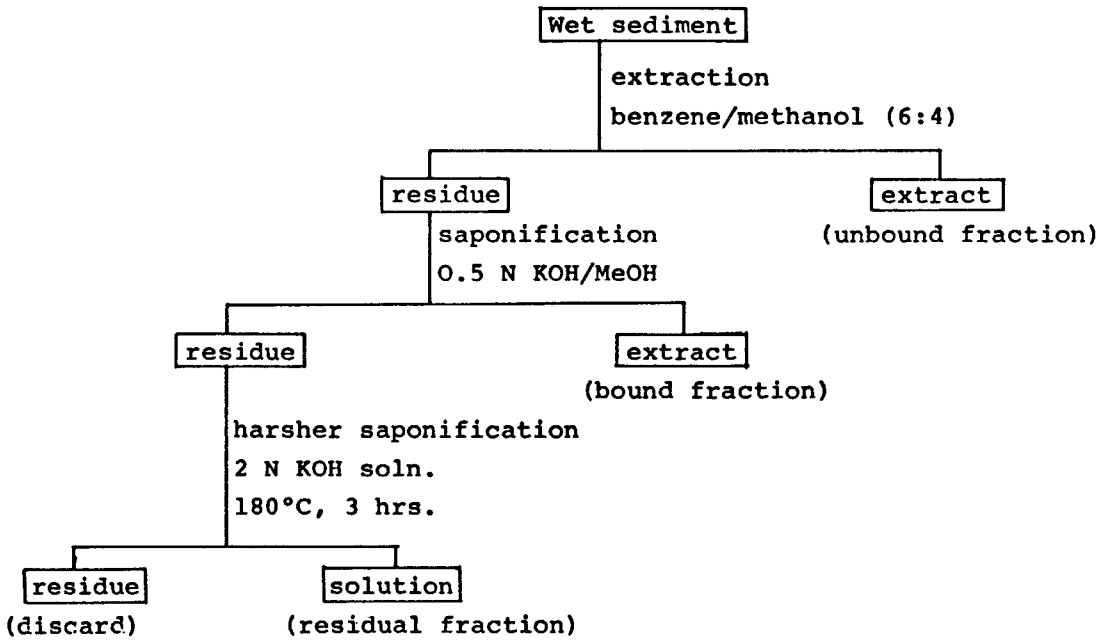fractions from sediments.
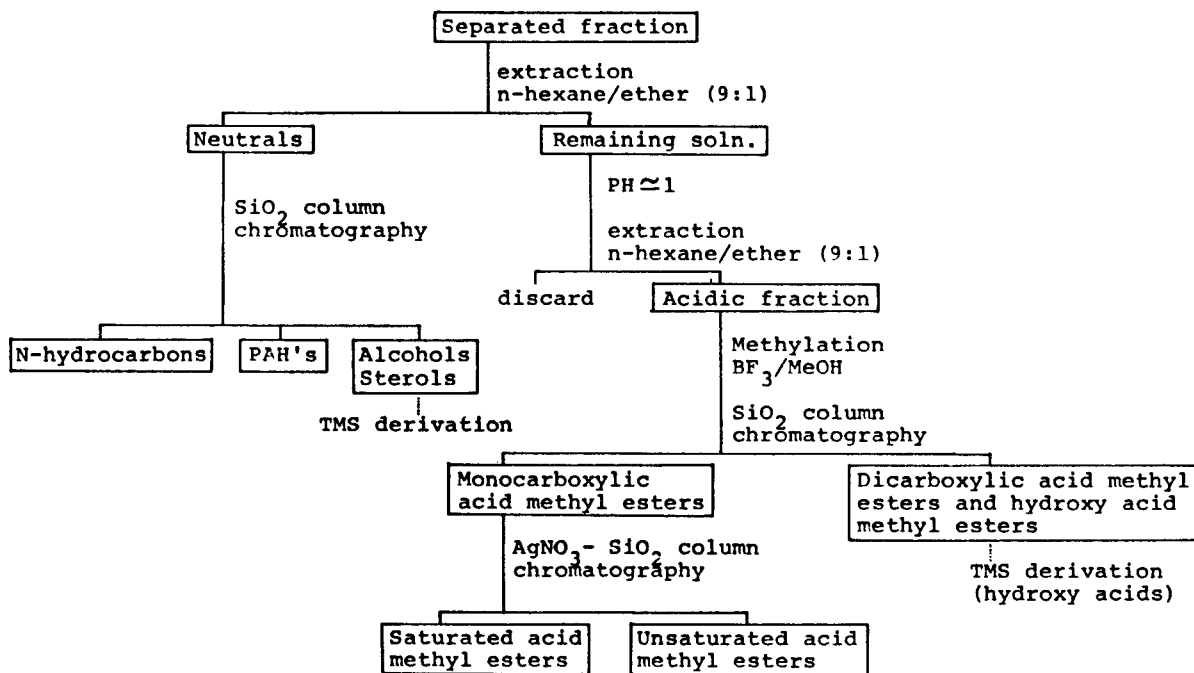
Fig. 3 Fractionation of organics into individual compound groups
for GC analyses

Bound fraction : The pre-extracted sediments were then saponified
with 50 ml 0.5 N KOH/methanol (containing 5 % H_2O) for 2 hours. The
alkaline solution was separated by filtration (GF/C) and concentrated.
Thus separated lipids were named as "bound fraction" and the fatty acids
in this fraction were called "bound fatty acids" or "fatty acids in
bound form".

Residual fraction : The residual sediments, in which unbound and
bound fractions do not exist, were subjected to harsher saponification
procedure. The residual sediments (0.50 g) was taken in a pyrex tube
(12 mm x 15 cm), which contains 5 ml 2 N KOH solution. The tube was
sealed and then heated at 180°C for 3 hours. After heating, the alkaline
solution was separated by filtration (GF/C). Thus separated fraction
was named as "residual fraction" and the fatty acids in this fraction
were called "fatty acids in residual form".

1.2.2. Extraction of organic compounds and further fractionation by silica gel column chromatography

Neutral compounds : Neutrals were extracted three times with 20 ml
n-hexane/ether (9:1) from three fractions. The extracts were washed with
distilled water and concentrated. The concentrated neutral fractions
were separated into three fractions, i.e. n-hydrocarbon, polynuclear
aromatic hydrocarbon (PAH) and alcohol + sterol fractions on a silica
gel colum containing 5 % H_2O. N-hydrocarbons were eluted by n-hexane,
PAH's were by n-hexane/benzene (9:1) and alcohols and sterols were by
benzene/ethyl acetate (1:1). The former two fractions were concentrated
to 50 µl in a 1 ml glass ample and subjected to GC and GC-MS analyses.
The organic solvents in the latter fractions were evaporated and the
alcohols and sterols were transformed to TMS ether derivatives in a
1 ml glass ample. The derivatives were analyzed by GC and GC-MS.

Acidic compounds : The remaining solutions, which contain organic
acids, were acidified by conc. HCl. Acidic fractions were extracted
three times with 20 ml n-hexane/ether (9:1) and then methylated using
14 % methanolic BF_3. Monocarboxylic acid methyl esters were eluted with

n-hexane/benzene (9:1) and dicarboxylic acid methyl esters and hydroxy acid methyl esters were eluted with benzene/ethyl acetate (1:1) on a 5 % water containing silica gel column. The former fraction was then separated into saturated and unsaturated fraction by means of $AgNO_3$-silica gel column chromatography. The saturated and unsaturated fractions were concentrated in a 1 ml glass ample and subjected to GC and GC-MS analyses. The hydroxy acid methyl esters were transformed to TMS ether derivatives in a 1 ml glass ample and then subjected to GC and GC-MS analyses.

1.2.3. GC and GC-MS analyses of organic compounds

Saturated fatty acid methyl esters were analyzed with a Shimadzu GC-4BM or GC-6A gas chromatograph on a 2 m x 3 mm glass column packed with 1.5 % OV-1 on Chromosorb W (AW, DMCS). The column temperature was programmed from 100 to 295°C at 5°C/min. Unsaturated fatty acid methyl esters were analyzed with a Shimadzu GC-4BM, GC-5A or GC-6A gas chromatograph on a 2 m x 3 mm glass column packed with 3 % DEGS on Chromosorb W (AW, DMCS). The column temperature was programmed from 120 to 200°C at 3°C/min. The esters were identified with a Shimadzu-LKB 9000 gas chromatograph-mass spectrometer (GC-MS), which was operated at an electron energy of 70 eV, and an accelerator voltage of 3.5 kV and an ion source temperature of 330°C. The molecular separator was maintained at 300°C. Gas chromatograms were recorded by use of total ionization current monitor (TICM) at 20 eV.

α,ω-Dicarboxylic acid methyl esters and hydroxy acid methyl ester TMS ethers were analyzed with a GC-MS at same conditions for fatty acid analyses. Dicarboxylic acids were quantified based on the peak height of the mass fragmentogram at m/e 98. The hydroxy acids were quantified based on the peak height of gas chromatogram (TICM). Identification of these compounds were performed by comparing their gas chromatographic retention times and mass spectra with those of authentic standards or those of literatures (Eglinton et al., 1968; Boon et al., 1977).

Normal hydrocarbons were analyzed with a GC-MS and GC on a same

column for fatty acid analyses. The column temperature was programmed
from 100 to 300°C at 8°C/min.

PAH's were analyzed with a GC-MS on a same column condition as
that for fatty acid analyses. The column temperature was programmed
from 180 to 280°C at 8°C/min. Quantification of PAH's was performed
based on mass fragmentography at m/e of individual molecular ions.

Alcohol TMS ethers were analyzed with a GC-MS on a 1.5 % OV-1 column.
The column temperature was programmed from 100 to 280°C at 5°C/min.
Quantification of the alcohols was performed based on the mass fragmento-
graphy at M-15 of individual molecules.

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CHAPTER 2

VERTICAL PROFILES OF CARBON, NITROGEN, HUMIC COMPOUNDS AND
MINERALS IN THE 200 M CORE OF LAKE BIWA

2.1. INTRODUCTION

The lacustrine sediment is a complicated mixture of organic and
inorganic materials. The organic matter, which originates in terrestrial
plants and/or soil organic matter and in autochthonous organisms such
as phytoplankton, may recieve biological attack and chemical changes
during sedimentation process in water column. They may interact with
inorganic materials which were input from the drainage area through the
inflow rivers and/or supplied by aquatic organisms such as diatom. So,
the distributions of individual organic molecules in the mixture (sedime-
nt) may be dependent on many factors. Therefore, the author thinks it
valid to study the fundamental indices of carbon, nitrogen, C/N ratio,
humic materials and mineral composition, which may be closely related to
the origin and history of the sediments, before individual organic
molecules are studied in detail.

2.2. EXPERIMENTAL

Approximate 60 sediment samples from the 5 and 200 m cores were
used in the present study.
Carbon and nitrogen : Total carbon and nitrogen contents of the samples
were measured by a Yanagimoto MT-2 C,H,N corder. The errors were within
1 % based on triplicate analyses of Lake Biwa sediments(#211-1, 89.0 m
in depth). Since carbonate carbon is less than about 1 % of the total

carbon content in samples of the lake sediment (Horie et al., 1971),
total carbon content is thought to approximate total organic carbon
content.

Humic compounds : Humic compounds were analyzed by the method of
Ishiwatari et al. (1980). The residual sediment samples (200 mg),
from which both unbound and bound fractions were removed, were extracted
with 10 ml 0.5N NaOH solution at 60°C for an hour. The alkaline solution
was separated by decantation, centrifuged at 8000 rpm for 20 min, and
subjected to Hitachi EPS-3T spectrophotometer to obtain the visible
spectra. The humic compounds which contain both fulvic and humic acids
were quantified at an absorbance of 400 nm by using the humic acid
solution from Lake Haruna (Ishiwatari et al., 1980) as standard.

Mineral composition : The mineral composition of the samples was
analyzed with a Rigaku X-ray diffraction computer system. The analytical
errors were within 10 %.

2.3. RESULTS

Carbon and nitrogen contents, and C/N ratios were in the ranges of
8.42-21.6 mg/g dry sediment, 1.16-2.77 mg/g dry sediment, and 5.17-10.4,
respectively. Figures 4 and 5 show a vertical profile of carbon content
and C/N ratio, respectively. Total carbon contents show a large peak
in the 1-15 m sediments. Higher values were also observed in the 55-90
m sediments. The C/N ratios (Fig. 5), which showed a higher value in
the surface sediment, appeared to decrease with depth in the 0-30 m
sediments, exhibiting some peaks.

The concentrations of humic compounds were in the range of 1.75-8.80
mg/g dry sediment. They comprise 9.2-28 % of total organic matter (TOM:
TOM = total carbon x 1.67). Fig. 6 shows a vertical profile of humic
compounds in the 200 m core. A large peak is recognized in the 1-15 m
sediments and also relatively higher values were observed in the 60-90 m
sediments. These sediment layers seem to be consistent with the layers
where higher values of total carbon contents were recognized.
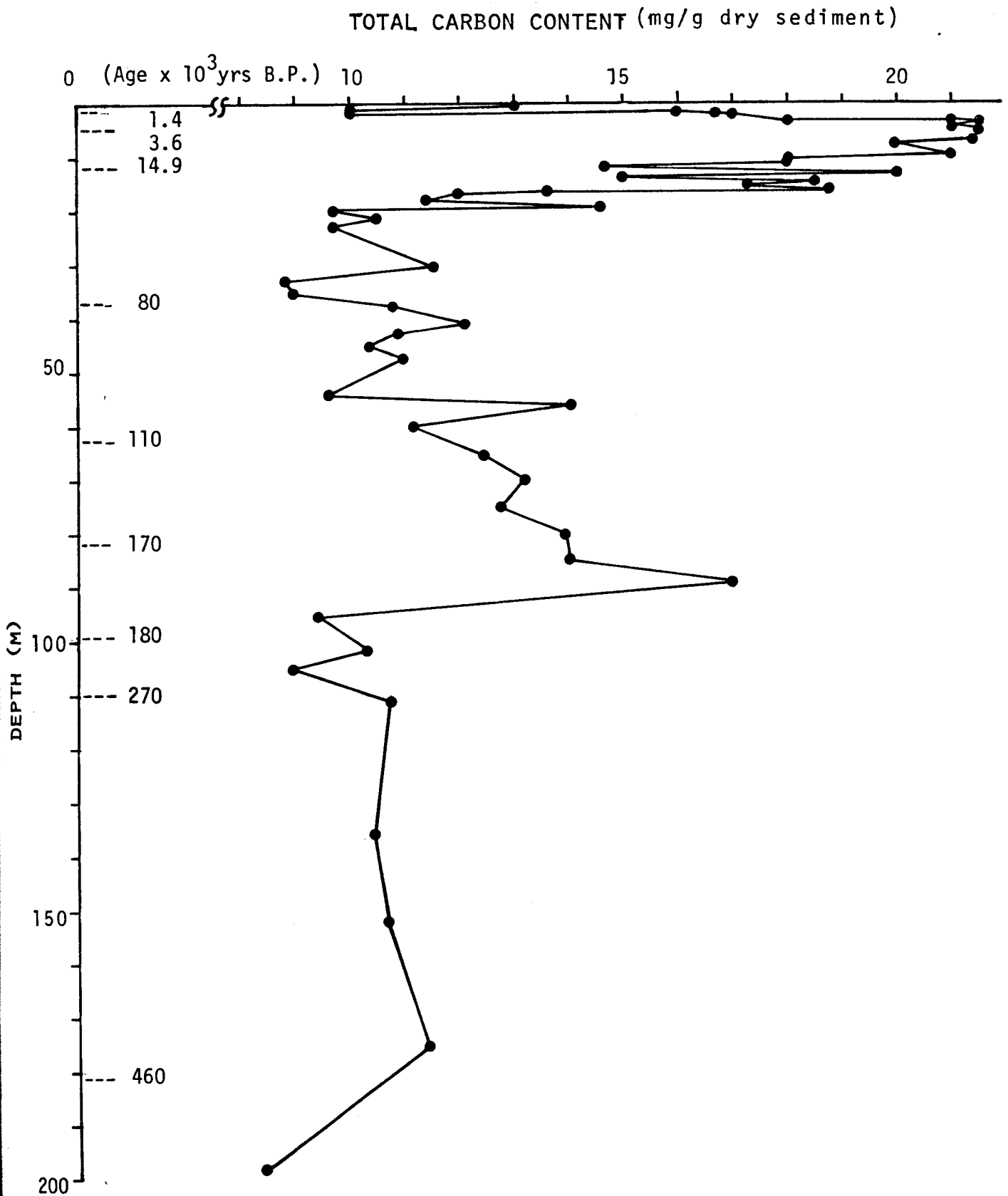
Fig. 4 Vertical profile of total carbon content in a 200 m core of
        Lake Biwa
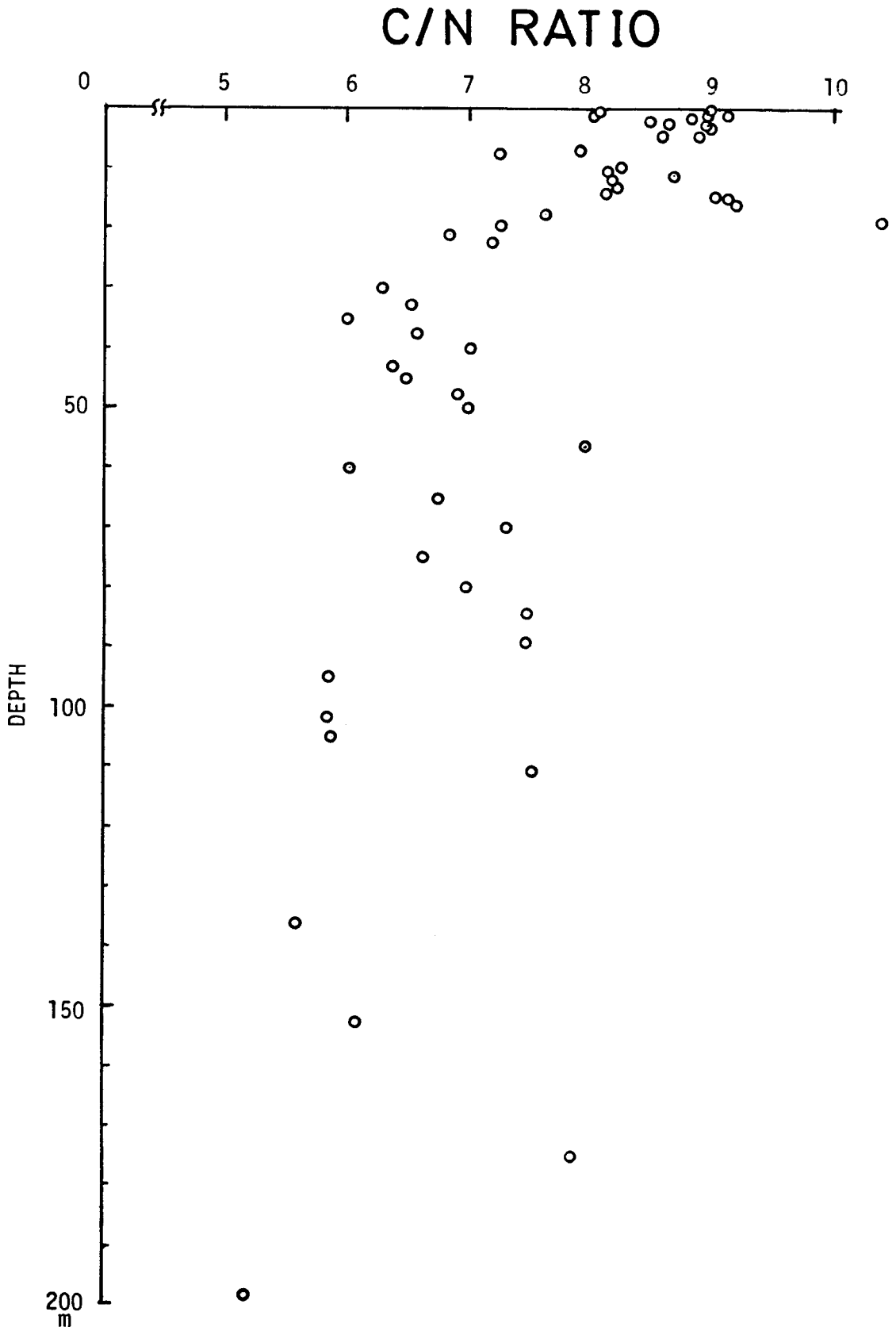
Fig. 5   Vertical profile of C/N ratio in a 200 m core
of Lake Biwa.
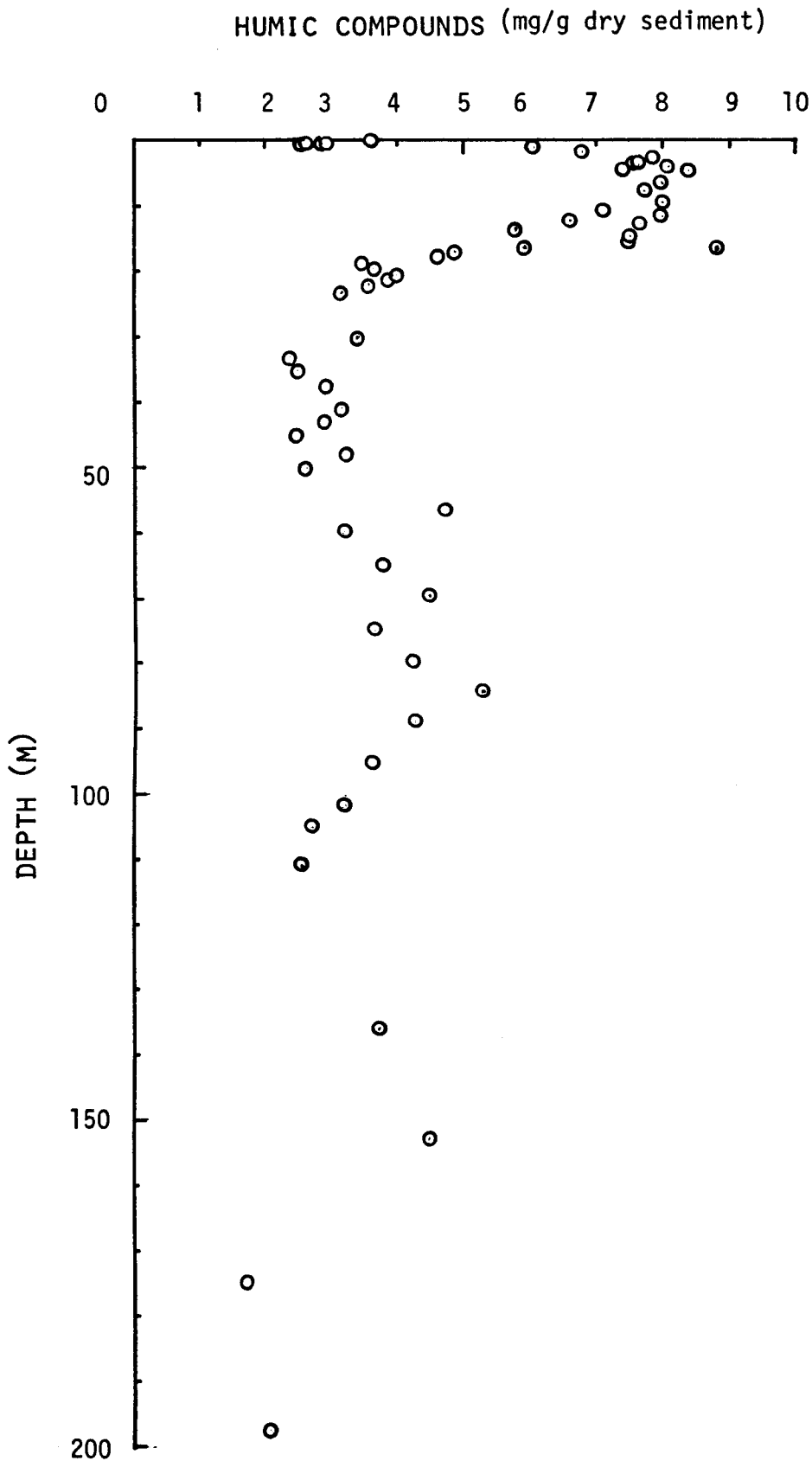
Fig. 6 Vertical profile of humic compounds in a 200 m
core of Lake Biwa.

The results of mineral analyses showed the presence of quartz, montmorillonite, illite, kaolinite or chlorite, christbarite and feldspar in the sediment samples. Table 1 shows the analytical results of minerals. The compositions appear to be almost constant throughout the core. Total amounts of these minerals account for 25-41 % of the dry sediments. Quartz, which was a major componest of minerals throughout the core, comprised 14-25 % of the dry sediments. Fig. 7 shows a vertical profile of quartz content in the sediment core. The content appeared to increase with depth in the 0-40 m sediments and decrease slightly in deeper sediments, exhibiting some peaks.

2.4. DISCUSSION

The author reported that total carbon content in the surface sediments (0-7 cm) of Lake Biwa decreased from 26.8 (0-1 cm) to 18.1 mg/g dry sediment (3-4 cm) and was almost unchanged in deeper sediments (Kawamura et al., 1980). This suggests that, although total organic matter is actively decomposed at the top surface sediments, they are not so decomposable in deeper layers. Therefore, the vertical variation in total carbon contents of the 200 m core may be dependent upon the input of the carbon to the lake bottom in the past. Fig. 8 shows a good correlation between total carbon content and the numberes of diatom remains in the upper 20 m sediments. This suggests that main sources of sedimentary organic matter are autochthonous phytoplanktons, although the contribution of allochthonous organic matter can not be excluded since the regression line has a positive value of the intercept (see Fig. 8). Therefore, the peak of carbon content in the 1-16 m sediments may be attributed to an increase in the primary production which have occurred in the past probably with environmental changes.

As stated above, the C/N ratios decreased with depth in the 0-30 m sediments and showed lower values in deeper sediments. This phenomenon may be due to the difference in a diagenesis between carbon and nitrogen, or to the changes in the C/N ratios of the organic matter supplied to

Table 1. The analytical results of clay composition in a
200 meter sediment core taken from Lake Biwa

| Sample No | Depth(m) | percentage of the dry wt. sediment | | | | | | | | ( )* |
		Mont	Ill	K	(or Chl)	Crist	Qtz	Al	Total	
	surface	6.5	2.1	12.5	2.3	1.3	16.2	2.6	41.2	31.0
1-1	0	4.8	1.2	7.4	1.4	1.3	17.1	2.3	34.1	28.1
1-11	0.5	4.8	1.2	7.9	1.5	1.1	17.4	2.1	36.2	28.1
2-1	1.5	8.3	1.9	10.0	1.8	0.8	13.6	1.5	36.1	27.9
2-21	2.5	7.4	2.2	7.9	1.5	1.4	18.0	2.1	39.0	32.6
3-9	3.0	5.2	1.6	9.3	1.7	1.5	19.7	2.1	39.4	31.8
3-19	3.5	4.7	1.6	10.0	1.8	1.3	13.7	1.6	32.9	24.7
4-7	4.0	5.5	2.0	8.8	1.6	1.3	16.0	1.8	35.4	28.2
4-17	4.5	5.5	1.7	8.8	1.6	1.6	18.3	1.9	37.0	29.8
4-21	4.8	5.5	1.6	9.3	1.7	1.3	18.0	2.4	38.1	30.5
9-2	6.6	8.3	1.6	5.0	0.9	1.4	14.1	2.0	32.4	28.3
11-2	7.7	6.3	1.4	8.4	1.5	1.4	18.4	2.2	38.1	31.2
14-2	9.5	7.0	1.5	-	-	1.3	16.8	1.9	28.5	28.5
16-2	10.6	7.7	2.2	10.7	2.0	1.3	18.4	2.5	42.8	34.1
17-2	11.2	7.4	2.7	14.4	2.6	1.4	17.9	2.2	44.3	32.5
18-2	11.7	5.9	2.5	13.0	2.4	1.4	17.8	2.0	42.6	32.0
21-2	12.5	6.6	2.1	7.9	1.5	1.3	16.5	2.3	36.7	30.3
23-2	13.4	6.3	1.9	12.1	2.2	1.4	17.4	1.7	40.8	30.9
25-2	14.6	6.6	2.3	12.6	2.3	1.7	21.0	2.4	46.6	36.3
26-2	15.2	6.3	2.2	12.6	2.3	1.3	20.2	2.2	44.8	34.5
27-2	15.8	6.5	2.3	12.2	2.2	1.0	15.7	2.5	40.2	30.2
31-2	17.1	5.9	2.4	13.0	2.4	1.8	20.5	2.4	46.0	35.4
33-2	17.5	7.0	1.7	10.7	2.0	1.5	25.4	2.3	48.6	39.9
35-2	18.5	7.4	2.2	13.0	2.4	1.4	20.9	2.1	47.0	36.4
37-2	19.5	7.7	2.5	9.8	1.8	1.9	17.8	2.1	41.8	33.8
40-2	21.0	6.6	2.1	14.0	2.6	1.1	20.3	2.2	46.3	34.9
47-2	22.8	6.6	1.2	10.7	2.0	1.5	18.4	2.1	40.5	31.8
57-2	26.6	7.4	2.8	10.7	2.0	1.5	22.6	2.4	47.4	38.7
65-2	30.2	5.5	1.9	11.6	2.1	1.3	21.6	2.5	44.4	34.9
72-1	32.4	6.6	2.8	11.6	2.1	2.0	23.0	2.6	48.6	39.1
81-1	35.1	7.4	3.6	16.3	3.0	1.5	22.1	2.6	53.5	40.2
86-1	37.5	7.4	2.3	13.5	2.5	1.6	24.4	2.8	52.0	41.0
95-2	40.6	7.7	2.0	12.6	2.3	1.5	24.7	2.5	51.2	40.7
103-1	42.7	8.1	2.4	12.6	2.3	1.7	24.4	2.5	51.4	41.1
107-1	45.2	6.3	2.6	14.4	2.6	1.1	23.5	2.6	50.5	38.7
113-2	47.8	6.3	2.1	7.9	1.5	1.3	21.9	2.1	41.6	35.2
120-1	50.4	5.2	2.0	10.7	2.0	1.3	20.8	2.5	42.5	33.8
143-1	59.8	5.9	2.1	11.2	2.1	1.5	19.6	2.0	42.3	33.2
160-1	65.1	4.8	2.2	10.2	1.9	1.4	21.8	2.1	42.5	34.2
172-1	69.7	-	1.9	13.5	2.5	1.2	22.0	2.3	40.9	29.9
190-1	79.7	7.4	2.6	14.9	2.7	1.4	20.6	2.1	49.0	36.8
211-1	88.9	5.2	1.7	9.3	1.7	1.0	18.6	2.0	37.8	30.2
244-1	101.9	7.4	2.7	12.6	2.3	1.6	20.6	2.3	47.2	36.9
269-1	110.9	5.2	2.7	14.4	2.6	1.3	22.8	2.6	49.0	39.2
336-1	136.3	6.3	2.3	14.4	2.6	1.5	17.5	2.0	44.0	32.2
379-2	152.5	6.6	2.4	15.8	2.9	1.6	17.4	1.7	45.5	32.6
438-1	174.6	6.6	3.6	14.0	2.6	1.2	19.2	2.2	46.8	35.4
498-1	197.7	10.3	3.8	15.4	2.8	1.2	14.3	2.1	49.2	36.6

* The value was calculated on an assumption of the presence of
chlorite in the sediment samples.

QUARTZ

Fig. 7 Vertical profile of quartz content in a 200 m core
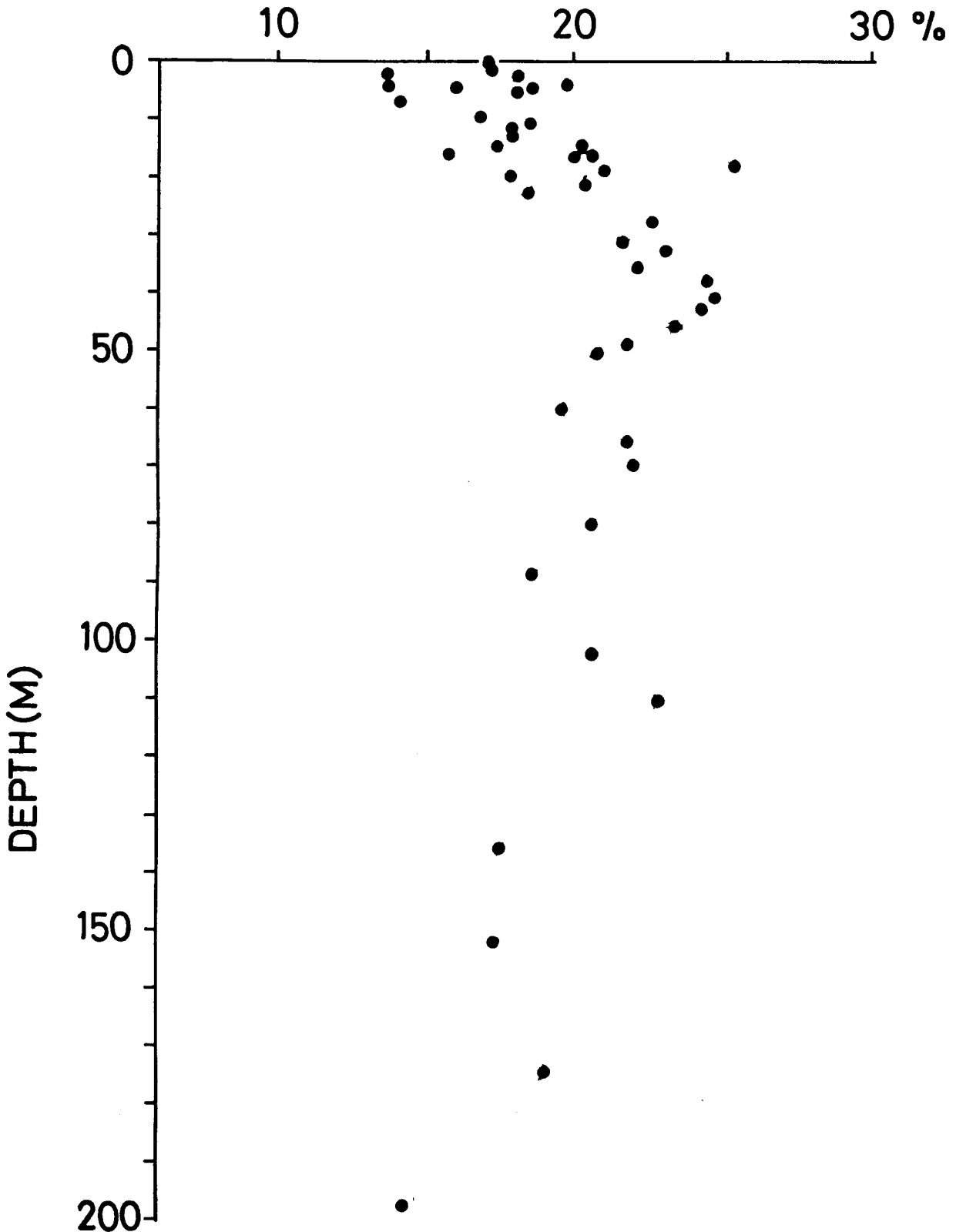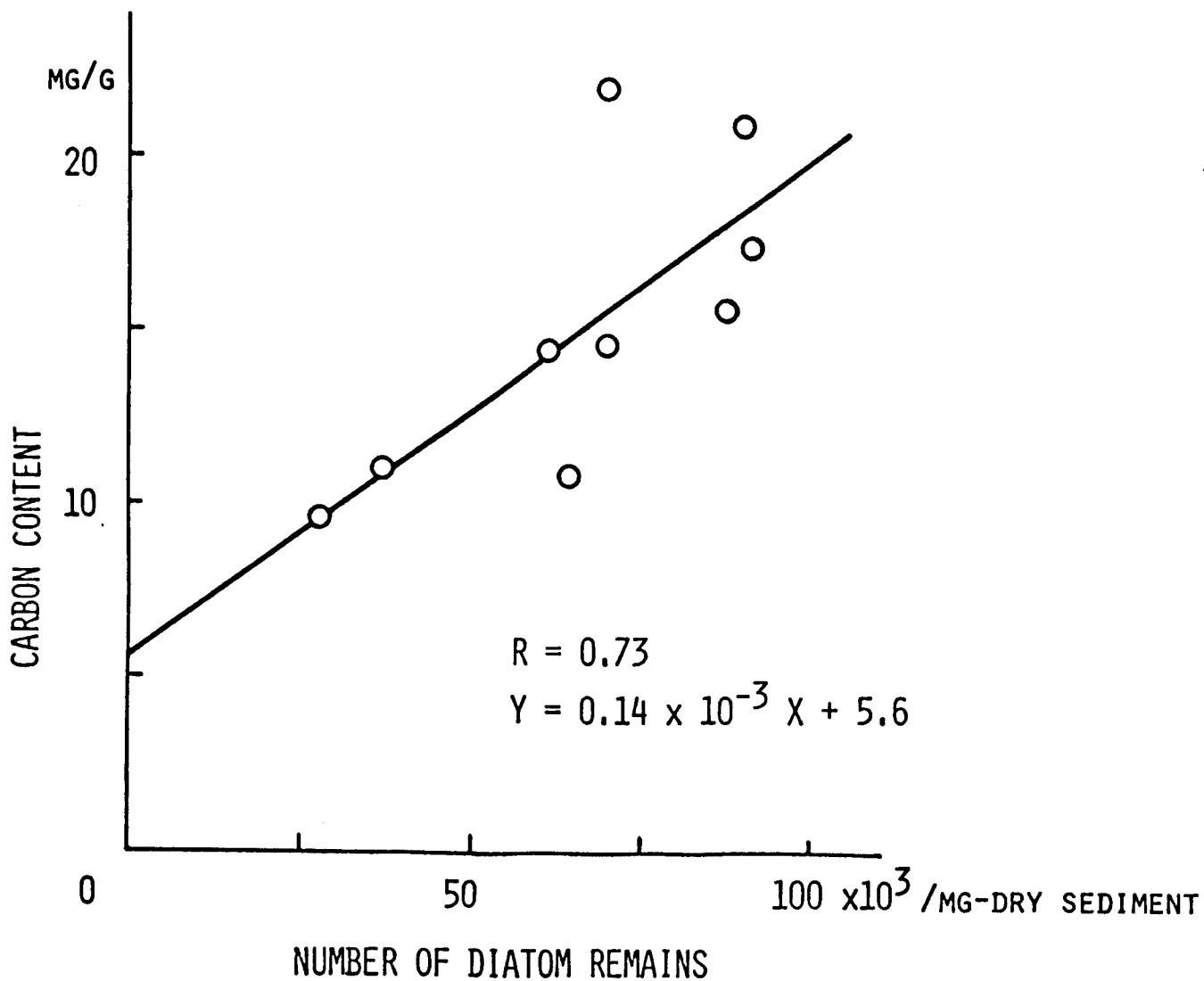of Lake Biwa.

Fig. 8 A correlation of the total carbon content (K. Ogura, personal communication) and the number of diatom remains (S. Mori, personal communication) in upper 20 m sediment layers in Lake Biwa. R is a correlation coefficient. Y is an equation of the regression line. X is the number of diatom remains.

sediments. If the difference in the diagenesis were the case, the C/N ratios should increase with depth, since nitrogen compounds are more liable to degradation (Montani et al., 1980). However, such an increase was not observed in the surface sediments. Therefore, the vertical profile of C/N ratios may be attributed to the changes in the quality of the organic matter supplied to sediments.

There is a possibility that the C/N ratios may be affected by the relative contribution of autochthonous organic matter (C/N: 5.5-6.8, Koyama, 1971; Ogura, 1975) and allochthonous organic matter (C/N: 15-20, Ishiwatari, 1967). However, this may be unlikely in the sediments of Lake Biwa, as discussed below. The concentration of quartz, which was obviously drived from surroundings, appeared to increase with depth in the 0-50 m sediments, as shown in Fig. 7. If the behavior of allochthonous organic matter in the lake environments were similar to that of minerals, the C/N ratios should become larger in the 0-50 m sediments with depth. However, such a result was not obtained in the core. This discrepancy, therefore, suggests that the phenomenon on the C/N ratios was not caused by the changes in the relative contribution of allochthonous and autochthonous organic matter.

Smith and Morris (1980) reported, based on an incubation experiment of a phytoplankton, that lipid content increase in a lower water temperature, while amino acids and carbohydrate contents increase in a higher water temperature. Therefore, it is reasonable to consider that the C/N ratios of organic matter produced by phytoplanktons may increase with a decrease in water temperature. There is a possibility that it have been cooler or colder than other ages in the time corresponding to the sediment layers in which higher C/N ratios are recognized. This may be supported by the following fact. As shown in Chapter 5, the author proposed the $C_{18:2}/C_{18:0}$ ratio (fatty acids) as a possible indicator of paleo-water temperature and considered that it had been cooler or colder in the ages, corresponding to the sediments of the depths of 0.2, 1-5, 11-12, and 15-16 m. The C/N ratios showed fairly high values in the sediments of 1-5, 10-14 and 14-16 m in depth. These sediment layers are consistent with the layers in which high $C_{18:2}/C_{18:0}$

ratios were observed. This agreement suggests that vertical variation of the C/N ratios may be related to environmental changes such as lake water temperature.

As stated above, the vertical profile of humic compounds is seemingly similar to that of total carbon content (see Fig. 4 and Fig. 6). Fig. 9 shows clearly a good correlation between humic compounds and total carbon. This is similar to that reported by Boldovskiy (1965) in the marine sediments of Bering Sea. Since total carbon is in most part of autochthonous orgin, this correlation suggests that humic compounds are mostly of autochthonous origin and produced in water column and/or top surface sediments during the sedimentation process of dead cells of phytoplanktons. And this correlation also suggests that post-depositional changes of humic compounds are not so remarkable in the sediments of several handreds thousands years.
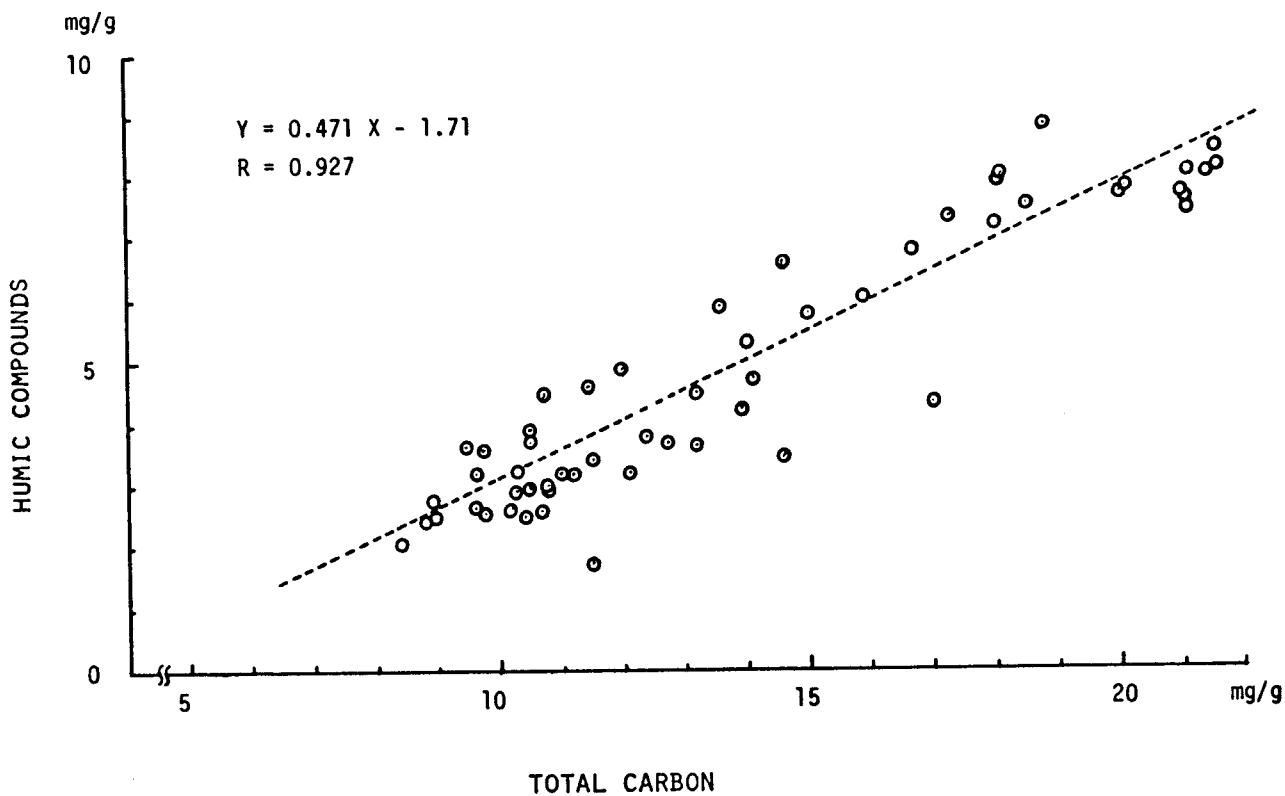
Fig. 9 A relationship between humic compounds and total carbon in a 200 m core of Lake Biwa.

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CHAPTER 3

VERTICAL DISTRIBUTION OF FATTY ACIDS IN THE 200 M CORE
OF LAKE BIWA

3.1. INTRODUCTION

Observational studies on fatty acids in the 200 m core of Lake
Biwa have been conducted by Ishiwatari and Hanya (1973, 1975). They
studied unbound fatty acids in 11 sediment samples of the core.
However, the numbers of the samples studied are too small to elucidate
the diagenetic process of fatty acids. Their bound and residual
fractions were not studied. Therefore, we have studied in more detail
fatty acids in the 200 m core. In this study, about 60 samples were
studied with a special attention to the unbound, bound and residual
fractions of fatty acids. Their vertical distributions were interpreted
in terms of their diagenesis and changes in their input to the bottom
sediment in the past.

3.2. EXPERIMENTAL

The analytical methods of fatty acids are presented in Chapter 1.
Twenty nine samples in the upper 20 m sediment layers were studied
for both unbound and bound fatty acids. For the samples in deeper
layers than 20 m in depth, direct saponification procedure was used
and unbound + bound fatty acids were studied. Fourteen samples of
the 200 m core were subjected to harsher saponification procedure and
residual fractions were studied.

3.3 RESULTS

Normal saturated C_{12}-C_{32} fatty acids (FA's), branched iso/anteiso C_{13}, C_{15} and C_{17} acids, mono- and poly-unsaturated FA's were detected in unbound and bound fractions. The results of mono- and poly-unsaturated FA's are presented and discussed in Chapter 5. The analytical results of unbound and bound saturated FA's are shown in Table 2.

The concentrations of total (unbound + bound) n-saturated FA's were 21.8-113 μg/g dry sediment. The total FA's comprised 0.15-0.35 % of the total organic matter (see p. 24). N-saturated fatty acid distribution showed bimodal pattern with peaks at C_{16} acid and C_{22}-C_{28} acids throughout the sediment core. Fig. 10 shows a typical distribution of these acids. Their distribution patterns appeared to shift from lower molecular weight FA predominance to higher molecular weight FA predominance with increasing depth. Fig. 11 shows a vertical profiles of individual fatty acid concentrations relative to TOM in a 200 m core. LFA's (lower M.W. FA's : C_{12}-C_{19}), in particular C_{16} acid, appeared to decrease drastically with depth in the surface sediments (0-5 m in depth). C_{20} and C_{22} acids seem to be almost constant throughout the core. C_{24}-C_{32} acid concentrations appeared to be relatively small in the upper 20 m layers and become large in deeper layers.

The concentrations of branched chain fatty acids were 0.46-8.73 μg/g dry sediment, as shown in Table 2. The percentages of these acids to TOM were in the ranges of 0.0024-0.027 %. Fig. 12 shows a vertical profile of the concentrations of branched acids to TOM. The values did not decrease smoothly with depth. This trend is quite different from that of LFA's which decreased drastically with depth in the upper region sediments.

The bound fatty acids account for 33-75 % of the total (unbound + bound) fatty acids in the 0-20 m sediments. These values are comparable to those of 32-65 % for Narragansett Bay (Farrington and Quinn, 1971), 65-71 % for Buzzards Bay core (Farrington et al., 1977), and 14-61 % for Lake Haruna 1.2 m sediments (Ishiwatari et al., 1977). Fig. 13 shows a vertical variation of the ratios of concentrations of bound

Table 2   Analytical results of fatty acids in the 200 m sediment core taken from Lake Biwa.

Sample No.	Depth (m)	C (mg/g)	N (mg/g)	C/N	LFA a*	LFA b*	HFA a	HFA b	BrFA a	BrFA b
1-1	0	13.2	1.47	8.98	36.3	14.8	18.7	8.73	2.5	3.63
1-3	0.1	10.5	1.30	8.08	8.59	9.14	9.27	6.46	1.08	2.59
1-5	0.2	10.3	1.28	8.05	10.7	9.55	14.2	5.70	1.04	2.88
1-7	0.3	10.2	1.27	8.03	9.19	6.25	10.1	6.11	0.96	2.77
1-9	0.4	9.73	1.16	8.39	6.74	8.71	9.87	6.02	0.87	2.65
1-11	0.5	10.5	1.17	8.97	12.3	14.6	2.57	14.2	0.69	3.44
1-21	1.0	15.9	1.73	9.19	9.00	17.4	10.5	36.1	0.63	4.52
2-1	1.5	16.7	1.90	8.79	10.4	11.3	9.29	8.23	0.92	3.04
2-11	2.0	17.0	2.01	8.46	10.8	18.4	8.41	28.1	0.69	4.29
2-21	2.5	18.1	2.10	8.62	17.8	16.7	24.2	11.9	1.33	4.14
3-9	3.0	21.0	2.34	8.97	10.3	10.2	17.3	18.0	0.72	2.58
3-19	3.5	21.1	2.35	8.98	15.4	18.8	25.7	13.6	1.39	4.19
4-7	4.0	21.6	2.41	8.96	9.77	11.3	17.1	16.3	0.88	3.31
4-17	4.5	21.2	2.47	8.58	14.4	15.8	22.3	20.7	1.37	3.68
4-21	4.7	21.6	2.43	8.89	14.6	11.8	30.1	13.7	1.46	3.20
9-2	6.6	21.4	2.70	7.93	9.07	14.6	14.2	26.6	2.13	4.90
11-2	7.7	20.1	2.77	7.26	8.66	16.2	11.9	24.0	2.51	6.22
14-2	9.5	21.1	2.56	8.24	9.40	12.6	16.4	21.2	2.33	4.60
17-2	11.2	18.1	2.08	8.70	9.11	10.8	17.4	11.6	1.65	2.78
18-2	11.7	14.7	1.79	8.21	4.60	7.75	8.62	12.6	1.31	2.71
21-2	12.4	20.0	2.43	8.23	8.22	10.9	17.1	13.1	1.88	3.55
23-2	13.4	15.0	1.84	8.15	7.63	8.48	12.8	17.8	1.31	2.06
25-2	14.6	18.5	2.05	9.02	6.92	8.58	12.8	22.4	0.83	1.87
26-2	15.2	17.3	1.89	9.15	7.11	7.87	18.1	14.0	1.25	1.64
27-2	15.7	18.8	2.04	9.22	7.96	7.99	9.92	26.4	1.65	1.89
28-2	16.2	13.6	1.81	7.51	4.41	9.54	6.80	18.8	0.78	2.09
31-2	17.1	12.0	1.59	7.55	3.23	6.91	3.60	13.4	0.39	1.26
33-2	17.5	11.4	1.51	7.55	4.93	4.80	10.1	15.5	0.82	1.31
35-2	18.5	14.6	1.41	10.4	3.39	3.82	6.90	7.68	0.32	0.64
37-2	19.5	9.72	1.34	7.25	16.9**		76.4**		3.0**	
40-2	21.0	10.5	1.53	6.86	12.3		48.0			
47-2	22.8	9.66	1.34	7.21	8.16		31.4		2.09	
65-2	30.2	11.5	1.83	6.30	12.0		60.9		1.76	
72-1	32.5	8.82	1.35	6.53	6.79		35.7		1.79	
81-1	35.1	8.94	1.48	6.04	6.10		22.8		1.62	
86-1	37.5	10.8	1.63	6.60	7.14		50.6		1.71	
95-2	40.7	12.1	1.72	7.04	17.7		60.9		3.39	
103-1	42.8	10.8	1.70	6.38	8.43		57.2		2.83	
107-1	45.2	10.4	1.59	6.51	8.66		64.0		3.49	
113-2	47.8	11.0	1.58	6.96	12.1		66.1		3.81	
120-1	50.4	9.59	1.37	7.00	7.89		41.4		2.17	
129-2	56.3	14.1	1.77	7.97	15.2		97.8		3.13	
143-1	59.8	11.2	1.85	6.04	7.52		33.7		0.77	
160-2	65.1	12.4	1.84	6.76	6.49		73.0		1.55	
172-1	69.7	13.2	1.79	7.37	8.6		61.7		2.07	
181-1	74.8	12.7	1.91	6.65	10.0		61.5		2.85	
190-1	79.8	13.9	1.99	6.99	10.3		84.7		2.68	
198-2	84.2	14.0	1.87	7.49	9.30		75.1		2.20	
211-1	89.0	17.0	2.27	7.48	10.3		82.0		2.11	
229-2	95.1	9.41	1.61	5.84	8.02		93.1		1.23	
244-1	102.0	10.3	1.77	5.84	8.22		61.5		3.08	
255-1	105.4	8.91	1.52	5.86	5.07		42.2		2.10	
269-1	110.9	10.7	1.42	7.54	4.59		36.7		1.19	
336-1	136.3	10.5	1.85	5.65	7.91		43.5		2.60	
379-2	152.5	10.7	1.75	6.11	5.02		45.8		1.37	
438-1	176.1	11.5	1.45	7.90	5.70		21.1		0.46	
493-1	197.3	8.42	1.63	5.17	3.54		33.2		1.18	

*a : unbound fatty acids

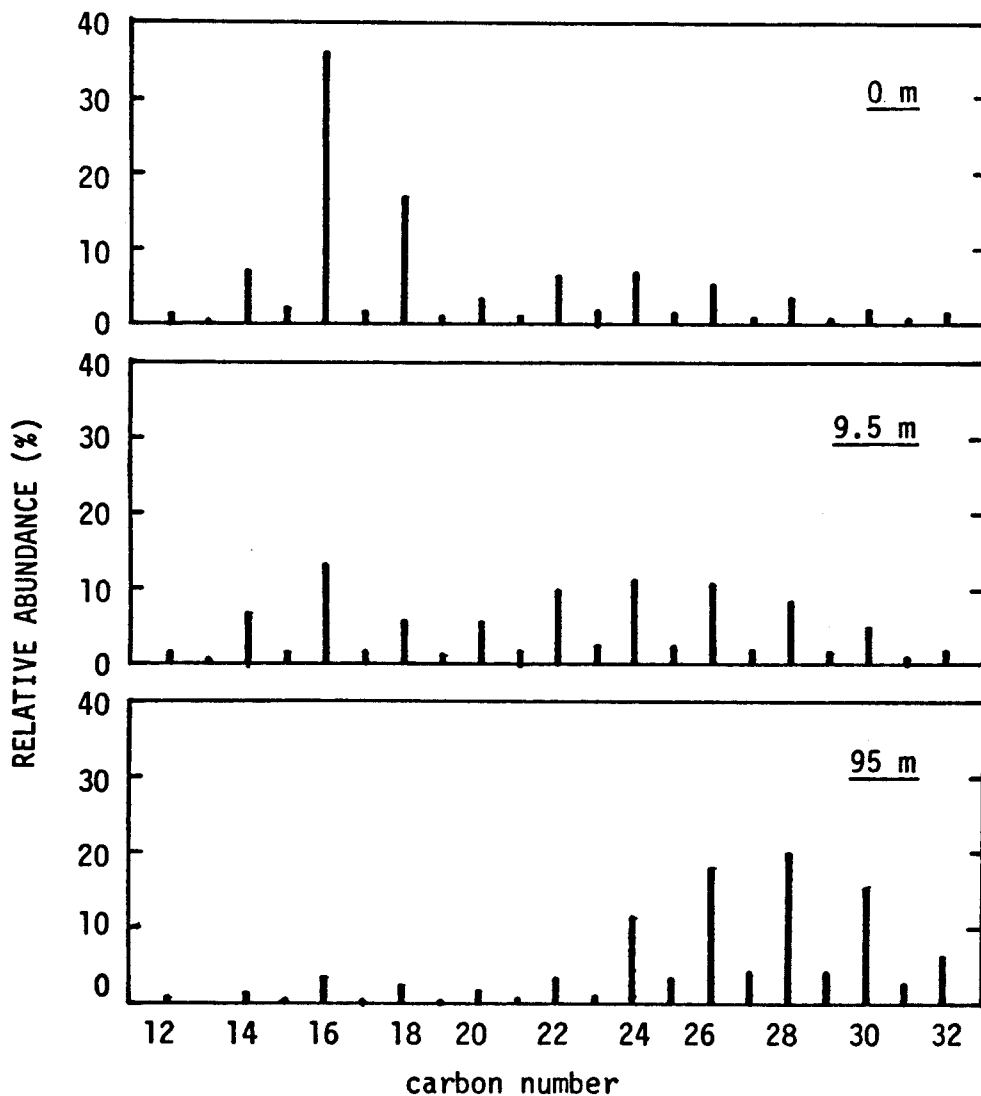*b : bound fatty acids

** : unbound + bound fatty acids

Fig. 10

REPRESENTATIVE DISTRIBUTION OF FATTY ACIDS IN A 200 M
SEDIMENT CORE FROM LAKE BIWA

FA/TOM(×10⁻² %)

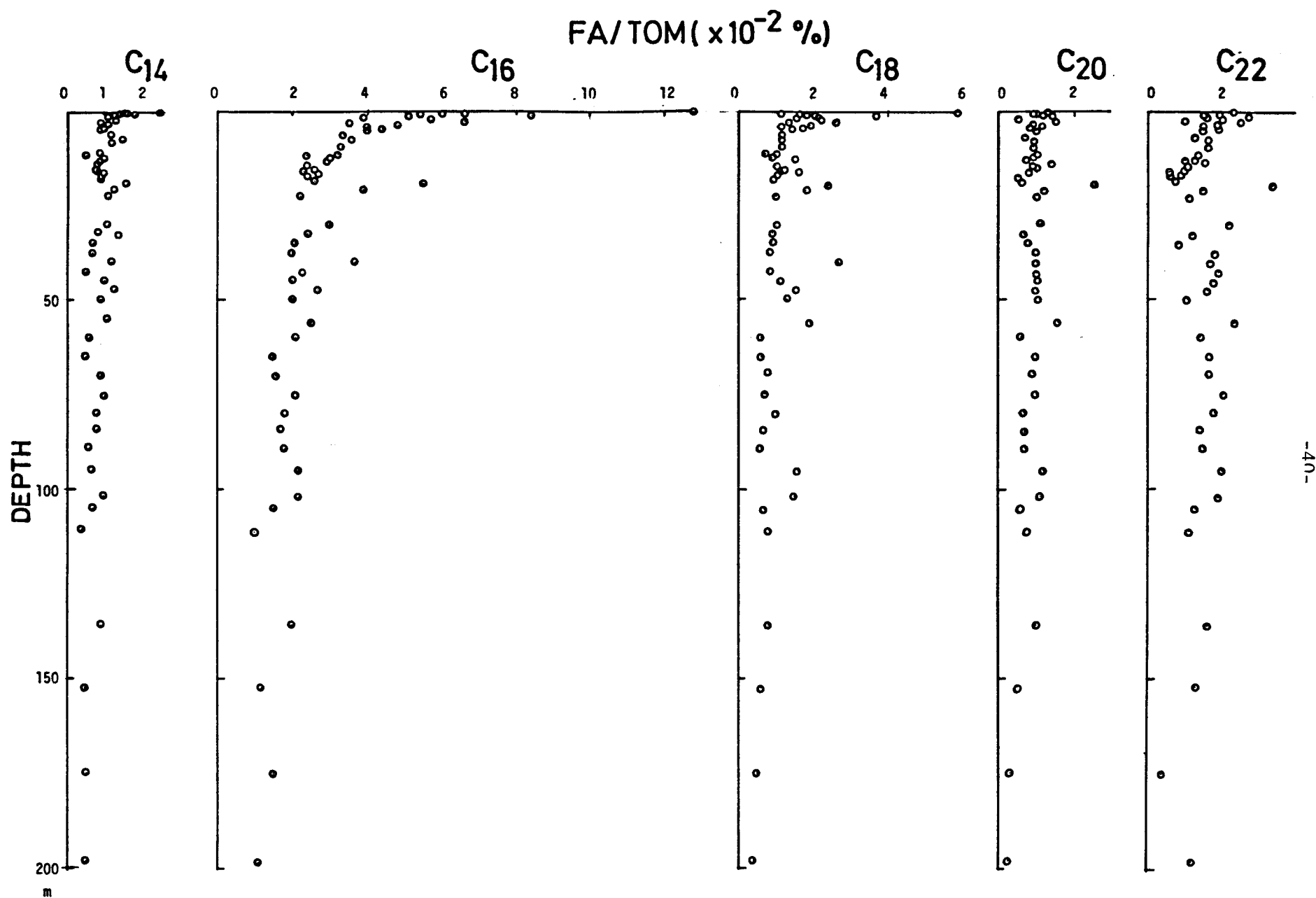Fig. 11 (a) Vertical profiles of individual fatty acidsconcentrations relative to TOM in a 200 m core 1f Lake Biwa.
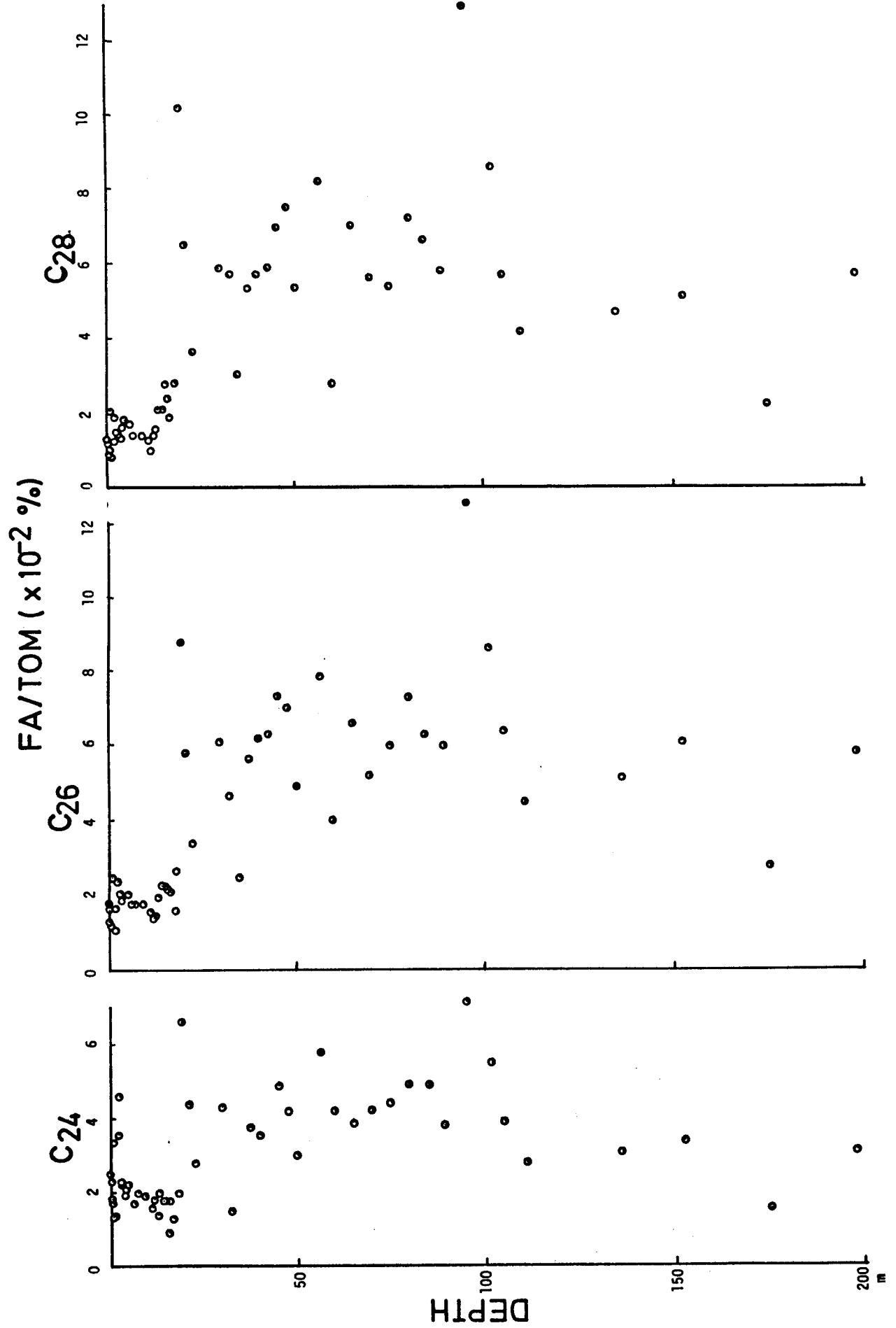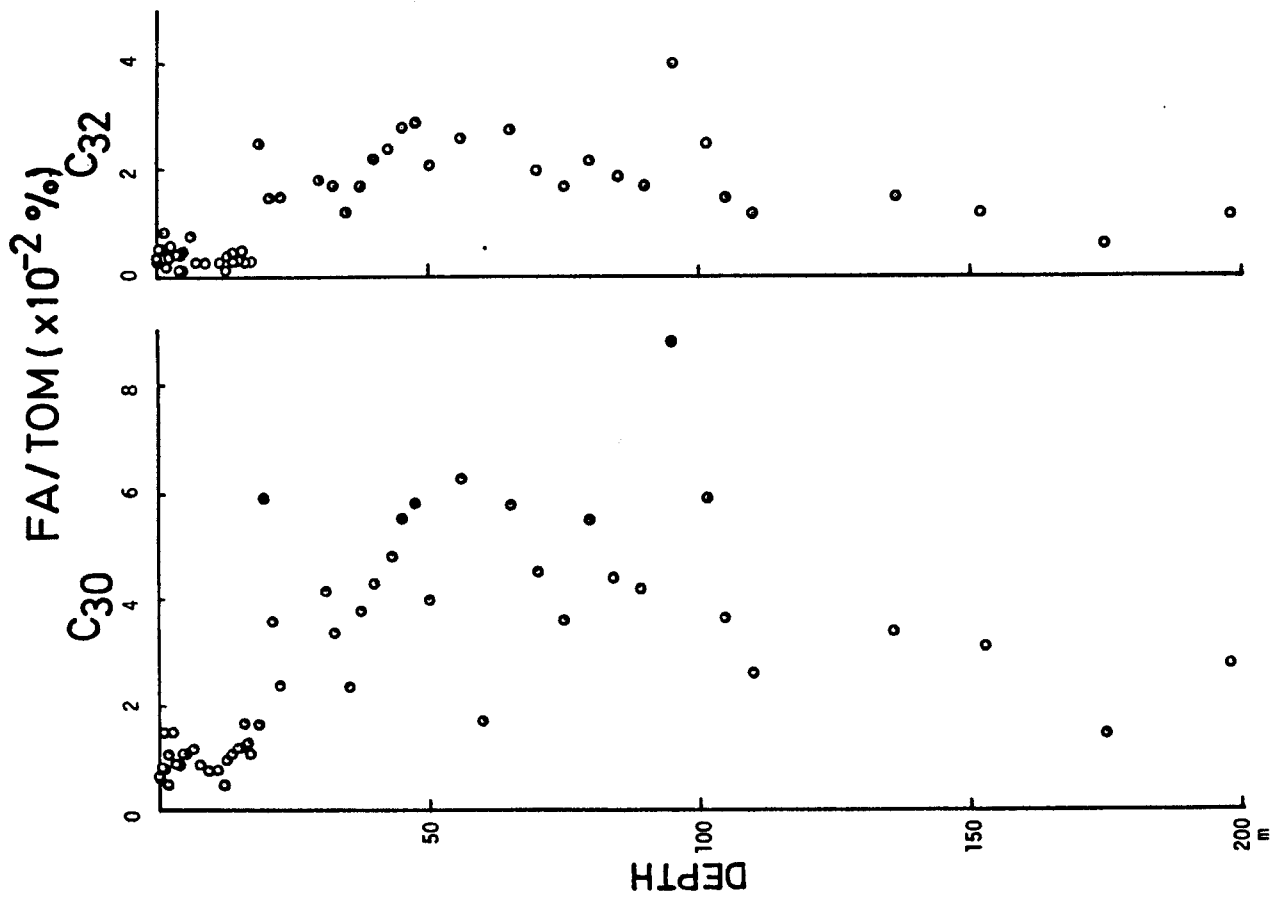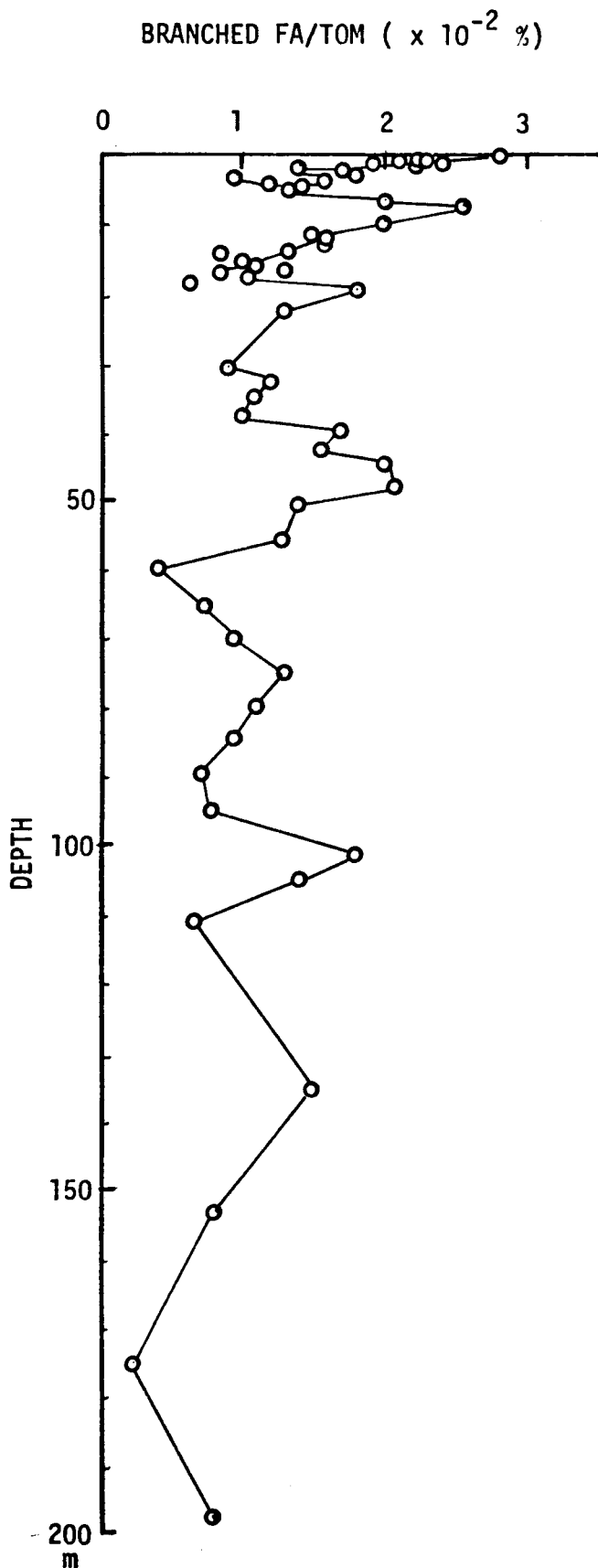
Fig. 11 (b)

Fig. 11 (c)

Fig. 12   Vertical profile of the concentrations
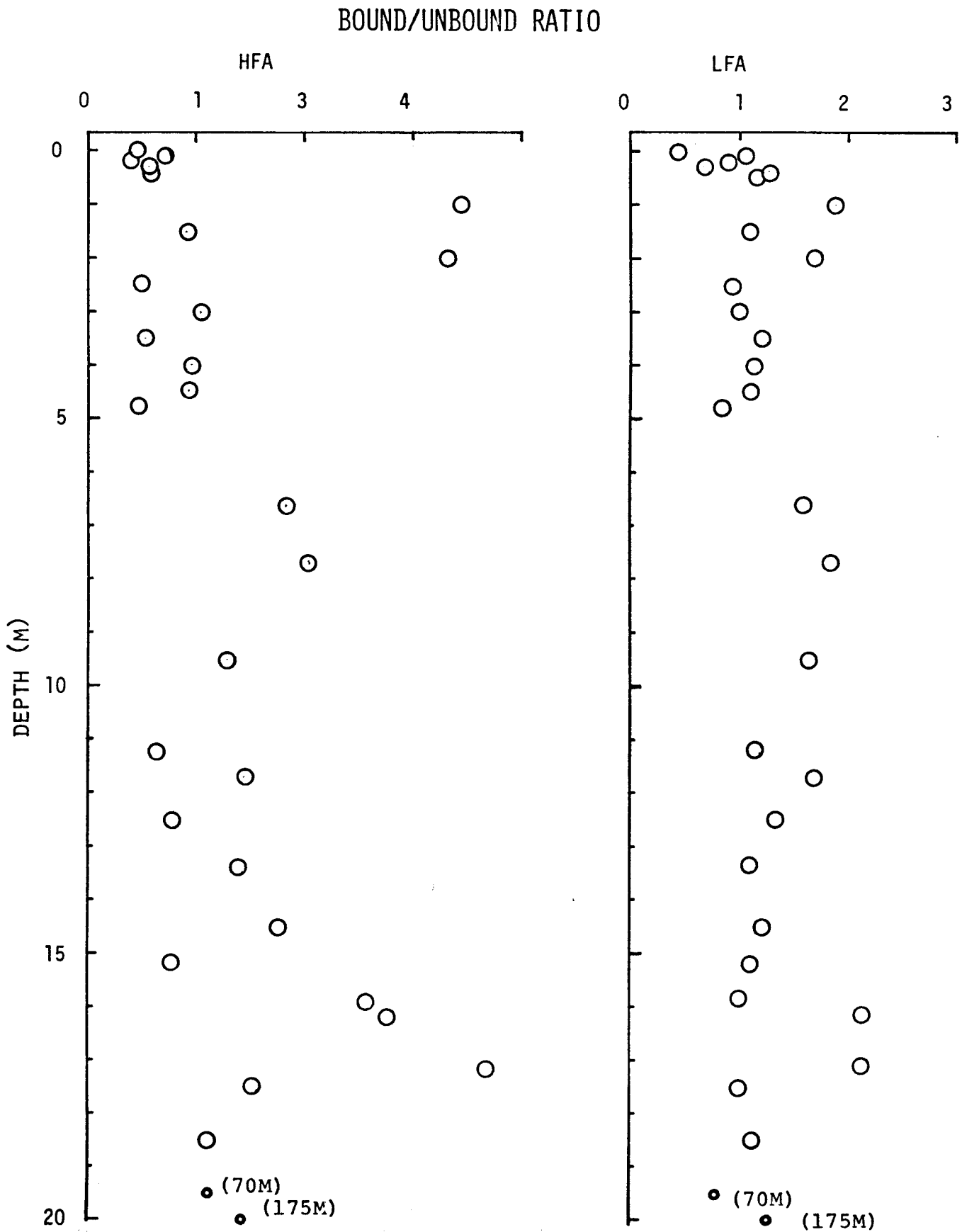of branched fatty acids to TOM in a 200 m core of
Lake Biwa.

Fig. 13 Vertical variation of the ratios of concentration of bound fatty acids to those of unbound acids for LFA's and HFA's in the upper 20 m sediments of Lake Biwa.

fatty acids to those of unbound acids for both LFA's and HFA's (higher
M.W. FA's : C_{20}-C_{30}). The ratios for LFA's increased in the 0-1 m
sediment layers from 0.4 to 1.9, and varied in the ranges of 0.8-2.0
in deeper sediments. The ratios for HFA's, which indicate 0.5 at the
top surface sediment, appeared to increase with depth and became ca. 1
in the 15-18.5 m sediments, exhibiting some peaks.

The concentrations of bound branched acids were larger than those
of unbound acids throughout the 0-20 m sediments. This is consistent
with the results of Lake Haruna sediments (Ishiwatari et al., 1977).
Fig. 14 shows a vertical variation of the ratios of bound to unbound
branched acid contents. It is of interest to note that the ratios showed
a large peak in the 0-3 m sediments.

Harsher saponification procedure of the residual sediments released
the following fatty acids: normal C_{12}-C_{30} saturated fatty acids, branched
chain C_{13}, C_{15} and C_{17} fatty acids. Fig. 15 shows a typical mass
fragmentogram of fatty acid methyl esters (m/e 74) obtained by harsher
saponification of the residual sediment (#2-21) of Lake Biwa. Although
n-fatty acids showed bimodal distribution with peaks at C_{16} and C_{22} acids
which is similar to that of unbound and bound fatty acids, LFA's were
remarkable dominant throughout the core. The analytical results are
shown in Table 3. The concentrations of LFA's were in the range of
4.51-22.94 µg/g dry sediment, showing higher values in the layers of
2-10 m sediments. On the other hand, those of HFA's were in the range
of 0.18-8.59 µg/g dry sediment, showing higher values in the same
layers. The concentrations of branched FA's were 0.28-2.12 µg/g dry
sediment.

3.4. DISCUSSION

3.4.1. Possible degradation mechanisms of LFA's in the upper region sediments

As shown in Fig. 11, LFA's decreased drastically with increasing
depth, whereas HFA's did not. This trend is more clearly presented by

BOUND/UNBOUND RATIOS OF
BRANCHED FATTY ACIDS
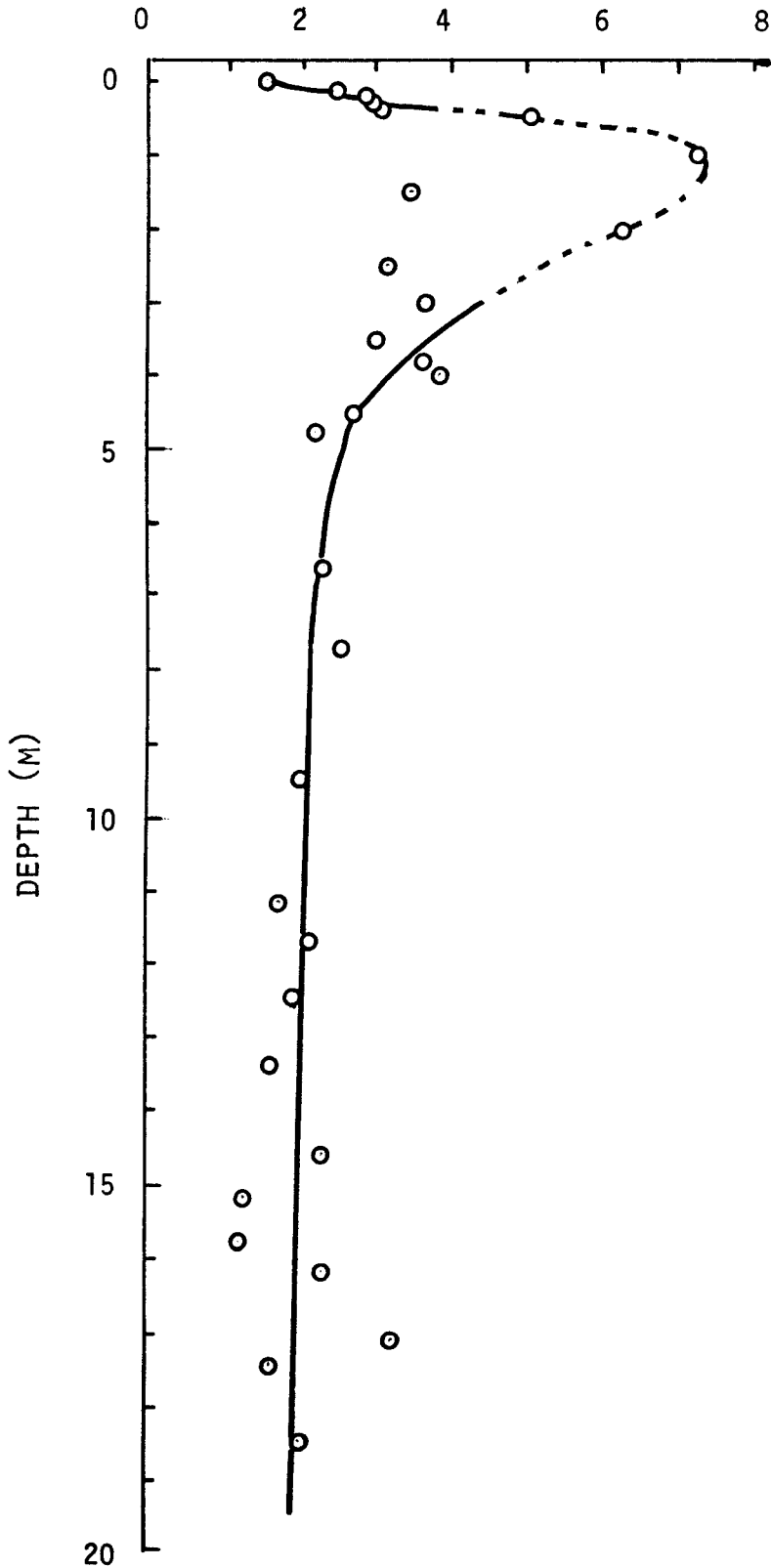
Fig. 14 Vertical fluctuation of the ratios of
bound to unbound branched acid contents in a 200
m core of Lake Biwa
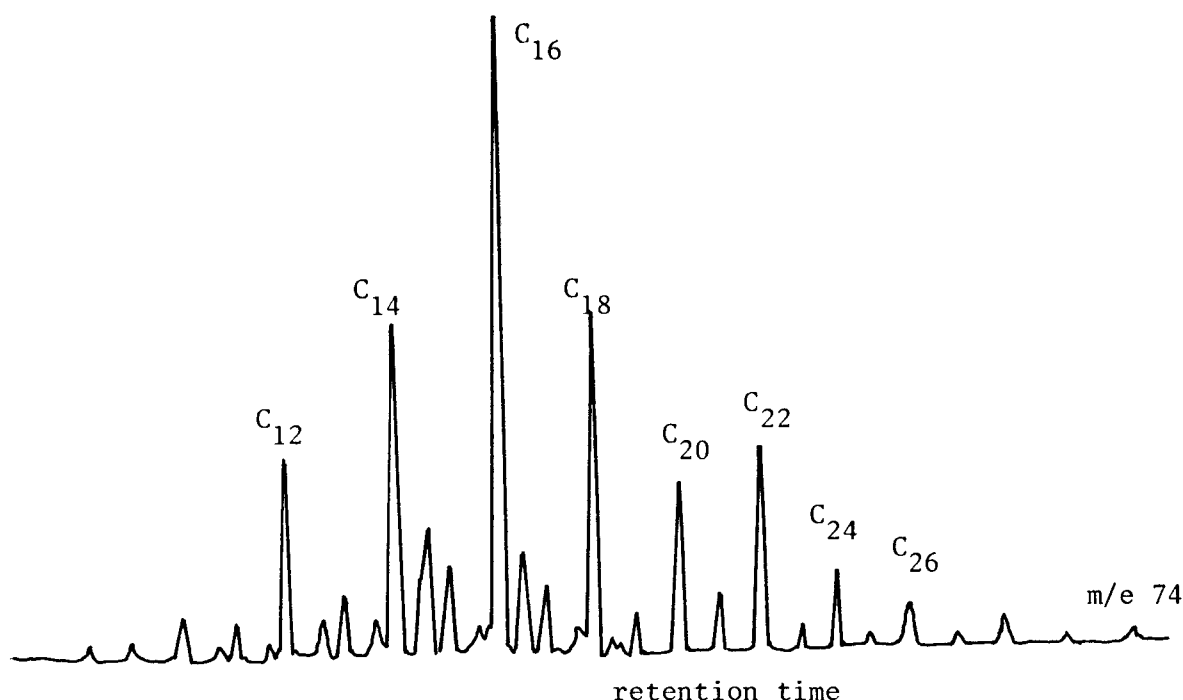
Fig. 15 Typical mass fragmentogram of fatty acid methyl esters (m/e 74) obtained by harsher saponification of the residual sediment (#2-21) of Lake Biwa.

Table 3 Fatty acids separated by harsher saponification of residual sediments

(μg/ g dry sediment)

Sample No.	Depth(m)	n-LFA	n-HFA	Branched FA
1-1	0	11.65	1.99	1.46
1-9	0.4	6.83	0.58	0.84
1-21	1.0	10.21	2.18	1.57
2-21	2.5	13.42	4.91	2.12
4-7	4.0	15.85	6.68	2.60
9-2	6.6	22.94	8.59	1.88
17-2	11.2	12.51	3.82	1.58
27-2	15.7	9.24	4.38	1.23
35-2	18.5	8.09	0.87	0.64
65-2	30.2	5.76	1.03	0.45
107-1	45.2	7.18	0.65	0.42
229-2	95.1	4.51	0.18	0.56
379-2	152.5	5.36	0.53	0.46
498-1	197.8	4.35	0.36	0.28

Fig. 16, which shows a vertical variation of the ratios (L/H ratios) of LFAs' contents to HFAs' contents in the 200 m core. The ratios decrease exponentially with depth in the upper 50 m sediment layers. In deeper layers, the ratios are almost constant. Such a decreasing trend in the ratio is consistent with the results by Matsuda and Koyama (1977), who observed fatty acid distribution in a sediment core (1.5 m) of Lake Suwa. They considered that the phenomenon that LFA's decrease preferentially with depth was caused by bacterial selective degradation of LFA's is sediments, although a direct evidence to support the consideration was not presented.

We consider that if fatty acids degraded, we could detect the degradation produces. We believe that β- and ω-hydroxy acids and α,ω-dicarboxylic acids are the degradation products, hence, we studied these compounds in the 200 m core. Although detailed analytical data of these compounds will be presented and discussed in Chapter 4, a part of the data is cited here to interpret the degradation mechanism of LFA's. Figure 17 shows the vertical variations of the concentrations of C_{16} fatty acid, ω-hydroxy acid, β-hydroxy acid and α,ω-dicarboxylic acid relative to TOM in upper 20 m sediment layers. In the upper sediment layers where the fatty acid decreases with depth, β- and ω-hydroxy acids and α,ω-dicarboxylic acid appear to increase with depth. This suggests that these compounds may be produced in the sediments through β- and ω-oxidation of C_{16} fatty acid. Figure 18 summarizes the possible mechanisms for the decrease of C_{16} fatty acid with depth. Since β- and ω-oxidations have been proposed to occur in microorganisms (Kester and Foster, 1963), it is reasonable to consider that the above processes are operative in the upper sediment layers. If this were the case, ca. 30 % of C_{16} fatty acid which disappeared in sediments with depth could be explained by β- and ω-oxidative degradation processes. The remaining parts may be explained by further degradation of the degradation products and consequently the fatty acids with shorter chain length may be produced, although other mechanisms such as trap or incorporation into organic or inorganic matrices in sediments could not be excluded.

These mechanisms seem to be supported by the following evidences.
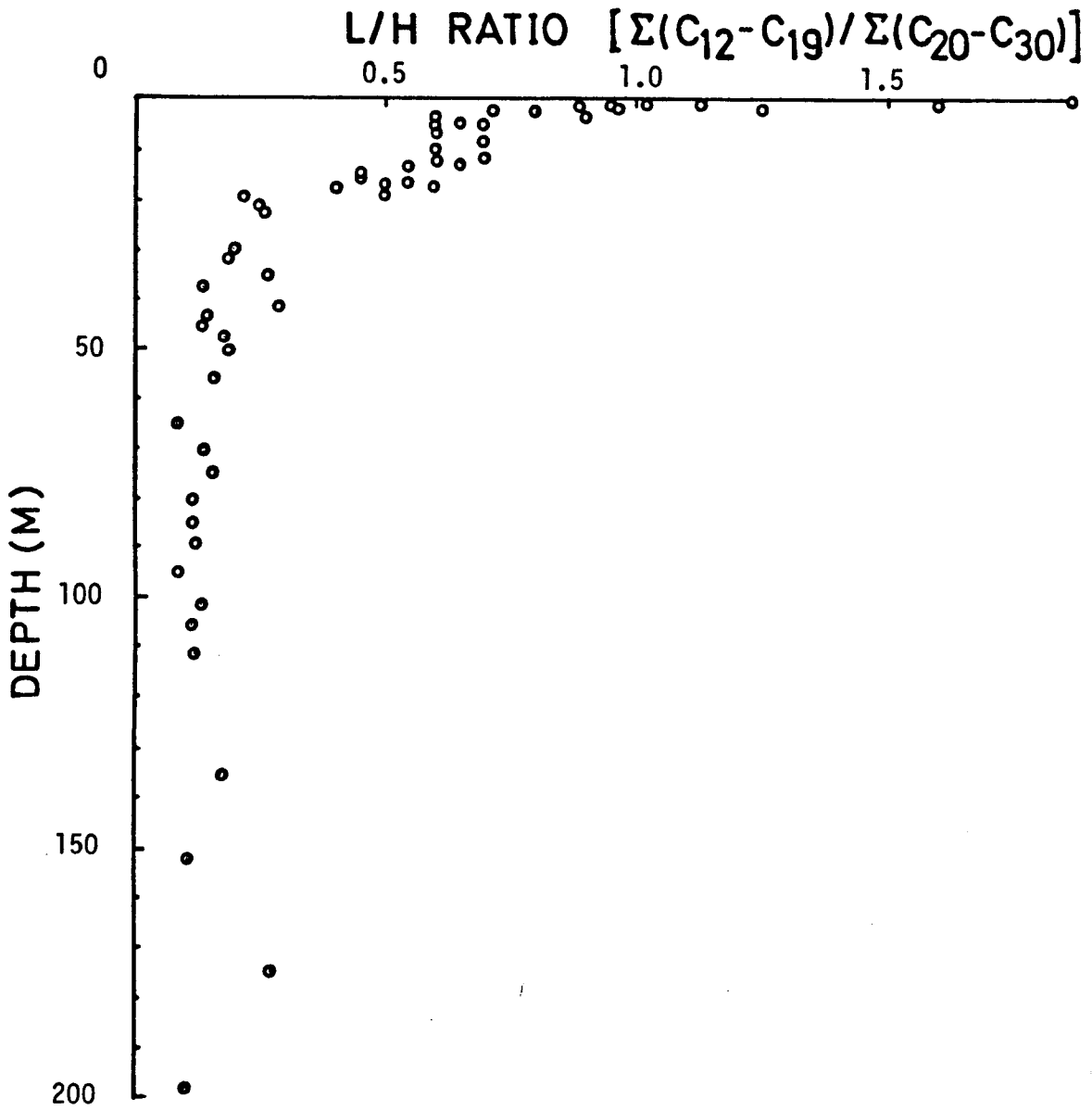
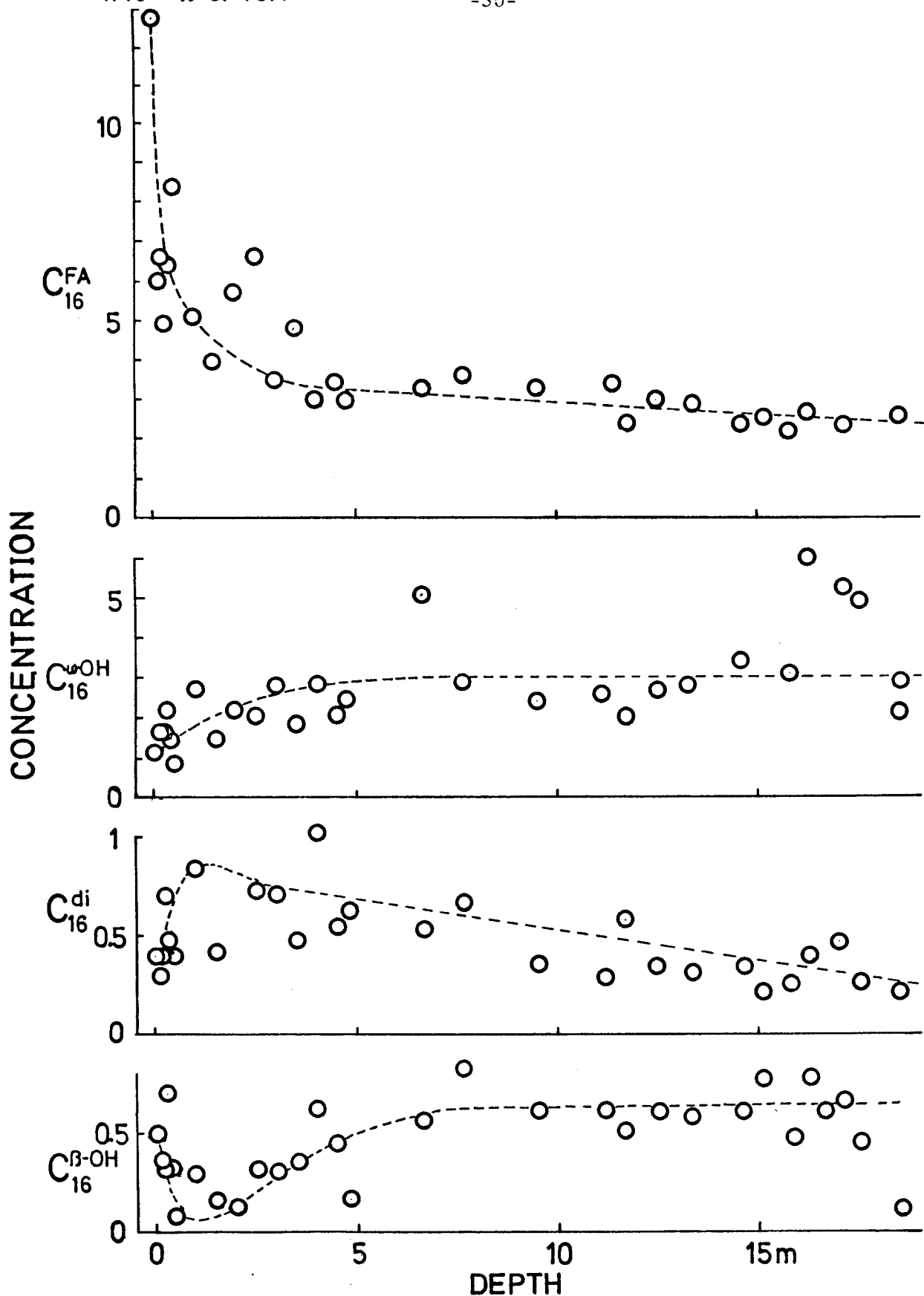Fig. 16 Vertical variation of the ratios of LFA's content to HFA's content in a 200 m core of Lake Biwa.

Fig. 17 Vertical distribution of the concentration of C_{16} fatty acid, ω-hydroxy acid, α,ω-dicarboxylic acid and β-hydroxy acid to TOM in the upper 20 m sediments of Lake Biwa.
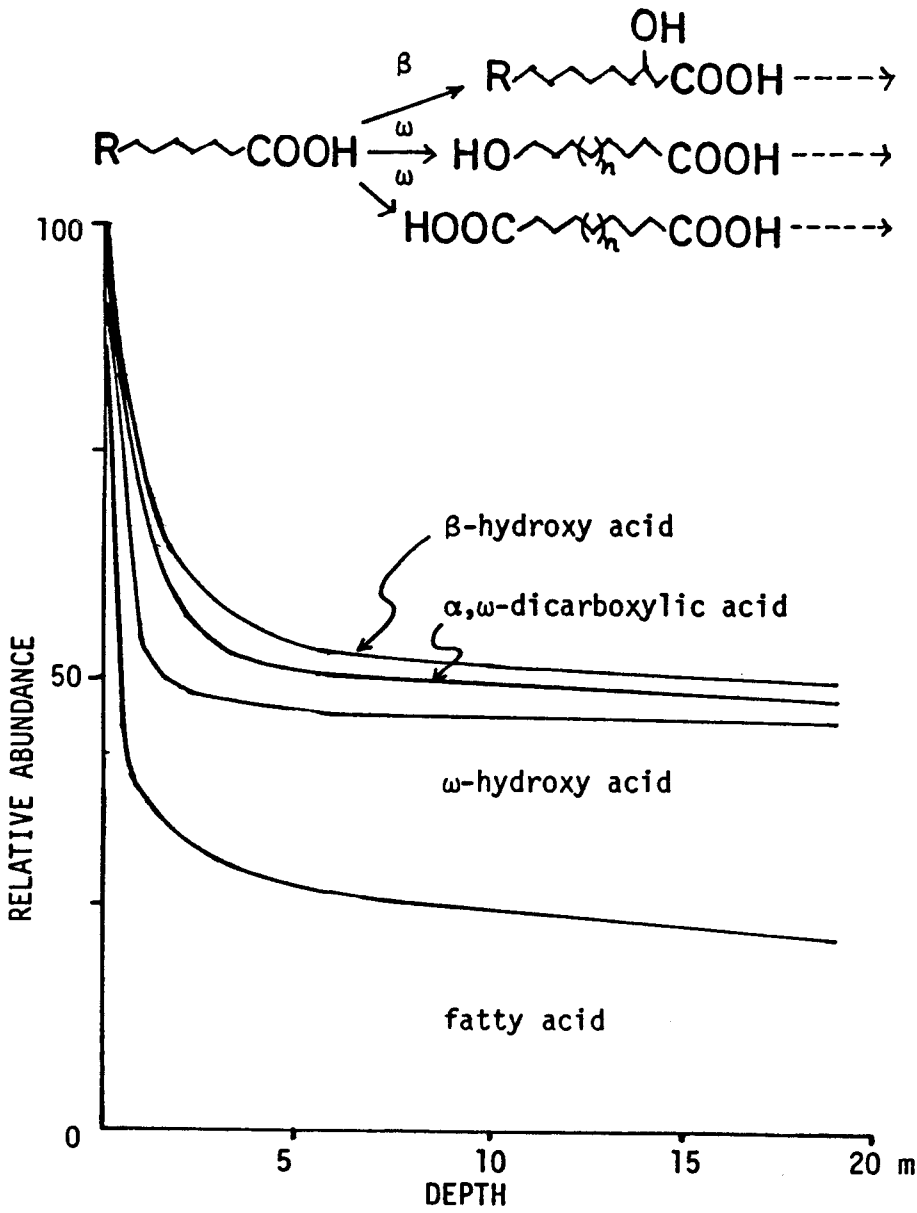
Fig. 18 POSSIBLE MECHANISM OF OXIDATIVE DEGRADATION OF C_{16}
FATTY ACID IN SEDIMENT

Figure 19 shows a vertical variation of the C_{n-2}/C_n ratios for fatty acids. The C_{14}/C_{16} ratios appear to increase with depth. The C_{16}/C_{18} ratios also appear to increase slightly with depth. These phenomena suggest that C_{n-2} acids are produced from C_n acids, supporting that β-oxidation mechanism is operative in the sediments. But, the C_{12}/C_{14} ratios do not show such a trend. This may be explained by large changes in the input of C_{12} acid to the sediments in the past. Figure 20 shows a good correlation between C_{12} acid and $C_{18:2}$ acid. Since $C_{18:2}$ acid showed a characteristic fluctuation in the 0-20 m sediment layers and the acid was proposed as a possible indicator of paleoclimatic change (see Chapter 5), this correlation suggests that the concentration of C_{12} acid may have been largely affected by the environmental changes of the lake in the past.

The ratios of C_{13}/C_{14}, C_{15}/C_{16} and C_{17}/C_{18} also seem to increase in the upper region sediment layers, as shown in Fig. 21. These phenomena suggest that α-oxidation of fatty acids also operative in the sediments, although α-hydroxy acids were not detected in the sediments.

Eglinton et al. (1968) detected α- and β-hydroxy acids in a 5000 yr-old lacustrine sediment and concluded that these acids were derived from the fatty acids by α- and β-oxidation based on the wide variation in chain length (C_{10}-C_{24}) and on the parallel distribution of chain length of the α- and β-hydroxy acids and the fatty acids. Johns and Onder (1975) reported the presence of dicarboxylic acids in recent sediments and considered that these acids have dual origins : (1) deposition by mangroves and (2) in situ production by sedimentary organisms. α-, β-, and ω-Oxidation mechanisms which were proposed to interpret the decrease of LFA's in the sediments are seemingly consistent with that of Eglinton et al. (1968) and Johns and Onder (1975).

LFA's were still detected in the sediment layer at 200 m in depth. This indicates that they are partly preserved in the deeper sediments without recieving microbial attack and chemical degradation.

3.4.2. Controlling factor of the vertical fluctuation of HFA's content

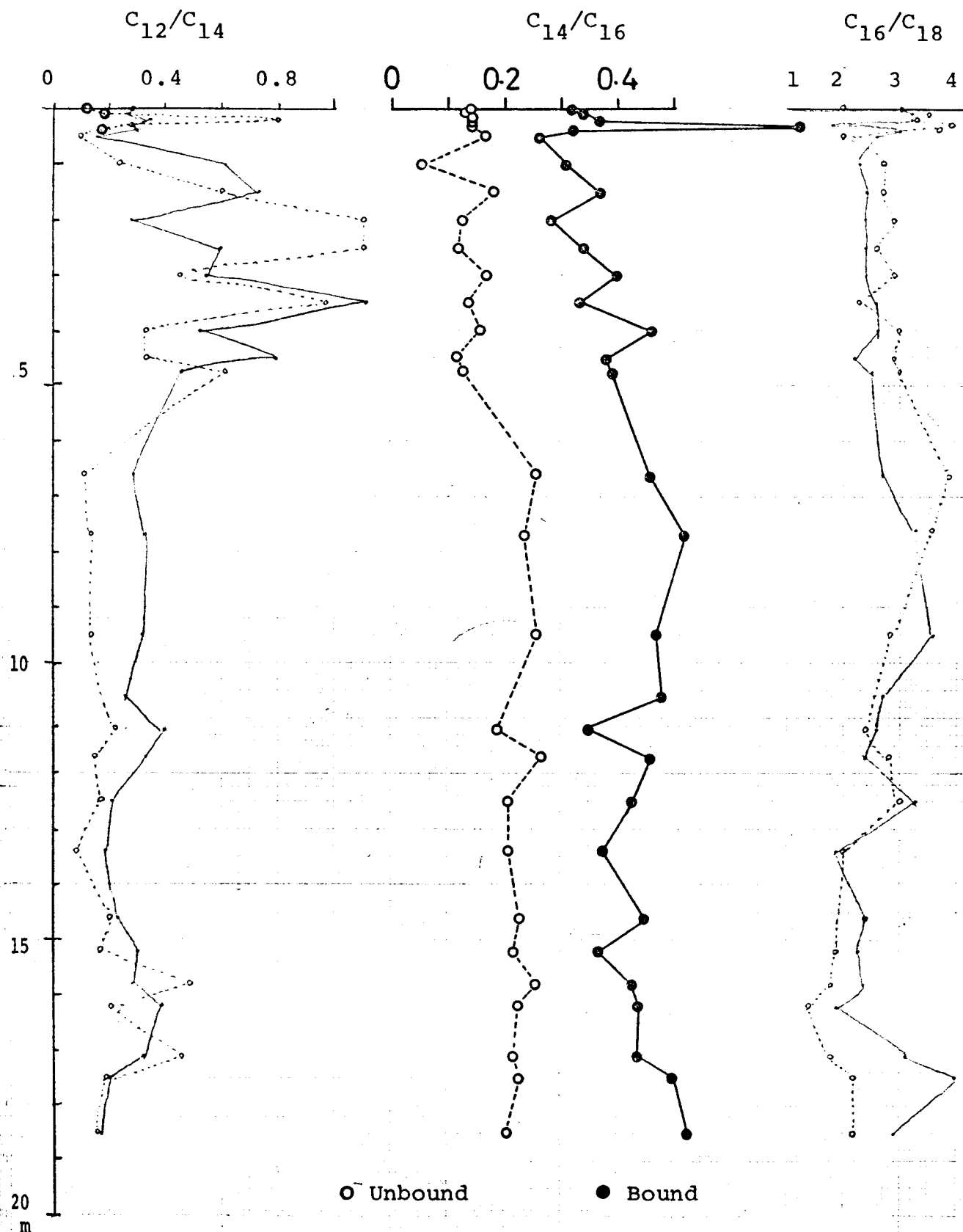As shown in Fig. 11, the concentrations of HFA's relative to TOM

Fig. 19 Vertical variation of the C_{12}/C_{14}, C_{14}/C_{16}, and C_{16}/C_{18} ratios for fatty acids in the upper 20 m sediments of a 200 m core of Lake Biwa.
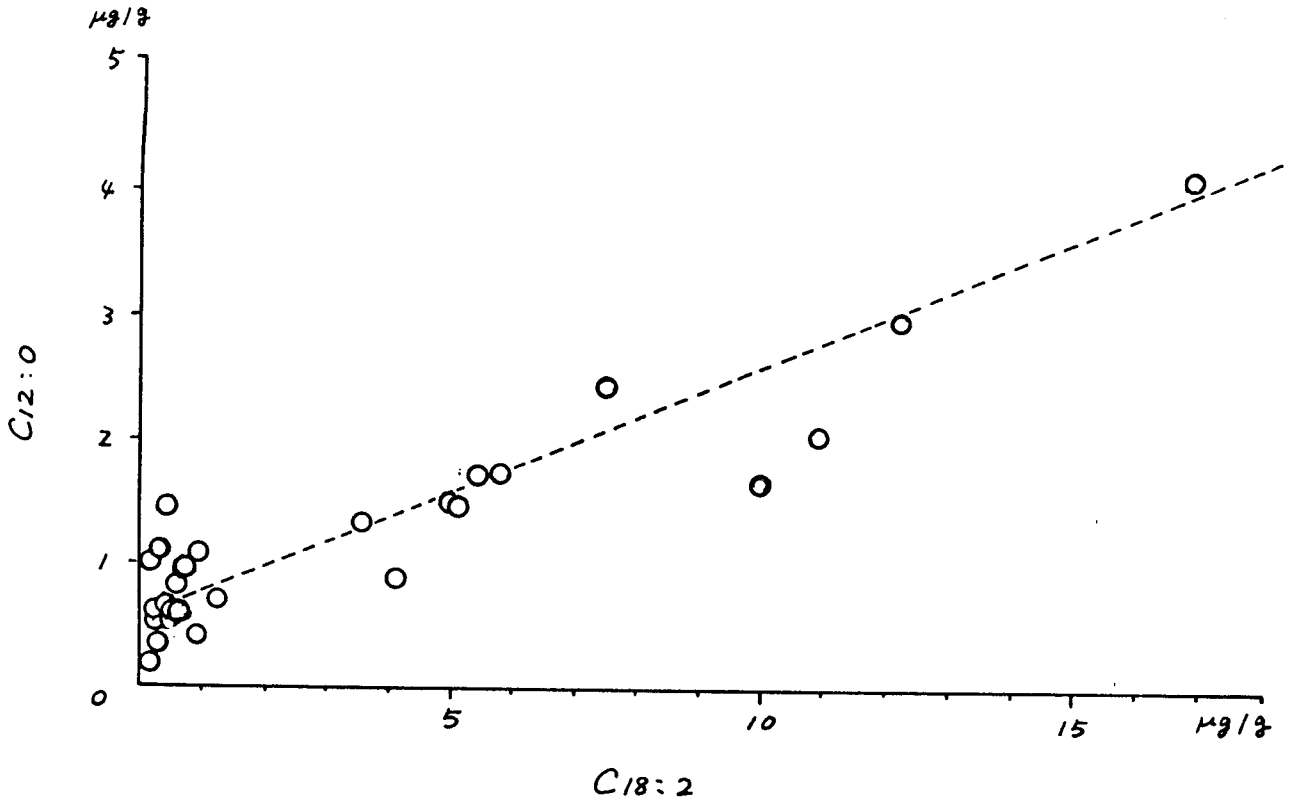
Fig. 20 A correlation between the concentration of $C_{12:0}$ and $C_{18:2}$
acids in the upper 20 m sediment layers of Lake Biwa.

in the upper 20 m sediment layers are seemingly low. These lower values are due to the higher concentration of total carbon in these layers. However, it is apparently recognized that the concentration of HFA's increased with depth in the 0-100 m sediment layers. Figure 22 shows a vertical profile of HFA's (C_{20}-C_{32}) concentration. The concentrations in the 50-100 m layers are 3 or 4 times larger than those in the surface sediments and 100-200 m sediment layers. This phenomenon may be caused by the change in the input of HFA's to the sediment in the past, as follows.

Since Ikan et al. (1975) reported that isoprenoid acids are derived from the oxidation of the corresponding alcohol (phytol) in an early diagenesis, there is a possibility that fatty acids may be produced from corresponding fatty alcohols in sediments (Ishiwatari and Kawamura, 1978). In order to test whether or not the possibility is the case, C_{14}-C_{30} alcohols were analyzed in the 200 m core. The concentrations of C_{20}-C_{30} alcohols were 1.2-14.6 µg/g dry sediment, which are lower than those of HFA's (14.6-97.8 µg/g dry sediment) by a factor of 5-10 in the 200 m core, although the percentage of C_{20}-C_{30} alcohols to TOM appears to decrease with depth in the 0-50 m sediment layers, as shown in Fig. 23. Therefore, it is unlikely that HFA's in the sediments were mostly produced from alcohols, although the oxidative process could not be excluded to occur in the sediments.

Baedecker et al. (1977) reported that extractable (unbound) fatty acids in marine sediment increased upon heating at 200°C for 64 days by a factor of 12. They considered that FA's which were released on heating have been trapped in sediment or humic materials. Harrison (1978) reported that considerable amount of extractable FA's was obtained by heating the pre-extracted sediments. He considered that those acids released upon heating have been incorporated in kerogen. These studies caused us to consider that HFA's might be released from the matirces in the sediment, i.e. humic materials or kerogen during diagenesis. Therefore, we conducted a thermal alteration experiment of the surface sediment and the sediment sample from 21 m in depth of Lake Biwa in order to evaluate the potentiality of the sediments
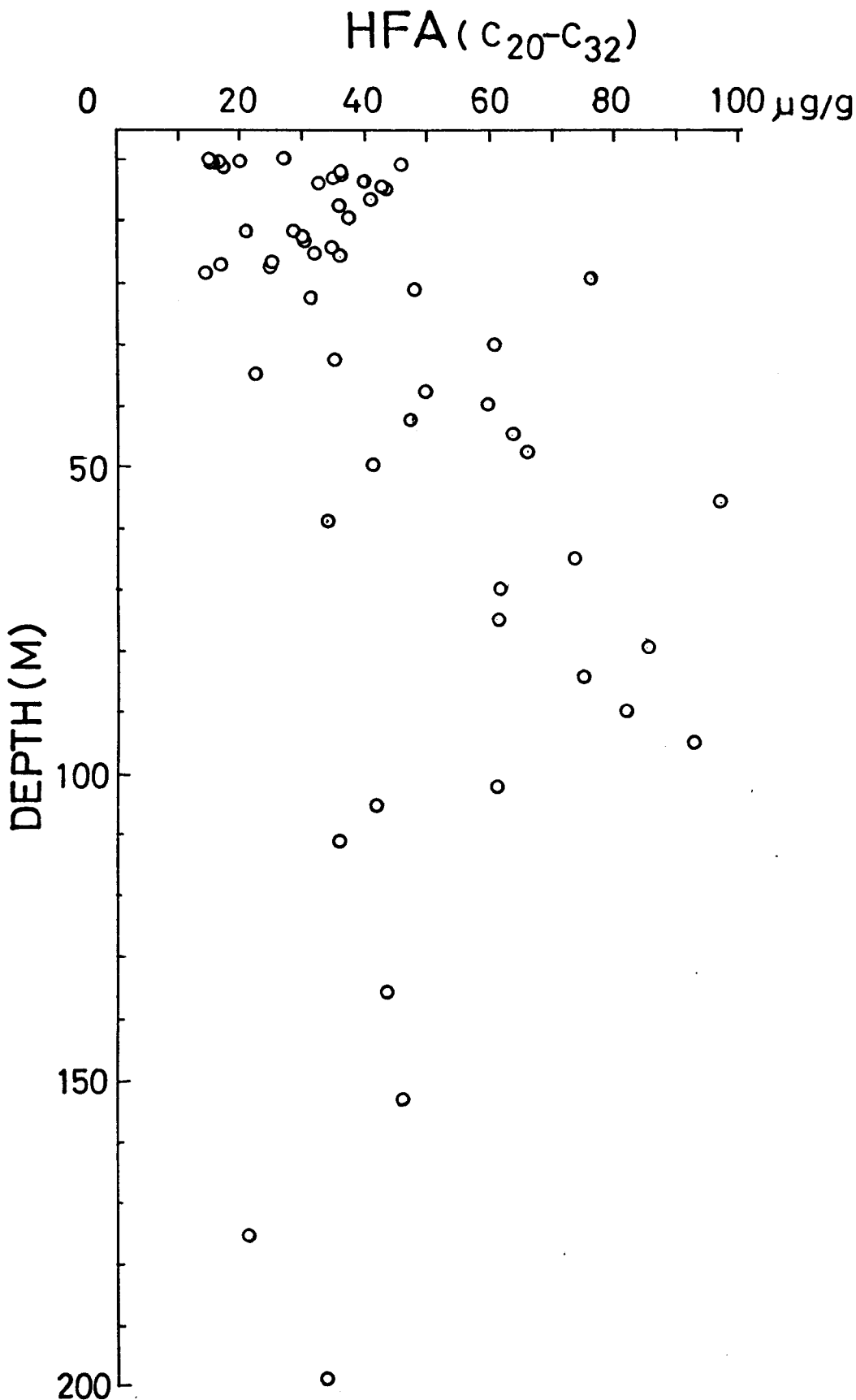
Fig. 22 Vertical pofile of the concentrations of HFA's
(unbound + bound) in a 200 m core of Lake Biwa.

ALCOHOLS
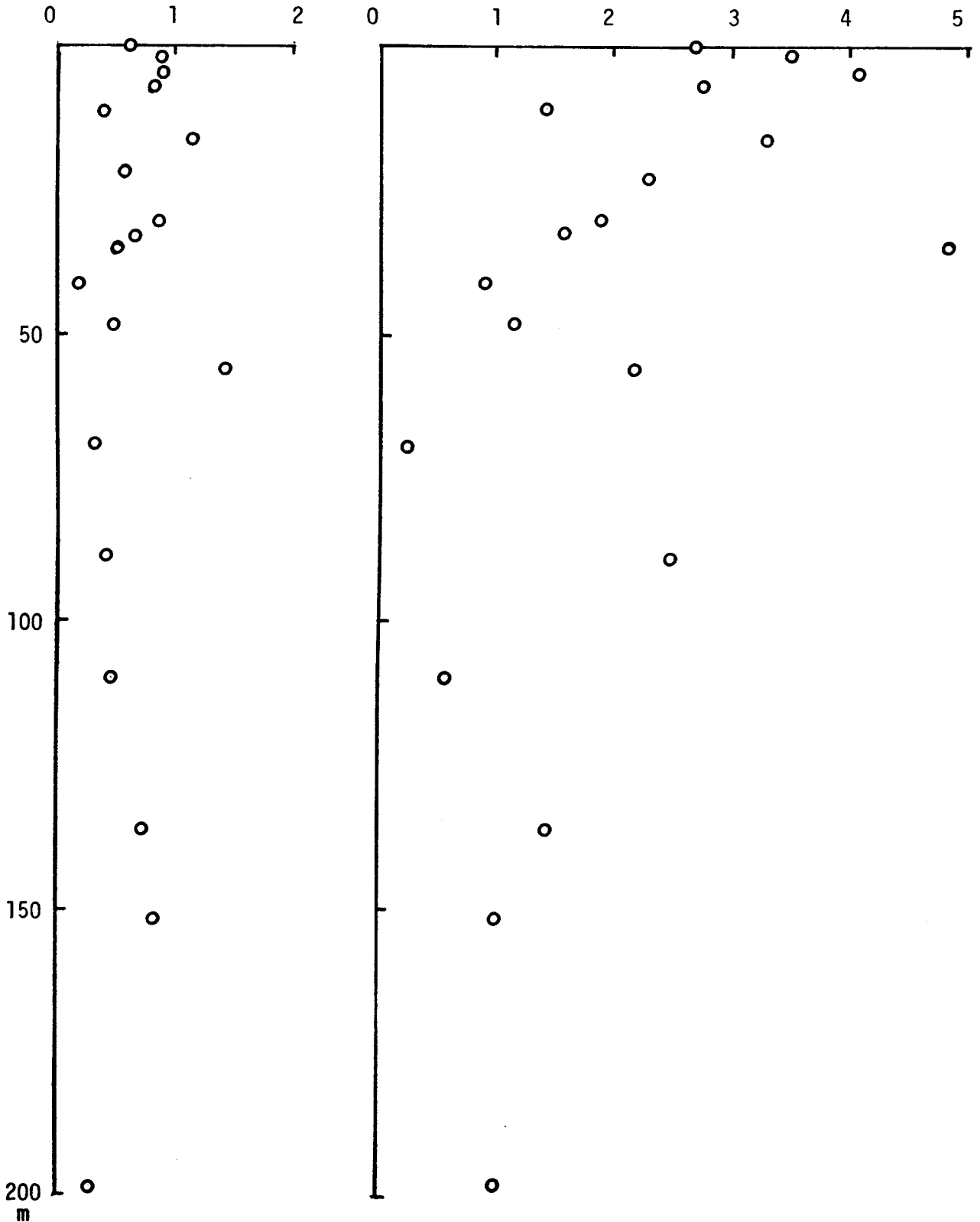
Fig. 23 Vertical variation of the percentage of alcohols to TOM in the 200 m core of Lake Biwa.

Table 4 Concentrations of fatty acids in heated sediment samples of Lake Biwa

(μg/g dry sediment)

Sample		Unheated	Heated (200°C, 1 day)	Increasing rate (%)
Surface	LFA's	88.5	111.1	25
	HFA's	93.7	113.6	21
21 m	LFA's	12.3	16.3	32
	HFA's	50.0	53.6	8

to produce fatty acids in diagenetic processes. The samples were heated at 200°C for 24 hours in a pyrex tube in wet condition. The unbound + bound fatty acids in the heated samples were analyzed in a way as stated in Chapter 1. Table 4 shows the experimental results.

The increasing rates of HFA's in the surface and 21 m sediments are 21 and 8 %, respectively. These results suggest that the sediments have a potential to release HFA's from organic and/or inorganic matrices in sediments during a diagenetic process. However, these values are too low to explain the phenomena observed in the 200 m sediment core. Consequently, we may conclude that the increase in the concentrations of HFA's in the 20-100 m sediment layers were not caused by diagenesis but by the change in the input of HFA's to sediment in the past. The increase in the input of HFA's may be concerned with an increase in the relative contribution of allochthonous organic matter to the sediment in the past, since HFA's are characteristic of terrestrial higher plants.

3.4.3. Vertical distribution of branched fatty acids

The fluctuation of branched fatty acids, as shown in Fig. 12 (see p. 43), may be associated with paleoenvironmental conditions. Since branched fatty acids are characteristic of microorganisms such as bacteria and fungi (Kaneda, 1967; Kikuchi et al., 1973), their distribution may reflect the microbial activity in the water column and surface

sediment in the past. The bacterial activity may be depressed in cool or cold water conditions and so less amount of branched acids may be supplied to sediment. While bacterial activity may become more active in temperate conditions and the branched acids may be more produced, consequently more abundant branched acids may be supplied to surface sediments. If this is true, it can be proposed that branched fatty acids may be a possible indicator to estimate the paleo water temperature.

As shown in Fig. 12 (p. 43), lower values of the concentrations of branched acids to TOM are observed in the 1-5 m and 15-18 m sediments. The ages corresponding to the sediment layers might have been cooler or colder. Since higher values are observed in the 6-9 m sediments, the ages might have been relatively warm. These inferences are seemingly consistent with the paleoclimate estimated by using the $C_{18:2}/C_{18:0}$ ratio in the 0-20 m core of Lake Biwa, in which the ages corresponding to 0.2, 1-5, 11-12 and 15-16 m sediment were suggested to be cooler or colder than other ages (see Chapter 5).

3.4.4. Unbound and bound fatty acids in the 0-20 m sediment layers

Fatty acids in algae, which are a major source of sedimentary organic matter, are present for the most part in unbound form. For example, 98 % of fatty acids in a fresh water green alga, Chlosterium (Lake Biwa) was in unbound form (Kawamura, unpublished data) and 94 % of fatty acids in a green alga, Gloetricia echinulata, was present in unbound form (Cranwell, 1979). In spite of extremly low concentration of bound fatty acids in algae, a considerable amount of bounf fatty acids has been detected in surface sediments of Lake Biwa and other surface sediments (e.g. Farrington et al., 1973; Ishiwatari et al., 1977). In addition, it was found that these bound fatty acids contain more abundant branched fatty acids which are characteristic of microorganisms such as bacteria than unbound fatty acids contain (see Table 2, p.38). These facts suggest that the bound fatty acids are formed by microbial activity in a sedimentation process and/or an early stage of diagenesis after deposition (Cranwell, 1978).

It is of interest to note that the ratios of bound LFA's to unbound LFA's appeared to increase with depth in the 0-50 cm sediments, as shown in Fig. 13 (p. 44). This fact suggests that transformation of unbound LFA's to bound LFA's is also operative in the near-surface sediments after deposition as well as in water column. In deeper sediments, the ratios varied in the ranges of 0.8-2.3, which are comparable with those of Lake Haruna sediments (Ishiwatari et al., 1977). Since the ratios did not show an increasing or decreasing trend in the 1-20 m sediments, the ratios may be primarily determined by some factors including bacterial activity in water column and surface sediments, and may be preserved in the sediment core.

On the other hand, the ratios for HFA's appeared to increase slightly with depth in the 0-20 m sediments, as shown in Fig. 13. This suggests that a part of unbound HFA's change to bound HFA's in a diagenetic process. The difference of the vertical trends in the ratios between LFA's and HFA's might be due to the difference in their origin and diagenetic history. However, decisive explanation for the difference can not be presented now. The peaks of the ratios for HFA's, observed in the layers of 1.0, 2.0 and 17.1 m, suggest that the ratios are determined at a stage before the organic matter is supplied to the sediments and that the values may reflect the diagenetic history in soils. Since HFA's are of allochthonous origin and may be subjected to bacterial attack, bound HFA's may be associated with bacterial activity in soils. The peaks of the ratios might reflect the relatively high activity of bacteria in soils.

A slight difference seems to be present between the vertical distributions of unbound and bound fatty acids. Figure 24 shows the carbon numbers of unbound and bound HFA's showing the highest concentration in the upper 20 m sediment layers. The carbon numbers for unbound and bound HFA's shift from C_{24} to C_{26} and from C_{22} to C_{28} with increasing depth, respectively. The shift may be explained by an unstability of lower carbon numbered fatty acids relative to higher carbon numbered ones. The difference in a manner of the shift between unbound and bound HFA's suggests that bound HFA's are more likely transformed than unbound HFA's.
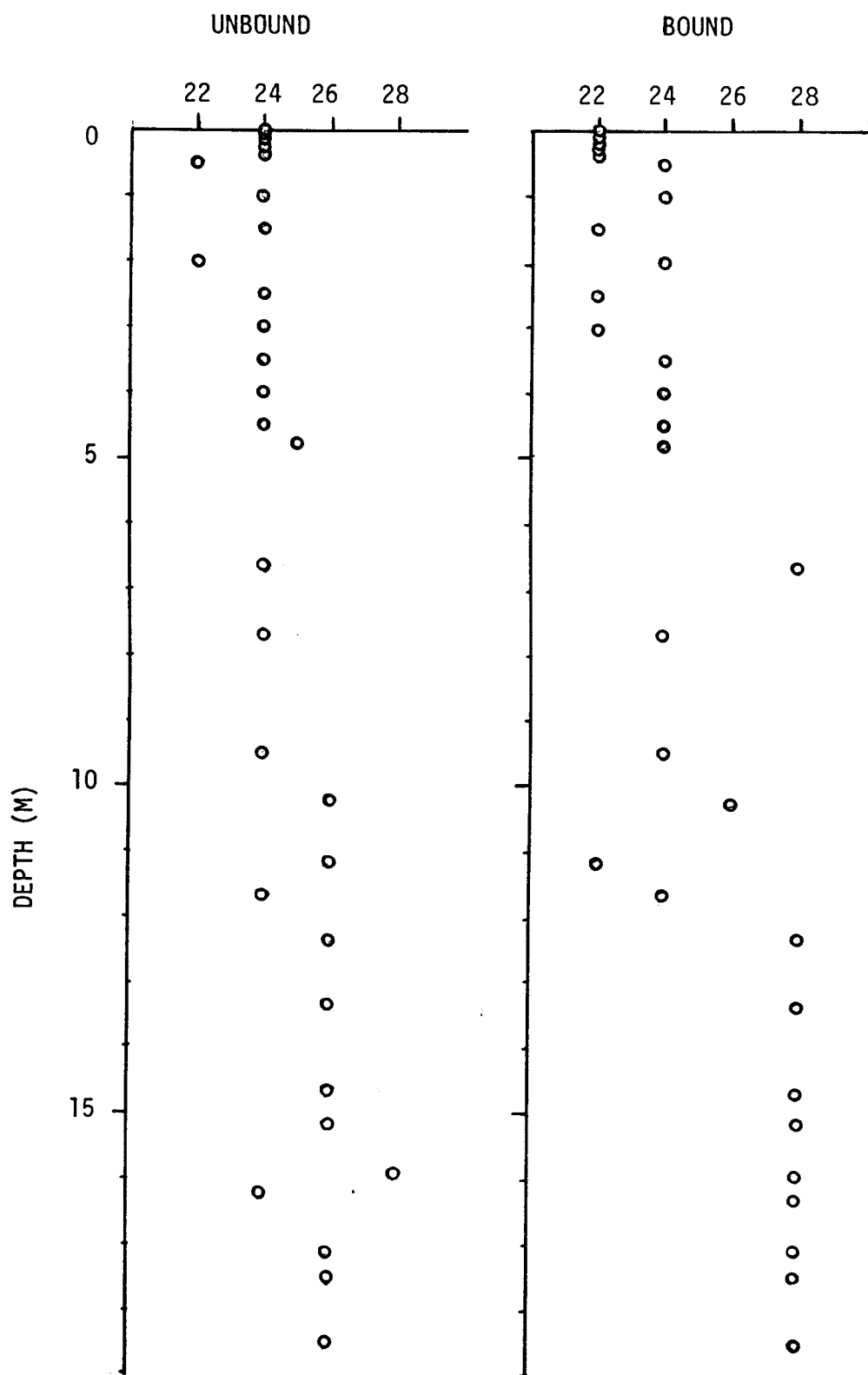
Fig. 24 Carbon numbers of unbound and bound HFA's showing the highest concentration.

This is reasonable because bound fatty acids may be associated with organic and/or inorganic matirces in sediments and so they are more likely to interact with the matrices.

As shown in Fig. 14 (p. 46), the ratios of bound branched acids to unbound ones showed a large peak in the 2-3 m sediment layers. The increasing trend of the ratios is also recognized in the surface sediments of Lake Haruna (Ishiwatari, unpublished data), as shown in Fig. 25, giving a vertical profile of the ratios of bound branched C_{15} to unbound one. The ratios increase with depth in the 1.2 m sediments, except for the sediment layers which contain volcanish ash. Such an increase in the ratios may be caused by an early diagenesis rather than changes in the relative input of unbound and bound branched acids. Since branched acids are of bacterial origin, it is reasonable to consider that bound branched acids may be transformed from unbound ones after the death of bacteria by enzymatic condensation and association with organic and/or inorganic matrices, and a part of the resulted bound acids, in turn, may change to unbound acids or be incorporated into geopolymer.

3.4.5. Fatty acids in residual fractions and their origin

Schnitzer and Neyround (1975) reported that a considerable amount of fatty acids was released by a harsher saponification procedure (170°C, 2 hours) of soil humic and fulvic acids. They considered that these acids are constituents of the humic and fulvic acids. A harsher saponification procedure may make it possible to break the ester linkages in the structures of organic matter in which fatty acids are incorporated. Therefore, it is reasonable to consider that fatty acids obtained by the procedure have been constituents of geopolymers (humic compounds and kerogen).

Although the concentraions of fatty acids in the residual fractions are lower than those of unbound + bound fatty acids (the former acids comprise 4.6-49.4 % of the latter), their distributions are quite different from those of unbound and bound FA's, as shown in Fig. 15 (p. 47). Figure 26 shows a vertical profile of the ratios of LFA's in

RATIO (bound / unbound, branched C_{15})
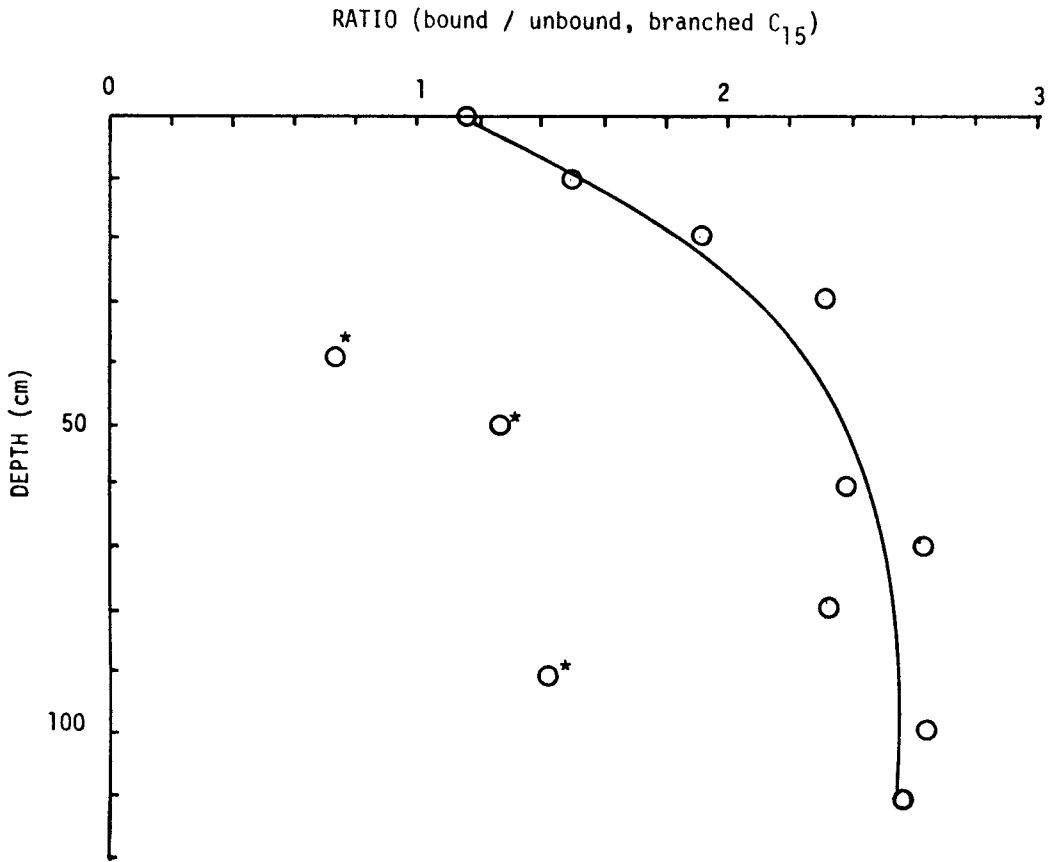
Fig. 25 Vertical profile of the ratios of bound branched C_{15} fatty acid to unbound one in Lake Haruna (Ishiwatari, unpublished data).
*: volcanic ash layer
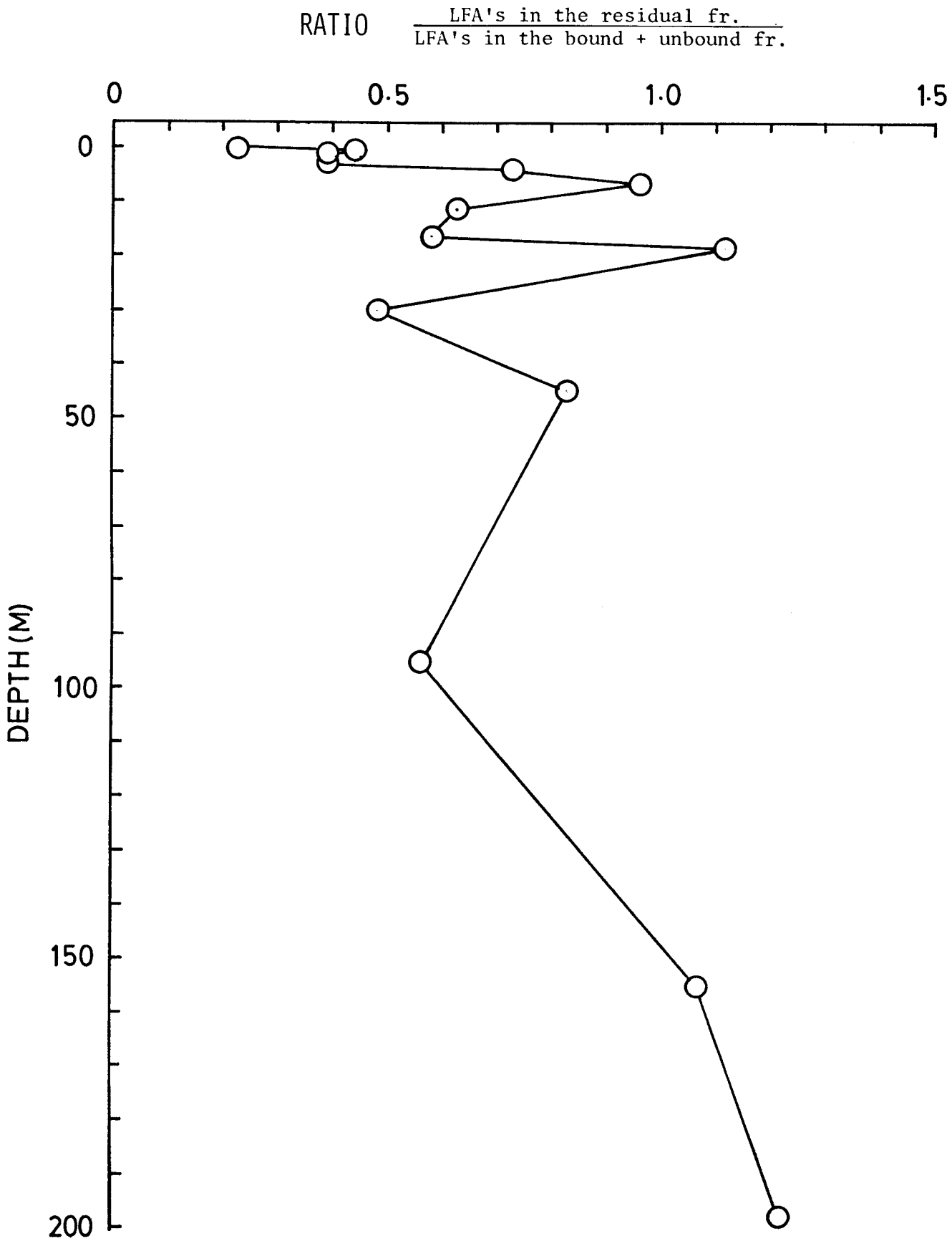
Fig. 26 Vertical profile of the ratios of LFA's in the residual fraction to those in the unbound + bound fraction in the 200 m core of Lake Biwa.

the residual fractions to those in the unbound + bound fractions in the 200 m core. The ratio, which showed 0.22 at the top surface sediment, largely increased with depth in the upper 10 m sediment layers and slightly increased in deeper sediment layers. This indicates that LFA's in the residual fraction are more stable than LFA's in the unbound + bound fractions. Since those LFA's may be incorporated in organic matrix, they may be less likely to be subjected to microbial attack than unbound and bound fatty acids.

Figure 27 shows a vertical profile of the concentrations of LFA's and HFA's in the residual fractions. A peak is recognized in the 1-20 m sediments, in which a large peak of total carbon contents was also observed (see Fig. 4, p. 25). Figure 28 shows a relationship between the concentration of LFA's in the residual fractions and the total carbon content. A good correlation indicates that these acids and the total carbon are of similar origin. Since the total carbon was suggested to be of autochthonous origin (see Chapter 2), fatty acids in the residual fractions may be also autochthonous. This is reasonable because the fatty acids in this fraction showed LFA predominant distribution, which is characteristic of aquatic organisms. Since these acids were actually detected even in the top surface sediment and they may be associated with geopolymers, it is probale that fatty acids in the residual fractions are formed from the dead cells of phytoplanktons during an early stage of diagenesis, probably in water column, where geopolymers may be also formed.

CONCENTRATION (μg/g dry sediment)
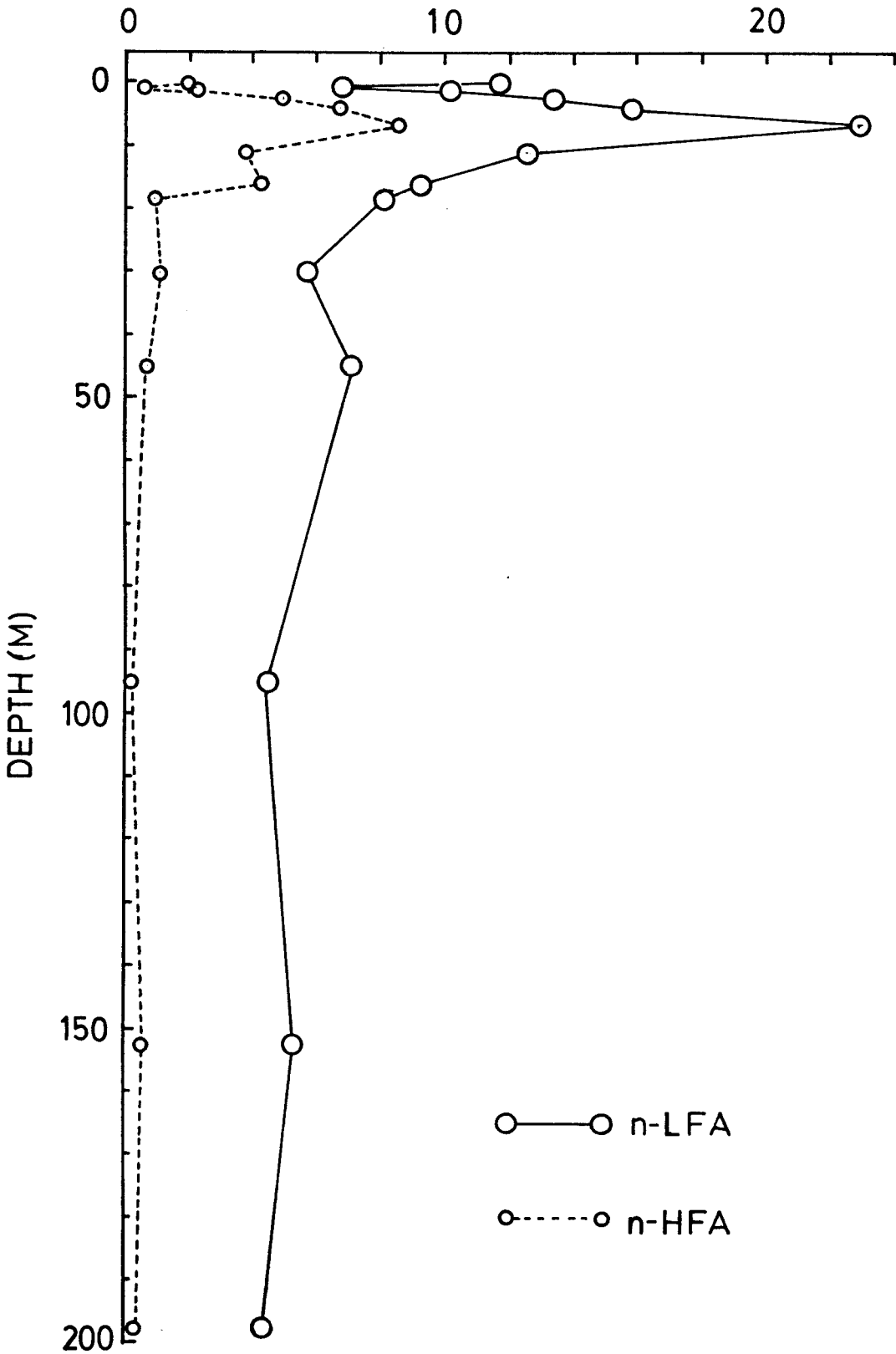
Fig. 27 Vertical profile of the concentrations of LFA's and HFA's in the residual fraction in the 200 m core.
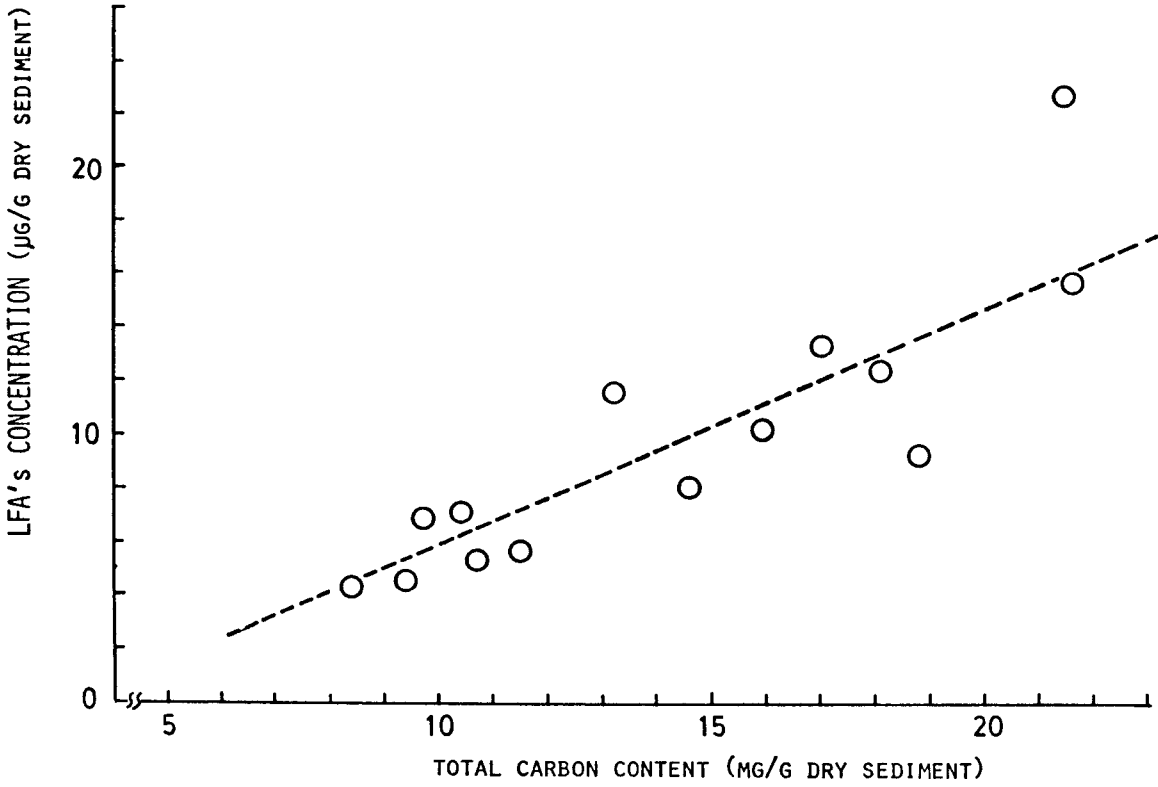
Fig. 28 A relationship between the concentration of LFA's in residual fraction and the total carbon content.

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CHAPTER 4

VERTICAL DISTRIBUTION OF HYDROXY ACIDS AND DICARBOXYLIC
ACIDS IN A 200 M CORE OF LAKE BIWA

4.1. INTRODUCTION

Hydroxy acids and dicarboxylic acids are geochemically interesting
compounds, as follows. (1) Since they are not present in phytoplanktons
which are a major source of organic matter in sediments but present
in bacteria and higher plants, these compounds are a possible indicator
for the microbial and/or allochthonous contribution to the sedimentary
organic matter. (2) Since their skeltons are similar to those of fatty
acids, the behavior of these compounds may strongly relate with that of
fatty acids in sediments. However, the reported data on hydroxy acids
and dicarboxylic acids are limited, as described in General Introduction.

In this chapter, hydroxy acids and dicarboxylic acids in ca. 60
samples of the 200 m core were studied with a special attention to
unbound, bound and residual fractions. The vertical distributions were
interpreted in terms of their diagenesis and a change in the input of
them to the bottom sediments in the past.

4.2. EXPERIMENTAL

The samples studied were the same as those used for fatty acid
analyses (Chapter 3). The analytical methods of hydroxy acids and
dicarboxylic acids were described in Chapter 1.

4.3. RESULTS

β-Hydroxy acids (C_{10}-C_{18}), ω-hydroxy acids (C_{12}-C_{30}), ω-1 hydroxy acids (C_{24}, C_{26}, C_{28} and C_{30}) and α,ω-dicarboxylic acids (C_9-C_{30}) were detected in unbound, bound and residual fractions of the sediments. It is important to note that these compounds were recoginzed to exist in the residual fractions of sediments, which has not been published yet. Figure 29 shows a typical gas chromatogram and a mass fragmentogram of hydroxy acids and dicarboxylic acids in bound fraction of Lake Biwa

RETENTION TIME
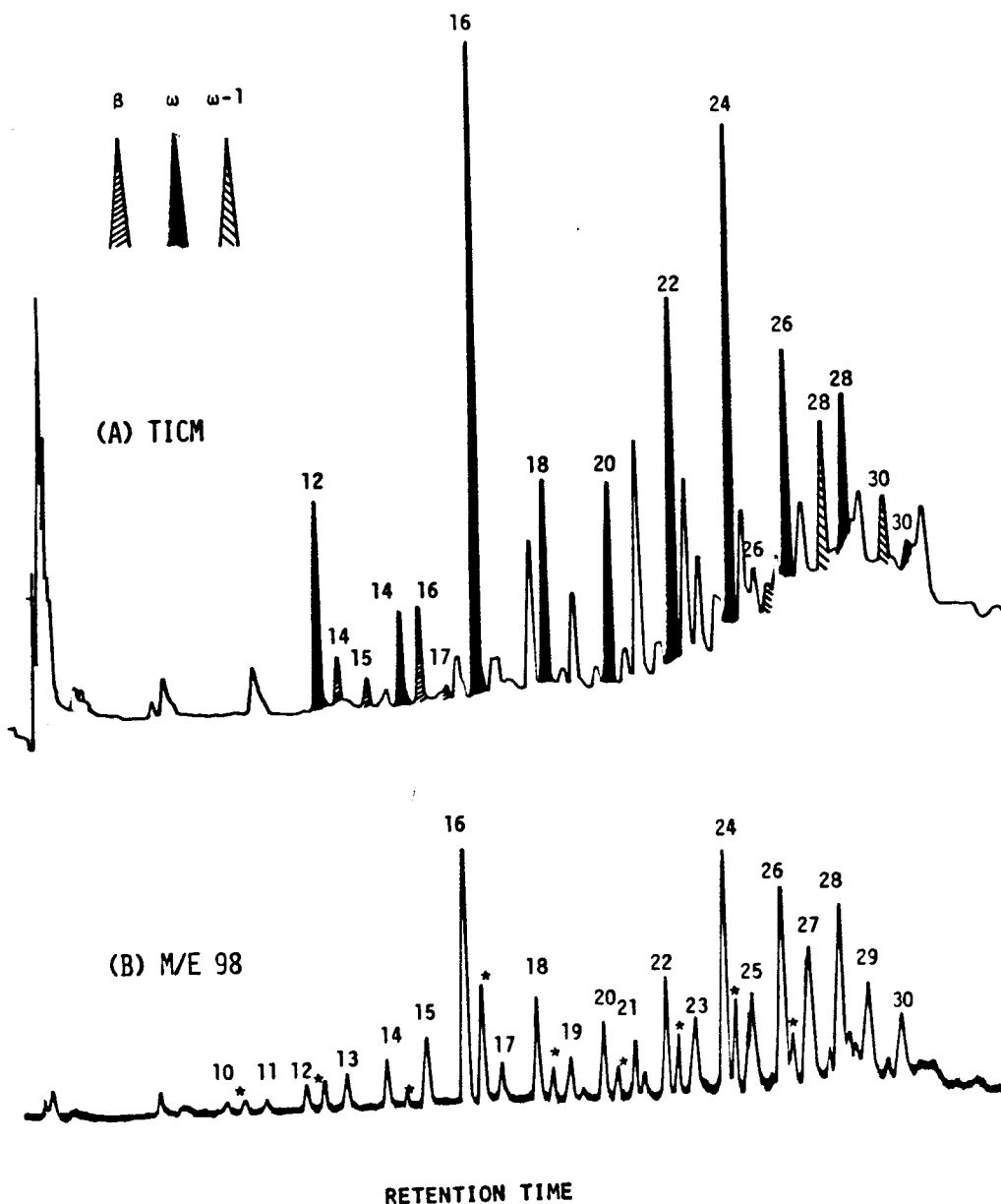
Figure 29. Typical gas chromatogram (TICM) of hydroxy acid methyl ester TMS ethers and mass fragmentogram (m/e 98) of dicarboxylic acid methyl esters in the sediment sample of Lake Biwa (# 160-1, 65.1 m in depth).

sediment. Figure 30 shows histograms of β- and ω-hydroxy acids and α,ω-dicarboxylic acids in the three fractions (unbound, bound and residual). Tables 5 and 6 show the analytical results.

Fig. 30 Distributions of β- and ω-hydroxy acids and α,ω-dicarboxylic acids of three fractions (unbound, bound and residual) in the sediment of Lake Biwa (#1-21, 1.0 m in depth).

Table 5. Analytical results of β-hydroxy acids, ω-hydroxy acids, and α,ω-dicarboxylic acids in unbound + bound fractions and residual fractions in the 200 m core of Lake Biwa

(µg/g dry sediment)

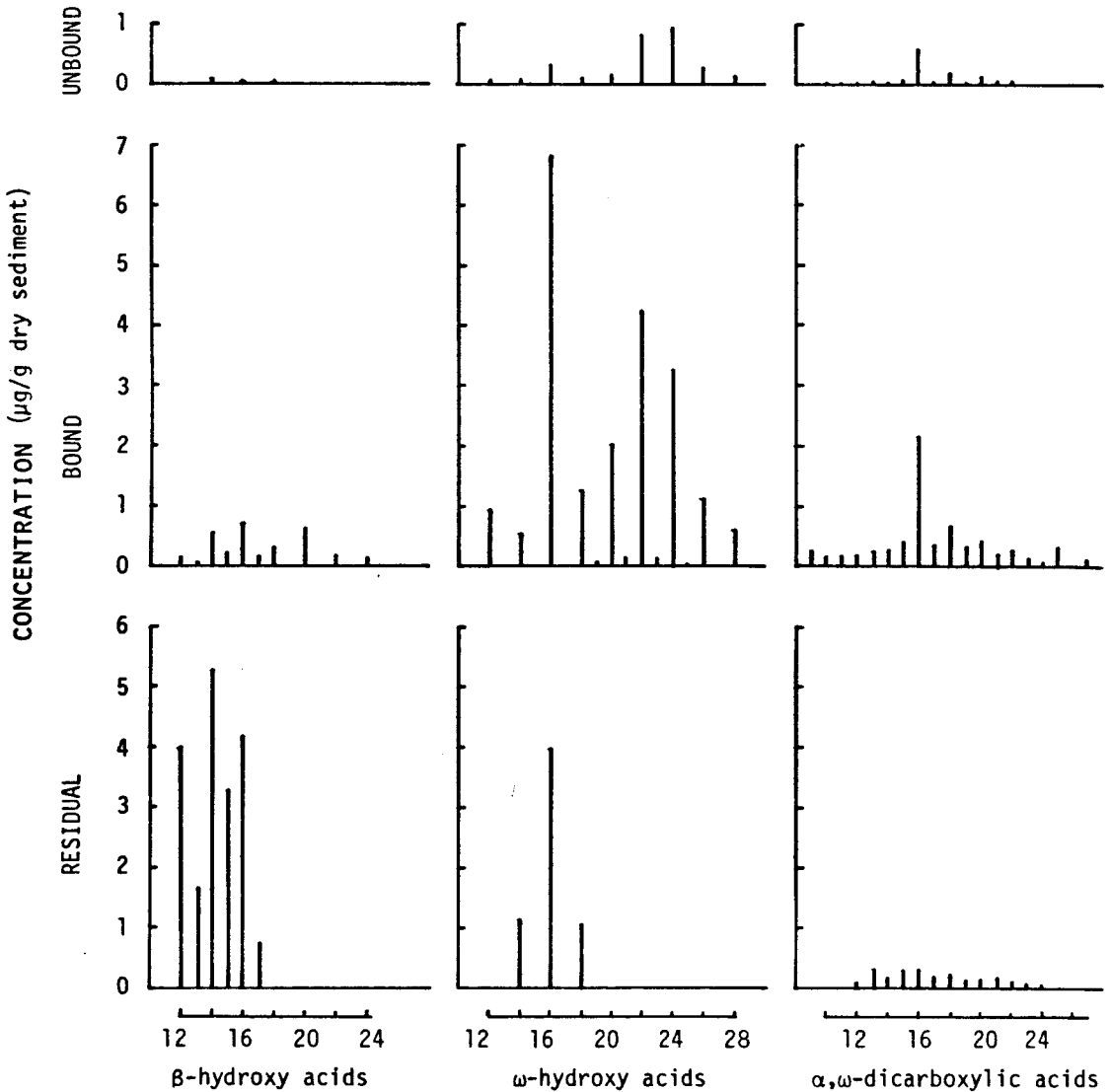| Sample No.	Depth (m)	Unbound + bound fraction			Residual fraction		
		β-OH	ω-OH	Di-acids	β-OH	ω-OH	Di-acids
1-1	0	4.09	5.27	2.24	19.56	5.65	2.82
1-3	0.1	2.12	8.29	1.86	-**	-	-
1-5	0.2	1.37	7.80	2.02	-	-	-
1-7	0.3	3.71	9.65	4.95	-	-	-
1-9	0.4	1.81	6.35	2.80	12.61	5.51	1.94
1-11	0.5	0.42	4.98	-	-	-	-
1-21	1.0	2.54	23.98	15.58	19.13	6.24	2.31
2-1	1.5	2.27	7.70	3.00	-	-	-
2-11	2.0	1.11	18.48	-	-	-	-
2-21	2.5	3.02	17.52	5.33	60.52	7.97	9.03
3-9	3.0	3.03	24.74	4.91	-	-	-
3-19	3.5	3.80	19.07	5.49	-	-	-
4-7	4.0	6.91	28.86	9.32	32.82	9.49	5.88
4-17	4.5	4.69	20.21	5.22	-	-	-
4-21	4.7	1.80	23.08	5.99	-	-	-
9-2	6.6	5.70	40.76	14.13	25.43	7.97	2.46
11-2	7.7	8.40	23.13	10.74	-	-	-
14-2	9.5	6.45	12.49	4.75	-	-	-
16-2	10.6	1.75	2.01	3.87	-	-	-
17-2	11.2	6.05	16.82	5.44	22.59	7.61	3.57
18-2	11.7	3.68	11.92	4.71	-	-	-
21-2	12.5	5.89	19.47	9.67	-	-	-
23-2	13.4	4.02	19.84	5.13	-	-	-
25-2	14.6	4.88	47.29	9.95	-	-	-
26-2	15.2	2.36	28.13	4.06	-	-	-
27-2	15.8	4.32	51.10	9.07	20.28	6.82	2.95
28-2	16.3	4.89	45.11	10.14	-	-	-
31-3	17.1	4.09	41.64	9.13	-	-	-
33-2	17.5	2.84	32.44	4.81	-	-	-
35-2	18.5	0.87	22.97	3.76	27.89	6.01	2.56
47-2	22.8	2.37	15.69	6.24	-	-	-
65-2	30.2	2.18	29.09	8.99	14.20	3.40	1.51
72-1	32.5	1.97	15.34	5.92	-	-	-
81-1	35.1	2.06	17.32	7.57	-	-	-
86-1	37.5	2.88	23.26	9.94	-	-	-
95-2	40.7	2.24	19.99	7.76	-	-	-
103-2	42.8	3.49	19.62	8.11	-	-	-
107-1	45.2	2.47	23.89	7.57	6.15	4.78	0.17
113-2	47.8	1.85	9.96	8.61	-	-	-
120-1	50.4	1.02	19.76	4.71	-	-	-
129-2	56.3	2.21	47.75	11.38	-	-	-
143-1	59.8	1.20	11.44	-	-	-	-
160-1	65.1	1.46	18.67	6.62	-	-	-
172-1	69.7	1.54	43.16	9.63	-	-	-
181-1	74.8	1.81	27.51	8.10	-	-	-
190-1	79.8	2.03	50.34	10.20	-	-	-
198-2	84.2	1.74	49.93	11.27	-	-	-
211-1	89.0	2.45	15.46	7.68	-	-	-
229-2	95.1	1.62	38.12	9.69	11.97	6.16	1.26
244-1	102.0	0.72	22.06	7.90	-	-	-
255-1	105.4	1.21	9.08	6.85	-	-	-
269-1	110.9	tr*	7.13	2.33	-	-	-
336-1	136.3	tr	4.77	2.24	-	-	-
379-2	152.5	1.07	31.00	6.33	7.32	5.51	1.22
438-1	174.6	0.35	10.72	-	-	-	-
498-1	197.8	0.57	4.69	3.25	5.59	5.07	0.80

*: trace; ** not analyzed.

Table 6. The concentrations of β-hydroxy acids, ω-hydroxy acids and α,ω-dicarboxylic
acids in the unbound (A), bound (B) and residual (C) fractions in the 0-20 m
sediments. (μg/g dry sediment)

Sample No.	Depth (m)	β-hydroxy acids			ω-hydroxy acids			α,ω-dicarboxylic acids		
		A	B	C	A	B	C	A	B	C
1-1	0	0.39	3.91	19.56	1.37	3.89	5.69	tr*	2.24	2.82
1-9	0.4	tr	2.18	12.61	1.63	4.73	5.51	0.47	2.80	1.94
1-21	1.0	0.17	3.42	19.13	3.06	21.45	6.24	1.34	15.58	2.31
2-21	2.5	0.50	2.53	60.52	5.52	11.00	7.97	na**	5.33	9.03
4-7	4.0	0.91	7.14	32.82	4.17	24.7	9.47	na	9.32	5.88
9-2	6.6	1.08	7.13	25.43	5.42	35.44	7.97	na	14.13	2.46
17-2	11.2	1.19	5.48	22.59	4.63	12.64	7.61	na	5.44	3.57
27-2	15.8	0.95	3.73	20.28	6.57	45.96	6.82	5.36	9.07	2.95
35-2	18.5	0.49	0.38	27.89	5.34	18.8	6.01	na	3.76	2.56

*: trace;   ** not analyzed

4.3.1. β-Hydroxy acids

β-Hydroxy acids showed unimodal distribution with a peak at C_{14} or
C_{16} acid. The distributions were characterized by that even carbon
numbered acids were almost normal chain while odd carbon numbered acids
were almost iso/anteiso branched chain. Although the qualitative
distribution in three fractions (unbound, bound and residual) were
similar each other, a significant difference was observed in the
quantitative distributions, as shown in Fig. 30 and Table 6. The
concentrations of β-hydroxy acids in three fractions seemed to increase
in the order: unbound < bound < residual fractions. The concentrations
of the acids in unbound fractions were smaller than 1.68 μg/g dry
sediment. Those of bound fractions were 0.38-7.82 μg/g dry sediment,
which are far larger than those of unbound acids in the 0-20 m sediment
layers. On the other hand, the concentrations of the acids in residual
fractions were 5.6-60.5 ug/g dry sediment, which correspond to 71.3-95.0
% of the total (unbound + bound + residual fractions) β-hydroxy acids
in the 200 m core.

Figure 31 shows a vertical profile of β-hydroxy acids in unbound +
bound fractions. Higher concentrations were recognized in the 0-20 m

ω-HYDROXY ACIDS

0 10 20 30 40 50

α,ω-DICARBO-
XYLIC ACIDS

0   10   20

β-HYDROXY
ACIDS

0   10 µg g^{-1}
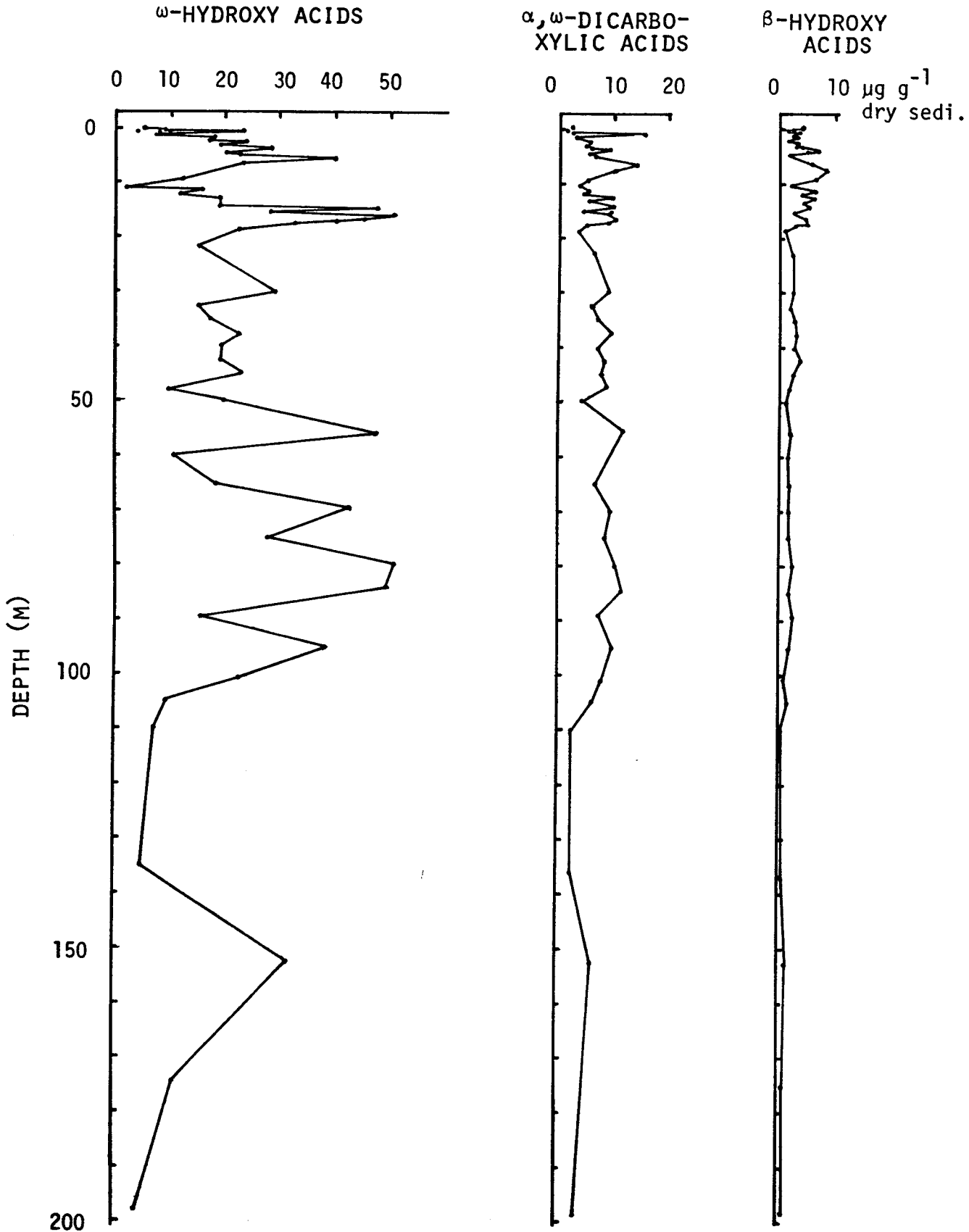   dry sedi.

DEPTH (M)

0

50

100

150

200

Fig. 31   Vertical profile of the concentrations of ω-hydroxy acids,
α,ω-dicarboxylic acids and β-hydroxy acids in a 200 m core lf Lake
Biwa.

sediment layers. These acids appeared to decrease with depth in deeper
layers showing minimum values in the 110-136 m sediments. The concent-
rations of the acids in the residual fractions showed higher values
in the 0-20 m sediments and then appeared to decrease in deeper sediment
layers (see Table 6).

4.3.2. ω-Hydroxy acids

ω-Hydroxy acids in unbound and bound fractions showed bimodal
distribution with maxima at C_{16} and C_{22} or C_{24} acids. The distribution
was characterized by that even numbered acids are predominant and
the concentraions of odd numbered acids are extremly low. Their distri-
butions in unbound and bound fractions seemed to be similar each other.
ω-Hydroxy acids were more concentrated in bound fractions than in
unbound fractions. The concentrations of the acids in unbound fractions
were 0.81-19.68 µg/g dry sediment, which are lower than those in
bound fractions (3.86-49.94 µg/g dry sediment) in the 0-20m sediment.
The percentage of bound acids comprised 44-100 % of the unbound + bound
acids.

On the other hand, the residual fractions were found to contain
a considerable amount of ω-hydroxy acids, which showed unimodal distri-
bution in the range of C_{14}-C_{18} acids with a peak at C_{16} acid. Their
concentrations ranged from 3.4 to 9.5 µg/g dry sediment, which are
corresponding to 11.5-51.7 % of the total (unbound + bound + residual)
concentrations.

As shown in Fig. 31, unbound + bound ω-hydroxy acids showed a
vertical fluctuation, having some peaks at 1-6, 15-18, 55, 70-85, 95
and 152 m sediments. On the other hand, those acids in the residual
fractions showed a maxima at 2-16 m sediments (see Table 5).

4.3.3. α,ω-Dicarboxylic acids

α,ω-Dicarboxylic acids in unbound and bound fractions showed
bimodal distributions with maxima at C_{16} and C_{22} or C_{24} acids. The
distributions were characterized by even carbon numbered predominance,
although odd carbon numbered dicarboxylic acids were also present

relatively abundantly. The concentrations of bound acids were higher than those of unbound acids throughout the upper 20 m sediment layers. The concentraiotns of unboud + bound acids were 1.86-15.58 µg/g dry sediment. Higher concentrations were recognized in the layers of 5-7, 12-17, and 30-100 m in depth (see Fig. 31).

α,ω-Dicarboxylic acids in residual fractions showed neither bimodal distribution nor even carbon numbered predominance, which are quite different from the distribution of the acids in unbound and bound fractions. The concentraions of these acids varied in the range of 0.17-9.03 µg/g dry sediment. Higher concentraiotns were recognized in the 0-20 m sediments.

4.4. DISCUSSION

4.4.1. Existence forms of the organic compounds in sediments

Although differences in the chemical states of hydroxy acids and dicarboxylic acids in three fractions (unbound, bound and residual) are not clearly presented, their existence forms can be tentatively differentiated by separation procedure. Unbound fraction, which is extracted by organic solvents, may contain free compounds and/or esters. Bound fractions, obtained by saponification of pre-extracted sediment, may contain organic compounds which have been combined to surface of organic matrix such as kerogen by ester linkage and or been trapped in inorganic matrix (Farrington et al., 1977). The compounds obtained by harsher saponification procedure are considered to have been incorporated in humic materials and kerogen matrix, and may be released through breakdown of the linkages in the polymeric structures.

β-Hydroxy acids

Since β-hydroxy acids are constituents of bacterial cell walls (Boon et al., 1977), the presence of these acids in sediments indicates that bacterial activities are involved in the formation of sedimentary organic matter. The fact that the concentrations of β-hydroxy acids in bound fractions are higher than those in unbound fractions suggests

that the former fractions are more associated with bacterial activity
than latter fractions. This is consistent with the statement that
fatty acids in bound fractions are more associated with microbial
activity than those in unbound fractions, inferred from the fact that
branched fatty acids are more concentrated in bound fraction than in
unbound fractions (Cranwell, 1978; see Chapter 3, p. 60).

Higher percentage of β-hydroxy acids in the residual fractions
(76-97 % of the total concentration) suggests that the formation of
geopolymers (kerogen and humic compounds) is involved in the microbial
activity since the geopolymers exist in the pre-extracted sediment
residue. In addition, since a considerable amount of these acids in
the residual fractions exists even in the top surface sediment,
it is reasonable to consider that bacteria in water column may be closely
associated with the formation of the geopolymers: probably bacterial
biomass may directly transform to the geopolymers after their death.
This is consistent with a consideration that the geopolymers are of
autochthonous origin, which was suggested from the fatty acid distribu-
tion in the residual fractions (see Chpter 3, p.66). Furthermore,
the above consideration is also consistent with the existence of a
good correlation between the concentration of humic compounds and
the total carbon contents (see Fig. 9, p. 34).

ω-Hydroxy acids

ω-Hydroxy acids in sediments have been considered to be derived
from cuticular wax and suberin of higher plants (Eglinton et al., 1968;
Cardoso et al., 1977) and/or from an aerobic microorganism (Boon et al.,
1977). So, ω-hydroxy acids in Lake Biwa sediments may have a dual
origin. As shown in Table 6 (p. 75), ω-hydroxy acids in unbound, bound
and residual fractions comprise 10.0-22.5 %, 35.7-72.6 % and 11.5-51.7 %
of the total contents (unbound + bound + residual fractions) in the
sediment, respectively. Since these acids in higher plants construct
the network structure of cutin and suberin through ester linkage with
other compounds (Hunneman and Eglinton, 1969; Kolattukudy, 1980), it is
reasonable that ω-hydroxy acids in bound fractions show higher percentages.
The results suggest that the net work structures which consist of

ω-hydroxy acids are fairly preserved in sediments. However, the fact that unbound fractions contain cutin acids suggests that these acids are formed by hydrolysis of bound ω-hydroxy acids during sedimentation in water column.

On the other hand, ω-hydroxy acids in residual fractions were of C_{14}, C_{16} and C_{18} predominance (Fig. 30, p.73). Their qualitative distributions are quite different from those of unbound and bound fractions. This difference suggests that the origin of ω-hydroxy acids in residual fractions are different from those in unbound and bound fractions. The acids in unbound and bound fractions may originate in both cutin and suberin, and in microorganisms. But the acids in the residual fractions may originate mainly in aerobic micro-organisms such as yeast, because yeast contains these acids (Stodola et al., 1967). This consideration suggests that the residual fractions are formed by microbial attack of the dead algae in water column.

α,ω-Dicarboxylic acids

The concentrations of α,ω-dicarboxylic acids are higher in bound fractions than in unbound fractions, as shown in Fig. 30. This may be related with that cutin and suberin contain these acids in their biopolymers (Kolattukudy, 1980). The unbound dicarboxylic acids may be derived from a hydrolysis of the biopolymers in water column and/or microbial oxidation of hydroxy acids or fatty acids.

4.4.2. Vertical distribution of hydroxy acids and dicarboxylic acids in the 200 m core

In general, the vertical distribution of organic molecules in sediments is dependent firstly on the input of them to the lake bottom and secondly on their post-depositional changes (diagenesis).

A large peak has been observed in the total carbon content in the upper 20 m sediment layers (see Fig. 4, p.25). The peak was considered to be caused by the increase in the primary production in the past, based on a good correlation between the numbers of diatom remains and total carbon content, as stated in Chapter 2 (p. 28). Therefore, it is reasonable to consider that the change in the primary

production reflects   on the input of hydroxy acids and dicarboxylic acids in sediments.

β-Hydroxy acids

As shown in Fig. 31 (p. 76), β-hydroxy acids in unbound + bound fractions showed relatively high concentrations in the 0-20 m sediment layers. These peaks may be caused by the increase in the input of these acids, because the layers correspond to the sediments where a large peak of carbon content was recognized. Figure 32 shows a good correlation between the total carbon contents and the concentrations of C_{16} β-hydroxy acid in the residual fractions. The good correlation indicates that β-hydroxy acids are closely related with the total carbon content, which may depend on the primary production in the past. It is, therefore, reasonable to consider that bacterial activity which is associated with β-hydroxy acids may increase generally in accordance with an increase in the primary production.
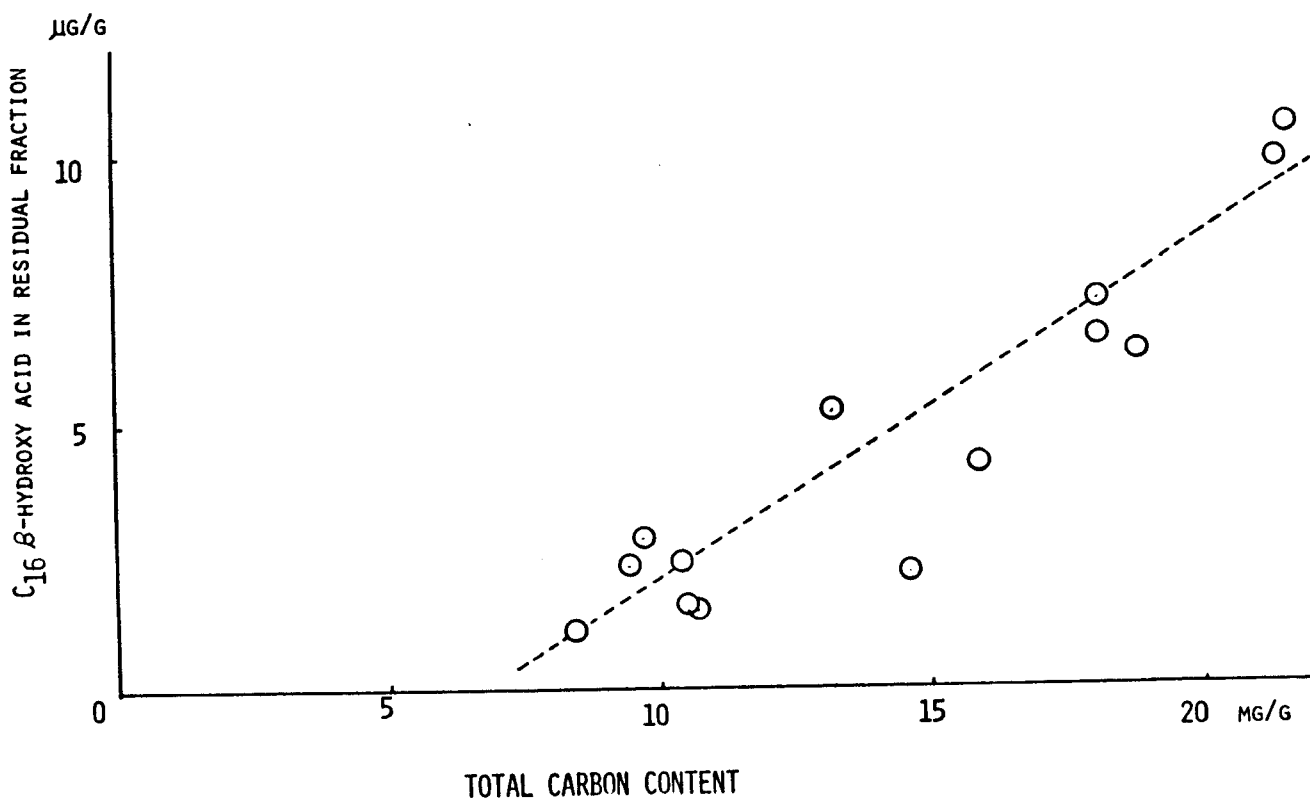

Fig. 32 Relationship between total carbon content and the concentrations of C_{16} ω-hydroxy acid in residual fraction in the 200 m core.

As stated in Chapter 3 (p. 48), C_{16} β-hydroxy acid, ω-hydroxy acid and
α,ω-dicarboxylic acid increased in the ca. 0-10 m sediment layers, in
which C_{16} fatty acid decreased drastically with depth. This fact
suggests that these compounds are produced by β- and ω-oxidation of
corresponding fatty acids in the sediments. However, the production
of β- and ω-hydroxy acids and α,ω-dicarboxylic acids by post-depositional
change of fatty acids in sediments seems to be less remarkable relative
to the input of these acids through water column. On the other hand,
in the sediments deeper than 50 m in depth, the concentrations of β-hydroxy
acids in unbound + bound fractions appeared to decrease with depth (see
Fig. 31, p. 76). These observations seem to be consistent with those
by Eglinton et al. (1968), who reported that these acids decreased
with depth in the 5000 year old sediments.

In addition, β-hydroxy acids in the residual fractions, in which
majority of the acids exists, seemed to decrease with depth in the
sediment layers deeper than 20 m in depth (see Table 5, p. 74). These
facts indicate that the degradation and/or polymerization of these acids
occur in the sediment core. This suggests that β-hydroxy acids are
unstable compounds in sediments. This suggestion seems to be supported
by the results of thermal alteration experiments (see Chapter 8), which
showed that β-hydroxy acids are chemically more labile compounds than
ω-hydroxy acids, α,ω-dicarboxylic acids and monocarboxylic acids in
sediments.

ω-Hydroxy acids and α,ω-dicarboxylic acids

Ishiwatari (1976) reported that one of the possible decomposition
pathways of monocarboxylic acids is omega oxidation producing α,ω-
dicarboxylic acids in the upper 100 m sediment layers of Lake Biwa,
based on higher values of $(C_{16} + C_{18})$di / $(C_{16} + C_{18})$mono ratio in
the 26-85 m sediments. Although the present author also considered
that the omega oxidation mechanism is operative in the sediments, he
believes that main zone where the oxidation is operative is upper 20 m
sediment layers and, in deeper layers, such a mechanism is less operative
(see p. 48). Since the ratios stated above suddenly became small at
the sediment of 100 m in depth (Ishiwatari, 1976), the author considers

that the higher values in the 26-85 m sediments may be caused by the increase in the input of dicarboxylic acids in the past rather than by diagenesis.

As shown in Fig. 33, the peaks of ω-hydroxy acids shifted from C_{22} to C_{24} or C_{26} acids with depth. Those of α,ω-dicarboxylic acids shifted from C_{22} to C_{28} acid, gradually with depth. These trends are seemingly consistent with that of n-saturated fatty acids, whose maxima in the C_{20}-C_{30} range shifted from C_{24} to C_{26} or C_{28} acid with depth. These phenomena may be due to diagenesis and mean that the lower molecular weight compounds are likely to degrade and the higher molecular weight compounds are likely to be preserved in sediments.

As shown in Fig. 31 (p. 76), ω-hydroxy acids and α,ω-dicarboxylic acids showed many peaks in their concentrations. These peaks may be caused by the increase in the input of these compounds in the past. However, these inputs seem to be independent on the primary production. Because, in the 3-10 m sediments where primary production was considered to have increased in the past (see Chapter 2, p. 28), the concentrations of these compounds are not necessarily higher than those of other sediments. This fact suggests that most parts of these acids are not produced inside the lake but are derived from the lake surroundings. These consideration may be reasonable since ω-hydroxy acids and α,ω-dicarboxylic acids are constituents of cutin and suberin of higher plants (Hunneman and Eglinton, 1968; Kalattukudy, 1980). The presence of these acids indicates that allochthonous organic matter is also supplied to bottom sediments together with autochthonous organic matter, although the former contribution is not so large. The vertical distribution of these compounds might reflect the paleoenvironmental conditions.

On the other hand, ω-hydroxy acids in the residual fractions showed a peak in the 4 m sediment, as shown in Table 6 (p. 75). Figure 34 shows a relationship between total carbon contents and concentrations of ω-hydroxy acids in the residual fractions. This relationship suggests taht ω-hydroxy acids in the residual fractions are associated with the primary production. Therefore, it is reasonable to consider that these acids in the residual fractions are formed by microbial
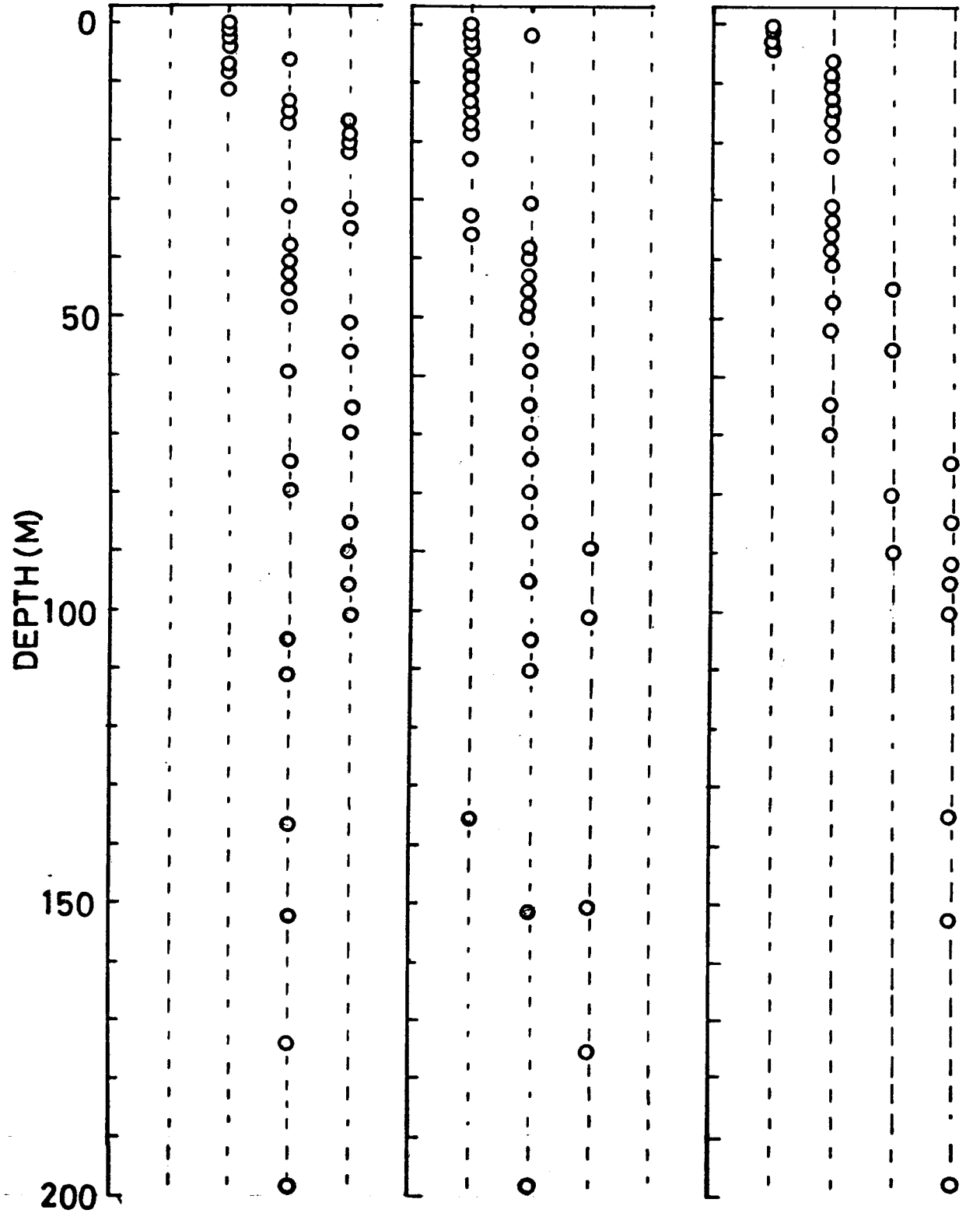
Fig. 33 Carbon numbers of the highest peak in the range of $C_{20}-C_{30}$ for fatty acids, ω-hydroxy acids and dicarboxylic acids in the 200 m core of Lake Biwa.
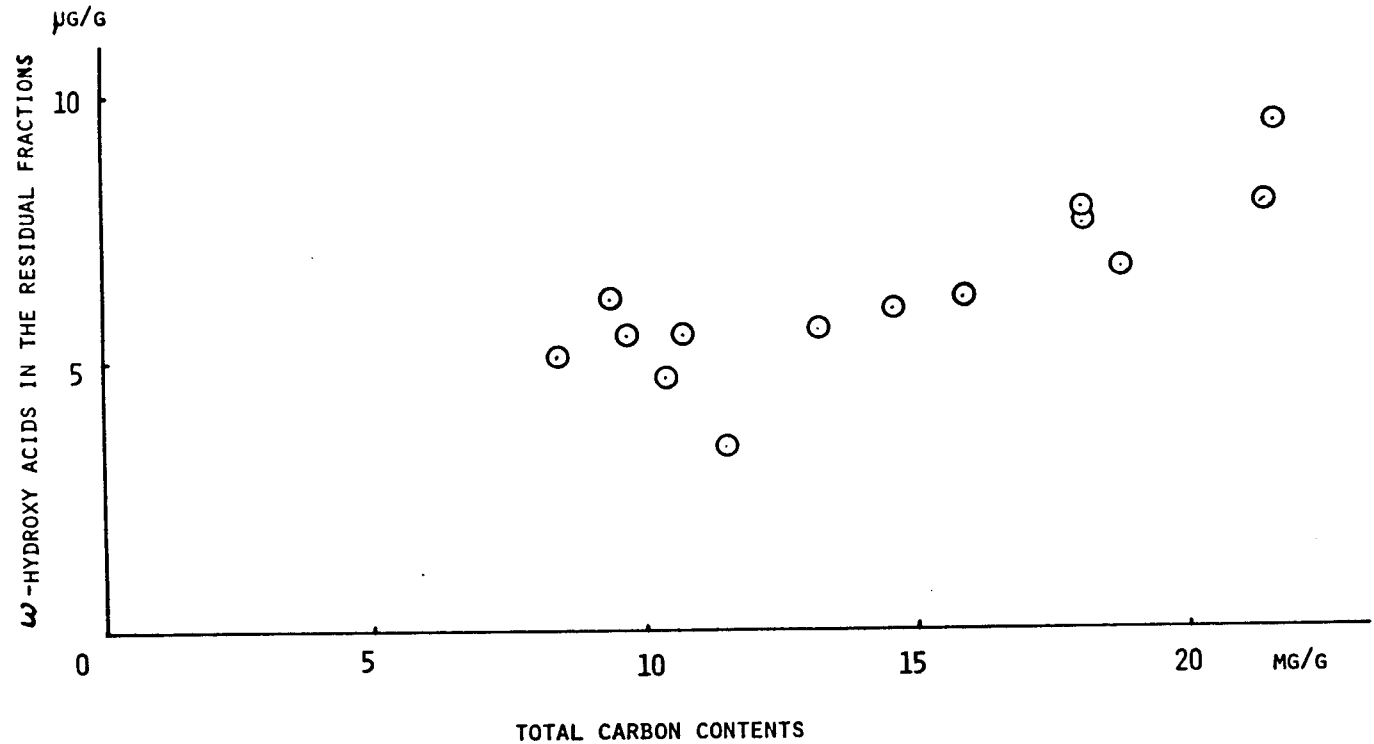
Fig 34 A correlation between the total carbon contents and the concentrations of ω-hydroxy acids in residual fractions of the 200 m core.

degradation of the dead algae in the water column. This consideration also suggests that geopolymers which are present in the pre-extracted sediment residue are formed by microbial attack on the dead algae.

4.4.3. Autochthonous/allochthonous contribution of organic compounds based on a single correlation coefficient

Table 7 shows single correlation coefficients among the organic compounds in the 20-200 m sediments of Lake Biwa. The samples which were used for statistical treatment were lower molecular weight (lower than C_{20}) ω-hydroxy acids (L-ωOH), lower molecular weight dicarboxylic acids (L-diacids), higher molecular weight (higher than C_{20}) ω-hydroxy acids (H-ωOH), and higher molecular weight n-saturated fatty acids (H-FA) in the 26 samples from the 200 m sediment core, except for the upper 20 m sediments in which diagenetic changes in organic matter may be conceivable, as suggested in Chapter 3. Lower M.W. fatty acids (C_{12}-C_{19}) were omitted in Table 7, because majority of these acids is

Table 7. Single correlation coefficients among the organic
compounds in the 20-200 m sediments of Lake Biwa.

	L-ωOH	L-diacids	H-ωOH	H-diacids	HFA
L-ωOH	-	0.87	0.50	0.31	0.47
L-diacids	-	-	0.52	0.47	0.47
H-ωOH	-	-	-	0.78	0.75
H-diacids	-	-	-	-	0.67
HFA	-	-	-	-	-

considered to have degraded in the surface sediments (see Chapter 3).

As shown in Table 7, the coefficients for the combinations of
H-diacids vs. H-FA, H-ωOH vs. H-FA, and H-diacids vs. H-ωOH are 0.67,
0.75 and 0.78, respectively. These higher values indicate good corre-
lations among three compounds, suggesting that they originate from
same or similar sources and that they behave in a similar manner in
aquatic environments. These results and considerations are reasonable,
since H-FA are essential constituent of higher plant wax esters and
H-ωOH and H-diacids are associated with cuticular wax and suberin of
higher plants as stated before (p. 83). Consequently, this suggests
that they are   valid indicators for an evaluation of the contribution
of allochthonous organic matter to the sediments. This idea is
consistent with that of Cranwell (1974) and Cardoso et al. (1977).
If this were the case, there should exist some evidences that support
the above idea.

Since quartz which is a major mineral component of the sediments
is obviously of terrestrial origin, it is a possible marker of the
contribution of allochthonous organic matter. The correlation between
quartz content and the concentrations of H-FA, H-ωOH and H-diacids were
0.26, 0.54 and 0.51, respectively. Relatively good correlation except
for H-FA supports the above idea that these compounds are of allochtho-
nous origin. However, the lower value for the combination of quartz

and H-FA may be caused by the production of H-FA in water column probably through an elongation of lower carbon numbered fatty acids by microorganisms such as yeast (Fulco, 1967).

On the other hand, the lower molecular weight compounds showed the following correlation coefficients: 0.87 (L-ωOH vs. L-diacids), 0.45 (L-FA vs. L-diacids) and 0.25 (L-FA vs. L-ωOH). A high value for the combination of L-ωOH and L-diacids strongly suggests that these compounds are of same origin and/or that the former acids are produced by the oxidation of the latter acids in water column and surface sediments. Since these compounds are present in microorganisms such as bacteria and yeast (Kester and Foster, 1963; Stodola et al., 1967), major parts of them have been derived from the microorganisms and preserved in the sediments. The values for other combinations are relatively low. This may be due to that, since the concentrations of L-FA have decreased in the surface sediments by microbial degradation, the concentrations in the deeper sediments do not indicate the net amounts of L-FA which were input to the bottom sediments. Therefore, these results do not deny that the L-ωOH and L-diacids have been generated by the oxidation of L-FA in water column and surface sediments by microorganisms. This is consistent with the conclusion (β- and ω-oxidation of fatty acids), which was proposed based on the vertical distribution of these compounds in the 0-20 m sediment layers of Lake Biwa.

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CHAPTER 5

A POSSIBLE INDICATOR OF PALEOCLIMATE: POLYUNSATURATED FATTY
ACIDS IN THE SEDIMENT CORE OF LAKE BIWA

5.1. INTRODUCTION

Polyunsaturated fatty acids have been reported to be present in
recent sediments, although their concentrations are low (Poltz, 1972;
Volkman and Johns, 1977; Cranwell, 1978; Thompson and Eglinton, 1978).
These acids are major components of aquatic organisms and their concent-
rations vary depending on temperature, light and nutrient conditions
(Morris, 1980). Moreover, these acids are liable to degrade or polymerize
after the death of organisms (Abelson, 1967; Rhead et al., 1971, 1972;
Gaskell et al., 1976), and their reaction rate is considered to depend
on environmental conditions (e.g. redox condition, Kawamura et al., 1980).
Therefore, polyunsaturated fatty acids in sediments would provide
information on source material and environmental conditions such as
paleotemperature.

This Chapter deals with the study of the presence and distribution
of polyunsaturated fatty acids in the upper 20 m sediment layers of the
200 m core of Lake Biwa and discusses their paleoenvironmental signifi-
cance.

5.2. EXPERIMENTAL

In this study, 15 samples from the 5 m core and 15 samples from
the upper 20 m of the 200 m core sample were subjected to analysis
of unbound and bound fatty acids. The analytical procedures are given
in Chapter 1.

5.3. RESULTS

Mono- and poly-unsaturated fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3\omega3}$ and $C_{18:3\omega6}$) were detected in the samples. Table 8 shows the analytical results. The relatively high concentration of $C_{18:2}$ and $C_{18:3\omega3}$ acids were observed in the sediment layers of 0.2, 1-5, 11-12 and 16 m in depth.

Table 8. Analytical results of unsaturated fatty acids in the sediment layers of Lake Biwa

sample No.	depth (m)	carbon	nitrogen	C/N	fatty acids (µg/g-dry wt. sediment)					
		(mg/g-dry wt. sedi.)			16:0	16:1	18:0	18:1	18:2	18:3
1- 1	0	13.2	1.47	8.98	28.4	2.37	13.3	4.88	0.12	0.12
1- 3	0.1	10.5	1.35	8.08	10.5	0.87	3.09	1.15	0.38	0.08
1- 5	0.2	10.3	1.28	8.05	11.5	1.35	3.48	2.67	3.52	0.38
1- 7	0.3	10.2	1.27	8.03	8.31	1.62	2.82	2.25	1.20	0.13
1- 9	0.4	9.73	1.16	8.39	8.79	1.08	2.62	1.26	0.43	0.05
1-11	0.5	10.5	1.17	8.97	14.7	1.24	6.53	3.84	0.88	0.10
1-21	1.0	15.9	1.73	9.17	13.4	1.88	5.45	4.02	5.42	0.56
2- 1	1.5	16.7	1.90	8.79	11.0	2.10	4.19	5.32	10.9	1.04
2-11	2.0	17.0	2.01	8.64	16.0	1.92	6.17	5.09	9.97	1.16
2-21	2.5	18.1	2.10	8.62	18.0	1.42	7.22	5.22	12.2	1.24
3- 9	3.0	21.0	2.34	8.97	10.7	1.76	4.00	2.87	5.09	0.62
3-19	3.5	21.1	2.35	8.98	16.9	3.17	6.85	7.11	16.9	2.03
4- 7	4.0	21.6	2.41	8.96	10.9	1.24	3.93	3.28	4.96	0.50
4-17	4.5	21.2	2.47	8.58	15.7	1.71	6.19	3.69	7.49	0.89
4-21	4.8	21.6	2.47	8.89	14.3	1.51	5.13	2.97	5.76	0.67
9- 2	6.6	21.4	2.70	7.93	11.8	1.24	3.81	1.29	0.13	tr
11- 2	7.7	20.1	2.77	7.26	12.2	1.16	3.53	1.12	0.38	tr
14- 2	9.5	21.1	2.56	8.24	11.0	1.13	3.44	1.08	0.30	tr
17- 2	11.2	18.1	2.08	8.70	10.3	1.11	4.08	1.48	0.70	tr
18- 2	11.7	14.7	1.79	8.21	5.92	0.67	2.37	0.80	0.33	tr
21- 2	12.5	20.0	2.43	8.23	9.76	1.18	3.09	0.97	0.17	tr
23- 2	13.4	15.0	1.84	8.15	7.18	0.63	2.61	0.66	0.11	tr
25- 2	14.6	18.5	2.05	9.02	7.46	0.76	3.06	1.01	0.20	0.03
26- 2	15.2	17.3	1.89	9.15	7.42	1.12	3.58	1.17	0.44	tr
27- 2	15.8	18.8	2.04	9.22	7.22	1.41	3.50	1.93	4.07	0.23
28- 2	16.3	13.6	1.81	7.51	6.16	0.77	3.58	1.16	0.54	tr
31- 2	17.1	12.0	1.59	7.55	4.90	0.55	1.98	0.76	0.64	tr
33- 2	17.5	11.4	1.51	7.55	4.00	0.59	1.77	0.76	0.27	tr
35- 2	18.5	14.6	1.41	10.4	2.86	0.35	1.43	0.43	0.10	tr

5.4. DISCUSSION

Diatoms are one of the phytoplanktons which contritute to sedimentary

organic matter. Since their shells are preserved in water column and sediment, they are a possible indicator of primary production. Although other organisms should also contribute to organic matter in sediments, their skeletal debris are hardly recognized.

The stratigraphical distribution of diatom remains in the 0-20 m depth interval of a 200 m core sample indicates that Stephanodiscuss carconensis and Melosira solida were major species throughout the layers (Mori and Horie, 1975). As shown in Fig. 8 (p. 31), a good correlation is interpreted to exist between total carbon content and the number of diatom remains in the sediments. This suggests that organic matter in the sediments is largely authochthonous. This is consistent with the observation that the C/N ratios are low in the core samples (7.3-10.4, average value 8.5, Table 8). The C/N ratios are close to those for phytoplankton (e.g.,6.8, Ogura, 1975) rather than those for soil organic matter (e.g. 15, Ogura, 1975) or for soil humic acids (15.6, Ishiwatari, 1967).

The present study indicates that polyunsaturated fatty acids have been preserved in the sediment for a fairly long time (approximately 20,000 yrs B.P. at the depth of 16 m; Horie et al., 1971). This is consistent with the results for Black Sea sediments (Peake et al., 1974), which showed the presence of $C_{18:2}$ and $C_{18:3}$ acids at a depth of 3 m (ca. 13,000 yr BP; Ross and Degens, 1974).

As shown in Fig. 35, the ratios of unsaturated acids to saturated acids ($C_{18:1}/C_{18:0}$ and $C_{18:2}/C_{18:0}$) show maxima at depths of 0.2 m, 1-5 m, 11-12 m and 16-17 m. These features could be explained by changes in the input of polyunsaturated acids relative to saturated acids to the sediments or by changes which occurred during early diagenesis. Since mineral compositions are similar throughout the sediment core (see Table 1, p. 29), the sedimentary environment may have not varied appreciably during the last 20,000 yr. Therefore, the maxima of the ratio of polyunsaturated fatty acids to saturated ones (PU/S ratio) may be attributed to the increase in the relative contribution of the former acids to the sediments at the time of deposition. The following factors are suggested to explain the above phenomenon:

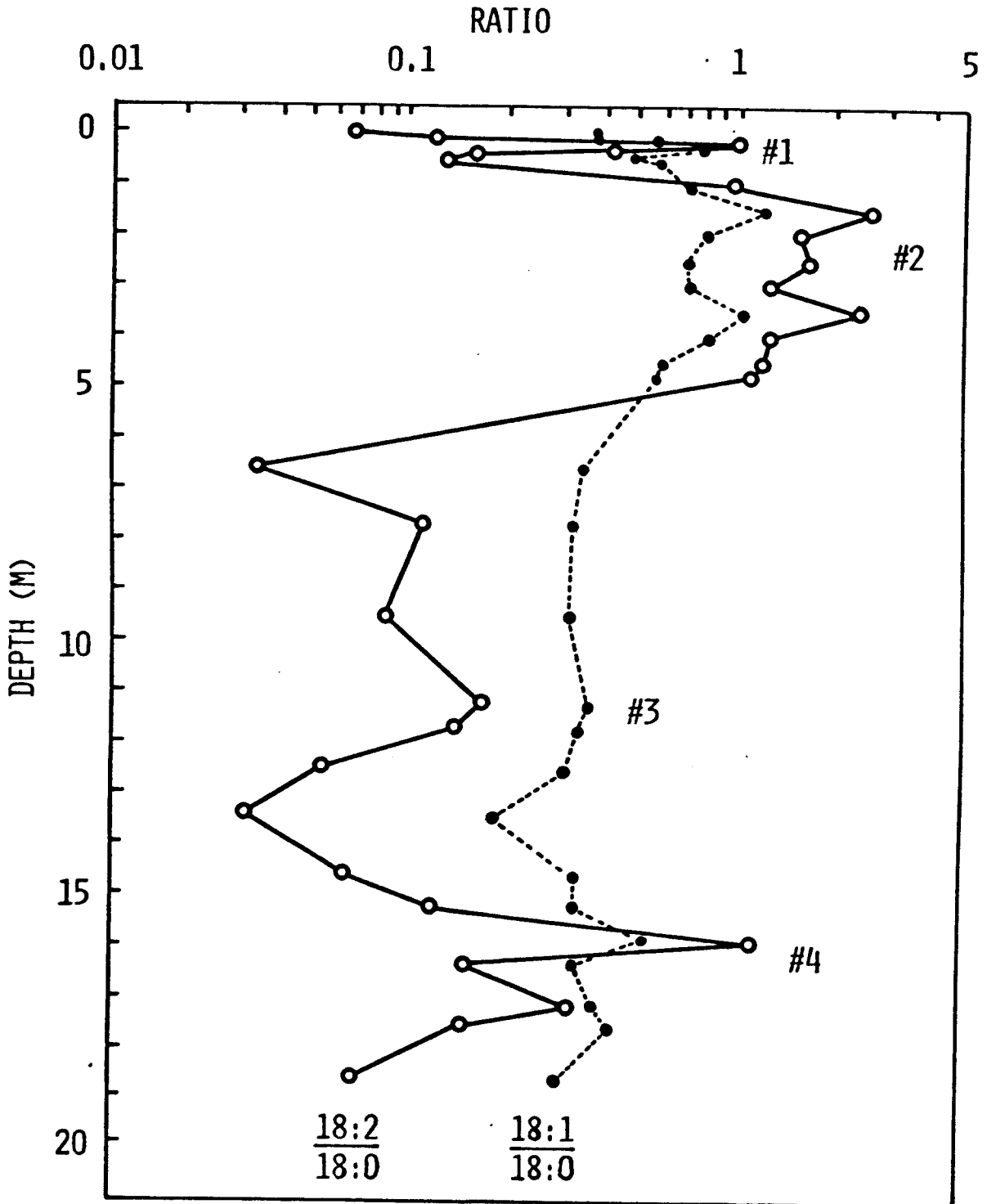(1) An increase in the concentrations of polyunsaturated fatty

Fig. 35 Vertical variation of the ratios ($C_{18:2}/C_{18:0}$ and $C_{18:1}/C_{18:0}$) of fatty acids in the upper 20 m sediments of Lake Biwa.

acids relative to those of saturated acids in aquatic organisms
may favor the above phenomenon,

and/or

(2) A decrease in the degradation rates of polyunsaturated fatty
acids relative to saturated acids in water and/or in surface
sediments may also favor the above phenomenon.

No obvious variation in fossil diatom composition was observed
throughout 20 m in depth (Mori and Horie, 1975). However, there is
evidence to indicate an increase of population of other organisms
during the past 20,000 yr. Kadota (1974) observed the vertical
distribution of animal microfossils in the same core, where animal
microfossils (Arthropoda Bosmina is most abundant) gradually decrease
from the surface to a depth of 17 m, exhibiting peaks in the sediments
at 5 m, 10.5 m and 15 m in depth. These sediments seemingly correspond
to sediments in which relatively high PU/S ratios were observed. On
this basis, it is possible that the increase in the PU/S ratios is
attributable to the increase in the population of zooplanktonic organisms
characterized by high concentration of polyunsaturated acids. If this
were the case, we could obtain the evidence of an increase in the
$C_{18:3\omega6}$ acid in those layers, because this acid is known to be present
specifically in animals (Fulco, 1974). However, since these tri-enoic
acid contents were too low to be quantified, we are unable to determine
at present whether or nor the increase in the PU/S ratios is due to an
increase in the populations of zooplanktons.

It is also probable that the polyunsaturated fatty acids in some
organisms became high at the particular time of the lake history. Based
on a incubation experiment of phytoplankton, Simith and Morris (1980)
claimed that lipid synthesis prevails over polysaccharide-metabolite
synthesis with decreasing temperature. Therefore, the concentration of
lipid containing polyunsaturated fatty acids in an organisms may increase
with decreasing temperature. It is also suspected that unsaturated fatty
acids in an organism tend to increase with decreasing temperature (Marr
and Ingraham, 1962; Holton et al., 1964). These compositional changes
have been interpreted by that orgamisms have a function to favor poly-

unsaturated fatty acids to maintain the membrane fludity at low temperatures (Sinensky, 1971; Fulco, 1974). In addition, according to Fukushima et al. (1976), the percentage of polyunsaturated acids ($C_{18:2}$ and $C_{18:3}$) in the total phospholipids of Tetrahymena Pyriforms increase from 39 % to 51 % with decreasing temperature from 39.5 °C to 15 °C. For the sunflower seed, the ratio of unsaturated C_{18} acid to saturated C_{18} acid was reported to increase by a factor of 3.5 with decrease in cultivated temperature from 15°C to 10°C (Harris and James, 1969).

Nutrient conditions can affect lipid content or fatty acid composition. Harris et al. (1965) reported that a rapid increase in the contents of $C_{18:2}$ and $C_{18:3}$ acids of Chlorella vulgaris was observed when C. vugaris was transfered from media containing organic carbon compounds to phosphate buffer (pH, 7.4) under illumination. However, there is no evidence at present to show that a drastic change of nutrient conditions have occurred in Lake Biwa for a long period.

The considerations of temperature effect on PU/S ratios are seemingly consistent with the results of Jeffries (1970). He found that lipid content in zooplankton and phytoplankton/microzooplankton in Narragansett Bay (Massachusetts) is higher in winter than in summer, and reported that the U/S ratios (mono- and poly-unsaturated fatty acids) are higher in winter to spring than in summer to fall.

Accordingly, it is likely that the lower the temperature of lake water in which phytoplanktons grow, the higher is the ratio of polyunsaturated to saturated fatty acids supplied to surface sediment. A possible increase in the population of zooplankton having abundant polyunsaturated fatty acids as mentioned above, may also be related to the decrease of lake water temperatures.

The degradation of polyunsaturated fatty acids which are less stable than saturated acids in the water column may be depressed by decreasing temperature and consequently this may favor for the preservation of unsaturated fatty acids. Thus, if polyunsaturated fatty acids or the PU/S ratios are preserved without change in sediments for a long period, we can estimate the temperature changes of lake water in the

past period based on changes in those ratios. However, the PU/S ratio is considered to decrease with depth since polyunsaturated acids are less stable than saturated ones. Consequently, the apparent amplitude of the variation of the PU/S ratio would be smaller than the true one.

As shown in Fig. 35, maxima in the PU/S ratio are observed in the layers of 0.2 m (#1), 1-5 m (#2), 11-12 m (#3) and 16-17 m (#4). The approximate ages of those layers are 200, 1000-4000, 15,000 and 20,000 yr BP according to the average sedimentation rate determination (Horie et al., 1971). If our inference on PU/S ratio is correct, the period mentioned above would have been cooler or colder than other ages.

Our inference seems to be supported by the palynological studies of Fuji (1976a) on the same core, where he estimated paleoclimatic conditions during the past 100,000 yr. Figure 36 shows a pollen diagram of boreal conifers which are Pinus haploxylon-type, Larix, Abies, Picea and Tsuga, and a vertical variation of PU/S ratios. These genera are
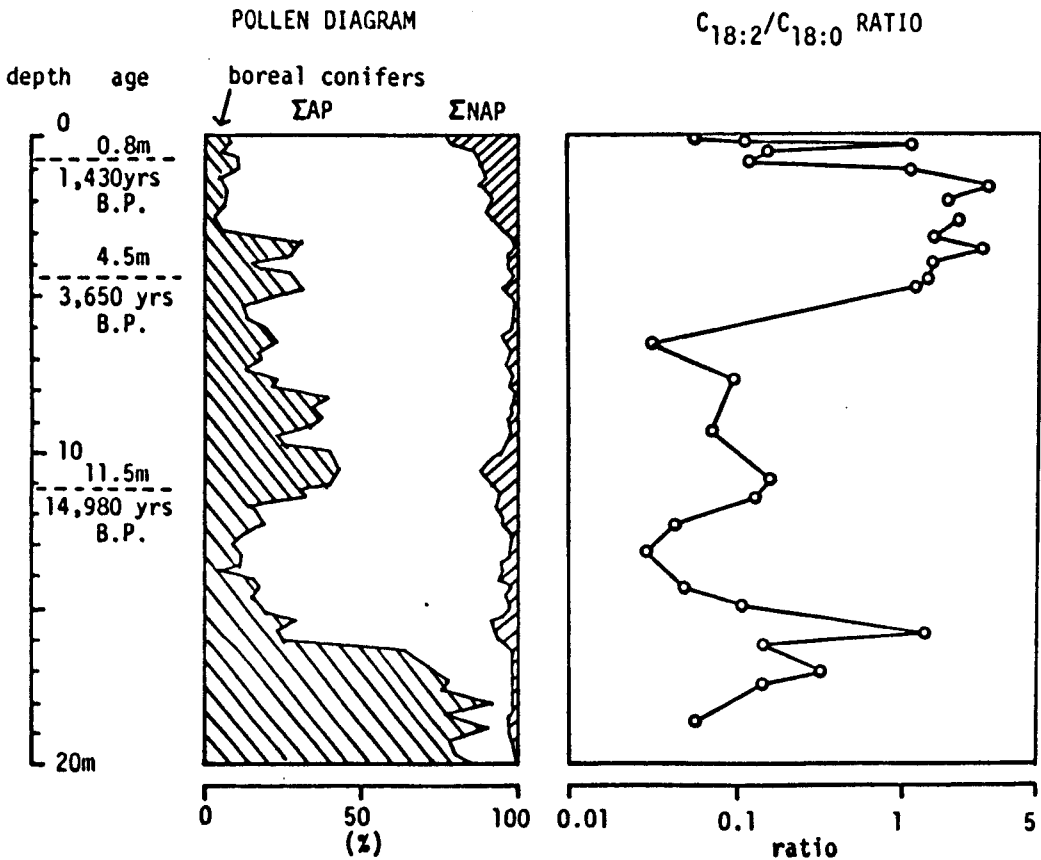

Fig. 36 A pollen diagram of boreal conifers and a vertical variation of $C_{18:2}/C_{18:0}$ ratios.

Table 9. Comparison of the paleoclimatic estimation basen on pollen analyses (Fuji, 1976a) and PU/S ratios.

zone[*]	depth (m)	pollen analyses[**]	PU/S ratios
Z-4-e	0 - 3.0	?	colder
Z-5-a	3.0- 4.5	cooler	colder
Z-5-b	4.5- 8.0	warmer	-
Z-6-a	8.0- 9.8	cooler	-
Z-6-b	9.8-12.1	colder	cooler
Z-6-c	12.1-16.3	cooler	-
Z-6-d	16.3-21.3	colder	colder

* Zonig is from Fuji (1976a).
** compared to the present climatic condition.

growing in the Subalpine or Subpolar zone, in which annual mean temperature is 2-6 °C (Fuji, 1976b). Table 9 shows a comparison of the paleoclimatic estimation based on pollen analyses and PU/S ratios. The estimations generally coincide. Good agreement is observed for the layers of #2 (3.0-4.5 m), #3 (9.8-12.1 m) and #4 (16-21 m). Although such agreement is not apparent for the layers at 8-9.8 m and 12-16 m in depth, a coincident trend is recognized in the times which correspond to the above layers, when it have been warmer than at the times correspond to the #3 and #4 layers. For the 0-3 m layer in which higher PU/S ratios were observed at 0.2 m and 1-3 m in depth, Fuji (1976a) did not give a paleoclimatic estimation because, in this layer, natural vegetation has been strongly affected by human activity (e.g. rice cultivation). In the layers deeper than 16-17 m in depth, the percentage of boreal conifer pollen is continuously high but PU/S ratios decrease with depth. This discrepancy might be due to the unstability of polyunsaturated acids.

Other evidence also seems to support our inference. Nakai and Shirai (1971) estimated the climatic changes by measuring ozygen isotopic ratios ($^{18}O/^{16}O$) of speleothem $CaCO_3$ from the Otaki cave (Gifu

Prefecture) near Lake Biwa. They claimed that cold ages were in the ages of 12,000 and 17,000 yr BP. These ages seem to correspond to #3 and #4 layers of Lake Biwa, in which PU/S ratios were higher and those times were considered to have been cooler or colder. They also claimed that a temperature maximum occuured in the age of 7000-8000 yr BP. These ages seem to correspond to 6-7 m layers of a sediment core in Lake Biwa, where PU/S ratios were very low and those times were considered to have been relatively warm or milder.

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CHAPTER 6

SUMMARY AND CONCLUSION OF PART I

[1] Total carbon comprised 0.84-2.2 % of the 200 m core sediment
samples taken from Lake Biwa. The total carbon contents showed
high values in the 1-15 m sediments. The high values were considered
to be due to an increase in primary production in the past.

[2] The concentrations of humic compounds are in the range of 1.75-8.80
mg/g dry sediment, which correspond to 9.2-28 % of total organic
matter. A good correlation was observed between the humic compounds
and the total catbon, suggesting that humic compounds are mostly of
autochthonous origin.

[3] The minerals (quartz, montmorillonite, illite, kaolinite or chlorite,
christbarite and feldspar) are present in the concentrations of 23-41
% of the dry sediments. Quartz is a major component, comprising
14-25 % of the dry sediments.

[4] The concentrations of normal saturated fatty acids in <u>unbound +</u>
<u>bound</u> fractions were in the range of 21.8-113 µg/g dry sediment.
They showed even carbon numbered predominance and bimodal distribution
with peaks at $n-C_{16}$ and $n-C_{22}-C_{28}$ acids. Fatty acids were also present
in the fractions obtained harsher saponification of the pre-saponified
sediment residue. Their concentrations ranged from 4.7 to 31.5 µg/g
dry sediment, and their distributions showed lower M.W. $(C_{12}-C_{19})$
predominance.

[5] <u>Unbound + bound</u> lower M.W. $(C_{12}-C_{19})$ fatty acids decreased
drastically in the upper sediment layers (0-20 m). More than 30 %
of the fatty acids which disappeared there was explained in terms of
α-, β- and ω-oxidative degradation. Thus, they converted into β- and
ω-hydroxy acids and α,ω-dicarboxylic acids. On the other hand, lower
M.W. fatty acids obtained by harsher saponification did not decrease
with depth, suggesting that they are more stable than the <u>unbound +</u>

bound fatty acids.

[6] Higher M.W. (C_{20}-C_{30}) fatty acids in unbound + bound fractions increased with depth in the 0-100 m sediment layers. This may be due to the increase in supply of these acids in the past, since the possibilities of the production of the fatty acids from the corresponding alcohols and of their release from the organic and/or inorganic matrices in sediments were inferred to be low by the laboratory heating experiments.

[7] A considerable amount of β-hydroxy acids was obtained by harsher saponification of the pre-saponified sediment residues. The author considered that they are of microbial origin and that the microbial activities are associated with the formation of geopolymers which exist in the sediment residue.

[8] C_{20}-C_{30} ω-hydroxy acids and α,ω-dicarboxylic acids in unbound + bound fractions showed vertical fluctuations. The author considered that their fluctuations were caused by the changes in their input to the sediment in the past , based on the good correlation among the C_{20}-C_{30} ω-hydroxy acids, α,ω-dicarboxylic acids and fatty acids in the 20-200 m sediment layers, which are all of allochthonous origin.

[9] The author proposed polyunsaturated fatty acid ($C_{18:2}$) as a possible indicator of paleoclimate. Based on the vertical changes in the $C_{18:2}/C_{18:0}$ ratio, the times of 200, 1000-4000, 15,000 and 20,000 yr BP were inferred to have been cooler or colder than other times. This hypothesis was supported by other evidence obtained from pollen analyses of the sediment core sample of Lake Biwa, and from oxygen isotopic analyses of speleothem $CaCO_3$ near Lake Biwa.

PART II

THERMAL ALTERATION EXPERIMENTS OF A RECENT SEDIMENT

CHAPTER 7

LABORATORY DIAGENESIS OF FATTY ACIDS IN SEDIMENTS:
CHANGES IN THEIR EXISTENCE FORMS UPON HEATING.

7.1. INTRODUCTION

Thermal alteration experiments of sedimentary organic matter
in laboratory conditions have been conducted by many workers (Douglas
and Eglinton, 1968; Eglinton, 1972; Ikan et al., 1975a, 1975b, 1975c;
Baedecker et al., 1977; Harrison, 1978; Sklarew, 1979). These
experiments have contributed for better understanding of diagenetic
processes of sedimentary organic matter and of their existing state.

Baedecker et al. (1977) reported that extractable fatty acids (FA's)
increased considerably when marine sediment samples were heated at
150°C for 64 days. Harrison (1978) obtained considerable amount of
extractable FA's on heating the lipid-extracted sediments. These
experimental results are of interest, because they indicate the
possibility of transformation of non-extractable FA's into extractable
FA's on heating. However, since only extractable FA's were analyzed
in the above experiments, the origin of FA's released on heating is
not clear.

The purpose of the present chapter is to trace the form of FA's
in a recent sediment sample on heating (120°C to 198°C for 48 hrs),
with a special attention to the behavior of bound FA's. Here, we
define the FA's extracted with common organic solvents to be "unbound
FA's" and the FA's extracted by saponification of pre-extracted
sediments to be "bound FA's" (Farrington and Quinn, 1971a). This
saponification procedure is more common method to separate the FA's
combined with organic and/or inorganic matrix in sediments (e.g.
Farrington et al., 1977; Ishiwatari et al., 1977a) than other methods,
e.g. acid hydrolysis (Cranwell, 1978) and organic solvent extraction

after HCl/HF treatment of sediment (Cranwell, 1977).

7.2. EXPERIMENTAL

A surface sediment sample was taken with an Eckman dredge near the center of Lake Biwa (Ie-1, see Fig. 1). The organic carbon content was 2.1 % and the clay mineral content was 31 % of the dry sediment (Kawamura et al., 1980). The sample was homogenized throughly by using a homogenizer (Nihonseiki, AM-1) and used for the thermal alteration experiment. A wet sediment sample (ca. 20 g; 60 % water content) was taken in a pyrex tube. After filling with N_2 gas, the tube was sealed and heated at 120°C to 198°C for 48 hrs.

Unbound and bound FA's in the unheated and heated samples were analyzed in the same way as stated in Chapter 1.

7.3. RESULTS

The following unbound and bound monocarboxylic acids were detected in the unheated and heated samples: C_{12}-C_{32} normal chain saturated FA's, C_{13}, C_{15} and C_{17} iso/anteiso branched chain FA's, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ unsaturated FA's. Qualitative data are shown in Table 10.

7.3.1. Normal saturated fatty acids

Figure 37 shows the molecular distributions of both unbound and bound normal saturated FA's of the unheated and heated samples. The distributions show a bimodal pattern with peaks around C_{16} acid and C_{22} or C_{24} acids. On heating, the concentration of bound lower molecular weight (C_{12}-C_{19}) fatty acids (LFA's) decreased considerably and that of bound higher molecular weight (C_{20}-C_{30}) fatty acids (HFA's) decreased slightly. On the other hand, the concentrations of both unbound LFA's and HFA's increased except for heating at 120°C.

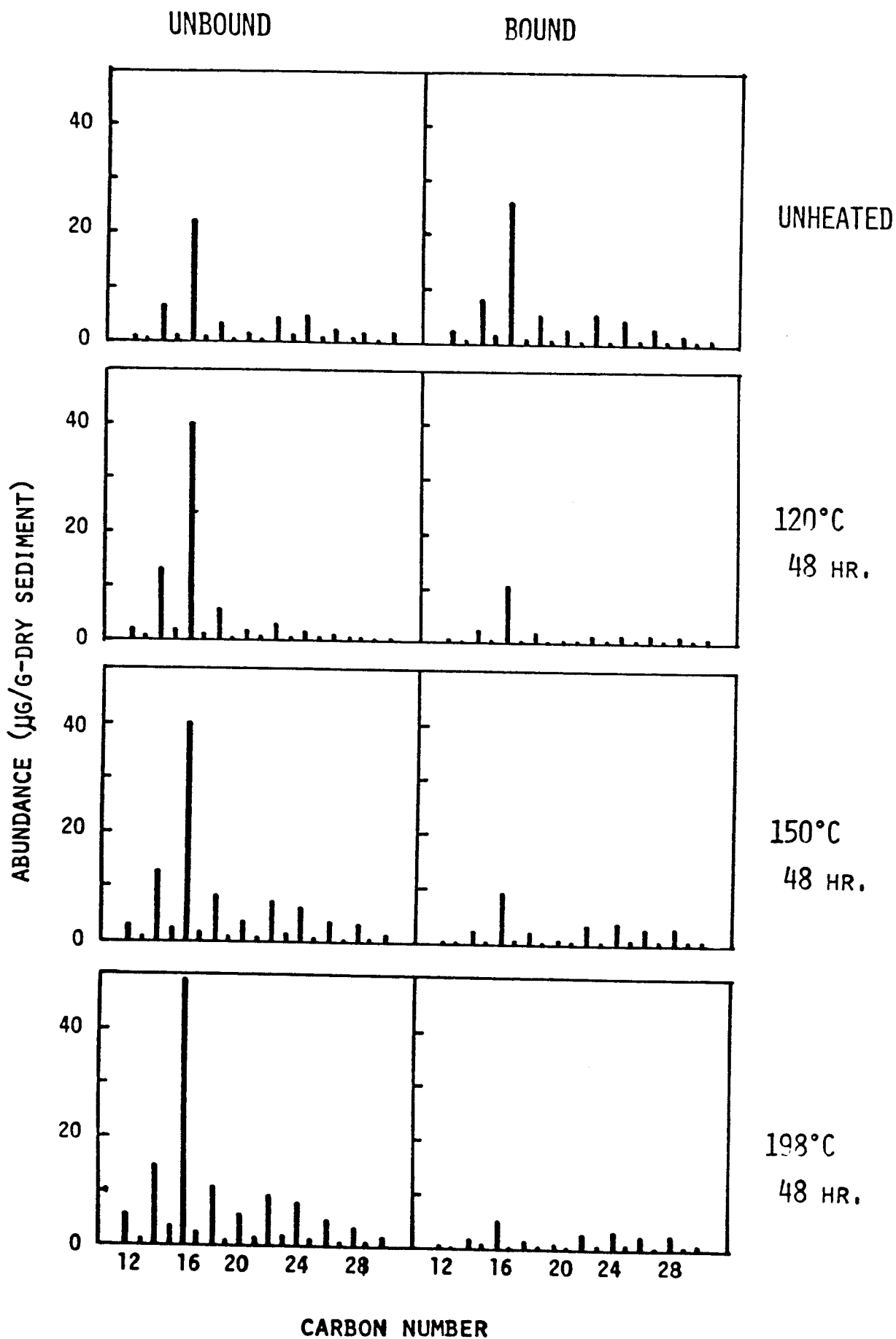As shown in Table 10, the total (unbound + bound) LFA's increased

Fig. 37 Molecular distribution of both unbound and bound
n-saturated fatty acids in the unheated and heated sediments.

Table 10. Concentrations of fatty acids in the unheated and heated sediment samples.

Fatty acids	Unheated			120°C, 48 hr.			150°C, 48 hr.			198°C, 48 hr. (μg/g dry sediment)		
	unbound	bound	total	unbound	bound	total	unbound	bound	total	unbound	bound	total
Saturated												
n-LFA (C_{12}-C_{19})	36.5	44.0	80.5	65.5	16.9	82.4	68.2	14.4	82.6	86.3	8.2	94.5
n-HFA (C_{20}-C_{30})	17.3	18.9	36.2	10.3	7.4	17.7	27.2	17.0	44.1	35.4	15.2	50.6
total	53.8	62.9	116.7	75.8	24.3	100.1	95.4	31.3	126.7	121.7	23.3	145.1
L/H ratio	2.1	2.3	2.2	6.4	2.3	4.7	2.5	0.85	1.9	2.4	0.54	1.9
CPI*(LFA)	12.5	16.3	14.3	14.1	18.2	14.8	13.1	20.8	14.0	10.8	12.4	11.0
CPI (HFA)	6.27	8.55	7.31	7.69	10.1	8.56	7.18	8.69	7.70	6.12	6.30	6.17
branched C_{13}	0.53	1.07	1.60	1.38	0.27	1.65	1.36	0.17	1.53	2.25	0.07	2.32
C_{15}	8.94	10.8	19.8	18.5	2.96	21.4	17.1	2.87	20.0	21.1	1.32	22.4
C_{17}	1.68	1.72	3.4	3.4	0.61	4.01	4.3	0.67	4.97	5.31	0.39	5.70
total	11.2	13.6	24.8	23.3	3.84	27.1	22.8	3.71	26.5	28.7	1.78	30.4
Unsaturated												
16:1	11.8	13.3	25.1	16.2	1.88	18.0	12.1	3.19	19.3	8.86	0.92	9.78
18:1	6.58	7.34	13.9	7.96	1.92	9.88	6.02	2.22	8.24	5.10	0.80	5.90
18:2	1.65	1.94	3.59	2.73	0.49	3.22	2.07	0.57	2.64	0.26	0.08	0.34
18:3	1.94	1.53	3.47	1.52	0.27	1.79	1.06	0.18	1.24	0.10	0.02	0.12
(16:0)	22.5	26.3	48.8	39.9	10.4	50.3	39.6	9.40	49.0	49.3	5.11	54.41
(18:0)	3.55	4.97	8.52	5.80	1.89	7.69	8.07	1.73	9.80	10.2	1.15	11.35

*CPI: The definition is given in the text.

gradually on heating, but the total HFA's first decreased at 120°C and then increased at higher temperatures. The latter phenomenon was confirmed by the later experiment (see Chapter 8). This phenomenon may be interpreted by that a portion of the HFA's originally present in unbound and bound forms was transformed into a non-extractable form when heated at 120°C, which then became extractable at the higher temperatures. The further explanation for this phenomenon can not be offered at present. Since CPI values [$(C_{12}$-$C_{18})_{even}/(C_{13}$-$C_{19})_{odd}$ for LFA's and $(C_{20}$-$C_{30})_{even}/(C_{21}$-$C_{29})_{odd}$ for HFA's] observed at 198°C are only slightly lower than those for the unheated sample, as shown in Table 10, chemical degradation of the unbound and bound FA's has not yet occurred notably at this temperature. This is also supported by the fact that total FA's did not decrease on heating at 198°C.

Behavior of LFA's : The amount of unbound LFA's in the unheated sample is 36.5 µg/g dry sediment. Upon heating at 120°C, their concentration increased to 65.5 µg/g dry sediment. With raising the temperature of heating from 150°C to 198°C, their concentration increased to 83.6 µg/g dry sediment, which corresponds to 2.4 times that of the initial sample.

In contrast to the behavior of unbound LFA's, the bound LFA's showed a drastic decrease from 44.0 µg/g dry sediment to 8.2 µg/g dry sediment with increasing the temperature to 198°C.

Behavior of HFA's : The unbound HFA's first decreased at 120°C and then increased with temperatures. Their amount at 198°C is 2.0 times that of the unheated sample. The behavior of the bound HFA's is more complicated than the unbound HFA's. Their total concentration showed a minimum at 120°C and then increased at 150°C, but the heating at 198°C did not produce more amount of bound HFA's than at 150°C.

7.3.2. Branched fatty acids .

As shown in Table 10, iso/anteiso C_{15} fatty acid was the most abundant in the branched FA's in the unheated and heated samples. Being similar to the behavior of normal LFA's, the unbound branched FA's increased and the bound ones decreased with increasing the temperature of heating.

7.3.3. Unsaturated fatty acids

The concentrations of both unbound and bound unsaturated FA's decreased generally on heating, except for an increase in the concentrations of unbound $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ acids in the sample heated at 120°C. The ratios of unsaturated to saturated FA's decreased on heating, suggesting that unsaturated fatty acids are thermally more labile compounds than saturated ones. The decreasing rate of unsaturated FA's with temperature is in the order: $C_{16:1} \simeq C_{18:1} < C_{18:2} < C_{18:3}$.

The increase of unbound FA's except for $C_{18:3}$ acid at 120°C suggests that the unbound FA's were formed from the bound ones. Bound $C_{16:1}$ acid decreased at 120°C and then increased slightly at 150°C (see

Table 10). This may be explained by that a part of bound $C_{16:1}$ acid changed into the residual form (see Discussion Section) at 120°C and then they returned partly to the original (bound) form on further heating.

7.4. DISCUSSION

7.4.1. Behavior of FA's

The present study showed that heating wet sediment at relatively low temperatures (150°C-198°C) produced 2.0 to 2.4-fold increase of unbound LFA's and HFA's, relative to the amount present in the unheated sediment. This is consistent with the results of Baedecker et al. (1977) and Harrison (1978). In the study of Baedecker et al. (1977), the amount of unbound n-FA's obtained by heating wet or dry sediment at 150°C for 64 days was approximately 12 times that of the unheated sample (1.7 ug/g dry sediment). They considered that the unbound n-FA's obtained from thermally treated sediments came from sediment surfaces or by cleavage of complexes of fatty acids with humic compounds. Harrison (1978) found that the amount of unbound FA's became about 1.6 to 1.9 times that (2.8 µg/g dry sediment) of the unheated sediment, when dried estuarine sediment was heated at 160°C and 240°C for 50 hrs. The results have been interpreted by that FA's incorporated intact in the kerogen matrix were liberated under thermal conditions.

On the basis of the present study, it is likely that there are three forms of FA's in sediments: (1) unbound form, (2) bound form and (3) residual form. "Residual form" means the FA's which can not be recovered by saponification of the unheated sediment but become extractable only after the thermal treatment. We have estimated the amount of the FA's transformed from bound and residual forms into unbound form, as shown in Table 11. The amount of unbound FA's generated from bound FA's was calculated subtracting the amount of bound FA's of a heated sediment from that of the unheated sediment. The amount of unbound FA's generated from residual form was estimated from the difference in the total amount of FA's between heated and unheated sediments.

Table 11. Estimates of possible sources of unbound fatty acids generated on heating wet sediment at 150°C and 198°C.

Temperature of heating	150°C			198°C		
Possible source*	Bound form	Residual form	Total	Bound form	Residual form	Total
	(μg/g	dry sediment)		(μg/g	dry sediment)	
LFA's	29.6 (94%)**	2.1 (6%)	31.7	35.8 (72%)	14.0 (18%)	49.8
HFA's	1.9 (19%)	7.9 (81%)	9.8	3.7 (21%)	14.4 (79%)	17.7
Total FA's	31.5 (76%)	10.0 (24%)	41.5	39.5 (58%)	28.4 (42%)	67.9
L/H ratio	15.6	0.26	3.2	9.7	1.0	2.8
Branched FA's	9.8 (85%)	1.7 (15%)	11.5	11.8 (68%)	5.6 (32%)	17.4

* Deifinition of bound and residual forms and the procedure of estimation are given in the text.
**of the total (Bound + Residual).

As clearly shown in Table 11, 76 % (at 150°C) and 58 % (at 198°C) of the unbound FA's generated on heating have come from the bound FA's. When examined in more details, we can point out the great difference in the behavior on heating between LFA's and HFA's. On heating at 150°C, for example, 94 % of LFA's liberated to unbound form comes from bound LFA's whereas only 19 % of the total HFA's is transformed from bound form to unbound form and most of unbound HFA's are derived from residual HFA's. The results of heating at 198°C are essentially similar to those at 150°C. The difference in the behavior on heating between LFA's and HFA's may reflect the difference in their existing state which is closely related with their origin. FA's in bound form may consist of FA's present in non-extractable lipids and/or trapped in or linked via ester or other bonds to inorganic and/or organic matrix (Farrington and Quinn, 1973; Farrington et al., 1977). FA's in residual form may exist in the sites in orgainic and/or inorganic matrix where saponification reagent can not act, or they are tightly linked with

organic and/or inorganic matrix (Shnitzer and Neyround, 1975). It
seems reasonable that most of unbound LFA's generated on heating
originate in bound LFA's, because bound LFA's are mainly of autochtho-
nous origin and are considered to be mostly present in dead cells and
sediment surface (Cranwell, 1979). Consequently, they are easily
released on heating. A possible interpretation for that most of unbound
HFA's generated on heating come from residual HFA's is as follows.
HFA's are mainly allochthonous and derived from higher plants (Kvenvolden,
1967; Brooks et al., 1976; Cranwell, 1974, 1978; Ishiwatari and Hanya,
1973; Ishiwatari et al., 1980; Matsuda and Koyama, 1977). They may have
been subjected to microbial attack at the soil surface. As a results,
a part of unbound + bound HFA's originally present in soil may have
been lost or converted into the residual form prior to reaching the
sediment.

7.4.2. Geochemical implication

Fatty acids are believed to be one of the most important precursors
of hydrocarbons in petroleum. Therefore, it is quite important to
determine the absolute amount of FA's in sediments and sedimentary rocks
in order to draw the picture of transformation of FA's in sediments to
petroleum hydrocarbons. FA's in sediments have been quantified by
many workers (e.g. Parker and Leo, 1965; Farrington and Quinn, 1971b,
1973; Farrington et al., 1977; Brown et al., 1972; Boon et al., 1975;
Ishiwatari et al., 1980). Reported data of unbound and bound FA's in
sediments show that a quite large percentage of the FA's are in the
bound fraction, that is, 32-65 % for Narragansett Bay (Farrington and
Quinn, 1971a), 65-71 % for Buzzards Bay core (Farrington et al., 1977),
14-71 % for Lake Haruna sediments (Ishiwatari et al., 1977a) and 54 %
for this lake. If our statement of the presence of FA's with residual
form is correct, bound plus residual FA's account for a larger percentage
of FA's in sediments than that previously determined. This indicates
the importance of quantifying bound and residual FA's in sediments.
Since our method for estimating residual FA's is only tentative, further
work must be done to develop the method (determination after thermal

treatment) and/or establish a new method.

Figure 38 gives the relative percentage of three forms of FA's from the unheated and heated (at 198°C) sediments. Under the thermal conditions of this study (below 200°C), hydrocarbons are not generated in a large amount (Shimoyama and Johns, 1971; Ishiwatari et al., 1977b). Therefore, if the changes in existence forms of FA's which were observed in this study take place actually in natural condition, we may observed such changes in sedimentary rocks at the stage when hydrocarbons are not yet generated extensively.

UNHEATED   198°C

LFA   U   B   14.8   U   B
        38·6 46·6   R   91·3   8·7

HFA   U   B   U   B
        34·2 37·4   R   70·0   30·0
              28·4
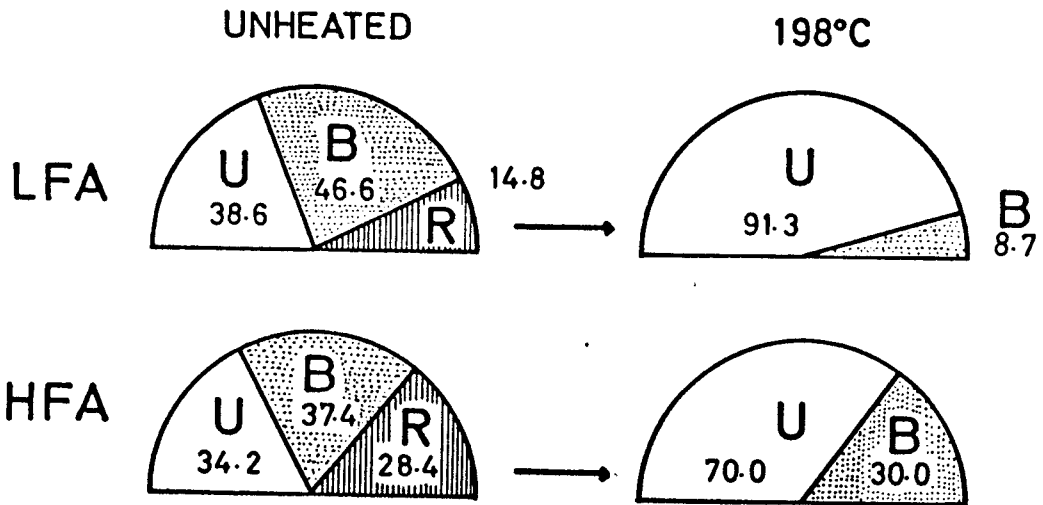
Fig. 38 Changes in the existence forms of fatty acids on heating at 198°C. U: unbound fatty acids, B: bound fatty acids, R: residual fatty acids.

7.5. REFERENCES

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CHAPTER 8

LABORATORY DIAGENESIS OF MONO- AND DI-CARBOXYLIC ACIDS
AND HYDROXY ACIDS IN SEDIMENTS

8.1. INTRODUCTION

In Chapter 7, a thermal alteration experiment of surface sediment
was conducted and the changes in the existence forms of fatty acids
was proposed to occur in the sediment upon heating. However, more detail
experiments were needed to be conducted in order to interpret the
vertical distributions of fatty acids and related compounds in a 200 m
core, and to predict their fates in the deeper sediments.

In previous thermal alteration experiments, the conditions such
as temperature and time have been limitted (Baedecker et al., 1977;
Harrison, 1978). In addition, dicarboxylic acids and hydroxy acids have
not been studied yet in heated sediment samples. It is of interest
to trace these compounds together with fatty acids.

The purpose of the study in this chapter is to trace the behavior
of fatty acids and the related compounds in sediments heated at various
conditions.

8.1. EXPERIMENTAL

A surface sediment taken from the bottom of Lake Biwa (Location
No., Ie-1) was used in this study. The sediment was throughly mixed
by a homogenizer (Nihonseiki, AM-1). About 5 or 10 g of the wet
sample was taken in a pyrex tube (12 mm x 15 cm). After filling with
nitrogen gas, the tube was sealed and subjected to thermal alteration
experiments.

Experiment 1

The sets of samples were heated at conditions of 68, 88, 112, 129, 140, 154, 179, 204, 254, 279, 300 and 325°C for 24 hours. The unheated and heated samples were saponified by 50 ml 0.5 N methanolic KOH (containing 5 % water) for 2 hours. The alkaline solution, which contains unbound + bound fractions, was separated by filtration and concentrated to about 5 ml. Mono- and dicarboxylic acids and hydroxy acids in the solutions were separated and analyzed as described in Chapter 1.

Experiment 2

The pre-extracted sediment residues, from which unbound + bound fractions were removed, were subjected to elemental analysis and alkaline permanganate oxidation after dryness of the samples at 105°C for one hour. Total carbon and nitrogen contents in the residues were measured as stated in Chapter 1. The 0.50 g of the sediment residue was oxidized with 10 ml $KMnO_4$/1 % KOH solution at 60°C for 2 hrs in an incubator (Taiyo, M-100). These conditions were determined based on the examination, which is shown in Appendix at the end of this chapter. The organic acids in the oxidation products were separated by extraction with ethyl acetate (Machihara and Ishiwatari, 1980) and methylated with 14 % BF_3/methanol. The methyl esters were analyzed with GC, as described in Chapter 1.

Experiment 3

Sets of samples were heated at 65°C and 83°C for 1, 2, 5, 10, 22, 54, 114, and 248 days. The heated samples were analyzed for fatty acids in a same way as stated in Chapter 1.

8.3. RESULTS

C_{12}-C_{32} n-Saturated fatty acids, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ unsaturated fatty acids, C_{12}-C_{24} β-hydroxy acids, C_{12}-C_{30} ω-hydroxy acids, C_{24}, C_{26}, C_{28} and C_{30} ω-1 hydroxy acids and C_9-C_{30} α,ω-dicarboxylic acids were detected in the unheated and heated sediment samples.

The distributions of these compounds are characteized as follows.

Fatty acids,ω-hydroxy acids, α,ω-dicarboxylic acids showed bimodal distribution with peaks at C_{16} and C_{22} in the samples. They all showed even carbon numbered predominance, which seems to be more marked in the order: ω-hydroxy acids $>$ fatty acids $>$ α,ω-dicarboxylic acids. β-Hydroxy acids showed an unimodal distribution with a peak at C_{16}. The odd carbon numbered acids were,for the most part, iso/anteiso branched chain. ω-1 Hydroxy acids seem to be characterized by an unimodal distribution with a peak at C_{26} or C_{28} and an even carbon numbered predominance.

The concentrations of these compounds changed depending on temperature and time.

8.3.1. Experiment 1

Table 12 shows the concentrations of saturated and unsaturated fatty acids, β-, ω- and ω-1 hydroxy acids and α,ω-dicarboxylic acids in the unheated and heated sediment samples at 68°C to 325°C for 24 hrs.

Table 12. Analytical results of saturated and unsaturated fatty acids, β-, ω- and ω-1 hydroxy acids and α,ω-dicarboxylic acids in the unheated and heated sediment samples at 68°C to 325°C for 24 hrs.

Temp.(°C)	LFA	HFA	UFA	β-OH	L-ωOH	H-ωOH	(ω-1)-OH	(μg g^{-1} dry sediment) L-diacids	H-diacids
Unheated	88.45	93.7	28.2	17.2	12.0	6.2	1.57	5.81	9.11
68	82.2	81.2	-*	7.4	-	-	-	-	-
88	66.6	45.9	-	19.4	-	-	-	-	-
112	87.5	55.0	-	21.4	-	-	-	-	-
129	96.4	82.4	25.8	30.7	12.5	9.5	3.63	6.03	10.26
140	87.7	121.4	-	32.0	-	-	-	-	-
154	97.8	103.2	21.3	53.5	11.8	11.4	6.39	6.67	10.18
179	117.3	121.1	22.2	37.0	10.9	13.8	8.48	6.59	10.41
204	111.0	113.6	12.9	6.4	11.4	10.5	5.10	9.06	14.87
229	111.2	123.9	13.7	6.1	17.8	5.7	1.67	11.35	22.28
254	113.6	121.9	0.9	3.3	8.6	1.7	tr	10.90	23.01
279	115.0	117.5	tr**	2.7	8.4	1.9	nd	12.41	24.13
300	135.8	130.9	nd***	1.1	4.2	1.3	nd	11.81	17.67
325	140.4	125.9	nd	0.6	1.3	0.4	nd	10.54	19.29

* Not analyzed, ** Trace, *** Not detected

Figure 39 shows changes in the concentrations of n-saturated fatty acids upon heating at 68-325°C for 24 hours. Both LFA's $(C_{12}-C_{19})$ and HFA's $(C_{20}-C_{30})$ firstly decreased at the temperatures of 68-88°C and then increased at higher temperatures. Their concentrations became larger than the initial concentration (unheated) at 129°C for LFA's and at 140°C for HFA's. They increased with temperature and became 1.6 times (for LFA's) and 1.3 times (for HFA's) larger than the initial concentrations at 325°C. Although these results are generally consistent with the previous results (see Chapter 7), this experiment clearly shows a decrease of both LFA's and HFA's upon heating at lower temperatures.
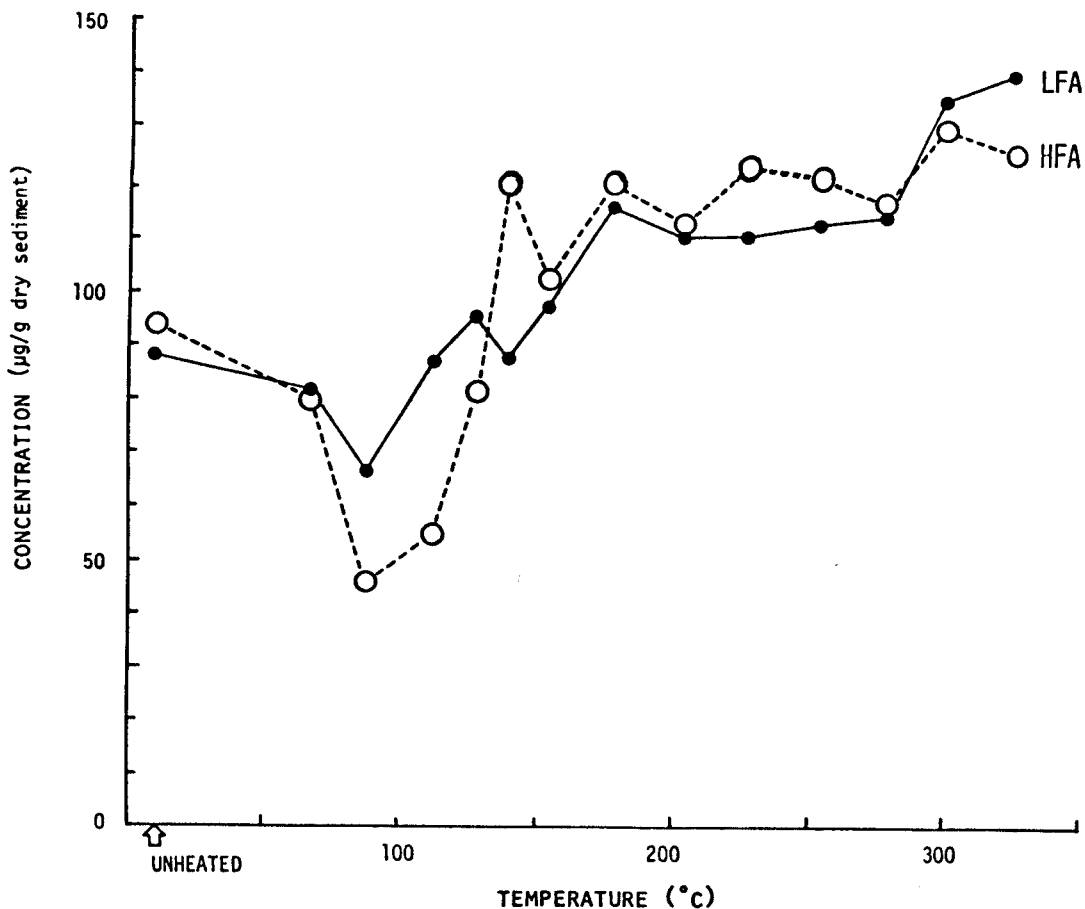
Fig. 39 Changes in the concentrations of fatty acids upon heating at different temperatures for 24 hours.

Figure 40 shows the concentrations of unsaturated fatty acids
upon heating. Unsaturated fatty acids decreased with depth and dis-
appeared at the temperatures higher than 200°C. Mono-, di- and tri-
enoic acids disappeared at 279°C, 229°C and 204°C, respectively.
This indicates that unsaturated acids with higher degree of unsaturation
are less stable.
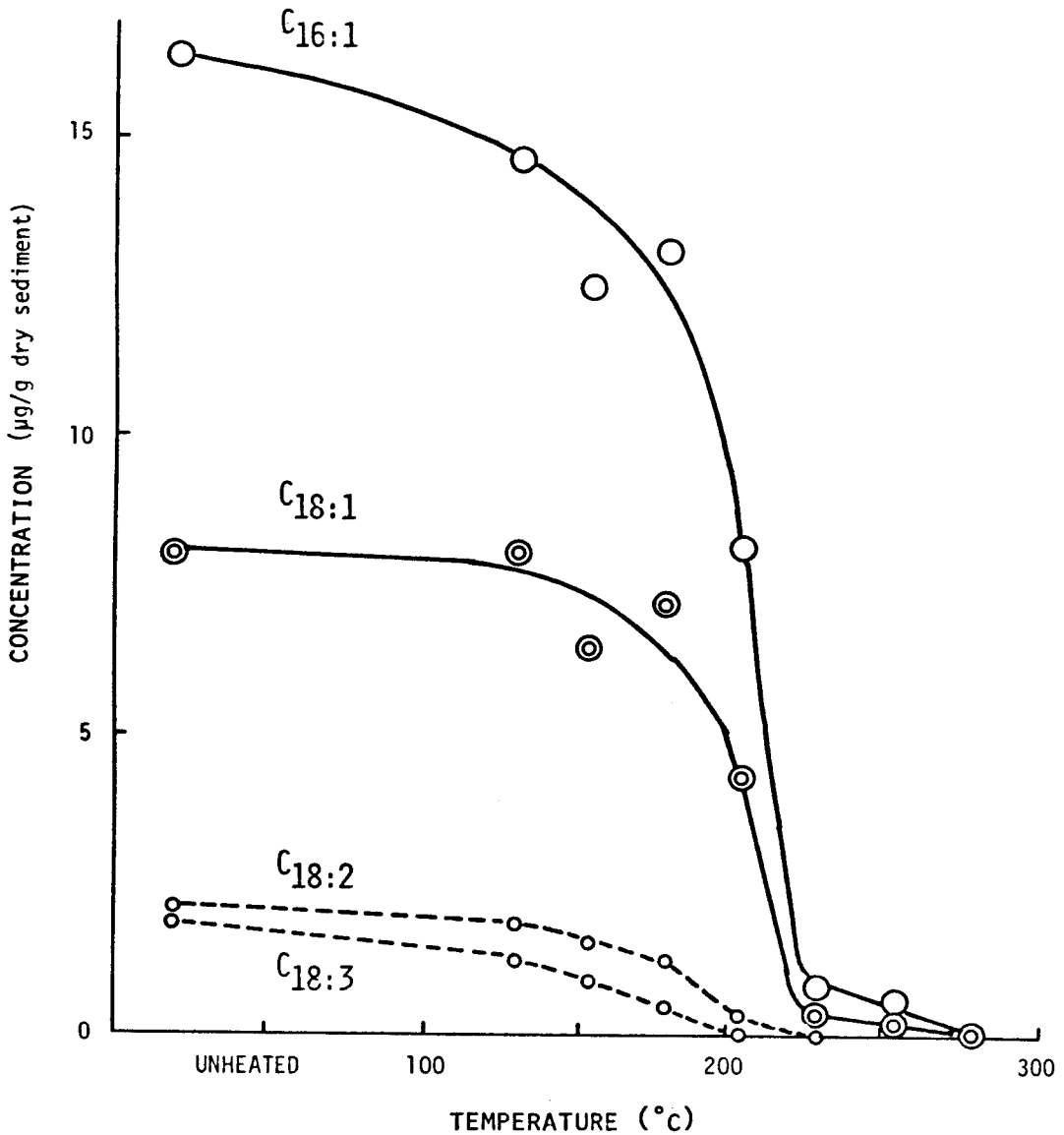
Fig. 40 Concentrations of unsaturated fatty acids in the unheated
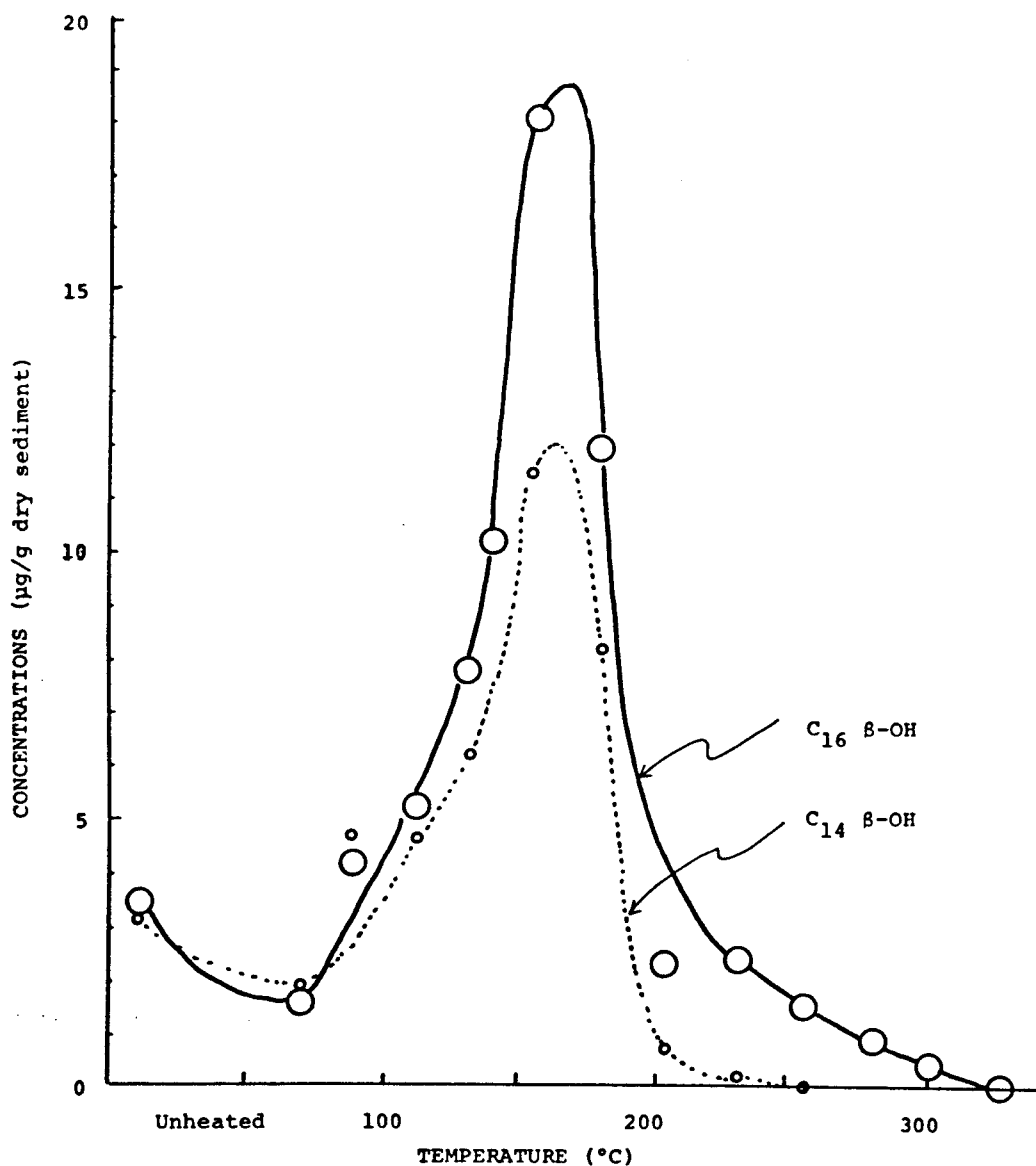and heated sediment samples.

Fig. 41 Changes in the concentrations of C_{14} and C_{16} β-hydroxy acids upon heating.

β-Hydroxy acids increased and showed maximum concentrations at 154°C, which are higher than the initial amounts (unheated) by a factor of three, and then they decreased drastically at higher temperatures. Figure 41 shows changes in the concentrations of C_{14} and C_{16} β-hydroxy acids in the unheated and heated sediment samples. These hydroxy aicds seemed to decrease at 68°C and then largely increased. The increasing rate of the C_{16} hydroxy acid is 410 % at 154°C.

Lower M.W. ω-hydroxy acids (C_{12}-C_{18}) appeared to decrease gradually
with tempearture, except for the heating at 229°C, and showed the
lowest concentration at 325°C, which is 10 times smaller than the
initial concentration. On the other hand, higher M.W. ω-hydroxy acids
(C_{20}-C_{30}) increased with temperature and showed maximum concentration at
179°C, which is ca. two times larger than that of unheated sample.
At higher temperatures, these hydroxy acids decreased and almost
disappeared at 325°C (see Table 12 and Fig. 42).

Figure 42 shows changes in the concentrations of higher M.W. (C_{22}-
C_{28}) ω-hydroxy acids, ω-1 hydroxy acids and α,ω-dicarboxylic acids
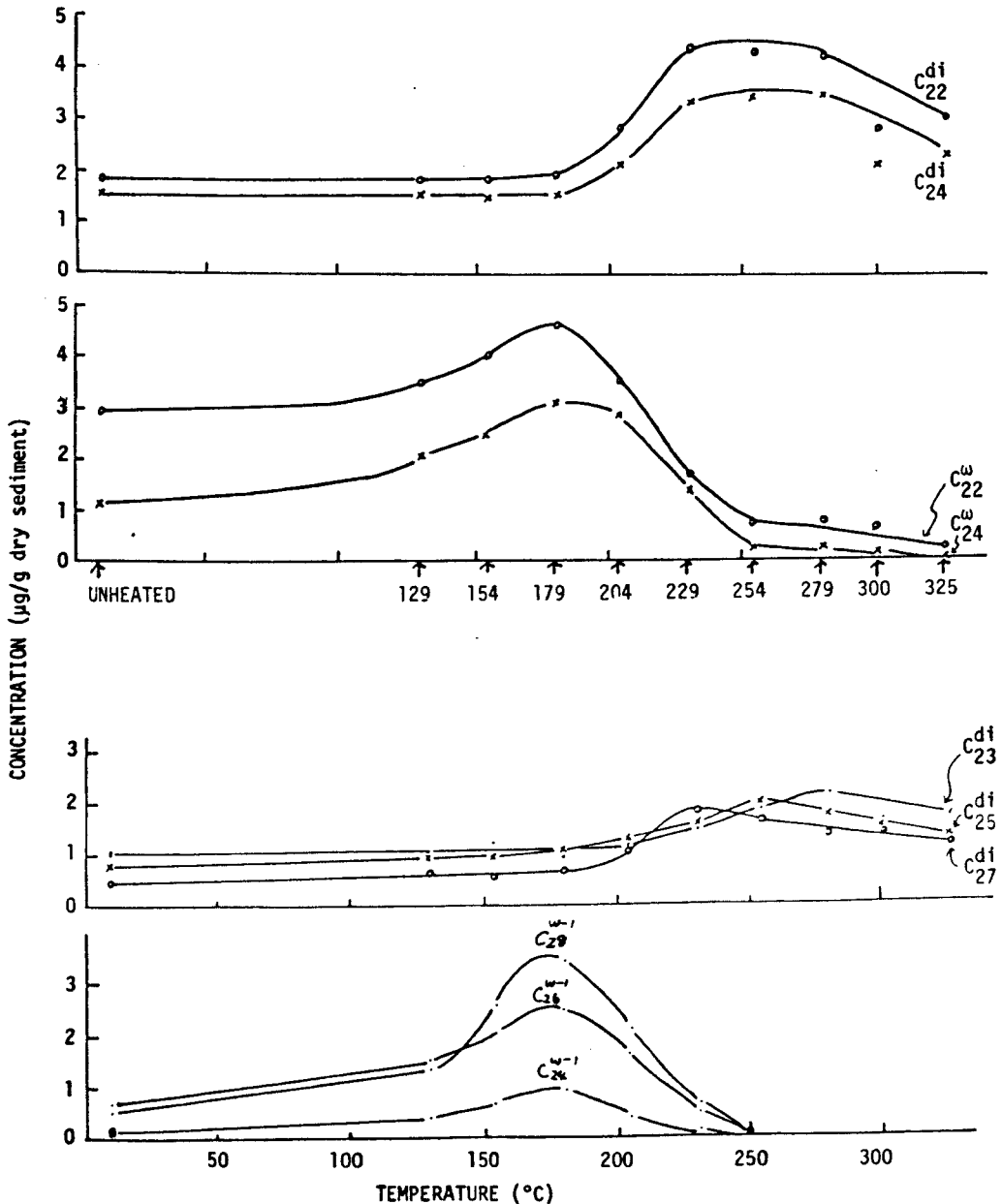
Fig. 42 Changes in the concentrations of ω- and (ω-1)-hydroxy
acids and dicarboxylic acids in the unheated and heated sediments.

upon heating. ω-1 Hydroxy acids showed a behavior similar to that of C_{20}-C_{30} ω-hydroxy acids: the concentration increased with temperature and became 5 times larger than initial concentration. At higher temperatures, these hydroxy acids decreased drastically and disappeared at 279°C.

α,ω-Dicarboxylic acids increased at the temperatures higher than 204°C and showed maximum concentration at 279°C, which is 2.5 times larger than initial concentration. At the temperatures higher than 300°C, they seemed to decrease, as shown in Fig. 43.
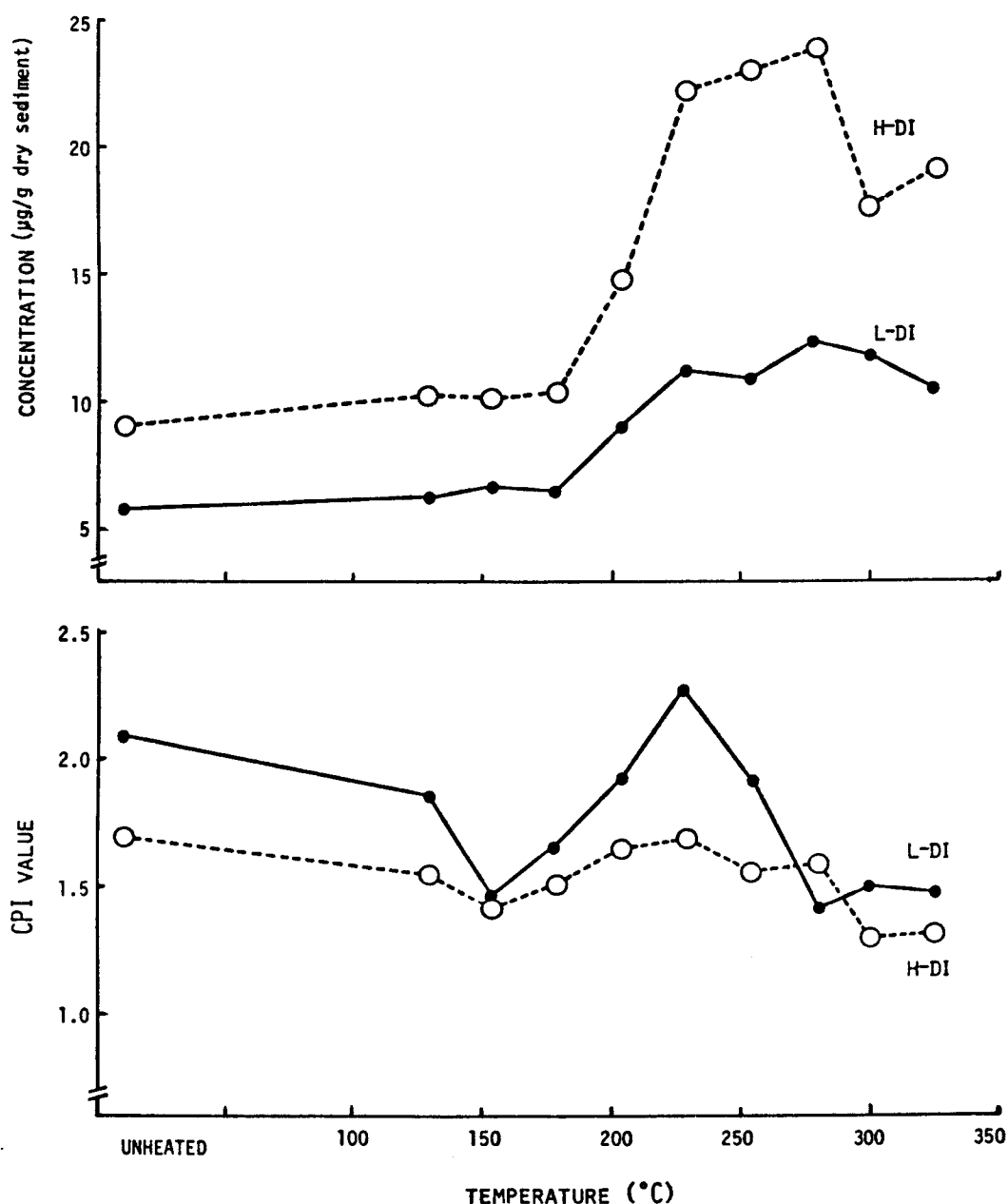
Fig. 43 Changes in the concentration of dicarboxylic acids in the unheated and heated sediments and their CPI values.

Table 13 Elemental composition of the extracted residue and
α,ω-dicarboxylic acids in the oxidation products of the extrac-
ted residue of the unheated and heated sediments at 68°C to 325
°C for 24 hrs.

Temp. (°C)	Carbon	Nitrogen	C/N	α,ω-dicarboxylic acids* (C_7-C_{20})	(C_7-C_9)
Unheated	16.9	1.93	8.76	206.4	110.7
68	17.0	2.03	8.38	-	-
88	17.1	1.93	8.83	181.5	94.2
112	17.3	1.94	8.90	-	-
129	-	-	-	276.7	153.2
140	15.7	1.64	9.60	-	-
154	13.9	1.10	12.6	257.2	149.1
179	14.2	1.07	13.3	298.5	151.5
204	13.2	0.94	14.0	262.2	152.8
229	13.0	0.90	14.4	-	-
254	14.1	0.99	14.2	219.8	117.7
279	14.2	0.97	14.6	-	-
300	11.7	0.91	12.9	115.3	59.8
325	10.2	0.94	10.9	-	-

* $\mu g \ g^{-1}$ dry sediment

8.3.2. Experiment 2

Table 13 shows the analytical results of elemental carbon and
nitrogen in the pre-extracted sediment samples, and α,ω-dicarboxylic
acids in the oxidation products of the samples. Figure 44 shows the
changes in carbon and nitrogen contents and C/N ratios upon heating.
Carbon and nitrogen contents decreased with temperature and became
60 % and 43 % of the initial contents (unheated) at 325°C, respectively.
The C/N ratio, which was 8.76 for unheated sample, increased with
temperature and showed maximum (14.6) at 197°C and then decreased at
higher temperatures. This indicates that nitrogen containing compounds
such as amino acids in the pre-extracted samples are likely released

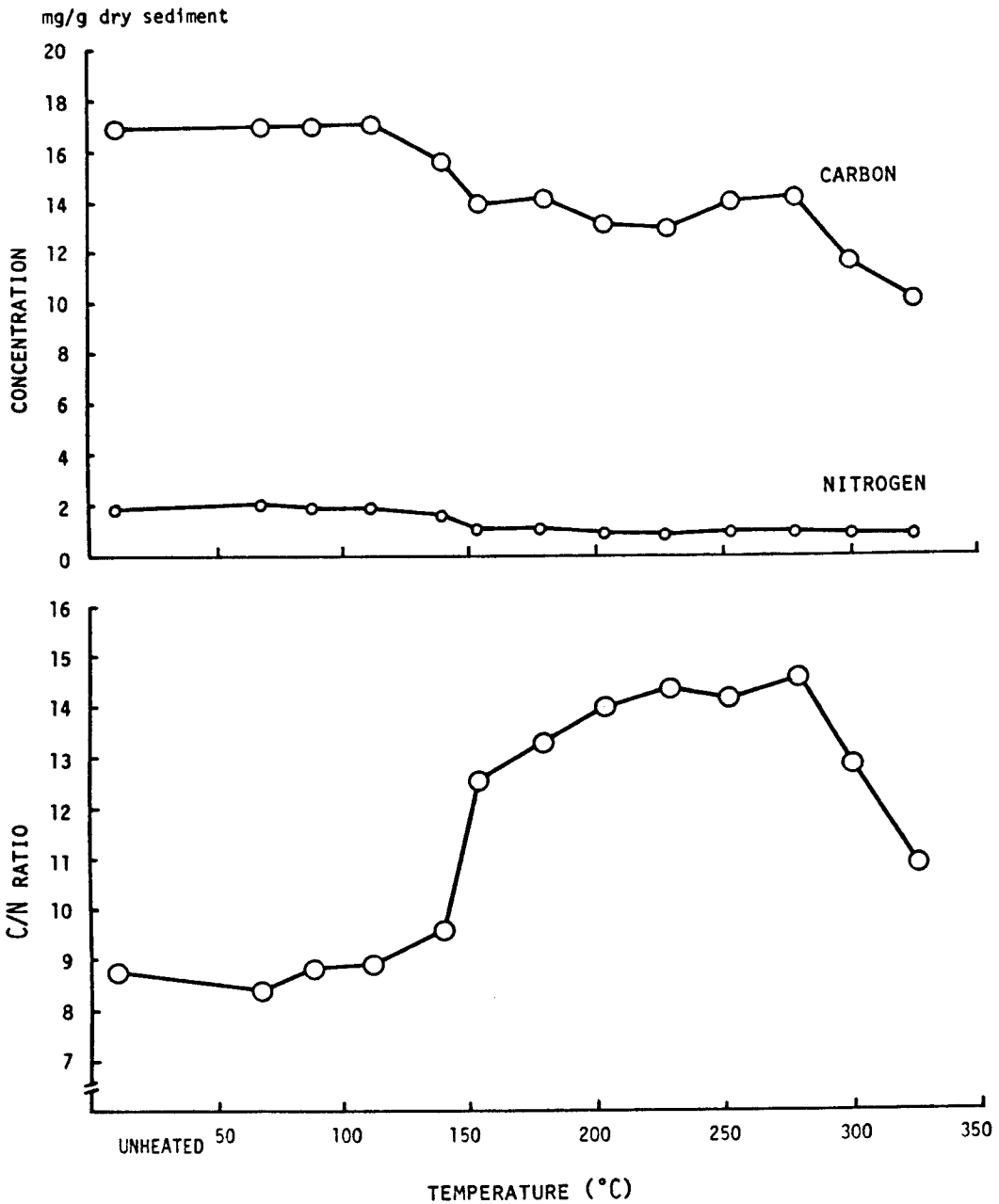than carbon rich compounds at the temperatures of 140°C to 279°C.

Fig.44 Change in carbon and nitrogen content and C/N ratio in
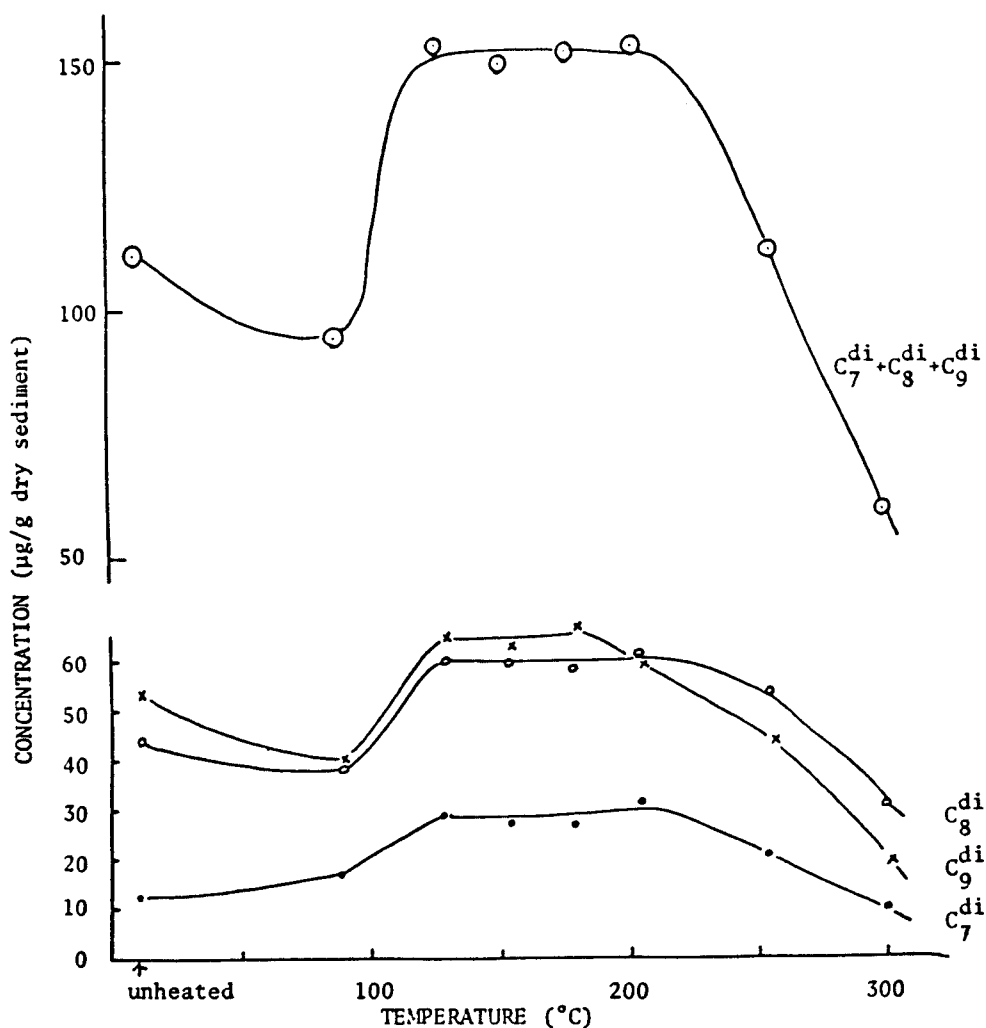the heated sediments at 68-325°C for 24 hrs.

Fig. 45 Concentrations of α,ω-dicarboxylic acids in the oxidation products of sediment residues of the heated samples.

As shown in Fig. 45, the concentrations of α,ω-dicarboxylic acids in the oxidative degradation products increased at 129°C to 204°C. The increasing rates for C_7-C_9 dicarboxylic acids were 35-38 % of initial amount (unheated). But, at higher temperatures, the concentrations decreased drastically and became 54 % of initial amount at 325°C.

8.3.3. Experiment 3

Table 14 shows the analytical results of fatty acids in the unheated and heated sediment samples at 65°C and 83°C for one day to 248 days.

Table 14   The analytical results of fatty acids in the unheated and heated sediments at 65°C and 83°C for 1-248 days.

Temp. (°C)	Fatty acids	Unheated	Time (day) ($\mu g \ g^{-1}$ dry sediment)							
			1	2	5	10	22	54	114	248
65°C	ns-LFA*	100.1	73.3	57.4	65.5	82.3	91.6	108.1	82.6	112.9
	ns-HFA**	123.5	107.9	60.3	98.1	85.6	84.9	82.8	32.7	58.8
	total***	223.6	181.6	117.7	163.6	167.9	176.5	190.9	115.3	171.7
	$C_{16:1}$	25.1	29.2	28.5	26.3	28.5	25.8	34.1	26.9	30.4
	$C_{18:1}$	13.9	13.8	12.9	14.4	13.7	13.9	17.8	12.0	16.0
	$C_{18:2}$	3.59	3.20	3.20	3.74	2.80	4.03	4.62	3.61	4.45
	$C_{18:3}$	3.47	2.46	2.23	2.53	2.40	3.02	3.08	2.41	2.74
83°C	ns-LFA	100.1	90.3	-****	91.0	92.5	88.8	99.9	92.8	95.6
	ns-HFA	123.5	114.1	-	113.5	111.6	91.2	136.1	106.7	129.9
	total	223.6	204.4	-	204.5	204.1	180.0	235.9	199.5	225.5
	$C_{16:1}$	25.1	17.4	-	10.1	3.94	13.7	16.1	16.3	17.4
	$C_{18:1}$	13.9	3.22	-	1.26	0.56	4.17	5.41	4.30	4.19
	$C_{18:2}$	3.59	4.15	-	3.53	3.26	4.71	3.80	3.31	3.68
	$C_{18:3}$	3.47	3.32	-	2.90	2.14	2.82	2.53	1.98	2.29

* ns-LFA: normal saturated fatty acids (C_{12}-C_{19}),   ** ns-HFA: normal saturated fatty acids (C_{20}-C_{30})
*** total: ns-LFA + ns-HFA,   **** not analyzed.

Figure 46 shows changes in the concentration of LFA's (C_{12}-C_{19}) and HFA's (C_{20}-C_{30}) upon heating at 65°C. LFA's decreased with time and showed minimum concentration in two days, which is 57 % of initial amount. In longer time, their concentration gradually increased and became slightly larger than initial concentration in 248 days. On the other hand, on heating at 83°C, fatty acid concentration did not show a drastic change as observed on heating at 65°C (see Table 14).

Figure 47 shows concentrations of unsaturated fatty aicds upon heating at 65°C and 83°C for 1-248 days. At 65°C, their concentration seemed to be unchanged. However, at 83°C, the concentration of $C_{16:1}$ and $C_{18:1}$ monounsaturated fatty acids firstly decreased drastically and then increased, whereas $C_{18:2}$ and $C_{18:3}$ polyunsaturated fatty acids did not show a drastic change.
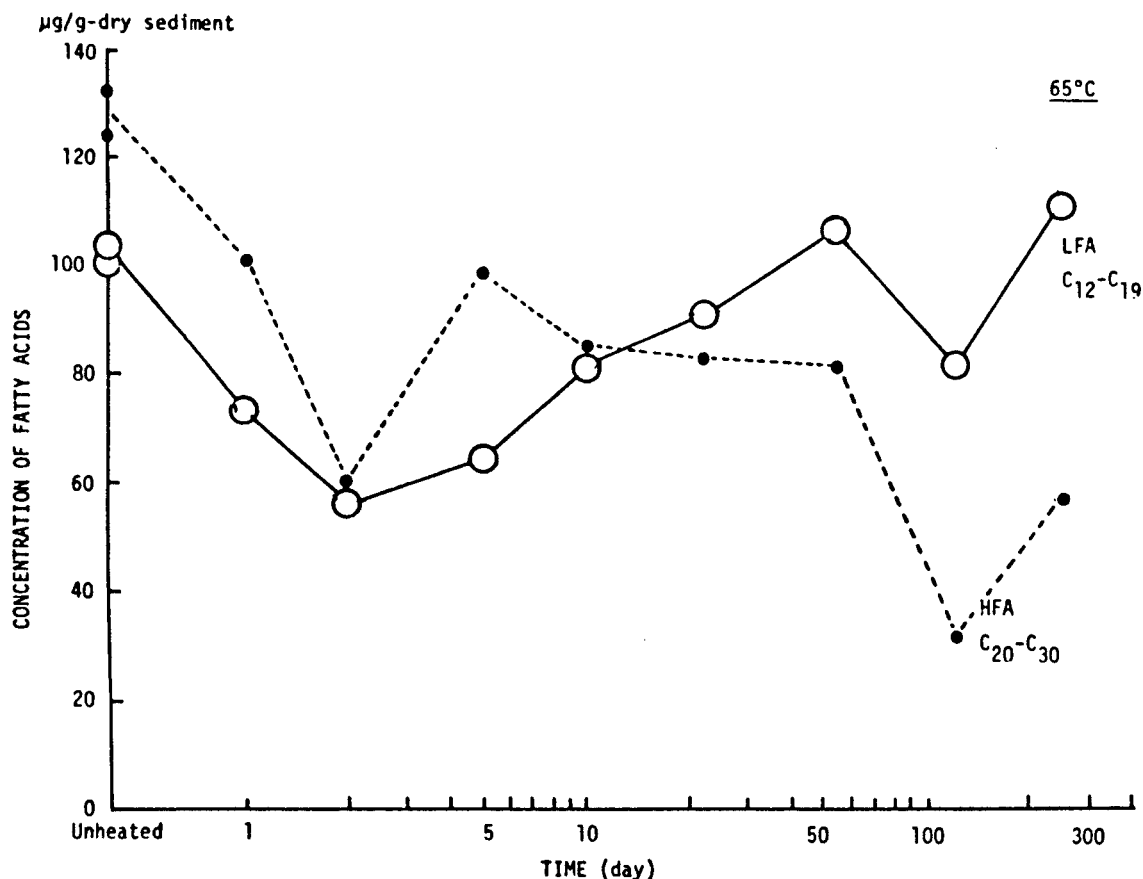
Fig. 46 Changes in the concentration of LFA's and HFA's upon heating
at 65°C for 1-248 days.

8.4. DISCUSSION

8.4.1. Disappearance of saturated fatty acids at lower temperatures

It is clearly observed, in experiments 1 and 3, that the concentrations
of saturated fatty acids decreased upon heating at lower temperatures.
The author believes that this phenomenon was not caused by the degradation
of these compounds, since their concentration increased at higher
temperatures. Therefore, it is reasonable to consider that this phenomenon
is associated with the changes in the existence states of these compounds
in sediments. There seems to exist two possible pathways for the changes
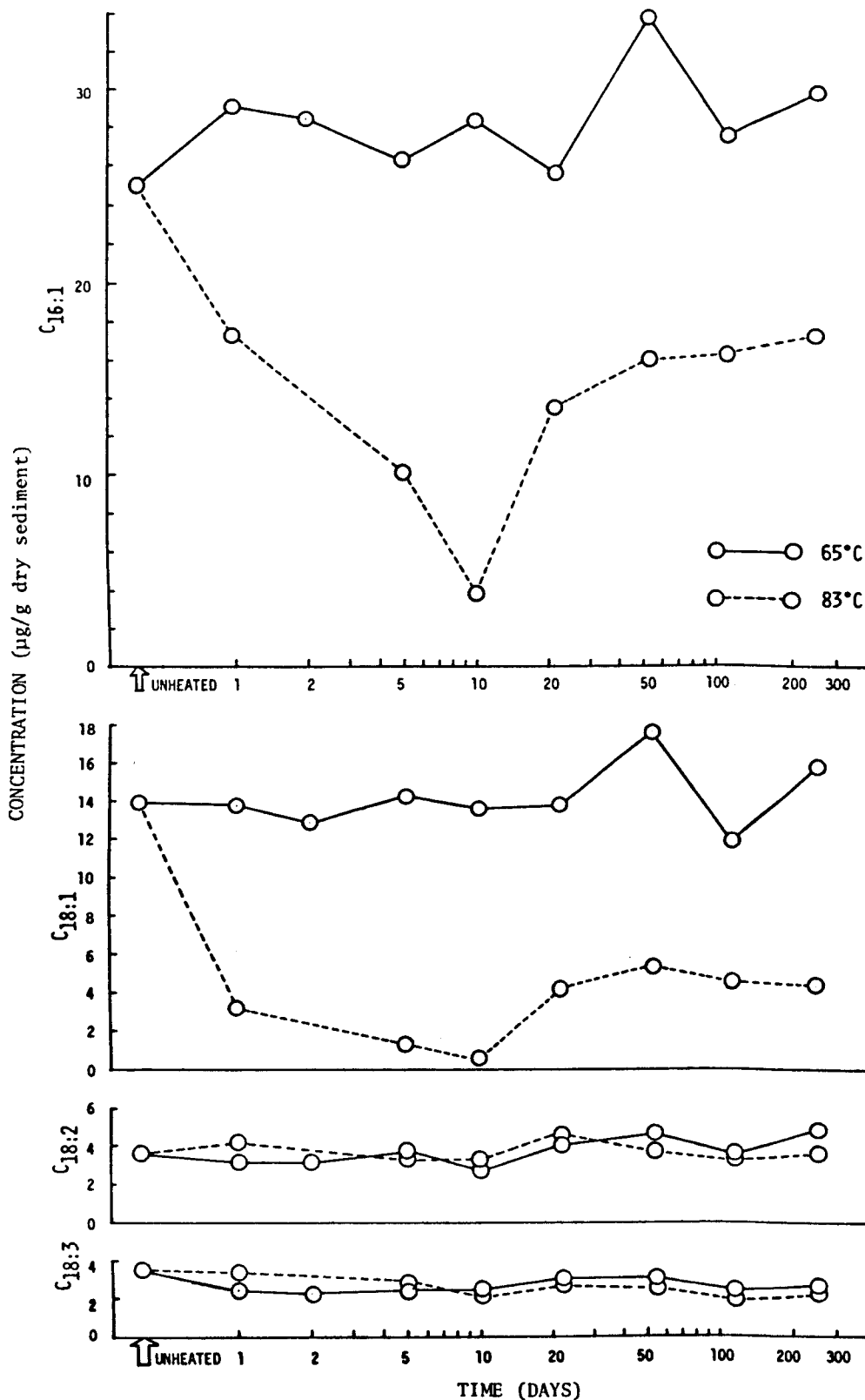
Fig. 47 Changes in the concentrations of unsaturated fatty acids upon heating at 65°C and 83°C for 1-248 days.

from analyzable to non-analyzable form upon heating as follows.

(1) Fatty acids in analyzable form may change to fatty acids which can be separated by saponification procedure but can not be analyzed.

(2) Fatty acids may transform to organic and/or inorganic matrices in sediment residue, from which unbound + bound fractions were removed.

These possibilities can be tested by studying (i) the lipids extracted by saponification, (ii) the pre-extracted sediment residue, of the heated samples at lower temperatures (68-112°C for one day, or 65°C for 2-5 days). If these possibilities were the case, the fatty acids which disappeared upon heating could be obtained by heating (i) or (ii) at the temperatures higher than 200°C, where fatty acid concentrations increased. If a considerable amount of fatty acids were obtained by heating (i), the fatty acids which disappeared might have polymerized in the lipid fractions. If a considerable amount of fatty acids were obtained by heating (ii), the fatty acids which disappeared might have been trapped into clay minerals and/or geopolymers (humic compounds and kerogen). These studies would give us more fruiteful information on the existence forms of organic compounds and their changes in sediments.

There seems to be present a difference in the decreasing rate between LFA's and HFA's (see Fig. 39 and Fig. 46). As shown in Table 15, the decreasing rates for HFA's are more ramarkable than those for LFA's. Such a difference may be caused by a slight difference in the chemical states between LFA's and HFA's, which may affect on the behavior of the fatty acids on heating at milder condition. A possible explanation of the phenomena is as follows. The polymer forming reaction, e.g. Maillard Reaction, might be operative in the heated sediments at milder condition. The fatty acids which exist in different chemical states may be trapped

Table 15   Decreasing rates of LFA's and HFA's in the heated sediment samples

	Exp. 1 (88°C, 1 day)	Exp. 3 (65°C)
LFA's	25 %	43 % (2 days)
HFA's	51 %	74 % (114 days)

LFA's : C_{12}-C_{19} fatty acids, HFA's : C_{20}-C_{30} fatty acids

and become non-extractable or non-saponifiable forms. In this process, fatty acids probably behave in different manners depending on their chain length and/or existing states.

8.4.2. Release of saturated fatty acids at higher temperatures

The concentrations of fatty acids increased and became larger than the initial concentrations at 140°C (see Fig. 39). This phenomenon may be explained by that the fatty acids, which were once trapped in organic and/or inorganic matrices on heating at milder conditions, may have been released by breakdown of the bondings between fatty acids and the matrices.

At higher temperatures than 140°C, the concentrations of fatty acids became larger than initial ones. LFA's and HFA's at 325°C are 159 % and 134 % higher than the initial amount, respectively. As stated in Chapter 7, fatty acids which increased upon heating might have come from the fatty acids which are probably incorporated in geopolymers.

Figure 48 shows CPI values (carbon prefer index) for LFA's $[\Sigma(C_{12}\text{-}C_{18})_{even}/\Sigma(C_{13}\text{-}C_{19})_{odd}]$ and for HFA's $[\Sigma(C_{20}\text{-}C_{30})_{even}/\Sigma(C_{21}\text{-}C_{29})_{odd}]$. The CPI values for both LFA's and HFA's are almost constant at the temperatures lower than 200°C, indicating that chemical degradation does not occur yet. At higher temperatures, the values decrease. This indicates that a part of the C_n fatty acids degraded to the C_{n-1} fatty acids probably through α-oxidation. It may be reasonable because a hydrogen atom at α position of fatty acids is likely to be activated. Since the concentration of fatty acids did not decrease at the temperatures where CPI values decreased, the release of fatty acids from geopolymers are superior to the disappearence of fatty acids by degradation. An extrapolation of CPI indicates that the CPI value for LFA's become 1 at lower temperature than that for HFA's. This suggests that LFA's are more liable to degradation than HFA's.

The concentrations of n-alkanes in the heated sediment samples largely increased at the temperatures higher than 279°C (see Chapter 9). However, fatty acids did not decrease at those temperatures. This suggests that sedimentary fatty acids are not a precursor of n-alkanes generated upon heating at those temperatures. This suggestion does not
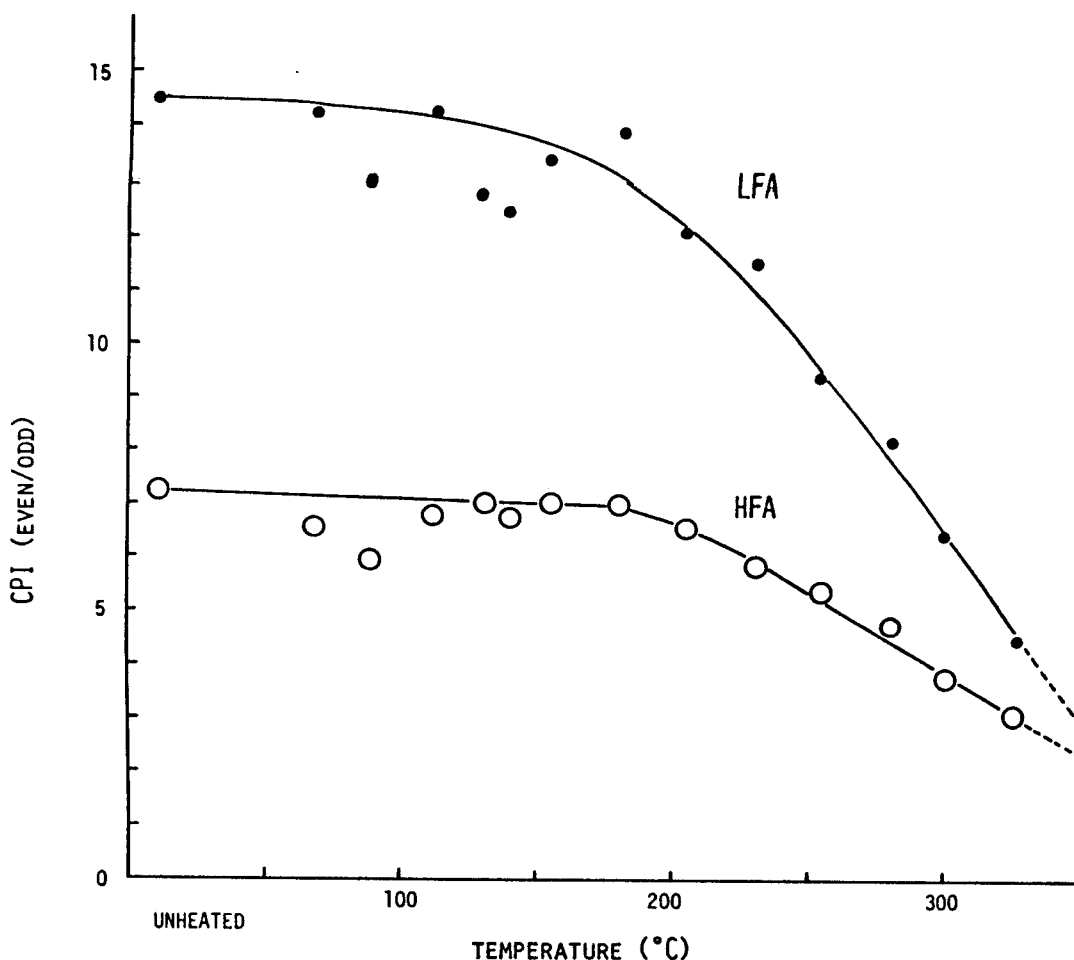
Fig. 48 Changes in the CPI values of LFA's and HFA's in the heated sediment samples for one day.

agree with a hypothesis that petroleum hydrocarbons are produced by the decarboxylation of fatty acids (Cooper and Bray, 1963).

8.4.3. Fate of unsaturated fatty acids

Unsaturated fatty acids decreased and disappeared at the temperatures higher than 200°C, whereas saturated ones did not decrease at those temperatures. This indicates that unsaturated acids are thermally less stable than saturated ones. There seems to exist two possible ways for the disappearance of unsaturated fatty acids, as follows.

(1) Unsaturated FA's may have degraded probably through the intermediates such as hydroxy acids and peroxides, although the intermediates were not detected.

(2) Unsaturated FA's may have polymerized and become constituents of geopolymers.

We can discuss the fate of these acids in the heated samples as follows. Unsaturated FA's (and a part of their degradation products) are considered to correspond to α,ω-dicarboxylic acids in the oxidation products of the heated sediments. C_7-C_9 α,ω-Dicarboxylic acids were main oxidation products of the residual sediments, as shown in Table 13. The C_7-C_9 dicarboxylic acids seem to decrease slightly at 88°C, as shown in Fig. 45. But, they increase at higher temperatures and become constant at 129-204°C. The amount of dicarboxylic acids which appeared at 129-204°C is 38.4-42.5 µg/g dry sediment. Those amounts are larger than those of unsaturated FA's (28.2 µg/g) in unheated sediment. Furthermore, at 129°C, the amount of unsaturated acids which disappeared was 2.4 µg/g dry sediment. These facts suggest that α,ω-dicarboxylic acids released on heating did not only originate from the disappeared unsaturated acids but from other precursors.

The author considers that unbound + bound fractions contain the precursors which produce α,ω-dicarboxylic acids on oxidation and those precursors transform to the geopolymers upon heating. This idea may be supported by the fact that α,ω-dicarboxylic acids in oxidative degradation products of lipid fractions are more abundant than the unsaturated fatty acids in the lipids (Machihara and Ishiwatari, 1981). Although unsaturated fatty acids drastically decreased at the temperatures of 179°C to 204°C (Fig. 45), the α,ω-dicarboxylic acids in the oxidation products did not increase at those temperatures. Furthermore, at higher temperatures, the dicarboxylic acids decreased. These facts indicate that the amount of precursor which produce dicarboxylic acids upon oxidation of the pre-extracted sediment residues decreased on heating.

As shown in Fig. 47, the concentration of monounsaturated fatty acids slightly increased with time at 65°C. On the contrary, at 83°C, these acids considerably decreased. Although this phenomenon may be involved in many factors, the following two pathways are possible. In the first, a part of these acids may change their existence forms from extractable to non-extractable forms and then change to the extrac-

table forms when heated for longer period. This pathways is similar to that for the decrease of saturated fatty acids at lower temperatures (see Fig. 39 and Fig. 46). In the second, those acids may degrade into other chemical compounds, e.g. hydroxy compounds or peroxides.

Polyunsaturated $C_{18:2}$ and $C_{18:3}$ fatty acids were almost unchanged upon heating at milder conditions, as shown in Fig. 47. Their behavior is quite different from that of monounsaturated ones under this experimental conditions. Such a difference may be associated with (1) the difference in the numbers of double bonds of unsaturated fatty acids, and/or (2) the difference in their existence states in sediments.

8.4.4. Release of β-hydroxy acids from matrices upon heating

As shown in Fig. 41, β-hydroxy acids increased drastically on heating and showed maximum at 154°C. Possible explanations for the phenomenon are: (1) the β-hydroxy acids might be originated from the corresponding fatty acids by their β-oxidation; (2) those acids might be released from geopolymers such as kerogen and humic compounds.

If β-hydroxy acids were produced by the oxidation of fatty acids, their concentration should decrease at the temperature of 154°C. However, such a decrease was not observed (see Fig. 39, p. 118). Therefore, the mechanism (1) is not probable. This is also supported by the following fact. n-C_{17} Fatty acid was heated in a pyrex tube with water and Ca-montmorillonite at 160°C, 202°C and 248°C for 1 day. β-Hydroxy acids were not detected in the heated slurries. This result suggests that β-hydroxy acids which appeared on heating the sediment were not produced by the oxidation of fatty acids.

It is probable that the β-hydroxy acids were released from sediment where β-hydroxy acids exist in geopolymers such as kerogen and humic compounds. This is supported by the following fact, as already described in Chapter 4. The pre-saponified (0.5 N KOH/methanol) sediment of Lake Biwa 200 m core was subjected to a harsher saponification procedure (2 N KOH soln., 180°C for 3 hrs) and considerable amounts of β-hydroxy acids (5.6-60 ug/g) were obtained, which are 5-20 times larger than the β-hydroxy acids in unbound + bound fractions (see Table 5, p. 74).

This fact indicates that a considerable amount of β-hydroxy acids exists in the pre-saponified sediments. Therefore, it is reasonable to consider that β-hydroxy acids were released from the geopolymers which may have trapped or incorporated a considerable amount of these acids in non-extractable forms.

β-Hydroxy acids are not present in algae, but present in bacteria as constituents of cell walls (Boon et al., 1977). The present results suggest that bacterial activities considerably contribute to a formation of geopolymers. It is possible that photosynthesized organic matter is subjected to bacterial degradation and the bacterial biomass may be transferred to geopolymers after the death of organisms.

As shown in Fig. 41, β-hydroxy acids drastically decreased at the temperatures higher than 154°C. This suggests that these acids are less stable than fatty acids, since the latter did not decrease at the above temperatures. C_n β-Hydroxy acids may degrade into the corresponding C_{n-2} fatty acids by β-oxidation. This is supported by the fact that C_{12} and C_{14} fatty acids increased at 179-204°C, where C_{14} and C_{16} β-hydroxy acids drastically decreased, as shown in Table 16.

Table 16 Concentrations of fatty acids and β-hydroxy acids in the unheated and heated sediment samples (μg/g dry sediment)

Compounds		unheated	Temperature 154°C	179°C	204°C	229°C
FA	C_{12}	3.1	3.4	4.7	5.8	5.3
	C_{14}	13.8	16.2	20.2	20.9	18.9
β-OH	C_{14}	4.2	11.5	8.2	0.7	0.3
	C_{16}	3.4	18.2	12.0	2.3	2.4

8.4.5. Behavior of ω- and ω-1 hydroxy acids

There seems to be a difference in the behavior between lower M.W. C_{12}-C_{18} ω-hydroxy acids and higher M.W. C_{20}-C_{30} ω-hydroxy acids, as shown in Table 12. The former acids did not increase upon heating, except for an increase of C_{16} acid at 129°C. Their behavior is quite different from that of β-hydroxy acids. This result suggests that major

parts of these acids are present in extractable or saponifiable forms
and that minor parts are trapped or incorporated in geopolymers.

On the other hand, C_{20}-C_{30} ω-hydroxy acids showed maximum concentra-
tion at 179°C, as shown in Fig. 42 (p. 121)   Their behavior is similar
to that of β-hydroxy acids. C_{24}-C_{30} ω-1 Hydroxy acids showed a similar
behavior to that of ω-hydroxy acids. Since these ω- and ω-1 hydroxy
acids are known to be constituents of cuticular wax and suberin of
higher plants (Kolattukudy, 1980) and to be produced by yeast (Stodola
et al., 1967), it is possible to consider that a part of these acids
which appeared on heating have polymerized during diagenesis in soils
and aquatic environments, and were released from the polymers. At the
temperatures higher than 179°C, ω- and ω-1 C_{20}-C_{30} hydroxy acids decreased,
whereas the corresponding α,ω-dicarboxylic acids increased. This
suggests that these hydroxy acids undergo ω- and ω-1 oxidation to
produce corresponding α,ω-dicarboxylic acids upon heating.

8.4.6. Behavior of α,ω-dicarboxylic acids

As shown in Fig. 42, the concentration of α,ω-dicarboxylic acids
were almost unchanged at the temperatures lower than 179°C, and increased
at higher temperatures showing maximum at 279°C. These acids appeared
upon heating seem to have dual origins. Firstly, a part of these acids
might be released from geopolymers such as kerogen and humic compounds.
Secondly, the remaining parts might be produced by the oxidation of ω-
and ω-1 hydroxy acids as stated before.

The second consideration is supported by the change in CPI value of
α,ω-dicarboxylic acids upon heating. As shown in Fig. 43, CPI value
(even/odd) for C_9-C_{19} dicarboxylic acids showed a minimum at 154°C
and a maximum at 229°C. The maximum of CPI value is caused by the
increase of the dicarboxylic acids (C_{14}, C_{16} and C_{18}), which may be
derived from corresponding ω-hydroxy acids by oxidation. The decrease
in CPI value at the temperature higher than 279°C can be explained by
the fact that C_{16} dicarboxylic acids decreased and odd carbon numbered
dicarboxylic acids increased. The odd numbered acids may be produced
by oxidative degradation of even carbon numbered dicarboxylic acids.

On the other hand, the minimum of CPI value can not be clearly explained now, although there is a possibility that a mass fragmentogram of odd carbon numbered dicarboxylic acid methyl esters scanned at m/e 98 is partly overlapped by that of β-hydroxy acid methyl ester TMS ether, whose concentration become maximum at 154°C.
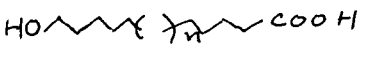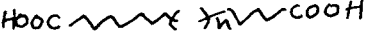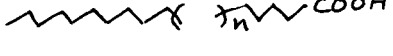
On the other hand, the change in CPI of C_{20}-C_{30} α,ω-dicarboxylic acids was not so remarkable as that of C_9-C_{19} dicarboxylic acids. This is explained by that the former acids are produced by the oxidation of ω- and ω-1 hydroxy acids, which are even carbon numbered predominant.

α,ω-Dicarboxylic acids were decreased at the temperatures higher than 279°C, probably by their degradation.

8.4.7. Comparison of thermal stability among fatty acids and related compounds

There is a variety in the behaviors of long chained compounds in the heated sediment samples, as shown in Table 17. Such a variety may

Table 17 Temperatures where organic compounds became maximum and disappeared in the heated sediment samples (68-325°C for 1 day)

Compounds		Temperature (°C)	
		Max. Conc.	Disapperaed
Poly-UFA $C_{18:3}$		-*	204
Poly-UFA $C_{18:2}$		-	229
Mono-UFA $C_{16:1}$, $C_{18:1}$		-	279
β-Hydroxy acids	R~~~~~~COOH (OH)	154	325
ω-1 Hydroxy acids	(OH)~~~~)n~~~COOH	179	279
ω-Hydroxy acids	HO~~~~)n~~~COOH	179	325
Alcohols	HO~~~~~~R	229	325
α,ω-Dicarboxylic acids	HOOC~~~~)n~~COOH	279	--**
Monocarboxylic acids	~~~~)n~~COOH	325	--
n-Alkanes	~~~~R	325	--

*: Max. Conc. was not observed upon heating. **: The compounds did not disappear in the heated samples.

be related to the thermal stability of these compounds, which is involved in functional groups and their position in a molecular skeleton. Thermal stabilities of these compounds can be compared each other on the basis of the temperatures where the compounds became maximum and disappeared.

It is obvious that double bonds are less stable than single bonds, since unsaturated fatty acids disappeared at 204-279°C, where saturated ones did not disappear. With an increase in the numberes of double bonds, unsaturated fatty acids become unstable.

Hydroxy acids are more stable than unsaturated fatty acids but less stable than alcohols and saturated mono- and di-carboxylic acids. Among hydroxy acids, the stabilities become higher in the order : β- $<$ ω-1 $<$ ω-hydroxy acids.

Alcohols are more stable than hydroxy acids but less stable than mono- and di-carboxylic acids.

α,ω-Dicarboxylic acids are more stable than alcohols but less stable than monocarboxylic acids.

Monocarboxylic acids (saturated) are most stable among these compounds. In the present experimental condition ($<$325°C), their concentration continuously increased. It is of interest to test how stable monocarboxylic acids are in the heated sediments at higher temperatures and in longer time.

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APPENDIX

This figure shows an effect of the time on the amounts of α,ω-dicarboxylic acids in the oxidation products of the pre-extracted sediments at 60°C.
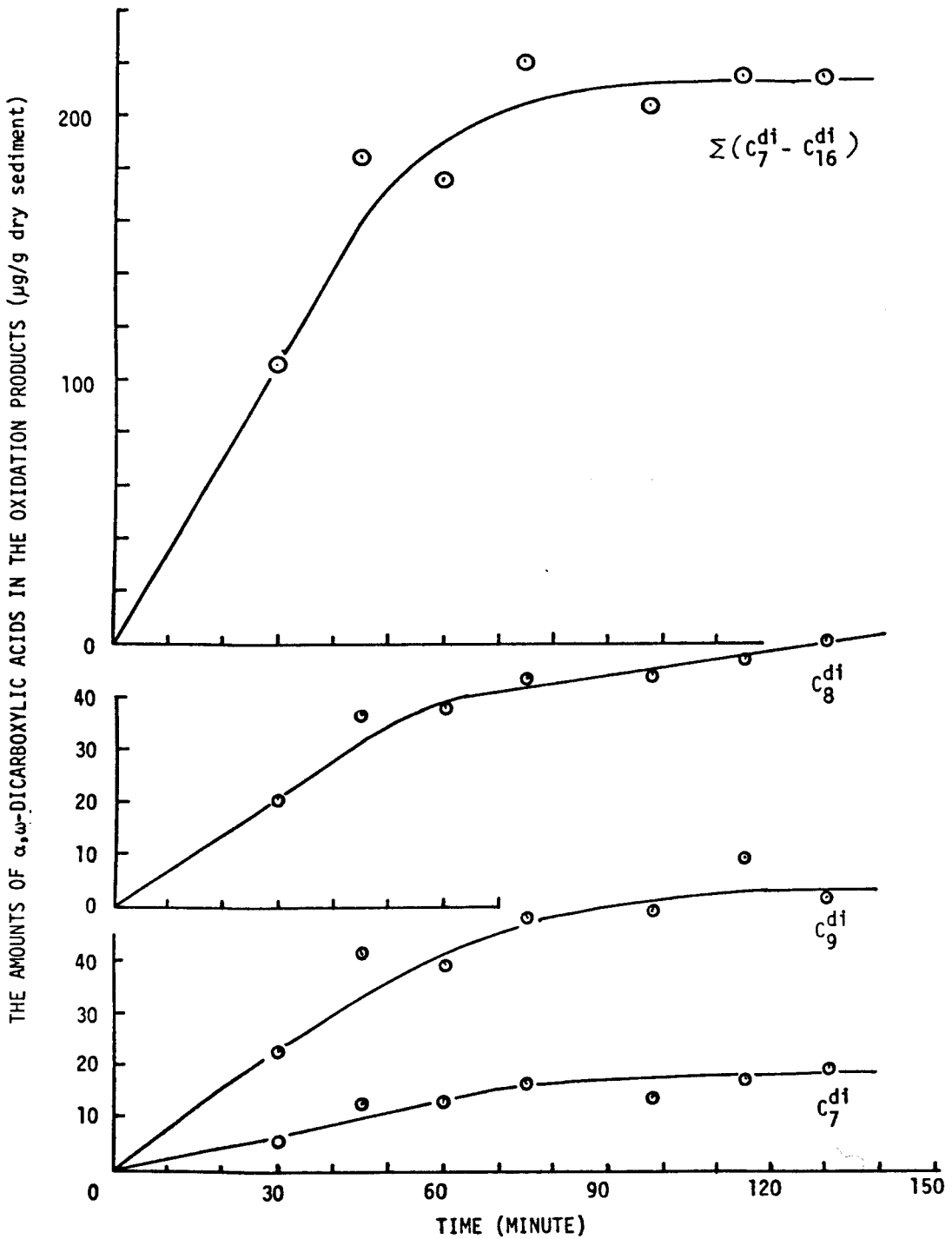
CHAPTER 9

GENERATION OF NORMAL ALKANES UPON HEATING YOUNG SEDIMENTS

9.1. INTRODUCTION

Fatty acids in sediments have been believed to be a precursor
of petroleum hydrocarbons (e.g. Cooper and Bray, 1963). This hypothesis
has been supported by the comparative observational study of fatty
acids and hydrocarbons in recent and ancient sediments (Cooper, 1962;
Kvenvolden, 1968; Douglas et al., 1968) and by the facts that
hydrocarbons were generated by heating fatty acids (Jurg and Eisma,
1964, 1968; Shimoyama and Johns, 1971, 1972). However, a clear
explanation for the above hypothesis has not been presented yet.
For example, fatty acids have not been reported to decrease in the
heated sediments at temperatures in which petroleum hydrocarbons are
generated. Therefore, there is still a question whether or not fatty
acids are indeed a precursor of petroleum hydrocarbons. Harrison
(1978) conducted a thermal alteration experiment of young sediment.
In his heating experiment, fatty acids did not so drastically decrease
at the condition where n-alkanes increased. Since the temperature
range was limitted to three points (80°C, 160°C and 240°C for 50 hrs),
it is needed to study the generation of n-alkanes in the heated sediment
samples in more detail together with fatty acids and related compounds
at more various temperature conditions.

The purposes of this chapter are to examin whether or not fatty
acids are actually a precursor of n-alkanes generated in heated
sediment samples and to explain the precursors of the n-alkanes
based on the analytical results of possible precursors, i.e. fatty
acids, hydroxy acids, dicarboxylic acids and alcohols.

9.2. EXPERIMENTAL

The samples were same as those which were used in Chapter 8. Sets of samples in a pyrex tube were heated at 68-325°C for 24 hrs. The neutral fractions were separated and then n-alkanes and alcohols were purified by silica gel column chromatography. The analytical methods are given in Chapter 1.

9.3. RESULTS AND DISCUSSION

Figure 49 shows the mass fragmentograms of n-alkanes in the
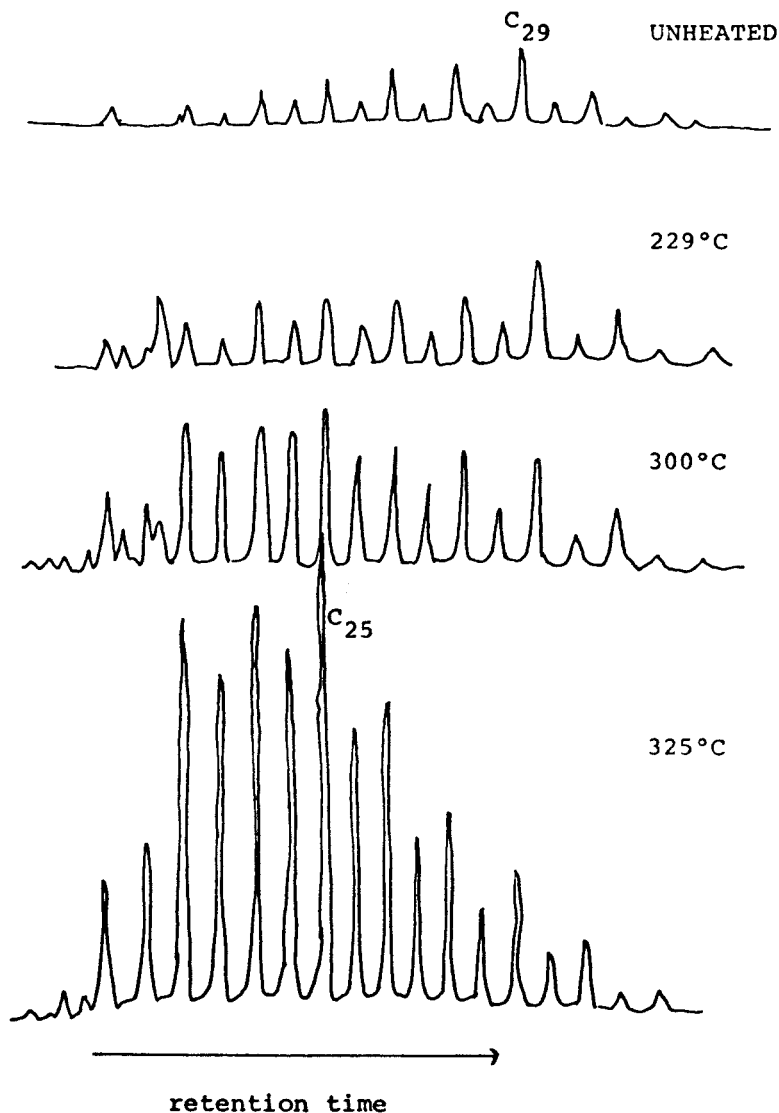

FIG. 49 Mass chromatograms (m/e 57) of n-alkanes in unheated and heated sediment samples.

unheated and heated sediment samples. With increasing temperature, the maximum peak appeared to shift from C_{29} to C_{25} and the CPI values seems to decrease approaching 1.

Fig. 50 shows the concentration of n-alkanes in the unheated and heated sediment samples at 68-325°C for 24 hrs. The concentrations are almost constant at the temperatures below 200°C. n-Alkanes increased at temperatures higher than 200°C. At 325°C, their amount became 110 μg/g dry sediment, which is ca. 20 times larger than that of the unheated sample (6.8 μg/g). This indicates that the sediment has a considerable amount (at least 100 ug/g dry sediment) of the precursors of n-alkanes upon heating. As the precursors, the following compounds with normal molecular chain are cited: fatty acids, hydroxy acids, dicarboxylic acids, alcohols and others (kerogen).
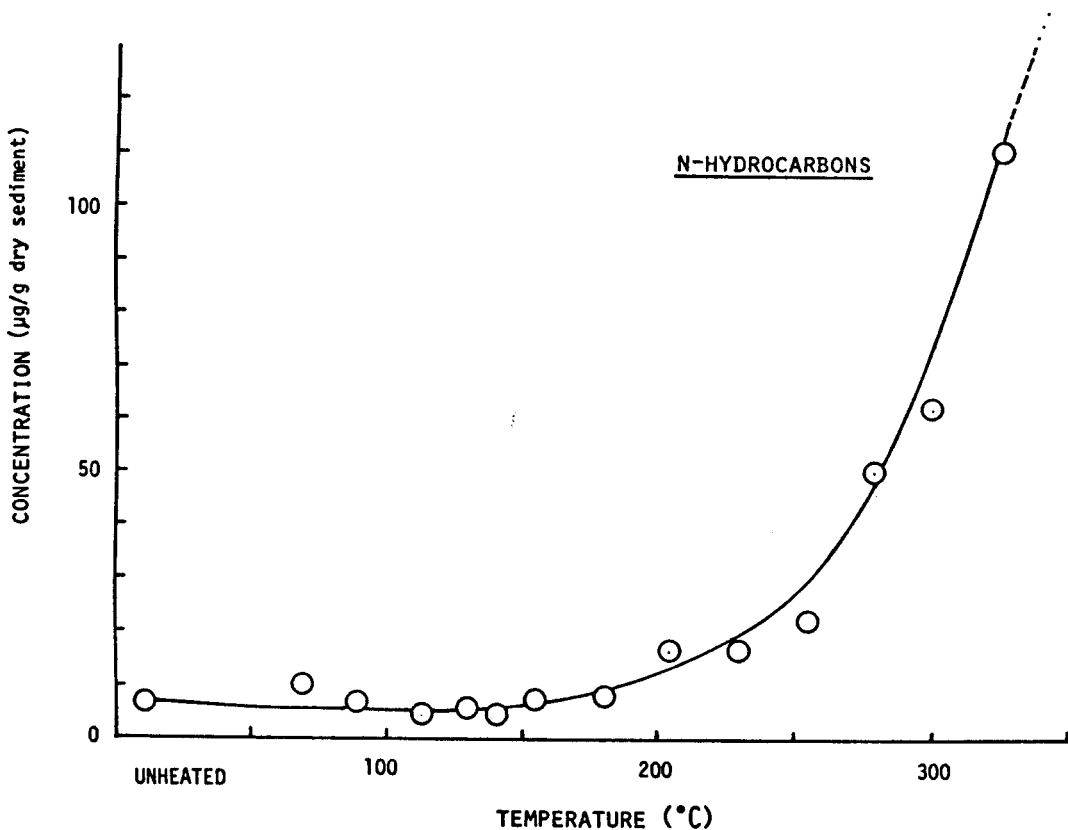

Fig. 50 Concentrations of n-hydrocarbons in the unheated and heated sediments at 68-325°C for 24 hrs.

At the temperatures higher than 280°C, where n-alkanes were drastically generated, fatty acids did not decrease (Fig. 39, p.118). This suggests that fatty acids are not a precursor of the generated n-alkanes. This suggestion does not agree with a hypothesis that petroleum hydrocarbons are produced by decarboxylation of fatty acids (Cooper and Bray, 1963).

Hydroxy acids which increased at 154-179°C disappeared at the temperatures higher than 200°C (Fig. 41, p. 120). But the temperature is low as compared to the temperature at which a considerable amount of n-alkanes was generated. This suggests that the potential of these compounds to produce the n-alkanes is low, although there is still a possibility that the skeletons of the disappeared hydroxy acids may remain in the heated sediments and produce n-alkanes at the temperatures higher than 200°C.

Dicarboxylic acids decreased slightly at the temperatures higher than 300°C (Fig. 43, p. 122). But the amounts of these acids disappeared at the temperatures were ca. 10 µg/g dry sediment, which is 10 times lower than the amount of the generated n-alkanes. Therefore, the potential of the dicarboxylic acids to produce n-alkanes may be low.

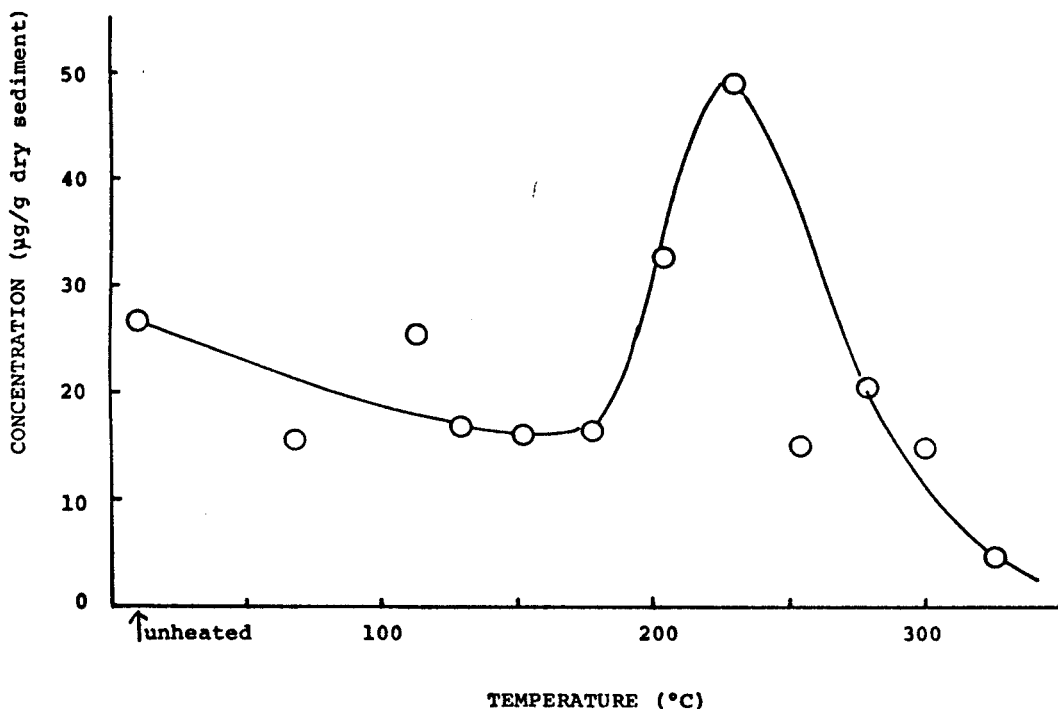On the other hand, normal alcohols increased upon heating the

Fig. 51 The concentrations of primary alcohols (C_{14}-C_{30}) in the unheated and heated sediment samples at 68-325°C for 24 hrs.

sediment at 229°C. Figure 51 shows the concentrations of the alcohols
(C_{14}-C_{30}) in the unheated and heated sediment samples. The concentra-
tions became 50 µg/g dry sediment at 229°C and then decreased at
higher temperatures. The range of the temperature, in which alcohols
decreased, correspond to the temperatures where n-alkanes were generated.
This fact suggests that the alcohols may be a precursor of n-alkanes
generated. This seems to be supported by the following consideration.
Since alcohols are even carbon number predoninance, they probably
produce even numbered hydrocarbons predominantly by dehydration,
consequently, the CPI values of the n-alkanes generated may be decreased.

However, the maximum concentrations of the alcohols in the heated
samples were 50 µg/g dry sediment, which is a half of the amounts of
n-alkanes generated. Therefore, other precursors of n-alkanes should
be present in the sediments. Geopolymer such as kerogen is a possible
precursor, since kerogen comprise majority parts of the organic matter
in sediments and has a high potential to produce n-alkanes upon heating
(Ishiwatari et al., 1977).

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CHAPTER 10

SUMMARY AND CONCLUSION OF PART II

[1] The heating experiments showed that lower M.W. (C_{12}-C_{19}) fatty
acids are present in unbound (39 %), bound (46 %) and residual (15 %)
forms, whereas higher M.W. (C_{20}-C_{30}) fatty acids exist in unbound (34 %),
bound (32 %) and residual (29 %) forms in the surface sediment of Lake
Biwa. The bound and residual forms changed to the unbound form upon
heating at 200°C for 48 hrs.

[2] The unbound + bound fatty acids in the sediment samples firstly
decreased (ca. 50 % of the initial amount) upon heating at 68-112°C,
and then increased at higher temperatures. The unbound + bound fatty
acids may have changed into the residual form (were trapped in organic
and/or inorganic matrices) and then released on heating at the higher
temperatures.

[3] Unbound + bound mono- and poly-unsaturated fatty acids in the heated
sediments decreased with temperatures and disappeared at 204-279°C.
These acids are thermally less stable than saturated ones. At the
milder conditions (83°C), monounsaturated fatty acids firstly decreased
and then increased with time. These acids may have been trapped in
the matrices and then released. However, polyunsaturated fatty acids
were almost unchanged at the same conditions.

[4] Unbound + bound β-hydroxy acids increased with heating temperatures
and showed maximum concentration at 154°C, which is three times larger
than the initial concentration (unheated). They decreased at higher
temperatures and almost disappeared at 204°C. It is probable that
β-hydroxy acids were released from sediments where these acids exist in
geopolymers such as kerogen and humic compounds. Since β-hydroxy acids
are characteristic of bacteria, microbial activity may considerably
contribute to the formation of geopolymers.

[5] At the temperatures where β-hydroxy acids and ω- and ω-1 hydroxy

decreased upon heating, the corresponding momocarboxylic acids and dicarboxylic acids increased. These acids which have increased may have been generated by the oxidation β-, ω- and ω-1 hydroxy acids.

[6] Based on the temperatures where organic compounds showed maximum concentrations and disappeared upon heating, thermal stability of the compounds is suggested to be in the order: polyunsaturated fatty acids $<$ monounsaturated fatty acids $<$ β-hydroxy acids $<$ ω-1 hydroxy acids $<$ ω-hydroxy acids $<$ alcohols $<$ α,ω-dicarboxylic acids $<$ saturated fatty acids.

[7] A considerable amount of C_{17}-C_{33} n-alkanes (100 μg/g dry sediment) were generated by heating the sediment at 325°C for a day. The amount of fatty acids (266.3 μg/g dry sediment at 325°C) did not decrease with the increasing temperature. This suggests that the n-alkanes were not generated from the fatty acids.

[8] Fatty alcohols, whose maximum concentration was ca. 50 μg/g dry sediment, disappeared upon heating at the temperatures higher than 200°C, where n-alkanes were generated. Although alcohols are one of the possible precursors of the n-alkanes, other precursors (e.g. kerogen) should be present in sediments.

PART III

GENERAL DISCUSSION AND FUTURE PROBLEMS

We discuss here on the relation between observational
study (PART I) and experimental study (PART II) and discuss geochemical
implications of the present thermal alteration experiment comparing
the distributions of organic compounds between heated sediments and
ancient sediments or rocks. We also deal with the future problems.

[1] Evaluation of diagenetic potential of HFA's

A heating experiment ($\simeq 200°C$) was proved to provide a helpful clue
for a explanation of the observational result, as follows. Although
HFA's (C_{20}-C_{30}) increased with depth in the 20-100 m sediment layers
of Lake Biwa, it was difficult to explain the phenomenon only by
their vertical distribution. There seemed to three possible explanations
for the increase of HFA's with depth.

(1) The fatty acids may be produced by the oxidation of corresponding
fatty alcohols in sediments.

(2) The fatty acids may be released from organic and/or inorganic
matrices in sediments.

(3) A considerable amount of the fatty acids may have been supplied
to the sediment in the past.

We conducted the analyses of alcohols in the 200 m core to
evaluate the first possibility and compared their vertical distribution
with that of HFA's. Based on the comparison, we considered that the
first possibility is low. Then, we conducted thermal alteration experiment
to evaluate the second. We considered that, if the precursors which
generate HFA's are present in the sediments, they may be released
upon heating, and that we evaluate the diagenetic potential of HFA's
in sediments. The results made us to believe that the second possibility
is also low. Consequently, we concluded that the third is most probable.
In this way, we could differentiate a factor (change in the input of

organic compounds in the past) from another factor (diagenesis).

[2] Trap of FA's upon heating and decrease of LFA's in the 0-20 m sediment layers of Lake Biwa

Unbound + bound LFA's and HFA's have decreased with temperature and time upon heating at milder conditions (65°C-112°C). On the other hand, LFA's concentrations decreased with depth in the 0-20 m sediment layers (ca. 20,000 yrs) of Lake Biwa 200 m core. These two results seemingly agree with each other. If the heating experiment accelerate the diagenetic changes which occur in actual sediments, the heating condition (65°C, 2 days) where LFA's decreased corresponds to geological time of n x 10^4 years. This consideration means that such a milder condition is appropriate to simulate a diagenesis of organic compounds which occur in sediments of recent to n x 10^4 years old.

These results also suggest that a considerable amount of FA's is released upon heating the ancient sediments, which may have trapped those acids abundantly.

[3] Difference in the behaviors between LFA's and HFA's in heated sediments and actual sediments

Upon heating at 65°C for 1-248 days, LFA's firstly decreased in 2 days and then increased in longer time whereas HFA's seemed to decrease continuously with time, consequently, FA's distribution became LFA predominant with time. If such a change have occurred in actual sediments, LFA predominant distribution should be observed in ancient sediments. Douglas et al. (1968) reviewed FA's in Green River Shale (Eocene, n x 10^7 yrs old), in which FA's showed LFA predominant distributions (C_{16} or C_{18}) with higher even/odd ratio. This fact is consistent with the present experimental result. Therefore, the fatty acid distribution (LFA predominant) in Green River Shales may have been due to the selective trap of HFA's to the matrices in sediments during diagenesis, although HFA's might have been originally scant in the sediments.

[4] Release of β-hydroxy acids upon heating

Upon heating at the temperatures higher than 129°C, a considerable amount of β-hydroxy acids was released from the matrices in sediments.

Although such a release was not observed in the 200 m core of Lake Biwa, a high concentration of unbound + bound β-hydroxy acids may be observed in deeper layer sediments or ancient rocks. However, β-hydroxy acids have not been analysed in ancient sediments. Since β-hydroxy acids disappeared at temperatures higher than 200°C probably by decomposition, these compounds may be a possible marker to judge a thermal history of sediments.

[5] Themal degradation of FA's and ancient rocks

Upon heating at the temperatures higher than 200°C, CPI values of FA's decreased approaching 1. This indicates that FA's degrade to C_1 or C_2 shorter chained FA's through α- and β-oxidation. If these mechanisms were operative in sediments, ancient rocks should show a fatty acid distribution with lower CPI values (≃1) and LFA predominance. Burlingame et al. (1971) reported the distribution of fatty acids in ancient rocks of Alaskan Tasmanite (Late Jurassic to Early Cretaceous age, 130-190 M yrs old) and Tasmanian Tasmanite (Permian, 220-275 M yrs old). Those acids showed the distributions of LFA predominance ($n-C_{11}$, $n-C_{12}$ or $n-C_{16}$ predominant) and lower CPI value (≃1). This result is consistent with our consideration based on the experimental results. The temperature condition (>325°C) may correspond to a geological time of Cambrian age or later ages.

Further work is necessary to interpret geochemical fates of fatty acids and the related compounds in sediments and apply the methodology of organic geochemistry to paleo-environmental studies, as follows.

[1] It is valid to study a long sediment core in marine areas to confirm the general aspects of the diagenetic mechanisms (α-, β- and ω-oxidation of LFA's) and the hypothesis ($C_{18:2}/C_{18:0}$ ratio as a possible indicator of paleo-climate) which were proposed in this study.

[2] The factors which control the distributions of organic compounds in surface sediments should be studied. The changes in the chemical states of organic molecules, which may occur mainly in water column, are very important to elucidate their distributions and fates in

sediments.

[3] More detail and systematical studies of thermal alteration
experiments and also laboratory incubation experiments of organic
matter would provide us more fruitful informations which may be powerful
clue to interpret the observational results and to produce experimental
or observational programmes.

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