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FURTHER STUDY ON THE VERTICAL DISTRIBUTION OF ORGANIC CONSTITUENTS IN AN ANTARCTIC LAKE: LAKE VANDA

Genki I. MATSUMOTO[1], Kunihiko WATANUKI[1] and Tetsuya TORII[2]

[1]*Department of Chemistry, the College of Arts and Sciences, the University of Tokyo, 8–1, Komaba 3-chome, Meguro-ku, Tokyo 153*
[2]*Chiba Institute of Technology, 17–1, Tsudanuma 2-chome, Narashino 275*

Abstract: Total organic carbon (TOC), dissolved organic carbon (DOC), and organic constituents (hydrocarbons, fatty acids and hydroxy acids) in the particulate matter ($\geqq 0.6$ μm) and sediment sample of Lake Vanda in southern Victoria Land of Antarctica were studied to elucidate their features in relation to stratification of lake water and microbial distribution. The TOC and DOC concentrations increased with depth and reached the maximum values of 48 and 38 mgC/*l*, respectively, in the bottom water (69.0 m). The contents of particulate fatty acids between depths of 5.0 and 50.0 m were near constant but increased abruptly from a depth of 52.5 m and attained the maximum value of 130 μg/*l* at a depth of 57.5 m, then decreased to the bottom. This fatty acid profile probably reflects the distribution of photosynthetic plankton in the lake. Vertical distribution of branched fatty acids in the water column is consistent with that of bacterial population. Hydrocarbons were found only in the bottom water (67.5 and 69.0 m) and the sediment. The most dominant hydrocarbon was 2,6-dimethylhexadecane (tentatively identified) in all the samples, along with the abundance of long-chain *n*-saturated fatty acids and 3-hydroxy acids ($\geqq C_{20}$) in the sediment sample, indicating the presence of unknown microorganisms, such as fungi and bacteria in the lake bottom. The high TOC and DOC concentrations in the bottom water imply the concentration of refractory organic matter over a long period of time after degradation of labile organic constituents.

1. Introduction

There are a number of ice-free areas in and around the coastal regions of Antarctica, although most of the continent is covered with thick ice sheet. The McMurdo Oasis of southern Victoria Land is the largest ice-free area in Antarctica extending 4000 km^2. Lake Vanda, a meromictic lake, lies in the depression of the Wright Valley of the McMurdo Oasis (77°32′S, 161°34′E). The lake is known as a natural solar energy trap with density stratification by salt water. The bottom temperature of the lake attains to 23.8°C (*e.g.*, MATSUMOTO *et al.*, 1985). The major salt in the lake is calcium chloride with the highest chlorinity of 4 times in magnitude greater than that of seawater (TORII *et al.*, 1975). Much attention has been paid to these unusual physical and chemical properties of the lake concerning the stratification and salt origin of lake water, but information of organic constituents in the lake is still scanty. MATSUMOTO and his co-workers have reported vertical distribution of organic con-

stituents, including total organic carbon (TOC), fatty acids and sterols in lake water and sediment and found that organic constituents in the lake are markedly different with depth (MATSUMOTO and HANYA, 1977; MATSUMOTO *et al.*, 1979, 1982b, 1984). In order to obtain more detailed information of Lake Vanda, here we first studied dissolved organic carbon (DOC) and applied capillary gas chromatography-mass spectrometry for the analysis of hydrocarbons, fatty acids and 3-hydroxy acids in particulate matter with close intervals of the lake and bottom sediment, and discuss their relation to stratification of lake water and microbial distribution.

2. Materials and Methods

2.1. Samples

Lake Vanda is 5.6 km long and 1.5 km wide with the maximum depth of approximately 70 m. The lake has no outlet and the lake level is balanced between the glacial meltwater from the Onyx River and the ablation losses of the lake ice. The lake surface is covered perennially with thick ice. After drilling into lake ice of 2.80 m thick using a SIPRE ice auger, water and sediment samples were collected on 18 December 1985 with a KITAHARA-type water sampler (1 *l*) at the deepest point (J point) of the lake (69.9 m). To determine DOC and organic constituents in particulate matter, the lake water (0.80–5.0 *l*) was immediately filtered through the glassfiber filter with a pore size of 0.6 μm (GB-100R, TOYO Roshi Co.). Water samples for TOC and DOC analysis were transferred into 50 m*l* polyethylene bottles and acidified with concentrated hydrochloric acid (pH$<$2). The glassfiber filter was enveloped with aluminum foil. All the samples were kept frozen until analysis.

2.2. Analysis

TOC and DOC were determined by the method of MENZEL and VACCARO (1964). The analytical methods of organic constituents on the glassfiber filter (particulate matter) are described elsewhere in detail (MATSUMOTO *et al.*, 1987). Briefly, the glassfiber filters were refluxed with 0.5 M potassium hydroxide in methanol (80°C, 2 h) and extracted with ethyl acetate after acidification. The ethyl acetate extracts were chromatographed on a silica gel column (160$\times$5 mm i.d., 100 mesh, 5% water). Hydrocarbons, fatty acids and 3-hydroxy acids were eluted with 2 column volumes of hexane, 3 column volumes of benzene: ethyl acetate (95:5) and 3 column volumes of ethyl acetate, respectively. The fatty acid and hydroxy acid fractions were methylated with 14% borontrifluoride methanol. Hydroxy acid methyl esters were further treated with 25% *N*,*O*-bis (trimethylsilyl) acetamide in acetonitrile solution to obtain their trimethylsilyloxy ether derivatives. To ascertain the presence of long-chain unsaturated fatty acids in the sediment sample, hydrogenation with hydrogen gas and platinum dioxide was performerd (MATSUMOTO *et al.*, 1987). Hydrocarbons and fatty acid methyl esters were analyzed using a SHIMADZU GC-8A gas chromatograph (FID) equipped with a HEWLETT-PACKARD fused silica capillary column (No. 2, 17 m$\times$2 mm i.d.). Column temperature was programmed from 80 to 300°C at 10°C/min. The temperature of the injector and the detector was maintained at 330°C during analysis. The flow rate of helium carrier gas was 1.0 m*l*/min. Trimethyl-

silyloxy ether methyl esters of hydroxy acids were analyzed using a SHIMADZU QP 1000 GC/MS equipped with the fused silica capillary column same as that used in the gas chromatography. Splitless mode was used. The column temperature was programmed from 60 to 120°C at 30°C/min, then 120 to 300°C at 10°C/min. Mass spectra were obtained every 1.5 s continuously at an ionization energy of 70 eV. The identification of each organic compound was made by comparison of retention sequences and mass spectra with those of authentic standards and the elucidation of mass spectra (MATSUMOTO *et al.*, 1979; MATSUMOTO and NAGASHIMA, 1984).

3. Results and Discussion

3.1. Physicochemical properties

Vertical profile of physicochemical properties of Lake Vanda has been studied by many researchers (*e.g.*, TORII *et al.*, 1975; MATSUMOTO *et al.*, 1984, 1985). Temperature of the surface water was near zero, rose stepwise with depth and reached the maximum value of 23.8°C at the bottom, but temperatures between depths of 8–18 m (5.1°C) and of 24–42 m (7.2°C) were constant, implying the circulation of lake water

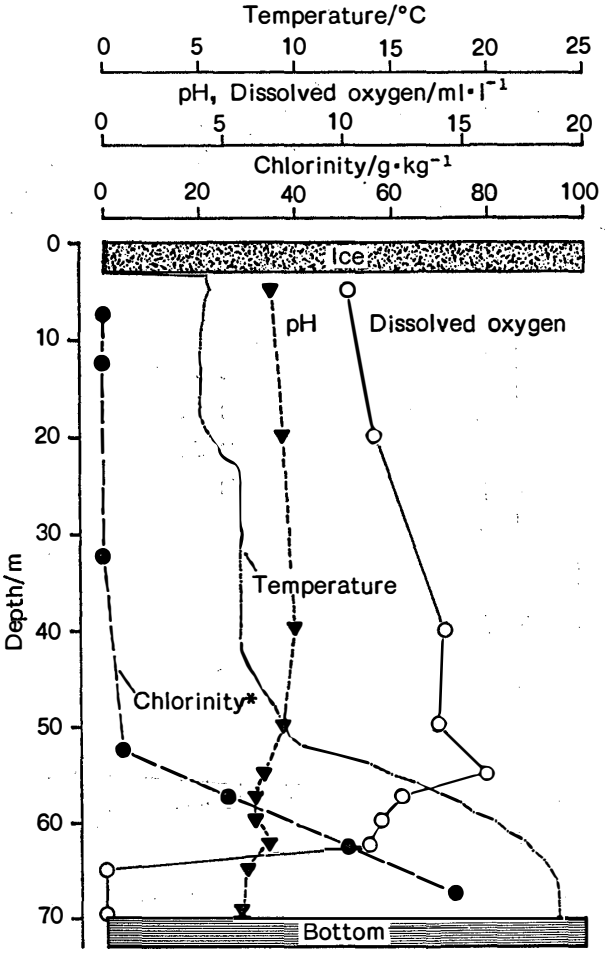

*Fig. 1. Physicochemical properties of the water of Lake Vanda of southern Victoria Land, Antarctica. * December 13–14, 1976 (after MATSUMOTO et al., 1984).*

at the respective depths (Fig. 1). Chlorinity increased with depth and attained to 72 g/kg at the bottom, indicating that the lake water is strongly stratified except for the depths described above. Dissolved oxygen contents above a depth of 62.5 m were near saturated or supersaturated (10–15 m*l*/*l*), while those below were anoxic and hydrogen sulfide was found. The dissolved oxygen peak at a depth around 55 m may be attributable to photosynthetic activity. The pH value of the surface water was 7.2, increased slightly to a depth of 40.0 m (7.5) but decreased to the bottom (5.9).

3.2. TOC, DOC

The TOC values between depths of 5.0 and 55.0 m were near constant, reflecting the possible circulation of lake water, but rose abruptly from a depth of 57.5 m to the bottom and attained the maximum value of 48 mgC/*l* (Fig. 2). This profile is similar to that of the previous study, although the maximum value was much higher than the previous results (25 mgC/*l*; MATSUMOTO *et al.*, 1984). It may be attributable to the differences of sampling depths: the previous samples have been collected on 13–14 December 1976 at the maximum depth of 66.4 m, while the present samples were taken at the maximum depth of 69.9 m. Indeed, the TOC values of this study are consistent with those of the previous study as shown in Fig. 2.

The DOC values were determined between depths of 55.0 m and 69.0 m, increased

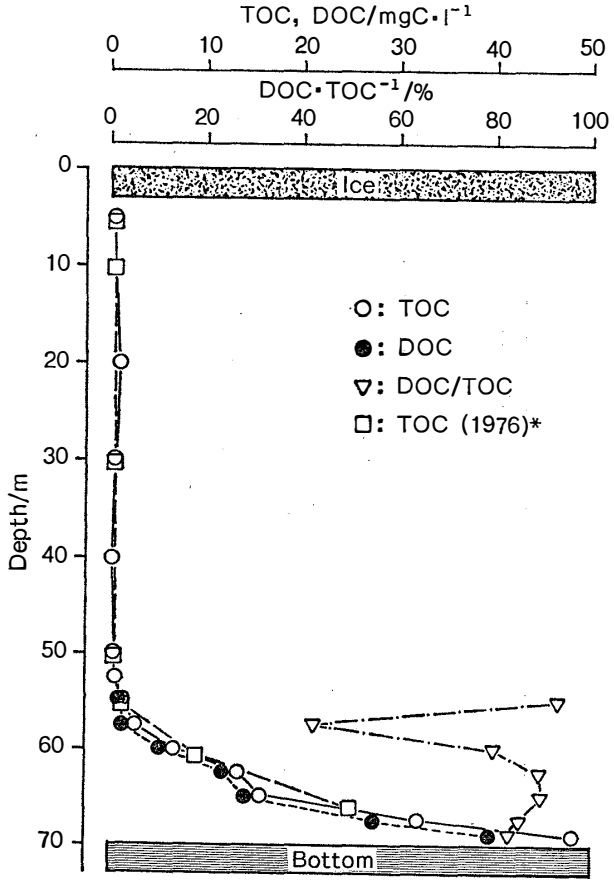

*Fig. 2. Vertical distribution of TOC and DOC in the water of Lake Vanda. * December 13–14, 1976 (after MATSUMOTO et al., 1984).*

with depth as in the case of the TOC values, and reached the maximum value of 39 mgC/*l* at the bottom (Fig. 2). The DOC/TOC percent ratios revealed, however, the minimum value at a depth of 57.5 m, suggesting high abundance of particulate planktonic materials as discussed below. High TOC and DOC contents at the lake bottom can be explained by the accumulation of refractory organic matter during a long period of time after degradation of labile organic constituents by microorganisms in the warm anoxic bottom water (MATSUMOTO *et al.*, 1984).

3.3 Hydrocarbons

Capillary gas chromatogram of the hydrocarbon fraction from the bottom sediment is shown in Fig. 3. Normal alkanes ranging in carbon chain length from nC$_{14}$ to nC$_{29}$ were found on the gas chromatogram with the dominance of odd-carbon numbers, along with alkenes C$_{17:1}$ (carbon chain length: number of unsaturation), C$_{20:1}$ and hop-22(29)-ene (30-triterpene) as well as branched alkanes bC$_{17}$, bC$_{18}$, bC$_{19}$ and bC$_{20}$. Interestingly, long-chain *n*-alkanes (nC$_{20}$–nC$_{29}$) were identified in the sample. The structures of bC$_{18}$ and bC$_{19}$ have been tentatively identified to be 2,6-dimethylhexadecane and 2,6,10-trimethylhexadecane, respectively (MATSUMOTO *et al.*, 1984).

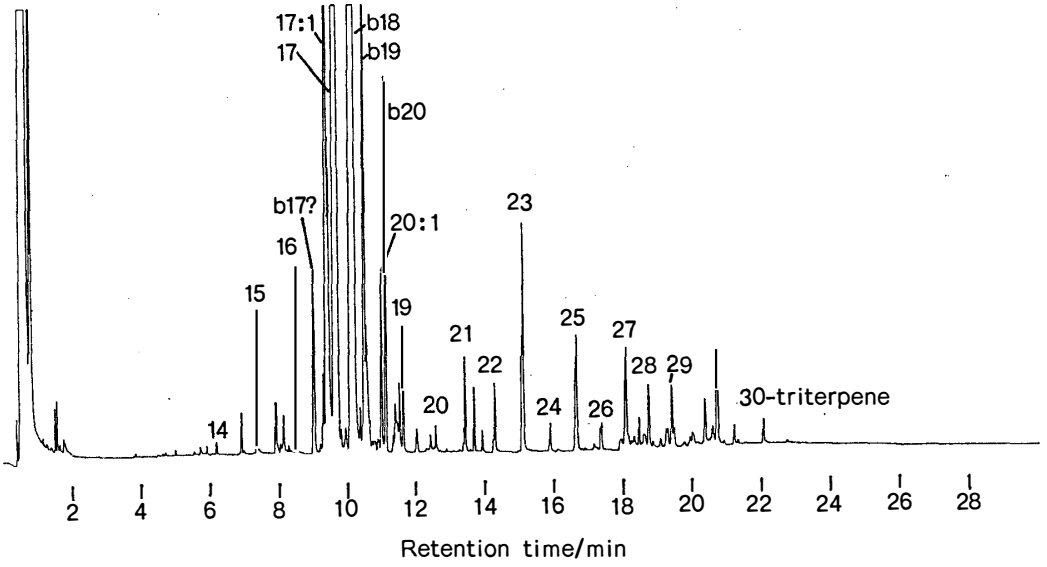

Fig. 3. Capillary gas chromatogram of the hydrocarbon fraction from the bottom sediment of Lake Vanda. Arabic figures on the peaks denote carbon chain length of n-alkanes. b: Branched alkanes. m: n=carbon chain length: number of unsaturation.

The concentrations of hydrocarbons in the oxic layer were extremely low and could not be found throughout the water column (5.0–62.5 m). The major hydrocarbons found in the bottom water (67.5 and 69.0 m) and the sediment were bC$_{18}$, nC$_{17}$ and bC$_{19}$ (Table 1). The most dominant component was bC$_{18}$ in all the samples as reported before (MATSUMOTO *et al.*, 1984). The bC$_{18}$/nC$_{17}$ and bC$_{19}$/nC$_{17}$ ratios at a depth of 57.5 m were 9.9 and 0.60, respectively, and decreased markedly with depth and attained to 1.4 and 0.083, respectively in the sediment. These results indicate that their source microorganisms are much different between the bottom water and the sediment.

Table 1. Hydrocarbons found in the particulate matter from the bottom waters and sediment of Lake Vanda, Antarctica.

Sample	Total conc.	Major constituents (%)				bC_{18}/nC_{17}	bC_{19}/nC_{17}
		nC_{17}	bC_{18}*	bC_{19}*	$bC_{20:1}$		
Water							
67.5 m	0.12 μg/l	8.7	86.1	5.2	0.0	9.9	0.60
69.0 m	0.95 μg/l	17.7	76.2	3.9	0.0	4.3	0.22
Sediment							
69.9 m	2.9 μg/g	35.0	48.5	2.9	4.6	1.4	0.083

* bC_{18} and bC_{19} were tentatively identified to be 2,6-dimethylhexadecane and 2,6,10-trimethylhexadecane, respectively.

The lake bottom is anoxic, hence algae and cyanobacteria should be non-existent. No hydrocarbons were detected in the oxic layers in spite of the occurrence of a significant amount of fatty acids as discussed below. Algae and cyanobacteria are unlikely sources of our hydrocarbons found in the bottom water and the sediment. Therefore, these hydrocarbons can be attributable to microorganism such as bacteria and fungi. Long-chain *n*-alkanes may be due to fungi (WEETE, 1976). Hop-22(29)-ene was first identified in the sediment of Lake Vanda, but it has been detected in sediment samples from Ace Lake of the Vestfold Oasis (VOLKMAN *et al.*, 1986) and Lake Fryxell (MATSUMOTO *et al.*, 1987). Bacteria are the most likely source of this triterpene, because hopanoids are widely distributed in bacteria (OURISSON *et al.*, 1979). Of special interest is the occurrence of the branched alkanes, such as bC_{18} and bC_{19} other than commonly known isoprenoids as the principal constituents. Their occurrence in the bottom water and sediment indicates the presence of some unknown microorganisms in the lake bottom.

3.4. *Fatty acids*

Capillary gas chromatogram of the fatty acid fraction from the sediment sample is shown in Fig. 4. Normal saturated fatty acids in carbon chain length ranging from nC_{12} to nC_{30} were detected with the predominance of even-carbon numbers, together with branched (*iso* and *anteiso*) and even-carbon numbered unsaturated fatty acids. The presence of long-chain unsaturated fatty acids of $nC_{20:1}$, $nC_{22:1}$, $nC_{24:1}$, $nC_{26:1}$ and $nC_{28:1}$ was confirmed by their mass spectra. Hydrogenation results of the unsaturated fatty acids also support the validity of their identification. That is, after hydrogenation the unsaturated fatty acid peaks were disappeared and the peaks of *n*-saturated fatty acids were found on the chromatogram. *Iso*- and *anteiso*-branched, and long-chain *n*-unsaturated fatty acids in the lake were first isolated and identified by this study. Unsaturated fatty acids of $nC_{16:n}$ and $nC_{18:n}$ were comprised of mono-, di- and trienoic acids, whereas the long-chain unsaturated acids were all mono-enoic ones. These long-chain unsaturated fatty acids were not found in the water column. The major constituents of the acids in the water column were nC_{16}, nC_{18}, $nC_{16:n}$ and/or $nC_{18:n}$ in all the samples.

The contents of short- (nC_{12}–nC_{19}) and long-chain (nC_{20}–nC_{28}) *n*-saturated, branched and unsaturated fatty acids in the particulate matter between depths of 5.0 and 50.0 m were near constant, but increased abruptly with depth and attained the maximum

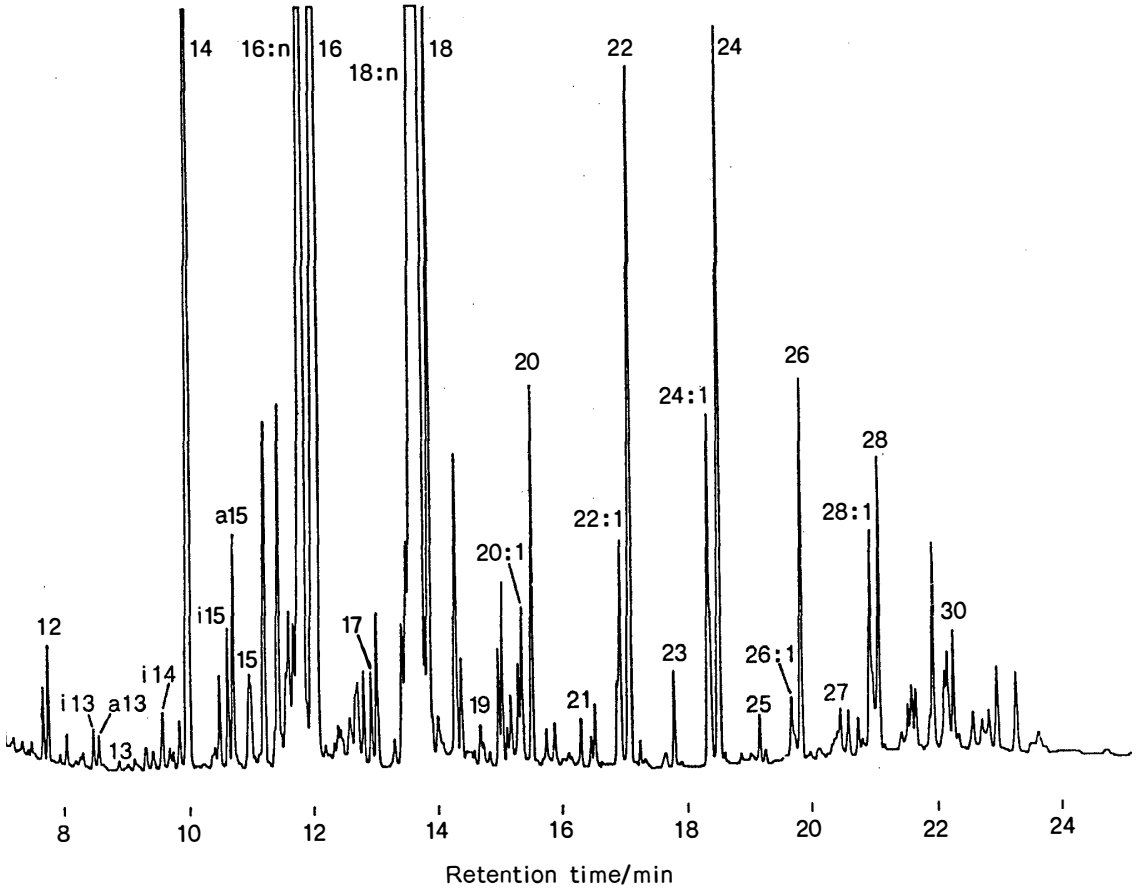

Fig. 4. Capillary gas chromatogram of the fatty acid fraction from the bottom sediment of Lake Vanda. Arabic figures on the peaks indicate carbon chain length of n-saturated fatty acids. i and a=iso- and anteiso-branched fatty acids, respectively. $C_{m:n}$ *=carbon chain length: number of unsaturation.*

values at a depth of 57.5 m, then decreased quickly to the bottom (Fig. 5). The increase of the contents of the fatty acids is mainly due to the increase of unsaturated and short-chain *n*-saturated fatty acids. These results are generally similar to those of total fatty acids distribution (MATSUMOTO *et al.*, 1984), although the maximum value of the total fatty acids has been obtained at a depth of 55.4 m. It is most likely that the maximum fatty acids peak has been missed in the previous study, because the sampling interval was rough and no sample around a depth of 57 m was collected. In addition, increasing lake depth during these 9 years due to the increase of inflow water through the Onyx River might also be responsible for the difference of the maximum fatty acids depth. That is, the maximum lake depths in December 1976 and in January 1986 were 68.5 and 69.9 m, respectively.

According to VINCENT *et al.* (1981), the maxima of photosynthesis and DNA synthesis in the lake have been obtained at about 57 m deep. Thus our fatty acids contents reflect probably the abundances of photosynthetic plankton. Numerous planktonic forms, generally less than 20 μm in size, including cocoid blue-green algae (cyanobacteria) and phytoflagellates have been found in the water column (GOLDMAN *et al.*, 1967). They must be important as primary producers in the lake. At this

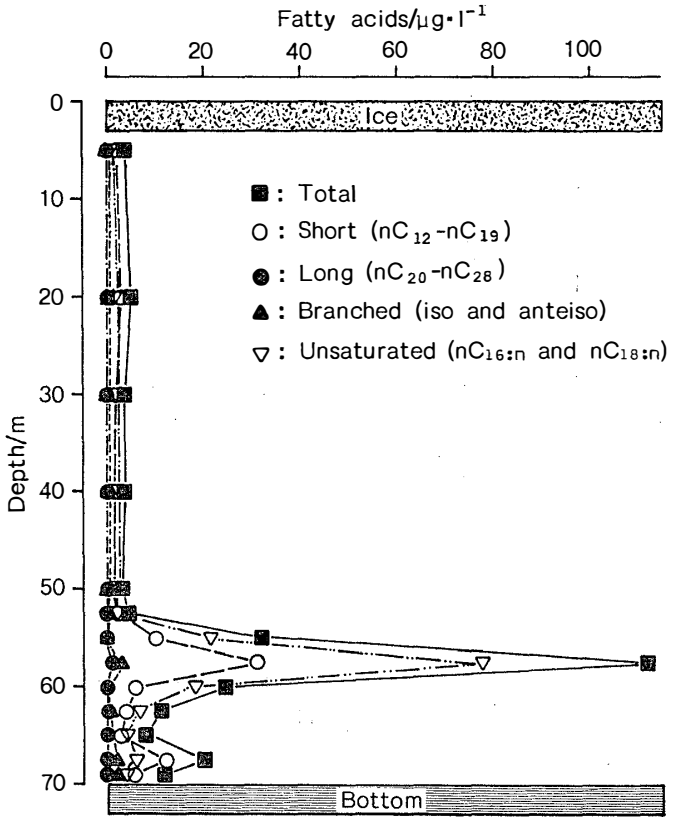

Fig. 5. Vertical distribution of the particulate fatty acids in Lake Vanda.

depth the maxima of NO_2-N and NO_3-N were also observed (Fig. 6). Consequently, around this depth (57 m) is the most biologically active layer throughout the water column. The supply of nutrients from the bottom may be important for the activity of photosynthetic plankton rather than *in situ* water temperature and light intensity as suggested by VINCENT (1981).

The ratio of the particulate fatty acid-carbon (PFAC) to TOC contents for depths between 5.0 and 52.5 m ranged from 0.4 to 0.9%, rose sharply with depth and reached the maximum value of 3.0% at a depth of 57.5 m, then decreased abruptly to the bottom (less than 0.1%, Fig. 7). The high PFAC/TOC ratio at a depth of 57.5 m should be mainly due to active photosynthetic plankton, while the extremely low values in the bottom water reflect the abundance of non-living organic matter, *viz.* the accumulation of refractory organic matter. This profile is similar to that of the total fatty acid-carbon/TOC ratios, though the PFAC/TOC ratios were generally low (MATSUMOTO *et al.*, 1984).

The composition of fatty acids in the particulate matter showed that short-chain *n*-saturated and unsaturated fatty acids constituted of the major portions at all the depths (Fig. 8). The high abundances of labile unsaturated fatty acids between depths of 30 and 62.5 m revealed that most of fatty acids were due to living plankton. Branched acids were relatively abundant in both the upper and bottom layers, reflecting the abundances of bacterial populations, because it is well known that branched acids are dominant in bacterial lipids (*e.g.*, LEO and PARKER, 1966; KANEDA, 1967).

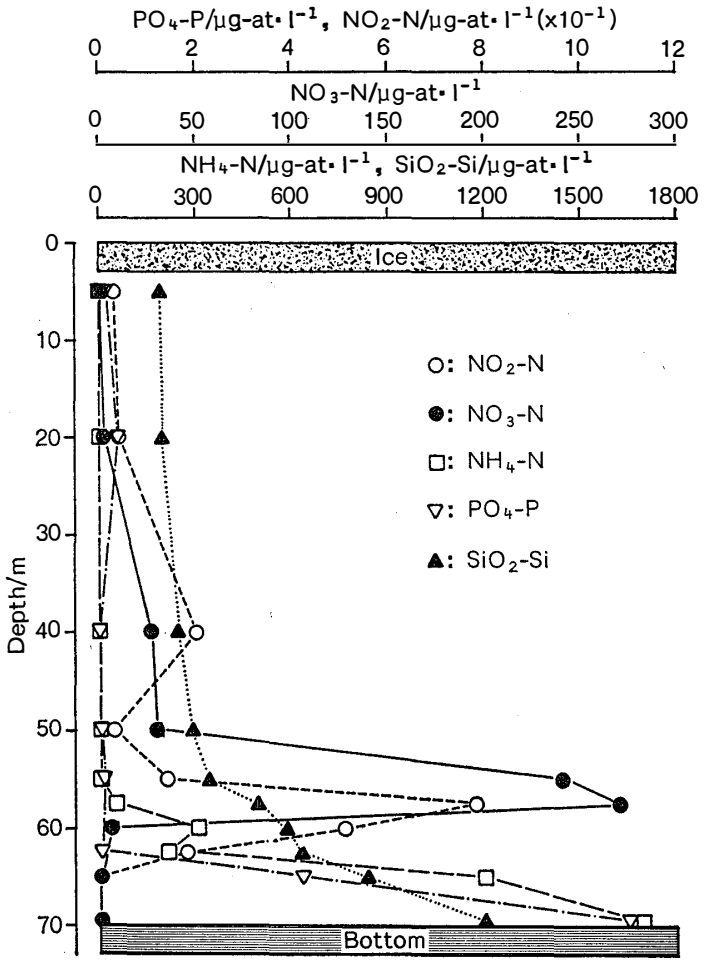

Fig. 6. Vertical distribution of nutrients in Lake Vanda.

This is consistent with the total bacterial numbers determined by acridine orange epifluorescence direct count method (TAKII *et al.* 1986; KONDA *et al.*, 1987). The composition of total fatty acids was also similar to that of the particulate fatty acids (Fig. 9; MATSUMOTO *et al.*, 1984).

Long-chain *n*-saturated fatty acids were not abundant throughout the water column, but significant amounts of these acids were found in the sediment sample. These results indicate that source organisms of fatty acids in the water column and the sediment are quite different. Of special interest is the occurrence of long-chain *n*-saturated fatty acids in the bottom sediment in relation to their source organisms, because vascular plants are absent in the studied areas. Long-chain *n*-saturated fatty acids have also been found in sediment samples from Lake Joyce of the McMurdo Oasis, Lakes Ô-ike, Itiziku and Skallen Ôike in the Syowa Oasis (MATSUMOTO *et al.*, 1981; MATSUMOTO, 1987) and Ace Lake (VOLKMAN *et al.*, 1987). Microorganisms such as fungi and bacteria are believed to be sources of these long-chain *n*-saturated fatty acids in Antarctic lakes (MATSUMOTO *et al.*, 1987), although VOLKMAN *et al.* (1987) suggested bacteria as their sources. These results suggest, *vice versa*, that microorganisms are important sources of long-chain *n*-saturated fatty acids other than the waxes of vascular plants in environments in both the mid and lower latitudes.

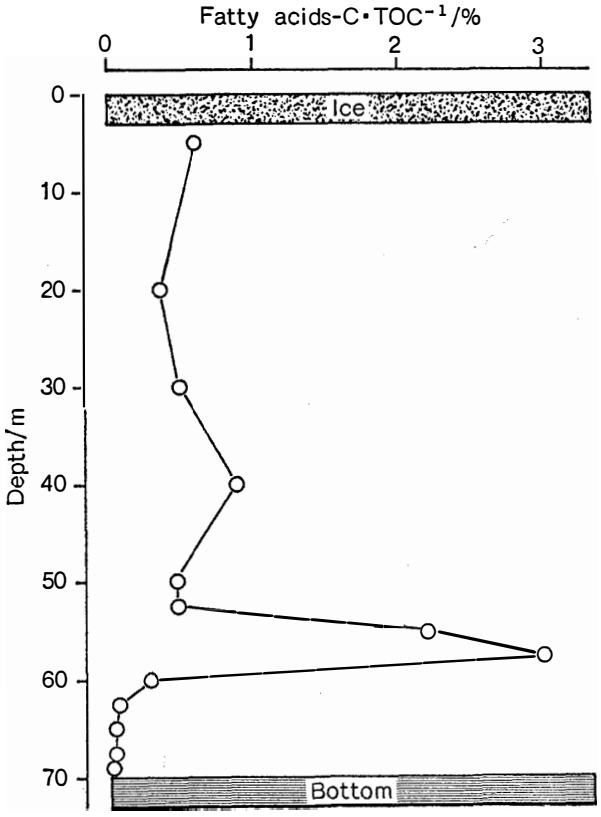

Fig. 7. Vertical distribution of the particulate fatty acid-carbon/TOC ratios in Lake Vanda.

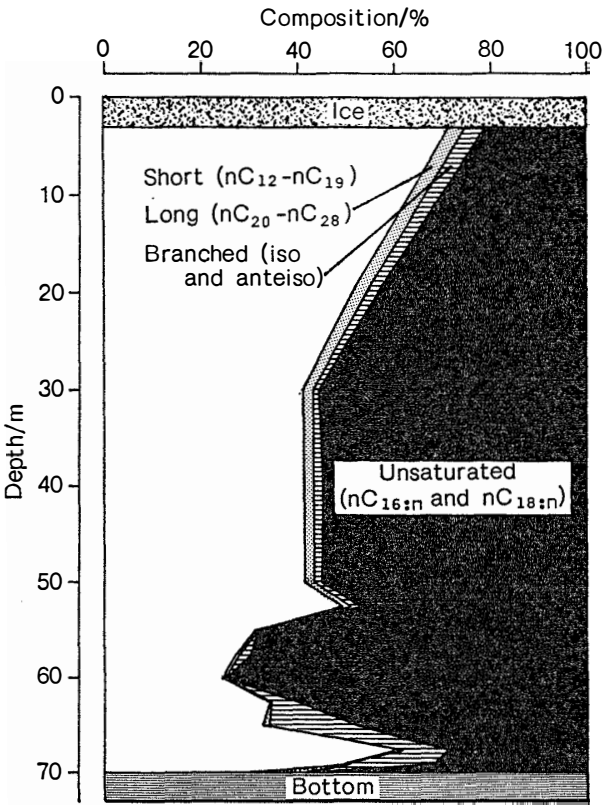

Fig. 8. Vertical distribution of the particulate fatty acid composition in Lake Vanda.

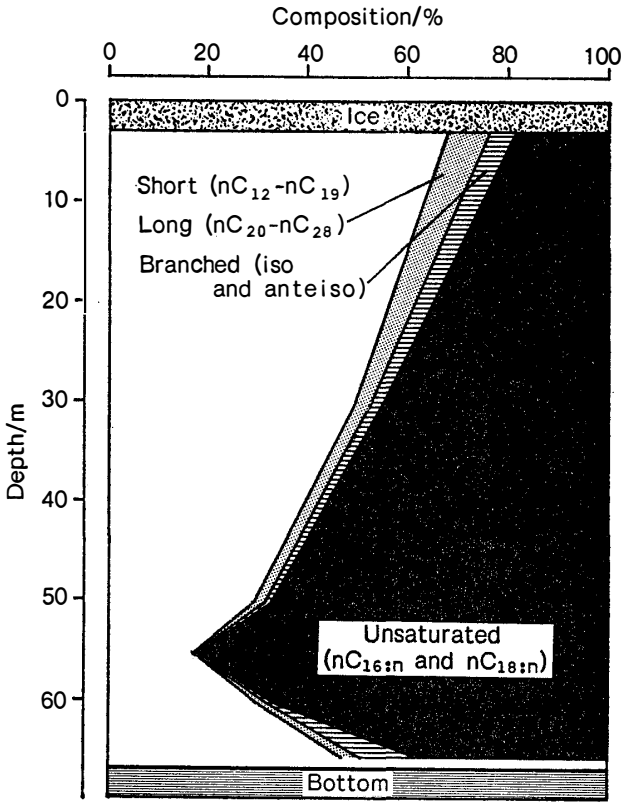

Fig. 9. Vertical distribution of the total fatty acid composition in Lake Vanda (data from MATSUMOTO *et al., 1984).*

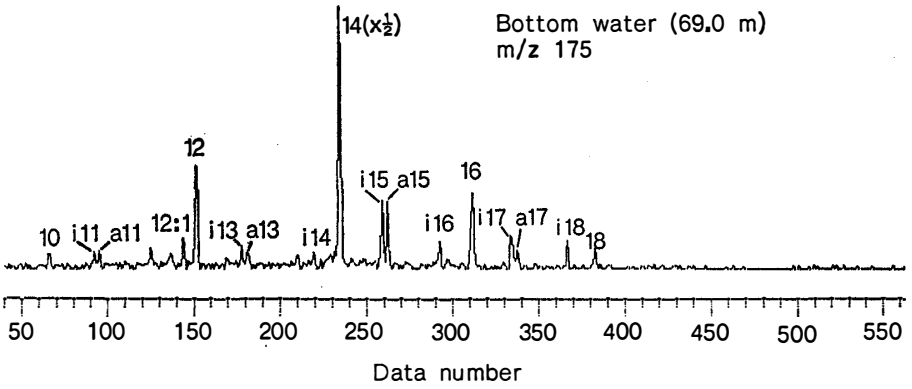

Fig. 10. Capillary mass chromatogram of the 3-hydroxy acid trimethylsilyloxy ether methyl esters (m/z 175) from the bottom water (69.0 m) of Lake Vanda. Arabic figures on the peaks denote carbon chain length of normal 3-hydroxy acids. i and a=iso- and anteiso-branched 3-hydroxy acids, respectively. $C_{m:n}$=carbon chain length: number of unsaturation.

3.5. Hydroxy acids

3-Hydroxy acids were studied only in the bottom water (67.5 and 69.0 m) and sediment samples. Figure 10 illustrates the capillary mass chromatogram of the 3-hydroxy acid trimethylsilyloxy ether methyl esters (m/z 175) from the bottom water (69.0 m). Normal 3-hydroxy acids having carbon chain length from nC_{10} to nC_{18} were found in the bottom water sample with the dominance of even-carbon numbers,

Table 2. 3-Hydroxy acid composition for the particulate matter from the bottom waters and sediment of Lake Vanda, Antarctica (%).

Sample	Short*	Long**	*Iso*	*Anteiso*	Unsaturated
Water					
67.5 m	71.0	0.0	19.6	9.4	0.0
69.0 m	74.8	0.0	12.8	9.6	2.8
Sediment					
69.9 m	34.3	45.9	9.6	4.1	6.1

* Short-chain *n*-saturated 3-hydroxy acids (nC_{10}–nC_{19}).
** Long-chain *n*-saturated 3-hydroxy acids (nC_{20}–nC_{26}).

together with mainly odd-carbon numbered *iso*- and *anteiso*-branched, and $C_{12:1}$ monounsaturated 3-hydroxy acids. The most dominant component is normal 3-hydroxy-C_{14} acid. The composition of 3-hydroxy acids in the sediment sample was much different from that of the bottom water (Table 2). Especially long-chain 3-hydroxy acids (nC_{24} and nC_{26}) were present only in the sediment sample as the major components. These results indicate again that the source organisms in the bottom water and the sediment are significantly different.

3-hydroxy acids are widely distributed in microorganisms involving bacteria, fungi and yeasts, and vascular plants, but little is known of their occurrence in cyanobacteria and algae (*e.g.*, MATSUMOTO and NAGASHIMA, 1984). In our sample vascular plants are unlikely sources. Fungi including *Aspergillus* spp. and *Penicillium* spp., yeast, actinomycetes and bacteria have been detected in both the water column and sediment of the lake (SUGIYAMA *et al.*, 1967; GOTO *et al.*, 1969; TAKII *et al.*, 1986). Thus 3-hydroxy acids may have come from various microorganisms, such as bacteria, fungi and yeasts in the lake bottom. Also cyanobacteria settling through the water column are possible sources.

4. Conclusions

High TOC and DOC contents of the bottom water of Lake Vanda revealed that concentration of refractory organic matter has occurred during a long period of time after microbial degradation of labile organic constituents. The maximum content of the particulate fatty acids was obtained at a depth of 57.5 m, reflecting probably the abundance of photosynthetic plankton. Around this depth is the biologically most active layer in the water column of the lake. The composition of organic constituents in the water column and the sediment was much different, showing the difference of source organisms. Vertical profile of branched fatty acid contents was consistent with distribution of total bacterial number. Branched alkanes as well as long-chain *n*-saturated fatty acids and 3-hydroxy acids were found in the sediment sample. They are believed to be due to certain microorganisms, such as fungi and bacteria.

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