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COMPARISON OF MICROBIAL COMMUNITY STRUCTURE IN SURFACE SEDIMENTS OF SAROMA KO LAGOON WITH SETO INLAND SEA IN WINTER

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Abstract: Microbial community structure in surface sediments of Saroma Ko lagoon, as estimated by phospholipid ester-linked fatty acids (PLFA), has been compared with eutrophic bays (Osaka Bay and Hiroshima Bay) and a lake (Lake Kojima) in the Seto Inland Sea of Japan during winter. Sixty PLFAs were identified in sediments. The abundant PLFAs included 14:0, i15:0, 16:1d9c, 16:1d11, 16:0, 10Me16:0, a17:0, 18:1d9, 18:1d911, 18:0 and 20:5. Biomarker fatty acids of sulfate reducing bacteria (SRB) were also detected. High amounts of fatty acids in the range of C_{12-19} demonstrated that the prokaryotes were a significant component of sediment organic matter. The mean total PLFA concentration varied from 0.6 (Osaka Bay) to 28.6 μg/g dry weight of the sediment (Saroma Ko lagoon). High total PLFA concentration in the sediments of Saroma Ko lagoon indicated significantly high amounts of microbial biomass.

From the presence of the bacterial biomarker fatty acids in sediments, the distribution of different microbial groups in sediments can be defined. In sedimentary microbial community structure of the study areas, the relative dominance of the microbial groups is in the following descending order: 1. Aerobic prokaryotes and eukaryotes, 2. Gram-positive bacteria and anaerobic bacteria, 3. SRB and other anaerobic bacteria, 4. Microeukaryotes. The aerobic prokaryotes and eukaryotes were dominantly present in Saroma Ko lagoon, whereas microeukaryotes, and gram-positive bacteria and anaerobic bacteria were abundantly present in Hiroshima Bay and Osaka Bay. The characteristic fatty acids of SRB and other anaerobic bacteria in Lake Kojima were higher than in other areas. The variation in the relative proportion of these microbial groups in sediments has been discussed in relation to the environmental conditions and pollution.

1. Introduction

Microbial communities in sediments play an important role in the mineralization of organic matter and degradation of pollutants. It is imperative to characterize the microbial community structure for understanding their role in these processes. Since the traditional methods for determining the diversities of microbial communities are limited only to cultured microorganisms, efforts have been made to develop methods to characterize microbial communities which do not necessitate culturing of microorganisms. One of the chemical methods, lipid analysis, has

proved very useful in microbial taxonomy and identification. This approach allows comparative analysis of microbial communities in various regimes and can also be used to monitor changes in microbial community composition in response to perturbation. Lipid analysis, especially of phospholipid ester-linked fatty acids (PLFAs), has been extensively employed in sediments of different environmental regimes (BAIRD and WHITE, 1985; BAIRD *et al.*, 1985; BOBBIE and WHITE, 1980; MANCUSO *et al.*, 1990; RAJENDRAN *et al.*, 1992a, b, c, d, 1993a, b, 1994; WHITE *et al.*, 1979). WHITE (1988) reviewed the validations of the PLFA analysis to determine the microbial community structure in sediments.

Research works were carried out in the first year sea ice region in Saroma Ko lagoon in Hokkaido, Japan as part of the Japan–Canada Saroma–Resolute (SARES) program. As part of the program, sediment samples were collected from Saroma Ko lagoon in February 1992 to know the microbial community structure under the first-year ice by PLFA analysis. The objectives of the present study are to determine the microbial community structure in sediments of Saroma Ko lagoon, and to compare with the microbial community structure in other eutrophic areas such as Osaka Bay, Hiroshima Bay and Lake Kojima in Japan. In Seto Inland Sea of Japan, Osaka Bay and Hiroshima Bay are reported to be highly contaminated by pollution and eutrophication. As a result of the acute eutrophication problem in Lake Kojima, the lower layer of the water column in certain parts of the lake is anoxic throughout the year. Comparison of the microbial community structure in these different environmental regimes will provide useful information about the response of microbial communities.

2. Materials and Methods

2.1. Sampling

Sediment samples were collected from Saroma Ko lagoon in the northeastern part of Hokkaido, Japan in February 1992 (Fig. 1). The surface of the lagoon was covered by fast ice (30 cm thickness). The average depth of the lagoon is about 8 m. After cutting the surface ice layer (1×1 m), surface sediment samples were collected by using an Ekman grab sampler. The top 2 cm of the sediment sample was sampled and stored at -20°C until fatty acid analysis. Sediment samples were collected in winter from Hiroshima Bay, Osaka Bay and Lake Kojima (Fig. 1) and were analyzed for PLFA composition. The description of these study areas and the sampling procedure have been extensively described earlier (IMAMURA, 1991; RAJENDRAN *et al.*, 1992c, 1994).

2.2. PLFA analysis

Freeze-dried sediment samples were used for lipid extraction. Lipid extraction was carried out as described elsewhere (BAIRD and WHITE, 1985). The extracted lipid was fractionated into neutral, glyco- and phospholipids by using silicic acid column chromatography. The phospholipid fraction was converted into fatty acid methyl esters by mild alkaline methanolysis (WHITE *et al.*, 1979). Thin layer chro-

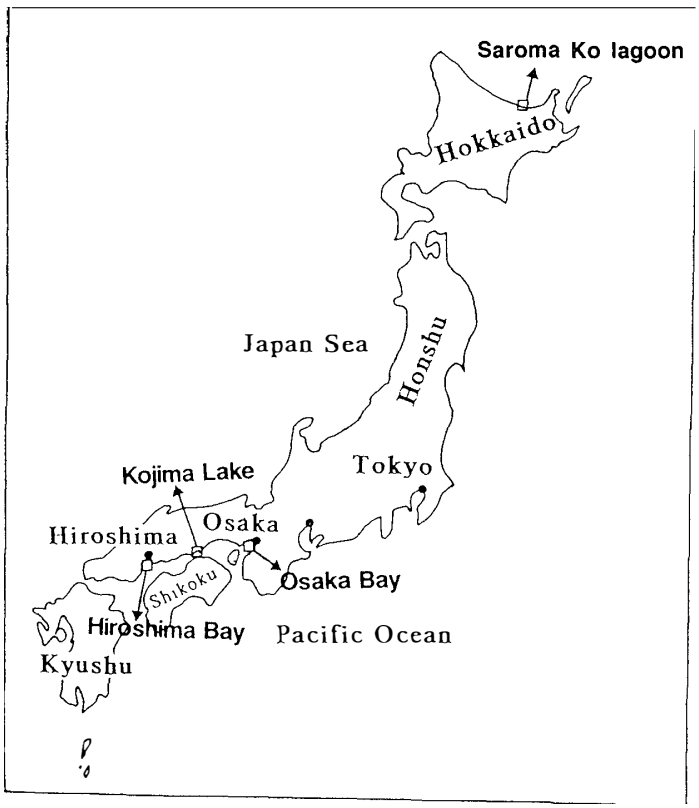

Fig. 1. Map showing the location of Hiroshima Bay, Osaka Bay, Lake Kojima and Saroma Ko.

matography was employed to purify the methyl esters, as described by RAJENDRAN *et al.* (1992c).

2.3. Gas chromatography (GC) and GC-mass spectrometry (GC-MS)

PLFAs were separated with a Hewlett Packard 5890 GC equipped with a FID and 25 m cross-linked 5% phenyl methyl silicone capillary column (0.2 mm i.d.). The sample was injected using an auto sampler (HP 7673A) and the injection was splitless at 250°C for 30 s. The oven temperature was programmed to be 50°C for 1 min, to increase at a rate of 20°C min^{-1} for 3 min, to increase at a rate of 3°C min^{-1} to 300°C and then to be isothermal for 10 min. Helium was used as the carrier gas. Authentic fatty acid methyl ester standards (Supelco Inc., U.S.A.) were used for fatty acid identification. Methylnonadecanate was used as the internal standard. GC-MS was performed with an HP 5890 instrument equipped with an HP 5970 series mass selective detector and the collected data were analyzed by using an HP chemstation. The abundances of all the identified fatty acid methyl esters were summed to yield total PLFA concentration. The positions and geometries of double bonds in the monoenoic fatty acids were determined by subjecting the fatty acids to dimethyl disulfide reaction (NICHOLS *et al.*, 1986), and then analyzed by GC-MS.

2.4. Statistical analysis

The means of the PLFA groups and the difference in the relative proportions of

the microbial groups of the study areas were compared by Tukey's Honestly Significant Difference (HSD) test. This test was carried out with a HITAC M-680H/180E program and a mainframe computer at Hiroshima University Information Processing Saijo Center, Higashi-Hiroshima.

2.5. Nomenclature

PLFA are designated as numbers of carbon atoms: the number of double bonds and the number of carbon atoms of the closest double bond from the carboxyl end (d) of the molecule are given. The prefixes "i" and "a" indicate iso and anteiso branching, respectively; the suffixes "c" and "t" indicate *cis* and *trans* geometry, respectively. Methyl branching is indicated as the position of the additional methyl carbon from the carboxylic end. "Cy" indicates the presence of a cyclopropane ring.

3. Results and Discussion

3.1. PLFA composition

Mean percentages and standard deviations of PLFA in surface sediments of the four study areas are presented in Table 1. Sixty PLFA were detected in the sediments collected from the study areas ranging from C_{12}-C_{25} (Table 1). The PLFAs 14:0, i15:0, 16:0, 16:1d9, 16:1d11, 10Me16:0, 18:0, 18:1d9, 18:1d11 and 20:5 were predominant (Table 1). They consist of saturated, branched, monounsaturated and polyunsaturated PLFAs. Mean percentages and standard deviations of different PLFA groups are shown in Table 2. Even numbered saturated PLFAs ranged from 24.3% (Hiroshima Bay) to 25.6% (Osaka Bay) of the total PLFA. The odd numbered saturated PLFAs were in the range of 2.9% (Osaka Bay) to 6.1% (Hiroshima Bay). The branched PLFAs (iso, anteiso and methyl branched PLFA) varied from 24.3% (Saroma Ko) to 30.9% (Lake Kojima), whereas the monounsaturated PLFAs (<20) ranged from 30.2% (Hiroshima Bay) to 41.6% (Saroma Ko). Polyunsaturated fatty acids (PUFAs), characteristic fatty acids of microeukaryotes, were in the range of 1.8% (Lake Kojima) to 8.2% (Hiroshima Bay). Low amounts of monounsaturated PLFAs (>20) and long chain fatty acids (>20) were detected in all of the areas investigated; they varied from 0.6% (Lake Kojima) to 1.0% (Osaka Bay), and from 0.9% (Lake Kojima) to 4.2% (Osaka Bay), respectively (Table 2). Sediments contained high proportions of biomarker fatty acids of microorganisms, as the fatty acids ranging from C_{12} to C_{19} are commonly present in microorganisms (LECHEVALIER, 1977). Large amounts of branched fatty acids in sulfate reducing bacteria (SRB), anaerobic bacteria and gram positive bacteria have been reported by many investigators (BOON *et al.*, 1977; EDLUND *et al.*, 1985; GILLAN *et al.*, 1983; KANEDA, 1977; TAYLOR and PARKES, 1983, 1985). Monounsaturated fatty acids have also been reported as biomarker fatty acids of prokaryotes and eukaryotes (GUCKERT *et al.*, 1987; PERRY *et al.*, 1979; VOLKMAN *et al.*, 1980; PARKES and TAYLOR, 1983). Significant amounts of both branched and monounsaturated fatty acids have also been detected in sediment samples of different environmental regimes (BAIRD and

Table 1. Mean percentages and standard deviations (in parentheses) of PLFA in sediments.

Fatty acid	Hiroshima Bay*	Osaka Bay**	Lake Kojima**	Saroma Ko lagoon**
12:0	— —	0.4 (0.6)	— —	0.2 (0.1)
i13:0	— —	— —	0.1 (0.1)	— —
a13:0	— —	0.8 (0.5)	0.1 (0.1)	— —
br14:0	— —	0.9 (0.8)	0.1 (0.1)	1.1 (0.7)
i14:0	0.8 (0.3)	0.9 (0.7)	1.3 (0.5)	1.1 (0.5)
14:1d7	0.1 (0.3)	0.6 (0.8)	0.5 (0.4)	0.3 (0.1)
14:1d9	— —	0.4 (0.6)	0.3 (0.3)	0.3 (0.3)
14:2	0.8 (1.1)	1.1 (1.0)	0.4 (0.6)	0.3 (0.3)
14:0	3.6 (1.0)	4.9 (2.7)	4.0 (0.9)	4.9 (0.8)
br15:1	0.1 (0.1)	0.2 (0.6)	0.6 (0.5)	0.6 (0.1)
br15:1	— —	— —	0.8 (0.4)	0.1 (0.1)
a15:1	1.2 (0.7)	— —	0.5 (0.1)	0.7 (0.2)
i15:0	5.5 (0.3)	5.1 (0.6)	6.3 (1.4)	3.9 (0.4)
a15:0	8.5 (1.1)	7.0 (1.0)	7.5 (1.6)	8.4 (1.0)
15:1	— —	— —	0.2 (0.2)	0.4 (0.2)
15:1d9	0.1 (0.1)	0.3 (0.4)	0.1 (0.1)	0.7 (0.0)
15:1	— —	0.1 (0.2)	0.2 (0.2)	0.1 (0.1)
15:0	2.2 (0.6)	1.5 (0.3)	1.4 (0.1)	2.6 (0.2)
br16:1	0.3 (0.2)	— —	0.2 (0.2)	0.3 (0.0)
16:2	1.3 (0.1)	— —	0.2 (0.3)	0.8 (0.3)
i16:0	2.4 (0.1)	2.3 (0.3)	1.9 (0.1)	1.8 (0.2)
16:1d6	— —	0.1 (0.2)	— —	— —
16:1d7	— —	1.1 (0.9)	— —	— —
16:1d9c	8.4 (3.7)	8.6 (2.0)	14.0 (2.9)	17.2 (2.1)
16:1d9t	1.2 (0.7)	1.2 (0.2)	2.1 (0.4)	1.5 (0.5)
16:1d11c	2.3 (0.1)	1.7 (0.2)	2.7 (0.9)	2.2 (0.2)
16:1d11t	0.1 (0.1)	— —	0.5 (0.2)	2.3 (0.9)
16:0	17.6 (1.2)	16.9 (2.4)	18.3 (1.4)	17.5 (1.2)
br17:1	1.4 (1.3)	0.2 (0.2)	1.7 (1.4)	1.2 (0.2)
10Me16:0	2.4 (1.1)	4.6 (0.6)	2.1 (2.0)	0.7 (0.3)
br17:1	0.4 (0.2)	0.2 (0.3)	0.2 (0.2)	0.7 (0.4)
br17:1	0.1 (0.2)	1.1 (0.8)	0.3 (0.2)	0.6 (0.0)
i17:0	1.3 (0.1)	1.3 (0.3)	0.9 (0.1)	0.8 (0.1)
a17:0	2.7 (0.4)	2.3 (0.4)	1.3 (0.3)	1.3 (0.3)
17:1d8	0.9 (0.1)	0.8 (0.3)	0.5 (0.1)	1.3 (0.6)
17:1d10	1.0 (0.6)	0.8 (0.4)	1.8 (0.4)	1.5 (0.1)
cy17:0	— —	0.1 (0.1)	0.2 (0.1)	0.1 (0.1)
17:0	3.9 (2.4)	1.4 (0.2)	2.1 (0.8)	1.3 (0.2)
18:2	2.5 (2.5)	1.8 (0.7)	0.6 (0.7)	1.2 (0.4)
18:1d9	7.4 (2.2)	1.3 (0.4)	6.6 (1.7)	4.6 (1.7)
18:1d11	8.3 (4.4)	5.2 (1.3)	7.3 (0.4)	8.9 (2.1)
18:1d11	0.4 (0.3)	9.1 (3.2)	0.4 (0.2)	0.3 (0.2)
18:1	0.1 (0.1)	0.6 (0.4)	0.2 (0.1)	— —
18:1d13	— —	— —	— —	0.2 (0.2)
18:0	3.2 (0.3)	3.4 (0.7)	2.3 (0.5)	1.9 (0.1)
br19:1	0.4 (0.1)	1.0 (0.4)	0.3 (0.1)	0.3 (0.3)
10Me18:0	0.5 (0.0)	0.3 (0.2)	0.6 (0.1)	0.2 (0.1)
i19:0	— —	— —	2.6 (1.0)	— —
cy19:0	0.7 (0.6)	0.7 (0.8)	0.8 (0.2)	0.4 (0.0)
20:5	1.9 (0.4)	0.4 (0.4)	0.2 (0.3)	1.0 (1.2)

*n=5; **n=6.

Table 1. (Continued)

Fatty acid	Hiroshima Bay*	Osaka Bay**	Lake Kojima**	Saroma Ko lagoon**
20:4	1.7 (0.4)	1.0 (0.2)	0.3 (0.6)	0.4 (0.1)
20:1d11	0.4 (0.1)	0.3 (0.3)	0.4 (0.3)	0.6 (0.2)
20:1	0.3 (0.2)	0.3 (0.4)	0.2 (0.1)	0.3 (0.1)
20:0	0.3 (0.3)	0.6 (0.2)	0.3 (0.1)	0.4 (0.1)
22:1	0.2 (0.3)	0.2 (0.3)	— —	— —
22:0	0.2 (0.1)	— —	0.1 (0.1)	0.3 (0.2)
24:0	0.1 (0.1)	— —	0.1 (0.1)	0.3 (0.1)
25:0	0.1 (0.1)	3.6 (3.0)	0.1 (0.2)	0 1 (0.0)

*n=5; **n=6.

Table 2. Mean percentages and standard deviations (in parentheses) of PLFA groups in sediments.

Fatty acid	Hiroshima Bay*	Osaka Bay**	Lake Kojima**	Saroma Ko lagoon**
Even numbered saturated PLFA (<19)	24.3 (2.1)	25.6 (4.0)	24.6 (1.9)	24.5 (1.4)
Odd numbered saturated PLFA (<20)	6.1 (2.3)	2.9 (0.4)	3.5 (0.7)	4.0 (0.3)
Branched PLFA	29.3 (2.5)	29.0 (3.3)	30.9 (2.2)	24.3 (2.0)
Monounsaturated PLFA (<20)	30.2 (7.5)	33.1 (6.1)	37.6 (4.1)	41.6 (2.1)
PUFA	8.2 (3.2)	4.3 (1.0)	1.8 (2.4)	3.8 (1.1)
Monounsaturated PLFA (>20)	0.7 (0.3)	1.0 (0.6)	0.6 (0.2)	0.8 (0.2)
Long chain PLFA (>20)	0.1 (0.6)	4.2 (2.9)	0.9 (0.4)	1.1 (0.3)
Total PLFA concentration (μg/g)	0.9 (0.5)	0.6 (0.3)	2.6 (0.8)	28.6 (14.0)
Monounsaturated PLFA (<20)/branched PLFA	1.1 (0.3)	1.2 (0.3)	1 2 (0.2)	1.7 (0.2)
t/*c*16:1d9	0.1 (0.1)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
i+a15:0/16:0	0.8 (0.1)	0.8 (0.2)	0.8 (0.1)	0.7 (0 1)

*n=5; **n=6.

WHITE, 1985; BAIRD *et al.*, 1985; GILLAN and HOGG, 1984; MANCUSO *et al.*, 1990; RAJENDRAN *et al.*, 1992a, b, c, d, 1993a, b, 1994). Although the characteristic fatty acids (PUFAs) of microeukaryotes were present in sediments, their relative proportions in PLFA profiles were quite low (Table 1 and 2). Large amounts of bacterial biomarker fatty acids and low amounts of microeukaryotic biomarkers in the PLFA profiles of the sediment samples indicate that the sedimentary microbial community structure was dominated by prokaryotes.

3.2. PLFA ratios

A perusal of literature showed that the branched fatty acids are characteristic of gram-positive bacteria and anaerobic bacteria, and monounsaturated fatty acids are abundantly present in aerobic prokaryotes and eukaryotes. The ratio of these two PLFA groups may indicate the relative dominance of these microbial populations in sediments. These ratios ranged from 1.1 (Hiroshima Bay) to 1.7 (Saroma Ko), indicating that the aerobic prokaryotes and eukaryotes were predominant in the sediments of the areas investigated (Table 2). The ratio of *trans* and *cis* isomers of 16:1d9 will also provide information about the nutritional status of the microorganisms, since these ratios tend to increase as the bacteria starve (GUCKERT *et al.*,

1986). These ratios in the present study areas were 0.1, suggesting that the microorganisms were not suffering from nutritional deprivation (Table 2).

3.3. *Total PLFA concentration*

The mean highest concentration of total PLFA (28.6 μg/g dry weight) was measured in the sediments of Saroma Ko; the lowest concentration of total PLFA (0.6 μg/g dry weight) was observed in the Osaka Bay sediments (Table 2). In the sediments of Lake Kojima and Hiroshima Bay, the mean total PLFA concentrations were 2.9 and 0.9 μg/g dry weight, respectively (Table 2). The variation in the total PLFA concentration in these areas indicates the difference in the microbial biomass as the total PLFA concentration in sediments is an indication of the microbial biomass (WHITE, 1988). The total PLFA concentration in the Saroma Ko sediments is significantly higher than the reported values in other coastal areas in Japan (RAJENDRAN *et al.*, 1992a, c, d, 1993a, 1994).

3.4. *Microbial community structure*

The reported biomarker fatty acids were used to classify the microbial groups and their relative distribution in microbial community structure (DOBBS and GUCKERT 1988a, b; FINDLAY *et al.*, 1990; RAJENDRAN *et al.*, 1993a, 1994). Based on the presence of biomarker fatty acids in microorganisms, they were classified into four microbial groups such as aerobic prokaryotes and eukaryotes (mono-unsaturated fatty acids), gram-positive bacteria and anaerobic bacteria (branched fatty acids in the range of C_{14} to C_{16}), SRB and other anaerobic prokaryotes (branched fatty acids in the range of C_{16} to C_{19}) and microeukaryotes (poly-unsaturated fatty acids) by FINDLAY *et al.* (1990). The PLFA profiles of the sediments were used to determine the relative dominance of the microbial groups in the microbial community structure and the variation in the community structure of the study areas. The distributions of different microbial groups in the sediments are shown in Fig. 2. In all the four areas, aerobic prokaryotes and eukaryotes (32.1 to

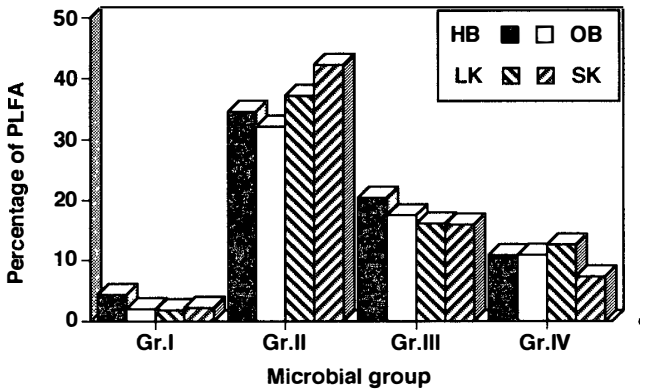

Fig. 2. Relative distribution of microbial groups in sediments of the study areas (HB: Hiroshima Bay; OB: Osaka Bay; LK: Lake Kojima; SK: Saroma Ko lagoon; Gr. I: Microeukaryotes; Gr. II: Aerobic prokaryotes and eukaryotes; Gr. III: Gram-positive bacteria and anaerobic bacteria; Gr. IV: SRB and other anaerobic bacteria).

42.3%) were the major group and microeukaryotes (1.8 to 4.3%) were the minor group (Table 3). The relative abundance of gram-positive bacteria and other anaerobic bacteria group (15.9 to 20.4%) was higher than the microbial group, SRB and other anaerobic bacteria (7.3 to 12.6%). Variations in the relative proportions of these microbial groups among the study areas reveals the difference in the microbial community structure in sediments.

The results of Tukey's HSD test also revealed the significant difference in PLFA groups and microbial groups of sediments (Table 4). The even numbered saturated PLFA (<19) and long chain saturated PLFA (>20) did not show significant difference among the study areas. The odd numbered saturated PLFA and PUFA were significantly present in sediments of Hiroshima Bay. Although the differences in branched PLFAs of Osaka Bay, Hiroshima Bay and Lake Kojima

Table 3. Mean percentages and standard deviations (in parentheses) of different microbial groups in the sediments of the study area.

Microbial group	Hiroshima Bay*	Osaka Bay**	Lake Kojima**	Saroma Ko lagoon**
Gr. I. Microeukaryotes	4.3 (1.1)	2.0 (0.6)	1.8 (1.9)	2.2 (1.1)
Gr. II. Aerobic prokaryotes & eukaryotes	34.6 (4.6)	32.1 (6.3)	37.2 (3.7)	42.3 (3.0)
Gr. III. Gram-positive & other anaerobic bacteria	20.4 (2.0)	17.5 (2.3)	16.1 (1.8)	15.9 (1.9)
Gr. IV. SRB & other anaerobic bacteria	10.8 (1.2)	10.9 (2.1)	12.6 (2.0)	7.3 (0.3)

*n=5; **n=6.

Table 4. Tukey's significant difference maps generated from Tukey's HSD test (HITAC M680H-VOS3), family-wise error rate set at alpha=0.05 for logarithmically transformed data); treatments for each PLFA group increase from right to left and those means connected by a common line are not significantly different.

	Low			High
PLFA group:				
Even numbered PLFA <19	HB	SL	LK	OB
Odd numbered PLFA <20	OB	LK	SL	HB
Branched PLFA	SL	OB	HB	LK
Monounsaturated PLFA <20	HB	OB	LK	SL
PUFA	LK	SL	OB	HB
Monounsaturated PLFA >20	LK	HB	SL	OB
Long chain PLFA >20	LK	SL	HB	OB
Microbial group:				
Microeukaryotes	LK	OB	SL	HB
Aerobic prokaryotes & eukaryotes	OB	HB	LK	SL
Gram-positive bacteria and anaerobic bacteria	SL	HB	OB	LK
SRB & anaerobic bacteria	SL	HB	OB	LK
Total PLFA concentration	OB	HB	LK	SL

HB: Hiroshima Bay; OB: Osaka Bay; LK: Lake Kojima; SL: Saroma Ko lagoon.

were not significant, these values were significantly higher than that observed in Saroma Ko. Monounsaturated PLFA in Saroma Ko were significantly higher that in Hiroshima Bay. The levels of total PLFA in sediments were significantly higher in Saroma Ko than in other areas. However, total PLFA concentration in Osaka Bay, Hiroshima Bay and Lake Kojima did not show significant difference, indicating the absence of difference in the microbial biomass of these three areas.

The difference in the relative proportions of PLFA characteristic of microbial groups in sediments of these study areas were also determined by Tukey's HSD test (Table 4). The characteristic fatty acids of microeukaryotes in Hiroshima Bay were significantly higher than that in Lake Kojima. The microbial group, aerobic prokaryotes and eukaryotes in Saroma Ko was more dominant than in Osaka Bay. Gram-positive and other anaerobic bacteria were significantly present in Hiroshima Bay and Osaka Bay. The fourth microbial group, SRB and other anaerobic prokaryotes, was significantly lower in Saroma Ko than in the other three areas. These significant differences in the relative proportions of PLFA groups and microbial groups indicate the variation in the composition and structure of the sedimentary communities.

From these results, it can be concluded that the relative proportions of microeukaryotes in Hiroshima Bay, aerobic prokaryotes and eukaryotes group in Saroma Ko, gram-positive and anaerobic bacteria in Hiroshima Bay and Osaka Bay, and SRB and other anaerobic bacteria in Lake Kojima were significantly higher than that observed in other areas. Low amounts of the microeukaryotic biomarkers (PUFAs) in sediments can be attributed to the environmental conditions. In polluted and contaminated sediments, the absence of microeukaryotic biomarkers has been reported (SMITH *et al.*, 1985; RAJENDRAN *et al.*, 1992b, 1993b). Similarly, low amounts of PUFAs in sediments that are subjected to environmental pollution have also been reported. The predominance of gram-positive bacteria and anaerobic bacteria in Osaka Bay and Hiroshima Bay might be due to organic pollution, as these two bays receive a larger amount of pollutants than the lakes. Significant amounts of SRB and other anaerobic bacteria in Lake Kojima could be attributed to the reduced condition of the sediments as they are reported to be euxinic with very low values of oxidation-reduction potentials and high values of organic matter, COD and sulfide. The relative predominance of prokaryotes and eukaryotes in Saroma Ko lagoon indicates that Saroma Ko lagoon may not have been exposed to the extent of pollution prevailing in other study areas. The variation in microbial community structure of the study areas may be due to the environmental parameters as the Saroma Ko lagoon experiences extremely low temperature than the other three study areas in winter.

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Saroma Ko, respectively.

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