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Sterols of sewage indicators in marine sediments of Jinhae Bay, Korea

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The Δ^5 sterols were measured in bed sediments of Jinhae Bay surrounded by industrial cities to assess the sewage-derived contamination. The total concentrations of total sterols ranged from 2.03 to 19.56 μg/g dry wt. The principal sterol was cholesterol with a contribution of more than 50% to total sterols. Coprostanol, providing an indication of long-term sewage loads, was found in all sediment samples and the concentrations were 0.03–3.86 μg/g dry wt, accounting for 1–26% to total sterols. The cluster analysis of sampling stations indicated that the sewage-derived contamination was localized in inner Masan Bay.

Key words: Sterol, Sediments, coprostanol, Sewage-derived contamination

INTRODUCTION

Municipal wastes are often discharged into the marine environment and it therefore becomes important to determine the distribution and fate of these contaminants in the aquatic systems. Excess quantities of untreated sewage could have detrimental effects in important fishing grounds and reduce the aesthetic quality of the bay. Fecal coliform bacteria, ammonia, or biodegradable organic matter have been used as tracers for effluent in the water column or sediments (Sherwin *et al.*, 1993; Seguel *et al.*, 2001). However, most approaches suffer from shortcomings such as lack of specificity or sensitivity, or the need to complete analyses within a few hours of discharge or collection (Goodfellow *et al.*, 1977). Coprostanol concentrations are unaffected by various treatment such as chlorination or aeration of overlying water and it persists in anoxic sediment (Venkatesan and Kaplan, 1990). Coprostanol, which is produced in the digestive tract of humans by enteric microbial reduction of cholesterol (McDonald *et al.*, 1983), is a major faecal sterol present in human waste (Brown and Wade, 1984; Sherwin *et al.*, 1993; Leeming and Nichols, 1996). Coprostanol has been shown to be an indicator of sewage pollution because this marker remains viable for some considerable time in the marine systems unlike bacteria (Grimalt *et al.*, 1990; Sherwin *et al.*, 1993). This approach can help in distinguishing between different sources of faecal pollution. There is no consensus among the researchers about how much coprostanol exists in sediments under natural, pristine conditions and which levels are necessarily pointing at perturbed, polluted sites (Gonzalez-Oreja and Saiz-Salinas, 1998). In this work, the distribution of Δ^5 sterols including coprostanol in sediments of Jinhae Bay was studied. The water quality of Jinhae Bay has deteriorated in recent year due to input of the increased terrestrial pollution load by intense economic activity. The inner bay is very anoxic. There was one previous study on coprostanol in the seawater investigated at three sites of Jinhae Bay (Kang, 1997). However, when faecal matter is introduced to aquatic environment, hydrophobic faecal sterols such as coprostanol are strongly bound to particulate matter and can be easily deposited into sediments (Hatcher and McGillivary, 1979). Therefore, it is important to assess the spatial extent of these contaminants deposited on the sediments. This study provides the first survey on coprostanol distributions as a sewage marker in sediments from the Korean coastal areas and these results will be useful in environmental management such as sewage plan for public health.

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MATERIALS AND METHODS

Study area

Jinhae Bay consists of several small embayments such as Masan Bay, Haengam Bay, Jindong Bay, Wonmun Bay and Gohyeon Bay with a total area of nearly 637 km^2. Average depths range from <5 m in the inner bay to 5–20 m in the central part. Jinhae Bay has been receiving a variety of wastes including municipal sewage and industrial wastewater for more than 30 years. Masan, Changwon and Jinhae cities surrounding Jinhae Bay are heavily populated areas. Total population around Jinhae Bay is about 1 million. Hundreds of industrial plants, which are mainly concentrated in Masan and Changwon cities, introduce significant amounts of organic pollutants. Deokdong sewage treatment plants have been built to serve nearby coastal cities (Lee and Kwon, 1994). The velocities of tidal currents are strong in the entrance channel of the bay, whereas the velocities are relatively weak in the western (below 30 cm/sec) and northern parts (below 10 cm/sec) of the bay (Kim *et al.*, 1994).

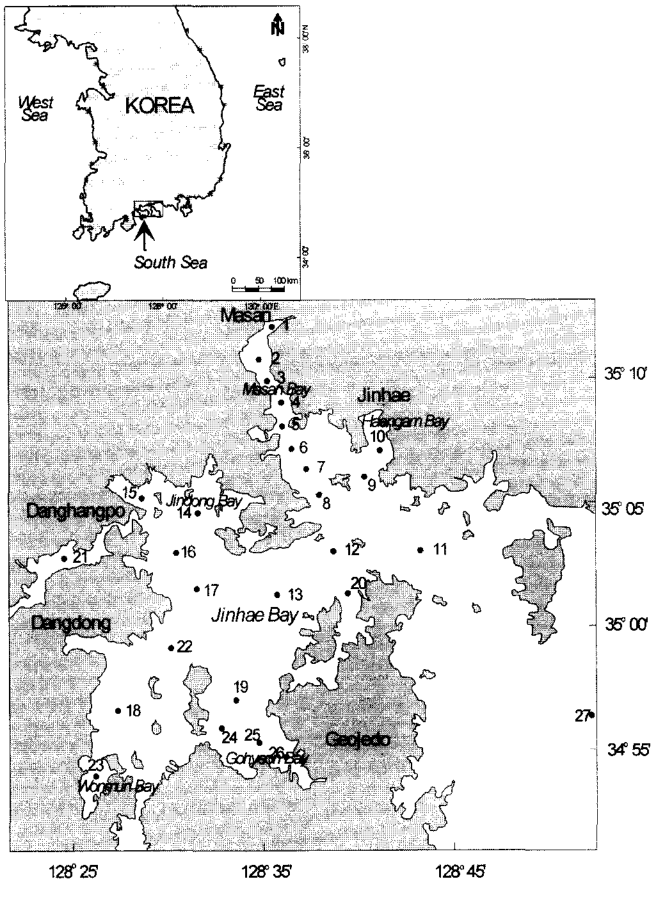

Fig. 1. Sampling stations on Jinhae Bay.

Sampling and Analysis

Surface sediments (ca. top 3–4 cm) were collected using box cores throughout Jinhae Bay in May and in August 2001 (Fig. 1). Each sediment sample was freeze-dried, ground and then sieved at 2 mm. Approximately 10 g of sediments were extracted with 20 mL of methylene chloride-chloroform (1:1) using mechanical shaking for 1 hour and extracts were centrifuged at 3000 rpm for 15 min. A surrogate standard (1-nona-decanol) was added to the samples prior to extraction. The supernatant was concentrated until almost dryness using Turbo Vap concentrator under 50°C and 11 psi and the solvent was exchanged into methylene chloride. The concentrated extracts (ca. 1 mL) were applied to the glass column with 10 g of silica gel. The sterol fraction was eluted with 50 mL of 10% methanol in chloroform after the removal of the less polar lipids using 50 mL of 40% hexane in chloroform. All solvents were pesticide or HPLC grade. The isolated sterols were derivatized with Sylon BFT (bis (trimethylsily) trifluoroacetamide (BSTFA): trimethylchlorosilane (TMCS), 99:1, Supelco), followed internal standard (5α-cholestanol) addition. Sterols were analyzed by Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector. The sterols were separated on a CP-Sil 8CB-MS column (30 m×0.32 mm i.d., 0.25 mm film thickness, Chrompack). The quantified and identified external sterols were coprostanol (5β-cholestan-3β-ol), epicholestanol (5α-cholestan-3α-ol), epicoprostanol (5β-cholestan-3α-ol), cholesterol and cholestanol (5α-cholestan-3β-ol). The sterol standards were purchased from Research Plus for coprostanol, epicoprostanol and epichoestanol, Fluka for 5α-cholestane, cholestanol and choesterol and Merck for 1-nona-decanol. The detector was set at 300°C. Oven temperature was programmed from 50°C to 210°C at 4°C/min, and to 280°C at 2°C/min, then to 320°C at 8°C/min, where it was held for 30 minutes. Recoveries of these sterols on the spiked reference materials were more than 90% and standard deviation of duplicate analysis was less than 10%. The detection limits for coprostanol, epicholestanol, epicoprostanol, cholesterol and cholestanol were 0.02, 0.01, 0.02, 0.05, 0.04 μg/g dry wt. respectively. Total organic carbon was determined by CHN analyzer (Perkin Elmer 2400).

RESULTS AND DISCUSSION

Distribution of sterols

The concentrations of sedimentary sterols can be seen in Table 1. The concentrations of total sterols were in the range of 2.03–10.77 μg/g dry wt in May and 2.10–19.56 μg/g dry wt in August. The concentrations of sterols among 16 stations except stations 2, 3 and 4 located inner bay showed no significant difference between May and August (t-test, $p>0.05$). The levels of sterols at stations 2 and 3 were lower in May than in August, while that at station 4 higher in May than in August. The dilutions by current and freshwater inflows were likely to be the major factors of difference between two months. The dilution factor in summer was reduced to 1/2 of dilution in spring (Kang *et al.*, 1999), while freshwater inflows were increased in summer (KMA, 2001). Coprostanol, cholesterol and cholestanol were found in all sediment samples. Epicholestanol and epi-coprostanol were also identified in almost all the sediments except one or two samples. Of the Δ^5 sterols, cholesterol was the principal compound, accounting for an average of more than 60% (1.16–7.69 μg/g dry wt). Coprostanol levels varied between 0.03 and 3.86 μg/g dry wt (1%–26%) depending on the sites. The detection of coprostanol in all samples indicated that sewage derived material is present in the sediment. Other identified steroids were cholestanol (0.20–2.75 μg/g dry wt; 8–29% of total sterols) and epicoprostanol (nd–1.78 μg/g dry wt; 0–23% of total sterols) with intermediate values and epicholestanol (nd–0.67 μg/g dry wt; 0–7% of total sterols) as minor components. The use of coprostanol

Table 1. Sterol concentrations (μg/g dry wt) in sediments from Jinhae Bay 2001

St.	Coprostanol		Epicholestanol		Epicoprostanol		Cholesterol		Cholestanol		Total sterol		OC[b] (%)
	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.	
1		3.86		0.67		0.94		7.05		2.30		14.82	
2	0.32	1.32	0.04	0.19	0.18	0.54	3.94	2.17	0.38	1.13	4.85	5.36	1.21
3	0.47	1.83	0.21	0.61	ND	1.78	5.40	5.15	1.19	2.75	7.27	12.12	
4	2.34	0.80	0.30	0.14	1.40	0.52	4.77	2.41	1.96	1.19	10.77	5.06	
5		0.91		0.28		0.61		3.31		1.31		6.42	1.87
6		0.92		0.24		0.45		3.18		1.29		6.08	1.14
7	1.49	1.10	0.20	0.24	0.91	0.67	3.38	2.25	1.64	1.23	7.61	5.48	1.65
8	0.21	0.45	0.05	0.10	0.11	0.31	1.32	2.76	0.34	0.62	2.03	4.24	1.55
9	0.16		0.07		0.19		2.79		0.56		3.76		
10		0.19		0.06		0.23		3.32		0.57		4.36	
11	0.16	0.16	ND[a]	0.04	0.38	0.36	3.41	4.34	0.37	0.51	4.33	5.42	0.96
12	0.15	0.15	0.06	0.03	0.32	0.36	3.74	3.92	0.48	0.53	4.74	5.00	1.27
13	0.18	0.09	0.07	0.15	0.50	0.48	3.62	3.55	0.57	0.52	4.94	4.78	1.63
14	0.26	0.20	0.17	0.12	0.35	0.27	2.44	2.88	1.29	1.28	4.50	4.75	1.65
15	0.13	0.24	0.08	0.15	0.34	0.40	5.82	2.36	0.91	1.29	7.29	4.43	2.08
16	0.11	0.15	0.07	0.21	ND	0.62	1.43	3.85	0.62	1.15	2.23	5.98	1.73
17	0.25	0.23	0.28	0.23	0.59	0.77	5.56	7.69	1.27	1.78	7.95	10.70	1.51
18	0.15	0.20	ND	ND	0.75	1.06	2.21	2.57	0.75	0.88	3.86	4.71	2.00
19	0.18	0.13	0.32	0.12	0.62	0.45	2.75	1.94	0.75	0.78	4.62	3.42	1.58
20	0.03	0.11	0.02	0.08	ND	0.48	2.15	2.28	0.31	0.35	2.51	3.29	1.38
21		0.18		0.11				4.47		0.85		5.60	
22		0.27		0.42		0.75		4.66		1.85		7.94	2.21
23		0.10		0.10		0.57		1.73		0.48		2.97	2.48
24		0.20		0.19		0.55		2.05		0.86		3.85	1.79
25		0.18		0.08		0.29		1.16		0.43		2.14	1.98
26		0.51		0.11		0.43		1.88		0.80		3.74	
27		0.09		0.04		0.31		1.65		0.20		2.29	

[a] ND=not detected

[b] OC=organic carbon

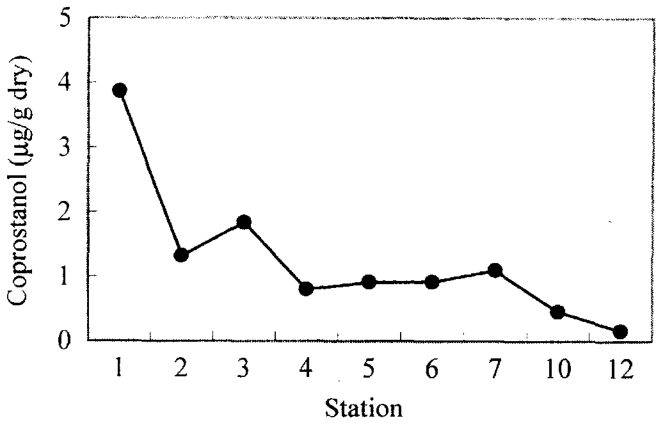

Fig. 2. Horizontal profiles of coprostanol concentration from inner site to outer site of Masan Bay in August 2001.

as faecal stanol is now a generally accepted approach for long-term investigation of sewages dispersion in the environment (Leeming *et al.*, 1996; Nichols *et al.*, 1996; Seguel *et al.*, 2001). Coprostanol concentrations reported in the literature for aquatic sediments range from below detection limits in remote areas (Hatcher *et al.*, 1977) to 293 µg/g dry wt at Kaiku sampling station in the Bilbao Estuary (Gonzalez-Oreja and Saiz-Salinas, 1998). The concentrations of coprostanol of Jinhae Bay were comparable to those of Santa Monica Basin (Venkatesan and Kaplan, 1990; 1.1–5.1 µg/g dry wt), Venice Lagoon, Italy (Fattore *et al.*, 1996; 0.04–4.41 µg/g dry wt) and the southeastern waters of Hong Kong (Chan *et al.*, 1998; 0.27–0.09 µg/g dry wt). Nichols *et al.* (1996) proposed that a level greater than 0.5 µg/g indicated significant sewage pollution. On this basis, higher levels found at stations 4 and 7 in May and at stations 1-7 and 26 in August were enough to point to significant sewage polluted sites. The concentrations of coprostanol appeared to decline progressively seaward (Fig. 2). The model application results on the nutrient and COD (chemical oxygen demand) also revealed that water quality concentrations in most of sites adjacent to land and river inflow are high, but rapidly decrease along the seaward direction (Cho and Chae, 1999). These results might reflect the direct input of untreated sewages being discharged into Masan Bay from the industrial cities via more than 10 small streams as well as treated sewages though sewage treatment plants have been built to serve nearby coastal cities such as Masan, Changwon and Jinhae. According to Cho and Chae (1998), the COD pollution loads of Jinhae Bay were 57% from the Masan and Changwon cities and 27% from multiport diffuser. The effluent from Deokdong sewage treatment plant discharges into Okgae site (station 8) close to the mouth of Masan Bay through the submerged multiport diffuser. The concentration of coprostanol at this site was low due to rapid dilution by strong current. From these results, the contribution of coprostanol from the ocean outfall does not seem to be predominant in the study area. Sedimentary coprostanol level obtained at the remote control site (station 27) of this study area was lower than that at a reference stations located in the open Adriatic Sea (0.2 µg/g dry wt; Sherwin *et al.*, 1993) and the southeastern waters of Hong Kong (0.34–.46 µg/g dry wt; Chan *et al.*, 1998). Theoretically, unpolluted sediments do not contain coprostanol. However, Hatcher and McGillivary (1979) suggested a threshold of ~0.01 µg/g even though there are no detailed literature data available on the levels of coprostanol in natural pristine coastal sediment. It means that sewage-derived contaminant is present in all samples from Jinhae Bay. Many authors reported that coprostanol partitioning favored the particulate phase according to its high particle affinity and physico-chemical properties characteristic of a neutral hydrophobic molecule (Brown and Wade, 1984) and there were a positive correlation between corostanol concentrations and organic carbon contents in sediment (Venkatesan and Kaplan, 1990; Sherwin *et al.*, 1993; Saiz-Salinas and Gonzalez-Oreja, 1997). However, the sediments of Jinhae Bay exhibited a poor correlation between the concentrations of coprostanols and total organic carbon contents (R^2=0.08) due to a similar distribution of total organic carbon content samong stations in Jinhae Bay (0.96-2.48%) with a mean of 1.67%.

Sterols ratios as pollution indexes

The sterols ratios are presented in Table 2. The interpretations based on the application of fecal stanols alone might easily be interfered with by other physicochemical factors, such as sediment organic carbon content and particle size distribution, because of the correlations among these parameters. The fecal sterol ratios in the sediments could be adopted as a better parameter for comparison and trend analysis purpose without being dependent on organic carbon fraction and particle size (Mudge and Lintern, 1999; Seguel *et al.*, 2001). The cop/total sterol ratios should reflect the degree of sewage contamination in the marine sediments. The reported values for cop/total sterols in the highly sewage-polluted surface sedi-

Table 2. The sterol ratios in sediments from Jinhae Bay in August 2001

Station	cop[a]/total sterol	5β[b]/ 5β+5α[c]	5β/ 5α+chol[d]	cop/chol	cop/ epi-cop[e]
1	0.26	0.63	0.41	0.55	4.13
2	0.25	0.54	0.40	0.61	2.43
3	0.15	0.40	0.23	0.36	1.03
4	0.16	0.40	0.22	0.33	1.54
5	0.14	0.41	0.20	0.27	1.48
6	0.15	0.41	0.20	0.29	2.06
7	0.20	0.47	0.31	0.49	1.64
8	0.11	0.42	0.13	0.16	1.48
10	0.04	0.25	0.05	0.06	0.82
11	0.03	0.24	0.03	0.04	0.45
12	0.03	0.23	0.03	0.04	0.43
13	0.02	0.15	0.02	0.03	0.18
14	0.04	0.14	0.05	0.07	0.75
15	0.05	0.16	0.07	0.10	0.60
16	0.03	0.12	0.03	0.04	0.25
17	0.02	0.11	0.02	0.03	0.30
18	0.04	0.19	0.06	0.08	0.19
19	0.04	0.14	0.05	0.07	0.28
20	0.03	0.23	0.04	0.05	0.22
21	0.03	0.17	0.03	0.04	0.00
22	0.03	0.13	0.04	0.06	0.36
23	0.03	0.17	0.04	0.06	0.17
24	0.05	0.19	0.07	0.10	0.37
25	0.09	0.30	0.12	0.16	0.63
26	0.14	0.39	0.19	0.27	1.19
27	0.04	0.30	0.05	0.05	0.28

[a] cop=coprostanol
[b] 5β=5β-cholestan-3β-ol
[c] 5α=cholestanol (5α-cholestan-3β-ol)
[d] chol=cholesterol (cholest-5-en-3β-ol)
[e] epi-cop=epi-coprostanol (5β-cholestan-3α-ol)

ments were in the range of 0.10–0.15 in New York Bight (Hatcher and McGillivary, 1979) and 0.28–0.42 in lagoonal sediments of Venice (Sherwin *et al.*, 1993). The cop/total sterols ratios at stations 1–7 and 26 of Jinhae Bay closer to potential sources of sewage treatment plant and municipal waste were comparable to those results, ranging from 0.14 to 0.26. This was well below the 0.37–0.38 described by Quemeneur and Marty (1994) as typical of raw sewage effluent. A decline in percent coprostanol seaward from inner sites of Masan Bay was noticed, suggesting the dilution by uncontaminated sediment. However it was difficult to assume that there were processes such as dilution of coprostanol by degradation of coprostanol due to anoxic conditions of dissolved oxygen level <3 mg/L in this sites (Kim and Lee, 1994). From urban sewage pollution, cop/chol (coprostanol/cholestol), 5β/(5β+5α){5β-cholestan-3β-ol/(5β-cholestan-3β-ol+5α-cholestan-3β-ol)} and 5β/(5α+chol) {5β-cholestan-3β-ol/(5α-cholestan-3β-ol+cholesterol)} ratios were considered. The cop/chol ratio ranged 0.03 to 0.60 and 5β/(5β+5α) ratio was between 0.11 and 0.63. High ratios were found in stations 1–7 and 26 adjacent to populated areas receiving a more direct deposition of coprostanol. Cop/chol ratio greater than 0.2 and 5β/(5β+5α) ratio in the range 0.7–1.0 are indicative for urban polluted sediments (Grimalt *et al.*, 1990; Mudge and Seguel, 1999). In this study area, 5β/(5β+5α) ratios did not exceed 0.7 and did not agree with the ratio criterion suggested by Grimalt *et al.* (1990). The intermediate values of the 5β/(5β+5α) ratios of >0.4 allowed an assessment of the urban sewage pollution. Similar behavior has also been observed in the southeastern waters of Hong Kong (Chan *et al.*, 1998) and Kaohsiung Harbour (Jeng and Han, 1994). According to Writer *et al.*

Table 3. Corretaion among sterol ratios in 26 sediment samples of Jinhae Bay in August 2001 (p>0.01)

	coprostanol	cop/total sterol	5β/5β+5α	5β/5α+chol	cop/chol	cop/epi-cop
coprostanol	1.000					
cop[a]/total sterol	0.827	1.000				
5β[b]/5β+5α[c]	0.745	0.797	1.000			
5β/5α+chol[d]	0.843	0.994	0.800	1.000		
cop/chol	0.809	0.988	0.785	0.996	1.000	
cop/epi-cop[e]	0.892	0.918	0.793	0.911	0.876	1.000

[a] cop=coprostanol

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[b] 5β=5β-cholestan-3β-ol
[c] 5α=cholestanol (5α-cholestan-3β-ol)
[d] chol=cholesterol (cholest-5-en-3β-ol)
[e] epi-cop=epi-coprostanol (5β-cholestan-3α-ol)

(1995), the sites with 5β/(5α+chol) ratio of around 0.06 are effected on a smaller scale by small wastewater treatment plant discharges and runoff from pastures and feedlots. 5β/(5α+chol) ratios of >0.06 were also found at stations 1–8, 15, 18 and 24–26. In particular, the elevated ratios (>0.2) at stations 1–7 of inner Masan Bay and 26 of Gohyeon Bay adjacent to populated areas could be explained by the input of terrestrial sewage-derived materials through small streams. The cop/epi-cop ratio can be useful for differentiating human and other mammalian feces, since epicoprostanol has not been found in human feces (Venkatesan and Santiago, 1989). The ratios presented were between 0.17 and 4.13. The elevated ratios of >1.0 at stations 1–8 and 26 confirmed the waste input into those sites. These ratios were comparable to those in the human polluted sediments from Kaoshiung Harbour, Taiwan (Jeng and Han, 1994; 1.6–6.0). The correlations among the six variables were significant (p<0.01) (Table 3). In particular, correlation coefficients among cop/total sterol, cop/chol and 5β/(5α+chol) ratios were close to +1 (p<0.01), indicating that the indices are equally suitable for monitoring coprostanol. Dendrogram from cluster analysis of sampling stations is shown in Fig. 3. All stations were grouped into three clustered areas by the six variables of coprostanol levels, cop/total sterols, cop/chol, 5β/(5β+5α), 5β/(5α+chol) and cop/epi-cop ratios. According to the means comparison (Table 4), only station 1 was classified with cluster 1 due to

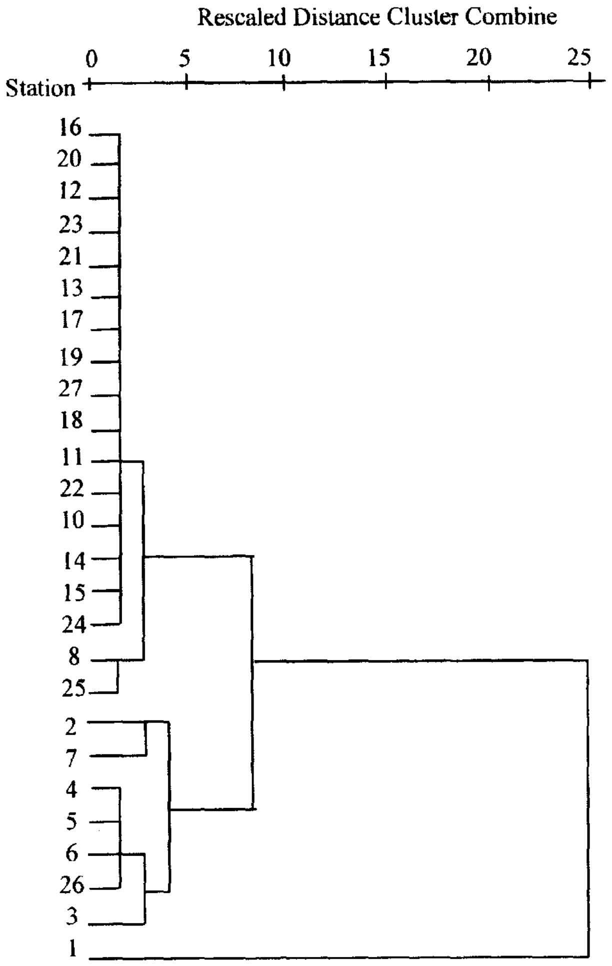

Fig. 3. Dendrogram by cluster analysis of sterol ratios in sediments of Jinhae Bay.

Table 4. Mean and standard deviation among clustered groups by the means comparison

Cluster		coprostanol	cop[a]/total sterol	5β[b]/5β+5α[c]	5β/5α+chol[d]	cop/chol	cop/epi-cop[e]
1	Mean	3.86	0.26	0.63	0.41	0.55	4.13
	N	1	1	1	1	1	1
	SD						
2	Mean	1.06	0.17	0.44	0.25	0.37	1.62
	N	7	7	7	7	7	7
	SD	0.42	0.04	0.05	0.08	0.13	0.50
3	Mean	0.18	0.042	0.2	0.052	0.069	0.43
	N	18	18	18	18	18	18
	SD	0.08	0.02	0.08	0.03	0.04	0.34
Total	Mean	0.56	0.08	0.28	0.12	0.17	0.89
	N	26	26	26	26	26	26
	SD	0.81	0.07	0.14	0.12	0.17	0.93

[a] cop=coprostanol
[b] 5β=5β-cholestan-3β-ol
[c] 5α=cholestanol (5α-cholestan-3β-ol)
[d] chol=cholesterol (cholest-5-en-3β-ol)
[e] epi-cop=epi-coprostanol (5β-cholestan-3α-ol)

extremely high levels of coprostanol, expressed as both coprostanol concentrations and sterols ratios. Cluster 2 (station 2–7 and 26) was characterized by the coprostanol levels and sterols ratios exceeding criteria. Cluster 3 (stations 8–25 and 27) showed very low coprostanol levels and sterol ratios. From these results, it could be assumed that the sewage-derived contamination was localized in inner Masan Bay.

CONCLUSION

Coprostanol levels varied between 0.03 and 3.86 μg/g dry wt (1%–26%) depending on the sites. All sediment samples in Jinhae Bay were shown to contain coprostanol of sewage-associated geochemical indicator. Of the Δ^5 sterols, cholesterol was the principal compound, accounting for an average of more than 60% (1.16–7.69 μg/g dry wt). The fecal serol ratios such as cop/total sterol, cop/chol and 5β/(5α+chol) ratios in the sediments provided suitable parameters for the comparison of sewage contamination in sediments. The cluster analysis from the different sterol ratios revealed that a fecal sterol signature in sediments might be highly localized at stations 1–7 and station 26 adjacent to land and river inflow.

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