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Fecal Contamination Associated with Local Reclamation Activity in the Han River Estuary

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Vertical distributions of coprostanol (5β-cholestan-3β-ol) and other sterols were investigated in the intertidal sediment of Shinbul island in the Han River estuary to estimate the short-term variations of fecal contamination in association with reclamation activity which caused a construction of tidal barrier and emigration of residents from the island. Quantitative contributions of coprostanol in total sterol (9.87–15.84%) and in total organic carbon (82.0–157.7 μg g^{-1} OC) implied that a substantial amount of organic matter associated with fecal pollutants was introduced into the sediment. The highest contribution of coprostanol to organic carbon that was observed between 0.3-0.9 cm depth seemed to be associated with increased human activities for the reclamation project of the island. The ratio of coprostanol to organic carbon decreased within 0.3 cm depth, which indicated decreased fecal contamination after the emigration of residents from the island. The results suggested that measurement of coprostanol could relevantly reflect short-term fluctuation of fecal contamination in the sediment of the Han River estuary.

Key words: Coastal sediment, Coprostanol, Fecal pollution

INTRODUCTION

The Han River estuary, Yellow Sea is an area overloaded with nutrient-rich fluvial waters from Han River (approximately 19 km^3 per year) which passes through Seoul-Incheon metropolitan area with the population over fifteen million (Hong and Yoo, 1996; KORDI, 1991). Because of the excess input of industrial and domestic pollutants, the estuary has suffered substantial anthropogenic environmental impact, resulting in highly eutrophic conditions along the estuary and its adjacent coastal areas (Chung and Park, 1988; Hyun *et al.*, 1999a, 1999b; Jeon *et al.*, 1994; Kang *et al.*, 1992). In addition, another factor for worsening environmental conditions for biological resources in the area is recent releases of landfill leachate from Kimpo landfill which is receiving tremendous volume of domestic wastes (22,000 tons per day) (Park *et al.*, 1999). Since sewage effluents may contain many pollutants including pathogenic bacteria and viruses associated with human wastes (Moe, 1997), it is important to identify the principal areas of sewage contamination and to estimate the relative amounts of anthropogenic components in the sedimentary organic matter.

Both biological and chemical indicators of sewage contamination have been considered to identify areas of contamination and potential health hazard. Generally, microbiological assay of fecal coliforms including *E. coli* and other indicator microorganisms have been used to delineate the fecal contaminated areas (Cooper and Danielson, 1997; Dufour, 1976). However, limitations of the coliform tests as adequate assays of sewage contamination have been pointed out because of the environmental stress (i.e., temperature, sunlight, and grazing, etc.) on the enteric bacteria (Anderson *et al.*, 1983; Barcina *et al.*, 1991; Dutka *et al.*, 1974; Fujioka *et al.*, 1981; Rhodes and Kator, 1988). As a result, fecal contaminated areas might show no evidence from coliform test while in reality they are heavily impacted. This is potentially important for urbanized areas along the coastal zone where coliform survival would be expected to be low. In addition, the presence of coliforms also does not allow a measure of historical records of fecal con-

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tamination in the sediment.

Several results have demonstrated that coprostanol (5β-cholestan-3β-ol), which is a fecal sterol of higher animals, can be used as a quantitative indicator of fecal presence (Goodfellow *et al.*, 1977; Hatcher and McGillivary, 1979; Jeng and Han, 1994; Maldonado *et al.*, 2000; Sherwin *et al.*, 1993; Venkatesan and Kaplan, 1990). Coprostanol is formed only by the bacterial reduction of cholesterol (Cholest-5-en-3β-ol) in the intestine of higher animals (Eyssen *et al.*, 1973). In addition, because it persists in anoxic sediment (Bartlett, 1987; Hatcher and McGillivary, 1979), coprostanol concentration can be a direct indicator of fecal contamination in the organic-rich anoxic sediment. In spite of its potential relevance, environmental evaluation using coprostanol as a fecal indicator has not been attempted in the Han River estuary area. The purposes of this study are: (1) to investigate the vertical distributions of organic carbon, ATP (adenosine-5triphosphate) and sterols with discussions on their probable sources; (2) to estimate degrees of fecal contamination; and (3) to discuss short-term variations of anthropogenic impact in the coastal sediment of the Shinbul Island in association with the construction of tidal barrier for the reclamation and the migration of local resident.

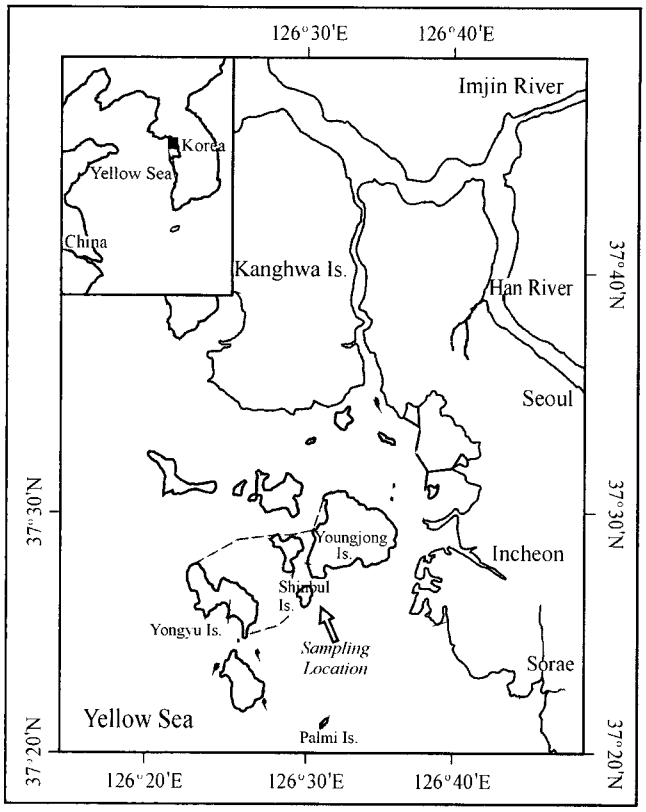

Fig. 1. A map showing study area. Dotted lines denote an area reclaimed for the construction of Incheon international airport complex.

MATERIALS AND METHODS

Study area

In June of 1996, sediment samples were collected at the intertidal flat of Shinbul Island located at the southern part of the Yongjong Island (Fig. 1). The whole Shinbul Island was included in the reclamation plan for the construction of Incheon International Airport. Samplings were carried out during the reclamation activities; a fisheries port near the sampling area was closed and all the residents in the vicinity of the port were moved out of the place several months prior to the sampling. At the seaward site near the low tide level, tidal barriers were under construction, which substantially reduced the exchanges of seawater of the Han River estuary. Consequently, although organic matter from the water column would still be trapped during low tide, the tidal flat was considered to be less impacted by recent fecal pollutants from the land-based sewage effluents. Under this circumstance, it was speculated that short-term variations of fecal pollution associated with human activities during the construction of tidal barrier and emigration of residents from the island might be detected from vertical profiles of coprostanol.

Sampling

Sediment samples were collected in two sedimentary habitats with different grain size. Station 1 was located near mid tide level with fine sediment size (clayey silt; normally smaller than 63 μm diameter), and Station 2 was located near high tide level with coarse sediment (sandy silt; normally larger than 63 μm diameter). Because of the construction of tidal barriers at low tide level, it was expected that both stations were less influenced by the tidal disturbance. Sediment conditions below 1–2 cm depths were considered as anoxic because of its black precipitates of metalsulfides (i.e., FeS_2). Approximately 10-cm of upper sediment was sampled using a plastic syringe core. The cores were kept in vertical position and both ends were sealed with silicon stopper for transportation back to laboratory. The cores were sliced into eight sections in 0–0.3, 0.3–0.6, 0.6–0.9, 0.9–2, 2–3, 3–4, 4–5 and 5–6 cm depth intervals, and

samples were stored in freezer at 20°C until analysis.

Organic Carbon

Sedimentary organic carbon (OC) was quantified using a CHNS element analyzer (CE Instrument, EA1110) after acidification of the bulk sediment with 1 N HCl solution (Iperen and Helder, 1985). BCSS-1 was used as a standard reference sediment for the quality control of the element analysis, and the error range was kept within 1% (Berman, 1987).

ATP (adenosine-5-triphosphate)

Sediment samples were placed into 10 mL phosphoric acid (0.5 M), and ATP was extracted for 10–15 min at 4°C (Karl, 1993). Extracted ATP was separated from sediment using centrifugation at 2500 rpm for 15 min. One-mL of Na_2-EDTA solution (0.048 M) in phosphate buffer (60 mM) was added into 5 mL ATP solution, pH was adjusted to 7.4 with NaOH (1 N), and then the samples were frozen at −20°C until further laboratory analysis. Concentrations of ATP were determined from luciferine-luciferase reaction using Luminometer (Turner designs, model 20e).

Sterol analysis

All glassware was pre-combusted at 450°C for 4 hours. All other components were washed in low residue detergent (Pierce RBS-35), followed by an acid wash with 10% HCl and solvent rinse prior to use. Sediment samples after weighing were transferred into glass test tubes with teflon-lined screw caps. All samples were extracted twice in dichloromethane (CH_2Cl_2): methanol (MeOH) (1:1, v/v) for 30 min. with ultrasonic bath at 20W. Total extracts were concentrated by rotary evaporation. Lipids were separated by the addition of water to arrive at overall ratio of 1:1:0.8 (CH_2Cl_2:MeOH:H_2O) (Bligh and Dyer, 1959). The lower organic phase containing lipids was collected and solvent from the total lipid extracts was evaporated by rotary evaporation. These extracts were subject to mild alkaline hydrolysis using 6 mL methanolic 0.2 N KOH and heating at 70°C for 30 min in a heating block. Samples were cooled, additional water was added, and the neutral fraction containing sterols and alcohols was partitioned three times into 2 mL of hexane:diethyl ether (9:1, v/v) mixture. An internal standard, 5α-cholestane, was added to samples. The neutral fraction was dried under nitrogen, and treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 50°C for 15 min to convert free alcohols to respective trimethylsilyl (TMS) ethers.

Sterols were quantified using capillary gas chromatography (Hewlett Packard 5890A) with flame ionization detector (FID). Separations were conducted in the splitless mode using a (5%-phenyl) methyl silicone fused capillary column (DB-5, 30 m length, 0.32 μm I. D., 0.25 mm film thickness, J & W Scientific) with hydrogen gas as a carrier gas (Harvey, 1994). A two stage temperature program used was from 50 to 120°C at a rate of 10°C min^{-1}, followed by 4°C min^{-1} to 300°C, and an isothermal hold at 300°C for 20 min. All data were processed using a dedicated data system (HP-Chemstation) with quantification based on peak area response compared to the internal standard. For structural identification, electron impact mass spectra were obtained using a GC interfaced to a mass selective detector (Hewlett Packard MSD 5970B) and the same conditions and column as for GC analysis with helium as the carrier gas. The MSD was operated at 70 eV with acquisition over the range of 50–600 amu. Structural identification of sterols was determined by comparison with reference and/or literature spectra and mass spectral interpretation.

RESULTS AND DISCUSSION

Organic carbon

Because major fractions of organic matter are sorbed to mineral grains, mineral surface areas play an intrinsic factor controlling the concentrations of organic matter and microbial biomass in the sediment (Keil *et al.*, 1994; Mayer, 1994; Meyer-Reil, 1986). Organic carbon from the Station 1 with fine grain size (i.e., clayey silt) showed higher concentrations than that in Station 2 with coarser grain size (sandy silt) (Fig. 2). Vertical distributions of organic carbon in the sediment ranged 4.9–9.5 mg g^{-1} dry sediment at Station 1 (average 6.7) and 2.6–5.0 mg g^{-1} dry sediment at Station 2 (average 3.1). A maximum concentration of organic carbon occurred in 0.3-cm depth, indicating that the sediment water interface receives a substantial amount of organically enriched living and non-living particles from the water column (Mayer, 1993).

ATP distribution

Because of the difficulties in microscopic enumeration of sedimentary microbial communities, var-

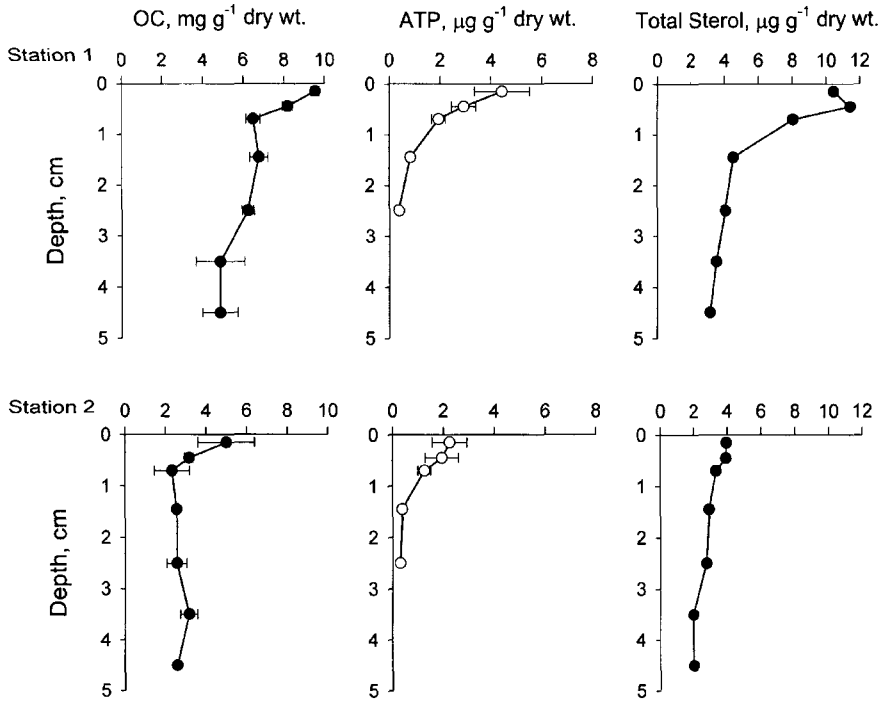

Fig. 2. Veretical distributions of organic carbon (OC), total microbial biomass measured by ATP, and total sterol at two stations with different grain size. Note that the grain size of Station 1 was fine (clayey silt), while the grain size of Station 2 was relatively coarse (sandy silt).

ious biochemical assays on the cellular components have been adopted to measure total microbial biomass in the sediment (Karl, 1986). Among those components, ATP has a short turnover time (i.e., high biological lability) in the environment, and is possible to analyze at low concentrations (down to 10^{-12} M). Therefore, ATP has been used extensively for the measurement of total microbial biomass in various aquatic and sedimentary environments (Karl, 1993). ATP concentrations ranged from 0.30 to 4.44 μg g^{-1} dry sediment, and were higher at Station 1 with high organic carbon than that of Station 2 with low organic content (Fig. 2). Highest ATP concentrations were observed within top 1-cm depth of the sediment, indicating that most eukaryotic and prokaryotic microbial populations reside in the surface aerobic condition.

Sterol distribution

Concentrations of total sterols ranged from 1.8 to 11.4 μg g^{-1} dry sediment which accounted for 0.06 –0.14% of total organic carbon (Table 1). Higher concentrations of total sterol appeared within top 0 –0.6 cm depth of the sediment, which is consistent with the vertical distribution patterns of organic carbon (Fig. 2). Sediment with high organic content (Station 1) was higher in total sterol concentration than that with low organic content (Station 2) (Fig. 2). A regression analysis between total sterol and organic carbon content showed a significant relationship (Fig. 3, R^2=0.78, $p<0.0001$), suggesting that sterols, with a comparatively long residence time in the sediment, can be an indirect parameter of organic content in the sediment.

The concentrations and relative abundance of individual sterols are presented in Table 1 and Fig. 4 respectively. Mixtures of sterols from marine, terrestrial and anthropogenic sources were observed. Cholest-5-en-3b-ol (Cholesterol) comprised a major fraction of total sterols with 11.3–29.6%. Cholesterol has been regarded an indicative sterol of zooplankton and benthic invertebrates, although a small amount is reported in several algal taxa (Volkman, 1986). Therefore, higher relative abundance of the cholesterol within in the top 1-cm of the sediment (Fig. 4) indicated that most benthic faunal distribution is concentrated in the aerobic surface sediment. Coprostanol (5β-cholestan-3β-ol) accounted for 9.9–15.8% in total sterol (Fig. 4). Similar range was found by LeBlanc *et al.* (1992) who reported that coprostanol contributed 2.5–19.2% of total sterol in surface sediment in the urbanized Narragansett Bay. High concentrations of coprostanol were observed within 1 cm depth of sediment, and were higher at Station 1 than at Station 2 (Table 1). Relative contribution of coprostanol in total sterol was constant at both Station 1 and 2, which indicated

Table 1. Composition and concentrations (μg/g dry sediment) of sterols and their carbon based concentrations.

St. 1		Depth range (cm)							
	Neutral lipid	0.0–0.3	0.3–0.6	0.6–0.9	0.9–2.0	2.0–3.0	3.0–4.0	4.0–5.0	5.0–6.0
	Cholest-5-en-3β-ol	3.10	2.77	1.78	0.88	0.63	0.54	0.36	0.40
	5β-cholestan-3β-ol	1.03	1.29	0.94	0.57	0.63	0.49	0.47	0.47
	24-methylcholesta-5,22-dien-3β-ol	1.26	1.19	0.86	0.44	0.39	0.32	0.28	0.26
	24-methylcholest-22-en-3β-ol	0.57	0.89	0.56	0.34	0.36	0.28	0.25	0.25
	24-methylcholest-5-en-3β-ol	0.68	0.57	0.54	0.32	0.26	0.22	0.21	0.20
	24-methylcholestan-3β-ol	1.01	1.06	0.79	0.46	0.23	0.36	0.33	0.15
	24-ethylcholesta-5,22-dien-3β-ol	0.70	0.74	0.54	0.37	0.35	0.29	0.28	0.28
	24-ethylcholest-5-en-3β-ol	1.42	1.68	1.27	0.70	0.71	0.60	0.59	0.55
	24-ethylcholestan-3β-ol	0.69	1.26	0.78	0.47	0.49	0.41	0.41	0.42
	Total sterol	10.46	11.44	8.05	4.53	4.05	3.52	3.16	2.98
	Total sterol, μg mg^{-1} Total carbon	1.11	1.40	1.24	0.67	0.65	0.73	0.65	–
St. 2		Depth range (cm)							
	Neutral lipid	0.0–0.3	0.3–0.6	0.6–0.9	0.9–2.0	2.0–3.0	3.0–4.0	4.0–5.0	5.0–6.0
	Cholest-5-en-3β-ol	1.06	1.03	0.79	0.59	0.53	0.34	0.31	0.43
	5β-cholestan-3β-ol	0.45	0.45	0.39	0.32	0.33	0.26	0.24	0.25
	24-methylcholesta-5,22-dien-3β-ol	0.46	0.47	0.41	0.34	0.31	0.23	0.23	0.27
	24-methylcholest-22-en-3β-ol	0.22	0.19	0.18	0.17	0.16	0.13	0.14	0.14
	24-methylcholest-5-en-3β-ol	0.21	0.18	0.15	0.16	0.14	0.11	0.11	0.12
	24-methylcholestan-3β-ol	0.38	0.36	0.31	0.27	0.25	0.20	0.20	0.21
	24-ethylcholesta-5,22-dien-3β-ol	0.36	0.41	0.35	0.34	0.33	0.25	0.25	0.27
	24-ethylcholest-5-en-3β-ol	0.64	0.50	0.45	0.44	0.40	0.26	0.32	0.33
	24-ethylcholestan-3β-ol	0.17	0.33	0.30	0.29	0.27	0.20	0.23	0.25
	Total sterol	3.95	3.93	3.33	2.93	2.75	1.99	2.02	2.27
	Total sterol, μg mg^{-1} Total carbon	0.99	0.96	1.28	1.08	0.73	0.71	0.79	–

that a substantial amount of organic matter associated with fecal contamination was introduced into the sampling area. Brassicasterol (24-methylcholesta-5, 22-dien-3b-ol), which is mainly found in diatom (Nichols *et al.*, 1990), was also abundant (8.8–12.3% of total sterol). The occurrence of 24-methylcholest-5-en-3β-ol (Campsterol), 24-ethylcholesta-5,22-dien-3β-ol (Stigmasterol) and 24-ethylcholest-5-en-3β-ol (β-Sitosterol) which commonly occur in vascular plants (Akihisa *et al.*, 1991; Goad and Goodwin, 1972) suggested that some of the organic matters in the sediment are terrestrial origin. Combined phytosterols accounted for 32.6–50.2% of total sterols.

Variations of fecal contamination

Coprostanol as a percentage of total sterols can be used as a degree of fecal contamination by eliminating the sediment grain size dependence (Hatcher and McGillivary, 1979; Maldonado *et al.*, 2000). Based on the significant positive correlation between total sterol and organic carbon content in the sediment (Fig. 3), percent coprostanol to organic carbon also reflected the degree of sewage contamination in the sediment (Hatcher and McGillivary, 1979). The surface concentrations of coprostanol (0.2–1.3 μg g^{-1} dry sediment) and its quantitative contribution to organic carbon (82.0–157.7 μg g^{-1} organic carbon) appeared within the ranges reported in other fecal contaminated sedimentary environments (Table 2). Generally, the coprostanol concentrations and its contributions in the sedimentary organic carbon show a decreasing gradient away from the point source of sewage effluent (LeBlanc *et al.*, 1992). Therefore, if we consider that our sampling stations are far away from major waterways of the Han River estuary, the concentrations and relative abundance of coprostanol in the vicinity of the wastes landfill area are assumed to

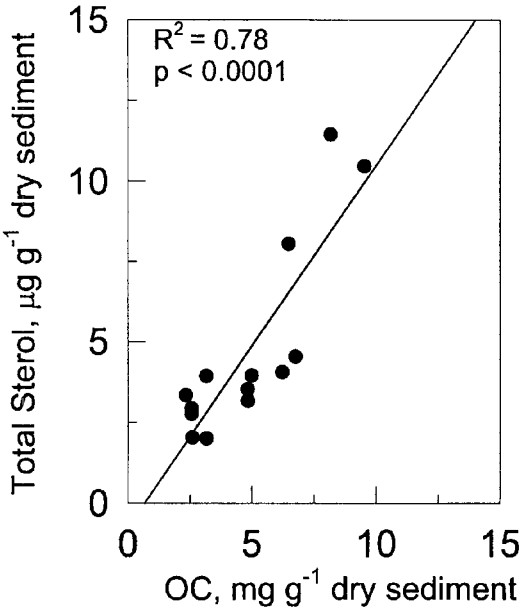

Fig. 3. Relationship between total sterol and organic carbon in the sediment.

be higher than that reported in this study site.

Vertical profiles of coprostanol to organic carbon ratios also provide records of the historic events on the fecal contamination. For example, Venkatesan and Kaplan (1990) in Santa Monica Basin reported that the decreasing coprostanol/organic carbon above 2 cm depth sediment was associated with the improvements in wastewater treatment introduced in 1960s to 1970s. Coprostanol concentrations in organic carbon were accounted for 0.08–0.17 μg mg^{-1} total organic carbon (Fig. 5). Maximum ratios of coprostanol in total organic carbon were observed between 0.3–0.6 cm depth, and striking decrease of coprostanol in total organic carbon occurred at the surficial sediment within 0.3 cm depth. In interpreting the profile, it must be considered that the area was in the process of reclamation, and the coastline had been no longer impacted by the anthropogenic contaminants from the resident of the island after their emigration from the island for previous 6 months. Therefore, it is plausible to interpret that decreased coprostanol/organic carbon at the surficial sediment indicates that recent accumulation of coprostanol has been decreasing since the migration of the residents in the island. On the other hand, maximum ratio of coprostanol to organic carbon in 0.3–0.9 cm depth (Fig. 5) suggested that

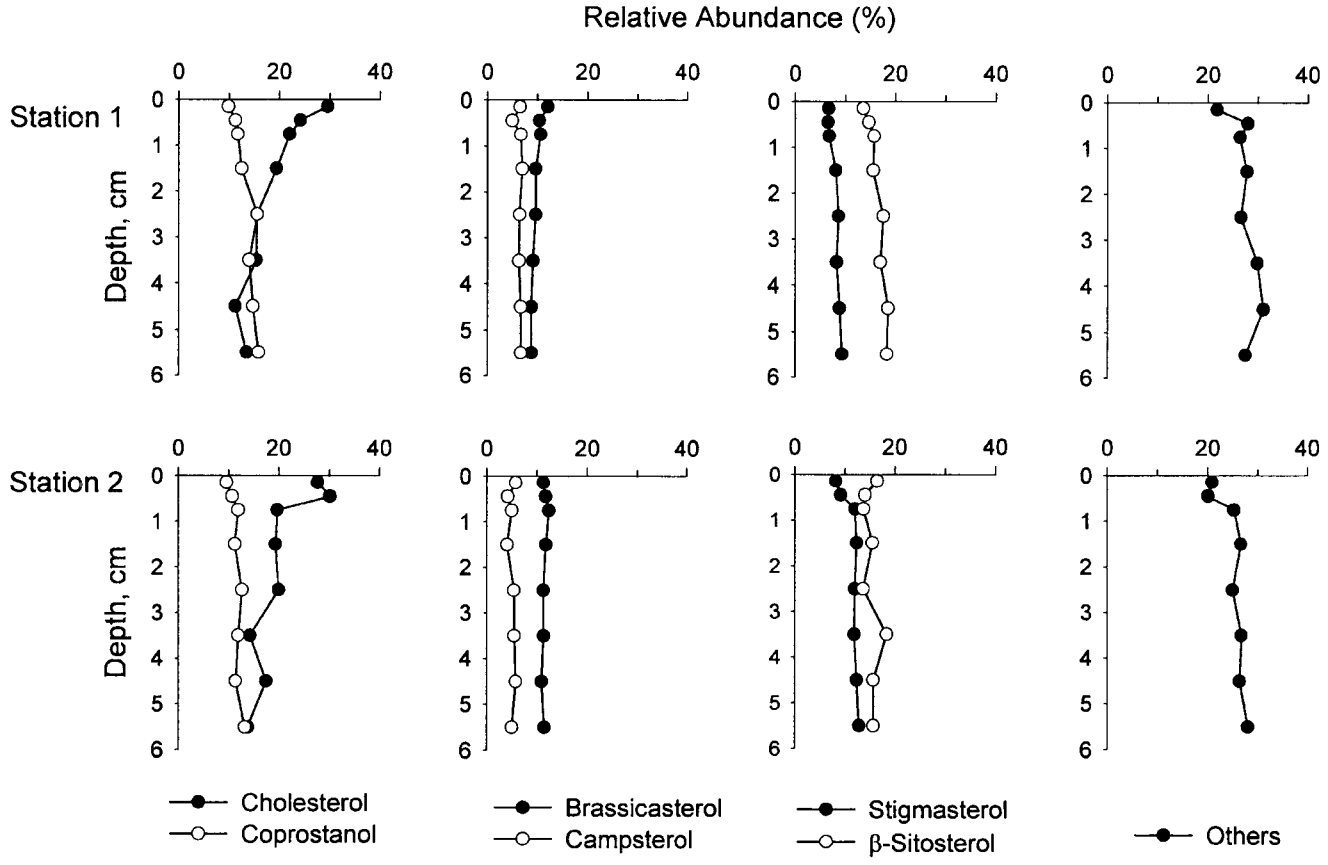

Fig. 4. Vertical profiles of relative abundance of individual sterol in total sterol.

Table 2. Coprostanol concentrations and its abundance in organic carbon in the surface sediments of the Han River estuary and other sedimentary environments.

Study area	Coprostanol μg g^{-1} dry wt.	Coprostanol μg g^{-1} OC	Reference
Han River Estuary, Korea	0.2–1.3	82.0–157.7	This study
Port Phillip Bay, Australia	0.004–2.6	1.2–187.1	OLeary *et al.* 1999
Sydney, Australia	0.01–2.9	0.1–69.3	Nichols *et al.*, 1993
Naragansett Bay, USA	0.1–39.0	19.0–642.0	LeBlanc *et al.*, 1992
Santa Monica Basin, USA	0.5–5.1	45.3–122.0	Venkatesan & Kaplan, 1990
New York Bight, USA	0.06–5.2	29.4–330.7	Hatcher & McGillivary, 1979

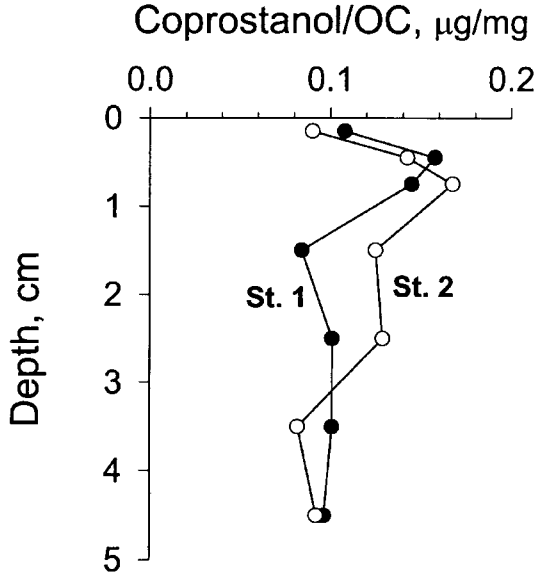

Fig. 5. Vertical profiles of the ratio of coprostanol to organic carbon.

the degree of fecal contamination had been increasing as a result of increased human activities associated with reclamation and emigration during the reclamation activity. In addition, tidal barriers were under construction toward seaward side, which resulted in decreased material flux between intertidal flat and the estuarine seawater. Reduced tidal action generally enhances the accumulation of coprostanol in the sediment (Maldonado *et al.*, 2000).

In evaluating the historic event of fecal contamination, it is essential to collect undisturbed sediment cores, and to measure sedimentation rate. We did not observe any bioturbation in our core samples. The vertical profile of ATP and organic carbon content showing an exponential decrease with depth (Fig. 2) also indicated that the sediment condition was physically stable (i.e., had not been disturbed by the tidal action) probably due to the construction of tidal barrier. We could not measure actual sedimentation rate because sensitive estimation of sedimentation rate within 2 cm depth of the sediment was not possible. However, if we consider annual sedimentation rates of 1–2 cm (Shin, personal communication), vertical profiles of coprostanol/organic carbon within 0.5 cm depth from surface has been formed for last 3–6 months. Our results suggested that the analysis of coprostanol together with a relevant measurement of sedimentation rates can be a useful parameter for determining historic input of fecal (or sewage) contamination. Further investigations on the coprostanol together with microbiological parameters can provide both detailed delineation of fecal contamination and useful information for effective management of coastal sediment and waste dumping sites in the offshore region.

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