

## Pollution History of Fecal Sterols in Dated Sediment Core from Masan Bay, Korea

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Fecal sterols have been used as a molecular marker for domestic pollution because of their abundance in human waste, high affinity to organic matter and persistence in anoxic sediment. Dated sediment core was collected near a wastewater treatment plant (WWTP) in Masan Bay, Korea, to investigate the historical trend of fecal sterols. The highest concentration of fecal sterols in the sediment core was found at the surface layer (dated as 2005). Rapid change in the concentrations of fecal sterols coincides with the establishment and operation of a WWTP; this suggested that discharges from WWTP contributed to contamination by fecal sterols in the bay. Analysis of data by non-parametric multidimensional scaling ordination showed the pollution history of fecal sterols associated with WWTP operation. Inventories and fluxes of fecal sterols have rapidly increased since establishment of the WWTP, indicating that the discharge of WWTP is an important source of sediment contamination in aquatic environment.

**Key words :** Fecal sterols, WWTP, Vertical profiles, Inventories, Fluxes

### 1. Introduction

Masan Bay is a semi-enclosed bay with a slow rate of water exchange. Approximately 1300 industrial complexes, including petrochemical, heavy metal, electrical, and plastic industries are distributed along the coast of Masan Bay. Previous studies have reported on the distribution and characteristics of toxic organic contaminants such as dioxins in surface sediments from Masan Bay.<sup>1-4)</sup> These studies showed that Masan Bay is highly contaminated by toxic organic contaminants because of local discharges from industrial complexes and because of slow water exchange in the bay.

Wastewater treatment plants (WWTPs) are considered as a major source of organic and estrogenic contaminants in coastal environments worldwide.<sup>5-6)</sup> In 2005, there were 294 WWTPs in Korea, the treating capacity of which was approximately 22.5 million tons/day.<sup>7)</sup> These WWTPs produced approximately 2.6 million tons of sludge, most of which (about 78%) was

released into the aquatic environment.<sup>7)</sup> The Korean Ministry of Environment has regulated the discharge of various toxic chemicals in wastewaters by using only the water quality-based control approach.

Fecal coliform bacteria such as *Escherichia coli* are traditionally used as indicators of sewage contamination because they appear to be specific to sewage pollution, are present in large numbers and are relatively easy to quantify.<sup>8)</sup> However, the method has some difficulties such as rapid degradation in the marine environment. In recent years, fecal sterol such as coprostanol are generally used as molecular marker for domestic pollution because of their abundance in human waste, high affinity to organic matter and persistence in anoxic sediment.<sup>9-10)</sup> Coprostanol is produced in the digestive tract of humans by the enteric microbial reduction of cholesterol.<sup>11)</sup> Li et al.<sup>12)</sup> and Choi et al.<sup>13)</sup> have reported that the distribution of fecal sterols in sediment from Masan Bay. There is, however, insufficient information on the pollution history and fluxes of fecal

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sterols in Masan Bay. A few earlier studies have reported on the vertical distribution of fecal sterols by sewage pollution in the coastal areas in the world.<sup>14-15)</sup> Historical trend studies are useful to identify and characterize the sources and to establish strategies to control and manage sources of contamination. To our knowledge, the present study is the first effort on vertical distributions of fecal sterols in Korean coastal waters. The objectives of this study were to describe the vertical distribution of fecal sterols and to determine the impact of continuous WWTP discharges on sediment contamination into Masan Bay, Korea.

## 2. Materials and Methods

### 2.1. Sample collection

The sediment core were collected at the location of 1

km from the outfall of a WWTP (35°09'07"N, 128°35'82"E) in May 2005 (Fig. 1). Core sample was taken using acryl tubes (length 150 cm, internal diameter 11.3 cm) by SCUBA divers. The collected core was immediately sectioned at 2 cm intervals using stainless steel plates. After sampling, all sectioned sediments were transported to the laboratory where they were stored in a freezer at -20°C until further analysis.

### 2.2. Chemical analysis

Sediment samples were freeze-dried and sieved through a 2 mm sieve. The chemical structure of coprostanol, which is a representative compound in various fecal sterols, is presented in Fig. 2. The experimental procedures in sediments were performed following the methods described elsewhere.<sup>12-13)</sup> In brief, the sediment core samples were placed in a 50

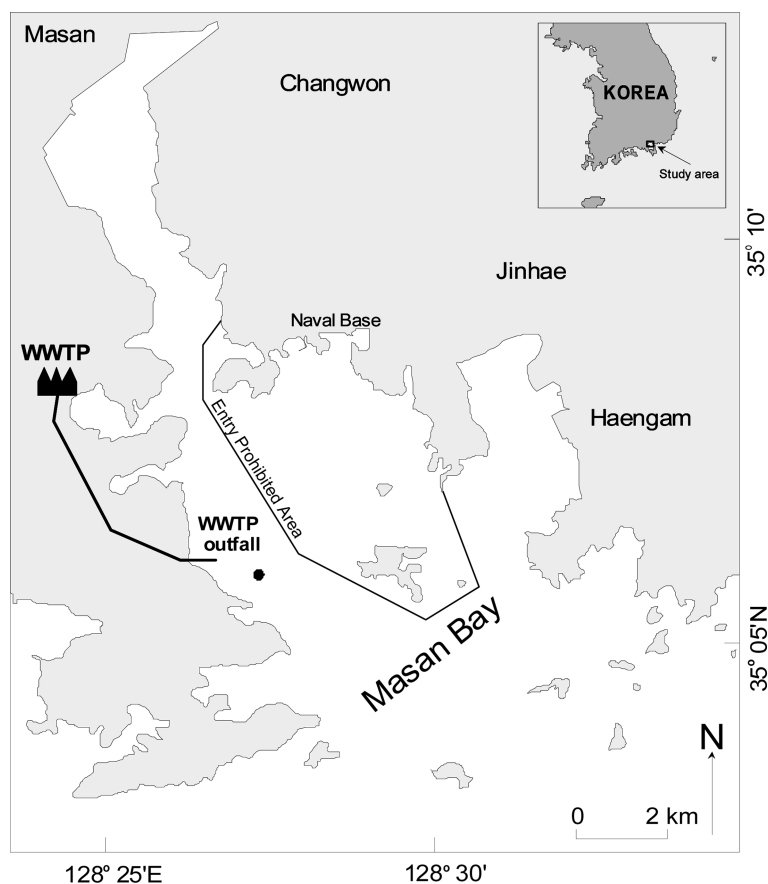
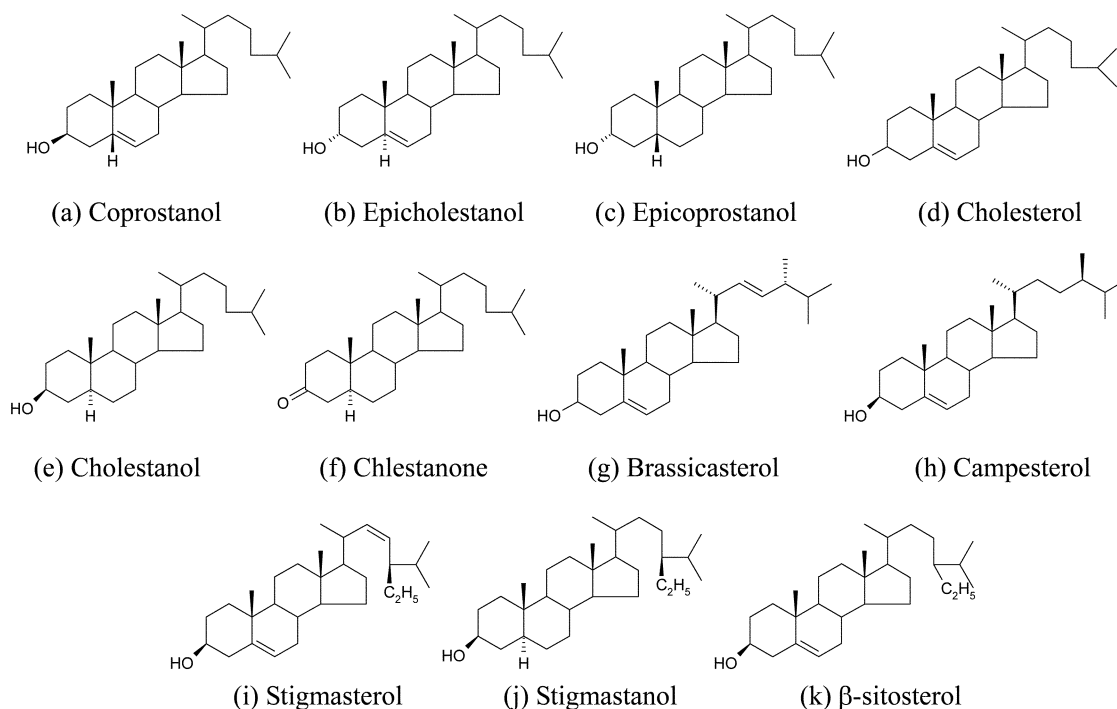


Fig. 1. Map showing sampling location (●) of a sediment core near wastewater treatment plant (WWTP) outfall in Masan Bay, Korea.



**Fig. 2.** Chemical structure of fecal sterol compounds analyzed in the present study.

mL Teflon centrifuge tube with a Teflon cap and then digested by mechanical shaking using 20 mL of 50% methylene chloride (ultra residue analysis, J.T. Baker) in chloroform (Merck, Hohenbrum, Germany), after spiking surrogate internal standards (1-nonadecanol; Dr. Theodor Schuchardt & Co, Hohenbrum, Germany). The extract was reduced to 1 mL under gentle nitrogen flow and transferred to hexane (ultra residue analysis; J.T. Baker). The extract was cleaned by passing through a florisil column, with successive

elutions with 60 mL of 40% hexane in methylene chloride and 40 mL of 20% methanol (ultra residue analysis, J.T. Baker) in chloroform. The second fraction was concentrated and derivatized using BSTFA (Sigma-Aldrich, St. Louis, MO, USA), then used for the analysis of the fecal sterols.

Detailed descriptions of instrumental analyses are summarized in Table 1. Briefly, identification and quantification of fecal sterols were carried out using a gas chromatograph with mass spectrometer detector (GC/

**Table 1.** Instrumental conditions of GC/MSD for fecal sterol analysis

Items	Conditions
Instrument	Agilent 6890 GC/Agilent 5973N MSD
Capillary column	DB-5MS (30 m length, 0.25 mm inner diameter, 0.25 $\mu$ m film thickness, J&W Scientific, Folsom, CA, USA)
Injection volume	1 $\mu$ L
Carrier gas	Helium, 1.2 mL/min
Injector temperature	280°C
Oven program	85°C for 3 min, 10°C/min to 130°C, 3°C/min to 310°C for 7 min
Ion source temperature	230°C
Quadruple temperature	150°C
Ionization mode	Positive electron impact (EI+)
Detection	Selective ion monitoring (SIM)

MSD). The MSD was operated in an electron impact ionization mode, and ions were monitored by selected ion monitoring. A DB5-MS column was used for the analysis of fecal sterols in the sediments. Eleven fecal sterol compounds were analyzed in this study. These include coprostanol, epicholestanol, epicoprostanol, cholesterol, cholestanol, cholestanone, brassicasterol, campesterol, stigmasterol, stigmastanol, and  $\beta$ -sitosterol. Total organic carbon (TOC) content in sediments was analyzed using a CHN elemental analyzer (Perkin Elmer, Model 2400; Boston, MA, USA), after removal of calcium carbonate with 1 N HCl. The recovery of the spiked surrogate internal standards of fecal sterols was  $77\% \pm 14\%$  (average  $\pm$  standard deviation). The limit of detection (LOD) of fecal sterols calculated as three times the signal-to-noise ratio was from 4 to 14 ng/g dry weight.

### 2.3. Sediment dating

One sub-core was used for dating the sediment by measuring specific activities of  $^{210}\text{Pb}$ .<sup>16)</sup> Sectioned sediments were dried, ground, and sieved through a 2 mm mesh. Three g of sediment was transferred to a plastic scintillation vial. Sediments were analyzed after 30 days using a well-type purity Germanium (HPGe) detector with an ultra-low background cryostat and 10' lead shield. The depositional flux and sedimentation rate for sediment core collected near the outfall of a WWTP was determined to be 1.09 g/cm<sup>2</sup>/yr and 1.99 cm/yr. The sedimentation rate in the present study was twice that in a previous study in Masan Bay (about 1 cm/yr).<sup>2)</sup> This finding can be explained by the presence of WWTP outfall and an advection effect of the bottom current in the bay.<sup>17)</sup>

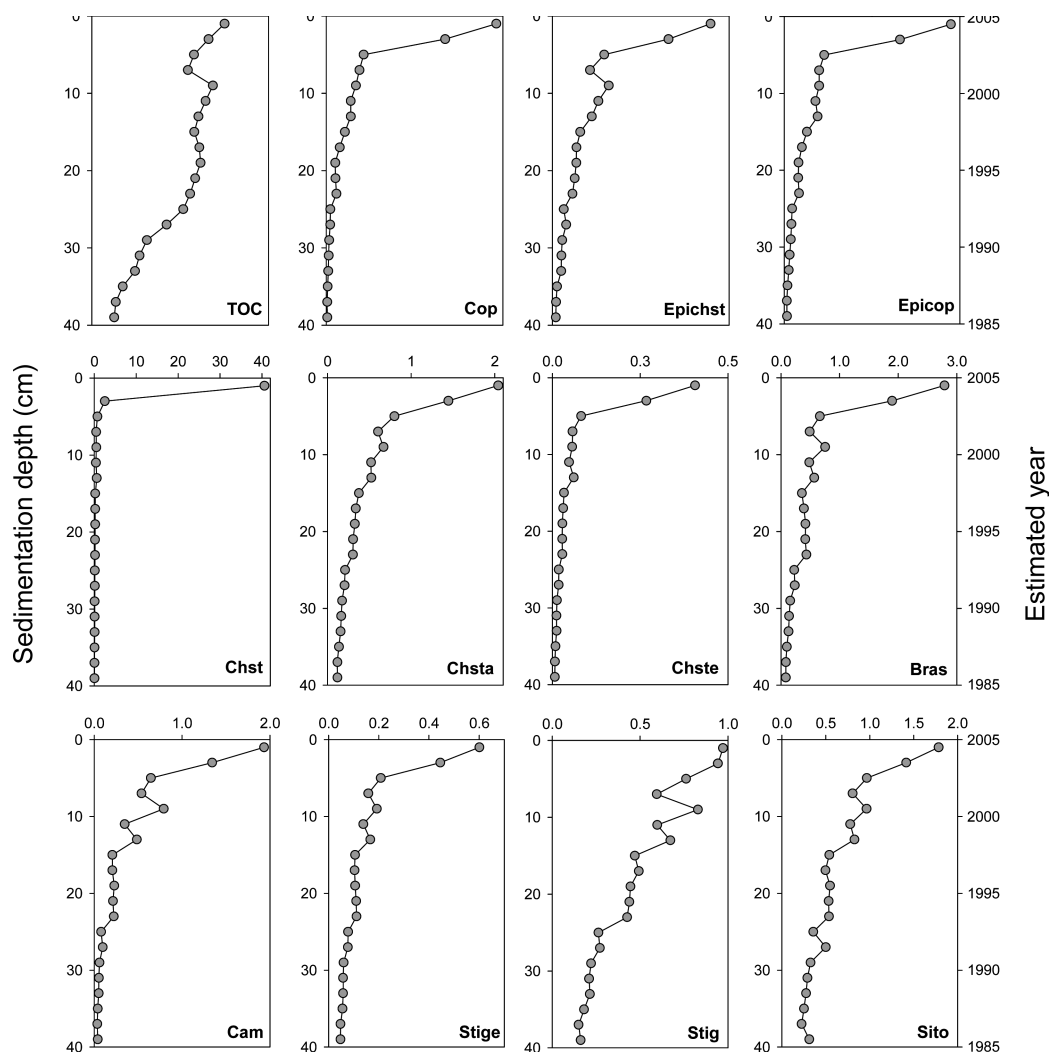
## 3. Results and Discussion

### 3.1. Temporal trends

Vertical profiles of TOC and each sterol compound in sediment core near a WWTP outfall are presented in Fig. 3. TOC contents in sediment core collected in the present study ranged from 1.13% to 2.13%. Total fecal sterol concentrations in sediment core ranged from 0.75

to 53  $\mu\text{g/g}$  dry weight. Overall vertical profiles of TOC and fecal sterol compounds were relatively similar. The highest concentrations of TOC and all of the fecal sterols were found at the surface layer (dated as year 2005), suggesting ongoing discharges of these contaminants into the bay. The concentrations of all of the fecal sterols generally showed an increasing trend from a depth of 20 cm ( $\sim$  year 1995) to the surface layer ( $\sim$  year 2005). The concentrations of most of the sterols below 20 cm depth were close to the detection limit, and remained constant. However, stigmastanol and  $\beta$ -sitosterol showed slightly different patterns compared to other compounds, because these compounds derive from terrestrial sources.<sup>18-19)</sup> The vertical profiles of fecal sterols in the sediment core were coincident with establishment and operation of the WWTP. The WWTP considered in the present study was established in 1994. Therefore, the WWTP activity contributed to the sediment contamination by fecal sterols including coprostanol in Masan Bay.

The concentrations (1,155 ng/g dry weight) of coprostanol in the surface sediment of a core were similar to the concentrations ( $900 \pm 960$  ng/g dry weight) reported for the inner part of Masan Bay.<sup>13)</sup> The coprostanol concentrations in surface sediments from Mokpo coastal waters ranged from 43 to 38,108 ng/g dry weight,<sup>20)</sup> similar to our study. The coprostanol concentrations from Ulsan Bay sediments varied from 141 to 8,257 ng/g dry weight.<sup>21)</sup> These data included the locations near a WWTP from each bay or coastal area. For foreign countries, the concentrations of coprostanol in sediments adjacent to a sewage outfall in San Pedro Shelf, Spain ranged from LOD to 919 ng/g dry weight.<sup>14)</sup> The coprostanol concentration in sediment from Macao estuary of southern China was 8,300 ng/g dry weight.<sup>15)</sup> Therefore, the moderated concentrations of coprostanol in surface sediment near the WWTP outfall may be the reduction in suspended particles in WWTP effluents following activated sludge process. Some studies have reported that fecal sterols including coprostanol are mostly eliminated during the WWTP treatment processes.<sup>22-23)</sup>



**Fig. 3.** Vertical distributions of TOC and fecal sterol compounds in a sediment core near WWTP outfall in Masan Bay, Korea. Concentration units for each of the compounds were % for TOC and  $\mu\text{mug/g}$  for individual fecal sterols. TOC: total organic compounds; Cop: coprostanol; Epichst: epicholestanol; Epicop: epicoprostanol; Chsta: cholestanol; Bras: brassicasterol; Cam: campesterol; Stige: stigmasterol; Stig: stigmastanol; and Sito:  $\beta$ -sitosterol.

### 3.2. Evaluation of wastewater pollution

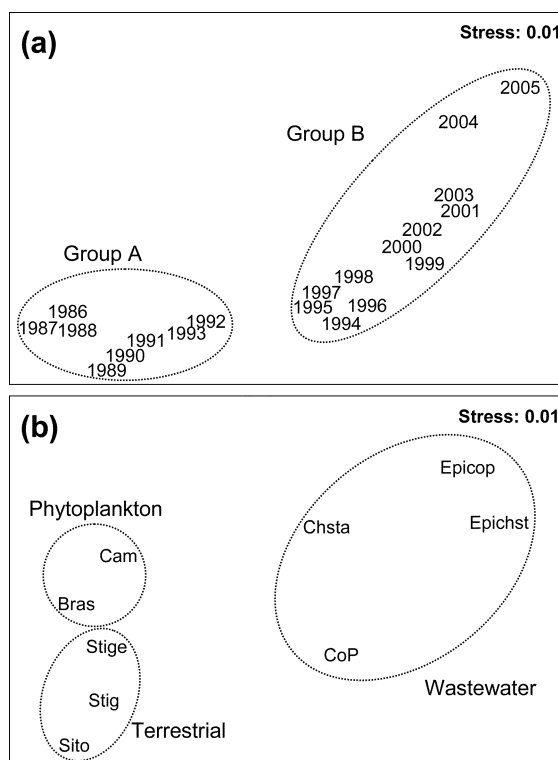
Tracking the sources of fecal sterols alone may be interfered by many physicochemical factors, such as sediment carbon content and particle size distribution.<sup>20</sup> Previous studies have evaluated the source impact by WWTP activities using various sterol ratios such as coprostanol/total sterols.<sup>20, 24-26</sup> However, there have some limitations to evaluate the extent of municipal wastewater contamination in sediments using these ratios. In the present study, to further

characterize historical trends on the sources of fecal sterols, two-dimensional ordination by non-parametric multidimensional scaling (MDS) was performed using PRIMER for Windows (PRIMER Version 5.2.9, Plymouth, UK). The half-lives of individual fecal sterol in marine sediments were not considered for the source characterization of fecal sterols.

This multivariate statistical technique has been used to determine the variability of chemical composition in environmental matrices such as sediments.<sup>3-4,26</sup> The

results of non-parametric MDS ordination of fecal sterols for each sedimentation year, using the Bray-Curtis similarities calculated from square-root transformed data are shown in Fig. 4. The plots had stresses of 0.01, with values less than 0.1 representing a good ordination with little chance of misinterpretation.<sup>27)</sup> Circles on the variable plot (Fig. 4a) were distinguished by two cluster groups of sedimentation years.

The first cluster (Group A) composed of core segments with the sedimentation years of 1986 to 1993, which is before establishment of a WWTP in the bay. This group was corresponded to some sterols such as brassicasterol, campesterol, stigmasterol, stigmastanol, and  $\beta$ -sitosterol (Fig. 4b). Brassicasterol and campesterol have mainly phytoplankton origin<sup>28)</sup> and stigmasterol, stigmastanol, and  $\beta$ -sitosterol are derived from terrestrial sources.<sup>29)</sup> Therefore, sediment contamination before establishment of a WWTP seems to be affected by inputs from marine and terrestrial activities in the bay. The second cluster (Group B) composed of the core segments with recent sedimentation years of 1994 to 2005, which is after establishment of a WWTP. Group B comprised of coprostanol, epicholestanol, epicoprostanol, and cholestanol, which are indicative compounds of wastewater pollution (Fig. 4b).<sup>26)</sup> The WWTP considered in the present study receives wastewaters from households and various industrial complexes located in the cities of Masan and



**Fig. 4.** Non-parametric multidimensional scaling (MDS) ordination plots of sedimentation years (a) and fecal sterol compounds (b) measured for each sediment segment near a WWTP outfall in Masan Bay, Korea.

Changwon. Therefore, a WWTP discharge contributed to a source shift from marine and terrestrial pollution to wastewater pollution.

**Table 2.** Comparison of inventories and fluxes (average  $\pm$  standard deviation) of individual fecal sterols before and after the establishment of a WWTP in 1994

	Inventories (ng/cm <sup>2</sup> )		Fluxes (ng/cm <sup>2</sup> /yr)	
	Before 1994	After 1994	Before 1994	After 1994
Coprostanol	137 $\pm$ 119	1725 $\pm$ 1908	28 $\pm$ 25	355 $\pm$ 392
Epichloestanol	96 $\pm$ 58	523 $\pm$ 398	20 $\pm$ 12	107 $\pm$ 82
Epicoprostanol	105 $\pm$ 66	773 $\pm$ 749	22 $\pm$ 13	159 $\pm$ 154
Cholesterol	599 $\pm$ 292	24,665 $\pm$ 67,044	123 $\pm$ 60	5,073 $\pm$ 13,788
Cholestanol	1004 $\pm$ 363	4,051 $\pm$ 2,940	206 $\pm$ 75	833 $\pm$ 605
Cholestanone	81 $\pm$ 42	568 $\pm$ 670	17 $\pm$ 8.6	117 $\pm$ 138
Brassicasterol	1058 $\pm$ 684	4667 $\pm$ 4283	218 $\pm$ 141	960 $\pm$ 881
Campesterol	477 $\pm$ 375	3,567 $\pm$ 2,979	98 $\pm$ 77	734 $\pm$ 613
Stigmasterol	372 $\pm$ 125	1,179 $\pm$ 882	77 $\pm$ 26	242 $\pm$ 181
Stigmastanol	1,345 $\pm$ 540	3,593 $\pm$ 1,017	277 $\pm$ 111	739 $\pm$ 209
$\beta$ -sitosterol	1,916 $\pm$ 626	4,838 $\pm$ 2,164	394 $\pm$ 129	995 $\pm$ 445
Total sterols	7,192 $\pm$ 3,235	50,148 $\pm$ 82,367	1,479 $\pm$ 665	10,313 $\pm$ 16,940

### 3.3. Inventories and fluxes

The fluxes and inventories of each sterol compound were calculated using the equation;  $F = C_i R$  and  $I = \sum C_i \rho_i d$ , where  $C_i$  (ng/g dry weight) is the concentration in sediment segment  $i$ ,  $R$  (g/cm<sup>2</sup>/yr) is the sedimentation rate,  $\rho$  (g/cm<sup>3</sup>) is the density of sediment segment, and  $d$  is the thickness of sediment. Overall, the inventories and fluxes of fecal sterols have rapidly increased since operation of the WWTP, similar to vertical profiles of the fecal sterols. To investigate the contribution of WWTP to environmental release of fecal sterols, we estimated inventories and fluxes in sediment core before and after the establishment of the WWTP in 1994 (Table 2). Inventories of total fecal sterols before and after a WWTP establishment were  $7,192 \pm 3,235$  ng/cm<sup>2</sup> and  $50,148 \pm 82,367$  ng/cm<sup>2</sup>, respectively. The fluxes of total fecal sterols before and after a WWTP discharge were  $1,479 \pm 665$  ng/cm<sup>2</sup>/yr and  $10,313 \pm 16,940$  ng/cm<sup>2</sup>/yr, respectively. The inventories and fluxes of coprostanol, known as indicator of human feces, were approximately 7 times higher after the establishment of WWTP. Specifically, inventories and fluxes of cholesterol were approximately 40 times higher after the establishment of WWTP. Consequently, WWTP discharge into Masan Bay is an important source of sediment contamination by fecal sterols including coprostanol.

### 4. Conclusion

Vertical profile of fecal sterols in dated sediment core near the WWTP outfall in Masan Bay, Korea varied according to the dated years. Rapid increase in the concentrations of fecal sterols in the sediment core coincides with the establishment and operation of a WWTP. In addition, inventories and fluxes of fecal sterols have rapidly increased since establishment of the WWTP. Therefore, the present study suggests that WWTP discharge into Masan Bay is an important source of sediment contamination, as evidenced by historical trends of fecal sterols in the bay.

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