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Original paper

VERTICAL EXISTENCE OF COPROSTANOL IN A SEDIMENT CORE FROM SEMARANG COASTAL WATERS, CENTRAL JAVA, INDONESIA

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ABSTRACT

Coprostanol has been proposed as an indicator of domestic (sewage) pollution by researchers because constraint of using coliform bacteria as the indicators of domestic pollution in the environment with high environmental stress, such as urban coastal waters. Increasing the volume of industrial wastes, toxic and heated, the changing of water salinity from low (freshwater) to high (sea water), and decreasing of dissolved oxygen (DO) in the waters, are the constrain factors of bacteria growth. How ever, all the researches have been done in the temperate (high latitude) regions. Information existence of coprostanol in tropical region, especially in Indonesia is still very poor. To understand the existence of coprostanol in the sediments, one core sediment sample (60 cm) was collected from Semarang coastal water adjacent to Banjir Kanal Timur which is the main drainage system of the East Semarang municipal district in Central Java by using a small gravity corer in July 2001. The core sediment sample was divided into 12 sections (5 cm each) for analyzing the concentration of coprostanol, grain size, and TOC. The result shows that coprostanol could be detected in all sample sections (vary from 1.06 to 2.94 μg/g). Coprostanol has significant positive correlation with TOC, but not significant with grain size. Coprostanol has very significant negative correlation with the depth of core. Based on the potency of sedimentation rate analysis on Banjir Kanal Timur Semarang coastal waters (0.35 cm/month), the 60 cm core sediment was predicted as a result of 14 –16 year sedimentation. All of these facts show that coprostanol has an excellent persistence in the sediment of tropical environment, and reflect that coprostanol has a potency as an alternative indicator of domestic waste pollution in urban tropical coastal waters.

Key words : Coprostanol, core, domestic, indicator, sediment, waste.

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INTRODUCTION

Fecal coliform bacteria have been widely used as an indicator for the sanitary quality of water or fecal pollution. Recently, various workers (Dutka 1973, Dutka *et al.* 1974) have seriously questioned the use of these organisms as a indicator of fecal pollution. This is mainly due to increasing the volume of toxic and heated industrial wastes, the subsequent change of salinity

from freshwater to seawaters, chlorination of wastewaters, and low dissolved oxygen are the constrains for the existence of coliform bacteria (Walker *et al.* 1982, Bartlett 1987, Bachtiar, 2002).

Detection of sewage pollution in the environment is considerable importance for health, aesthetic, and ecological reasons, especially in urban coastal ecosystem, such as the Semarang coastal waters where the water is used for multiple purposes. The use of coprostanol was reviewed by Walker *et al.* (1982) and it has been applied to water column and sediments (Kirchmer 1971, Dutka *et al.* 1974, Goodfellow *et al.* 1977, Hatcher *et al.* 1977, Hatcher and McGillivary 1979, Brown and Wade 1984, Düreth *et al.* 1986, Holm and Windsor 1990, Coakley and Poulton 1991, Coakley *et al.* 1992, Bachtiar *et al.* 1996, Jeng *et al.* 1996, Takada *et al.* 1997). These studies indicate that the use of this fecal sterol shows great promise as an indicator of fecal pollution.

Various environmental factors, such as chlorination of wastewater, toxic and heated industrial wastes, which accentuate the many shortcomings of coliform enumeration method, were found to have no effect on coprostanol concentrations (Kirchmer 1971). Several biodegradation studies of coprostanol in the environment found that coprostanol was aerobically degraded by bacteria naturally present in sewage and natural waters (Switzer-Howse and Dutka 1978, Kirchmer 1971, Hassett and Lee 1977). However, coprostanol, along with cholesterol and cholestanol, is quite persistent in anoxic sedimentary environments (Hatcher and McGillivary 1979, McCalley *et al.* 1980, Readman *et al.* 1986, Bartlett 1987). However, almost all of the studies had been conducted in fresh water environment at temperate regions. Therefore the information related to coprostanol in tropical region, especially in Indonesia coastal waters, is still very poor. The differences on physical conditions will affect the physical, chemical, and biological processes, as a result those would affect existence and persistence of coprostanol.

The objective of this work is to examine the vertical existence of coprostanol in the coastal water sediments by analyzing the sediment core sample. The vertical existence of coprostanol in a sediment core sample also reflect the persistence of coprostanol in the nature where both are the requirements of using coprostanol as an alternative indicator of domestic waste pollution.

MATERIALS AND METHODS

a. Study area

A study was carried out on the coastal water of Banjir Kanal Timur, Semarang (**Figure 1**). The coastal water also received the effluents from two other streams: Kali Banger (Tambak Lorok) and Kali Tenggang. Semarang is the provincial capital city and the largest city in Central Java located on the north coast of Central Java at 6°55′ S and 110°24′ E. Population of Semarang is approximately 1.3 million people. The climate is tropical with the average annual temperature is 28°C. In general, west winds are dominant during rainy season from November to April, and east winds during the dry season from May to October.

b. Sample collection

Because vertical existence of coprostanol was studied using a sediment core sample which represents a long process of sedimentation, it is unnecessary to do spatial and temporal sampling. A core sediment sample (60 cm) was collected using a small gravity corer with a 6-cm diameter core barrel. The position of sampling site was determined using GPS. In wet stable condition, the sediment core was divided into 12 sections (5 cm each). Each sediment sample was freeze-dried and stored until analysis.

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c. Coprostanol analysis

The analytical method for sediment samples is combined between the method used by Bachtiar *et al.* (1996) and Jeng & Han (1994). All solvent used were HPLC grade. All reagents employed were reagent grade, and all glassware was distilled. The analytical procedure is as follows.

Fig. 1. Core sampling site in Banjir Kanal Timur coastal waters, Semarang.

- *Soxhlet extraction*

About 5 to 10 g of dry sediments (depend on the grain size) were extracted with a mixture of benzene and methanol (1:1, v/v) in a Soxhlet apparatus for 24 h. Heptadecanol (C17:OH) was added to the extract as an internal standard.

- *Saponification*

The spiked extract was concentrated and saponified with 0.5 N methanolic KOH (0.5 N KOH in 95:5 methanol/H_2O (%)). The neutral lipid was extracted with n-hexane four times.

- *Column chromatography*

The extracted lipid was fractionated by silica gel (deactivated with 5% water) column chromatography. The less polar lipids were removed by elution with 40% hexane in chloroform, and the sterol-containing fraction was isolated using 10% methanol in chloroform. The fractions were collected in 9.5-dram vials. All samples were stored refrigerated until gas chromatography (GC) analysis.

- *Sample preparation for GC*

The isolated sterol was concentrated to near dryness, and latter transferred to HP septum-capped vial using 2 x 0.5 ml heptane. BSTFA (bis(trimethylsilyl)-trifluoro-acetamide) (100 µl), was added, and the samples were heated at 130°C for 15 minutes to make them more responsive on the GC capillary column. After cooling, the samples were ready for GC analysis.

- *Gas Chromatograph*

Analysis of coprostanol was carried on a Hitachi 263-50 gas chromatography equipped with SE-30 column and Flame Ionization Detector (FID). Nitrogen was used as the carrier gas. The injector and detector was set at 300°C. Oven temperature was programmed at 150°C - 280°C at 5°C min^{-1}. Coprostanol concentration was calculated based on relative response factor (RRF) from a reference solution containing 6 µg coprostanol and 6.9 µg reference standard (C18:OH). RRF was determined using the following formula:

RRF=[µg cop. (Std.)/area cop. (Std.)] x [area C18:OH (Std.)/ µg C18:OH (Std.)] ……... (1)

From (1), coprostanol concentration in samples can be determined using the following formula:

µg cop. = (RRF x area cop. x µg IS)/(area IS) ……. (2)

In addition to coprostanol, total organic content (TOC) and grain size of sediment were also determined. TOC was determined as loss on ignition from dried sediment samples (after removed of carbonate), placed in a furnace at 550°C for 2 hour.

RESULTS AND DISCUSSION

Result

The results of coprostanol analysis of the sediment cores were listed in **Table 1**. The data of TOC and grain size of sediment were listed in **Table 2**.

Table 1. Coprostanol Analysis of the Sediment Cores

Section Number	Sample ID	Weight of Sample (g)	RRF	IS (µg)	Area Cop.	Area IS	Weight of Coprostanol (µg)	Coprostanol Concentration (µg g^{-1})
1	C(0-5)	6.8793	1.033	3.980	867	215	16.5792	2.41
2	C(5-10)	6.9547	1.121	3.980	425	115	16.4884	2.37
3	C(10-15)	7.0126	1.033	3.980	893	178	20.6260	2.94
4	C(15-20)	6.6586	1.033	3.980	1270	347	15.0473	2.26
5	C(20-25)	6.8661	1.033	3.980	2542	692	15.1026	2.20
6	C(25-30)	6.9920	1.033	3.980	731	316	9.5107	1.36
7	C(30-35)	7.0211	1.121	3.980	598	217	12.2950	1.75
8	C(35-40)	7.0355	1.033	3.980	529	262	8.3011	1.18
9	C(40-45)	6.9581	1.136	3.980	449	275	7.3820	1.06
10	C(45-50)	6.8790	1.033	3.980	585	289	8.3223	1.21
11	C(50-55)	6.6615	1.121	3.980	585	316	8.2596	1.24
12	C(55-60)	7.0542	1.136	3.980	498	217	10.3760	1.47

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Table 2. TOC and Grain Size Analysis of the Cores Sediment

Section Number	Sample ID	TOC (%)	Percentage of Grain Size (Phi)			Grain Size	
			Sand (-1.0–4.0)	Silt (4.0–8.0)	Clay (8.0–12.0)	Mean (Phi)	Wentworth Size classes
1	C(0-5)	11.17	10.2	37.5	52.3	8.32	Clay
2	C(5-10)	9.47	6.5	26.4	67.1	7.85	Very fine silts
3	C(10-15)	9.96	8.9	20.7	70.4	7.94	Very fine silts
4	C(15-20)	12.04	2.1	16.7	81.2	8.76	Clay
5	C(20-25)	11.57	4.4	23.0	78.6	8.48	Clay
6	C(25-30)	6.78	23.2	7.1	69.7	4.87	Coarse silts
7	C(30-35)	9.79	25.6	2.3	72.1	6.53	Fine silts
8	C(35-40)	9.64	26.2	27.7	46.1	6.71	Fine silts
9	C(40-45)	7.99	29.5	12.1	58.4	5.83	Medium silts
10	C(45-50)	9.54	8.9	11.4	79.7	8.57	clay
11	C(50-55)	9.07	11.3	21.2	68.5	7.26	Very fine silts
12	C(55-60)	10.17	24.2	12.9	62.9	8.61	Clay

The results (**Table 1**) shows that coprostanol could be detected in all section of core sediment sample. The concentration of coprostanol was vary from top to bottom of sediment core sample with average value is 1.80 µg g^{-1} and range from 1.06 - 2.94 µg g^{-1}.

Discussion

In general, the concentrations of coprostanol decreased down the core to section 9 (C40-45), and then increase to section 12 (C55-60) (**Figure 2a**). This feature indicates that coprostanol is relatively stable after section 9 (depth more than 45 cm).

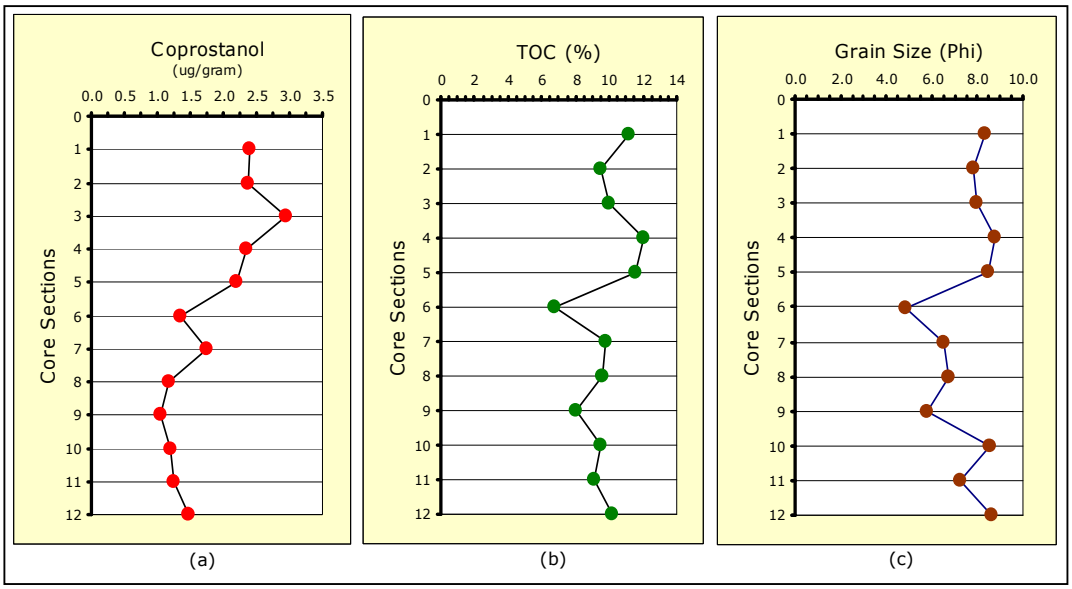

Fig. 2. Depth profile of coprostanol (a), TOC (b), and grain size of sediments (c).

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Depth profile of TOC (**Figure 2b**) shows a similar pattern with depth profile of coprostanol. The average value of TOC was 9.77 % which ranged from 6.78 - 12.04 %. Depth profile of grain size (**Figure 2c**) also shows a variation from the top to the bottom of sediment core sample. The grain size of sediment varies from coarse silts to clay.

Hatcher and McGillivary (1979) in their study on domestic wastes in New York Bight found that TOC and coprostanol had a positive correlation to the amount of sludge which dumped into New York Bight in 25 years. They also stated that beside mixing and dilution processes which could affect the content of coprostanol in sediment, characteristic sediments (grain size and TOC) were other factors that might also affect the distribution of coprostanol in sediment. (Coakley *et al.* 1992). To understand the correlation between coprostanol with TOC and grain size of sediments, correlation analysis has been done.

The correlation analysis of coprostanol and TOC shows a significant positive correlation with coefficient correlation 0.593* and significant correlation 0.042 (**Figure 3a**). Coprostanol and grain size have positive correlation but not significant with coefficient correlation 0.503 and significant correlation 0.096 (**Figure 3b**). Coprostanol and core depth have a very significant negative correlation with coefficient correlation -0.839** and significant correlation 0.001 (**Figure 3c**). TOC and grain size have a very significant positive correlation with coefficient correlation 0.850** and significant correlation 0.000 (**Figure 3d**).

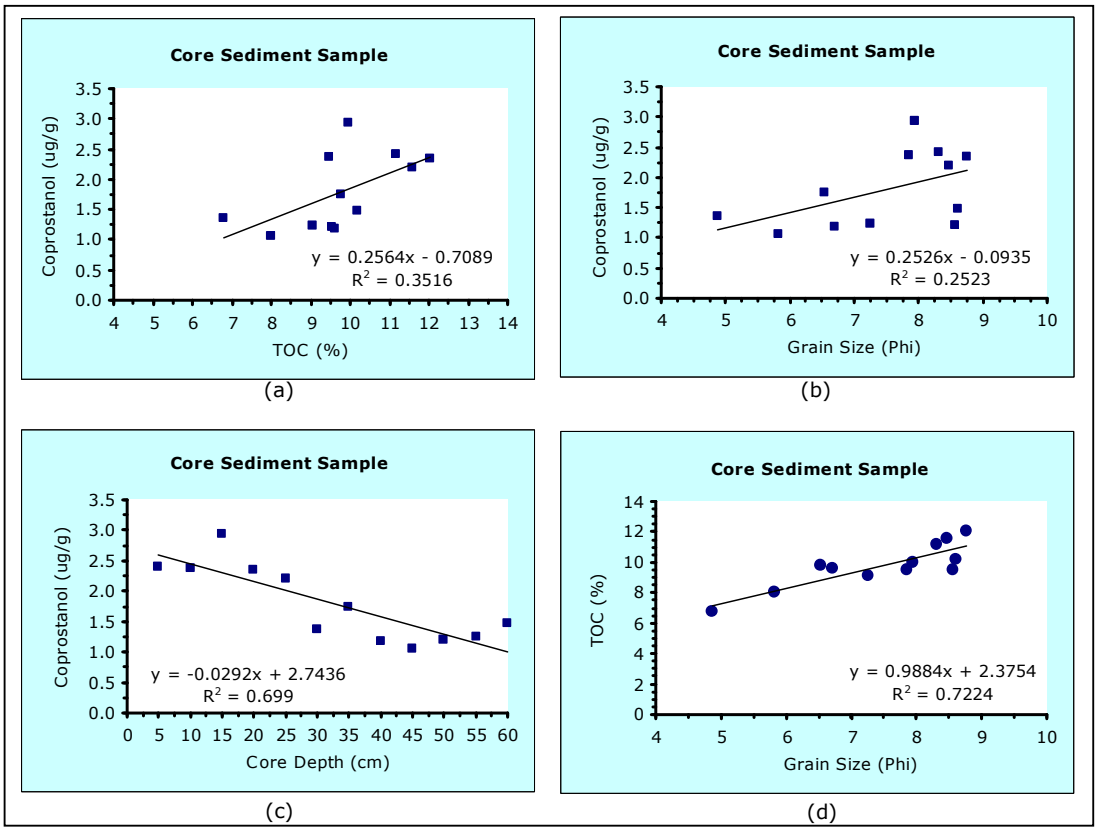

Fig. 3. Correlation coprostanol and TOC (a), and coprostanol and grain size (b).

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Jeng and Han (1996) in their work on coprostanol in a sediment core from anoxic Tan-Shui estuary found that at a depth of about 20 cm, the concentrations of coprostanol and cholestanol changed, which supposedly mark the time when a sewage treatment plant became operational in the estuary. In the upper layer (top 20 cm), the concentration of extractable coprostanol, normalized to TOC, increased down the core. This also indicates that anoxicity must have a crucial role to the preservation and diagenesis of sterols. Because the concentration of coprostanol was affected by TOC in sediment, therefore to obtain a clear depth profile of coprostanol, the concentration of coprostanol has to be normalized with the values of TOC. The depth profile of normalized coprostanol (**Figure 4**) shows that there are three patterns of coprostanol distribution in the core sediment sample. The profile was different compared to the profile found by Jeng and Han (.1996), affected by the operation of sewage treatment plant. First pattern is in the upper layer, range from top of core sediment sample to section three (0 – 15 cm depth). The values of normalized coprostanol in this pattern increase with increasing of depth, range from 0.22 to 0.30. This indicates as an unstable layer that could be affected by dynamic of coastal waters or activities of local fishermen. The second pattern was in the middle section of core sediment sample. The values ware relatively lower than the first pattern, ranged from 0.18 to 0.20. The third pattern was the bottom section of the core, started from section 8 to 12. The values of this pattern were lower than the second pattern with very small variation. This pattern indicates a stable concentration of coprostanol in sediment core sample, which maybe affected by a condition of anoxic sediments.

Bachtiar (2002) analyzed the potency of sedimentation rate on Banjir Kanal Timur Semarang coastal waters, and conclude that the sedimentation rate was 0.35 cm/month. Based on that potency of sedimentation rate, the 60 cm core sediment was predicted as a result of 14 – 16 year sedimentation.

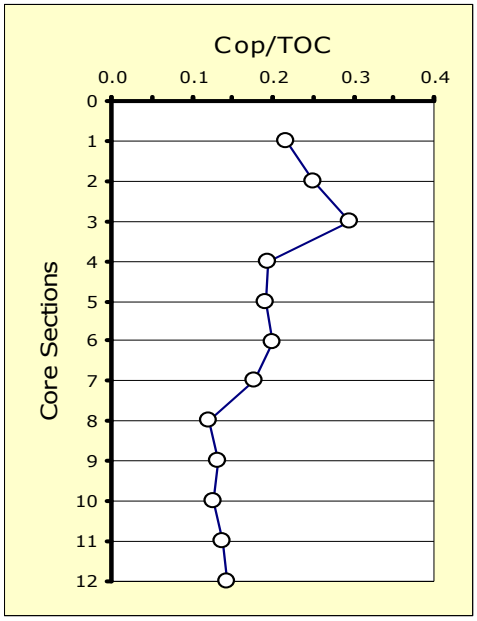

Fig. 4 Depth profile of normalized coprostanol

CONCLUSION

This study showed that coprostanol could be detected in the top to the bottom of 60 cm sediment core sample which collected from coastal waters of Banjir Kanal Timur, Semarang. It indicates that coprostanol has an excellent persistence in the environment of urban tropical coastal waters. Therefore, coprostanol has a potency as an alternative indicator of domestic waste pollution in urban tropical coastal waters.

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