Coprostanol as Sewage Indicator: A Case Study in Kuala Selangor, Selangor

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ABSTRACT: Surface sediment samples from 11 sites in Kuala Selangor, Selangor were analyzed for a range of sterol biomarkers to indicate sewage contamination. Cholesterol was the most abundant compound detected ranged from 3.19 to 2450.98 μgg^{-1} dry weight due to its variety of sources. The highest concentration of cholesterol at station S11 was probably due to high input from small rivers nearby. Coprostanol as a major fecal sterol was detected in all samples but in lower concentrations ranged from 0.38 to 10.76 μgg^{-1} dry weights. Coprostanol present in the aquatic environment has been proved as a very useful biomarker of sewage contamination. The value of coprostanol/cholesterol ratios calculated indicate no sewage contamination occurred and high input of cholesterol is produced from biogenic sources. Meanwhile, the relationship between epicoprostanol/coprostanol and coprostanol/cholesterol ratios showed only stations S2 and S8 have the most untreated sewage while stations S5 and S6 have the most treated sewage. However, it can be concluded that Kuala Selangor is not contaminate with sewage even though fecal sterols were present in all samples.

Keywords: sterol, coprostanol, biomarkers, sewage contamination, health

Introduction

Sterols have been used as indicator for different species of marine and terrestrial input (Mudge & Duce 2005) into aquatic systems and as an indicator for sewage pollution. This paper focuses on one of the most promising method for tracing sewage input and the use of sterol biomarkers. Biomarkers are organic compounds in the environment that retain sufficient structural integrity for their source to be recognized (Leeming et al. 1996); therefore they can be used as 'finger prints' of the pollutant.

Coprostanol (5β-cholestan-3β-ol) is the principal sterol in human and higher animal faeces (Walker et al. 1982). Recently, it has been suggested that coprostanol may be an unambiguous indicator of faecal contamination in seawater and marine sediments (Jeng & Han 1994; Mannino & Harvey 1999; Mudge & Lintern 1999; Peng et al. 2002). Coprostanol is produced mainly in the intestines of mammals (including man) by enteric microbial reduction of cholesterol, the main steroid found in the tissues of vertebrates (Escalona et al. 1980).

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The presence of this compound in natural environments is considered as a reliable indicator of mammalian faecal contamination because this process is the only known source of coprostanol (Yde et al. 1982). Coprostanol also has been shown to be a reliable marker of sewage pollution when coliform bacteria may have been destroyed due to high temperatures or due to the presence of toxic substances (Yde et al. 1982, Düreth et al. 1986).

The purpose of this study is to examine the presence of cholesterol, coprostanol, epicoprostanol and cholestanol in the sampling sites in order to identify sewage contamination in Kuala Selangor, Selangor.

Materials and Method

Study area

Eleven surface sediment samples were collected from Kuala Selangor, Selangor (**FIG. 1**). The methods used for extraction are adapted from procedures which have been used by Mudge and Norris (1997) and Masni & Mudge (2006).

Sterol analysis

Approximately 30-40 g wet weight of sediment was hydrolyzed with 50 ml of 6% potassium hydroxide in methanol. The samples were refluxed for 4 hours and centrifuged at 25000 r.p.m for 5 minutes. The supernatant was then funneled into the separating flask.

Non-polar lipids were extracted from the supernatant by liquid-liquid separation. 20 ml of hexane and 10 ml of double distilled water were added into the supernatant. The mixture was then shaken vigorously and the non-polar fraction was transferred into a florentine flask. The whole procedure was repeated to ensure a maximize extraction. Samples were evaporated at 40 ˚C in a rotary evaporator, redissolved in 2-3 ml of hexane and transferred into the 14 ml vial. Anhydrous sodium sulphate was added to remove any water and polar compounds left in the samples. The remaining solution was filtered through filter paper and blow dried under oxygen free nitrogen (OFN).

Samples derivatisation had to be done in order to permit analysis of compounds with the Gas Chromatograph. About 2-3 drops of bis-(trimethylsilyl) triflouroacetamide (BSTFA) were added into the samples and then heated in a heating block for 10 minutes at 60 ˚C. Finally, they were evaporated to dryness under OFN, and then redissolved in 1 ml of hexane.

A computerized gas chromatography-mass spectrometry (GC-MS) (Perkin Elmer Clarus 500) was used to analyze the sterols in the samples. The temperature program used started at 80 ˚C, increasing at 15 ˚C min^{-1} to 300 ˚C, then at 5 ˚C min^{-1} to a maximum of 350 ˚C for 10 minutes. Calibration was carried out by using cholesterol-TMS in order to quantify peaks obtained from the analysis.

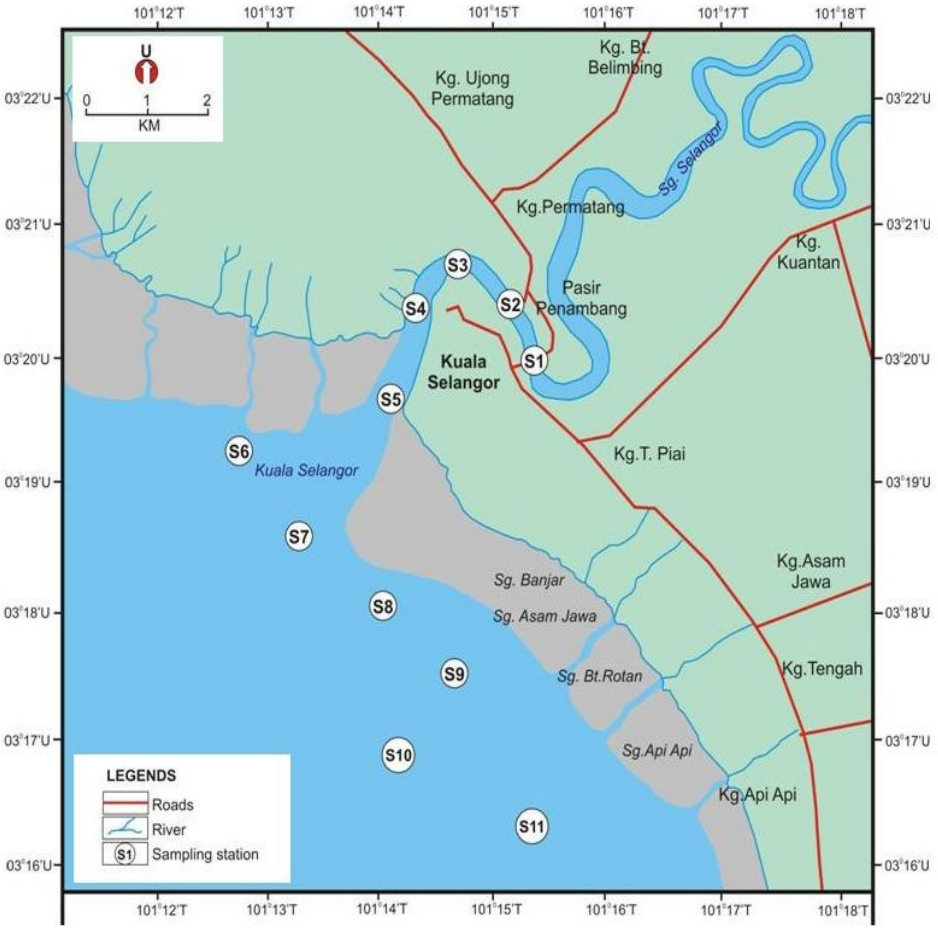

FIG. 1- Location of sampling stations

Results and Discussion

TABLE 1 shows the concentrations of four compounds analyzed from different sampling stations. Cholesterol was the most abundant compound and was present in all samples ranging from 3.19 to 2450.98 $\mu g g^{-1}$ dry weight. The highest concentration was detected at station S11 which received input from small rivers in the area (**FIG. 1**). However cholesterol cannot be used as individual biomarker due to its variety of sources including animals and sewage (Mudge & Lintern 1999), but it can be used in the form of ratios with other sterols

such as coprostanol to identify sewage contamination (coprostanol/cholesterol ratio).

Coprostanol, the major fecal sterol was present with a concentration ranged from 0.38 to 10.76 μgg^{-1} dry weight with the highest value from station St.7, which is in the middle of the river mouth of Kuala Selangor. It is reported that other than sewage input, coprostanol can be formed by the anaerobic hydrogenation *in situ* (Fattore et al. 1996) or from bacterial reduction of cholesterol (Bayona & Albaiges 2006). However individual concentration of coprostanol does not necessarily indicates sewage contamination (Schönning et al. 2002). Therefore it should be used in the form of ratios together with cholesterol, for example station S7 has the highest coprostanol content but the ratio does not indicate any sewage contamination occurring there. The epimer of coprostanol, epicoprostanol is a product formed during treatment of wastewater and digestion of sewage sludge (Mudge & Seguel 1999), so it can be used as an indicator of treatment.

Some ratios of sterol compounds are indicators to evaluate sewage contamination and the most useful is coprostanol/cholesterol ratio. Ratio of ≥ 0.2 indicates sewage contamination as suggested by Grimalt & Albaigés (1990). In this study, only stations S2 and S8 exhibit a ratio value slightly higher than 0.2 which is 0.22 and 0.26 respectively (**TABLE 1**). Overall, sewage contamination does not occurr in the sampling area based on the ratio calculated.

The coprostanol/cholesterol ratio can also be used to differentiate between biogenic sources of sterols and sewage source with the ratios of < 1 indicate biogenic source and >1 indicate sewage source (Leeming et al. 1996; Patton & Reeves 1999). Generally, all of the sampling stations have value < 1, which indicate high input of cholesterol from biogenic sources as mentioned by Fattore et al. (1996). The lowest ratio of coprostanol/cholesterol at station S11 was due to high concentration of cholesterol (**TABLE 1**). This might explain why there is no sewage contamination occurred in the study area.

The ratio between coprostanol and epicoprostanol can be used to indicate the degree of treatment that sewage has received in the system (Mudge & Duce 2005). A scatter plot of epicoprostanol/coprostanol ratios and coprostanol/cholesterol ratios (**FIG. 2**) showed that sites with high sewage content have a low epicoprostanol/coprostanol ratio. Stations S2 and S8 have the most untreated sewage while stations S5 and S6 have the most treated sewage with high epicoprostanol/coprostanol ratios compared to the rest of the samples. Individual concentrations of epicoprostanol for both S5 and S6 are much higher than coprostanol itself (**TABLE 1**).

TABLE 1- Concentrations of fecal sterols (μgg^{-1} dry weight) in Kuala Selangor

Station	TOC (%)	Cholesterol	Cholestanol	Coprostanol	Epi coprostanol	Cop/chol ratio
S1	8.4	21.32	12.77	0.78	2.28	0.04
S2	4.89	11.04	5.17	2.38	1.16	0.22
S3	4.65	14.87	0.97	2.15	0.85	0.14
S4	27.12	438.97	29.06	3.40	2.87	0.01
S5	8.39	22.90	9.49	1.18	19.59	0.05
S6	4.88	13.86	4.54	1.04	21.80	0.07
S7	8.00	67.55	22.23	10.76	23.48	0.16
S8	3.05	3.39	-	0.88	1.35	0.26
S9	7.56	3.19	2.18	0.38	1.36	0.12
S10	5.41	44.72	10.89	3.90	1.47	0.09
S11	7.08	2450.98	25.23	3.04	0.89	0.001

(Cop-coprostanol; chol-cholesterol)

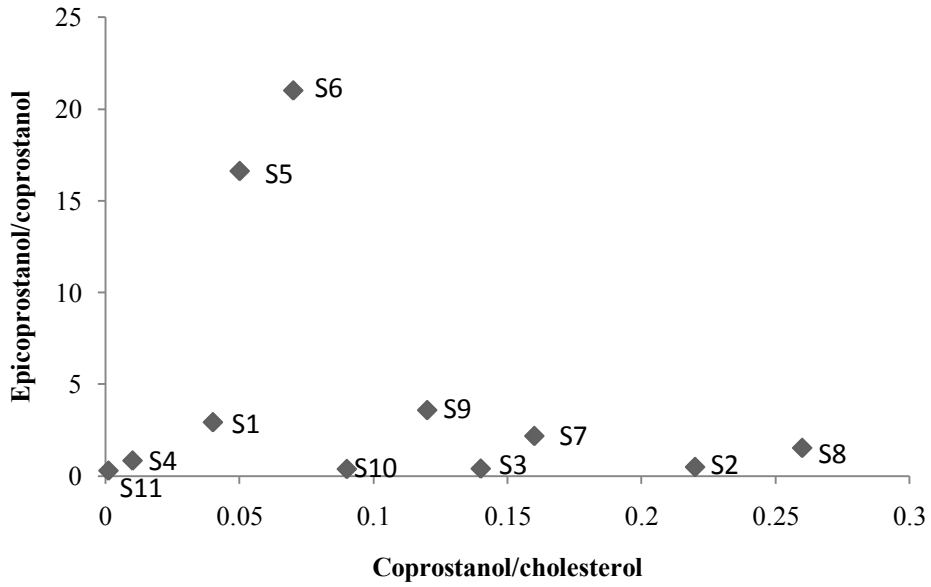

FIG. 2- Sewage biomarker ratio using epicoprostanol/coprostanol and coprostanol/cholesterol ratios in Kuala Selangor

Conclusion

The results of this study showed that Kuala Selangor is not contaminated with sewage based on the coprostanol/cholesterol ratio values calculated. Even though concentration of coprostanol alone is high at certain stations, it does not necessarily indicate sewage contamination. This ratio also indicates high input of cholesterol from biogenic sources in all sediments sampled. Among eleven sampling stations, only two stations showed high content of treated sewage, and another two showed high content of untreated sewage and the rest had moderate amounts of both compounds. Thus, it can be concluded that that Kuala Selangor is not contaminated with sewage based on concentration of faecal sterols detected and ratio values calculated.

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