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STEROL AS AN ANTHROPOGENIC MARKER IN SURFACE SEDIMENTS OF KAPAS ISLAND, TERENGGANU

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Abstract: Kapas Island is a little tropical island in the South China Sea which is well known as one of the main tourist attractions located in Terengganu, Malaysia. This study describes sterol distribution using sewage sterol data collected from surface sediments to indicate the presence of anthropogenic effects on the coastal environment of Kapas Island. Samples from a total of 15 sampling stations were collected, extracted and analysed using Gas Chromatography-Mass Spectrometry (GC-MS). Terrestrial and sewage inputs of 7 sterol compounds were identified to evaluate the impact of human disturbance in the marine sediment. The results indicate that the concentrations of sterol compounds ranged from 6.19 µg/g (dry weight) to 9.88 µg/g (dry weight) in the surface sediments. Faecal sterol content was used as a tool to identify sources of organic material by using the ratio between faecal sterols and terrestrial input caused by anthropogenic effects as a means to evaluate pollution levels and marine ecosystem stability. The results of the analyses indicated low concentrations and ratios of sewage sterols. Most sediment in the study area contains low levels of sewage contamination, which is probably due to the sand that characterizes the sediment. Sandy sediments are less likely to retain organic material, thus resulting in low concentrations of sterol compounds in the sediments. Nevertheless, the presence of sterol compounds is an indication of the human imprint/presence at Kapas Island.

Keywords: Sterol, Kapas Island, faecal sterol, sewage, GC-MS.

Introduction

There are increasing concerns over pollution, especially in coastal areas, which are alarming because of the excessive tourism activities in these coastal regions. This is also exacerbated by the lack of restriction by higher authorities to prevent the abundant overflow of effluents at certain times in certain areas, which has resulted in the discharge of substantial amounts of anthropogenic waste from human and industrial activities into the sea (Carreira *et al.*, 2004; Commendatore & Esteves, 2004; Samuel *et al.*, 2012). One of the coastal areas in Malaysia that is suffering from increasing amounts of pollution is Kapas Island. Kapas Island is a major attraction in Peninsular Malaysia for tourists who want to escape to an idyllic spot of beautiful white sandy beaches without having to spend a lot of money. During certain periods, particularly the monsoon season, the Island is closed for safety reasons. However, when it is open, the profusion of human activities inevitably affects the marine environment because of the direct discharge of effluents into the sea. A review of the literature indicates that studies related to environmental pollution at Kapas Island are scarce; hence, this study sought to gain a better understanding of pollution in the coastal area, especially sources of sterols and their roles as anthropogenic indicators (Howard *et al.*, 2006; Sojinu *et al.*, 2010; Samuel *et al.*, 2012). Sterol biomarkers have been shown to be reliable indicators of anthropogenic contamination in sediments because of their wide distribution in living organisms, their specific characteristics, and hydrophobic nature in addition to their ability to withstand long decay in the environment (Martins *et al.*, 2007; Gao & Chen, 2008; Wang *et al.*, 2008).

Lipid biomarkers, especially sterols, have been successfully used to investigate

waste discharge, faecal contamination and anthropogenic disturbances in aquatic environments in different regions around the world (Venkatesan & Mirsadeghi, 1992; Green & Nichols, 1995; Martins *et al.*, 2007). Specifically, the faecal sterols, coprostanol (5β-cholestan-3β-ol), coprostanone (5β-cholestan-3-one) and epicoprostanol (5β-cholestan-3β-ol) have been widely used as human waste pollution indicators because they are present in human faecal matter and thus can be used as an indication of human activities in adjacent areas (Mudge & Seguel, 1999; Mudge & Duce, 2005; Peng *et al.*, 2005; Cordeiro *et al.*, 2008). Coprostanol and epicoprostanol are the sterols most often cited in sewage impact studies due to the contributions of faecal sterol inputs that are not derived naturally from aquatic sediments (Venkatesan & Kaplan, 1990). Coprostanol is closely related to human sources and natural biomarkers because it is produced in human digestive tracts by the microbial degradation of cholesterol, which is persistent in the natural environment (Wang *et al.*, 2010). Coprostanol comprises 40-60% of the total sterol in human waste and has strong resilience to any degradation (Nichols *et al.*, 1996; Martin *et al.*, 2007). Therefore, any decline in concentration is attributed to physical sediment transport.

Kapas Island is famous as a budget destination among tourists, where its white sandy beaches are open only during the off-season monsoon from November to March. Apart from being famous as a tourist attraction, it is also well known for cuttlefish anchoring. However, the island can only accommodate up to 500 people at a time because its limited settlement distribution is focused on the west coast area of the island facing Peninsular Malaysia, while the rest of the island is not inhibited by humans due to its the exposure to the currents and waves of the South China Sea. The different demographic profiles for the two parts of Kapas Island clearly help to differentiate between the disturbance caused by human activities and the natural sterol present in the marine aquatic sediments.

The aim of this study is to identify and evaluate sewage pollution caused by human activities using faecal sterols as sewage biomarkers. The evaluation is based on analysis of sandy sediments using a GC-MS to trace pollution influenced by human disturbances. Although the concentration of organic material in sandy sediments is very low due to its porosity, it is still important as a tool to detect the level of environmental pollution.

Materials and Methods

Sampling Sites

Fifteen sampling sites surrounding Kapas Island, Terengganu were chosen for this study (Figure 1 and Table 1). The surface sediment samples were collected using a PONAR grab sampler and stored in glass jars. The samples were then refrigerated at 4 °C until further analysis.

Total Organic Carbon (TOC)

A CHNS analyser (Fison, EA 1108, Italy) was used to measure the percentage of TOC by directly measuring the percentage of total carbon, nitrogen and sulphur according to procedure established by Nelson & Sommers (1996). Samples were weighed and dried in a Memmert oven at 60 °C for a few days. The samples were pulverised using a mortar and pestle prior to the CHNS analysis. Approximately 2 ml of 1 M hydrochloric acid (HCl) was mixed with 1-1.5 grams of each dry sample to destroy any inorganic material that was present in the sediment sample. The samples were then dried in a Memmert oven at 105 °C for 10 hours to ensure that no HCl residue remained in the sediment sample. The samples were analysed using the CHNS analyser following the drying procedure. The standard used here was sulphanilamide methionine.

Sterol Analysis

Approximately 30-40 grams of each sample was hydrolysed using 50 ml of potassium

Figure 1: Sampling stations surrounding Kapas Island sediment

Table 1: Coordinates, water depth, temperature, pH and description of each sampling stations sediment

Station	Latitude (N)	Longitude (E)	Water Depth (m)	Temp. (^{O}C)	pH	Description
S1	05° 14.162’	103° 15.679’	17.0	29.4	8.19	Resort area
S2	05° 14.323’	103° 16.144’	25.2	29.6	8.18	Rocky Beach with terrestrial trees
S3	05° 14.031’	103° 16.296’	25.0	29.6	8.19	Rocky Beach with terrestrial trees
S4	05° 13.816’	103° 16.435’	24.7	29.3	8.18	Rocky Beach with terrestrial trees
S5	05° 13.356’	103° 16.547’	23.4	29.5	8.22	Rocky Beach with terrestrial trees
S6	05° 12.866’	103° 16.642’	22.8	29.5	8.15	Rocky Beach with terrestrial trees
S7	05° 12.356’	103° 16.470’	20.2	29.5	8.22	Rocky Beach with terrestrial trees
S8	05° 12.491’	103° 15.902’	11.0	29.5	8.23	Resort area with terrestrial trees
S9	05° 12.710’	103° 15.681’	9.5	29.5	8.24	Resort area
S10	05° 12.954’	103° 15.560’	11.3	29.2	8.27	Jetty and resort area
S11	05° 13.195’	103° 15.519’	10.5	29.3	8.25	Jetty and resort area
S12	05° 13.409’	103° 15.564’	10.8	28.9	8.27	Resort area
S13	05° 13.648’	103° 15.715’	5.9	29.5	8.25	Sandy bay
S14	05° 13.790’	103° 15.861’	3.5	29.7	8.25	Sandy bay
S15	05° 13.840’	103° 15.543’	11.5	28.9	8.25	Sandy near resort area

hydroxide (KOH) in methanol for 4 hours. This procedure was intended to break the ester/ether lipid linkages from the sediment (Mudge & Norris, 1997; Pereira, 1999). Then, the sample was centrifuged (Hettich Zentrifugen 320 R) for 3 minutes at a speed of 4000 rpm, yielding supernatant liquid. The supernatant was mixed with 20 ml hexane and 10 ml deionised water and then shaken vigorously in a separating flask, producing a free, non-polar lipid layer

(in the bottom part of the flask) used for sterol analysis and a polar layer in the upper part of the flask. The procedure was repeated 3 times to ensure maximum extraction. The bottom layer was then concentrated using a rotary evaporator at 40 °C, re-dissolved with 2-3 ml hexane and stored in a 14-ml vial together with anhydrous sodium sulphate to remove any polar traces remaining in the sample. The solutions were then filtered using filter paper (Number 2 Whatman), dried using nitrogen gas (OFN) and heated in an aluminium box with 2-3 drops of bis-(trimethylsilyl)trifluoroacetamide (BSTFA) for 10 minutes at 60 °C. The samples were then dried using OFN, re-dissolved with 1ml hexane and transferred into 1.5-ml vials prior to the GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

A gas chromatography mass spectrometry (Clarus 500, Perkin Elmer, USA) was used to analyse sterols from the sediments on a non-polar, DB5-HT capillary column (30m × 0.25mm × 0.10µm). The carrier gas used was helium with 99.996% purity to vaporize and carried the compounds through the GC-MS at a flow rate of 1 ml min^{-1} and column pressure of 50 kPa. The program used computerized temperatures, beginning at 80 °C and increasing at 15 °C min^{-1} to a maximum of 300 °C, and then increasing at 5 °C min^{-1} until the temperature reached a maximum of 350 °C for 10 minutes. Sterol compounds were calibrated using sterol standard solutions of cholesterol as external standards to establish a standard calibrating curve. Five calibration standards were used: 0.01, 0.05, 0.1, 0.5 and 1 ng ml^{-1}.

The spectrum of each sterol compound present in the samples was recorded directly into the GC-MS computer programme, Turbo Mass, to identify the compounds' spectrum based on the diagnosis of mass for each compound. Example of sterol chromatogram for one of the sample is shown in Figure 2.

To ensure efficiency of the entire process, standard methods and techniques were adopted throughout the experiment, beginning with the extraction procedure, which was repeated three times to ensure that no remaining sterol traces were detected in these latter extractions. All sample sediments were stored in glass jars; the apparatus for the entire experiment were all constructed from glassware to prevent the reaction of organic matter from the sediment, which would have disrupted its organic composition. Prior to the experiment procedure, all of the glassware apparatus and

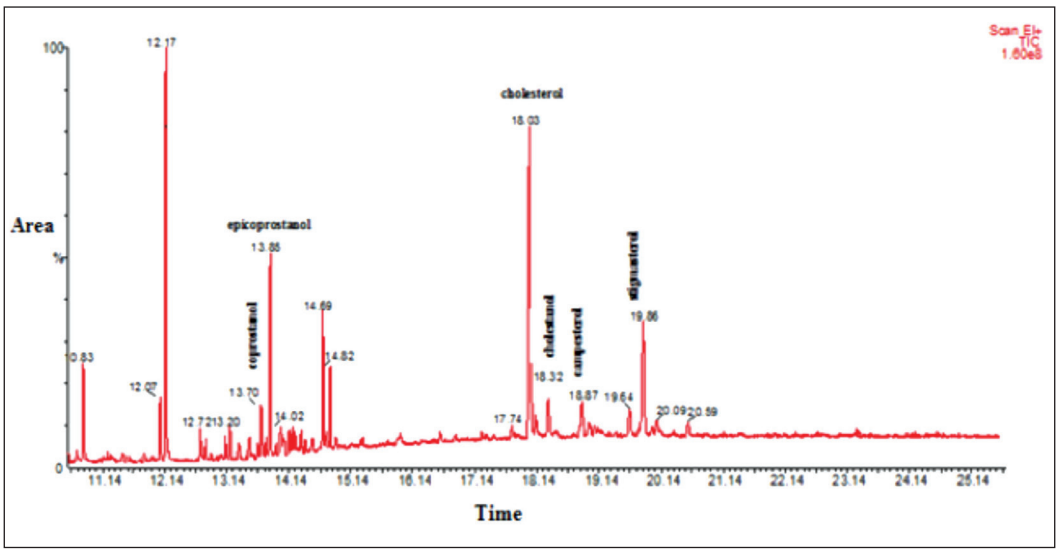

Figure 2: Example of sterol chromatogram from the GC-MS analysis

teflon-lined caps were rinsed with deionized-distilled water and an organic solvent, hexane, and then soaked with Decon-90 prior to the GC-MS analysis. To determine the accuracy and efficiency of the extraction procedure, triplicate samples ($n = 3$) with relative standard deviation less than 14% was performed. Blanks and calibration standards were used throughout the GC injections. A blank was injected, followed by the calibration standard. Five samples were injected afterwards, and followed by the blank and calibration standard again. Procedural blanks were also analysed and no compounds of interest were measured in any sample.

Results and Discussion

Total Organic Carbon (TOC)

Table 2 shows that the concentrations of TOC in the surface sediments from Kapas Island ranged from 0.46% to 10.21% for all 15 stations, with an average value of 4.71%. S14 had the highest TOC content (10.21%), while S2 recorded the lowest (0.46%). Based on the demographic profile (Figure 1), the TOC level is low for stations S1-S7, which are located in the South China Sea. It is possible that the low TOC content is due to the current, strong waves and greater water depth (Table 1) at those stations in comparison with the other stations, S8-S15. The high TOC level at S14 is possibly attributable to its shallow depth, which is the shallowest of all 15 sample locations, as well as its location, which is closest to the terrestrial land area of the island and in a bay protected from strong waves. The higher TOC levels are predominantly directly proportionate to the higher levels of nutrients as a result of the close proximity of sewage discharge, residential areas and industries (Froehner *et al.*, 2010; Adnan *et al.*, 2012). In contrast, station S2 recorded the

Table 2: Concentrations of individual sterols, selected ratios, and TOC in each sampling stations

Station	Cop (ng/g)	E-cop (ng/g)	Choles (ng/g)	Cholest (ng/g)	Brass (ng/g)	Camp (ng/g)	Stig (ng/g)	∑-ols	Cop/ Choles	Cop/ (Cop +Cholest)	E-cop/ Cop	TOC (%)
S1	6.19	7.37	6.24	6.28	6.30	6.30	6.28	43.80	0.99	0.49	1.00	2.34
S2	7.36	8.45	8.81	7.53	7.67	7.80	7.71	55.33	0.84	0.45	1.15	0.46
S3	7.11	6.85	7.36	7.59	7.44	7.60	7.59	51.54	0.97	0.48	0.96	1.26
S4	6.51	6.50	6.50	6.53	n.d	n.d	n.d	26.04	1.00	0.50	1.00	0.91
S5	6.37	6.38	6.45	6.37	n.d	n.d	n.d	25.57	0.99	0.50	1.00	0.62
S6	7.87	7.87	7.85	n.d	7.87	n.d	7.87	39.33	1.00	1.00	1.00	0.52
S7	6.82	6.82	6.82	6.82	6.82	n.d	n.d	34.08	1.00	0.50	1.00	2.11
S8	6.86	6.87	6.91	6.98	6.93	n.d	n.d	34.55	0.99	0.49	1.00	6.44
S9	7.24	7.23	7.28	7.27	n.d	n.d	n.d	29.02	0.99	0.50	1.00	7.49
S10	9.88	9.87	9.84	9.85	9.86	n.d	n.d	49.03	1.00	0.50	1.00	4.00
S11	7.26	7.25	7.26	7.26	n.d	n.d	n.d	29.03	1.00	0.50	1.00	7.00
S12	7.06	7.05	7.05	7.05	n.d	n.d	n.d	28.20	1.00	0.50	1.00	9.11
S13	7.20	7.20	7.23	7.24	7.22	n.d	7.20	43.29	1.00	0.50	1.00	8.77
S14	7.69	7.82	7.69	8.01	n.d	n.d	n.d	31.20	1.00	0.49	1.00	10.21
S15	7.69	7.69	7.72	7.69	n.d	n.d	n.d	30.79	1.00	0.50	1.00	9.48
∑-ols	109.10	110.04	110.99	102.48	60.11	21.69	36.64	551.06	14.77	7.90	15.13	10.71

n.d: not detected; cop: coprostanol; E-cop: epicoprostanol; Choles; cholesterol; Cholest: cholestanol; Brass: brassicasterol; Camp: campesterol; Stig: stigmasterol; ∑-ols: sum of sterols; Cop/Choles: coprostanol/cholesterol; Cop/(Cop+Cholest): coprostanol/(coprostanol+cholesterol); Choles/Cholest: cholesterol/cholestanol; E-epi/Cop: epicoprostanol/coprostanol; TOC: Total Organic Carbon

lowest TOC value because of its exposure to the strong, open ocean current, which transports the sediment containing organic material to other places. The TOC content was used as supporting data for the analysis of organic materials in the surface sediments. The TOC content is crucial in correlating the degree of pollution in the environment caused by organic material.

Sterol Distribution

Table 2 shows the individual concentrations of all selected sterols, including sewage markers (coprostanol, epicoprostanol and cholestanol), marine markers (brassicasterol) and terrestrial markers (stigmasterol and campesterol) in surface sediments surrounding Kapas Island. The sum of sterol concentrations at each station ranged from 55.33 ng/g to 25.57 ng/g dry weight. Cholesterol is the most abundant sterol detected at the sampling sites surrounding Kapas Island because cholesterol can be and is produced by almost all living organisms (Jeng *et al.*, 1996; Mudge & Norris, 1997; Seguel *et al.*, 2001). The total cholesterol concentrations ranged from 6.24 ng/g to 9.84 ng/g dry weight (Table 2). The highest cholesterol concentration was recorded at station S10. This station was located near the jetty area, which is the only location where larger ships and boats drop off or pick up passengers travelling to the island. The surrounding area is comprised of concentrated resort settlements that are densely populated and highly developed compared with the other parts of Kapas Island. Cholesterol is abundant at the sampling stations because it is the primary sterol compound produced by all organisms, including algae, diatoms, macrophytes, phytoplankton and zooplankton (Logan *et al.*, 2001; Peng *et al.*, 2005; Sojinu *et al.*, 2012). Thus, at almost all of the sampling sites surrounding Kapas Island, cholesterol is a major sterol, except for the area with sewage outfalls where coprostanol is the major sterol compound. The presence of cholesterol indicates a possible source from marine mammals and/or humans (Venkatesan *et al.*, 1986; Venkatesan & Santiago, 1989). Mudge & Seguel (1997) deduced that it is common for substantial concentrations of cholesterol to be found in most marine and estuarine sediments.

One faecal sterol often cited, epicoprostanol, is an isomer of coprostanol that is formed during treatment of wastewater and sewage sludge digestion. The presence of epicoprostanol is closely related to fully or partially treated sewage. A high concentration of epicoprostanol in faecal material indicates sewage that is fully treated. Epicoprostanol is the second most abundant sterol found at the sampling sites of Kapas Island, with concentrations ranging from 6.37 ng/g to 9.87 ng/g dry weight. The highest concentration was reported at station S10, which is located near the jetty area. The sampling site is in the vicinity of the local population where the resort is located. The possibility that a high amount of sewage wastage was discharged directly into the sea without any treatment could have contributed to the high concentration of epicoprostanol. The concentration of epicoprostanol is often used as an indicator of the level of treatment or faecal material age relative to the level of sewage pollution. Some sterol compounds have been successfully used to determine the sources of sterols and to evaluate the level of sewage contamination in sediments by calculating the sterol ratios (Grimalt *et al.*, 1990; Leeming *et al.*, 1996).

Sewage Sources

Coprostanol, epicoprostanol and cholestanol are identified as individual sewage sterols and have been used successfully as tracers for sewage contamination. Furthermore, unlike the cholesterol, cholestanol, campesterol, β-sitosterol and stigmastanol sterols that are present in the majority of marine sediments, the content of coprostanol is high mostly in the areas where there is sewage outfall and in polluted areas (Venkatesan & Kaplan, 1990; Green & Nichols, 1995). The individual sterol, coprostanol, can withstand anoxic conditions and can be preserved for a long time, which

facilitates the historical tracing of sewage pollution (Saliot *et al.*, 1991; Sicre *et al.*, 1994; Saliot *et al.*, 2002). Coprostanol concentrations of >500 ng/g are indicative of considerable sewage pollution, as demonstrated in a study conducted by González-Oreja and Saiz-Salinaz (1998). Because the detected concentrations of coprostanol at all of the Kapas Island sampling stations were comparatively lower than 500 ng/g, they are indicative of low sewage concentrations. In addition, a study by Grimalt *et al.* (1990) classified a coprostanol level of >100 ng/g as an indication of contaminated sewage. Therefore, none of the areas in which the Kapas Island sampling stations are located are considered to be contaminated by sewage based on the coprostanol concentration criteria proposed by Grimalt *et al.* (1990).

Rather than relying on only one single sewage sterol species, the degree of pollution can be evaluated more clearly and precisely by using a combination of sewage sterols. Evaluation of only one sterol, in this case coprostanol, is not sufficient for the effective detection and quantification of sewage pollution because it may generate questionable results regarding the source's contribution to sediments (Mudge *et al.*, 1999). Therefore, ratios of coprostanol/cholesterol, epicoprostanol/coprostanol and coprostanol/(coprostanol+cholestanol) were used to detect sources of sewage to evaluate the degree of pollution in the study area (Mudge & Norris, 1997). By combining the ratios of coprostanol/(coprostanol+cholestenol), coprostanol and epicoprostanol, the faecal material can be evaluated more precisely rather than based the evaluation on coprostanol alone, as proposed by Jeng *et al.* (1996), Chan *et al.* (1998) and Marvin *et al.* (2001).

Coprostanol/cholesterol ratios are used as indicators of domestic sewage and biogenic material discharged into the marine environment (Grimalt & Albaiges, 1990; Mudge & Bebiano, 1997). McCalley *et al.* (1981) proposed that the coprostanol/cholesterol ratio helps to elucidate whether the contributions are from biogenic sources or sewage sources. Ratios of <1 are reported for biogenic sources, while ratios of >1 indicate sewage sources (Fattore *et al.*, 1996; Nichols *et al.*, 1996; Reeves & Patton, 2005). Based on the coprostanol/cholesterol ratios listed in Table 2, the lowest value of 0.84 was recorded for station S2, whereas the coprostanol/cholesterol ratio values are close to 1 for the other 14 stations. The ratios of all of the stations listed in Table 2, which are <1, suggest the inputs are from biogenic sources. This implies that the locations of all sampling stations surrounding Kapas Island are dominated by biogenic sources, which suggests that macrophytes might be a secondary source in the organic matter of marine sediments (Reeves & Patton, 2005).

The ratio of epicoprostanol/coprostanol has been used to differentiate human waste from other coprostanol sources, in which ratios ranging from 1.55 to 6.00 are indicative of human waste (Venkatesan & Kaplan, 1990; Pratt, 2005; Wang *et al.*, 2008). Specifically, the ratio helps to provide useful information in assessing the degree of sewage treatment, as proposed by Mudge & Seguel (1999) and Mudge & Norris (1997). Mudge & Seguel (1999) suggested that ratios of <0.2 indicate untreated sewage, while ratios of >0.8 are indicative of partially treated sewage (primarily or secondary). Table 2 indicates that epicoprostanol/coprostanol ratios of >0.8 were measured at all stations in the study area, particularly in areas with inputs from partially treated sewage, such as station S2, where the highest ratio of 1.15 was recorded. This indicates that S2 (Figure 1) is located near the sewage outfall where the resort settlement is located at Gumia Island (see Figure 1), resulting in higher ratio values.

The stanol index, coprostanol/(coprostanol+cholestanol), was used to facilitate in the differentiation between sewage and biogenic sources (Grimalt *et al.*, 1990). A stanol ratio of >0.7 is characteristic of urban sewage pollution (Jeng & Han, 1994; Fattore *et al.*, 1996). The coprostanol/

(coprostanol+cholestanol) ratios listed in Table 2 indicate that the highest coprostanol/(coprostanol+cholestanol) ratio (1.00) was measured at station S6, identifying it as a sewage pollution area. The other 14 stations are considered as having intermediate ratio values (0.3-0.7) as a result of the mix between both biogenic and anthropogenic sources (Adnan *et al.*, 2012). Therefore, evaluations of coprostanol and coprostanol/(coprostanol+cholestanol) concentrations will facilitate the identification of sewage sources and the natural reduction of sewage to sedimentary sterols (Grimalt *et al.*, 1990; Fattore *et al.*, 1996; Reeves & Patton, 2005).

Conclusion

The study of faecal sterols and stanols has been proven to be useful in identifying sewage indicators that can be used to evaluate anthropogenic imprints in the surface sediments surrounding Kapas Island. The faecal sterol concentrations reported for our study showed that the areas in which the sampling sites were located could be considered as uncontaminated by sewage inputs, even though coprostanol was determined to be the second most abundant faecal sterol after cholesterol in the study area. In general, Kapas Island exhibits a mix of both biogenic and sewage sources for effluents of anthropogenic inputs. The study of sterol data will collectively contribute to the knowledge of sterol biomarkers as well as highlight the need to take measures to improve the environmental conditions on Kapas Island.

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