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Coprostanol in Siak River Sediments, E Sumatra, Indonesia

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Abstract To follow faecal pollution steroid compounds have been analysed in 106 sediment samples from the Siak River, E Sumatra, Indonesia. Coprostanol was detected in 40 of these. Contents ranged from 50 to 10,530 ng/g d.w. with a mean of 878 (TOC-normalised: range 7.4–393.0, mean 44.1 μg/g TOC). Total contents and the coprostanol/cholesterol ratio argue for a major contribution from untreated sewage which is also evident from field observations. The distribution along the river indicates the quantitatively dominant source to be the city of Pekanbaru with an estimated population of 1.5 million. Coprostanol contents decrease downstream indicating ongoing degradation either during transport or in the surface sediment. However, additional sources of coprostanol become evident further downstream. On the other hand, the 5β/(5β + 5α)-cholestan-3β-ol ratio versus cholesterol and a ternary plot using C27 sterols suggest that plant sources also contribute to the sedimentary coprostanol due to its formation by bacteria in suboxic/anoxic sediments.

Keywords Faecal contamination · Coprostanol · Plant source · River · Sumatra · Indonesia

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Introduction

Faecal sterols have been widely used to monitor the degree of pollution by sewage waste (e.g. Hatcher and McGillivary 1979; Walker et al. 1982; Leeming et al. 1996; Isobe et al. 2002, 2004; Peng et al. 2002, 2005). Coprostanols (coprostanol + epicoprostanol) in environmental samples arise from the anaerobic microbial reduction of cholesterol within the intestine of animals and man (Martin et al. 1973). Hence, faeces of higher animals including man contain 5β-cholestan-3β-ol (coprostanol) and its epimer 5β-cholestan-3α-ol (epicoprostanol) as specific markers. These compounds have hence been proposed as indicators of human faecal pollution. Coprostanol may constitute up to 60% of total sterols in human faeces and is the primary sterol present in human sewage (MacDonald et al. 1983). Relationships among coprostanol, epicoprostanol and other sterols present in animal and human faeces have been applied to the identification of faecal sources (Leeming et al. 1996).

In the present communication we examine coprostanol contents of surface sediments from the Siak River, E Sumatra, Indonesia. Here untreated sewage is discharged from numerous municipalities including the capital of Riau Province, Pekanbaru.

Materials and Methods

Surface sediments were obtained in March 2004, September/October 2005, February and September 2006 (Fig. 1). Sediments were sampled with a van Veen-type hand-operated grab (150 cm^2) from the tributaries of the Siak, Tapung kanan and Tapung kiri, to the estuary (Fig. 1).

After recovery sediments were first air-dried and, after transport to the home laboratory, freeze-dried and ground in

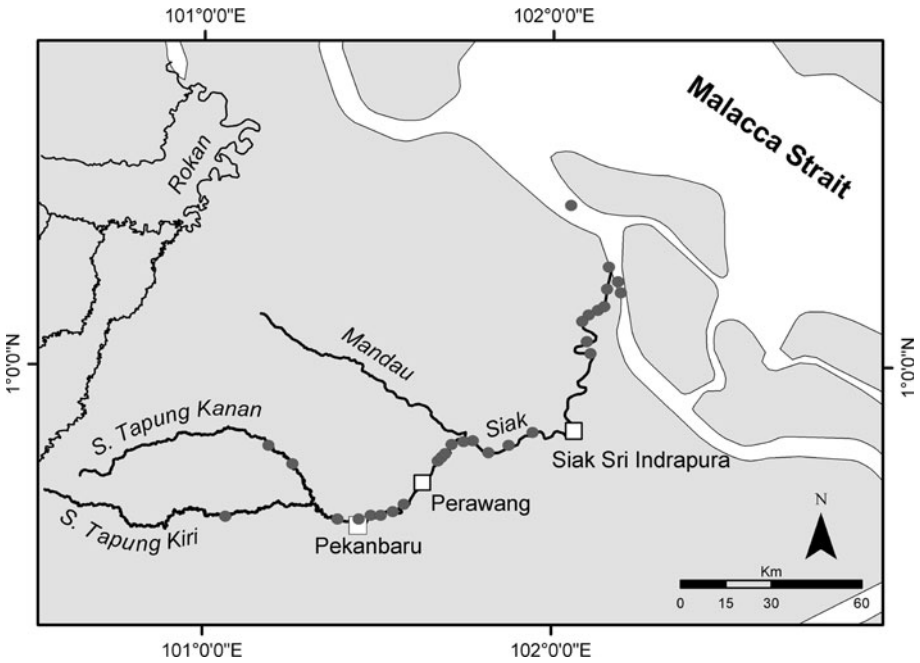

Fig. 1 Locations in the Siak River system where coprostanol was detected

an agate ball mill at 200 rpm for 30 min. Extracts were prepared by ultrasonic extraction using solvent systems of sequentially increasing polarity: 1. *n*-hexane, 2. *n*-hexane/dichloromethane (50:50 v/v), 3–5. dichloromethane/methanol (90:10 v/v) corresponding in polarity to hydrocarbons, alcohols and polar N,S,O compounds, respectively. The combined lipid extracts were rotary-evaporated to dryness and a mixture of squalane, 5α-androstanol, 5α-androstanone and erucic acid was added for internal standardisation. The *n*-alkanes were separated from the total extracts using a 1.0 * 20 cm glass chromatography column packed with activated silica gel (100–200 mesh). After adding an aliquot of the redissolved total lipid extract to the column, *n*-alkanes were eluted with 15 mL of hexane. Polar compounds were then eluted with a mixture of 40 mL dichloromethane/methanol (9:1 v/v) and analysed using an Agilent 5973 GC–MS System operating at 70 eV with a mass range of *m/z* 50–650 in the scan modus. The GC was equipped with a DB 5 capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). The temperature programme ran from 60 to 300°C at a rate of 6°C/min and a hold at 300°C for 30 min. Helium was used as carrier gas with a flow rate of 1.2 mL/min. Before measurement the polar compounds were derivatised to trimethylsilyl ethers by adding 50 µL of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) to each sample. Components were identified by comparison of their mass spectra and retention times with synthetic standards or published data. The different internal standards added prior to the sample extraction were used for quantification. Recoveries of these standards varied between 86 and 90%:

Total carbon (TC) was determined by high temperature combustion (LECO® SC 444), total inorganic carbon (TIC) by coulometry after acidification (UIC®). Total organic carbon (TOC) was calculated as difference TC–TIC. All values reported are means of duplicate measurements and have an average reproducibility of ±0.2%.

Results and Discussion

Coprostanol could be detected in 40 out of 106 samples analysed with contents ranging from 50 to 10,530 ng/g d.w. with a mean of 878 (TOC-normalised: range 7.4–393.0, mean 44.1 µg/g TOC). The distribution along the river indicates the quantitatively dominant source to be the city of Pekanbaru with an estimated population of 1.5 million. Coprostanol contents decrease downstream indicating ongoing degradation either during transport or in the surface sediment (Fig. 2). However, additional sources of coprostanol become evident further downstream, e.g. the cities of Perawang or Siak Sri Indrapura, and even the smaller villages in the estuary obviously contribute to the sewage burden of the river.

There are various suggestions as to the level of absolute coprostanol contents indicative of faecal pollution. González-Oreja and Saiz-Salinas (1998) suggested values >500 ng g^{-1} while Writer et al. (1995) considered levels above 100 ng g^{-1} to be positively associated with sewage input. Nichols et al. (1993) found values of coprostanol >500 ng g^{-1} in sediments near a sewage outfall, although much higher levels could be found in heavily contaminated sediments (e.g. >9,000 ng g^{-1}; Nichols and Leeming 1991). According to Martins et al. (2007) coprostanol levels above 1,000 ng g^{-1} and the vicinity to a potential

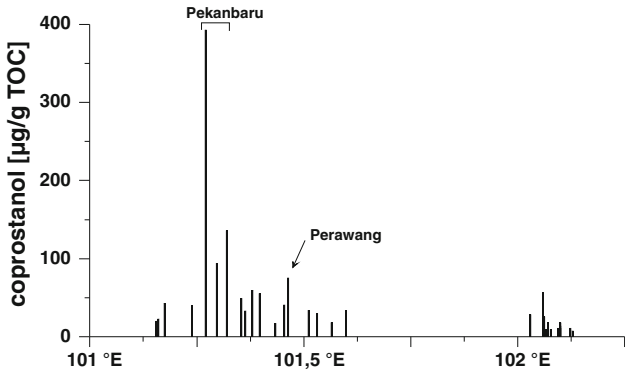

Fig. 2 Coprostanol contents in surface sediments of the Siak river and its tributaries

source suffice to indicate sewage contamination. This level was exceeded for the Siak sample suite by only eight samples while another 11 samples had contents between 500 and 1,000 ng g^{-1}.

The fact that only in about 40% of the samples analysed this marker for faecal contamination could be found is possibly related to the fact the that Siak River sediments are mostly silts to medium sands. Furthermore, the river bed morphology is not known. Hence, although attempts were made during sampling to retrieve sediments from the deepest part of the river it cannot be ruled out that samples were taken on the slope where deposition of finer material will less likely.

As Δ5-stenols can also be hydrogenated by anaerobic microbial activity in situ (Gasskell and Eglinton 1975; Mackenzie et al. 1982) these compounds may not be absolutely valid as sewage markers. In the present sample suite, however, truly anoxic conditions in surface sediments were not encountered despite low water column oxygen contents (Rixen et al. 2008). Based on the absolute coprostanol contents a sewage origin of coprostanol seems at first sight to be the most likely explanation for the presence of this compound in Siak River sediments.

Another criterion useful for the interpretation of coprostanol levels is to relate its concentration to cholesterol levels. Together with coprostanol, cholesterol is the main sterol in raw sewage. It is, however, not a specific sterol as it is produced by zoo- and phytoplankton too. Thus, the coprostanol/cholesterol ratio indicates how different biogenic sources contribute. This ratio ranged from 0.36 to 3.76 with a mean of 2.07 in Siak River sediments. A ratio >1 was found in particulate of raw sewage from Toulon, Morlaix and Brest sewage treatment plants (Quéméneur and Marty 1994) and 4 ± 0.2 for sewage sludge from New York City (Takada et al. 1994). Only 4 out of 36 samples containing coprostanol had values <1 all along the Siak River thereby indicating high and continuous sewage input.

In an environment without faecal contamination the hydrogenation of cholesterol to cholestanol (5α-cholestan-3β-ol) through 5α-cholestan-3-one seems to be favoured in comparison to hydrogenation of cholesterol to coprostanol which is the stereospecific product of the hydrogenation within the intestine. Thus, Grimalt et al. (1990) suggested that the 5β/(5β + 5α)-cholestan-3β-ol ratio can be used as indicator of urban sewage pollution. Siak samples had values from 0.33 to 2.30 with a mean of 1.19 (Fig. 3). When, based on the data of Grimalt et al. (1990), a value of >0.4 is adopted for sewage pollution then only a few Siak samples would be sewage contaminated while the majority falls into a mixing region where contributions from plants and sewage cannot be clearly distinguished. This is supported by a consideration of data presented by Furey et al. (2007) from New Orleans where faecal contamination is also evident from the presence of coliform bacteria and the data for faecal material from various sources (Leeming et al. 1996; Fig. 3). In both cases data points clearly fall into the region characteristic of sewage contamination. Thus, from the 5β/(5β + 5α)-cholestan-3β-ol ratio another coprostanol source for the Siak sediments appears likely.

To further investigate the possibility of a plant source a ternary plot of the C27 sterols is employed (Fig. 4). With the exception of station 116 the same stations that showed faecal contamination in the cholestanol ratio versus coprostanol plot (Fig. 3) also deviate from the majority of Siak samples. On the other hand, faeces and New Orleans data (Leeming et al. 1996; Furey et al. 2007) are clearly separated. This suggests that faecal contamination is

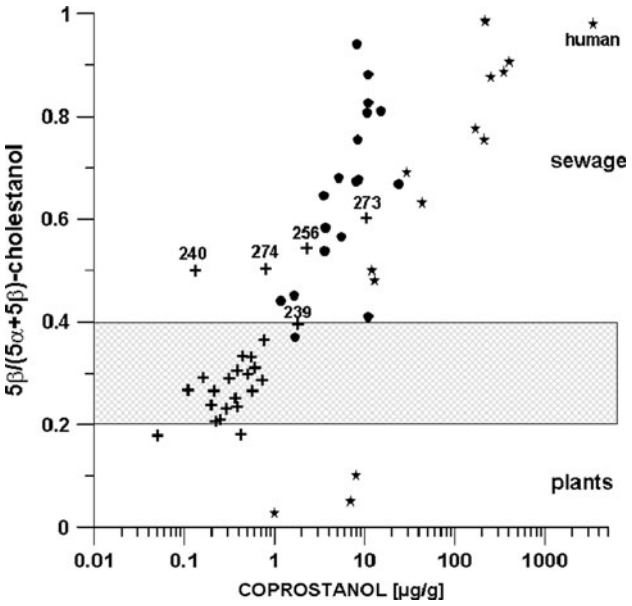

Fig. 3 Cholestanol ratio versus coprostanol content. Crosses–Siak samples; *dots*–recalculated from Furey et al. 2007; *stars*–recalculated from Leeming et al. 1996

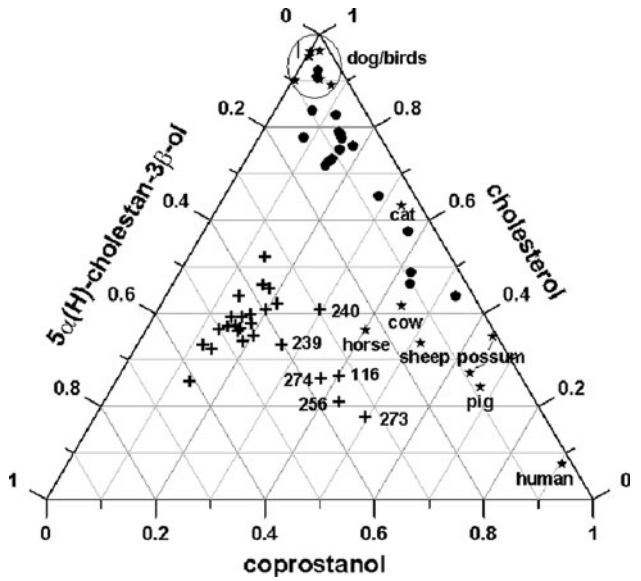

Fig. 4 Ternary plot of C27 sterols. lCrosses–Siak samples; *dots*–recalculated from Furey et al. 2007; *stars*–recalculated from Leeming et al. 1996

characterised by low amounts of 5α-cholestan-3β-ol and variable contributions of coprostanol and cholesterol. Thus, a ternary plot of the three C27 sterols is useful in distinguishing plant-derived from sewage derived compounds.

From both Figs. 3 and 4 other sources of coprostanol in the Siak are evident. It can be assumed that the origin of the cholestanols can be sought in plant-derived organic material that is abundant in river sediments. Although true anoxia was not encountered it is likely that the high organic matter load leads to suboxic conditions under which selective reduction of cholesterol to 5α(H)-cholestan-3β-ol may take place.

Although field observations attest to the large input of untreated sewage to the Siak sediment data do not unambiguously show a chemical signal from this source in the sediments. This suggests that the overwhelming proportion of this input is degraded in the water column. Here, the pronounced decrease in oxygen content downstream from Pekanbaru supports this assumption.

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