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Combined Analysis of Bile Acids and Sterols/Stanols from Riverine Particulates To Assess Sewage Discharges and Other Fecal Sources

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This investigation aimed to demonstrate the combined use of bile acids, stanols, and sterols to assess fecal matter inputs into aquatic environments. Bile acids, stanols, and sterols were determined in suspended particulates in water samples collected from sites in the vicinity of discharges from sewage treatment works along the course of the Avon River, Bristol, U.K. The concentrations of the major fecal bile acids [lithocholic (LCA) and deoxycholic (DOCA)] were determined using GC and GC/MS and found to increase along the course of the river. These results agreed with those obtained for coprostanol, the traditional indicator of fecal pollution and other related sterols and stanols. In contrast, sterols and stanols not originating from feces, i.e., 24-ethylcholesterol and 24-ethylcholestanol, tended to decrease in concentration as compared to coprostanol and other fecal markers in the lower reaches of the river. The increasing concentration of bile acids downstream of sewage discharges correlates with the coprostanol/(coprostanol + 5α-cholestanol) ratio of >0.7, thus supporting the use of bile acids as sewage pollution markers. Overall, it is demonstrated that a combined multimolecular approach involving bile acids, stanols, and sterols provides an enhanced means of assessing fecal matter inputs into aquatic environments.

Introduction

Fecal bacteria are associated with sediment and water particulates (*1*−*5*) and have been used since early this century to monitor water quality and discharge of sewage in freshwater and saline water (*6*−*14*). However, using bacteria as indicators of fecal contamination suffers from several disadvantages including extreme variability in their survival, culturability, and enumeration, particularly in saline environments (*11*, *15*−*17*). Conversely, coliform bacteria can give a false indication of sewage pollution due to their growth in water (*18*) or from the inclusion of coliforms of nonfecal origin (*19*).

The use of natural and synthetic organic compounds, inorganic substances, and various trace metals overcomes some of the problems associated with the use of fecal bacteria as pollution indicators but has several limitations including the following: (i) lack of specificity to fecal inputs, (ii) alteration by environmental microorganisms; (iii) their sensitivity and accuracy may be effected by the natural background levels, and (iv) some of the proposed chemical indicators require prohibitively expensive analytical techniques to detect them (*20*).

Coprostanol is one of the major fecal sterols excreted by man and some other higher animals (*14*, *21*−*26*), and its use has been shown to overcome many of the problems associated with other chemical indicators. This compound and related 5β-stanols have been used for the last 30 years as sensitive and reasonably specific indicators of sewage pollution in the environment (*11*−*14*, *23*, *27*−*30*). For example, Leeming and Nichols (*31*) have analyzed sediments collected from different locations of the Derwent Estuary, Australia, and found that the mid-estuary and parts of the upper estuary were more contaminated by sewage as compared to the lower parts. Similar studies were carried out for the Mississippi River (*32*), the Seine Estuary (*33*), and the Kaoping River (*34*). These studies showed that the concentration of coprostanol decreased with distance from the coast and with increasing sediment depth, probably due to decreased sewage input, dilution by uncontaminated water or sediment, dilution by biogenic sterols, and degradation (*34*−*36*).

Although coprostanol in aquatic systems has long been considered as an unambiguous indicator of fecal input, it is degraded rapidly by aerobic bacteria present in sewage and natural waters (*29*, *11*). Furthermore, cholesterol is reduced by microorganisms to 5α- and 5β-stanols in anoxic environments (*37*−*39*). In an attempt to improve the reliability of coprostanol as a fecal pollution marker, several suggestions have been made. For example, the importance of the relative proportion of coprostanol as compared to cholesterol in determining the source of sedimentary coprostanol has been emphasized (*11*). In a development of this approach, the ratio of coprostanol/(coprostanol + 5α-cholestanol) can be used for assessing fecal input in an anoxic environment where the ratio is >0.7 (*30*, *34*). Others have suggested the use of coprostanol/5α-cholestanol (*29*) or coprostanol/(5α-cholestanol + cholesterol) (*32*). For the use of coprostanol for sewage monitoring, the significance of the transformation of cholesterol to 5α− and 5β-stanols is still to be fully resolved.

The bile acids are major excretory products of body cholesterol together with the neutral stanols and sterols (*24*, *31*, *32*). The primary bile acids, chenodeoxycholic (CDOCA) and cholic acid (CA), are formed in the liver from cholesterol and secreted with the bile to the intestine. The microorganisms present in the intestine transform primary bile acids (CDOCA and CA) to secondary bile acids. Most of the secondary bile acids are absorbed in the intestine and returned to the liver with only a small but significant fraction excreted in the feces. Lithocholic acid (LCA) and deoxycholic acid (DOCA) are the major secondary bile acids excreted in the feces of humans and some higher animals (*35*, *40*). Therefore, the additional information gained from the analysis of bile acids in the environment provides a potentially new means of evaluating fecal pollution.

In our first contribution on the use of bile acids as sewage pollution indicators (*36*), bile acids were determined in sediment and particulates from raw and treated sewage effluent. In this study, fecal bile acids and sterols were determined in suspended particulates in water samples collected at locations in the vicinity of discharges from sewage treatment works along the River Avon, U.K. The River Avon was chosen for this study as it receives discharges from numerous sewage works; it is convenient for sampling; and the nature of the various sewage discharges is well-described

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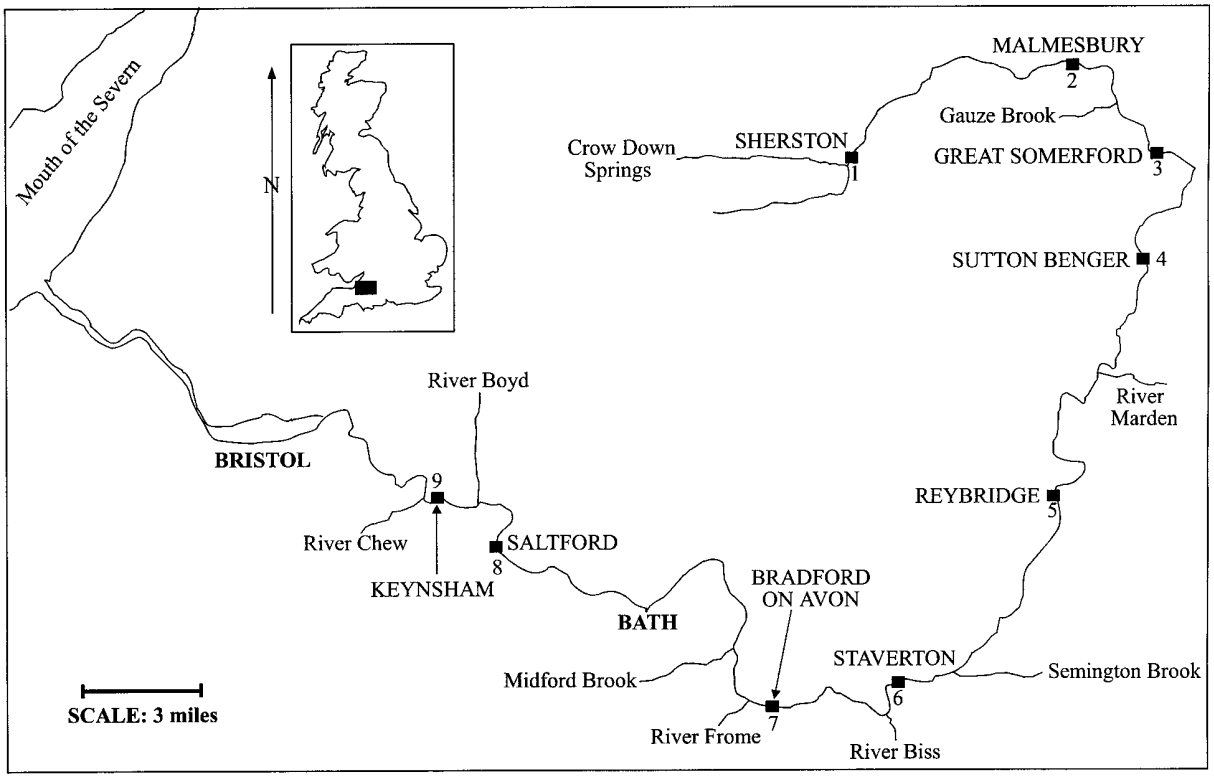

FIGURE 1. Sampling sites on the River Avon.

TABLE 1. Location of Sites along the River Avon from Which the Water Samples Were Collected for Determination of Bile Acids, Stanols, and Sterols

site no.[a]	location	O/S grid reference[b]
1	Sherston	(ST) 852857
2	Malmesbury	(ST) 944864
3	Great Somerford	(ST) 965832
4	Sutton Benger	(ST) 955776
5	Reybridge	(ST) 919692
6	Staverton	(ST) 855610
7	Bradford on Avon	(ST) 804600
8	Saltford	(ST) 693679
9	Keynsham (Bitton)	(ST) 65968

[a] Numbers are as shown in Figure 1 following the course of the River Avon. [b] Grid references for site locations obtained from U.K. Ordnance Survey map.

TABLE 2. Major Sewage Treatment Works Discharging Directly into the River Avon

city	population	daily water flow (m^3)	type of treatment
Bath	115 262	25 740	biological filter
Bradford on Avon	10 233	2 950	biological filter
Calne	18 408	3 400	biological filter
Chippenham	34 850	10 000	activated sludge
Keynsham	20 937	4 720	biological filter
Malmesbury	10 613	2 400	biological filter
Melksham	16 354	5 000	biological filter
Sutton Benger	6 621	1 635	biological filter
Thingley	19 236	3 750	activated sludge
Wootton Bassett	16 852	2 300	biological filter

in terms of their location, magnitude, and type of treatment process employed (Table 1) and has been the subject of an earlier investigation involving coprostanol (*41*).

Experimental Section

Sample Collection and Preparation. Surface waters were collected on the same day in May 1996 from nine sites along the River Avon as near the middle of the river as possible. Table 2 lists the sites, and Figure 1 shows their location along the River Avon. Samples were collected in glass bottles, and concentrated H_2SO_4 (5 mL L^{-1}) was added immediately to inhibit microbial activity (*11*, *12*, *41*). A control sample was collected from Sherston in the upper reaches of the river upstream of any of the major sewage works. Sample extraction and analysis were similar to that applied to sewage effluent samples (*36*), with the addition of steps required for stanol and sterol extraction. In summary, water samples (2 L) were filtered within 6 h of collection through glass fiber filters (Grade GF/C, Whatman, Maidstone, U.K.), and the trapped particulates were stored under N_2 at −20 °C (*37*) until analysis. After tearing the filters into 1 cm^2 pieces with stainless steel tweezers and scissors and the addition of the internal standards (hyocholic acid, HCA, and 5β-pregnanol), the particulates trapped on the glass fiber filters were Soxhlet extracted with 150 mL of DCM/MeOH (2:1 v/v) for 24 h. The extract was reduced to a small volume by rotary evaporation. An aliquot of the extract was saponified with 5 mL of 5 M KOH in 90% MeOH for 1 h. After being cooled to room temperature, 10 mL of water was added to the organic extract, which was then acidified to pH 3−4 with 6 M HCl. The neutral and acidic compounds were co-extracted with chloroform (3 × 10 mL). The combined chloroform extracts were again reduced in volume by rotary evaporation and then passed through an anhydrous sodium sulfate column to remove any water which may effect the efficiency of solid-phase extraction columns. The dried extracts were transferred to preweighed vials, reduced to dryness under a gentle stream of nitrogen, dissolved in a minimum volume of hexane by ultrasonication, and then applied to an aminopropyl column (500 mg of NH_2; Isolute Co.) that had been preconditioned with hexane (6 mL). The neutrals, including sterols, were

eluted with 6 mL of DCM/2-propanol (2:1 v/v), and the carboxylic acids were recovered by elution with 12 mL of 5% v/v acetic acid in diethyl ether.

Bile Acid Extraction. The free carboxylic acids recovered from aminopropyl columns were transferred to screw-capped vials, methylated by adding 10 mL of freshly prepared diazomethane in ether, and allowed to stand at room temperature overnight. Methylated acids were then dissolved in 1 mL of DCM/hexane (2:1 v/v) and fractionated on an activated (120 °C, overnight) silica gel column (150 × 8 mm; 0.6 g; 220−440 mesh). The column was eluted with 5 mL of DCM/hexane (2:1 v/v) and 5 mL of DCM/MeOH (2:1 v/v) to recover monocarboxylic fatty acid methyl esters and hydroxy carboxylic acid methyl esters (including the bile acids), respectively.

Sterol Extraction. The dried neutral sterols recovered from the aminopropyl column were further separated on a silica gel column after sonication in 1 mL of hexane for 5 min by loading onto an activated (120 °C, overnight) silica gel glass column (150 mm × 8 mm) and eluting the column with a series of solvents: hexane (3 mL), hexane/DCM (9:1 v/v; 1.5 mL), DCM (2 mL), DCM/MeOH (1:1 v/v; 2 mL), and MeOH (2 mL) to recover hydrocarbons, aromatics, ketones, alcohols, and polar compounds, respectively. The alcohol fraction obtained from the 'flash' column contains sterols and *n*-alcohols, and further separation of these compounds was carried out using urea adduction (*42*).

Derivatization. All fractions were evaporated to dryness and converted to their trimethylsilyl (TMS) ethers by adding 100 μL of silylating agent (dry pyridine/hexamethyldisilazane/trimethylchlorosilane; 9:3:1 v/v/v; Sigma Chemical Co.), sealed under nitrogen and allowed to stand at 70 °C for 1 h. The excess derivatizing reagents were removed with a gentle stream of nitrogen, and the derivatized bile acids were diluted with an appropriate volume of hexane prior to analysis by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Instrumental Analysis. Aliquots (0.5−1 μL) of all samples were injected manually into a Hewlett-Packard 5890 series II GC using on-column injection. The analytes were separated using a Chrompack CP-SIL-5 CB fused silica capillary column (50 m × 0.32 mm i.d.; 0.12 μm film thickness). The oven was held at 40 °C following injection and then temperature programmed from 40 to 230 °C at 20 °C min^{-1}, then to 300 °C at 2 °C min^{-1}, and held at that temperature for 20 min. Hydrogen was used as the carrier gas (10 psi head pressure), and a flame ionization detector (FID) was used to monitor the column effluent in GC analyses. GC/MS analyses were carried out using a Carlo Erba HRGC 5160 Mega series GC, comprising an on-column injector coupled to a single stage quadrapole mass spectrometer (Finnigan MAT 4500). The GC conditions were similar to those noted above. The temperature of the interface between the GC and MS was held at 300 °C. The MS operating conditions were as follows: ion source, 170 °C; filament current, 0.25 mA; electron voltage, 70 eV; m/z range, 50−650; scan rate, 1 scan s^{-1}; carrier gas, helium. Data acquisition and processing were carried out using an INCOS data system. Peak assignments were made by comparison of literature mass spectra and comparisons of retention times of authentic compounds, followed by co-injection. Quantification was based on GC-FID peak areas with reference to an internal standard (see above). Detection limits for both bile acids and sterols/stanols were 1 ng μL^{-1} injected on-column, which was well within the range of the amounts of analytes recovered from the majority of particulate samples.

Results and Discussion

The main sources of the organic matter in the River Avon are from human waste through discharge from treatment plants, animal waste from surrounding farms, natural terrestrial run-off, industrial waste from factories along the river, and the aquatic biomass. Water was collected from nine locations along the River Avon for bile acid analysis to examine the extent of the fecal pollution along the river and in particular to compare the data provided by stanols, sterols, and bile acid fecal indicators. The overall aim was to determine whether a range of chemical indicators associated with suspended particulates could be used to detect variations in the input of treated wastewater or natural drainage along the river's course in relation to the differing nature of the catchment, i.e., urban versus rural, and distance from source.

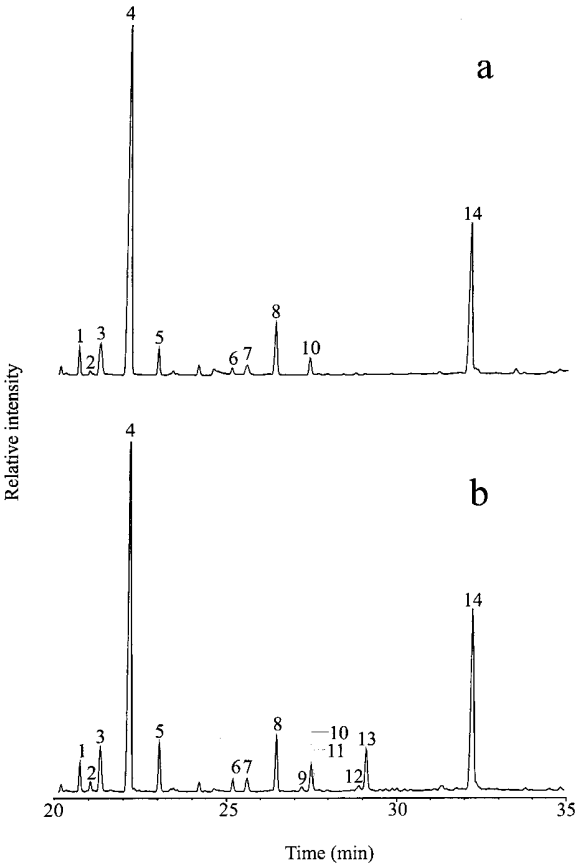

FIGURE 2. Partial GC profiles of the methyl ester−TMS ether derivatives of the hydroxy acid fraction of suspended particulates in water from the River Avon near its source at Sherston (site 1; a) and lower reaches at Keynsham (site 9; b).

Analysis of Bile Acids. The hydroxy acid profile of water particulates collected from the upper reaches of the River Avon (Sherston, site 1, Figure 1) is shown in Figure 2a,b as compared with a sample collected from Keynsham (site 9) at the lower reaches of the river. Table 3 lists the compounds identified by GC/MS in the particulates of the River Avon water. The ω- and α-hydroxy acids known to derive from higher plants and algae (*43*) dominate both samples. Significantly, however, no bile acids were detected in the sample collected from Sherston, a location where no sewage or other significant sources of fecal input occurs (Figure 2a). Hence, this sample effectively constitutes a 'control' for this study.

The GC profile obtained from the second water sample collected at Malmesbury contained an additional component that was identified as DOCA using GC/MS and co-injection with the authentic compound. DOCA was also detected in the sample collected from Great Somerford (site 3) together with LCA; its 3α-epimer, ILCA; and ADOCA. Interestingly,

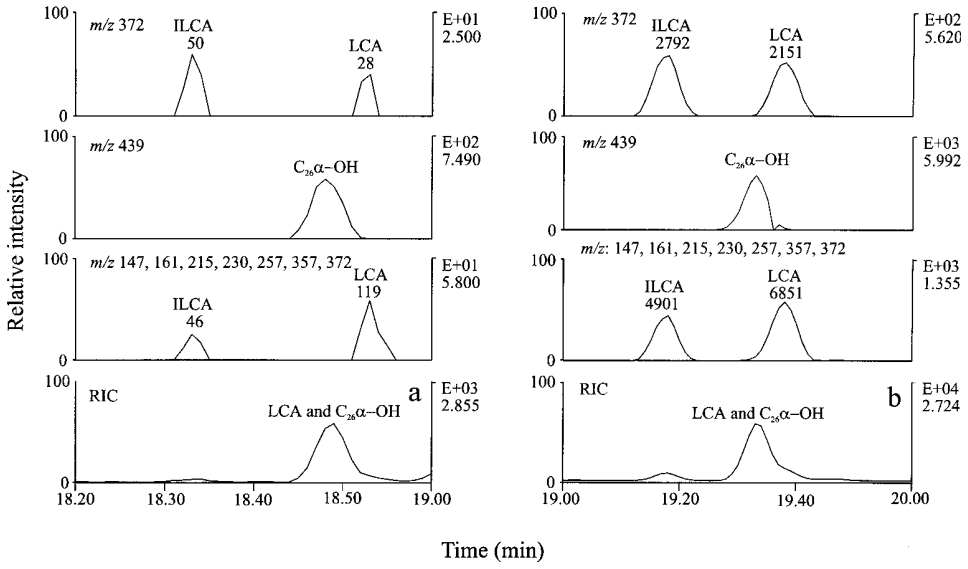

FIGURE 3. Partial RIC mass chromatograms of the hydroxy acid fraction of suspended particulates in water from the River Avon near Great Somerford (site 3; a) and Keynsham (site 9; b).

TABLE 3. Abbreviations and Peak Assignments for Bile Acids, Sterols, Stanols, and Other Compounds Referred to in Chromatograms and Text

compound assignment	peak assignments	abbreviations
C_{18}-monoacylglycerol	1	
$C_{23}\alpha$-OH[a]	2	
C_{22}DA[b]	3	
$C_{22}\omega$-OH[c]	4	
$C_{24}\alpha$-OH[a]	5	
$C_{25}\alpha$-OH[a]	6	
C_{24}DA[b]	7	
$C_{24}\omega$-OH[c]	8	
3β-hydroxy-5β-cholanoic	9	ILCA
$C_{26}\alpha$-OH[a]	10	
3α-hydroxy-5β-cholanoic	11	LCA
3β,12α-dihydroxy-5α-cholanoic	12	ADOCA
3α,12α-dihydroxy-5β-cholanoic	13	DOCA
3α,6α,7α-trihydroxy-5β-cholanoic	14	HCA
5β-pregnanol	a	internal standard
coprostanol	b	5βC_{27}
epi-coprostanol	c	5βC_{27} epi
cholesterol	d	$C_{27}\Delta^5$
5α-cholestanol	e	5αC_{27}
24-methyl-5β-cholestan-3β-ol	f	5βC_{28}
24-ethyl-5β-cholestan-3β-ol	g	5βC_{29}
24-ethylcholest-5-en-3β-ol	h	$C_{29}\Delta^5$
24-ethyl-5α-cholestan-3β-ol	i	5αC_{29}

[a] α-OH, α-hydroxy acids. [b] DA, diacids. [c] ω-OH, ω-hydroxy acids.

ILCA, LCA, and ADOCA were not detected in the water particulate samples collected from Malmesbury. Significantly, DOCA comprises ca. 80% of the total bile acids of cattle feces (*31*, *35*). The dominance of DOCA therefore may reflect the fact that fecal matter from herbivores, such as cattle, makes a significant contribution to the overall fecal load of the river at this site.

An important practical point concerns the coelution of LCA with the C_{26} α-hydroxy acid. Since these compounds were not separated using 'wet' chemical methods, the concentration of LCA was established using GC/MS to obtain the relative proportions of LCA and ILCA, as shown in Figure 3. As the *m*/*z* 372 chromatogram did not accurately reflect the distribution of LCA and its epimer, the summed mass chromatogram of the ions of *m*/*z* 147, 161, 215, 230, 257, 357, and 372 (selected since they carry the greater proportion of the ion current for these bile acids) was plotted to give an accurate representation of both LCA and ILCA. The latter compound (ILCA) presented no coelution problems; therefore, the ratio of the peak areas was obtained from the summed mass chromatograms, which reflects the relative proportion of LCA and ILCA. This ratio was then used to calculate the concentration of LCA using the peak area of ILCA determined from the GC-FID chromatogram. This correction was applied to all samples analyzed during this study. No such interference was observed during our previous study of raw and treated effluent taken directly from a sewage works where the concentrations of bile acids are significantly higher (ca. 2 orders of magnitude; *35*, *36*).

The concentrations of the four major bile acids, i.e., ILCA, LCA, ADOCA, and DOCA, increased along the entire course of the river (Figure 4). This increase probably reflects the increasing load of fecal material discharged into the river from the sewage treatment plants rather than agricultural inputs, which appear to be more significant in the upper reaches of the river. The absence of hyodeoxycholic acid (a bile acid known to be characteristic of pig feces) in all samples precludes significant pig fecal inputs to the River Avon (*40*). The higher bile acid concentration in the sample collected from Bradford-on-Avon probably relates to an influx of fecal material introduced by the River Biss. The latter river receives discharges from the Trowbridge treatment plant and joins the River Avon upstream of the sample site. The substantial increase in bile acid concentration in the river from sewage discharges associated with the major center of population, i.e., Bath, can be clearly seen in the samples collected from sites 8 and 9 at Saltford and Keynsham, which are located downstream of the city.

Analysis of Sterols. Results for sterols from the samples collected along the course of the River Avon support the observations made on the basis of the bile acid analyses. For example, the very low concentration of coprostanol (0.2 μg L^{-1}) and 24-ethylcholestanol (0.3 μg L^{-1}) at Sherston (site 1) about 1 mi from the source and significantly higher concentrations (5.5 and 2.7 μg L^{-1}) in the lower reaches reflect the increased discharges from the various sewage treatment works. Table 3 lists the identities of the major sterols and stanols detected in the particulates. Although cholesterol is produced by algae, 24-ethylcholesterol (e.g., sitosterol) is

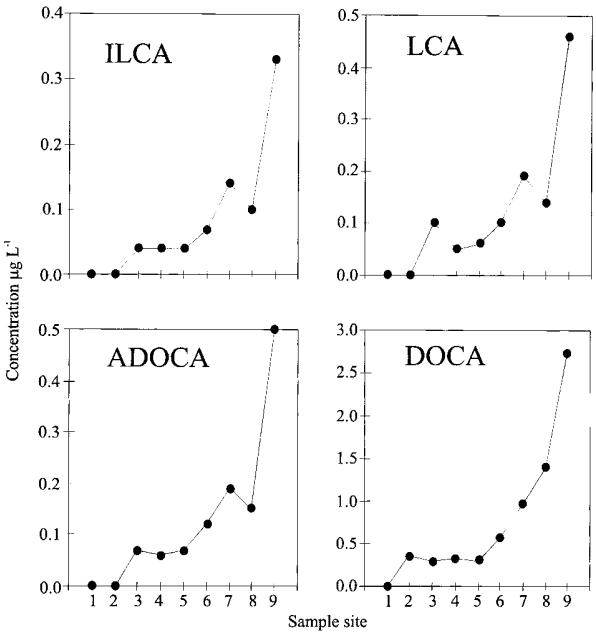

FIGURE 4. Plots of the concentrations (μg L^{-1}) of bile acids associated with suspended particulates in water samples collected along the course of the River Avon.

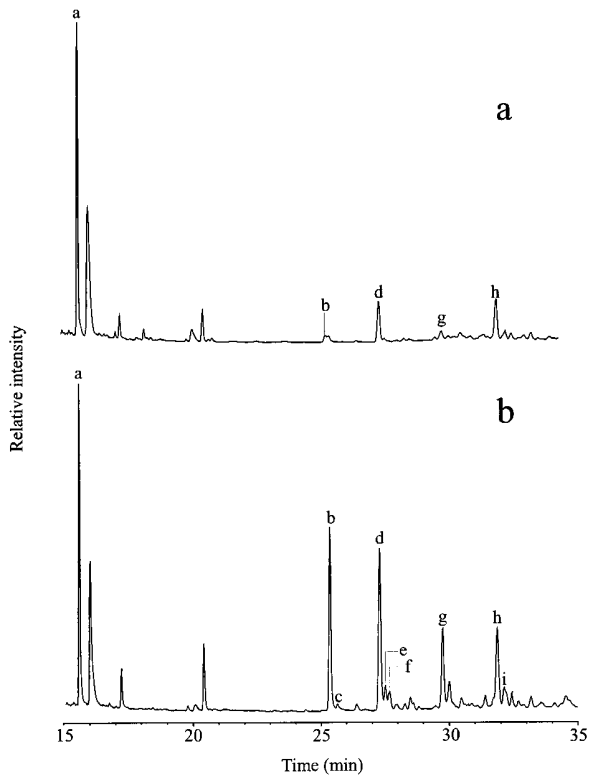

FIGURE 5. Partial GC profiles of the TMS ether derivatives of the stanol/sterol fraction of suspended particulate matter in water collected from the River Avon near the source at Sherston (site 1; a) and lower reaches near Keynsham (site 9; b).

generally regarded as a higher plant indicator. These were dominant in the sterol fraction of the Sherston sample (site 1) (Figure 5a), where they are presumed to derive largely from the aformentioned sources (44). However, the 2:3 ratio of the 5β-C_{27} to 5β-C_{29} stanols in the Sherston sample is consistent with an origin from herbivorous animals (29, 35). However, since no bile acids were detected in this sample, it may be that the lower amounts of 5β-stanols present derive from microbial reduction of algal or plant sterols rather than being of fecal origin (37). This is supported by recent work that indicates that bile acids are relatively more stable to degradation than 5β-stanols (36). However, since bile acids are present in feces at lower concentrations than 5β-stanols, failure to detect them may be due to their concentration in this sample being close to the limit of detection of this technique.

Figure 5b displays a partial GC chromatogram of particulate sterols obtained from site 9 at Keynsham; the high concentration of fecal material introduced into the river is evident as compared to the sample from Sherston (site 1). The sterol distribution in samples from various locations along the River Avon is shown in Figure 6. Variations in the abundance of the sterols along the river reflect changes in the contributions of the stanols and sterols introduced to the river from various sources. Figure 7 shows plots of the concentrations of eight stanols and sterols along the River Avon. The plots show similar trends of fecal input along the River Avon as observed for bile acids (Figure 4).

The relative concentrations (Figure 6) of the 5β-C_{27} to 5β-C_{29} stanols within water samples changes along the course of the river from 0.67 at Sherston to 2.05 at Keynsham. This trend is consistent with an increase in the proportion of human sewage relative to that from herbivorous animals (presumed to be predominantly cattle) along the length of the river. The concentration of 24-ethylcholesterols (derived from plant matter) and its reduced product 24-ethylcholestanol also decreased in the samples below Reybridge (site 5). This decrease probably relates to the relative decrease of higher plant input (i.e., greater urbanization in the lower reaches of the river) as compared to other components that are typically present in municipal sewage.

Increases in the concentration of sterols along the River Avon were observed by McCalley et al. (41), who measured 1 and 2 μg L^{-1} of coprostanol and cholesterol, respectively, in water samples collected above the Saltford sewage works as compared to 3 μg L^{-1} of coprostanol and cholesterol in samples from Keynsham below the sewage works. The data obtained in this study support the use of the ratio coprostanol/(coprostanol + 5α-cholestanol) recommended by Grimalt and co-workers (30) as a more reliable parameter for use in detecting sewage pollution. A plot of the variation of this ratio along the course of the river is shown in Figure 8. As can be seen from Figure 8, the proposed cutoff value for this ratio of 0.7 is achieved for all samples taken below points of sewage discharge, thus demonstrating that these discharges are dominated by human effluent. The concentration of bile acids increases more dramatically along the course of the river as compared with the 5β-stanols. For example, the ratio of coprostanol to DOCA is 5.75 at Malmesbury but decreases to 3.14 at Saltford and to 2.03 at Keynsham. This is presumed to result from combined effects of the greater stability of bile acids as compared with stanols and sterols, as demonstrated in our earlier investigation of a sewage treatment works (36) and laboratory degradation experiments (35). There appears to be little difference in the overall sensitivity of bile acids and coprostanol as sewage indicators. Indeed, DOCA would appear to have the potential to be a more sensitive indicator since its concentration varies over a wider dynamic range along the course of the river (Figure 4). The more minor bile acids show similar overall variation but with more fluctuation between sample sites. These fluctuations parallel those seen in coprostanol, e.g., a positive excursion occurs for ILCA, LCA, ADOCA, and coprostanol at site 7 (Bradford-upon-Avon) away from the otherwise gradual increase in their concentrations along the length of the river (Figures 4 and 6).

Statistical analysis was carried out for all the sterol and bile acid data in order to assess the degree of correlation

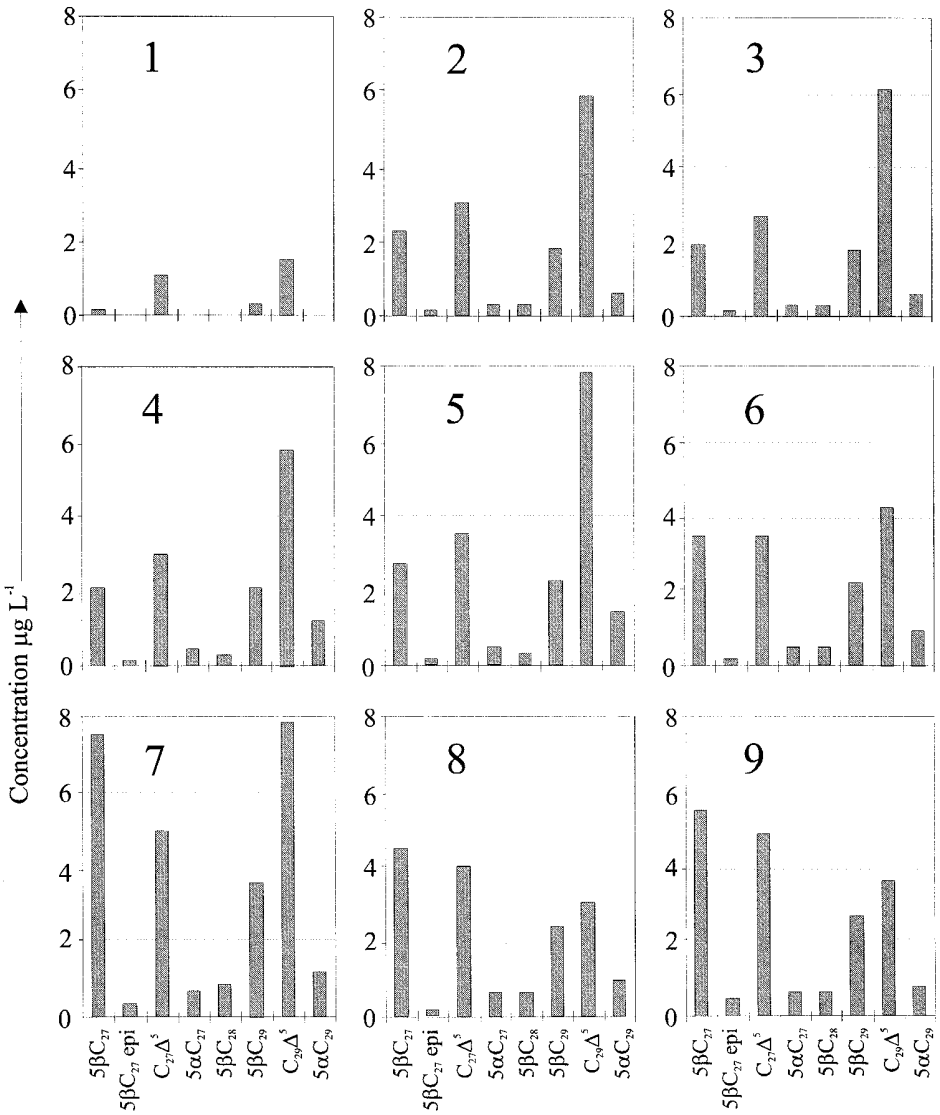

FIGURE 6. Distributions of stanol and sterol components in suspended particulates of water samples collected along the course of the River Avon.

between the various marker compounds and thereby establish whether they have a common origin. Hierarchical Cluster Analysis, Spearman Correlation Coefficients, and Factor Analysis (performed using the SPSS package) all revealed analogous trends. The bile acids all correlated well as a group (factor analysis correlation values range from 0.9512 to 0.9967), indicating that they all have a common origin. Likewise the fecal stanols (5β-stanols) show similarly close correlation between one another (coprostanol/5βC_{28}, 0.9140; coprostanol/5βC_{29}, 0.9315). A reasonable correlation was seen between the bile acids and fecal sterols (e.g., LCA/coprostanol, 0.6877; DOCA/coprostanol, 0.6928). The fact that the correlation between the bile acids and 5β-stanols is less significant than that obtained for components within their respective classes must relate to differences in their origins (e.g., human sewage discharges versus agricultural runoff), differing degradation rates, and dilution/partitioning effects. As expected, the major plant-derived sterol (i.e., sitosterol) and the corresponding 5α-stanols show poor correlations ($\leq$0.4, except for 0.55 in the case of 5βC_{29}) with all other fecal-derived bile acids and sterol derivatives, confirming that, for the most part, these two components do not derive from the sewage discharges into the river.

The results obtained in this investigation largely parallel those obtained reported by McCalley et al. (41) in their earlier study of coprostanol at various location along the River Avon. Although the results obtained from this work indicate that the total fecal input as measured by coprostanol appears to have reduced after 15 years, an accurate comparison cannot be made without more precise measurements of water depth, river flow rate, and precise sampling location. The application of a multimolecular approach, which includes bile acids, sterols, and stanols, has enhanced the specificity of the use of steroidal compounds as sewage pollution indicators and also provided a further important step in the validation of bile acids as a new group of sewage pollution indicators. Further work will focus on correlating the incidence of bile acids with other statutory indicators of sewage pollution, such as fecal coliform bacteria.

In summary, this paper provides the first demonstration of the complementary use of bile acids, stanols, and sterols as indicators of modern fecal inputs to an aquatic environment. The results of analyses of suspended particulates from sites along the course of the River Avon have revealed that:

(i) The major source of bile acids is human fecal matter discharged by treatment plants.

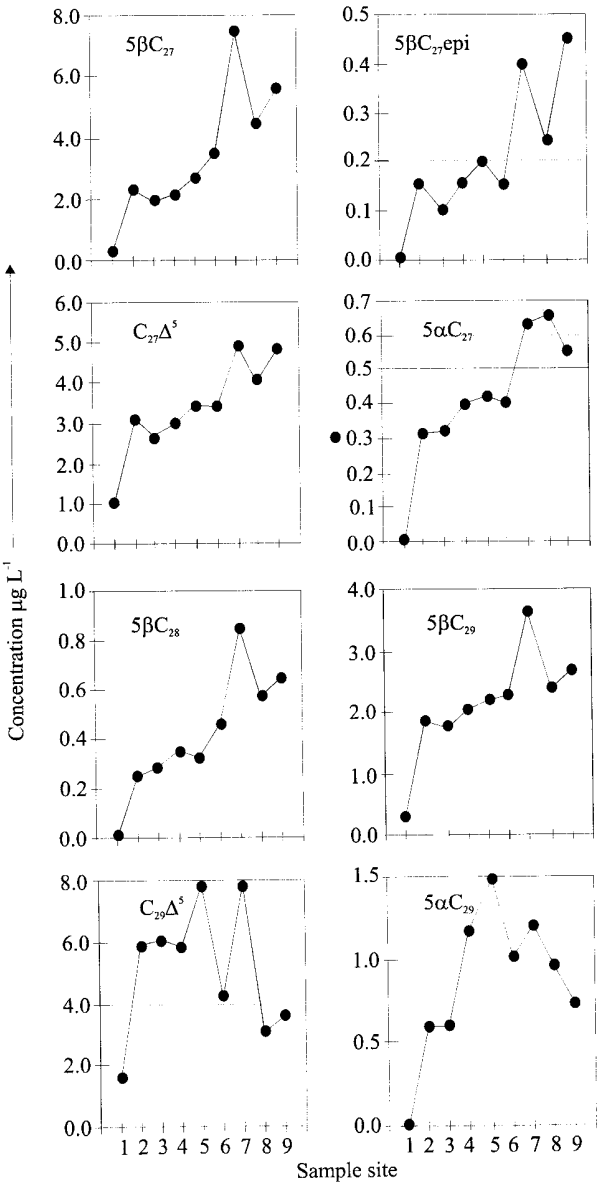

FIGURE 7. Plots of the concentrations (μg L^{-1}) of sterols in suspended particulates of water samples collected along the course of the River Avon.

(ii) Evidence from both the bile acid and stanol profiles indicate that agricultural inputs are more significant in the upper reaches, i.e., sites 1−4. The lack of hyodeoxycholic acid in any samples precludes a significant contribution from pig fecal matter to the River Avon.

(iii) The concentration of bile acids and stanols showed similar trends, increasing along the course of the river due to the cumulative effects of discharges of fecal matter by sewage works.

(iv) Use of the proposed ratio coprostanol/(coprostanol + 5α-cholestanol) (30) further supported increased fecal pollution following the course of the river.

(v) Comparison of the changes in the concentrations of DOCA and coprostanol along the course of the river confirm the greater stability of bile acids to degradation (36) as well as possible source differences.

(vi) The markedly different trends in the concentration of the 24-ethylcholesterol and 24-ethylcholestanol confirm their origin from nonfecal sources. The reduction in concentration, particularly of the 5α-C_{29} components, in the lower reaches

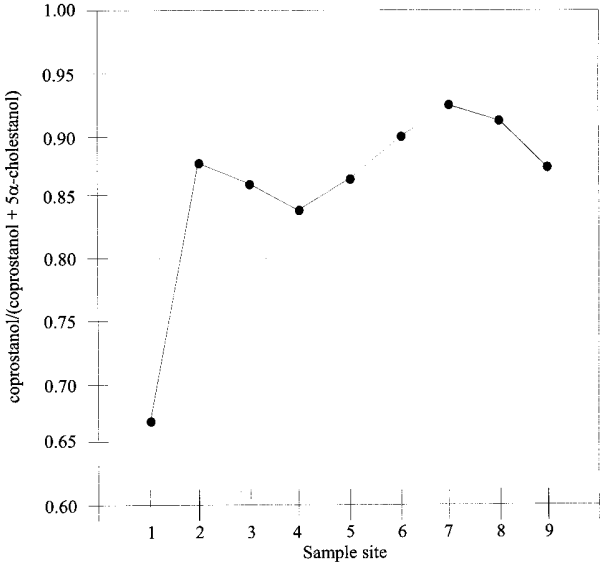

FIGURE 8. Ratio of coprostanol/(coprostanol + 5α-cholestanol in suspended particulates of water samples collected along the course of the River Avon.

may be the result of increased urbanization. These data confirm the potential to use bile acids along with stanols and sterols as indicators of sewage pollution. We will extend this approach to sediments and examine the minor bile acids to increase specificity and will correlate the occurrence of bile acids with microbial communities known to be associated with fecal sources.

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