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Determination of fecal contamination indicator sterols in an Australian water supply system

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Abstract This paper reports a reconnaissance survey of the concentrations of sterol compounds (as indicators of fecal contamination) in a large water supply system in southeast Australia comprising a network of rivers, channels, and drains. Levels of coprostanol and cholestanol were determined in surface water and bottom sediment using gas chromatography–mass spectrometry analysis across 17 strategic sampling sites and over 12 months. Clear differences in the levels of fecal contamination were observed among sites. Four sites routinely contained high levels of the fecal indicator sterols indicated from surface water and sediment sample analysis. Coprostanol concentrations at each location varied from 0 ng/L at the reference site to 11,327 ng/L in a surface water sample of a drain directly downstream of a knackery. The majority of the sites contained coprostanol in the range of 500 to 800 ng/L. Since no fecal-associated sterol compounds were detected at the external reference sites, these were assumed to be free from fecal contamination. Sewage water discharge and/or substantial water runoff maybe the principal factors contributing to fecal contamination of the supply drains and channels.

Keywords GC–MS · BSTFA · Faecal sterols · Catchment survey · Coprostanol · Fecal contamination

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Introduction

Appropriate management of the supply and quality of water, in terms of microbial risk, is an imperative for protecting human health both directly via water consumption and indirectly through food production and other water uses. A common risk identified is the contamination of water sources by water-borne pathogens through fecal contamination. These sources of contamination are classified into "nonhuman" fecal contamination (natural sources, e.g., migratory and local wildfowl, kangaroos and wallabies, sheep and cattle, effluent from dairies, poultry farms, piggeries, and slaughter houses) and "human" fecal contamination.

The nature and associated potential hazards to health of contamination may be assessed using biological and chemical indicators (Cooper and Danielson 1997; Isobe et al. 2002; Glassmeyer

and Shoemaker 2005). However, monitoring of contamination through assessing microbial (biological) indicators is limited by two factors, culturability and organism characterization (Pontius 2000; Solo-Gabriele et al. 2000; Dutka et al. 1974) leading to an inability to distinguish a particular source of contamination (Isobe et al. 2002; Hyun et al. 2002). Chemical markers such as fecal sterols can provide advantages in determining the level and extent of contamination as they have environmental stability, ease of detection and can be used to discriminate between human and nonhuman contamination (Isobe et al. 2002; Hyun et al. 2002; Ottoson and Stenström 2003; Noblet et al. 2004).

The sterol coprostanol (5-cholestan-3β-ol) is produced primarily in the intestines of higher animals by enteric microbial reduction of cholesterol (Readman et al. 2005) leading to hydrogenation of the double bond between C5 and C6 in the second hexane ring of cholesterol (carnivore/omnivore). The presence of coprostanol in water sources is considered as a specific indicator of fecal contamination (Gilli et al. 2006). Coprostanol has a low solubility in water and a tendency to associate with suspended particles and is incorporated into bottom sediments (Venkatesan and Kaplan 1990). Within anoxic sediments, it may be preserved up to 450 days at 15°C and with very limited degradation (Nishimura 1982). Moreover, the concentration of coprostanol in water and/or sediments is barely influenced by various treatments such as chlorination or aeration of overlying water, which makes it a powerful marker for fecal pollution monitoring (Leeming and Nichols 1996). In a similar fashion, differences in the abundance of 24-ethylcoprostanol and 24-ethylepicoprostanol can be used to identify differences between fecal contamination from carnivores or omnivores and herbivores (Leeming and Nichols 1996).

A large complex water supply system managing 70% of the stored water in Victoria, Australia within an extensive catchment covering approximately 68,000 km^2 and including 7,150 km of open channels was the focus of the study. The total water carried in the system equates to approximately 3.4 gal/year and is used for town and rural supply, for domestic, manufacturing, food processing, agricultural irrigation, and stock production as well as recreation. The purpose of this survey was to determine the concentrations of fecal sterols (especially coprostanol and cholestanol) in surface water of the supply system in order to monitor potential high input sites of fecal contamination. Measurement of fecal sterols was achieved through the following aims (1) to estimate the levels of fecal contamination indicators using a gas chromatography–mass spectrometry (GC–MS), (2) to assess variation in sterol indicators among reconnaissance sampling sites over a 12-month period, and (3) to establish a relationship between the observed fecal sterol profiles and the point source contamination.

Materials and methods

Study area and descriptions

The catchment and supply system contained a variety of water use requirements (human consumption, livestock, and other agricultural uses, food manufacturers, and environmental flows) within the catchment. The extent of the catchment is *approximately* 68,000 km^2, with 250 km of inflow from a major river and 7,150 km of open channels with an extensive associated network of drains.

The sampling sites were identified within the catchment on the basis of industry locations and a pilot microbial risk analysis study (Table 1). Each site was classified according to three groups; group 1, those associated with rivers (southeastern upland areas of the catchment) and offtakes from rivers; group 2, sites associated with channels (central region); and group 3, those associated with drains (northwestern catchment area). The sampling protocol was a "before–after control for impact." Samples were collected both upstream and downstream (300 m from outflows) of outfall drains, a piggery, a slaughterhouse, a dairy, a poultry farm, and a town sewage treatment plant. In addition to these locations, samples were collected from three sites external to the water supply system (group 1), which were assumed to have minimal direct impact from human or intensive industry-derived wastewater. Samples of water

Table 1 Specific identification of sampling locations the water supply system along with description of reason to include in the sampling plan

Cluster reference	Site ID	Description	Classification
1	001	Reference site 1: Kevington river	Group 1
2	007	Reference site 2: channel offtake river	
3	024	Reference site 3: natural wetlands	
4	010	Channels-piggery upstream	Group 2
5	011	Channels-piggery downstream	
6	017	Channels-poultry upstream	
7	018	Channels-poultry downstream	
8	045	Channels-sewage upstream	
9	048	Channels-sewage downstream	
10	008	Drains-outfall upstream	Group 3
11	009	Drains-outfall downstream	
12	013	Drains-knackery upstream	
13	014	Drains-knackery downstream	
14	015	Drains-dairy upstream	
15	016	Drains-dairy downstream	
16	019	Drains-dairy upstream	
17	020	Drains-dairy downstream	

were obtained, by grab sampling, from channel bank, or from bridges at a depth of 1 m when water was present in the watercourse. Grab samples (10 L) were taken from each site at monthly intervals from August 2006 to October 2007. The climatic conditions during this period are presented in Table 2 (Eildon (145° 84′ E 37° 21′ S), Tatura (145° 27′ E 36° 44′ S), and Kyabram (145° 06′ E 36° 34′ S) representing the major areas of Victoria associated with the catchment). Water temperature, pH, and turbidity (optical density) at collection was recorded. The samples were transported in cool boxes and stored at 4°C in a cold room at the University of Melbourne laboratories

Table 2 Temperature range (°C) and average monthly rainfall (mm), water temperature (°C), pH, and optical density (median) of water samples during study period (August 2006 to October 2007)

Site	Variable	Winter (June, July, August)	Spring (September, October, November)	Summer (December, January, February)	Autumn (March, April, May)
Eildon Fire Tower (group 1)	Rainfall	65.2	26.4	25.6	87.8
	Temperature	3.9–13.8	6.9–21.7	15.0–28.7	10.7–22.5
	Water temp	7.5	13.8	18.8	15.5
	pH	6.96	6.97	7.15	7.32
	OD	0.037 (0.006–0.095)	0.004 (0.000–0.012)	0.038 (0.009–0.101)	0.016 (0.006–0.044)
Tatura (group 2)	Rainfall	29.7	10.9	19.1	34.2
	Temperature	2.7–14.9	7.8–22.4	14.9–30.4	9.5–23.1
	Water temp	6.8	17.0	20.7	15.0
	pH	7.25	7.49	7.32	7.34
	OD	0.149 (0.048–1.528)	0.137 (0.049–0.312)	0.077 (0.07–0.326)	0.062 (0.027–0.970)
Kyabram (group 3)	Rainfall	27.4	10.7	12.0	37.5
	Temperature	3.2–15.0	7.1–22.9	15.2–31.4	10.9–23.6
	Water temp	6.4	17.5	19.9	16.7
	pH	7.61	7.67	7.44	7.58
	OD	0.336 (0.059–1.186)	0.430 (0.087–1.682)	0.231 (0.121–0.778)	0.219 (0.067–0.886)

at Parkville, Victoria. The samples were processed for sterol analysis within 48 h of collection.

Materials

All chemicals and solvents for sterol analysis were of analytical reagent grade except sterol standards (GC grade). Sterol standards, 3β-hydroxy-5α-cholestane (cholestanol), and 5β-cholestan-3β-ol (coprostanol) were purchased from Sigma-Aldrich (Australia). Pyrelene *d*–12 was used as an internal standard (Hanselman et al. 2006) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatization reagents were also obtained from Sigma-Aldrich (Australia).

Extraction and preparation

Water samples were prepared to yield two types of samples for sterol analyses (1) surface water and (2) bottom sediment (1–4 cm). Water samples were filtered using prebaked glass fiber filters to collect suspended particles (0.7 μm; Pall, USA). The filtrates and trapped particulate matter were analyzed on the same day. Calculations of fecal sterols were based on per liter of water samples. Sediment samples were collected from only four sites (008, 009, 014, and 016) for selected months only (May 2007 to October 2007—winter to spring period). Sediments were centrifuged at 2,000×*g* for 10 min. The supernatant was discarded; the residue was used for sterol analysis and the dry weights were determined.

Extraction and derivatization of fecal sterols

Methods for extraction and derivatization of fecal sterols from surface water and sediments were modified from Hyun et al. (2002). Suspended particles from surface water samples were collected on the prebaked glass fiber filter from a 350-mL volume. Retained particulates, along with the filter, were transferred into a glass tube with a Teflon-lined screw cap and extracted twice with dichloromethane (CH_2Cl_2)–methanol (MeOH; 1:1 *v*/*v*) for 30 min using an ultrasonic bath (40 kHz; model FXP8M, Unisonics, Australia). The extract was evaporated to dryness using a heating block (35°C). The extract was then subject to mild alkaline hydrolysis using 9 mL methanolic 0.2 N KOH and heating at 70°C for 30 min. The sample was cooled; additional water was added (3 mL) and the neutral fraction containing sterols and alcohols was partitioned three times into 5 mL of hexane–diethyl ether (9:1, *v*/*v*) mixture. The neutral fraction was dried under nitrogen and treated with BSTFA at 50°C for 15 min, to convert free alcohols to respective trimethylsilyl (TMS) ethers. For the sediment, 2 g (wet weight) was transferred into a glass tube with a Teflon-lined screw cap. The sample was then treated as described above for water samples.

The samples were analyzed using a Model 6850 series II Network GC with a 5973 network mass selective detector (MSD) controlled by MSD ChemStation software (G1701EA E.01.00.237, Agilent Technologies, Australia). Separations were conducted in the splitless mode using a (5% phenyl) methyl silicone fused capillary column (HP-5MS, 30-m length, 0.25-μm i.d., 0.249-mm film thickness; J and W Scientific, USA) with helium as the carrier gas. The volumes of derivatized samples (1.0 μL for sediment or 2.0 μL for filtered water) were injected using the autosampler (Agilent 6850 series). The analysis temperature was programmed as follows. The initial column temperature of 70°C was held for 1 min, after which an increase of 15°C/min was maintained until 250°C, when the rate of increase was reduced to 4°C/min to 310°C and held for 15 min. The transfer line was constant at 300°C. The ionization voltage was 70 eV. In the scanning mode, the mass range was 60–600 amu. Each eluted peak was identified using pure sterol standards and matching with mass spectra from a commercial library (NIST02.L, supplied by Agilent Technologies, Australia). The data were processed using a dedicated data system (MSD ChemStation software—data analysis applications) with quantification based on standard curves of known compounds.

Sterol identification and quantification

For structural identification, electron impact mass spectra were obtained using a GC interfaced to a mass selective detector (MSD 5973Network, Agilent Technologies, Australia) and the same

conditions and column as for GC analysis with helium as the carrier gas. Structural identification of sterols was determined by comparison with reference spectra (obtained from a commercial library database search) and mass spectral interpretation of the compound detected in the sample. Several ($n = 5$) samples were spiked with known amounts of sterol standards to confirm the identification of coprostanol and cholestanol. The detection limit for each sterol was calculated based on the weight giving a signal three times the peak-to-peak noise of the background signal. The method detection limit based on a signal/noise ratio of 3 was 2 ng/L with an instrument detection limit of 0.5 ng/L. The internal standard for sterol analysis was pyrelene $d-12$, a nonpolar saturated compound of similar chemical structure that elutes well before sterols in GC. For quantification, standard curves were generated using high-purity (>99%, GC grade) coprostanol and cholestanol. Known amounts were added to derivatized samples in BSTFA (0.5–250 ng of each standard—five-point calibration curve) and injected onto the GC column. Standard curves were constructed using the data obtained from duplicate injections of each dilution using ChemStation software. Coprostanol and cholestanol present in unknown samples were quantified using these standard curves.

Cluster analysis

Sahn clustering was performed using the site sterol profile data to determine similarities among sites. This was achieved using Genstat software to create a similarity–diversity pairwise matrix based on quantitative measures of coprostanol and cholestanol concentrations. Clustering was performed in a nonhierarchical manner using Genstat Version 8 software (Lawes Agricultural Trust 2003).

Results and discussion

Climatic data

The 30-year average rainfall at Eildon Fire Tower (in the region of the head water of the catchment) is 1,031 mm; however, the rainfall in 2006 was only 364.2 mm reflecting the intense drought conditions prevalent throughout southeast (SE) Australia. In 2007, the rainfall at the site was 907 mm or 88% of 30-year average. At Tatura, the rainfall in 2006 and 2007 were 199.6 and 374.8 mm compared to the 30-year average of 482 mm. In the area of Kyabram, the rainfall in 2006 and 2007 was 228.7 and 356.2 mm compared to an annual average of 356 mm (Table 2). The pH of water samples increased progressively across the catchment with the highest being observed in the drainage system in sites in group 3. Furthermore, the water turbidity increased across the catchment with water samples taken at the head water being of pristine quality compared to that being taken from drainage system in group 3.

Separation and validation of sterol derivatives

The pure standards, coprostanol and cholestanol, showed an excellent separation under the analytical conditions used in this study. The retention times of the corresponding TMS ethers were 23.668 and 25.090 min for coprostanol and cholestanol, respectively. These retention times matched (>99%) with the expected retention times under the same conditions (MSD ChemStation software, Agilent Technologies, Australia), validating their identity (Fig. 1).

In order to determine the resolution of response (background noise and elution patterns of the peaks), a blank sample, a representative environmental sample (sediment sample collected from site number 009 in July 2007), and a spiked environmental sample were used. A typical chromatogram of the blank sample (in BSTFA solvent) showed a clear background from the retention time of 18 min onwards demonstrating baseline stability, and no coelution or inference from other compounds (Fig. 2a). Injection of the derivatized sediment sample showed an elution pattern for a range of sterols with good separation of coprostanol and cholestanol (Fig. 2b). Verification of these two compounds was achieved by spiking the environmental sample with coprostanol and cholestanol (Fig. 2c shows the profile of the sample spiked with coprostanol). The linearity (correlation coefficient R) of the standard curves (0.5 to 250 ng/μL) was 0.998 and 0.999

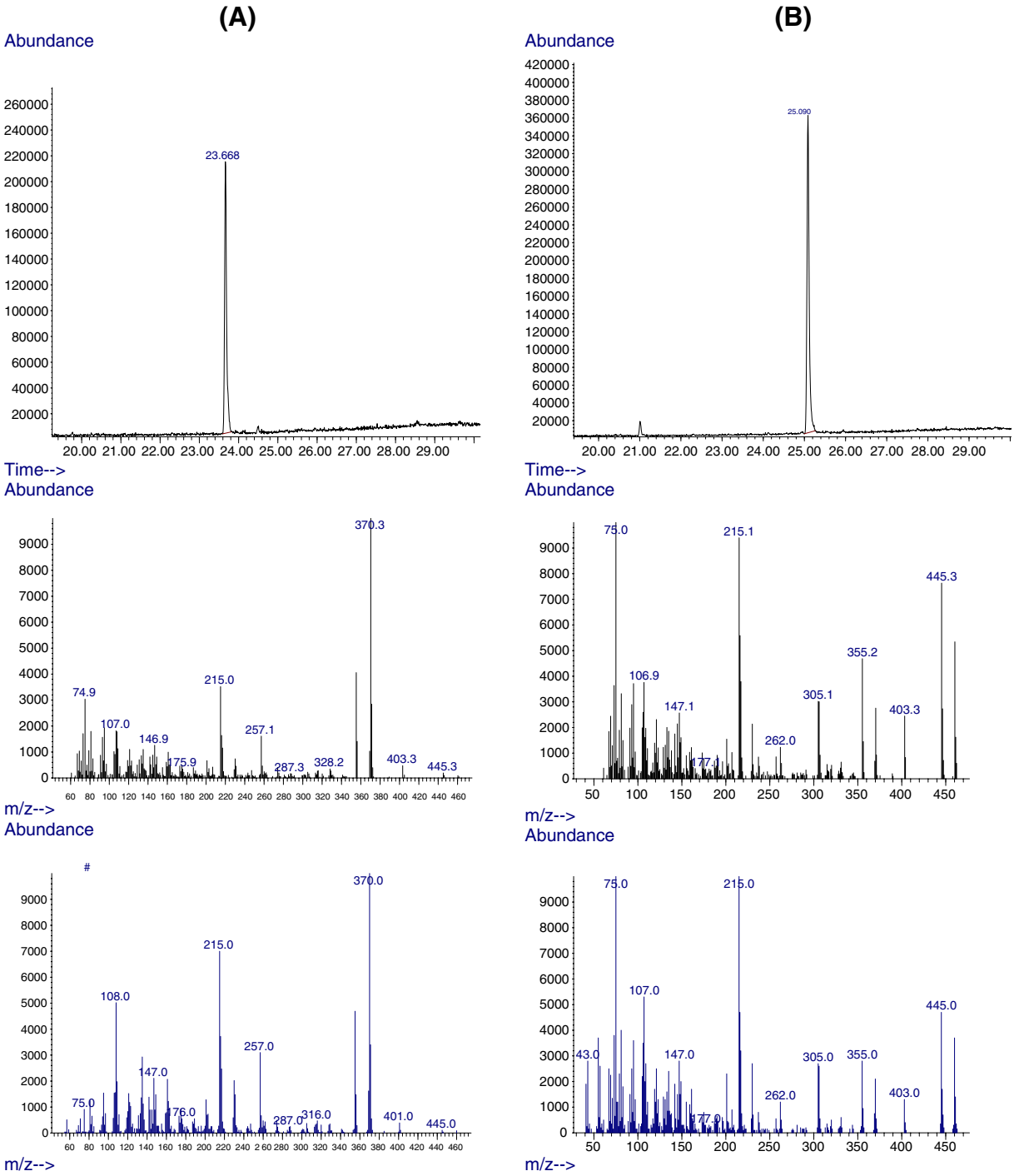

Fig. 1 Derivatized coprostanol (**a**) and cholestanol (**b**) standard peaks and relevant mass spectra identified using commercial MS database library search

for coprostanol and cholestanol, respectively. The detection limit was 0.5 ng/μL and the quantification limit was 2 ng/L in the final extract.

Cluster analysis (Fig. 3) assessed similarities in levels of coprostanol and cholestanol between sampling sites and suggested four statistically dif-

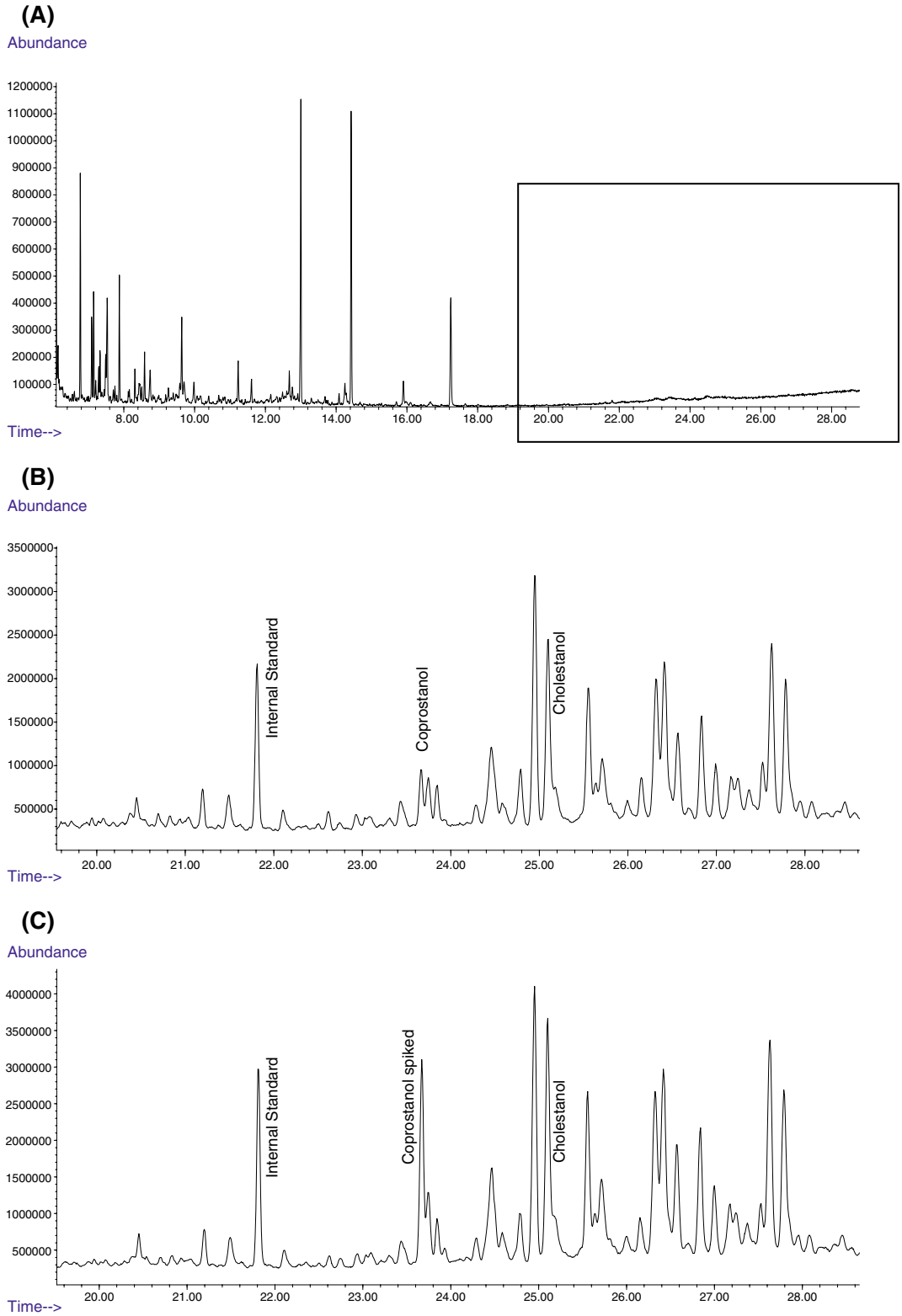

Fig. 2 Total ion current chromatograms: **a** blank BSTFA; **b** July 2007 sediment sample from site 009; and **c** July 2007 sediment sample from site 009 spiked with coprostanol

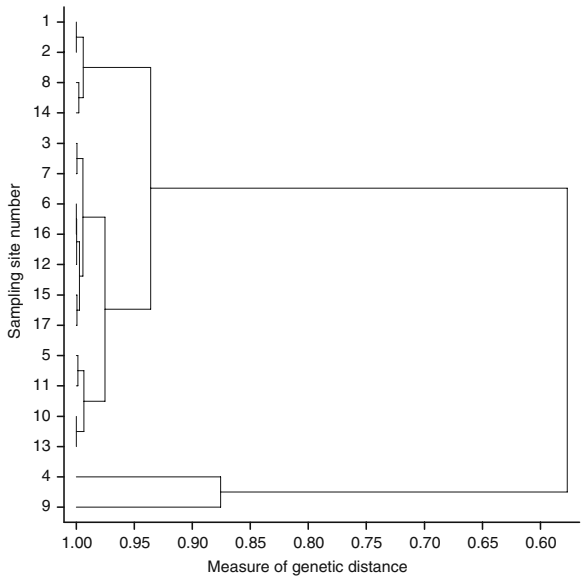

Fig. 3 Sahn clustering of sites according to coprostanol and cholestanol concentrations

ferent groups; Cluster 1, sites that were either reference sites or offtakes of catchment water into channels and drains (001, 007, 013, and 019); Cluster 2 contained channel and drain water with two statistically identifiable subclusters; 2A that contained predominately surface water (7/8 site representatives) and 2B that contained drain water from group 3 (3/4 site representatives). Cluster 3 contained drain sites immediately adjacent to industry.

Detection of coprostanol and cholestanol in surface water

Significant variation in the quantities of suspended particulates was detected among sites and collection dates (data not shown). All three reference sites (001, 007, and 024; group 1) and sites 010 and 011 had a relatively low sediment loading when compared to water sampled from sites 008, 009, 014, and 016. The concentrations of coprostanol and cholestanol in the filtered samples (Table 3) categorized sites into those containing high (reached up to >1,000 ng/L; sites 008, 009, 013, 014, and 016), average (400–800 ng/L; sites 010, 011, 015, 017, 018, 019, 020, 024, 045, and 048), and low (<400 ng/L; sites 001 and 007) levels of sterol indicators. The concentrations of coprostanol were higher in the winter samples (group 2 $P < 0.05$ and group 3 $P < 0.01$, respectively; Table 3).

Differences in coprostanol concentration among sites were also observed between the seasons and above and below enterprises in the area associated with drains in the northwest of the catchment (Wilcoxon's signed-rank test: group 3 area: $W = -338$ $n_{s/r} = 29$; $z = -3.65$; $P < 0.001$). These observations were not, however, observed in the group 2 area (Wilcoxon's signed-rank test: group 2 area: $W = 24$ $n_{s/r} = 15$; $z = 0.67$; $P > 0.05$), an area characterized by channels.

The concentrations of coprostanol detected in water were reported previously to correlate with indicators of microbial contamination (Leeming and Nichols 1996).

The Australian guidelines for drinking water supplies state a threshold of 700 ng/L of coprostanol (NHMRC 2004), which was detected at only two sites in the current study (Table 3). However, contact with water with slightly lower sterol indicator (microbial contamination) through recreational use and other downstream activities is of concern to public health. Indeed, a threshold concentration for coprostanol of 500 ng/L was proposed as a primary contact limit in Canadian waters (equating to 200 coliforms per 100 mL; Churchland et al. 1982). Previous concurrent analysis of coprostanol and bacterial indicators of sewage pollution showed that concentrations of coprostanol of only 60 ng/L corresponded to the primary contact limit for fecal coliforms (ANZECC 1992) and 400 ng/L corresponded to the secondary contact limit. Concentrations of coprostanol in water taken at certain times of year (especially winter) in the current study were above 500 ng/L at all sites except 001 and 007. The low (undetected) concentrations of coprostanol at sites 001 and 007 support previous studies of little or no detectable contamination in river water in pristine environments (Nichols et al. 1996). However, a moderate level of coprostanol was detected at the third reference site (natural wetlands, 024) indicating that fecal matter may have been flushed into this site during rainwater runoff from the surrounding environment.

Table 3 Concentrations (ng/L) of coprostanol and cholestanol in surface water samples collected from all sites during October 2006 to October 2007

Site		Nov 06	Dec 06	Jan 07	Feb 07	Mar 07	Apr 07	May 07	Jun 07	Jul 07	Aug 07	Sep 07	Oct 07
001	Cop	nd	–	–	–	nd	–	–	–	nd	–	–	nd
	Chol	nd	–	–	–	nd	–	–	–	nd	–	–	nd
007	Cop	nd	nd	nd	nd	nd	nd	nd	nd	308	nd	nd	nd
	Chol	nd	400	395	nd	nd	nd	nd	nd	205	nd	nd	nd
024	Cop	D	D	D	D	D	D	–	569	557	526	994	616
	Chol	D	D	D	D	D	D	–	420	389	351	664	406
008	Cop	792	748	D	D	D	531	519	4,039	1,210	1,933	1,147	729
	Chol	503	522	D	D	D	371	354	596	800	424	476	507
009	Cop	678	822	D	D	D	551	541	2,024	1,257	609	613	574
	Chol	460	262	D	D	D	420	400	479	817	449	407	407
010	Cop	600	623	nd	nd	557	nd	nd	739	524	524	nd	574
	Chol	402	402	395	nd	375	nd	nd	490	351	340	336	409
011	Cop	nd	622	nd	nd	nd	nd	nd	651	516	559	nd	517
	Chol	395	398	nd	nd	361	350	351	364	347	350	nd	341
013	Cop	D	D	D	D	D	D	D	D	910	D	–	–
	Chol	D	D	D	D	D	D	D	D	837	D	–	–
014	Cop	D	D	D	D	543	11,327	611	804	2,120	1,153	833	602
	Chol	D	D	D	D	390	837	341	647	1,353	687	543	408
015	Cop	723	617	685	638	459	–	530	524	652	536	529	nd
	Chol	465	413	545	482	320	–	373	366	452	371	396	nd
016	Cop	657	707	633	627	433	–	829	747	567	676	534	554
	Chol	427	527	447	437	298	–	481	527	396	581	366	383
017	Cop	607	nd	nd	nd	644	nd	521	516	514	519	472	nd
	Chol	395	397	400	nd	392	356	341	344	341	343	313	340
018	Cop	600	603	nd	610	543	nd	524	687	516	647	643	517
	Chol	403	420	415	428	386	379	380	757	360	553	457	347
019	Cop	647	620	nd	nd	nd	nd	–	D	D	D	nd	514
	Chol	395	435	400	nd	403	nd	–	D	D	D	291	350
020	Cop	605	600	620	605	564	516	D	533	520	514	nd	nd
	Chol	405	398	432	413	373	349	D	347	344	353	300	339
045	Cop	717	603	nd	620	500	514	–	nd	517	513	nd	nd
	Chol	640	398	403	400	333	329	–	349	424	344	299	339
048	Cop	nd	600	nd	607	549	514	nd	586	516	551	748	nd
	Chol	395	397	400	407	357	346	343	577	511	479	572	340

nd not detected, *D* site was dry, – sample was not available or collected

Detection of coprostanol and cholestanol in sediments

Concentrations of coprostanol and cholestanol within the dried sediments collected among the sites and months in the current study ranged from 478.1 to 2,735.3 and from 314.6 to 1,378.3 ng/g, respectively (Table 4). Even though sites 009 and 014 contained relatively higher concentrations of coprostanol and cholestanol compared to all other sites over the entire sampling period, there were no significant differences in concentrations of the two fecal sterols between sites, reflecting the lack of discrete patterns and high variability in concentrations. However, higher concentrations (numerically but not significant) were detected at all sites in August, September, and October 2007 in comparison to May, June, and July 2007. This may potentially be related to greater rainfall incidence between August and October, which may have flushed the fecal contamination into the drain and channel sample sites.

In a similar study, Chou and Liu (2004) found that areas known to have lower pollutant (raw

Table 4 Concentrations (ng/g dry) of coprostanol and cholestanols in sediments samples collected from four sites

Site	May 2007		Jun 2007		Jul2007		Aug 2007		Sep2007		Oct 2007	
	Cop	Chol	Cop	Chol	Cop	Chol	Cop	Chol	Cop	Chol	Cop	Chol
8	478.1	314.6	1,393.8	1,123.4	581.5	400.0	990.0	681.3	1,914.7	533.8	518.2	358.0
9	777.6	573.7	1,079.7	817.6	603.8	418.8	998.3	703.4	1,535.0	1,056.7	2,002.2	1,378.3
14	–	–	703.3	498.3	956.0	674.0	1,673.3	340.7	2,735.3	550.0	846.2	526.9
16	–	–	–	–	–	–	510.0	397.0	511.8	361.8	–	–

sewage) loading contained lower concentrations of indicator sterols. Concentrations of coprostanol (up to 134,918 ng/g) and cholestanol (up to 15,784 ng/g) were recorded in streams containing high effluent loadings and were much lower (up to 342 and 334 ng/g for coprostanol and cholestanol, respectively) in streams receiving less sewage wastewater. Several other reports have demonstrated that coprostanol in sediments can be used as a quantitative indicator of fecal presence (e.g., Maldonado et al. 1999; Geary et al. 2006) reflecting its persistence in anoxic sediment (Bartlett 1987).

In the current study, very high concentrations of coprostanol and cholestanol (in either surface water or bottom sediments) were recorded at four of the sampling sites. All of these were drains at which coliforms and other pathogenic indicator counts were previously found to be comparatively high (data not shown). Wastewater contribution in the catchment may be from on-site systems or runoff from the upstream area. Coprostanol and cholestanol ratios may be employed to indicate that fecal contamination from human sources may be present (Geary et al. 2006, 2007; Black et al. 2007; Tyagi et al. 2008). However, further investigations are required prior to concluding the definite origin of contamination source(s).

Conclusions

Fecal contamination levels in drains and channels of a water supply system in SE Australia were determined based on indicator sterol analyses. It is difficult to link fecal contamination with a definite source or factor or the potential risks associated with the levels of sterols identified *in situ*. However, it is assumed that runoff water, industrial or wild animal feces, and, potentially, illegal discharges of domestic sewage may contribute to the contamination. Improved management practices of agricultural land use are required to reduce these levels to protect human health. Further investigations to differentiate the definite origin of contamination (human or animal) would be useful for better management of these locations.

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