Coprostanol
Indicator
Faecal pollution
Sea

Coprostanol
Indicateur
Pollution fécale
Mer

The occurrence of coprostanol, an indicator of faecal pollution, in sea water and sediments

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ABSTRACT

The present study deals with the concentration of coprostanol and other sterols in sea water and sediments from the Ariake Sea and in water from the Anraku River.
After extraction of lipids with hexane, coprostanol and other sterols were isolated by column chromatography on alumina with hexane-benzene. The quantitative determination of sterols was carried out by gas-liquid chromatography on 1.5% OV-17, using cholestane as an internal standard.
Sea water and sediment samples from the Ariake Sea contained substantial amounts of coprostanol and a large amount of cholesterol. Coprostanol concentrations ranged from 0.06 to 1.05 µg/l of sea water, and from 0.02 to 1.77 µg/g of dry sediment, respectively. Coprostanol was also detected in the water of the Anraku River, polluted with the sewage from swineries upstream.
These data show how coprostanol may be used as an indicator for the detection of faecal contamination in marine environments.

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Le coprostanol, indicateur possible de la pollution fécale dans l'eau de mer et les sédiments

RÉSUMÉ

La présente étude concerne l'analyse de la concentration du coprostanol et d'autres stérols dans l'eau et les sédiments de la mer d'Ariake et dans les eaux de la rivière Anraku. Après extraction des lipides à l'hexane, le coprostanol et les autres stérols ont été isolés par chromatographie sur colonne d'alumine et élution à l'hexane-benzène. Le dosage des stérols a été réalisé par chromatographie gaz-liquide sur OV-17 1,5%, le cholestane servant de standard interne.
Les échantillons d'eau et de sédiments de la mer d'Ariake renferment des quantités non négligeables de coprostanol et de cholestérol. Les concentrations de coprostanol dans l'eau et les sédiments varient respectivement de 0,06 à 1,05 µg/l (eau de mer) et de 0,02 à 1,77 µg/g (sédiment sec). Le coprostanol a également été détecté dans les eaux de la rivière Anraku polluée par les ueaux usées d'une porcherie.
Ces données indiquent que le coprostanol peut être utilisé comme un indicateur pour la mise en évidence d'une contamination fécale dans l'environnement marin.

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INTRODUCTION

Sterols are synthesized from lower units in most animals, plants, and yeast, and occur widely in living organisms as lipid constituents. In higher animals such as man and other mammals, cholesterol, a major sterol, is metabolized to bile acids and steroid hormones. A certain amount of cholesterol is excreted into faeces after undergoing stereospecific reduction of the double bond to coprostanol (5 β-cholestan-3 β-ol) by intestinal microorganisms (Rosenfeld *et al.*, 1954; Rosenfeld, Gallagher, 1964). The faeces of man and mammals consequently contain large amounts of coprostanol as well as cholesterol (Gould, Cook, 1958; Wells, Makita, 1962; Eneroth *et al.*, 1964). All stanols occurring in natural sources have a *trans* fusion of rings A/B; exceptionally, coprostanol possesses a *cis* fusion of rings A/B.

Several workers have attempted to use coprostanol as an indicator of faecal pollution in water (Murtaugh, Bunch, 1967; Smith, Gouron, 1969; Ogura, Hanya, 1970; Reichert, 1971; Ogura, 1972; Tabak *et al.*, 1972). Since information concerning the occurrence of coprostanol in the marine environment is rare, it is the author's intention to obtain further knowledge on coprostanol as an indicator of faecal contamination in the sea.

The present study deals with the concentration of coprostanol and other sterols in the Ariake Sea and in the faeces-polluted water of the Anraku river.

MATERIALS AND METHODS

Water and sediment samples

Sea water (surface water) and sediment (0-10 cm depth) from the Ariake Sea, Kyushu, Japan, were collected at a number of stations during September 1971, and June 1972, shown in Figure 1. Water (surface water) samples from the Anraku River, polluted with excreta from swineries, were taken in January 1972. In this study, all water samples were unfiltered, and some plankton and suspended matter were present in the samples analysed.

Extraction and isolation of unsaponifiable matter from samples

Water samples (10 l) were acidified with hydrochloric acid to pH 2-3 and homogenized three times with 3 l of hexane by means of a large blender. In the case of sediments (1 kg in wet weight), lipids were extracted twice with 2 l of chloroform-methanol (2 : 1, v/v). The recovery of a known amount of coprostanol added to water and sediment samples was 97 to 98%. The hexane or chloroform-methanol extract was saponified with 10% ethanolic potassium hydroxide at 80°C for 3 hours. Unsaponifiable matter was isolated from the saponification mixture with ether in the usual manner.

Thin-layer chromatography (TLC) and alumina column chromatography

TLC on Kieselgel G (0.25 mm in thickness, Merck) with benzene-ethyl acetate (2 : 1, v/v) (Smith *et al.*, 1968) was carried out to check for the presence of 5 β-stanols. Column chromatography on alumina (Grade II, Merck) was conducted to separate coprostanol (5 β-stanol) from 5 α-stanols and unsaturated sterols. In the standard procedure, unsaponifiable matter (about 20 mg) was loaded on the column (12.5 × 1.0 cm i.d.) packed with alumina (10 g), and elution was carried out with 100 ml each of hexane, hexane-benzene (3 : 1), hexane-benzene (1 : 1), benzene, and benzene-ethyl acetate (1 : 1). The fraction (20 ml) was collected and monitored by gas-liquid chromatography (GLC). In this chromatography, coprostanol (fraction No. 12-14) was separated from sterols except 5 β-stanols (fraction No. 15-19).

Determination of coprostanol and other sterol concentrations

The concentrations of sterols were determined by GLC using the Shimadzu GC-3AF unit with hydrogen flame ionization detectors as described previously by Teshima and Kanazawa (1971). The columns used and operating conditions were as follows: 1.5% SE-30 on 60-80 mesh Chromosorb W (2.0 m long × 4 mm i.d., column temperature 220°C, flow rate of nitrogen 35 ml/min); 1.5% OV-17 on 80-100 mesh Shimalite W (3.0 m long × 4 mm i.d., column temperature 235°C, flow rate of nitrogen 35 ml/min). The identification of sterols was performed by comparing relative retention times (RRT) for cholesterol with those of reference sterols. In GLC on 1.5% SE-30, the peak of coprostanol was over-

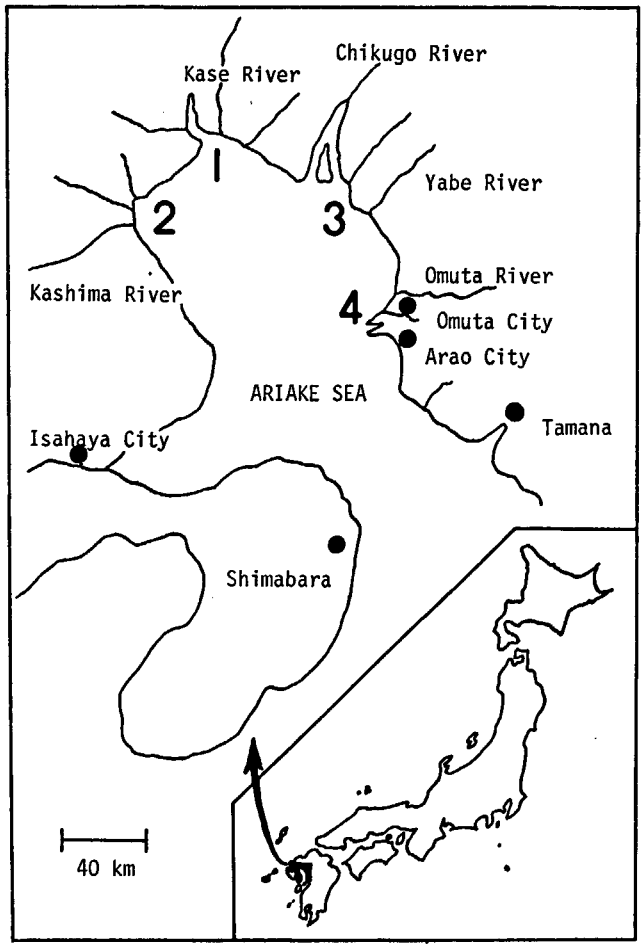

Figure 1
Sampling stations in the Ariake Sea.

Table 1

Lipid, unsaponifiable matter, and sterol (except 5 β-stanols) concentrations in sea water and sediments from the Ariake Sea.

Station No.	Date of sampling	Sea water			Sediment		
		Lipids (mg/l)	Unsap. matter (*) (mg/l)	Sterols (**) (μg/l)	Lipids (mg/g dry)	Unsap. matter (*) (mg/g dry)	Sterols (**) (μg/g dry)
1	September, 1971	8.3	6.1	10.2			
1	June, 1972	6.1	5.1	49.2	1.23	0.45	47.8
2	September, 1971	12.2	7.2	15.3			
2	June, 1972	7.2	6.7	52.5	1.30	0.47	43.3
3	September, 1971	11.7	8.2	6.2			
3	June, 1972	8.2	1.4	80.3	1.68	0.64	2.32
4	September, 1971	14.4	7.9	23.9			
4	June, 1972	7.9	6.0	84.5	1.80	0.77	65.4

(*) Unsaponifiable matter.
(**) Sterols except 5 β-stanol.

lapped by that of 22-dehydrocholesterol (22-*trans*-cholesta-5,22-dien-3 β-ol). The separation of both sterols was achieved by GLC on 1.5% OV-17; in this column the RRTs of coprostanol and 22-dehydrocholesterol were 0.86 and 0.92, respectively.

Determination of ammonia-N, COD (chemical oxygen demand), and BOD (biochemical oxygen demand) concentrations

The concentration of ammonia-N, COD, and BOD in water samples was estimated according to the standard methods devised by the American Public Health Association (1971).

Production of coprostanol by the intestinal microflora of fish

As part of the investigation of the possibility of coprostanol production by the intestinal microflora of aquatic animals, faeces of the teleost, *Tilapia zilli*, were analysed. Six *Tilapia*, 60 g in average body weight, were fed on the commercial rainbow trout pellet diet over 15 days. Faeces were collected daily, and subsequently analysed for coprostanol concentration.

RESULTS

Lipids, unsaponifiable matter, and sterols, with the exception of 5 β-stanols, in sea water and sediments from the Ariake Sea

In the present study, sea water and sediment samples were taken at stations near the estuary where an inflow of domestic sewage was suspected. Table 1 shows the lipid, unsaponifiable matter, and sterol (except 5 β-stanols) concentrations in the sea water and sediments collected. The sea water (1 l) contained 6.1 to 14.4 mg of lipids, 1.4 to 8.2 mg of unsaponifiable matter, and 6.2 to 84.5 μg of sterols. The sediments (1 g in dry weight) contained 1.23 to 1.80 mg of lipids, 0.45 to 0.77 mg of unsaponifiable matter, and 2.32 to 65.4 μg of sterols. The concentrations of lipids and unsaponifiable matter in the sea water varied with the location of sampling stations and seasons, whereas concentrations in the sediments did not vary so markedly. Sterol concentration showed a large variation from sample to sample in sea water and sediments alike. Table 2 shows the concentrations of sterol constituents from the sea water and sediments. In all stations except station 2, cholesterol was the major sterol (74.0 to 86.6% of total sterols except 5 β-stanols) in the sea water. Apart

Table 2

Sterol constituents in sea water and sediment samples obtained during June, 1972 from the Ariake Sea.

Sterol	Concentration (μg/l) in sea water — Station No.				Concentration (μg/g dry) in sediment — Station No.			
	1	2	3	4	1	2	3	4
24-Norcholesta-5, 22-dien-3 β-ol	0.91	4.90	1.20	2.70	0.60	0.66	0.09	0.92
22-*trans*-Cholesta-5, 22-dien-3 β-ol	0.18	0.58	0.10	0.01	1.02	0.56	0.10	1.46
Cholesterol (*)	40.1	14.8	69.6	62.5	21.9	15.7	0.63	26.3
24-Methylcholesta-5, 22-dienol	3.35	15.8	4.20	10.6	8.23	3.99	0.21	7.99
24-Methylcholesterol (*)	1.01	1.42	1.23	2.51	4.80	8.65	0.10	7.07
24-Methylenecholesterol	1.17	1.63	1.59	1.82	4.53	7.49	0.12	6.61
24-Ethylcholesta-5, 22-dienol	0.41	0.91	0.01	0.81	0.48	1.92	0.12	1.99
24-Ethylcholesterol (*)	2.07	9.72	2.40	3.51	6.00	3.92	0.73	12.7
Isofucosterol	0.01	2.70	0.01	0.01	0.21	0.40	0.21	0.40

(*) By GLC analysis used in this study, the separation of 5 α-stanols and monoene sterols was not completely achieved. Cholesterol, 24-methylcholesterol and 24-ethylcholesterol may therefore contain corresponding C_{27}, C_{28} and C_{29}-5 α-stanols.

from certain red algae, most marine algae and phytoplankton generally contain only small amounts of cholesterol in their tissues (Austin, 1970). It is thus reasonable to assume that cholesterol occurring in sea water from the Ariake Sea may be partly derived from terrestrial sources. In fact, Ogura (1973) has reported that the urban river, River Tamagawa, which is polluted with domestic sewage from a population of about 1.5 million, contains large amounts of cholesterol. The sterols from station 2 included large portions of 24-methylcholesta-5,22-dienol and 24-ethylcholesterol as well as cholesterol. The authors assume that both C_{28} and C_{29}-sterols come partially from phytoplankton, such as diatoms, present in the sea water sample. The sea water sample from station 2 also contained relatively large amounts of C_{26}-sterol (24-norcholesta-5,22-dien-3 β-ol). C_{26}-sterol was first isolated from the clam (Idler *et al.*, 1970) and later found to occur widely in marine organism; the origin of this sterol is, however still obscure. It may be worth noting that the occurrence of C_{26}-sterol in sea water was associated with a high concentration of 24-methylcholesta-5,22-dienol. In addition to the above-mentioned sterols, the sea water contained 22-dehydrocholesterol, 24-methylcholesterol, 24-methylenecholesterol, 24-ethylcholesta-5,22-dienol, and isofucosterol. In the sediments, as in the sea water, cholesterol was the prominent sterol (27.1 to 45.9% of total sterols except 5 β-stanols); other notable sterols included 24-methylcholesta-5,22-dienol, 24-methylcholesterol, 24-methylenecholesterol and 24-ethylcholesterol.

The sterols detected in sea water and sediments in the present study have also been reported elsewhere in sea water (Jeffrey, 1966; Matthews, Smith, 1968; Saliot, Barbier, 1973; Gagosian, 1975; Gagosian, 1976); in suspended matter (Kanazawa, Teshima, 1971); in marine sediments (Schwendinger, Erdman, 1964; Attaway, Parker, 1970; Ogura, 1972); and in benthic algae (Boutry *et al.*, 1976).

Coprostanol concentration in sea water and sediments from the Ariake Sea

The water from the Ariake Sea contained 0.06 to 1.05 μg of coprostanol/l as shown in Table 3. Since coprostanol occurs only in the faeces of mammals, its incidence in sea water is indicative of faecal contamination in the Ariake Sea. Coprostanol concentration appeared to be higher in samples collected in 1972 than in 1971, this suggests an increase in faecal pollution in the Ariake Sea, although the suggestion requires confirmation by future continuous investigation. Coprostanol concentration in the sea water seemed to be high at stations 1 and 4; COD value was also high at station 4. Station 4 is located near Omuta City and Arao City, which together have a population of about 30 thousand. It is therefore assumed that the high concentration of coprostanol at this station may be ascribed to domestic sewage. To understand the origin of coprostanol, sea water samples were further collected at seven stations situated at intervals of 1 km near the Omuta River estuary, and the distribution of the coprostanol concentrations was investigated. As shown in Figure 2, coprostanol concentrations ranged from 0.05 to 0.78 μg/l and appeared to be high in the mouth of the river and low off shore. The levels of coprostanol in the stations examined were not, however particularly high in comparison with those of other stations listed in Table 3. These results show that the Omuta River is not the sole source of faecal contamination.

With regard to the sediments, coprostanol concentration was high at station 1 (1.77 μg/g dry wt.) and station 4 (0.43 μg/g dry wt.), and low at stations 2 and 3. Coprostanol concentration in the sediments was remarkably high in comparison with the concentration in sea water. This suggests that coprostanol in water polluted with mammalian faeces moves to the sediments and is deposited there. Ogura (1972) has also observed that coprostanol concentration in Tokyo Bay is higher in sediments than in sea water. Moreover, Smith *et al.* (1968) have reported that coprostanol in polluted water may be removed by activated sludge treatments. This information would indicate that faecal contamination in aquatic environments can best be determined by analysis of coprostanol concentration in sediments, rather than in water samples.

Table 3

Coprostanol concentration in sea water and sediments from the Ariake Sea.

Station No.	Date of sampling	Sea water		Sediment
		Coprostanol (μg/l)	COD (ppm)	Coprostanol (μg/g dry)
1	September, 1971	0.16	2.6	–
1	June, 1972	0.82	–	1.77
2	September, 1971	0.20	2.8	–
2	June, 1972	0.91	–	0.06
3	September, 1971	0.06	2.5	–
3	June, 1972	0.35	–	0.02
4	September, 1971	0.32	3.6	–
4	June, 1972	1.05	–	0.43

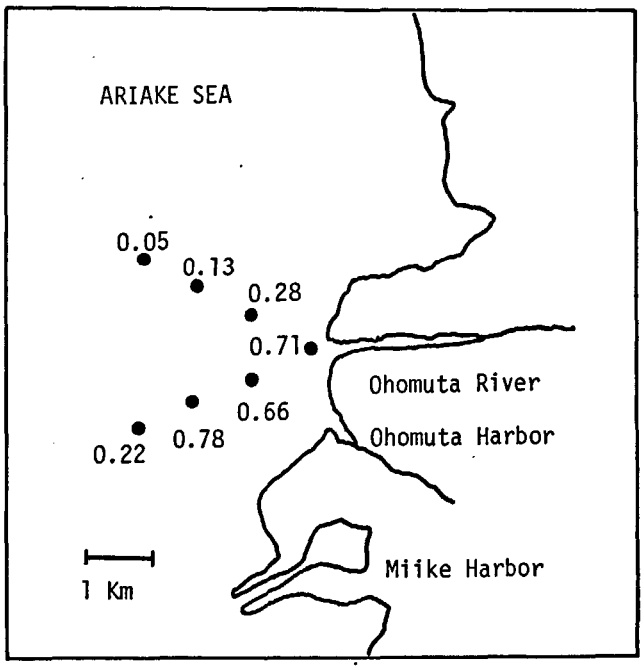

Figure 2

Coprostanol concentrations in sea water near the outfall of the Omuta River. Roman numerals indicate the concentrations (μg/l) of coprostanol.

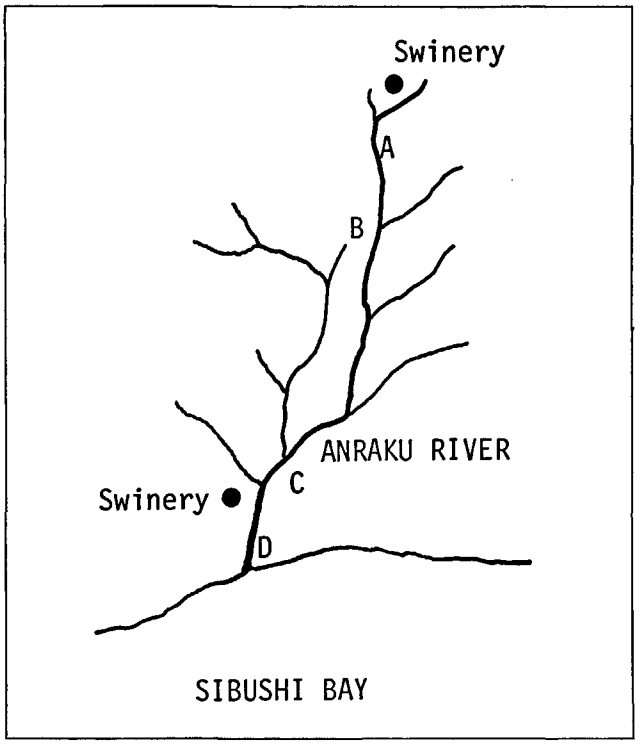

Figure 3
Sampling stations in the Anraku River.

Coprostanol concentration in water from the Anraku River

The Anraku River, Kagoshima Prefecture, was polluted with sewage from two swineries. To examine the transport of coprostanol into the sea, water samples were collected at four stations, as shown in Figure 3, and analysed for coprostanol concentration. As shown in Table 4, coprostanol concentration was high at stations A and D, downstream from the swineries, and this suggests that the Anraku River was polluted with the faeces of pigs from the swineries. The concentration of ammonia-N and COD was also high at station A. The concentration of ammonia-N, COD, and BOD of water from station D was, however, similar to the levels detected at stations B and C.

Production of coprostanol by fish

The occurrence of coprostanol in natural water has generally been recognized as indicative of pollution by the faeces of mammals. The possibility remains, however, that coprostanol may be derived from the excreta of aquatic animals. For clarification of this point, faeces of

Table 4
Coprostanol and cholesterol concentrations and other indices of pollution in water from the Anraku River.

Concentration	Sampling station A	B	C	D
Coprostanol (μg/l)	0.31	Trace	Trace	0.45
Sterols (*) (μg/l)	26.4	17.4	16.7	18.8
Cholesterol (μg/l)	6.4	5.4	3.6	13.0
Ammonia-N (mg/l)	4.2	1.5	1.3	1.1
COD (ppm)	1.4	0.5	1.1	0.6
BOD (ppm)	2.1	2.3	2.6	2.0

(*) Sterols except 5 β-stanols.

Tilapia fed on a diet containing cholesterol as a sterol source were analysed. In duplicated experiments, the authors could not detect coprostanol in the faeces of *Tilapia*. Although there is no information comparable with the present study, it is therefore postulated that fish may not excrete coprostanol in their faeces.

DISCUSSION

The presence of coprostanol in water has been considered to be a consequence of faecal contamination (Murtaugh, Bunch, 1967; Smith, Gouron, 1969; Ogura, Hanya, 1970). Little information is available, however, concerning the occurrence of coprostanol in marine environments. The present study describes the occurrence of substantial amounts of coprostanol in both sea water and sediments from the Ariake Sea and in water from the Anraku River. Table 5 shows the coprostanol concentrations of sea water and sediments reported by several workers. Coprostanol concentration in sea water from the Ariake Sea was lower than that reported for Galveston Bay, Tokyo Bay, and the Rhine River, which would suggest that faecal pollution is less-pronounced in the Ariake Sea than in the above three areas. Coprostanol concentration was also lower in sediment samples from the Ariake Sea than in sediment samples from Tokyo Bay.

The authors believe that the unambiguous detection of coprostanol in sea water and sediments could provide a method to complement bacterial coliform counting. Since coprostanol was mainly present in the sediments, the authors recommend the determination of coprostanol concentrations in sediments as a means of evaluating the degree of faecal contamination in marine environments.

Table 5
Comparison of coprostanol and cholesterol concentrations in water and sediments.

Concentration	Source	Coprostanol	Cholesterol	Reference
Water (μg/l)	Rhine River	1.20-3.20	–	Reichert (1971)
	Galveston Bay	1.0	1.0	Smith, Gouron (1969)
	Tokyo Bay	0.2-6.6	2.2-8.6	Ogura (1972)
	Ariake Sea	0.06-1.1	2.0-6.3	Present study
	Anraku River	Trace-0.45	3.6-13.6	Present study
Sediment (μg/g/day)	Tokyo Bay	0.10-48.0	0.45-29.2	Ogura (1972)
	Ariake Sea	0.02-1.7	0.63-26.3	Present study
	Kagoshima Bay	–	0.01-0.26	Unpublished data

As sea water generally contains extremely small amounts of coprostanol, analysis is difficult; the method employed in the present study required 10 or more litres of water sample for the quantitative determination of coprostanol. Recently, Matsushima *et al.* (1975) have described a method for the determination of trace amounts of coprostanol in water using the GLC with an electron capture detector. Such a method would permit the treatment of a larger number of samples, and rapidly provide data concerning the distribution of coprostanol in sea water.

CONCLUSION

The analysis of samples from the Ariake Sea reveals that coprostanol concentrations in sea water and sediments ranged from 0.06 to 1.05 μg/l and 0.06 to 1.77 μg/g dry wt., respectively. The occurrence of substantial amounts of coprostanol in the Ariake Sea suggests faecal pollution from terrestrial sources. Coprostanol was also detected in water from the Anraku River polluted with the sewage from the swineries.

These data show how coprostanol may be used as an index for the detection of faecal contamination in marine environments.

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