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Coprostanol and Bacterial Indicators of Faecal Pollution in the Scheldt Estuary

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Doubts on the validity of the coliform test to accurately indicate the presence of faecal pollution in water have stimulated research on the use of other parameters. Coprostanol (5β-cholestan-3β-ol) is one of the principal sterols in faeces of human beings and animals (MARTIN et al. 1973; MURTAUGH & BUNCH 1967; SMITH & GOURON 1969). Since faeces is the only known source of coprostanol, its presence in the aquatic environment is considered as a sign of faecal contamination, mainly from domestic and agricultural sewage. Coprostanol analyses may be particularly useful in detecting faecal pollution in outfalls low in microorganisms because of high temperatures or the presence of toxic substances. In the present investigation, samples from the Scheldt estuary were analyzed for coprostanol and bacterial faecal indicators in order to compare the sensitivity of the two methods. The Scheldt estuary was chosen because of its length; consequently the high water pollution at Antwerp decreases slowly seawards. Coliforms, faecal coliforms, faecal streptococci and sulphite reducing clostridia were chosen as bacterial indicators of faecal pollution.

METHODS

In June 1980 surface samples were collected from areas of the Scheldt estuary indicated at Fig. 1. Samples were refrigerated at 5°C. Bacteriological analyses were completed within 7 h of collection. For coliform, faecal coliform and faecal streptococci analyses, membrane filtration procedures were followed as described by YDE & DE MAEYER-CLEEMPOEL (1980). For the enumeration of the spores of sulphite reducing clostridia, samples were heated for 10 min at 80°C. After filtration the membrane filter was put upside-down on the surface of an sulphite-glucose-iron agar (3 g meat extract, 10 g peptone, 5 g NaCl, 20 g glucose, 15 g agar, distilled water to 1.000 mL, sterilized at 120°C for 20 min; 10 mL 10 % Na_2SO_3 and 10 mL 5 % $FeSO_4$-7 H_2O sterilized by membrane filtration). The plates were incubated anaerobically in GasPak jars (BBL) at 37°C for 24 h. All black colonies were counted.

For the determination of the coprostanol content, samples were preserved upon collection with 1 mL of 1 % $HgCl_2$ L^{-1} and refrigerated at 4°C until analyses. The water extraction was conducted according to the method described by DUTKA et al. (1974). However, no sample cleanup was performed. Instead, after drying with

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anhydrous Na_2SO_4, the sample was evaporated to dryness and treated with 50 uL BSA (N,O-Bis-(Trimethylsilyl) acetamide) for an initial volume of 2 L water to form trimethylsilyl derivatives. Gas chromatographic analyses were performed using flame ionization detectors and a 15 m OV-1 LL glass capillary column. Helium carrier gas flow rate was maintained at 3 mL min^{-1}. Injection port and detector temperatures were 275°C. Column temperature was 250°C. The detection limit was 0.10 ug L^{-1}.

RESULTS AND DISCUSSION

From TABLE 1 it can be seen that the faecal pollution of the Scheldt is important in the Antwerp area (sampling point 8). Bacterial indicater levels tend to decrease at increasing distance from Antwerp. This fact may be influenced by a diminution of the number of sewage outfalls downstream Antwerp and by an important dilution factor.

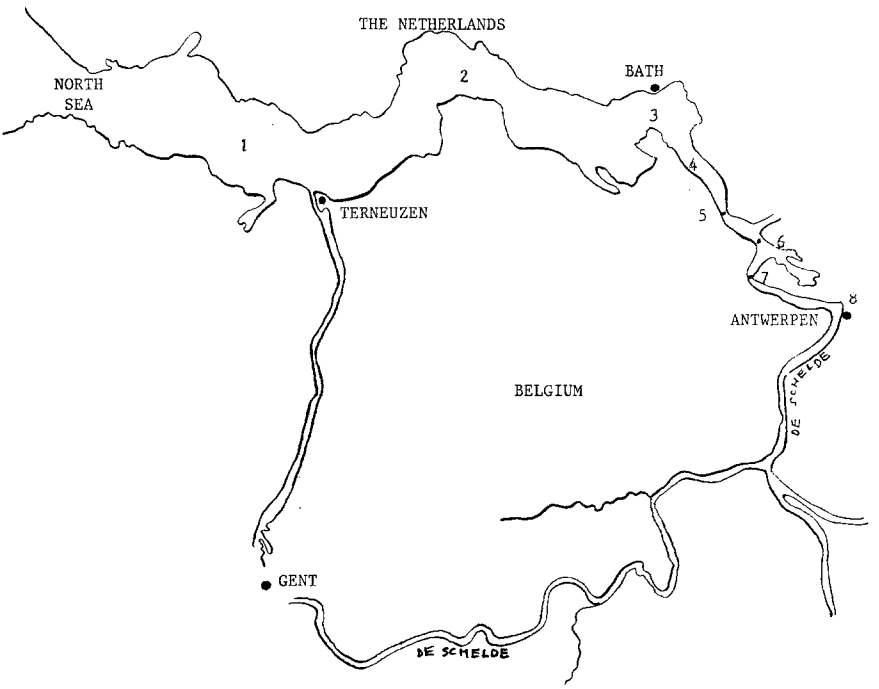

Fig. 1 : Sampling sites of the Scheldt (De Schelde)

Quantitative measurements of coprostanol were limited at 0.10 ug L^{-1}. The highest value (0.28 ug L^{-1}) was detected in the Antwerp area. In four sampling stations coprostanol concentrations remained under the detection limit. In the samples of the other sites, the levels of coprostanol were low. In a comparable study of the Clyde estuary (GOODFELLOW et al. 1977), concentration of coprostanol varied between 47.5 and < 0.1 ug L^{-1}. More definite values were

TABLE 1 : The levels of coprostanol and bacterial indicators in waters of the Scheldt estuary.

Sampling point	Coprostanol (ug/L)	10^{-3} x Total Coliforms/L	10^{-3} x Faecal Coliforms/L	10^{-3} x Faecal Streptococci/L	10^{-3} x Sulfite reducing Clostridia/L
1	< 0.10	1.2	0.4	< 0.1	1.7
2	< 0.10	6.0	1.6	2.2	3.0
3	< 0.10	15	1.2	2.4	22
4	0.25	39	1.4	1.8	45
5	< 0.10	15	0.8	0.4	37
6	0.19	190	4.0	4.0	40
7	0.12	100	10	10	120
8	0.28	1,200	40	<10	120

< = less than

TABLE 2 : Belgian cross-boundary waters, the levels of bacterial indicators.

Cross-boundary station	n(a)	TC/mL (b)		FC/mL (b)		FS/mL (b)		S.R.C./mL (b)	
		average	range	average	range	average	range	average	range
- Belgium - The Netherlands									
Maas	30	1,020	(5,300-65)	100	(500-10)	45	(170-0)	25	(220-0)
Channel Gent-Terneuzen	11	315	(1,300-10)	25	(94-0)	25	(240-0)	2	(7-0)
- Luxemburg-Belgium									
Sûre	12	530	(4,000-9)	40	(92-0)	30	(200-0)	4	(3-0)
- France-Belgium									
Meuse	9	320	(1,120-55)	35	(100-8)	25	(67-0)	4	(14-0)
Lys	12	29,600	(13.6×10^4-190)	9,300	(95×10^3-170)	530	(1,400-10)	70	(150-0)
Sambre	13	3,200	(11,600-220)	170	(500-27)	35	(107-0)	29	(120-0)
Escaut	9	5,600	(25,000-195)	170	(830-4)	640	(5,300-0)	86	(300-0)
Espierres Canal	12	95×10^4	(7×10^6-12×10^3)	72×10^3	(24×10^4-10^3)	26×10^3	(15×10^4-2×10^3)	1,800	(6,000-36)
- Estuary station 5 (c)	10	3,900	(32,100-73)	50	(170-0)	15	(33-1)	55	(210-0)

(a) : number of samples.
(b) : TC = total coliforms; FC = faecal coliforms; FS = faecal streptococci; S.R.C. = sulphite reducing clostridia.
(c) : see fig. 1.

found for the New York Bight sediment : 4.8 and 5.2 ppm (ESCALONA et al. 1980).

The low recovery of faecal sterols in the Scheldt estuary does not correlate well with the values for bacterial indicators, which were easy to determine at all sampling sites. Counts of total coliforms were greater than counts of faecal coliforms and streptococci, which are of comparable degree. On the other hand, faecal coliforms and streptococci are more reliable indicators of faecal pollution than total coliforms. Pseudomonads and nonfaecal strains of the family Enterobacteriaceae are able to mimic real faecal strains on the isolation media for total coliforms. Sulphite reducing clostridia are also considered as indicators of faecal contamination. As indicated at TABLE 1, the detection of sulphite reducing clostridia is remarkably sensitive. In the samples of all sites more clostridia were counted than faecal coliforms and streptococci. In addition, in 5 of the 8 sampling stations, more sulphite reducing clostridia were detected than total coliforms.

For inland surface waters (rivers, channels) the inverse situation is observed. This is well illustrated at TABLE 2. Eight Belgian cross-boundary rivers or channels and the Scheldt estuary sampling station 5 were sampled at regular intervals. Although great variations within the results, at all but one sampling site faecal coliforms and streptococci populations were more important than clostridia densities. At the Scheldt estuary station the mean clostridia count was found to be higher than the mean faecal coliform and streptococci count. It is known that the sea has a killing effect on bacteria from terrestrial origin (BARJA et al. 1977) (GAUTHIER et al. 1975). It is reasonable to accept that at some degree this effect is also applicable to estuary waters. As sulphite reducing clostridia are sporeforming bacteria, a higher resistance can be expected. This offers an explanation for the abundant clostridia populations within bacterial indicators in the Scheldt estuary. It would be interesting to check if these findings are also valuable for other estuary waters. It can be concluded from data in TABLE 1 that the determination of coprostanol is less sensitive than the detection of bacterial faecal indicators. It is possible to achieve a much better detection limit of coprostanol (0.01 ug L^{-1}) by the use of a multiple ion detection system in the gas chromatographic procedure. Consequently indefinite values of coprostanol in TABLE 1 could change into precise figures. There is little chance that the sensitivity of the coprostanol analysis could become superior to the sensitivity of the bacterial performance. Literature data support this concept. Comparative studies on the detection of coprostanol and bacterial indicators were performed for some Canadian rivers and lakes (DUTKA et al. 1974), coastal Baltic seawater near Lübeck (KUSSMAUL et al. 1978), waters of the Clyde estuary (GOODFELLOW et al. 1977). It is seen from these data that when coprostanol levels are no longer detectable, counting bacterial indicators is still possible. Unfortunately, clostridia densities were not reported.

Compared to the bacterial procedure, the technique for the copro-

stanol detection has some disavantages : a longer processing time, the need for sophisticated instruments, the more laborious performance, the higher costs. However in certain circumstances, the coprostanol technique is preferred. Where marine water samples can not be analysed immediately after collection, bacterial analysis is of no use because of a decrease of bacterial indicator populations. Coprostanol can be preserved at the time of sampling, making a delayed analysis possible. Coprostanol analysis may be particularly useful in detecting faecal contamination in thermal and chemical polluted waters, low in microorganisms because of high temperatures or the presence of toxic substances.

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