Bull. Environm. Contam. Toxicol. 24,289-295 (1980)

On the Presence of Fecal Steroids in Sediments from Two Mexican Harbors[1]

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Recently published papers (MURTAUGH & BUNCH 1964, KIRCHMER 1971, DUTKA et al. 1974, GOODFELLOW et al. 1977) have suggested that 5 beta-stanols, and in particular coprostanol (5 beta-cholestan-3 beta-ol) can be used as chemical indicators to detect fecal contamination of streams and estuaries. Coprostanol is produced in the intestine of mammals by the microbial reduction of cholesterol, which is the main steroid in the tissues of vertebrates (FRUTON & SIMMONDS 1958). Coprostanol is thus excreted by mammals along with cholesterol and other steroids, although coprostanol is generally the dominant one (ENEROTH et al. 1964). Since this is the only known source of coprostanol, its presence in measurable quantities in the aquatic environment (water and sediments) is considered a sign of fecal contamination, mainly from domestic sewage.

The distribution of fecal steroids in marine sediments has been studied by GOODFELLOW et al. (1977) in the Clyde estuary and the Firth of Clyde near the city of Glasgow, and by HATCHER & McGILLIVARY (1978) in the New York Bight. In both studies it was suggested that the concentration of coprostanol in marine sediments can be a useful indicator of accumulating fecal pollution in coastal environments. However, these authors pointed out that fecal steroids in sediments can be affected by a number of physical and biological factors which are difficult to assess.

In the present investigation, samples of sediments from the coastal cities of Veracruz, Ver. and Mazatlán, Sin. were analyzed for coprostanol and cholesterol in order to determine the degree of sewage pollution in these areas.

MATERIALS AND METHODS

Sediment samples were obtained by means of a Van Veen grab from the points indicated in figures 1 and 2, and stored in plastic bags. The Veracruz samples were frozen immediately after collection, while those from Mazatlán were air dried 1 h after collection.

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1. Contribution 203 of the Centro de Ciencias del Mar y Limnología, UNAM.

2. UNESCO/UNDP

0007-4861/80/0024-0289 $01.40

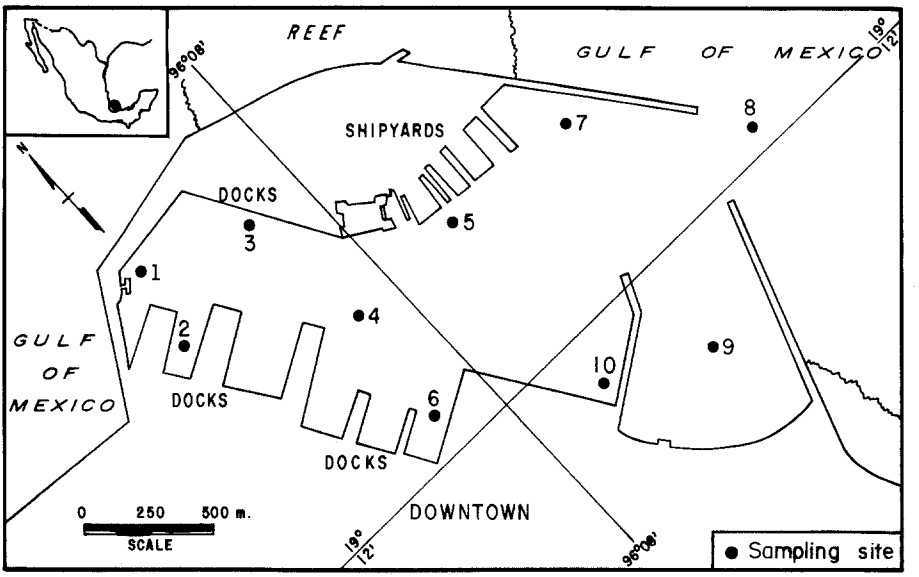

FIGURE 1: VERACRUZ HARBOR.

The samples were oven-dried at 80°C after establishing by means of cholesterol-fortified sediments that this procedure did not in any way alter the steroids present. The dry samples were refluxed for 4 h with a 1:1 mixture of benzene and methanol; the extracts were separated from the sediments by filtration, and their volume reduced to ca. 1 mL in a rotary evaporator.

Concentrated extracts were purified by column chromatography using 7 mm x 18 cm glass columns packed with alumina previously deactiva ted with 3 % by weight of distilled water. Hydrocarbons were eluted first, using 10 mL of hexane, followed by the alcohols which were eluted with 10 mL of ethyl ether. Samples were dissolved in carbon disulfide for glc analysis.

For all analysis, a gas chromatograph fitted with flame ionization detector and a stainless steel column (180 cm x 3 mm i.d.) packed with 3 % OV-101 on Chromosorb W-HP (80-100 mesh) was used. The operating conditions were: injection block: 260°C; column oven: 235°C detector: 260°C; carrier gas flow (nitrogen): 35 mL/min. For peak identification and quantification (made on the basis of peak area), standard solutions of coprostanol and cholesterol (Steraloids, Inc.) were used.

Additionally, the organic matter content of each sample was determined by the titration method of GAUDETTE et al. (1974), in which organic compounds in the sediment are oxidized with a potassium

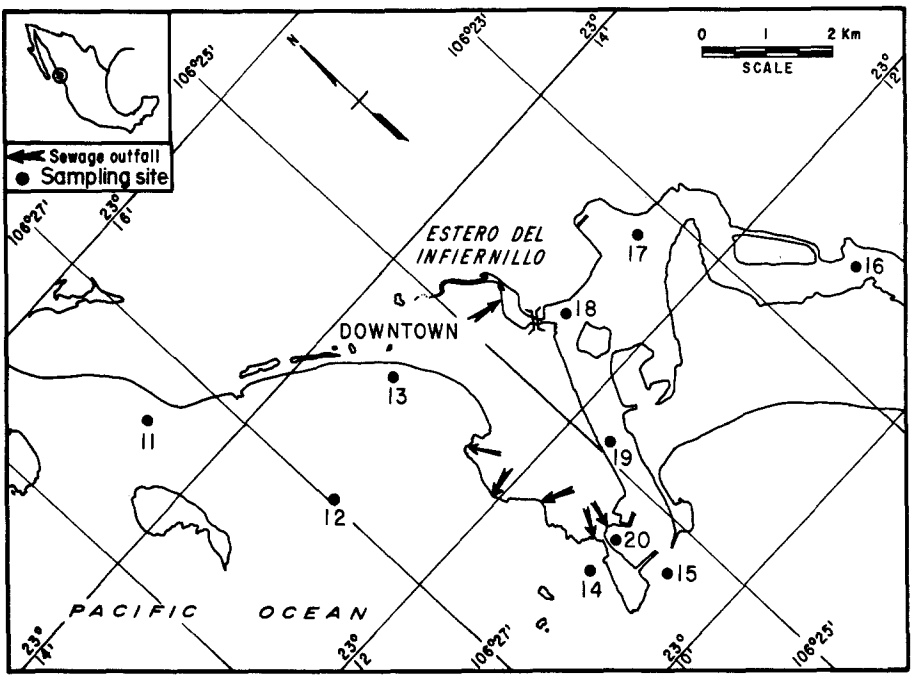

FIGURE 2: SURROUNDINGS OF THE CITY OF MAZATLAN, SINALOA

dichromate/sulphuric acid mixture and the excess dichromate titrated with ferrous sulphate solution. The results are expressed as total organic carbon (TOC) in per cent by weight of the dry sample.

RESULTS AND DISCUSSION

The concentrations of coprostanol determined in the sediments from the Veracruz harbor range from 0.006 to 0.44 ppm (Table 1). These values are relatively low compared to those reported by GOODFELLOW et al. (1977) for the Clyde estuary near the city of Glasgow (0.19 to 14 ppm) and by HATCHER & McGILLIVARY (1978) for the New York Bight (0.056 to 5.2 ppm). It is necessary to point out, however, that in these areas there is considerable sewage sludge dumping, while in the Veracruz harbor no direct sewage discharges from the city are reported. The highest coprostanol concentrations (more than 0.1 ppm) are found at sample points 4 (center and deepest part of the harbor), 1 (point farthest from the harbor's entrance) and 5 (near the shipyards). Levels of around 0.05 ppm are found near the docks, but these values fall off quickly towards the entrance of the harbor, where no steroids are detectable. The nature of the sediments in this area is also very different from the one found in the more protected parts of the harbor, being more sandy and quite low in organic matter.

TABLE 1

Results of Analysis on Samples from Veracruz Harbor

Sample No.	Sediment Appearance	Coprostanol (ppm) (1)	Cholesterol (ppm) (1)	TOC (2) %	Coprostanol/TOC
1	black sandy mud	0.14	0.80	1.50	0.09
2	black mud	0.05	1.1	3.20	0.02
3	black mud	0.04	0.64	2.40	0.02
4	black mud	0.43	0.76	2.70	0.16
5	black mud	0.13	0.28	3.70	0.04
6	black mud	0.05	1.33	3.0	0.02
7	brown sandy mud	0.01	0.05	2.20	0.01
8	brown sand	N.D.	N.D.	0.41	-
9	brown muddy sand	0.006	0.01	0.74	0.01
10	black mud	0.04	0.10	4.10	0.01

(1) micrograms per gram of dry sediment

(2) total organic carbon as per cent of dry sediment weight

N.D. = not detected

TABLE 2

Results of Analysis on Samples from Mazatlán, Sin.

Sample No.	Sediment Appearance	Coprostanol (ppm) (1)	Cholesterol (ppm) (1)	TOC (2) %	Coprostanol/TOC
11	brown sand	T	T	0.21	-
12	brown muddy sand	0.04	0.2	0.37	0.103
13	brown sand	0.03	0.02	0.23	0.13
14	brown muddy sand	0.06	0.13	0.32	0.18
15	brown sand	0.02	0.04	0.41	0.05
16	black colloidal mud	0.2	1.30	3.13	0.06
17	black sandy mud	0.03	0.24	0.87	0.03
18	black sandy mud	0.10	0.39	0.99	0.10
19	black muddy sand	T	N.D.	0.51	-
20	brown muddy sand	0.10	T	0.80	0.12

(1) micrograms per gram of dry sediment

(2) total organic carbon as per cent of dry sediment weight

T = trace

N.D. = not detected

In the light of these results, it seems reasonable to conclude: (1) that there is not a very high degree of fecal pollution in the Veracruz harbor, (2) that this pollution must have as its source small, unreported sewage outfalls (probably in the shipyards area) and possibly also refuse from the ships that dock in the harbor (although the high concentrations of cholesterol with respect to coprostanol found suggest that this refuse is primarily non-fecal in nature), and (3) that steroid levels are relatively high in areas where there is poor water circulation, as evidenced by the physical nature of the sediments (fine-grained, high organic content).

Table 2 shows the results obtained with the Mazatlán sediments. The city of Mazatlán has a population of ca. 200,000 inhabitants, and discharges a high percentage of its sewage, which is untreated, into the Estero del Infiernillo. This water body is connected by a narrow channel with the main estero near sample point 18. Other minor outfalls, marked in the map, correspond roughly to sample points 12, 13, 14 and 20. From table 2 it can be seen that the levels of coprostanol found correlate well with the known sources of fecal pollution, with the possible exception of sample 16, which has the highest coprostanol concentration (0.199 ppm) and was taken in the upper part of the estero, where there is almost no human habitation. The sediment here is composed of extremely small particles, very different from the rest of the samples. Throughout most of the year, freshwater input to the estero is negligible, and tidal currents are the main factor determining circulation patterns in this area. It is suggested that the fecal steroids, adsorbed to very fine particulates, are transported by tidal currents to this point, where they are finally able to settle to the bottom.

The coprostanol levels found in the rest of the Mazatlan samples range from 0.020 to 0.20 ppm. Thus, if fecal steroids are indeed reliable indicators of pollution from domestic sewage, it would seem that serious fecal pollution in the surroundings of the city of Mazatlán is perhaps restricted to the Estero del Infiernillo. However, it is suggested that further studies are necessary in order to ascertain the exact manner in which fecal steroids are removed from the environment, and if their removal means that also the harmful components of sewage, principally viruses and bacteria, are not present in significant levels. As far as the city of Mazatlán is concerned, it is likely that littoral currents in the bay area, tidal currents in the estero and bacterial action are the main agents responsible for the dilution, transport and degradation of coprostanol in the adjacent environment.

In tables 1 and 2 the coprostanol/TOC ratio for each sample is listed. It can be seen that these values are considerably higher in Mazatlán (mean: 0.096) than in Veracruz (mean: 0.040). This seems to indicate that the contribution of organic matter from sewage is more significant in the sediments from the port of Mazatlán than in those from the Veracruz harbor, despite the somewhat higher absolute levels of coprostanol found in the later area.

ACKNOWLEDGEMENTS

The authors thank Dr. Lawrence Mee for his help during the sampling and in the interpretation of the results and to Dr. Pat Hatcher of the Atlantic Oceanographic and Meteorological Laboratories in Miami Florida U.S.A. for providing the standards used in this work.

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