COPROSTANOL AS A POTENTIAL TRACER OF PARTICULATE SEWAGE EFFLUENT TO SHELF WATERS ADJACENT TO THE CHESAPEAKE BAY

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SUMMARY

Samples were collected in the Chesapeake Bay entrance and contiguous shelf waters and were subsequently analyzed for particulate coprostanol and cholesterol concentrations. Surface coprostanol concentrations were fairly uniform, with a slight increase with depth. This increase with depth may be due to sewage-associated particulates settling as they leave the Bay, or the resuspension of contaminated sediment. Preliminary findings indicate sewage-associated materials are being transported from the Chesapeake Bay to shelf waters, where they may have a detrimental affect on living marine resources.

INTRODUCTION

Man is continuously discharging sewage effluent into the marine environment. Sewer systems, generally, not only service individual homes, but also service various industries and most often storm drainage systems. Therefore, the influent to sewage treatment plants contains many constituents, including pathogenic bacteria and viruses, heavy metals, pesticides, and petroleum hydrocarbons, in addition to domestic sewage (refs. 1 to 4). Unfortunately, even secondary sewage treatment does not remove all of these contaminants (refs. 2 to 5). In a recent study, Van Vleet et al. (ref. 3) suggested that the amount of oil discharged into the U.S. coastal waters via wastewater effluents can be nearly as important as the amount released to coastal waters by direct spills. Sewage effluents, thus, contain materials that may adversely affect water quality, which in turn, may reduce the value of the marine resources impacted.

The enumeration of fecal coliform bacteria is routinely used as an indicator of fecal contamination (refs. 2, 6 and 7). Recent studies (refs. 5, 8 and 9) describe the limitations of the coliform test as an indicator of sewage contamination in the marine environment. The inadequacy of coliform enumeration has lead researchers to investigate other parameters that may be more accurate indicators of fecal pollution. One promising alternative is coprostanol.

Coprostanol (5β-cholestan-3β-ol) is thought to be formed exclusively by the enteric bacterial reduction of cholesterol in man and higher animals (refs. 10 to 13). Unlike cholesterol, coprostanol is not a naturally occurring sterol in the marine environment; therefore, the detection of coprostanol

would indicate fecal contamination from either domestic wastes or runoff from pastures and barnyards (ref. 13). Coprostanol has also been found to be resistant to microbial degradation (refs. 5, 14, 15 and 16). Hatcher and McGillivary (ref. 16) found coprostanol throughout a new bight core that spanned a 26-year period, therefore providing a historical measure of the degree of sewage contamination. Coprostanol has also been shown to be a reliable indicator of fecal pollution even when the effluent was chlorinated for the purpose of bacterial reduction (refs. 6 and 8). Although this disinfection procedure reduced the bacterial population, there was no detectable change in coprostanol structural configuration or concentration. Coprostanol has been shown to be an indicator of fecal contamination and there may be a direct relationship between coprostanol concentrations and the degree of water pollution (refs. 5, 6 and 13).

Coprostanol is found to associate with particulate matter. Sediments near effluent discharges have a much higher concentration of coprostanol than the overlying waters, indicating that much of the coprostanol is removed to the sediment near the sewage outfall (ref. 8). Van Vleet et al. (ref. 3) noticed a similar trend for petroleum hydrocarbons discharged from a sewage treatment plant. They reported that half of the hydrocarbons were deposited near the outfall and the other half were removed from the area. Although much of the coprostanol may be deposited near sewage outfalls, it has been detected in seawater far removed from any fecal input sites (ref. 5). Therefore, coprostanol isolation and identification may serve as a viable indicator of the fate of fecal pollution and associated toxic materials resulting from the discharge of sewage effluents into natural waters.

The NOAA/NASA Superflux program provided a unique opportunity to more thoroughly investigate the transport of sewage-associated materials, utilizing coprostanol, from the Chesapeake Bay system (i.e., rivers and tributaries) to adjacent continental shelf waters. Furthermore, data of this nature may enable us to better understand the fate of sewage-associated material in the Chesapeake Bay and contiguous waters.

MATERIALS AND METHODS

Water samples were collected from the entrance to Chesapeake Bay and adjacent shelf waters and analyzed for particulate coprostanol and cholesterol concentrations. A total of 59 samples, taken aboard the NOAA vessels Delaware II (June 17-23, 1980) and George B. Kelez (June 24-27, 1980) during the Superflux II cruise, were analyzed. Seven samples were also taken from the R/V Linwood Holton (June 19 and 24, 1980), which was participating in a program conducted by the Department of Oceanography at Old Dominion University called BAPLEX.

The water samples, approximately 16 liters, were collected at various depths and were filtered on shipboard, as soon after collection as possible, through a preignited Gelman A/E glass fiber filter. The filters were wrapped in aluminum foil and kept frozen until they were analyzed back at the laboratory. An internal standard, nonadecanol, was added to the filter which was then

saponified/extracted under reflux for 2 hours with 100 ml of 0.5 N methanolic/KOH and 10 ml of toluene. The extract was filtered and the filtrate was placed in a separatory funnel containing 100 ml of 10 percent NaCl solution (adjusted to a pH of less than 2 with HCl). Seventy milliliters of dichloromethane (CH_2Cl_2) were added to the separatory funnel, the contents shaken, and the organic phase removed. The aqueous fraction was extracted two more times with 70 ml CH_2Cl_2 each time. The combined CH_2Cl_2 extracts were evaporated to dryness, and the residue was eluted through an alumina-silica gel column to separate alcohols and sterols from other organics. This fraction was then analyzed on a Hewlett-Packard 5830 gas chromatograph (GC), equipped with a 25-m methylsilicone, fused silica, WCOT, capillary column. The analysis was done by temperature programming from 80° to 270° C at 10° C/min. The eluting materials were detected with a flame ionization detector, the response of which was recorded and integrated with a Hewlett-Packard model 18850A reporting integrator. Concentrations of coprostanol and cholesterol were calculated with respect to the internal standard. Procedural blanks and standards were run systematically in association with all analyses to determine background levels of coprostanol and also to insure that the GC was operating properly. The presence of coprostanol was confirmed by coinjection with authenic coprostanol and by formation and GC analyses of TMS-derivatives.

RESULTS AND DISCUSSION

Particulate coprostanol and cholesterol concentrations were measured in 59 samples collected on the Superflux II cruises and 7 samples collected on the BAPLEX cruises. The BAPLEX samples provide more synoptic data because all of the samples, except one, were taken within a 2-hour window. The Superflux II samples, on the other hand, were taken over a 10-day period.

Various Superflux II and BAPLEX station locations are shown in figure 1. In figure 2, surface coprostanol concentrations at these stations are shown. The coprostanol concentrations of the BAPLEX samples are fairly consistent with a slightly elevated concentration near Cape Henry. This high concentration at BAPLEX station 4 may be caused by influence from Lynnhaven Inlet, or by direct discharge from ships. It is important to note that during the time of sampling there were numerous coal colliers moored in the Chesapeake Bay entrance. The discharge from these colliers and the heavy shipping traffic may explain this and other highly localized coprostanol concentrations. The particulate coprostanol concentration for the Superflux II samples varied considerably. Superflux II station 800 was sampled twice, on June 17 and 24. The difference between the coprostanol concentrations in these samples taken 1 week apart and at different stages in the tidal cycle illustrates the complexity of the transport system of particulates in the Chesapeake Bay entrance. The interpretation of data obtained over such a time interval in a complex system becomes very difficult.

A summary of coprostanol and cholesterol concentrations for Superflux II and BAPLEX samples is given in table 1A. The average coprostanol concentration for the BAPLEX samples is 0.190 µg/ℓ. For Superflux II samples,

the average coprostanol concentration is 0.250 μg/ℓ. Since only surface samples were collected at the BAPLEX stations, the Superflux II samples were broken down into surface (-1 m) samples and samples at depth (>-3 m). The average coprostanol concentrations for the surface and depth samples are 0.200 μg/ℓ and 0.278 μg/ℓ, respectively. The average coprostanol concentration for the BAPLEX surface samples is approximately the same as for the Superflux II samples taken at a depth of 1 m, indicating that on an average, the coprostanol concentration in surface waters of the Chesapeake Bay entrance and contiguous waters is fairly uniform. The average coprostanol concentration with depth is somewhat higher than that found in the surface waters. This increase with depth may come from either sewage-associated particles settling out as they leave the Bay, or the resuspension of contaminated sediment. The average cholesterol concentration determined in these samples is approximately five times higher than the coprostanol concentrations. The higher concentration of cholesterol is probably due to naturally occurring cholesterol in the marine environment. Coprostanol and cholesterol concentrations found in this study agree well with those reported in the literature (see table 1B and refs. 17 and 18). The Chesapeake Bay entrance is such a dynamic system that we cannot be certain which processes are dominant without more detailed study.

CONCLUSION

Particulate-associated coprostanol detected in the Chesapeake Bay entrance may originate from the discharge of sewage treatment plant effluent, runoff from nearby lands, or direct discharge from ships in the area. The coprostanol concentration in the surface water of the Chesapeake Bay entrance and contiguous waters is fairly uniform. An increase in concentration is found with depth, indicating the sewage-associated particulates are settling as they exit the Bay or contaminated sediment is being resuspended. The extended and somewhat random sampling scheme of this complex area makes the interpretation of the data difficult. However, we may conclude from this preliminary study that sewage-associated materials are being transported from the Chesapeake Bay to adjacent shelf waters where they may have adverse effects on living marine resources.

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TABLE 1A.- SUPERFLUX II AND BAPLEX RESULTS (µg/ℓ)

Source	Samples	Avg. coprostanol	Range	Avg. cholesterol	Range
BAPLEX (surf)	7	0.190	0.111-0.400	1.144	0.490-1.950
Superflux (all)	59	0.250	0.072-1.042	1.056	0.215-5.267
Superflux (-1 m)	21	0.200	0.072-1.042	0.956	0.215-5.267
Superflux (-3 m)	28	0.278	0.077-1.014	1.111	0.435-5.065

TABLE 1B.- COMPARISON OF COPROSTANOL AND CHOLESTEROL CONCENTRATIONS

Source	Coprostanol (µg/ℓ)	Cholesterol (µg/ℓ)	Reference
Superflux II	0.072-1.042	0.215-5.267	Present study
Clyde estuary	0.1-47.5	--	(8)
Ariake Sea	0.06-1.1	2.0-6.3	(17)
Tokyo Bay	0.2-6.6	2.2-8.6	(18)

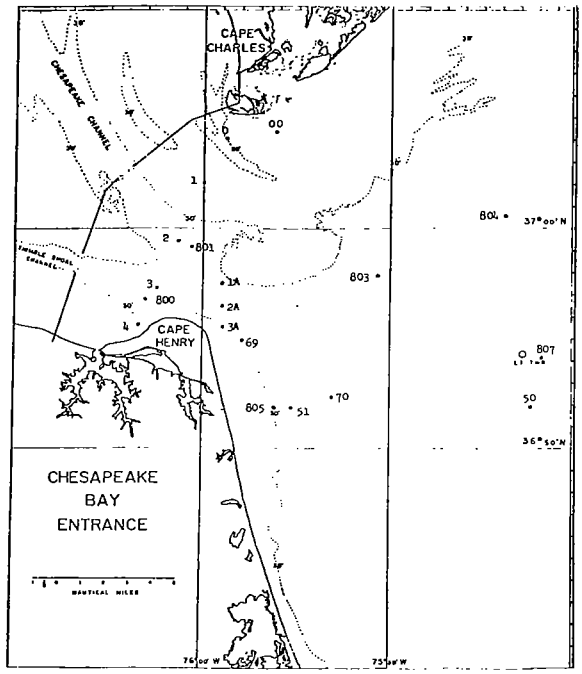

Figure 1.- Superflux II and BAPLEX sampling locations.

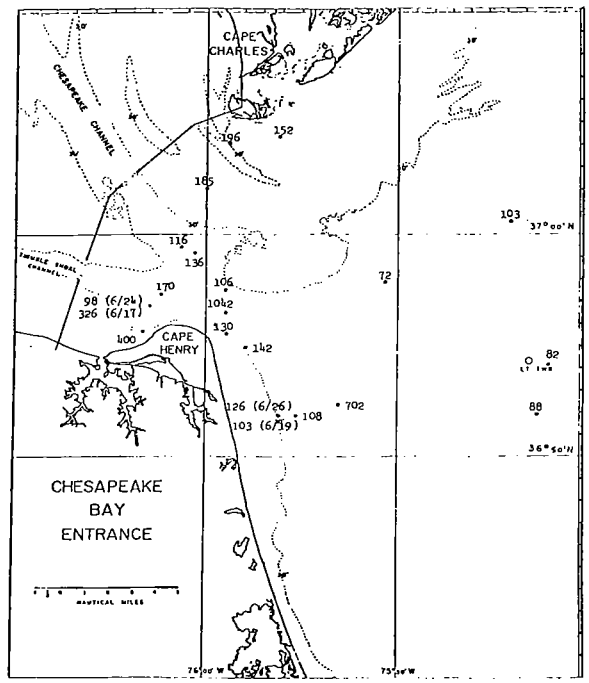

Figure 2.- Surface particulate coprostanol concentrations (µg/ℓ).