

## **Coprostanol Distribution from Sewage Discharge into Sarasota Bay, Florida**

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Distribution of the fecal sterol, coprostanol, was determined in sediment from forty-one sites throughout Sarasota Bay, Florida. This project was part of a water quality study to estimate the impact of sewage effluent discharged from the City of Sarasota's wastewater treatment plant into Sarasota Bay (Priede-Sedgwick, 1983). Coprostanol (5 $\beta$ -cholestan-3 $\beta$ -ol) is one of the principal sterols found in the feces of man and other mammals (Rosenfield, et al., 1954; Rosenfield and Gallagher, 1964; Teshima and Kanazawa, 1978), and has been shown to be a reliable marker of fecal pollution (Murtaugh and Bunch, 1967; Tabak et al., 1972; Goodfellow et al., 1977; Walker et al., 1982).

An important characteristic of coprostanol is that it is associated with fine particulate matter emanating from wastewater effluents, as are many other sewage-derived pollutants, such as pathogenic organisms, toxic metals and toxic organic chemicals (Dutka et al., 1974; Hatcher et al., 1977; Hatcher and McGillivary, 1979; Brown, 1983; Brown and Wade, 1981; 1983; Yde et al., 1982).

For this study, coprostanol was used as an indicator to estimate the extent to which sewage-derived particulate matter has been distributed within Sarasota Bay sediment.

## **METHODS**

Sediment samples were collected as a composite of at least three grabs with a petite Ponar sampler to provide 1 kg of sediment from each site. The top 5 cm from each grab was obtained to provide a uniform sample of surface sediment. Each composite was placed in pre-cleaned containers and stored frozen until analysis.

In preparation for analysis, sediment samples were thawed and then well mixed to ensure homogeneity. Each was air-dried to get rid of as much water as possible and the moisture content of the sediment was determined by drying a small subsample (ca. 1 g) at 1050-1100C to a constant weight. Weighed samples (ca. 100 g air-dried) were solvent-extracted through 300 cycles

on the Soxhlet extraction apparatus using a methanol:toluene (72.5/27.5, V/V) azeotrope. Following extraction, the azeotropic solution was concentrated to ca. 10 ml volume using a Kuderna-Danish concentrator.

Two aliquots were taken from the concentrated solution and an internal standard (cholesterol) added to one. Both aliquots were then saponified to remove interference from fats by combining with equal volumes of water and  $0.5\ \underline{N}$  potassium hydroxide in methanol and the solution was refluxed for two hours. Saturated NaCl solution (pH 2) was then added and the mixture extracted three times with dichloromethane (CH2Cl2). The CH2Cl2 extracts were combined and then reduced to ca. 5 ml volume with a Kuderna-Danish concentrator.

The sterols were separated from other organics in the sample by column chromatography on a glass column containing 1/1, V/V, silica gel over micron-neutral alumina (1 cm x 10 cm column tube). The column was successively eluted with three bed volumes of hexane, dichloromethane, and methanol. The first two eluents, containing hydrocarbons, were saved for future analyses. The methanol eluate containing sterols was evaporated under a stream of dry nitrogen and derivatives of the sterols were formed using N, 0-bis (Trimethylsilyl) acetamide (BSA) for gas chromatographic analysis. One out of every five samples was performed in duplicate to verify analytical precision for the environmental samples. Procedural blanks and standard recovery samples were incorporated with each set of five sediment samples analyzed.

Gas chromatographic analysis was performed with a Varian 6000 GC (Sunnyvale, California) equipped with dual FID using 25 m x 0.2 mm SE-30 fused silica columns. The GC was coupled to a VISTA-401 chromatography data system. Temperature programing was from 250°C to 280°C at 4°C min $^{-1}$ , with a 5 min. initial hold. The carrier gas was N2 with N2 make-up to the detector.

## RESULTS AND DISCUSSION

A contour of coprostanol concentrations in sediment throughout Sarasota Bay is shown in Figure 1. These results show very high concentrations (2,500 ng  $g^{-1}$  sediment) in Whitaker Bayou (site of sewage treatment plant outfall), indicating short range deposition of sewage-derived particulate matter. Concentrations decreased with distance into the Bay, eventually reaching our detection limit of 10 ng  $g^{-1}$ . Concentrations within Sarasota Bay exhibited a skewed distribution with the contours extending much farther from Whitaker Bayou in a north-south direction along the eastern shoreline than in a westward direction out into the Bay.

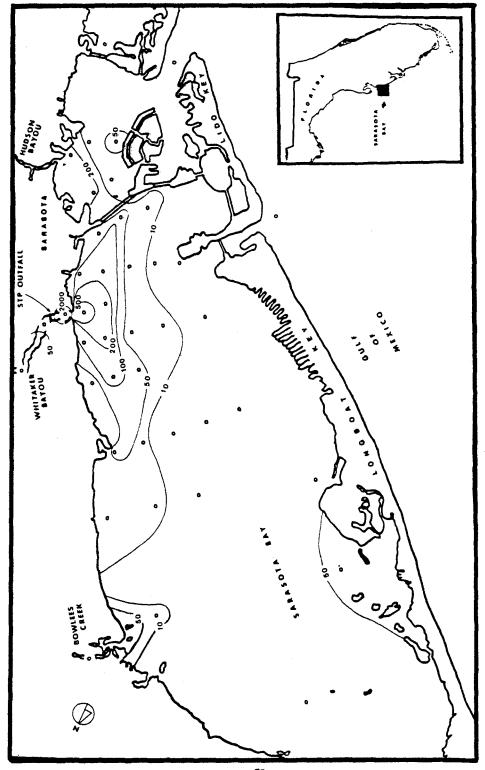
Comparison of these data with water current patterns of Sarasota Bay (Estevez, 1982) indicates that the dominant influence on distribution of sewage-derived particulates within the Bay is tidal action. The mode of flushing particles from Whitaker Bayou has not been investigated, however, one may hypothesize that enhanced runoff associated with storm events would be a major factor in sediment resuspension and transport into Sarasota Bay.

The extent to which coprostanol is distributed throughout Sarasota Bay provides an indication of the distribution of other sewage-derived pollutants (i.e. pathogens, toxic metals, petroleum, pesticides) that also are associated with particulate matter. However, additional studies are needed to determine the relationship of coprostanol with these other sewage-derived pollutants.

To estimate the area of Sarasota Bay impacted by the sewage effluent, we assume that a coprostanol concentration of >10 ng/g dry-weight sediment indicates the presence of sewage-derived material. Based on this information, the area affected by coprostanol-containing sediment was observed to be 14.4 km by 2.4 km, or about 36 km<sup>2</sup>. A major concern for estimating coprostanol input from the sewage outfall is the uncertainty about other coprostanol sources (marinas, other tributaries, septic tank leaching). To observe coprostanol input from non-sewage effluent sources, sediment was collected from the mouth of two tributary draining areas of land-use similar to Whitaker Bayou. These were: Bowlees Creek, approximately 7 km to the north; and Hudson Bayou, about 3.5 km to the south of Whitaker Bayou. Coprostanol concentrations observed in these tributaries were 88 ng  $g^{-1}$  and 221 ng  $g^{-1}$ respectively, or less than 10% of that observed in Whitaker Bayou. The relatively high coprostanol concentration found in Hudson Bayou also may be related to sewage due to periodic overflow that has been documented from sewer lines in that area.

Factoring out areas where coprostanol may have originated from other sources, the area containing coprostanol that may be considered to originate from the City of Sarasota wastewater discharge into Whitaker Bayou is approximately 7 km (N-S) by 2.2 km (E-W), or about 15.4 km². Since Sarasota Bay encompasses about 80 km², this study shows that approximately 20% of the Bay sediment has been impacted by sewage-derived particulates emanating from the Sarasota wastewater effluent. The relationships between coprostanol and other water quality parameters studied during this project are presently under investigation.

Acknowledgments: This investigation was funded as a joint effort from the U.S. Environmental Protection Agency, Section 201, with



Sarasota Bay sediment sample sites (o) with coprostanol concentration contours given as ng  $\mathfrak{g}^{-1}$  dry weight sediment. Figure 1.

matching funds from the City and County of Sarasota, administered through the Florida Department of Environmental Regulation. The authors would like to give special acknowledgment to Dr. Ernest D. Estevez, project coordinator for Mote Marine Laboratory.

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