

Potential Applications of Waste-Specific Molecular Markers

Upon release into the marine environment, wastewater particles are either dispersed and transported away from the site of discharge, incorporated into the biological cycle, or deposited on the sea floor. Because many of the most toxic constituents in effluents are associated with suspended particulate matter, the fate of wastewater particles has been of interest to marine environmental scientists for decades. Field studies offer an opportunity to measure the effects of waste discharge and the distribution of particle-associated contaminants. Unfortunately, interpretation of such measurements is often complicated by the interplay of natural processes and inputs from multiple sources.

Source-specific molecular markers can help track the movement and fate of waste particles. Molecular markers are substances whose structures are uniquely identified with a single source.

Municipal wastewater treatment plants discharge a complex mixture of synthetic and natural chemicals. If particle-bound substances persist in the environment, and if they enter the ocean only through waste discharge, they are potential waste-specific molecular markers. The presence of such compounds would probably be of little interest except for the fact that many toxic contaminants of concern (e.g. PCBs, PAHs, trace metals) reach the ocean from a variety of sources in addition to municipal wastes (e.g. surface runoff, atmospheric



Chemist Azra Khan evaluates data from samples analyzed for contaminants.

aerosols, oil seeps, tanker spills, etc.). Consequently, in urbanized coastal areas with multiple inputs, it is difficult to determine if an effect is attributable solely to waste discharge. Under these conditions, the presence of a waste-specific molecular marker in the environment is clear evidence that waste particles are present.

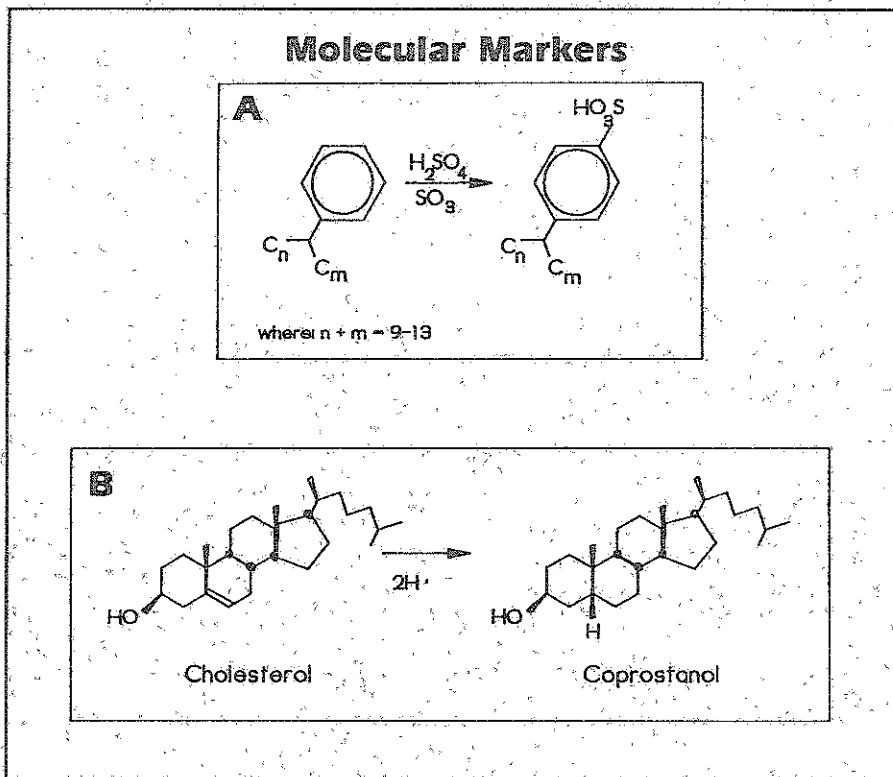
The purpose of this article is to provide background information on two classes of waste-specific molecular markers: long-chain alkylbenzenes and fecal steroids. We describe three applications of these compounds to illustrate their potential for answering questions related to the discharge of wastes in the marine environment. The treatment provided here is limited and the interested reader is referred to Eganhouse (1986a) and Walker *et al.* (1982) for more extensive discussions.

Molecular Markers

Alkylated benzenes with side chains of 10-14 carbons have been synthesized industrially since about 1950. They were used in the production of alkylbenzene sulfonates, the anionic surfactants most commonly used in commercial detergents. Alkylbenzenes produced between 1950 and the mid-1960s had highly branched side chains (tetrapropylene-based alkylbenzenes: TABs) that conferred stability upon the TABs and their sulfonated analogs (ABS). The persistence of these branched alkylbenzene sulfonates resulted in undesirable foaming problems in wastewater treatment plants and natural water bodies. Consequently, a more biodegradable substitute was sought that led to the development of the linear

Figure 1.

Waste-specific molecular markers: A) Synthesis of linear alkylbenzenesulfonates from linear alkylbenzenes, B) Conversion of cholesterol to coprostanol.



alkylbenzenes (LABs) (Figure 1).

The LABs are a mixture of 26 secondary phenylalkanes. They are present in minor amounts in commercial detergents (<1%) and in municipal wastewater effluents, usually from domestic inputs. However, industrial sources may be important in certain cases. The LABs are easily measured using conventional methods (Eganhouse 1986a, Takada and Ishiwatari 1985), and they occur in numerous waste-impacted riverine, marine, and estuarine environments (Eganhouse *et al.* 1983a, Murray *et al.* 1987, Takada and Ishiwatari 1987, Valls *et al.* 1989) including the tissues of organisms (Albaigés *et al.* 1987, Sherblom and Eganhouse 1991). No natural

sources of these compounds are known.

Fecal steroids, particularly coprostanol (Figure 1), are natural products that have been used for more than 30 years as molecular indicators of human fecal contamination. Coprostanol is produced principally from cholesterol, an ubiquitous steroid, by stereo-specific biohydrogenation reactions mediated by the enteric bacteria of mammals. Because mammals represent a quantitatively minor component of all but a few ecosystems, the presence of coprostanol in urbanized coastal areas is evidence of human fecal inputs. Coprostanol and other fecal steroids are usually among the most abundant organic constituents of municipal wastes.

They are easily determined in marine sediments and particles collected in sediment traps positioned near waste discharges. However, our understanding of the biodegradability and ultimate fate of fecal steroids in the marine environment is incomplete.

Materials and Methods

The methods used in this study are based on classical solvent extraction, adsorption chromatography, and either high resolution gas chromatography (HRGC) or HRGC/mass spectrometry. These procedures are described in Eganhouse (1982, 1986a) and Eganhouse *et al.* (1983b, 1988).

Results and Discussion

LABs as a "Molecular Clock"

The LABs are five groups of isomers with different chain

lengths (C_{10-14}). Within each group, there are four to six individual isomers that differ by the substitution position of the benzene ring on the alkyl chain (Figures 1 and 2). "Internal isomers" are LABs with the benzene ring substituted near the interior of the alkyl chain (e.g. the 6-phenylalkanes). "External isomers" are LABs with the benzene ring substituted near the end of the chain (e.g. 2-phenylalkanes).

The composition of the LABs found in commercial detergents, municipal wastewaters, and waste-impacted sediments differs significantly (Eganhouse *et al.* 1983a, 1983b). The relative abundances of internal and external isomers is similar in detergents and treated wastewater effluents (Figure 3a,b). Sediments affected by wastes, and particles collected in sediment traps, have greater relative amounts of the internal isomers (Figure 3c,d). This difference is due to selective microbial degradation of the

external isomers (rather than physical fractionation) (Bayona *et al.* 1986, Takada and Ishiwatari 1990). Particles collected in sediment traps, and surficial sediments collected near the Los Angeles County Joint Water Pollution Control Plant (JWPCP) outfalls, have a slightly lower degree of depletion of the external isomers than sediments deposited at greater sub-bottom depths (Eganhouse *et al.* 1983a). This suggests that LAB degradation occurs prior to and following sedimentation. Because most of the modification occurs prior to incorporation of LABs into sediments, degradation rates are probably greater in the oxidizing environment of the water column than in reducing sediments. This hypothesis is consistent with the results of laboratory degradation experiments conducted under aerobic and anoxic conditions.

Careful studies of the degradation kinetics of LAB isomers under environmentally relevant conditions have not been performed. However, if these degradation rates were known, isomer distributions might be used to infer the approximate age of the particles with which the LABs are associated. Degradation experiments, thus, represent a "calibration" of a short-term "molecular clock".

One application of such a clock is the analysis of LABs in microlayer samples such as those collected by the City of Los Angeles in their ocean monitoring program. The presence of LABs would be evidence of wastewater input to the ocean surface; the isomer distribution could provide information on the age of waste particles.

Figure 2.

Mass fragmentograms of linear alkylbenzenes in sludge samples from Nut Island Treatment Plant, Boston, MA. (after Eganhouse *et al.* 1988).

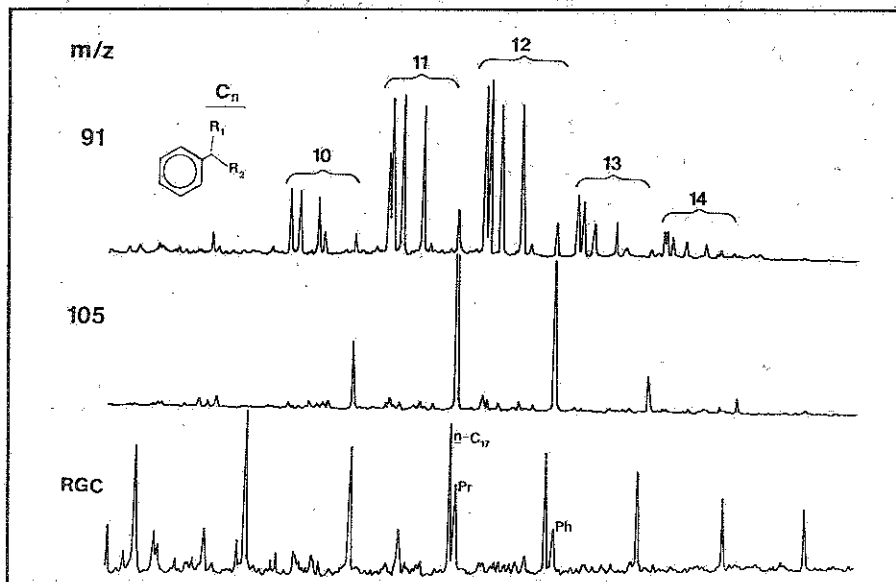
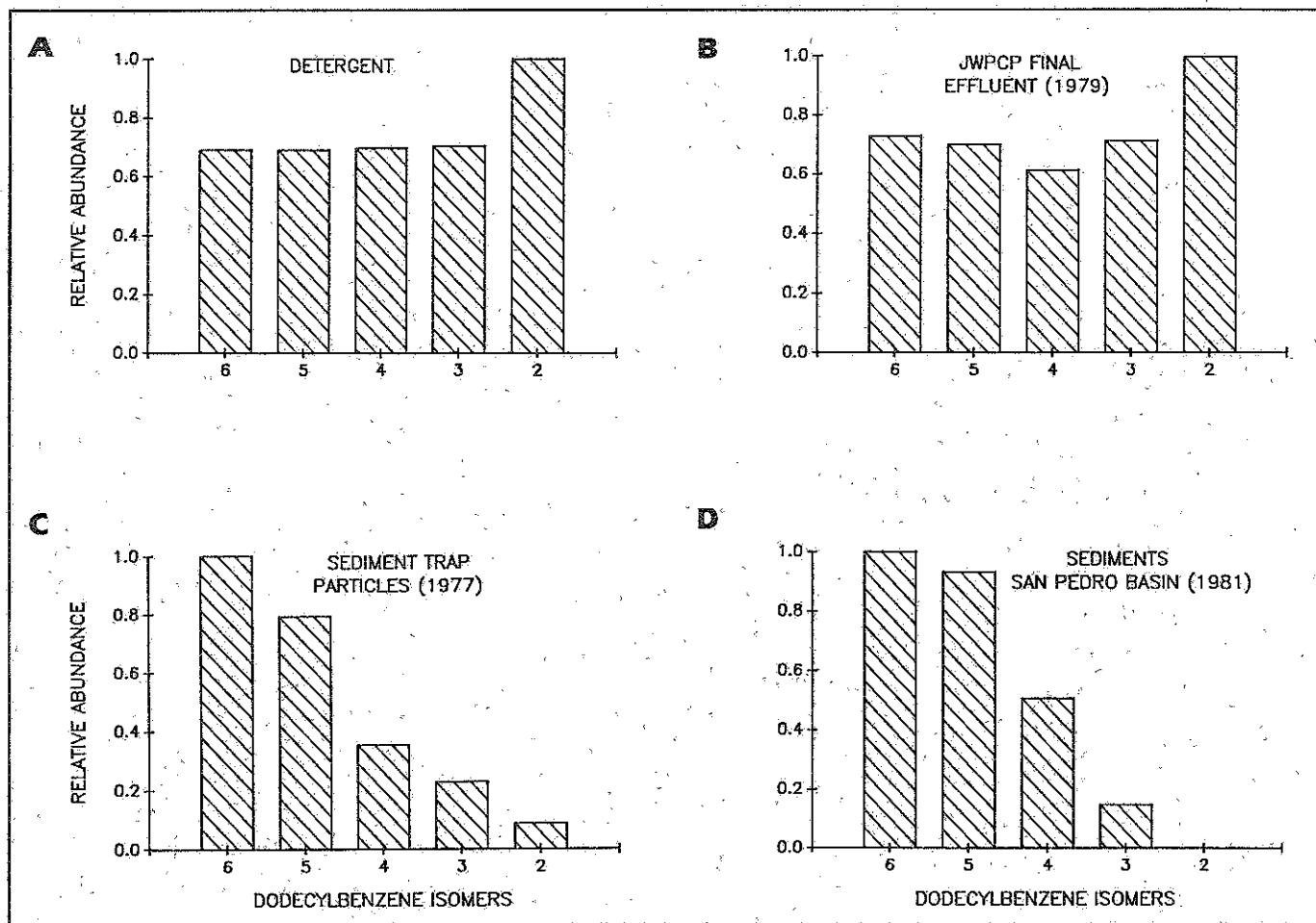


Figure 3.

A) Distribution of dodecylbenzene isomers in a commercial detergent, B) Joint Water Pollution Control Plant (Los Angeles County) final effluent (6/15/79), C) sediment trap particles from San Pedro Basin, and D) surface sediments from San Pedro Basin [after Eganhouse *et al.* 1983a].



Geochronology and Diagenesis

The TABs and their sulfonated derivatives dominated the anionic surfactant market until the mid-1960s when they were supplanted by the LABs. If long-chain alkylbenzenes survived transport to the sea floor, one should find them in sedimentary deposits affected by municipal waste discharges. A sediment core might bear the history of deposition of these compounds.

The trends in suspended solids emissions from the JWPCP outfalls from 1945 to 1981 (Figure 4a) are similar to the distribution of organic carbon in a core from Station 3C, 8 km down-current (Figure 4b). The correlation between these profiles suggests that sediments deposited at this site record the history of waste emissions. Similar core patterns occur for total nitrogen, total hydrocarbons, and other constituents known or believed to come from the JWPCP effluent

(Eganhouse and Kaplan 1988).

The usage rates of the alkylbenzene sulfonates from 1950 to 1981 (Figure 5a) are assumed to mirror the input of alkylbenzenes into waste treatment systems. Both types of long-chain alkylbenzenes are found in sediments at Station 3C (Figure 5b). The TABs are more abundant in the deeper sediment layers; concentrations decline below detection limits deeper than 24 cm. Most important, the TABs occur between 12 cm and the

sediment-water interface. By comparison, the LABs are most abundant in the upper sediment layers and decline below detection limits at about 18 cm. Unlike the historical usage trends of the sulfonated analogs (Figure 5a), the vertical sediment profiles of the long-chain alkylbenzenes overlap.

The presence of long-chain alkylbenzenes is unequivocal evidence of waste contamination at the site. The concentrations are

much higher than have been reported for other sites affected by municipal discharges. The presence of TABs in the surficial sediments is surprising because the core was collected in 1981. Even if some surface sediments were lost during coring, the presence of TABs in the surficial sediments cannot be attributed to contemporary inputs from JWPCP. They were not present in final effluent samples in 1979 (Eganhouse *et al.* 1983a). Be-

cause TAB production and use ended in the mid-1960s, and because significant amounts of TABs are found in the near-surface sediments, it appears that the sediments have undergone vertical mixing. However, the mixing rate(s), or mixing depths were not great enough to obliterate major features of the stratigraphy (Figure 4).

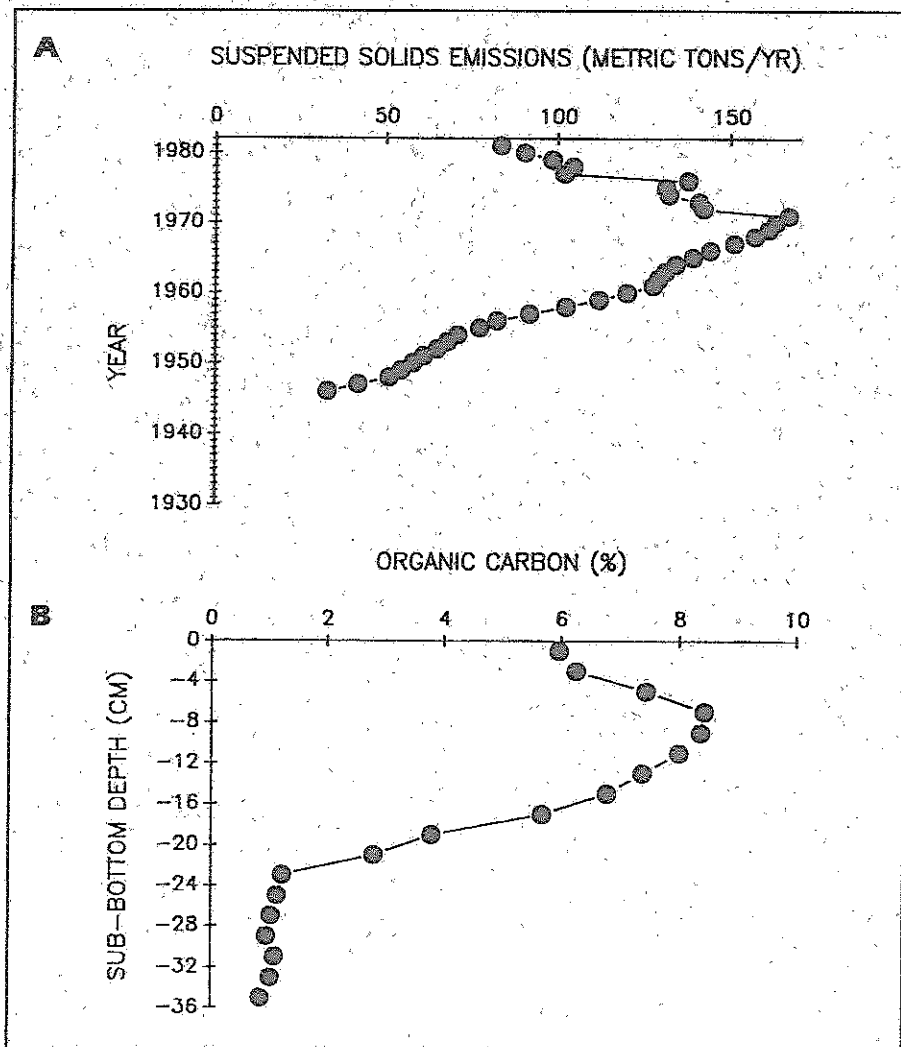
The co-occurrence of a TAB concentration maximum and the first detection of LABs probably represents the alkylbenzene changeover period (Figure 5b). This is consistent with other data that suggest that the 24 cm horizon is about 1950, the surface sediment (0-2 cm) is the late 1970s to 1981, and the 6-8 cm section is about 1970-71 (Eganhouse and Kaplan 1988). The long-chain alkylbenzenes provide information on the history of effluent deposition. Because these sediments were deposited under conditions that violate assumptions of radiometric methods of dating, the alkylbenzenes provide stratigraphic and diagenetic information that would otherwise be difficult to obtain.

Differentiating Inputs from Different Wastewater Sources

The most obvious application of molecular markers is in tracking waste particles in the environment. However, currents can move suspended particles great distances from their source as evidenced by the widespread contamination of the northern portion of the Southern California Bight by DDT. If effluent from one treatment plant were to be transported into a region affected

Figure 4.

A) Historical emission of suspended solids from the Joint Water Pollution Control Plant (Los Angeles County), and B) vertical profile of total organic carbon in sediments of Station 3C on the Palos Verdes Shelf in 1981 (after Eganhouse *et al.* 1988).



by another municipal discharge, it might be difficult to differentiate between their contributions. This situation exists in Santa Monica Bay. The predominantly northward flowing subsurface currents on the shelf transport effluent particles from the Palos Verdes area into Santa Monica Bay. Another example is Boston Harbor where two major treatment plants discharge municipal wastes into shallow water.

We became interested in this question when studies in Boston Harbor indicated that the sediments near a CSO (combined sewer overflow) had Σ LAB/coprostanol ratios that were different from the effluent particles discharged from the CSO. This suggested that despite their proximity to the outfall and their sewage origin, the accumulating sediments did not come from that CSO. The requirements for using this ratio to differentiate wastewater sources are: 1) municipal treatment plants have distinct Σ LAB/coprostanol ratios and 2) the ratios are conserved after the particles are discharged.

Requirement 1: The JWPCP receives influent from one of the few factories in the United States that synthesize LABs. This industrial source probably accounts for the exceptionally high LAB concentrations in the effluent (Table 1) and the inability to account for >85% of the LABs based on domestic detergent use (Eganhouse *et al.* 1983a). Although LABs have been detected in final effluent discharged by Hyperion Treatment Plant, the mean annual concentration, and its variation over time, is unknown. The LAB concentration in Hyperion effluent is probably lower than the concentration in

JWPCP effluent. We are currently conducting a survey of LAB and coprostanol concentrations in effluents from the four largest treatment plants in southern California in a project sponsored by the State Water Resources Control Board.

Requirement 2: The dominant source of contaminants to the sediments near the JWPCP and South Essex Sewage District (SESD, Salem, Massachusetts) outfalls are municipal effluents

(Eganhouse 1986b, Eganhouse and Kaplan 1988). Although Σ LAB/coprostanol ratios in effluents are variable, the ranges generally do not exceed a factor of two (Table 2). Sediments on the Palos Verdes Shelf have a mean ratio that is greater than the ratio for JWPCP effluent (Table 2). However, the 95% confidence intervals for the two groups overlap. The similarity between effluent and sediment LAB/coprostanol ratios at the SESD site is more remarkable.

Figure 5.

A) Usage rates of linear alkyl sulfonate (LAS) and alkylbenzene sulfonate (ABS) surfactants in the U.S., and B) vertical concentration profiles of linear (LAB) and highly branched (TAB) alkylbenzenes in sediments from Station 3C on the Palos Verdes Shelf (after Eganhouse *et al.*).

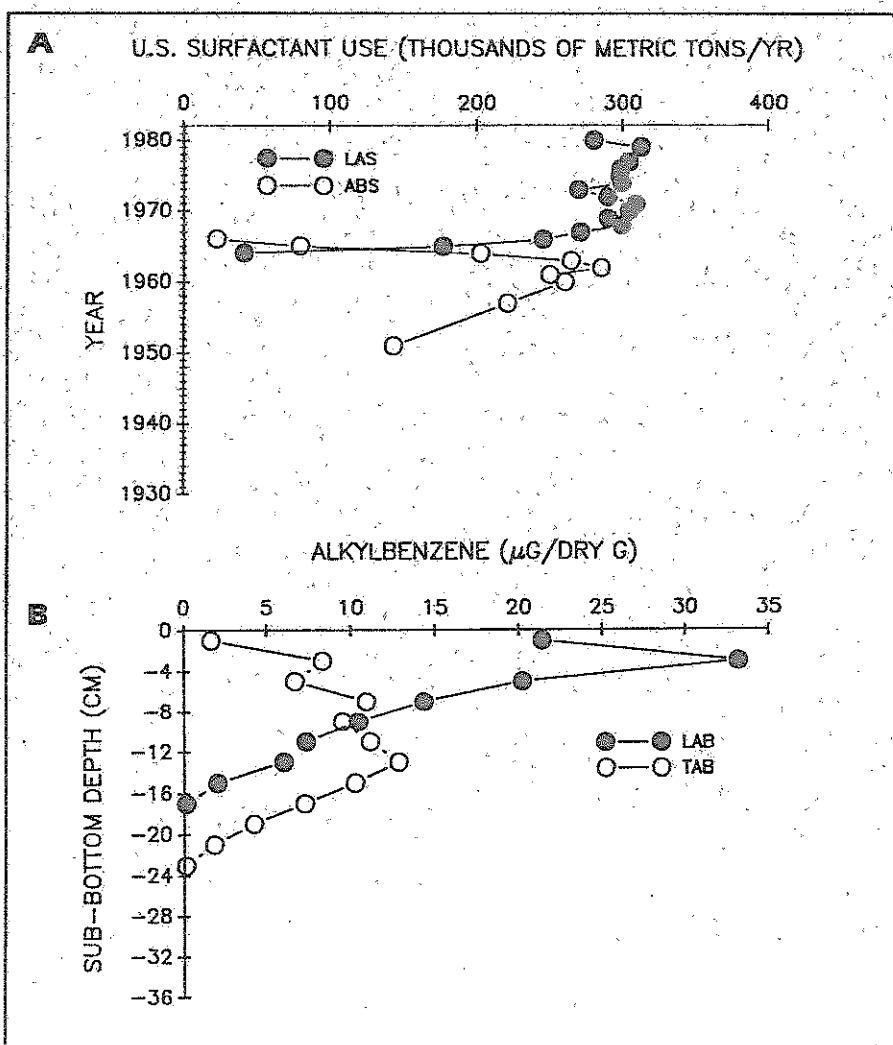


Table 1.

Linear alkylbenzene concentrations in final effluent from the Los Angeles County Joint Water Pollution Control Plant (JWPCP), South Essex Sewage District (SESD; Salem, MA), and the Fox Point Combined Sewer Overflow (FPCSO; Boston Harbor, MA).

	Date	Concentration	
		($\mu\text{g/liter}$)	($\mu\text{g/dry g}$)
JWPCP ^a	1/79	108	614
	2/79	242	1220
	3/79	98.0	612
	4/79	140	686
	5/79	78.4	472
	6/79	302	1340
	7/79	114	504
	8/79	142	651
	9/79	155	728
	10/79	87.2	484
	12/79	149	931
SESD ^b	2/17/86	5.30	nd ^c
	2/18/86	5.24	nd
	2/26/86	9.68	nd
	3/11/86	6.29	nd
	3/18/86	8.20	nd
	3/25/86	11.8	nd
	4/01/86	6.38	nd
FPCSO ^d	3/16/88	0.09	4.4
	4/01/88	0.22	10.6
	4/13/88	0.16	8.7
	7/12/88	0.85	15.9
	7/20/88	2.26	55.1

^aConcentrations reported by volume are for dissolved + particulate samples. Concentrations reported as dry solids assume that 100% of LABs are associated with suspended solids. Data from Eganhouse *et al.* (1983a)

^bConcentrations reported on a volume basis are for dissolved + particulate samples. Data from Eganhouse (1986b)

^cnd=not determined

^dConcentrations based on particles isolated by filtration through GF/C filters. Data from Eganhouse and Sherblom (1990)

Conclusions

The long-chain alkylbenzenes and fecal sterols are molecular indicators of municipal wastes.

These compounds may allow us to: 1) track waste effluent particles in the water column, 2) age-date recent versus historical effluents, 3) establish geochronology not amenable to other types of dating, and 4) differentiate

contributions made by different wastewater discharges to the environment. The extent to which these compounds can be exploited for these purposes will depend on their survivability in the marine environment. ■

Table 2.

The ratio of linear alkylbenzene (LAB) to coprostanol in final effluent samples from the Los Angeles County Joint Water Pollution Control Plant (JWPCP), the South Essex Sewage District (SESD), and sediments collected near the marine outfalls (Palos Verdes Shelf and Salem Harbor, respectively). The mean and standard deviation (in parentheses) are given for the ratios.

	Effluent		Sediments	
	Date	LAB/copros	Depth/Stn.	LAB/copros
JWPCP	1/79	0.72	0-2 cm	1.12
	2/79	1.06	2-4 cm	2.04
	3/79	1.02	4-6 cm	1.18
	4/79	0.73	6-8 cm	nd ^a
	5/79	0.23	8-10 cm	0.85
	6/79	0.76	10-12 cm	1.14
	7/79	0.40	12-14 cm	1.25
	8/79	0.74	mean	1.26 (0.40)
	9/79	0.66		
	10/79	0.88		
	12/79	1.03		
	mean	0.75 (0.26)		
SESD	2/17/86	0.082	1B	0.182
	2/18/86	0.084	1E	0.105
	2/26/86	0.141	1D	0.099
	3/11/86	0.088	mean	0.129 (0.046)
	3/25/86	0.167		
	4/1/86	0.158		
	mean	0.120 (0.04)		

^and=no data

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