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Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments

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ABSTRACT

Monitoring the water quality of recreational beaches is only one step toward understanding microbial contamination—the primary cause of beach closings. The surf zone sediment reservoir is typically overlooked and may also be important. This study involved monitoring the fecal indicator bacteria (FIB) levels in water and sediment at three ocean beaches (two exposed and one enclosed) during a storm event, conducting laboratory microcosm experiments with sediment from these beaches, and surveying sediment FIB levels at 13 beaches (some exposed and some enclosed). Peaks in *Escherichia coli* and enterococci concentrations in water and sediment coincided with storm activity at the two exposed beaches, while levels of both FIB were consistently high and irregular at the enclosed beach. Results from microcosm experiments showing similar, dramatic growth of FIB in both overlying water and sediment from all beaches, as well as results from the beach survey, support the hypothesis that the quiescent environment rather than sediment characteristics can explain the elevated sediment FIB levels observed at enclosed beaches. This work has implications for the predictive value of FIB measurements, and points to the importance of the sediment reservoir.

1. Introduction

Microbial contamination of surf zone water frequently causes southern California swimming advisories and beach closings, due primarily to stormwater runoff (Boehm et al., 2002a, b; Council, 2004; Kim and Grant, 2004; Kim et al., 2004; Reeves et al., 2004; Ahn et al., 2005; Grant et al., 2005; Jeong et al., 2005; Pednekar et al., 2005) and possibly to sewer leakage. Swimming near storm drains increases the risk of respiratory and gastrointestinal illness (Haile et al., 1999). Enclosed ocean beaches, popular for families with small children because they are sheltered, are particularly notorious for poor water quality.

In the Los Angeles area, water quality is monitored daily; however, the microbial quality of beach sand is overlooked. Studies have shown that sediments can act as an important reservoir for fecal indicator bacteria (FIB) in freshwater environments (LaLiberte and Grimes, 1982; Burton et al., 1987; Brettar and Holfe, 1992; Dan and Koppel, 1992; Davies et al., 1995; Blumenroth and Wagner-Dobler, 1998; An et al., 2002; Alm et al., 2003; Haack et al., 2003; Whitman and Nevers, 2003). While some marine sediments have also been studied (Gerba and McLeod, 1976; Davies et al., 1995; Craig et al., 2002, 2004; Desmarais et al., 2002; Anderson et al., 2005; Ferguson et al., 2005; Jeong et al., 2005), there are still significant gaps in our knowledge, and it is still assumed that enteric bacteria

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fail to thrive in most nonhost environments (Winfield and Groisman, 2003).

Marine sediment may be more conducive to FIB survival relative to the water column by reducing sunlight inactivation (Sinton et al., 1999), protecting against predators (Brettar and Holfe, 1992; Davies and Bavor, 2000), increasing nutrient and organic carbon availability (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982; Blumenroth and Wagner-Dobler, 1998; Craig et al., 2004), and providing colonizable surfaces (Brettar and Holfe, 1992; Davies et al., 1995). These surfaces facilitate biofilm formation, which plays a role in moderating nutrient accessibility through close proximity of organisms as well as temperature and condition fluctuations (Decho, 2000). Resuspension of FIB and pathogens in the sediment due to human and boat activity or natural turbulence may contribute to human health risk (An et al., 2002; Craig et al., 2004).

1.1. *Research goals*

This study examined the persistence and survival of FIB in the sediments of two unsheltered (exposed) beaches and one enclosed beach in Santa Monica Bay that have had frequent beach postings due to microbial contamination. We monitored FIB levels in both the sediment and water daily throughout a storm event, conducted laboratory microcosm studies using sediment from the three beaches, and surveyed 13 beaches (two enclosed and eleven open) for FIB levels in sediment.

We tested the following hypotheses:

- FIB concentrations persist longer in sediment than in water following the peak of a storm at our study sites.
- In laboratory microcosms, the sediments from the three beaches show differential FIB growth in sediment biofilms.
- The quiescent sediments at enclosed beaches retain higher FIB concentrations than those at open beaches.

2. Experimental methods and procedure

2.1. *Study sites*

The locations of the three sites chosen for this study include (A) Surfrider Beach, Malibu, an open beach sampled at the outlet of Malibu Lagoon; (B) Santa Monica Beach, an open beach, sampled at the outlet of the Arizona projection storm drain; and (C) Mother's Beach, an enclosed beach in Marina del Rey (Fig. 1). In 2003, Surfrider Beach was posted 35 times, Santa Monica Beach 62 times, and Mother's Beach 24 times due to concentrations of enterococci exceeding State standards (Council, 2004). In addition, to test our third hypothesis, we measured *E. coli* and enterococci in water and sediment at two enclosed beaches (Cabrillo and Mother's) and eleven open beaches in the Santa Monica Bay during dry weather.

2.2. *Storm sampling*

The three beaches were monitored during storm events from 9–17 February 2003, between 6:30 and 7:30 AM. At the peak of the storm, tide stage at 7 AM was approximately 1 m above MLLW. The tide was coming in, and peaked at 1.7 m above MLLW at noon. Water samples were collected at a depth of approximately 6 in using 125 mL polypropylene Nalgene bottles. Because FIB levels have been observed highest in the top layer of sediment (Desmarais et al., 2002; Alm et al., 2003), triplicate samples were obtained by skimming the top 1 cm of undisturbed sediment.

Measurements of turbidity, pH, temperature, dissolved oxygen, and salinity were obtained using a multi-parameter water quality probe (Hydrolab).

To quantify FIB attached to marine sediment, we repeated the following process three times: (1) washed 10 g samples thoroughly with 30 mL Milli-Q pore water by manually shaking them for 1 min; (2) performed sonication for 10 min (Craig et al., 2002); and (3) conducted another 1 min washing exactly as described in the first step. The washes were then pooled and enumerated using Colilert-18 and Enterolert (defined substrate technologies for *E. coli*, and enterococci, IDEXX). All water samples were diluted by at least ten-fold, according to manufacturer's instructions for *E. coli* and enterococci in marine water. Sample data were acquired in triplicate for all time points. Our preliminary studies showed equivalent results using both phosphate buffer and Milli-Q water as extractant for the sonication and shaking steps (data not shown).

2.3. *Laboratory microcosm experiments*

Laboratory microcosms to evaluate FIB's survival in overlying water and sediment contained approximately 10 g of autoclaved sediment (from Surfrider, Santa Monica and Mother's Beaches) and 20 mL of autoclaved ocean water. Control microcosms contained either ocean water alone or overlying commercially available washed sand (Fisher S25-500). Laboratory strains of *E. coli* (ATCC 12014) and *Enterococcus faecalis* (ATCC 11420), recently shown to be a dominant species in water and sediment at another southern California beach, (Ferguson et al., 2005) were used to inoculate the water in these microcosms. Microcosms were sacrificed, and FIB were enumerated using IDEXX in water and sediment after inoculation and on days 1, 2, 4, 6, and 9. Sediment bacteria were grouped into two categories depending on the degree of association: (a) "Loosely attached"—bacteria that were removed from the sediment by inverting the sand in extraction water several times and (b) "Tightly attached"—bacteria that were detached from sediment following 10 min of sonication (performed twice). This classification reflected how easily the sediment bacteria could be resuspended from biofilms into the aqueous phase.

To investigate the importance of organic content, a second set of microcosm experiments was conducted. Four types of microcosms were assembled in duplicate. The sediment was taken from both Mother's Beach and Santa Monica Beach and was analyzed "as is," and after the removal of organic content (by burning at 475 °C in a high-temperature furnace for more than 10 h). Each microcosm was inoculated with lab culture *E. coli* and wrapped in foil. After 3 days, the bacteria levels in the microcosms were tested in both overlying water and sediment (the two sediment extractions, loosely and tightly

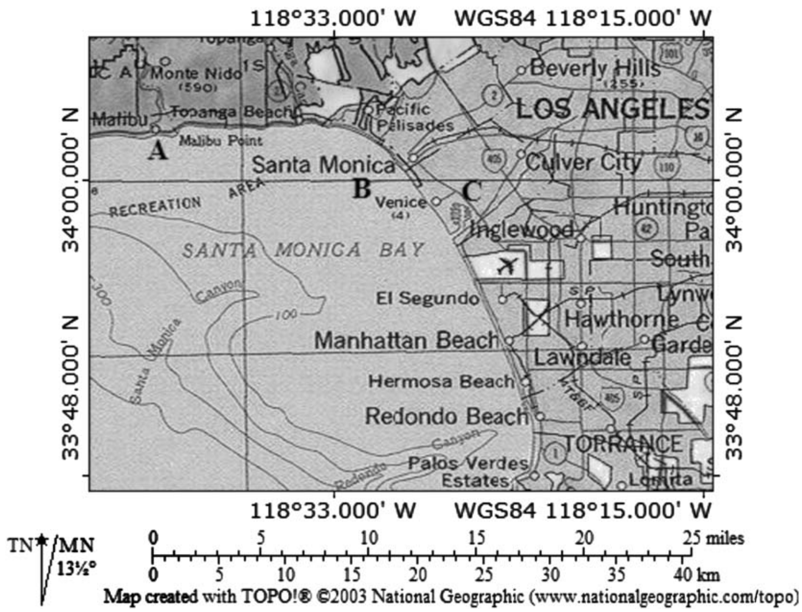


Fig. 1 – Map of Santa Monica Bay beach locations. Sampling sites were Surfrider Beach (A), Santa Monica Beach (B), and Mother's Beach (C).

bound, were pooled). Bacteria were extracted by the method described above, except with sea water as the extractant.

2.4. *Organic content analysis*

Organic content values were obtained by loss on ignition at 550 °C for 2 h (Clesceri et al., 1998).

2.5. *Particle size evaluation*

Particle size analyses were conducted by charging the particles in a sodium metaphosphate solution and taking hydrometer measurements at standardized time increments (Zedler, 2001).

2.6. *Beach survey*

E. coli and enterococci in water and sediment were measured at two enclosed (Cabrillo and Mother's) beaches and eleven open beaches in Santa Monica Bay on 22 July 2005 in dry weather. Sediment samples were collected in triplicate from each beach between 7 and 8 AM, and 15 g subsamples from each triplicate were extracted twice in series by 1 min of vigorous manual shaking in phosphate-buffered saline. Each extractant was then analyzed in triplicate, as described previously.

3. Results and discussion

3.1. *Storm sampling*

To test our first hypothesis—FIB levels in sediments after a storm persist longer than those in water—we measured FIB levels daily in water and in sediment at three beaches prior to, during, and following a storm event in February 2003. At the two exposed beaches, concentrations for both *E. coli* and enterococci reached maximum values between 10^3 and 10^4 MPN/100 mL in water and approximately 10^3 MPN/g in sediment during the onset and peak of the storm (Figs. 2 and 3). The occurrence of peak values during the period of highest rainfall has been observed elsewhere (Rees et al., 1998; Coelho et al., 1999; Solo-Gabriele et al., 2000; Crowther et al., 2001; Lipp et al., 2001; Craig et al., 2002; Pednekar et al., 2005). In this case, the peak may be attributed to the direct inflow of a large volume of stormwater runoff, which has been shown to be the major local source of pollution to local beaches (Haile et al., 1999). Also, resuspension of sediments as a direct result of storm events can lead to increased FIB concentrations in water (Coelho et al., 1999; An et al., 2002; Craig et al., 2004). Sediment FIB levels observed here are comparable to those previously measured in both freshwater (Davies et al., 1995; Desmarais et al., 2002; Alm et al., 2003; Whitman and Nevers, 2003) and marine sediments (Sanchez et al., 1986; Ghinsberg et al., 1994; Davies et al., 1995; Craig et al., 2002; Jeong et al., 2005).

We expected that FIB levels would persist longer in sediments than in water following a peak due to a storm event. However, at Surfrider and Santa Monica Beaches, the peaks for *E. coli* and enterococci in both water and sediment returned to prestorm levels within a week, with a slightly slower decline in sediments for enterococci. This is contrary to the somewhat more gradual decrease observed in sediments in another study (Craig et al., 2002).

In general, FIB levels in water and sand at Mother's Beach, the enclosed beach, were consistently high and did not appear well-correlated with the storm. Sediment levels for *E. coli* and enterococci averaged 10^4 MPN/g sediment. Enterococci exhibited a peak (10^6 MPN/g sediment) during non-storm conditions. These values are on average two orders of

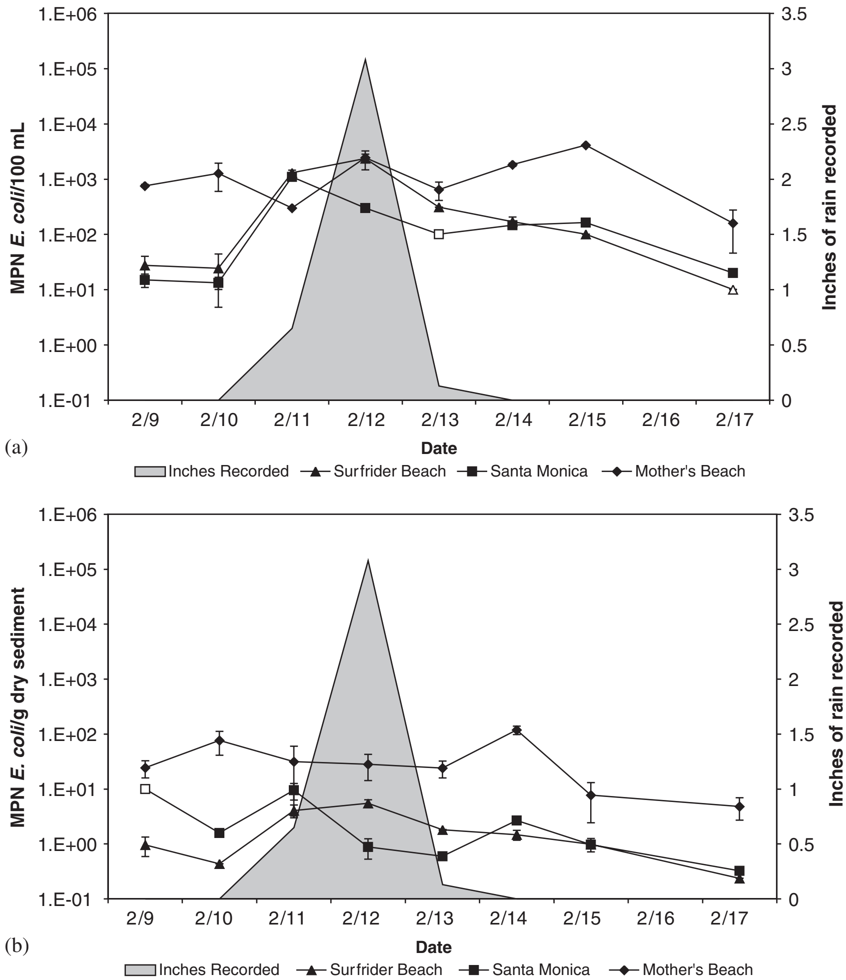


Fig. 2 – *E. coli* concentrations in (a) water and (b) sediment at three Santa Monica Bay beaches throughout the period of the storm.

magnitude higher than the background values seen at the two exposed beaches. The lack of a direct input of storm water to Mother's Beach contributes to the lack of correlation between FIB levels and the occurrence of the storm.

Salinity and turbidity results (data not shown) reflected a change during the storm peak, correlating with the peaks observed in FIB concentrations. Turbidity ranged between 10 and 30 NTU at all three beaches during the days before and after the peak of the storm, and reached a maximum during the storm of 50, 116, and 405 NTU at Surfrider, Santa Monica and Mother's Beaches, respectively. Salinity decreased during the peak of the storm from approximately 50 mS/cm to 5, 12, and 28 mS/cm at the three beaches, respectively.

3.2. *Microcosm experiments*

To explain observed differences between the various sediments' ability to support microbial growth and to test the hypothesis that the presence of sediment results in enhanced microbial growth, we inoculated and monitored laboratory microcosms containing autoclaved sediment from the three beaches and autoclaved Santa Monica Beach water. In the microcosm without sediment, *E. coli* levels decreased by three orders of magnitude over the first two days of the experiment (Fig. 4a), as expected (Gerba and McLeod, 1976; Sanchez et al., 1986; Burton et al., 1987; Brettar and Holfe, 1992; Davies et al., 1995; Craig et al., 2002, 2004). In contrast, in all treatments with sediment (even the control containing washed sand), *E. coli* levels (Fig 4a) in water increased by two to three orders of magnitude. While levels of *E. faecalis* remained fairly constant in the microcosm without sediment, as well as in water overlying Surfrider Beach sand and in the washed sand control, the level of *E. faecalis* increased by three orders of magnitude in the water overlying sediment at Mother's and Santa Monica Beaches (Fig. 4b).

Consistent behavior was observed in the levels of both *E. coli* and *E. faecalis* loosely attached to sediment (Fig. 5). Levels of *E. coli* easily rinsed from sediment increased considerably (to approximately 10^4 MPN/100 g after just one day and to 10^7–10^8 MPN/100 g after several days) and similarly in all four microcosms containing sediment. Levels of loosely attached *E. faecalis* increased more significantly in the microcosms containing Mother's and Santa Monica

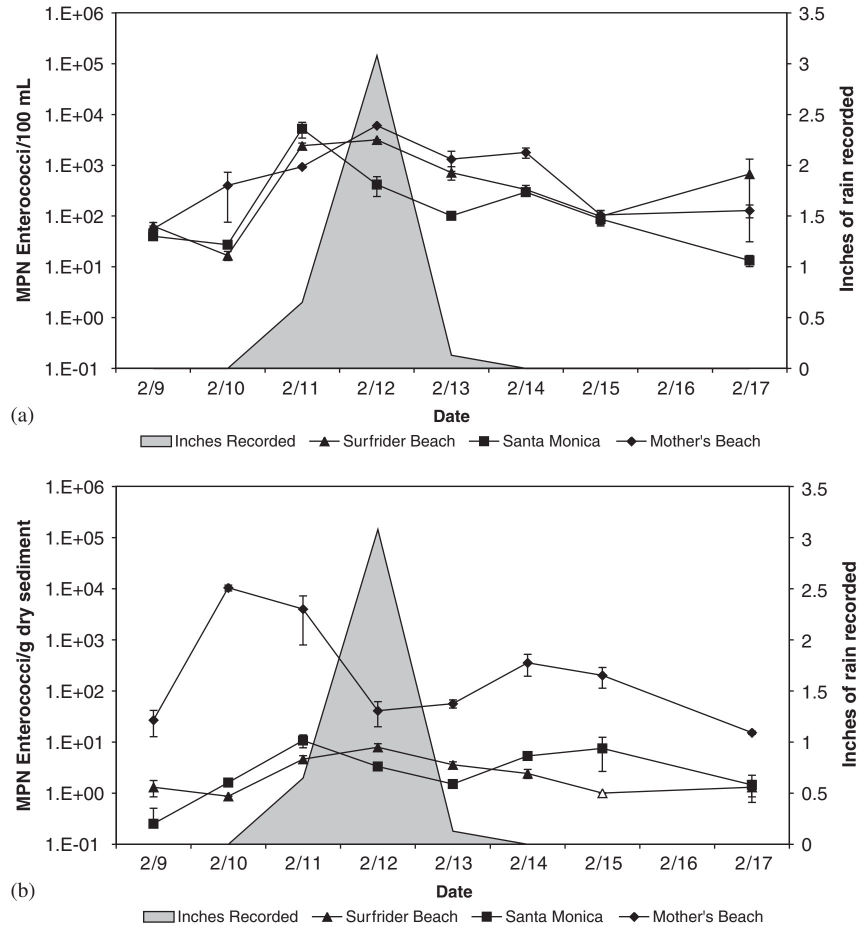


Fig. 3 – Enterococci concentrations in (a) water and (b) sediment at three Santa Monica Bay beaches throughout the period of the storm. Hollow symbols represent non-detect samples plotted at the detection limit.

Beach sediment (reaching approximately 10^8 MPN/100 g) compared to washed sand and Surfrider Beach sand (10^4–10^5 MPN/100 g).

In the tightly held fraction (Fig. 6), *E. coli* exhibited growth similar to that observed in the rinsed sediment fraction, reaching 10^7–10^8 MPN/100 g in all microcosms containing sediment. *E. faecalis*, on the other hand, again grew preferentially in Mother's and Santa Monica Beach sediment and quickly leveled off between 10^4 and 10^5 MPN/100 g in washed sand and Surfrider Beach sediment.

Significant persistence and/or growth of indicator organisms in overlying water were observed in the presence of sediment in all microcosms. In our experiments, *E. coli* flourished similarly in the presence of all sediment types, including the commercially available sand. This suggests that the particular characteristics of the surface may be less important. Surfaces facilitate the formation of biofilms, which can result in favorable growth conditions due in part to increasing accessibility of nutrients. Growth in sediment microcosms has been observed in inoculated sediment without active predators present (either after autoclaving or with predators inhibited) (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982; Davies et al., 1995; Desmarais et al., 2002), while in the presence of predators, persistence rather than growth in sediment microcosms has been observed (Burton et al., 1987; Davies et al., 1995; Craig et al., 2004).

Enterococci, on the other hand, grew differently in the presence of the various types of sediment; growth was much more dramatic in the presence of sediment from Mother's Beach and Santa Monica Beach. This may be explained by the somewhat higher levels of organic content measured at these two beaches (1.1 and 1.0% at Mother's and Santa Monica Beaches, respectively, compared with 0.7% at Surfrider Beach). Particle size distribution tests showed Surfrider and Santa Monica Beach sediment both consisted of 98% sand (>0.075 mm), and 2% silt (between 0.02 and 0.075 mm). While large differences between the beaches were not observed, Mother's Beach sediment was 97% sand, with somewhat higher levels of silt (2.5%), and clay (< 0.0015 mm, 0.5%).

To test the hypothesis that sediment organic content is an important determinant of FIB survival in overlying water, we evaluated the growth of *E. coli* in microcosms containing Santa Monica Beach and Mother's Beach sediments in the presence and absence of the natural organic matter. One day after inoculation, levels of *E. coli* in the duplicate microcosms were measured in overlying water and in the sediment. In all four microcosms that contained sediment with its original organic content, levels of *E. coli* in both matrices reached the

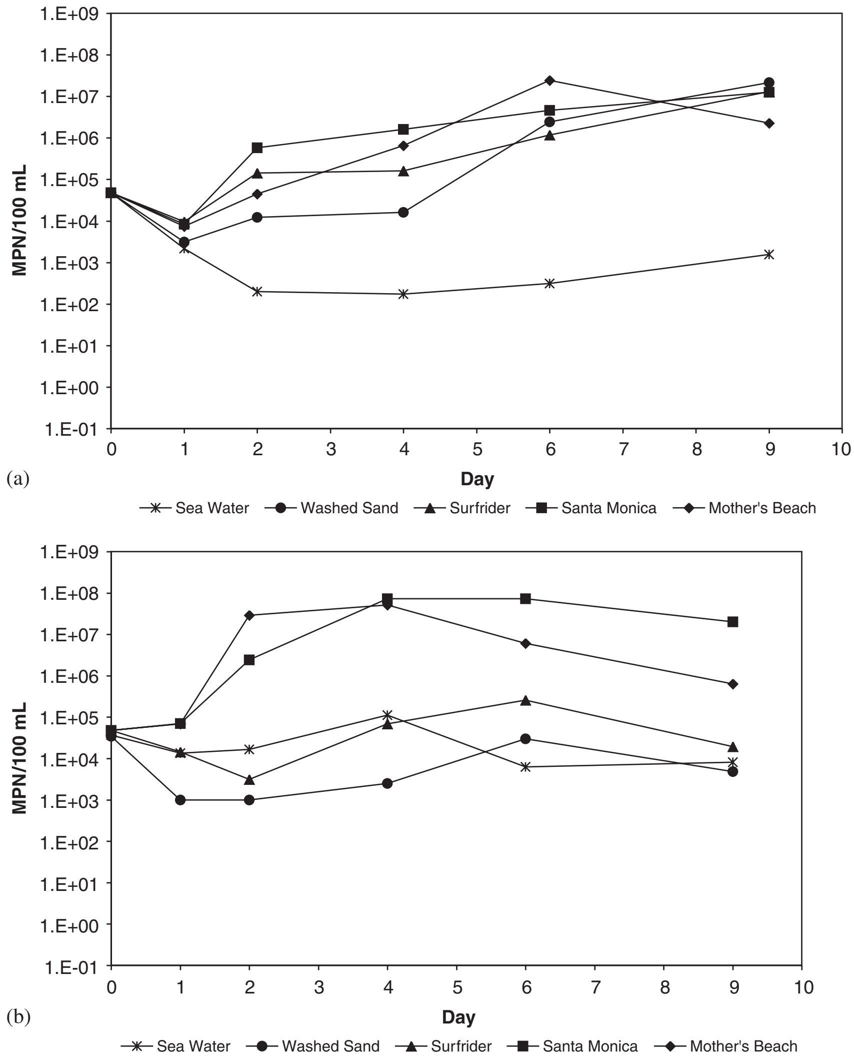


Fig. 4 – Concentrations of (a) *E. coli* and (b) *E. faecalis* in microcosm water overlying beach sediment from the three beaches.

maximum values for the analysis (2.4×10^4 MPN/100 mL in water and 1.5×10^5 MPN/100 g wet sediment). In contrast, the treated microcosms without sediment organic content had *E. coli* levels below the detection limit in both matrices (10 MPN/100 mL in water and 64 MPN/100 g wet sediment). These results show that the survival of FIB in water in the presence of sediment at Santa Monica and Mother's Beaches appears to depend on the organic content in the sediment. Other studies have demonstrated that the relative amount of organic matter in sediment can result in different degrees of survival (LaLiberte and Grimes, 1982; Burton et al., 1987; Craig et al., 2002, 2004; Desmarais et al., 2002).

3.3. *Beach survey*

In an effort to determine if the phenomenon of elevated FIB levels observed at Mother's Beach could apply to other enclosed beaches, we surveyed water and sediment from 13 beaches in southern California on one summer morning. Levels of enterococci at the two enclosed beaches were two to three orders of magnitude higher than all of the values observed at the eleven open beaches, which supports the notion that the physical environment at enclosed beaches is conducive to high FIB levels in sediments. For *E. coli*, levels at the two enclosed beaches were among the highest measured, although the relationship was less clear (Fig. 7).

The dilution of pollutants and transport of sediments at the beach shoreline due to the effect of high energy and wave-driven currents has been extensively documented (Boehm, 2003; Ahn et al., 2005; Boehm et al., 2005; Grant et al., 2005). High-energy waves, such as those at our open beach sites in Santa Monica Bay, increase resuspension and outward transport of sediments and microbial contaminants while the low-energy waves at Mother's Beach will not have nearly as significant a dilution factor in the surf zone. Also, the more stationary environment at the enclosed beaches may promote bacteria sorption and biofilm formation on sediment surfaces.

3.4. *Implications*

Because health standards for beach sediments have not been developed, it is difficult to evaluate whether the FIB levels

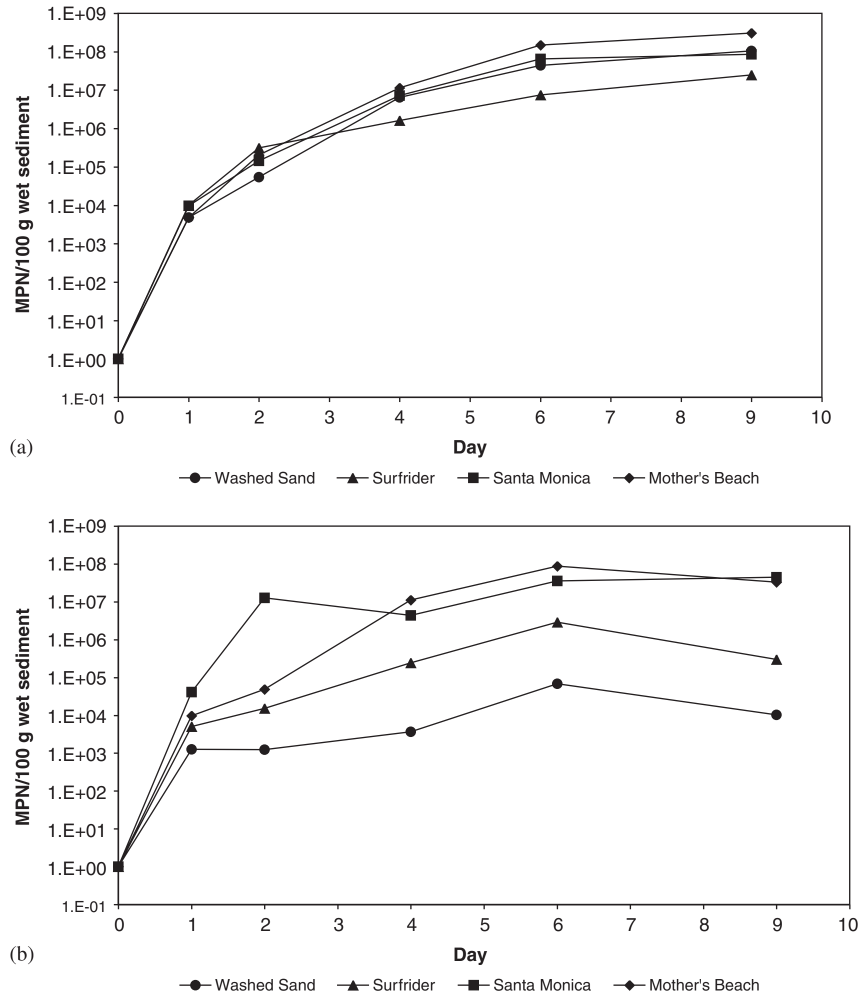


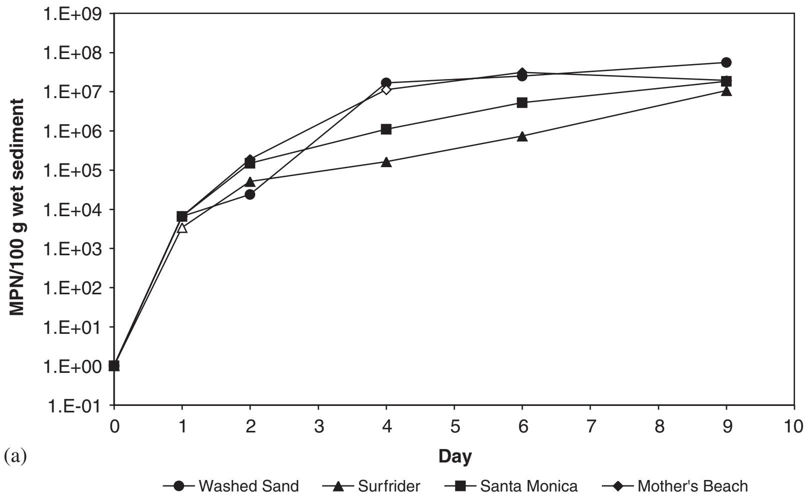
Fig. 5 – Loosely bound sediment concentrations of (a) *E. coli* and (b) *E. faecalis* in microcosms containing sediment from the three beaches. Bacteria were extracted by manually inverting sediment with water. Two sequential washes were pooled and analyzed in triplicate using IDEXX.

measured in sediments at the field sites pose a health risk. One approach is to consider a typical resuspension of 100 mg/L and determine whether the bacteria originating in that amount of sediment exceed health standards. The water quality health standards for *E. coli* and enterococci are 400 and 104 MPN/100 mL, respectively. Resuspension of 100 mg/L of sediment with levels in excess of 4×10^6 MPN *E. coli*/100 g and 1×10^6 MPN enterococci/100 g would lead to exceedances.

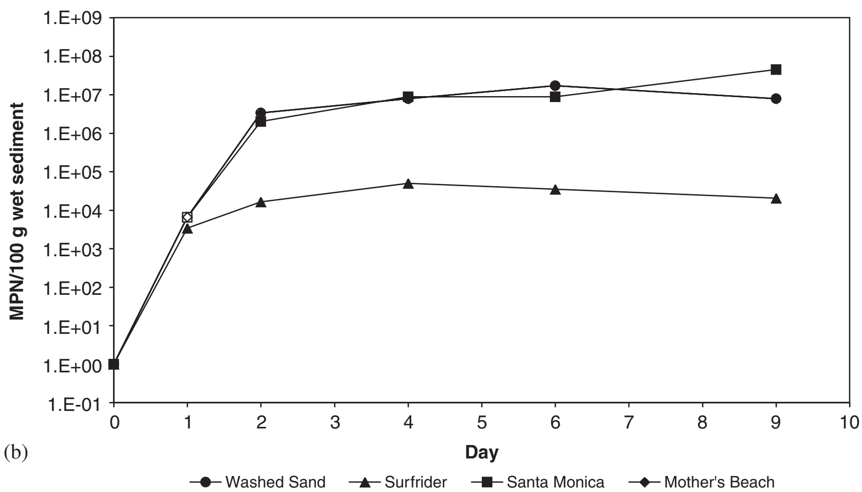
While FIB levels in water at Surfrider and Santa Monica Beaches exceeded health standards during the storm, sediment levels never rose above levels that would lead to exceedances directly by resuspension of 100 mg/L. On several days at Mother's Beach, sediment levels for *E. coli* and enterococci were above the levels that could result in overlying water concentrations above the health standard. This analysis does not consider resuspension of nutrients that may promote FIB growth.

Of course, this analysis does not account for children, who have more direct exposure to sediments. While studies to correlate levels of FIB in sediments with human illness have not been conducted, it may be useful to consider how exposures based on ingestion of sediment would compare with ingestion of water. For example, ingestion of 1 g of sediment containing 10^4 MPN/100 g enterococci (as seen at both enclosed beaches) would result in a comparable exposure to ingesting 100 mL of water at the health limit. It is important, however, to bear in mind that FIB may behave differently from their pathogen counterparts in sediments, calling into question the suitability of using these organisms as pathogen indicators.

Indeed, growth of indicator bacteria in the environment weakens the connection between FIB and pathogens and may lead to overestimation of risk. Notably, Boehm and Weisberg (2005) observed a strong correlation between water quality exceedances and the spring tide at many southern California beaches. One hypothesis proposed to explain this and other observations of tidal influence (Grant et al., 2001; Boehm et al., 2002a, b, 2004; Pednekar et al., 2005) is that FIB regrowth and remobilization of FIB from sand may result in erroneous water quality postings.



(a)



(b)

Fig. 6 – Tightly bound sediment concentrations of (a) *E. coli* and (b) *E. faecalis* in microcosms containing sediment from the three beaches. Bacteria were extracted by vigorous hand shaking and 10 min sonication in water. Two sequential washes were pooled and analyzed in triplicate using IDEXX.

4. Conclusions

The conclusions of this study are as follows:

- At the two beaches exposed to the ocean, FIB levels in sediment peaked along with, but did not persist significantly longer than, water column FIB concentrations during a storm. High levels of FIB were documented in the water sediments of the enclosed beach throughout the period of study.
- Laboratory microcosm studies showed that Santa Monica Bay beach sediments can provide a favorable environment for FIB survival and growth. This suggests that sediments may provide the same favorable environment for the pathogens for which FIB are meant to proxy. On the other hand, it may also indicate that FIB are not appropriate indicators of pathogens due to regrowth that may occur in sediments.
- The microcosm results, combined with results of a 13-beach survey showing elevated sediment FIB levels at enclosed beaches, indicate that the effect of physical layout may be more important than that of solid-phase characteristics on FIB persistence in sediments.

This study points to the importance of further research characterizing FIB as well as pathogen survival in sediments, particularly at enclosed recreational beaches.

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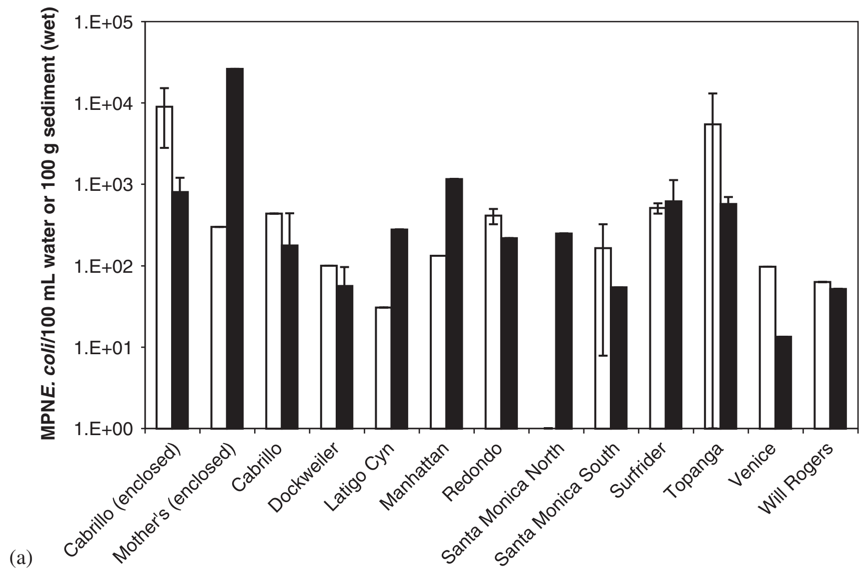

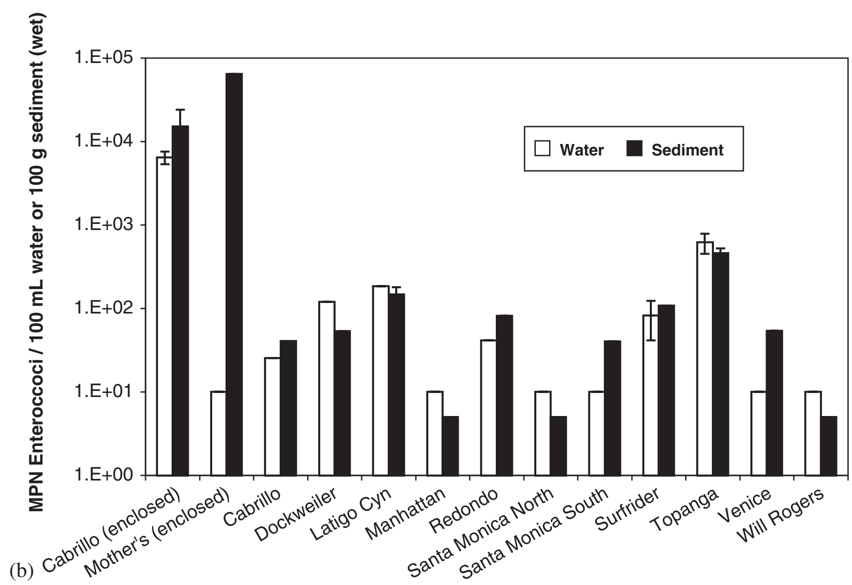

Fig. 7 – *E. coli* (a) and enterococci (b) in water and sediment at two enclosed (Cabrillo and Mother's) beaches and eleven open beaches in Santa Monica Bay on 22 July 2005. Sediment samples were collected in triplicate from each beach between 7 and 8 AM, and extracted two times in series by hand shaking in phosphate-buffered saline. Error bars indicate the standard deviation of triplicates, and columns without error bars indicate the average of two analyses.

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