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Distribution of genetic markers of fecal pollution on a freshwater sandy shoreline in proximity to wastewater effluent

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Abstract

Water, sand, and sediment from a Lake Superior harbor site continuously receiving wastewater effluent was sampled monthly for June to October 2010 and from May to September 2011. Understanding the dynamics of genetic markers of fecal bacteria in these matrices is essential to accurately characterizing health risks. Genetic markers for enterococci, total *Bacteroides*, and human-associated *Bacteroides* were measured in site-water, sand, and sediment and in final effluent by quantitative PCR. The similarity between the quantity of molecular markers in the water column and effluent indicated that the abundance of genetic markers in the water column was likely controlled by effluent inputs. Effluent turbidity was positively correlated ($p = 0.05$) with AllBac and HF183 in final effluent and AllBac in the water column. In sand and sediment, Enterol and AllBac were most abundant in the upper 1–3 cm depths, whereas HF183 was most abundant in the upper 1 cm of sand and at 7 cm in sediment. The AllBac and Enterol markers were 1- and 2-orders of magnitude more abundant in sand and sediment relative to the water column per unit mass. These results indicate that sand and sediment may act as reservoirs for genetic markers of fecal pollution at some freshwater sites.

Introduction

The degree of fecal contamination in surface waters is currently inferred by the presence and abundance of fecal indicator bacteria (FIB), mainly *E. coli* and enterococci. Fecal indicators can be enumerated by culture-based methods or molecular techniques that target and quantify genus, species, or strain-associated DNA sequences. Molecular methods such as quantitative PCR (qPCR) yield results in several hours, as opposed to 24 to 48 h for culture-based methods. In addition, the development of animal host-associated primers allows for identification of potential sources of fecal pollution^{1,2}. Consequently, there has been a shift from the use of culture-based methods to qPCR tools for water quality monitoring. The use of qPCR for bacteria within the genus *Enterococcus* is a promising method for identifying fecal contamination, as the abundance of the enterococci marker has been correlated to gastroenteritis disease risk in marine and freshwaters^{3,4}. Similar to this method, qPCR for

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SUPPORTING INFORMATION Further details on the methods and results, are included in Tables S1–S6 and Figures S1 and S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

bacteria within the genus *Bacteroides* indicates the presence of fecal contamination, as it is abundant in fecal matter of both humans and animals^{5,6}. *Bacteroides* sp. strains are obligate anaerobes, so their presence is thought to indicate recent contamination. At ambient temperatures (15–20°C), *Bacteroides* persists for approximately one week^{7,8}; however, in sterile environments, it can persist for up to 200 days⁹. Additionally, *Bacteroides* markers have been developed to target source-associated strains, such as those that identify contamination from human sources¹⁰.

The concentration of FIB in the water column can be influenced by sand and sediment bacterial concentrations in lake, river, and ocean environments^{11–13}. Bacteria from the water column can be deposited in sand from wave action or settle-out from the water column to the sediment¹⁴. As cultivable *E. coli* and enterococci are more abundant in shallow sand and sediment, resuspension may lead to water quality exceedances^{14–16}. Sand and sediments also offer protection from light, and may provide nutrients, potentially leading to growth of FIB^{17,18}.

The role of sand and sediment as a reservoir for genetic markers is not well understood. Molecular markers for enterococci and *Bacteroides* have been detected in sand and sediment, but their depth distribution has not been well-characterized. While the presence of *Bacteroides* genetic markers in freshwater streambed sediments and enterococci genetic markers in marine sand were found to vary with depth^{19,20}, their quantitative distribution in freshwater matrices has not been examined. In addition, the persistence of molecular markers in sand and sediment is not known. As qPCR can amplify DNA from live, dead, or dying cells, as well as free DNA^{21,22}, fecal contamination may potentially be detected long after it first occurs. Before there is widespread implementation of qPCR methods for monitoring water quality, the capacity of sand and sediment to serve as a reservoir of genetic markers of fecal pollution needs to be examined in more detail. Taken together, results from several studies suggest that there is a great potential for FIB to accumulate in sand and sediment. Consequently, the understanding of the dynamics of genetic markers and FIB in these matrices is essential to accurately characterizing the level of fecal contamination and associated health risks.

In the present study, culturable bacteria and molecular markers for enterococci, total *Bacteroides*, and human-associated *Bacteroides* were measured on a sandy shoreline site of Duluth-Superior harbor. In earlier studies done within this system, human fecal markers were not detected in sediment grab samples²³, or they were detected in less than 25% of sand and sediment 10 cm cores (Eichmiller, unpublished data). Therefore, the current study utilized a site with consistently high loading of markers via its proximity to an effluent outfall. The purpose of this study was four-fold. First, we determined how genetic markers of fecal matter are distributed in sand and sediment. Second, we examined the relationship between water column loading and sand and sediment genetic marker abundance. Our third goal was to examine the relationship between genetic marker concentration in untreated sewage, final effluent, and the site water column. Our final goal was to examine the correlations, if any, among culturable and molecular markers in final effluent, the water column, sand, and in sediment.

Materials and Methods

Study site and sample collection

The study site was located on a non-recreational shoreline site of Duluth-Superior Harbor in Duluth, MN (47°13'37"N, 91°54'2"W) on the property of Western Lake Superior Sanitary District (WLSSD) (Fig. 1). The sampling site is located approximately 100 m from an outflow pipe that discharges final effluent below the water's surface (Fig. 1). A 10–20 cm

berm was located approximately 30 cm from the shoreline, beyond which was heavily vegetated by grass.

Samples were collected monthly from June to October in 2010 and from May to September in 2011. The WLSSD final effluent received high doses of sodium hypochlorite periodically over the study period (J. Mayasich, personal communication; See Supplementary Information (SI) Table S1). At each sampling time, three replicate water, sand core, and sediment core samples were taken (See supporting information (SI) for further details). Three replicate final effluent samples were taken from the effluent sampling station at WLSSD. Three times over the 2 year study period (June and August 2010 and June 2011) three replicate samples of untreated sewage (i.e. raw influent) were taken with the assistance of WLSSD staff.

Sample processing and analyses

Cores were carefully removed from core tubes and divided into 1 cm slices. The top cm, 3, 5, 7, and 9 cm core depths were used for analyses in 2010, the 11 cm depth was also analyzed in 2011. For each depth fraction, percent moisture content was determined and total DNA was extracted for qPCR analysis (See SI). In 2011, culturable *E. coli* and enterococci were enumerated by standard methods (See SI). On October 8, 2012, triplicate and sediment cores were analyzed for total organic carbon, texture, oxidation-reduction potential, and oxygen content (See SI). Effluent flow and turbidity data were accessed online at <http://www.lakesuperiorstreams.org/streams/stream_data.html> through the data visualization tool for WLSSD on October 10, 2011.

Genetic markers for total enterococci (Enterol), total *Bacteroides* spp. (AllBac) and human-associated *Bacteroides* (HF183) were enumerated by quantitative PCR (qPCR) (Tables S2 and S3). For detailed qPCR methods, see SI.

Statistical analyses

The concentrations of indicators and markers were log-transformed for statistical analyses in order to approximate a normal distribution. Samples below the detection limit were assigned a value of one-half the limit of detection. Comparisons between colony forming units (CFU) and cell equivalents (CE) was done by assuming four copies of the Enterol marker per *E. faecalis* genome²⁴. A paired t-test was used to determine differences between water column and effluent indicator concentrations over the entire sampling period. Pearson product-moment correlation was used to determine significant relationships between indicators and markers in water, sand, and sediment and correlation between effluent characteristics and water and effluent molecular marker abundance. The relationship between depth of sand or sediment samples and concentration of indicators and markers was examined using simple linear regression analyses. Linear regression was also used to examine decreases in culturable indicators in sand and sediment in 2011. A two-way ANOVA was used to determine the effect of treatment month, genetic marker, and month by marker interaction on genetic marker reduction from sewage treatment. This analysis was also used to determine differences in indicator abundance between water column and effluent by sampling month, and determine the effects of sample type and analysis method on enterococci abundance in 2011. Post-hoc comparisons were conducted using Tukey HSD, at $\alpha = 0.05$. Statistical analyses were done using JMP®Pro version 9.0.2 (SAS Institute Inc., Cary, NC). Statistical significance was set at $\alpha = 0.05$ for all analyses.

RESULTS AND DISCUSSION

Indicators in sewage influent and final effluent

In untreated sewage influent, Enterol, AllBac, and HF183 genetic markers were found at average concentrations of 7.6 ± 0.7 (mean $\pm$ standard deviation), 8.8 ± 0.6 , and 7.0 ± 0.4 $\log_{10}$ target gene copies/100 mL, respectively. Final effluent contained Enterol, AllBac, and HF183 at average concentrations of 5.8 ± 0.4 , 6.4 ± 0.5 , 4.4 ± 0.6 $\log_{10}$ copies/100 mL, respectively. Within effluent, genetic markers were all positively cross-correlated, with similar R^2 (coefficient of determination) values (Table 1). Treatment of sewage resulted in a 2.5 ± 0.7 $\log_{10}$ average reduction in gene copy number, which is within the range observed in previous studies that reported reductions of 2.5 to 3.5 $\log_{10}$ ^{25–27}. Genetic marker reduction varied with sampling date and marker type (Table S4). The reduction in copy number was similar for HF183 and AllBac. In contrast, the reduction of Enterol was less dramatic on all three dates, with an average reduction that was 0.89 $\log_{10}$ copies/100 mL less than AllBac and HF183 ($p = 0.05$). Similarly, Srinivasan et al.²⁶ found that enterococci generic markers had a 0.91 $\log_{10}$ lower total reduction in markers throughout the sewage treatment process than did a human-associated *Bacteroides* marker.

Chlorination of the effluent affected the reduction of genetic markers. Reduction followed the trend: June 2011 > August 2010 > June 2010, with greater reduction on days with higher chlorination treatment (See SI). For Enterol and AllBac, the reduction in copies of molecular markers was distinct on all three dates ($p = 0.05$), whereas for HF183, the reduction in markers was lower in June 2010 ($p = 0.05$), with similar magnitudes of reduction in June 2011 and August 2010.

The concentration of culturable enterococci and *E. coli* in untreated sewage and final effluent was measured in June 2011. Treatment of sewage resulted in a 5.8 $\log_{10}$ and 4.7 $\log_{10}$ reduction in cultured enterococci and *E. coli*, respectively. The U.S. EPA and Minnesota state freshwater water quality standards specify that the geometric mean of culturable *Enterococcus* and *E. coli* spp. should not exceed 33 CFU/100 mL or 126 CFU/100 mL, respectively, and no single sample should exceed 235 CFU/100 mL for *E. coli* and 61 CFU/100 mL for *Enterococcus* sp.^{28,29} For final effluent in 2011, the geometric mean ($n = 5$) of cultured enterococci concentrations was 12 CFU/100 mL, 95% CI [1.2, 137], which is below the water quality standard. The geometric mean *E. coli* concentration ($n = 5$) in final effluent was 272 CFU/100 mL, 95% CI [52, 1427], which was above the standard. The single-sample water quality standard was exceeded two times for cultured enterococci and in three instances for cultured *E. coli*. It should be noted that WLLSD was operating its disinfection system as per the requirements of a variance from the MN secondary treatment standard for fecal coliform bacteria during the time frame of this study. That variance expired on February 29, 2012.

Indicators in the site water column and relationship to final effluent

Enterol, AllBac, and HF183 were detected in the water column at all sampling time points. On average, the AllBac marker was most abundant at 6.3 ± 0.5 (mean $\pm$ standard deviation) $\log_{10}$ copies/100 mL. The concentration of Enterol and HF183 was 5.4 ± 0.4 and 4.1 ± 0.8 $\log_{10}$ copies/100 mL, respectively. In the water column, Enterol and AllBac were both positively correlated to HF183, but Enterol and AllBac were not significantly correlated ($p > 0.05$) (Table 1). The geometric mean ($n = 5$) concentration of culturable enterococci and *E. coli* in the water column, in 2011 was 62 CFU/100 mL, 95% CI [32, 121] and 368 CFU/100 mL, 95% CI [187, 722], respectively. The geometric means of culturable indicators from the water column at the site were higher than those from the final effluent, possibly due to additional inputs from waterfowl that frequent the Duluth-Superior Harbor¹² and this site.

Additionally, some bacteria, such as *E. coli*, can become naturalized to and grow within secondary environments such as sands or soils¹². The single-sample enterococci water quality standard was exceeded twice, and the three water quality exceedances were observed for *E. coli*. The study site is not a recreational beach; therefore, it is not routinely monitored, and no advisories were issued over the course of the study period.

The concentration of Enterol and HF183 was slightly lower in the water column compared to final effluent (paired t-test, Enterol, $p = 0.006$; HF183, $p = 0.03$), but there was no difference between AllBac markers, culturable enterococci, or *E. coli* in the water column and final effluent (paired t-test, $p > 0.05$). Few differences between final effluent and site water column indicator concentrations were observed on individual sampling dates (Figs. 2 and S1). The abundance of HF183 in the water column and in the final effluent were positively correlated ($R^2 = 0.77$, $p = 0.0007$), whereas the abundances of Enterol and AllBac were not (both $p > 0.05$).

The effect of effluent turbidity, flow rate, and chlorination on genetic marker concentration in effluent and the water column was examined through linear regression. Effluent turbidity was positively correlated to effluent AllBac copy numbers ($R^2 = 0.80$, $p = 0.0005$) and HF183 ($R^2 = 0.49$, $p = 0.02$). AllBac concentration and effluent turbidity were also positively correlated in the water column ($R^2 = 0.40$, $p = 0.05$). The association of turbidity and bacterial genetic markers is not surprising as bacteria are often associated with suspended particles in environmental waters. High turbidity of wastewater effluent can result from a variety of reasons. However, previous research has shown that *Salmonella* abundance in final effluent was positively correlated with turbidity across four wastewater treatment plants of varying efficiencies³⁰. The effluent flow rate and sodium hypochlorite treatment level in the wastewater treatment plant was not significantly correlated to copy numbers of genetic markers ($p > 0.05$). There was no relationship between effluent flow and effluent turbidity, possibly due an observed lag time, of up to 10 h, between peaks in effluent flow and peaks in turbidity. Since some measurements were only taken in 2011, there were no significant relationships among culturable indicators, between culturable indicators and markers, or between culturable indicators and effluent turbidity or flow in water column samples.

Taken together, it appeared that effluent input largely controlled FIB cultivable and molecular marker concentrations in the water column at the study site. In this region, geese can also constitute a large potential source of FIB¹². In Duluth-Superior Harbor, however, the discharge of final effluent has been shown to result in a spike total *Bacteroides* and human-associated *Bacteroides* genetic markers within the water column near the two effluent outfalls that are within the harbor, the WLSSD and the City of Superior (WI) Wastewater Treatment Facility (SWTF)²³.

Distribution of indicators in sand and sediment

On average, there were no significant differences between molecular marker copy numbers in sand and sediment. Sand and sediment concentrations were 5.6 ± 0.2 and $5.3 \pm 0.6 \log_{10}$ copies/g for Enterol, 5.5 ± 0.2 and $5.4 \pm 0.8 \log_{10}$ copies/g for AllBac, and $2.1 \pm 0.1 \log_{10}$ copies/g for HF183 in both sand and sediment. Copy number of the HF183 marker fell below the limit of detection (30 copies/g) in 19 and 17% of sand and sediment samples, respectively. The geometric mean ($n = 30$, 5 sampling dates by 6 depths) concentration of enterococci in 2011 was 3.9 CFU/g, 95% CI [0.8, 19] in sand and 1.7 CFU/g, 95% CI [0.8, 3.8] in sediment. The geometric mean ($n = 30$) concentration of *E. coli* in 2011 was 4.0 CFU/g, 95% CI [1.2, 13] in sand and 3.9 CFU/g, 95% CI [2.5, 6.1] in sediment.

Copy numbers of the HF183 marker were 1 to 2 log₁₀ less abundant than Enterol and AllBac in the water column, whereas HF183 was 3.4 log₁₀ less abundant than the Enterol and AllBac markers in sand and sediment on a per mass basis. It may be possible that HF183 decays in the water column before it can be deposited in sand and sediment, or HF183 decays more rapidly than Enterol and AllBac following deposition. However, HF183 marker was detected in every core sample, so at sites with consistent human fecal inputs HF183 is likely to occur.

The high loading of genetic markers into the water column near the study site likely led to high abundances of these molecular indicators in sand and sediment. Proximity to a wastewater outfall was found to affect the lateral distribution of culturable *E. coli* and enterococci in sediments of a freshwater lake¹⁶ and *Clostridium perfringens* in ocean sediments near a research station in Antarctica³¹. Sand and sediment contained approximately 4.9 log₁₀ enterococci CE/g. There are no comparable studies of Enterol in freshwater sand, but the concentration of enterococci CE in sand and sediment at the study site is well above the range of 2.2 to 3.2 log₁₀ CE/g observed in marine beach sand^{18,32}. Few studies have quantified *Bacteroides* genetic markers in sand and sediment. However, a previous study found that the concentration of total *Bacteroides* in sediment in the Duluth-Superior Harbor was elevated near a wastewater outfall, but human-associated *Bacteroides* was not detected in sediment²⁸.

All genetic markers were most abundant in the upper 1 to 3 cm of sand (Fig. 3). Culturable enterococci and *E. coli* were also most abundant in the upper cms of sand and sediment (Fig. S2). In sediment, Enterol and AllBac were abundant in the upper cm, similar to the distribution in sand. HF183, however, was most abundant in the 5 and 7 cm portions of sediment. Enterol and AllBac concentrations in sand and sediment were negatively correlated with depth (both $p < 0.05$). Genetic markers decreased less with increasing depth in sand (regression slope, Enterol = -0.06; AllBac = -0.08) compared to sediment (regression slope, Enterol = -0.14; AllBac = -0.20). Although HF183 also decreased with depth in sand, the trend was not significant ($p = 0.23$). In sediment, % TOC decreased more dramatically with depth compared to sand (Table S5). Higher organic matter may facilitate the persistence of culturable *E. coli* and *Enterococcus* in freshwater sediments³³. Therefore, the distribution may be partially due to slower decay at lower depths in sand.

Previous studies have reported that *E. coli* and enterococci are most abundant in the upper layers of sediment in freshwater streams, rivers, lakes, and estuarine bays^{16,34,35}. In freshwater sand, however, *E. coli* was most abundant from 0 to 5 cm, and enterococci were most abundant from 5 to 10 cm depth³⁶. There was no decrease in the detection frequency of total *Bacteroides* and a source-associated genetic marker for humans with sediment depth in a freshwater stream in a presence/absence study, but human genetic markers were most frequently detected from 10 to 20 cm depth¹⁹. In this current study, the HF183 marker was found near the detection limit at 11 cm depth in sand and sediment. The decrease in HF183 with depth in sand, the peak in HF183 at 7 cm depth in sediment, and subsequent decrease in sediment with depth, makes it unlikely that this marker is most abundant from 10 to 20 cm depth at this site.

Resuspension of shallow sediments high in FIB can lead to spikes in water column concentrations¹⁴. Resuspension of sand and sediment at the study site could occur through surface runoff, a dramatic increase in the flow of the St. Louis River, or wave action from strong winds. However, wave height at the study site was never observed to exceed 5 cm, possibly, in part, due to the sheltered environment of the site. Nevertheless, resuspension of less than 0.5 cm depth of sediment can lead to water quality exceedances if sediment concentrations are high³⁴. Thus, factors that may lead to resuspension of sand or sediment

must be taken into consideration when using genetic markers for water quality monitoring or microbial source-tracking.

Correlations among indicators in sand and sediment

There were significant cross-correlations between culturable and molecular indicators in sand and sediment (Table 1). In all instances, indicator correlations in sand explained more variance compared to sediment. There was a strong relationship between AllBac and Enterol in both sand and sediment, but the comparison of AllBac and Enterol to HF183 explained little variance in sand and was insignificant in sediment. Similarly, culturable enterococci and *E. coli* were strongly correlated in both sand and sediment. Culturable FIB were either not significantly related to or did not explain much variance in HF183 abundance in sand and sediment. The abundance of Enterol, AllBac, and HF183 between sand and sediment were positively correlated ($R^2 = 0.24, 0.39, 0.27$, respectively, all $p < 0.05$). In addition, culturable FIB were positively correlated between sand and sediment ($R^2 = 0.77$ and 0.53 , respectively, $p < 0.05$).

The relationships between indicator abundances in water column and sand or sediment were dependent on the indicator and depth. The concentration of AllBac in sediment was not correlated with water column concentrations at any depth in sand or sediment ($p > 0.05$). While the concentration of HF183 in the water column and sand were not correlated, in sediment they were positively correlated at 1 cm ($R^2 = 0.48$) and 7 cm depth ($R^2 = 0.40$) (both $p < 0.05$). Surprisingly, Enterol abundance in the water column had a negative relationship with sand at 3, 5, 7, and 9 cm depth ($R^2 = 0.53, 0.43, 0.39$, and 0.44 , respectively; all $p < 0.05$) and to sediment depth at 1 cm ($R^2 = 0.41$). It is unclear why higher effluent concentration would lead to lower sand and sediment concentrations of the Enterol indicator. One possibility is that the abundance of Enterol may be more affected by shifts in environmental conditions or habitats than other markers, as evidenced by the lack of correlation between effluent and water column copy numbers and between water column and sand and sediment copy numbers. Concentrations of culturable FIB in the water column were not correlated to sand or sediment concentrations at any depth ($p > 0.05$).

Comparison of CE and CFU

Assuming four copies of the Enterol marker per *E. faecalis* genome²⁴, comparisons were made, in 2011, between the number of enterococci as measured by qPCR and culture methods (Fig. 4). There was a significant interaction between sample type and analysis method on enterococci abundance ($p = 0.04$). Enterococci CE were more abundant in sand and sediment than in water and effluent, whereas the concentration of culturable enterococci in sand and sediment relative to liquid matrices was similar. Overall, enterococci CE were 2.9 to 4.8 $\log_{10}$ greater than enterococci CFU on a per mass basis. The magnitude of the difference in CE and CFU of enterococci followed the trend: sand > sediment > effluent > sewage > water column. This observation may be due to the ability of extracellular DNA to persist for greater periods in marine and freshwater sediments, resulting, in part, from adsorption to particles that can protect DNA from degradative enzymes³⁷.

In accordance with other studies, enterococci abundance determined by qPCR and culture methods were positively correlated at the study site (Fig. 1)^{24,38}. Whitman et al.³⁸ found that, on average, CE and CFU approximated a 1:1 ratio; however, the concentration of enterococci CE by qPCR at the study site reported here was 3 to 5 $\log_{10}$ greater than enterococci CFU for all sample types. This is to some degree a result of the ratio of cells and free DNA in wastewater effluent, but other specific characteristics of the study site should not be discounted. There may also be differences between the persistence of enterococci genetic markers and culturable cells. Walters et al.³⁹ found that naked enterococci genomes

decayed slightly slower than intact cells, but the difference was not significant. Regardless, our results suggest that the use of qPCR for enterococci quantification in place of culture methods at this site may not be useful.

There are several techniques that can be used to target live cells and reduce or reveal the contribution from extracellular DNA. The use of DNA binding dyes, such as propidium monoazide (PMA), prior to qPCR analysis may partially resolve the discrepancy between enterococci CE and CFU at some sites²⁵. However, while chlorination treatment of effluent differentially reduces culturable and molecular indicators^{26,40}, PMA treatment of chlorinated effluent has been reported to not decrease the number of amplifiable copies of genetic markers⁴⁰. It is unknown whether this phenomenon is due to the presence of viable but not culturable cells or a change in cell permeability from chlorination treatment. Reverse transcription-quantitative PCR (RT-qPCR) can also be used to preferentially target rRNA from live bacterial cells^{41,42}. Molecular techniques are rapid and accurate; however, they can be extremely difficult to optimize⁴³. Alternatively, live/dead staining has been used in conjunction with genus-specific fluorescent *in situ* hybridization (FISH) probes to target *Bacteroides* spp. in human feces⁴⁴.

The distribution of molecular markers at a freshwater shoreline site that is continuously exposed to wastewater outflows may differ from unexposed freshwater beaches. The sheer number of bacteria that are introduced into the system on a daily basis favors selection for persistent strains. Evidence suggests that some enterococci may become naturalized to aquatic habitats, especially aquatic vegetation where they are orders of magnitude more abundant than in the water column⁴⁵. In addition, the high concentration of markers in the water column, coupled with the sheltered environment of the study site, may lead to higher settling rates than would be prevalent at beaches with more wave action. Nevertheless, these results clearly highlight the potential for sand and sediment to harbor high copy numbers of fecal genetic markers, especially for total *Bacteroides* and enterococci.

Fecal pollution of surface waters is a public health concern worldwide. Demand for fast and accurate methods for water quality monitoring will lead towards a shift from the use of culture-based methods to quantification of molecular markers of fecal pollution. Recent studies have focused on the dynamics of molecular indicators of fecal pollution in the water column, even though sand and sediment can contain a greater proportion of fecal indicators within the beach environment^{14,34}. In addition, exposure to beach sand is associated with risk of gastroenteritis, and illness is associated with increased abundance of genetic markers for enterococci and *Bacteroides*^{31,46}. Results from this study show that sand and sediment can act as a reservoir of molecular indicators of fecal bacteria. The use of genetic markers of fecal bacteria for beach monitoring and assessment should take into account the role of sand and sediment before management decisions are made.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Study site. (A) Inset shows the location of Duluth-Superior Harbor in Lake Superior. Arrows indicate wastewater treatment plant outflow locations for Western Lake Superior Sanitary District (WLSSD) and Superior Wastewater Treatment Facility (SWTF). (B) Detail of shoreline near WLSSD, and location of the sampling site relative to the final effluent outflow pipe.

Figure 2.

The concentration of Entero1 (A), AllBac (B), and HF183 (C) molecular markers of fecal contamination in final effluent (solid bars) and the site water column (open bars) in 2010 and 2011. Asterisks indicate significant differences between the concentration of markers on a sampling date based on Tukey's HSD ($\alpha=0.05$).

Figure 3.

Box plots of Enterol (A, D), AllBac (B, E), and HF183 (C, D) in sand (A, B, C) and sediment (D, E, F) across sampling dates from 2010 to 2011. The left boundary of the box indicates the lower quartile of the data, the right boundary indicates the upper quartile, and the line within the box indicates the median. Whiskers show the 90th and 10th percentiles. The dotted line indicates the limit of detection for the HF183 assay.

Figure 4. Enterococci colony forming units (light bar) and cell equivalents (dark bar) in 2011 samples. Sewage values are from June 2011. Error bars indicate the 95% confident interval of the mean. Bars with the same letter are not significantly different based on Tukey's HSD at $\alpha = 0.05$. Sewage was excluded from the analysis.

Table 1Adjusted R² values among indicators within samples types.

Variable	Water column		Effluent		Sand				Sediment			
	Entero1	AllBac	Entero1	AllBac	Entero1	AllBac	HF183	<i>E. coli</i>	Entero1	AllBac	HF183	<i>E. coli</i>
AllBac	0.31	–	0.46	–	0.85*	–	–	–	0.68*	–	–	–
HF183	0.62	0.46	0.41	0.50	0.13	0.17*	–	–	0.00	0.04	–	–
<i>E. coli</i>					0.65*	0.71*	0.02	–	0.19	0.37*	0.09	–
Enterococci					0.49*	0.47*	0.00	0.89*	0.13	0.24	0.20	0.59*

^a All correlations are positive* Bold, $p < 0.05$; Bold and italics, $p < 0.01$; Bold, italics, and asterisk, $p < 0.001$.