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Research papers Observations of transport of bacterial-like microspheres through beach sand



CONTINENTAL Shelf Research

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ABSTRACT

Often, there is an order of magnitude more fecal indicator bacteria (enterococci) in beach sand than in nearby water. Consequently, sand is considered a reservoir for these bacteria, potentially contributing to poor water quality, and raising questions regarding the human health risks associated with sand exposure. An integral aspect of the distribution and persistence of sand-associated enterococci is the transport of bacteria introduced into the beach environment. Here, plastic microspheres are used as a proxy to examine the wave-induced movement of bacterial-like particles through sand on an ocean beach. Laboratory tests suggest microspheres and bacteria move similarly through sand columns, and have qualitatively similar short-term adsorption-to-sand behavior. Microspheres buried ~0.05 m below the sand surface on an ocean beach moved rapidly $[O(10^{-3}) \text{ m/s}]$ away from their initial location, both vertically into the ground water below the sand and horizontally seaward within the sediment matrix in response to waves running up the beach face and percolating through the sand.

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1. Introduction

Marine beach swimming water quality is evaluated by using the concentration of the bacteria enterococci as a proxy for fecal contamination. Recently, non-fecal sources of enterococci have been documented in beach sand (Yamahara et al., 2007, Halliday and Gast, 2011) and decaying algal material (Whitman et al., 2003, Badgley et al., 2011, Imamura et al., 2011). Epidemiological studies have shown that enterococci concentration in swimming water is correlated with gastrointestinal illness in humans (Cabelli et al., 1982, Kay et al., 1994, Haile et al., 1999, Wade et al., 2003). The level of enterococci often is much higher in sands than in nearby waters (Table 2 in Halliday and Gast, 2011), and transport of bacteria harbored in sand can be a source of contamination to the water column.

The effects of current- and wave-induced transport on fecal indicator bacteria have been studied in fresh water at a beach in Lake Michigan (Ge et al., 2010, 2012). During the day, currents moved material offshore, and at night waves transported the material back to shore, increasing bacterial abundances. Model-data comparisons on a low-energy ocean beach suggest that enterococci levels also are sensitive to wave-induced sediment resuspension and tidal washing of intertidal beach sands (Feng et al., 2013).

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Through-beach transport driven by waves running up the beach face (swashes) and infiltrating into the sand and by submarine groundwater discharge also may be important mechanisms that move sand-associated enterococci to coastal waters (Russell et al., 2012). In particular, on the upper beach face, swashes can drive strong pressure gradients that result in flows through the beach that could transport bacteria (Turner and Masselink, 1998). Recent work indicates that indigenous enterococci were released from marine beach sand columns in response to fluid flowing through them, and that transport of enterococci through the sand likely was influenced by the movement of the liquid front and the result of air–water interface scouring (Phillips et al., 2011, Russell et al., 2012).

These and other laboratory-based studies of bacterial transport through idealized porous media identified additional factors that influence movement and retention, including media grain size and shape and the presence of organic material on the surface of the media (Camesano and Logan, 1998, Bradford et al., 2002, Salerno et al., 2006, Chen and Walker, 2007, Knappett et al., 2008, Weaver et al., 2013), as well as the biological factors of cell type, size, shape, surface charge, and motility (Simoni et al., 1998, 2000, Bolster et al., 2009, Johanson et al., 2011). However, information is limited regarding bacterial transport in the natural marine beach environment, where tidal cycles transiently saturate the media, and the ionic strength and composition of the fluids is variable (Russell et al., 2012). It is not known where bacteria introduced into beach sand go, nor how fast they move, where they stop, and if they reach the sub-marine ground water.

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To assess bacterial transport through a natural beach, a proxy that behaves similarly to enterococci in sand would be useful. Here, it is shown that the movement of microspheres is similar to the movement of enterococci through marine beach sand cores in the laboratory. In addition, the adsorption of microspheres is qualitatively similar to the adsorption of enterococci bacteria to sand particles in the laboratory. Subsequently, in a pilot field experiment microspheres were deployed in the sand near the high tide line on an ocean beach, and their movement into and through the beach sand was observed.

2. Materials and methods

Laboratory studies were used to examine the viability of using plastic microspheres as a proxy for microbes, and a field study was conducted to investigate the movement of microspheres through natural beach sands.

2.1. Laboratory sand column experiments

Qualitative comparisons of the movement of microspheres with the movement of bacteria through columns of undisturbed beach sand were performed in the laboratory. Carboxylated 1.0 μm microspheres with green fluorescence (Fluoresbrite; Polysciences) were diluted in 10 ml filtered seawater, and 10⁶–10⁹ total particles were placed on each sand column. The 1.0 µm size was selected because it is similar to that of enterococci $(0.5 - 1.0 \mu m)$. Overnight cultures of an environmental isolate of enterococci were labeled using the stain chloromethylfluorescein diacetate (CMFDA; Invitrogen) (First et al., 2012). This stain was chosen because the cells remain alive and their behavior should represent that of viable bacteria introduced into the environment. Briefly, CMFDA was added at a final concentration of 25 µM to 1 ml of liquid bacterial culture, and incubated for 3 hours in the dark at 42 °C. To remove extracellular stain, the culture was centrifuged at 10,000 g for 5 min, the supernatant was removed, and the culture was resuspended in 1 ml of $1 \times$ phosphate buffered saline (PBS). The culture was centrifuged and resuspended in 1 ml new $1 \times PBS$ 4 times. CMFDA-labeled enterococci were counted using a FACS-Caliber flow cytometer and diluted in 10 ml filtered seawater. The amount of bacteria used in different experiments ranged from 10^{6} – 10^{8} total cells. Labeled bacteria were prepared fresh for each experiment.

Undisturbed sand cores were collected during both summer and winter months from a local beach (Cape Cod, MA) prior to each experiment using clean plastic core tubes (0.05 m diameter, 0.20-0.25 m long). A total of 11 bacterial and 16 microsphere additions were performed on sand columns. To examine the variation between cores, duplicates were collected on the same day and run with the same type and amount of particle at least once in each season. Microspheres or bacteria were added to the surface of a column, followed by the addition of four 250 ml portions of seawater. The cap at the bottom of the column had a 0.03 m "X" cut into it to allow water to flow out. A 0.04 m square piece of 250 µm mesh Nitex membrane was placed between the sand at the bottom of the core and the cap to retain sand particles. To mimic the infiltration of seawater through the beach surface, the liquid was allowed to flow naturally through the sand and was collected from the bottom of the column after each addition. Bacteria or microspheres were detected and enumerated in replicate 2 ml aliquots of the water using flow cytometry. After the 4th addition of seawater, the sand was extruded and collected in 4 equal sections (0.03–0.05 m intervals). Each sand section was placed in a 500 ml flask with 250 ml of distilled water added, and agitated twice. Water was filtered through $20\,\mu m$ mesh Nitex membrane to remove large particles, and replicate 2 ml aliquots of the water were collected for enumeration. In one set of experiments the 3rd wash was replaced with 250 ml of distilled water to examine the effect of a freshwater spike, such as rain, on the spheres and bacteria.

Flow cytometry counts were converted to concentration (particles per ml) for each rinse or core section. To facilitate direct comparisons between the behaviors of bacteria and microspheres, the concentrations (*C*) of each particle type were normalized by the concentration of the first rinse or sand column (C_0) (e.g., C/C_0 ; the first sample is=1). All of the normalized results for each particle type were averaged and then plotted.

Sand grain size estimates were based on the ISO 14688-1 size range for fine (0.06–0.20 mm), medium (0.20–0.63 mm), and coarse sands (0.63–2.0 mm). The sand grain size distribution was similar for all of the cores tested, and showed fine sand on the top 0.02–0.04 m, with medium sand through the remainder of the core, and coarse sand occasionally at the bottom 0.02 m. Early in the testing, it was observed that layers of decaying macroalgae significantly reduced flow through the column, and thus cores with visible algal layers were not used.

2.2. Adsorption of microspheres and bacteria to sand

To compare the adsorptive loss of the two particle types to sand collected from the beach, 10 g of sand from either the top or the bottom of a core was added to flasks containing 100 ml of 0.2 μ m filtered seawater. Microspheres or bacteria were added to approximately $4-7 \times 10^5$ per ml concentration, and the flasks were incubated with gentle agitation (80 rpm, 22 °C). Control flasks did not contain sand, and each treatment was performed in triplicate. The water was sampled at 0, 1, 3, and 6 h, and counted by flow cytometry to determine the loss of particles to the sand. Counts were converted to normalized concentration (particles per ml divided by the initial concentration), and the replicates were averaged. Adsorption rates were estimated as the slope of the regression line between normalized concentration contration and time.

2.3. Movement of microspheres in the field

The migration of microspheres within beach sands in response to waves and currents was investigated on an ocean beach on 3 days in September 2010 at the US Army Corps of Engineers Field Research Facility in Duck, NC. To measure the fluctuating location of the water table (phreatic surface) under the sand (Raubenheimer et al., 1999) pressure gauges were buried beneath the water table (in the phreatic zone) along a transect from above the hightide line to below the low-tide line (Fig. 1). Waves running up and down the beach face (swashes) were measured by the buried pressure gages when the beach was saturated and with LIDAR. Tides, waves, and currents (Fig. 2) were measured with a pressure gage and a current meter deployed in approximately 1.7-m water depth offshore of the buried pressure gages. The change in beach elevation was mapped using both GPS and LIDAR surveys. Sand grain sizes in the swash were about 0.2-0.4 mm, and the hydraulic conductivity previously was estimated to be roughly 0.03 m/s (Turner and Masselink, 1998).

Fluorescently labeled microspheres $(1 \times 10^{12} \text{ total})$ were added to 100 g of sand and allowed to sit overnight before each experiment. This process allowed easy deployment of the microspheres into the beach. Immediately prior to high tide the inoculated sand was buried about 0.05 m beneath the sand surface, just below the predicted high-tide line. Sand cores were collected every 30 min over the following 1 to 2 h at 2-m intervals along cross-shore transects moving away from the planting sites during the receding



Fig. 1. Image of the beach near Duck, NC, in September 2010. Orange flags mark the position of buried (within the water table) pressure gauges, and the metal core tubes with yellow tape on top are at the sampling locations for the planting experiments (each cross-shore location was sampled at several different times). As the tide receded, sampling was extended towards the falling water line. A current meter and pressure sensor (to measure tide level, waves, and currents, Fig. 2) were deployed in 1.7-m water depth, along and offshore of the line of buried pressure gages. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

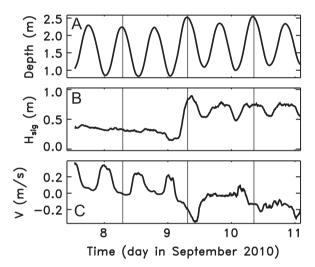


Fig. 2. (A) Water depth (tide level), (B) significant wave height (4 times the standard deviation of sea-surface elevation fluctuations), and (C) mean alongshore current (positive is flow to the north) versus time. Vertical lines are drawn near the times of the 3 microsphere plantings.

tide (Fig. 1) using 1-m long aluminum core tubes inserted 0.5-0.8 m into the beach. Core caps were applied to the top of each tube after insertion to prevent waves from entering. The cores were retrieved from the beach using a winch, and the sand was prevented from shifting inside by filling any empty space at the top of the tube with paper towels and recapping. Once all the cores were recovered, they were transported to the on-site laboratory where they were cut open with a table saw. The upper two thirds of each core consisted primarily of medium sized grains, and the lower one third of each core consisted primarily of coarse sized grains. This vertical structure varied slightly in the cross-shore, with the medium grain sand comprising a greater portion of the landward cores. Consecutive sand sections of 0.04-0.06 m were removed and processed by shaking with distilled water as in the laboratory core experiments to recover microspheres. Duplicate aliquots were stored in the dark at 4 °C and shipped back to Woods Hole where they were counted by flow cytometry.

3. Results and discussion

3.1. Laboratory sand column experiments

Overall, the movement of microspheres through undisturbed natural beach sand columns was similar to the movement of bacteria (Fig. 3, compare red with black curves). The fresh water pulse was not observed to increase microsphere or bacteria release from the column (not shown). There was variability in recovery between the individual experiments (error bars in Fig. 3), as might be expected from cores taken on different days and during different seasons. Although all of the cores had similar grain size distributions, small differences could impact particle movement, as could differences in attachment to sand grains, which was not examined. Another source of variability in particle movement could be the presence of a biofilm community in the sands. The biofilms of the experimental sand columns were not examined, but a previous study suggests that the presence of a biofilm in creek bed material increased the deposition of particles from the overlying water (Arnon et al., 2010). It also has been hypothesized

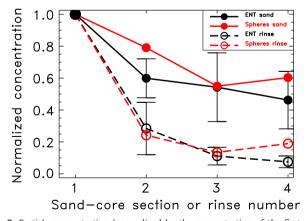


Fig. 3. Particle concentration (normalized by the concentration of the first sand core or rinse) *versus* sand-core section (agitated in 250 ml water) or rinse number (250 ml flow-through water). Sand cores are solid curves with closed circles, and water rinses are dashed curves with open circles, with black for CFMDA-labeled enterococci and red for microspheres. The average of all replicates (symbols) with 1 standard deviation error bars for the enterococci is shown [error bars for the spheres (not shown) are similar] (For interpretation of the references to color in this figure the reader is referred to the web version of this article).

that the positive correlation between percentage of bacteria released and the hydraulic conductivity of sand cores is related to biofilm biomass (Russell et al., 2012). Despite the variability, bacteria and microspheres exhibited qualitatively similar behavior through the sand columns (Fig. 3).

The primary argument against using an inert proxy such as microspheres is that they do not have the ability to mimic live bacterial biological processes (motility, growth and death, surface properties) (Sinreich et al., 2009). Studies that have examined the effect of increased ionic strength on the surface charge of enterococci and microspheres indicate that the charges become more neutral with increasing salinity (Knappett et al., 2008, Shapiro et al., 2009, Chen and Walker, 2012). Although cell membrane composition may continue to play a role, the reduced repulsive influence of surface charge decreases potential differences between the behavior of bacteria and microspheres. In the marine environment straining may have a larger impact than surface charge on the retention of bacteria and microspheres.

Microspheres have been used to examine the movement of particles through porous media, sometimes as proxies for microbes (Harvey and George, 1989, Harvey et al., 1993, Knappett et al., 2008, Shapiro et al., 2009, Sinreich et al., 2009), and at other times to examine the abiotic (physical) aspects of colloid movement (Li and Johnson, 2005, Salerno et al., 2006, Arnon et al., 2010). In some studies bacteria and microspheres have similar behavior (Sinreich et al., 2009), whereas in other studies their behavior differs (Harvey and George, 1989). The variability in behavior may be related to differences in the systems (soil and limestone in the first case, sandy aquifer in the second case), but the microbial sources also may have been important. Microsphere and bacterial behavior were similar when a single bacterial species was used (Sinreich et al., 2009), and different when mixed natural bacterial populations were used (Harvey and George, 1989).

3.2. Adsorption of microspheres and enterococci to sand

Both bacteria and microspheres rapidly adsorbed to the sand particles in the laboratory tests, with microspheres adsorbed at a slightly higher initial rate than the bacteria (Table 1, Fig. 4 where red curves are below black curves for time ≤ 3 h). The average adsorption rate of the normalized concentrations for the controls (not shown) deviated from the expected value of 0 by 13% (bacteria) and 16% (spheres) (Table 1), suggesting the range of expected errors in the surface and bottom samples. Differences between each of the three replicates were relatively small, although larger for the spheres than for the bacteria (Table 1). Student's ttests for independent samples assuming unequal variation indicate that the adsorption rates of bacteria and microspheres were not statistically different. Specifically, the null hypothesis that the rates of adsorption for bacteria and microspheres to surface or bottom sands are not different cannot be rejected (p=0.082 and p=0.848, respectively), consistent with the hypothesis that the adsorption behavior of the microspheres is similar to that of the bacteria.

The laboratory tests (Figs. 3 and 4) suggest that the movement of microspheres is similar to the movement of target bacteria

Table 1

Mean adsorption rates (*10⁵ per ml per h) and standard deviations for bacteria and microspheres from triplicate experiments.

	Bacteria	Microspheres
Control	0.013 ± 0.005	-0.016 ± 0.014
Surface sand	0.069 ± 0.003	0.049 ± 0.010
Bottom sand	0.057 ± 0.001	0.055 ± 0.019

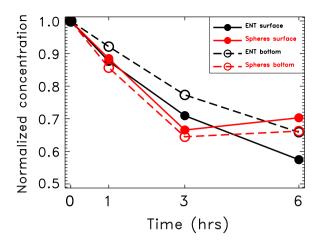


Fig. 4. Normalized (by the initial concentration) concentration *versus* time during adsorption-to-sand tests for enterococci (black) and microspheres (red) from surface (solid curves with closed circles) and bottom sand (dashed curves with open circles). The average of 3 replicates is shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

(enterococci), implying that microspheres could be used as a proxy for qualitatively studying the movement of enterococci through an ocean beach. It is important to emphasize that the enterococci and microspheres likely were not assimilated into the biofilm (if present), so the observed transport behavior approximates that of particles recently introduced to the media.

3.3. Movement of microspheres in the field

Offshore (1.7-m water depth) significant wave heights (Fig. 2B, H_{sig}) and alongshore currents (Fig. 2C, v) were weaker during the first field experiment (Sep 8, Fig. 5A and B, H_{sig} =0.35 m, v = -0.03 m/s) than during the second (Sep 9, not shown, H_{sig} =0.85 m, v = -0.25 m/s) and last experiments (Sep 10, Fig. 5C, H_{sig} =0.72 m, v = -0.12 m/s). In all three field experiments, the microsphere plant site was approximately 2 m seaward of the high tide runup limit. On Sep 8, 9, and 10, the number of swashes passing over the plant site was approximately 4 (with 3 in the initial 30 min), more than 75, and about 10, respectively. The water table level near the plant site during the hour-long sampling period was nearly constant on Sep 8, rose about 0.05 m then fell about 0.02 m on Sep 9, and rose about 0.05 m on Sep 10 (the initial water table level is shown in Fig. 5C). The maximum ocean water level was higher on Sep 9 and Sep 10 than on Sep 8 owing to higher tides and increased wave setup, and thus the water table level under the sand surface was higher (the blue water-table curve in Fig. 5C is higher than in Fig. 5A and B). There was little change in the beach surface elevation (erosion or accretion) during the first (Sep 8, Fig. 5A and B, black curves) and last experiments (Sep 10, Fig. 5C). During the second experiment (Sep 9, not shown) waves and currents were stronger, and caused approximately 0.2-0.3 m of erosion of the beach face.

In all three experiments, microspheres were present at or near the bottom of the cores (0.5–0.8-m below the sand level) (Fig. 5) indicating that they moved down through the sand matrix to the water table in response to waves running up the beach face and percolating down. The two peaks in microsphere abundance on Sep 8 (Fig. 5B, red contours near cross-shore distance=81 m) may be owing to retention of particles near the surface and changes in particle movement in the vadose and phreatic zones. In the laboratory experiments, some of the particles were retained near the surface of the core, while others moved rapidly through the sand and elute in the first rinse. In the field, the retained particles may result in high concentrations near the planting site. Once

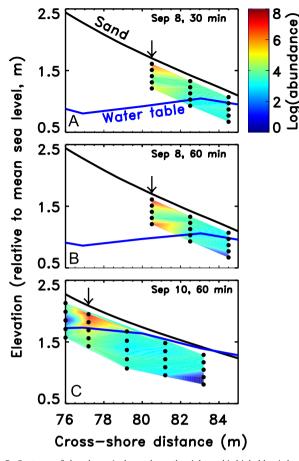


Fig. 5. Contours of abundance (color scale on the right, red is high, blue is low) as a function of cross-shore location and depth relative to mean sea level. Locations of samples in the cores are shown with filled black circles. The black curves are the sand surface, the blue curves are the water table elevation, and the arrows point to the planting site for (A) Sep 08 (30 min after planting at about 0700 h EDT), (B) Sep 08 (60 min after planting at about 0730 h), and (C) Sep 10 (60 min after planting at about 0830 h). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

within the beach environment the moving particles may slow at the water table surface owing to changes in vertical flow rates, changes in attachment properties owing to the fresher groundwater, or a combination of both leading to a secondary area of high concentrations near the water table. In the Sep 10 experiment (Fig. 5C), the water table was almost 1 m higher than it was on Sep 8 (Fig. 5A and B), and there were more than twice as many swashes crossing the plant site. The relatively short vertical separation between the planting location and the water table on Sep 10 may have blurred the distinction between the retained and moved populations, and the additional swash infiltration may have led to a more even distribution of the particles.

The time between the first wave to run up the beach and inundate the planting site and the time of arrival of spheres at sites seaward and at locations deeper in the cores provides a rough estimate that the rate of microsphere movement is $O(10^{-3})$ m/s, similar to prior estimates of instantaneous swash infiltration rates through saturated sands on this (Turner and Masselink, 1998) and other beaches (Baldock et al., 2001, Butt et al., 2001). These rates are higher than typical time-averaged field estimates of net swashgroundwater exchange and horizontal flow rates [$O(10^{-5})$ m/s] (Turner and Masselink, 1998, Raubenheimer et al., 1999), suggesting either that the microspheres are more easily swept downward by rapid infiltration than carried upwards by exfiltration, or that net infiltration rates through the partially saturated (vadose) zone above the water table are higher than those through the mostly saturated sands below the water table (in the phreatic zone) (Baldock et al., 2001). The microspheres also spread horizontally through the vadose zone (Fig. 5). The results of the second (not shown) and third (Fig. 5C) field experiments suggest that swashes were able to transport microspheres landward (away from the ocean) from the plant site. Swash-driven horizontal flows in the vadose zone on ocean beaches are not understood well. Prior field studies have suggested that instantaneous and timeaveraged horizontal flows in the phreatic zone are much smaller than instantaneous vertical flow rates. However, model simulations show a recirculation zone under bores in the swash in which instantaneous horizontal flow rates can be high (Li and Barry, 2000). Thus, the horizontal spreading of the microspheres could be owing to movement of particles in conjunction with sediment movement (Gast et al., 2011) or to through-beach movement of water under swash bores.

4. Conclusions

Laboratory comparisons of the movement of microspheres with that of target bacteria (enterococci) showed that the particles behave qualitatively similarly in beach sands, suggesting that microspheres could be used as a proxy for studying the movement and transport of bacteria on beaches. It is important to note that because the microspheres are inert, processes that affect the persistence of bacteria, such as photo deactivation, growth, and death cannot be addressed with this approach. Furthermore, in these experiments the enterococci and microspheres were not assimilated into the biofilm (if present), so the observed transport behavior approximates that of particles recently introduced to the media. Experiments on an ocean beach demonstrated that swashes and infiltration moved bacteriallike microspheres rapidly $[O(10^{-3}) \text{ m/s}]$ away from their initial location, both vertically through the vadose zone above the water table down to the ground water and horizontally within the sediment matrix, mostly toward the ocean water line, and possibly also away from the water line. Consistent with prior laboratory and modeling results, differences in the rate of movement could be owing to swash properties (e.g., water depth in the swashes, and frequency of swashes) that affect the movement of the liquid front or to differences in the saturation of the beach matrix that could affect the hydraulic conductivity or the air-water interface scouring. The results suggest that longer deployments could be performed to examine the movement of bacteria through the beach and into the ground water, and potentially out through the beach face at low tide. These longer deployments would allow testing of the hypothesis that during large tides or extreme storms with large waves, bacteria in contaminated sand near the high-tide line could be transported through the beach to the ocean and to the groundwater.

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