The Implications of High Numbers of Fecal Indicator Organisms in Sub-Tropical Beach Sands

M.L Cuvelier[1], K. L. Nowosielski[1], N. Esiobu[2], D. McCorquodale[1], A. Echeverry[2], R. Mohammed[2], A. Rogerson[1]

[1] Oceanographic Center of Nova Southeastern University, Dania Beach, Florida. [2]Florida Atlantic University, Davie, Florida.

Abstract:

Recreational beaches are periodically closed when high counts of fecal indicator bacteria are detected in the water. It is assumed that these high indicator counts imply the presence of pathogens that could pose health risks to bathers. An ongoing study has monitored the numbers of enterococci and *E. coli* in the water column, in wet sand, and in dry sand above the high tide level of three Florida beaches. Bacteria were enumerated by membrane filtration using mEI (enterococci) and mTEC (*E. coli*). The data show that sand concentrates indicator bacteria some 50 – 100 fold relative to water. There are two important questions arising from this result. Firstly, do these counts mirror the presence of pathogens or are the high counts misleading and due to the survival of 'environmentally adapted' indicators? Secondly, does the sand act as a sink for indicator organisms that could be flushed out on tidal cycles and after weather events? If so, then levels of indicator organisms in the water column, detected during routine sampling by water managers, could be misleading. The present study addresses both of these issues. The possible health aspects of high fecal indicator counts in the sand were investigated via a beach questionnaire and by the direct detection of bacterial pathogens by PCR (molecular work in progress). The role of sand as a sink for fecal indicator organisms was examined by measuring the 'washout' of enterococci and *E. coli* from sand in the swash zone, and by comparing the numbers of enterococci in near shore waters (within 10 m of the shoreline) with offshore waters. The results of the questionnaires suggest no increase in respiratory or enteric symptoms in beach users. However, the sample size is insufficient for robust statistical analysis and more data is being collected. Preliminary data on the 'washing out' of indicator organisms from sand suggest that counts in the water in the swash zone can, on occasion, be some 10 fold higher than in water 4 m offshore. The implications to water managers of these results will be discussed.

Introduction:

The US coastal areas have seen dramatic population shifts over the last few decades. Today, over half of the population lives in the coastal counties (Mallin *et al*, 2001). This, in turn, has led to increased environmental stress due to an increase in contamination from fecal organisms (Pathak and Bhattacherjee, 1994). When fecal bacteria are released to coastal waters, they may present a threat to the safety of swimmers (Rozen and Belkin, 2001). As part of an ongoing study at NSU and FAU (EPA R828830) on the survival of fecal organisms in shoreline water and beach sand, we found significantly higher numbers of enterococci (the recommended indicator for recreational waters) in sand relative to water (Figure 1).

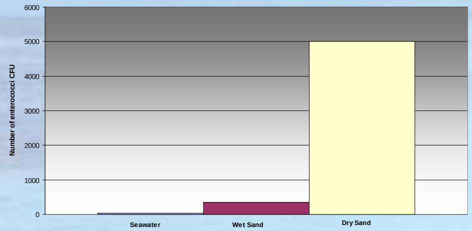


Figure 1. Distribution of enterococci in water, wet sand and dry sand. The 'dry' sand was above the high tide line and was moist but had about half the water content of wet sand. Data points are means (n=75). Samples were collected bimonthly between summer 2001 and spring 2002. Data courtesy of A. Hartz.

Ongoing work is attempting to determine whether these high numbers in the sand constitute a health risk to beach users. This involves mesocosm experiments (which are showing increased survival in the sand), molecular approaches (to distinguish sewage isolates from 'environmental' isolates) and a ground-truthing of the data via a beach questionnaire. Results to date strongly suggest that enterococci (and *E. coli*) organisms in the sand are not appropriate indicators of sewage contamination. For example, trapped fecal organisms show enhanced survival and reproduction in sand and the epidemiological study is not showing any obvious signs of increased illness in beach users exposed to beach sand (such as children playing in the sand).

A consequence of these high counts in the sand, and the focus of this present report, could be the movement of indicator organisms from the sand into the water column. If this is significant, it has important implications for water managers who routinely test the quality of bathing water by sampling indicator bacteria in the water immediately adjacent to the swash zone.

Aims:

- Determine the number of bacteria in the sand and water of a local beach.
- Determine the extent of washout of bacteria from sand into the water.
- Determine whether there are more indicator bacteria in shoreline waters than offshore waters (up to 3000 m offshore; 1.86 miles).

Materials and Methods:

Enumeration of indicator bacteria: Samples were collected from two beaches in south Florida. Hollywood Beach is a popular bathing beach close to Fort Lauderdale and John. U. Lloyd is a remote beach (without significant beach users) located one mile north of Hollywood.

1) Enterococci: a) Sand. 200 g of sand were shaken vigorously with 500 ml PBS for 1 min. Two aliquots (10 and 50 ml) were filtered onto a 0.45 µm filter, and incubated on mEI agar for 24 h at 41.5ºC. b) Water. 100 ml of water was filtered and processed as above. Samples were replicated 3 times and collected regularly between August 2002 and May 2003.

2) *E. coli*. Sand and water samples were processed as above except that the media used was mTEC. Samples were incubated at 44.5ºC for 24 h and confirmed on Urea Substrate Media.

Washout of bacteria from sand: a) Enterococci and *E. coli* were counted from water samples collected at distances of 0.1, 3.0, 5.0, 10.0 and 20.0 m from shore. Samples were replicated between 25 and 80 times and were processed as described above (1). All samples were collected between February and May 2003. Samples were collected both on the incoming and outgoing tide.

Washout of fluorescent beads from sand: Sand taken from a nearby beach in J. U. Lloyd State park was mixed with fluorescent beads (0.5 um diameter) to give a concentration of about 3.3x 10^6 beads per gram of sand. This seeded sand was placed in a designated area (20x20x10cm) on a private beach on the grounds of the Oceanographic Center. Sand samples were taken from the surface (0 cm) and with increasing depth to 10cm. Samples were taken at time zero and after each tidal cycle until no beads remained in the sand. For bead counting, sand (1 g) was shaken vigorously with distilled water and suspended beads were recovered on a 0.22 µm filter. The number of beads in replicate fields of view was counted by epifluorescence microscopy.

Washout of *E. coli* in a sand mesocosm experiment: Fluorescently tagged *E. coli* were added to sand 48 h prior to experiment to allow attachment. Previous studies by Aaron Hartz (pers. comm.) have shown that this bacterium attaches to sand particles within 24-48h. Sterile seawater was added to the sand and it was placed on a rocking platform used to mimic wave action (gentle, medium and high). Numbers of *E. coli* removed from the sand into the water column were monitored over 120 h by epifluorescence microscopy.

Offshore sampling: For all offshore samples, 200 ml of water was processed.

Results:

Beach Bacterial Densities: Numbers of enterococci and *E. coli* were obtained at Hollywood Beach, Florida (August 02 – May 03) and from a beach in John U. Lloyd State Park (October 02 – March 03). Samples were collected from the water and from the wet sand (midway between high and low tide). Figure 2 shows that both indicators were much more abundant in sand (CFU 100 g^{-1}) relative to water (CFU 100 ml^{-1}) and that bacteria were much more abundant in the sand of the heavily used beach. Surprisingly, there were statistically more *E. coli* ($p< 0.05$) and enterococci ($p < 0.1$) in the water at Hollywood beach relative to J. U. Lloyd beach suggesting that the numbers of bacteria in the sand had an influence on the numbers in the water. If there is significant wash out of bacteria from the sand into the water, this could give a false indication about the levels of sewage in the water, particularly since the beach 'indicators' may be replicating *in situ*.

To test this notion, several experiments were conducted to measure the scale of flushing of bacteria from sand by wave action. It was reasoned that if the beach was influencing the abundance of bacteria in the water column, higher densities would be obtained in (and close to) the swash zone where wave action would suspend bacteria. Figure 3 shows that the numbers of fecal indicator bacteria in the water decrease dramatically after 5 m from the shoreline. Undoubtedly, this was due to dilution effects, but it does illustrate that water close to the beach is being impacted by the high densities of indicators in the sand.

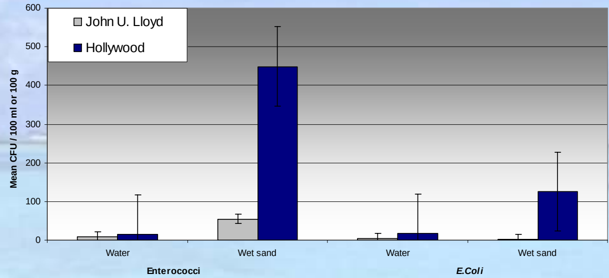


Figure 2. Comparison of numbers of enterococci and *E. coli* in the water and wet sand of two beaches. Hollywood beach (blue) is a heavily used beach and J. U. Lloyd beach (gray) is a lightly used beach. Data as means ±S.E.

Moving further offshore, the low densities of enterococci are maintained (data up to 3,000 m offshore). This is illustrated in Figure 4 where the high levels of indicators in the swash zone (mean = 13.6 enterococci 100 ml^{-1}; n = 545) rapidly fall off to a mean of 1.5 enterococci 100ml $^{-1}$ (n= 482). The few occasional high counts offshore represent rare patches of bacteria that may, or may not, be sewage derived. Again, these data suggest that most of the bacteria being sampled in close proximity to the beach are being washed from the beach rather than being pushed in by offshore waters.

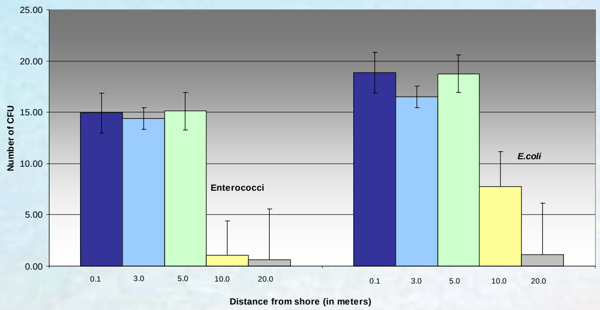


Figure 3. Numbers of enterococci and *E. coli* in the water moving out from the swash zone (0.1 m) to 20 m offshore. There is a dramatic decrease in the density of indicator bacteria due to dilution with increasing distance from the sand. Data as means ± S.E; n =100. [Note: water quality testing by the State is usually conducted at about 1 to 3 m from the beach.]

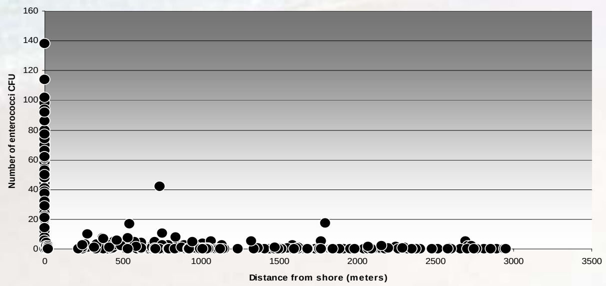


Figure 4. Numbers of enterococci (per 100 ml) in the waters adjacent to the beach (about 1 m from the sand) and in surface water extending 3000 m offshore. Data as means of 2 replicates. Clearly, there is a dramatic reduction in the numbers of fecal indicator organisms in open water.

The scale of this translocation of beach bacteria into the water was examined in a laboratory experiment and via a field experiment using bacterial-sized polystyrene particles (0.5 µm diameter). Figure 5 shows the results of the laboratory experiment that used fluorescently labeled *E. coli* and a rocking platform to mimic wave action. Bacteria were added to sand 48 h prior to experimentation to allow them to attach to sand particles. Previous experiments with labeled *E. coli* have shown that they attach to sand grains in seawater within 24 h (Hartz, pers. Comm.). Results of the three treatments (low, medium, high energy 'waves') showed that in all cases, *E. coli* were rapidly washed from the sand. Within 24 h, only 30% of bacteria remained in the sand under high energy conditions and around 50 – 60% for the low and medium energy conditions.

This dramatic removal of bacteria from the sand by wave action was also found in the case of the *in situ* mesocosm experiment using bacterial-sized fluorescent beads (Fig. 6). In just one tidal cycle, more than 85% of the particles were removed from the sand, irrespective of depth.

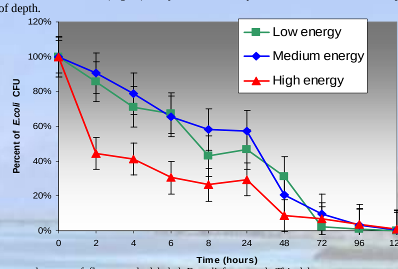
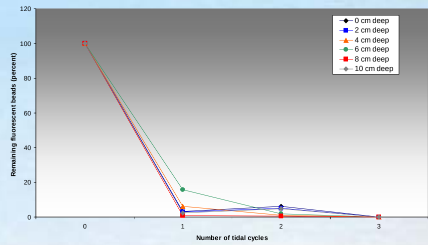
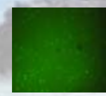


Figure 5. Percentage decrease of fluorescently labeled *E. coli* from sand. This laboratory mesocosm experiment used three artificial wave conditions (low, medium and high energy). In all cases, the 'washout' of indicator bacteria from the sand was dramatic. Data as means ± S.E.





For counting, beads were shaken from the sand into distilled water, collected on a filter and observed by epifluoresence microscopy.

Figure 6. Washout of fluorescently labeled beads (0. 5 µm diameter) from a beach mesocosm experiment. Wet sand located midway between high and low tide was seeded with around 3.3 x 10^6 beads per g sand. The numbers of beads remaining were counted every tidal cycle until all were removed. While it is true that these beads were not attached, and may not have behaved identically to fecal bacteria in the sand, the losses over just one tidal cycle imply significant washout of *in situ* bacteria. Data as means ± S.E; n=5

Discussion:

It is clear that sand contains much higher levels of fecal indicator organisms than seawater as evidenced by the data in Figure 1. As part of a two-year study into whether these higher abundances constitute a health hazard, the behavior of indicator organisms in sand has been studied with mesocosm experiments. These experiments have suggested that fecal organisms will replicate in the protected crevices afforded by sand particles. Bacteria also accumulate in the sand because of its filtering capability. Experiments in the laboratory have shown that autoclaved sand can remove 66% of fecal bacteria and non-sterile sand can remove 92% of bacteria from seawater. Similar results were reported by Gerba et al. (1975) who showed that filtering by soil could reduce the number of fecal bacteria by more than 50%. *In situ* mesocosm experiments have also demonstrated the filtering capacity of beach sand. For example, sterile sand on the beach accumulated 3,354 enterococci from the water after just 4 tidal cycles (Hartz, pers. comm.). It is likely, therefore, that the number of indicator bacteria in wet sand is influenced by the following equation:

Abundance = (number of bacteria surviving and growing + number being filtered) – (number being consumed + number washing out)

The present study focused on the possible loss of indicator bacteria from the sand into the adjacent water column (i.e. number washing out). If this is the case, and if many of these indicator bacteria are 'environmental' indicators, rather than true indicators of sewage contamination, their presence in the swash zone could influence the outcomes of routine beach testing. Traditionally, the water quality of recreational beaches relies on testing the numbers of indicator bacteria in the water a few meters offshore and the present study has shown that there are considerably more indicator organisms in the zone 0 to 5 m from the shore than in the water extending from 5 m to 3000 m offshore. This strongly suggests that the sand influences the bacterial counts in the water. Experimentally, we have confirmed that wave action can remove almost all the indicator bacteria from sand in one or two tidal cycles. In short, rain events, storms, and tidal wave action are probably washing fecal indicator bacteria from the sand to give misleadingly high counts in the nearshore water. This may be leading to unwarranted beach closures. Dorfman (2002) reported that elevated bacterial levels were responsible for 84% of Florida's beach closings/advisories in 2001. Of these, 89% were from unknown sources.

Of course, it must be confirmed that the fecal indicator counts in sand represent harmless 'environmental' bacteria rather than significant levels of sewage contamination. But work underway on the molecular makeup of beach *E. coli* and the results of an epidemiological study on incidences of illness after beach use are suggesting that present indicators are inappropriate and improved methods for recreational water quality testing are required.

Acknowledgments: We gratefully acknowledge financial support from the USEPA (Grant Award R828830)

References:

Gerba, C., Wallis, C., and Melnick, J. 1975. Fate of wastewater, bacteria and viruses in soil. Journal of the Irrigation and Drainage Division. September. pp. 157-174.

Mallin, M., Ensign, S., McIver, M., Shank, G., and Fowler, P. 2001. Demographic, landscape and meteorological factors controlling the microbial pollution of coastal waters. Hydrobiologia 460: 185-193.

Pathak, S., and Bhattacherjee, J. 1994. Effects of pollutants on survival of *Escherichia coli* in microcosms of river water. Bulletin of Environmental Contamination and Toxicology 53: 198-203.

Rozen, Y., and Belkin, B. 2001. Survival of enteric bacteria in seawater. FEMS Microbiology Reviews 25: 513-529.