Fecal Indicator Bacteria and Pathogen Persistence in Dry Beach Sand and Sediment Biofilms

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This year our goals were to: 1) investigate fecal indicator bacteria (FIB) dynamics in beach sand following sewage spills; 2) use traditional FIB measurements along with a novel rapid FIB method and Bacteroides levels in water and sand to conduct fecal source tracking and identification at three impaired beaches; and 3) continue to collaborate with the Southern California Coastal Water Research Project on an epidemiological study of adverse health impacts due to water and sand exposure.

Coastal sediments are increasingly recognized as important reservoirs of *E. coli* and enterococci, referred to as fecal indicator bacteria (FIB). The abundance of FIB in sediment can have two distinct implications for public health. On one hand, persistence and regrowth of non-pathogenic FIB in sediment weakens the relationship between FIB and the pathogens they are meant to proxy. On the other hand, sediments may provide a favorable environment for pathogens, as they do for FIB, and may be an unexplored route of exposure.

Importantly, both FIB and pathogens appear to have greater persistence in sediments than in water. In general, sediments may be more conducive to FIB survival relative to the water column due to reduced sunlight inactivation, protection from predators, nutrient and organic carbon availability, and the presence of a surface for the formation of biofilms.

Fecal pollution is a major cause of water quality impairment in coastal areas. However, our understanding of the fate of fecal pollution in coastal ecosystems, as well as our ability to identify and mitigate its sources, is greatly limited by uncertainties surrounding its behavior in two major reservoirs: wetlands and beach sediments.

Investigation of FIB dynamics in beach sand following sewage spills.

Factors affecting FIB and pathogen survival/ persistence in sand remain largely unstudied. This work elucidates how biological and physical factors affect die-off in beach sand following sewage spills.

Solar disinfection with mechanical mixing was pilot-tested as a disinfection procedure after a large sewage spill in the Los Angeles area. Effects of solar exposure, mechanical mixing, predation and/or competition, season, and moisture were tested at bench scale. Desiccation was a dominant factor for *E. coli* but not enterococci inactivation. Effects of season were investigated through a comparison of experimental results from winter, spring, and fall.

Moisture was the dominant factor controlling *E. coli* inactivation kinetics. Initial microbial community and sand temperature were also important factors. Mechanical mixing, common in beach grooming, did not consistently reduce bacterial levels. Inactivation rates are mainly dependent on moisture and high sand temperature. Chlorination was an effective disinfection treatment.

Use of traditional FIB measurements along with a novel rapid FIB method and Bacteroides levels in water and sand to conduct fecal source tracking and identification at three impaired beaches.

We completed laboratory and field testing of our rapid method for *E. coli* and enterococci based on immunomagnetic separation/ATP quantification (IMS/ATP).

One approach to addressing coastal water quality concerns is the use of source tracking and identification strategies, which have historically been based on FIB as a first tier to direct additional sampling efforts. This tiered approach is hampered by the temporal variability of FIB in aquatic environments. The tiered approach is further limited by the absence of an economical rapid detection method to serve as a middle tier that would allow investigators to obtain results from multiple samples in near real time and before the contamination source has dissipated.

The development of rapid detection assays, allowing enumeration of microbial contaminants as quickly as one hour and enabling a more diverse suite of organisms to be studied, has progressed significantly in recent years. While many of these assays are promising, only IMS/ATP currently incorporates a feasible on-site analysis that can be optimized to a 30 minute assay of multiple samples.

Until recently, impediments have obstructed the transition and application of IMS/ATP to measurement of water quality in marine systems. We have made significant improvements to the IMS/ATP protocol and its applications; this includes devising a more robust anti-*E.coli* biosorbent, testing the biosorbent in both freshwater and marine waters, and examining IMS/ATP as a rapid and potentially adaptive tier in microbial source tracking and pollution identification.

We devised a covalently-linked anti-*E.coli* bead complex that was used to measure water quality in freshwater and marine systems by IMS/ATP. We collected and analyzed samples from freshwater and marine systems. R^2 values for the correlation between traditional measurements and the rapid method were 0.87 and 0.94 in freshwater and marine, respectively. IMS/ ATP was also evaluated as a rapid, intermediate tier for a multi-tiered approach in source tracking and identification and was able to rapidly identify the presence of high *E. coli* loading in one of the two channels, which resulted in high levels at the confluence of the two channels.

We are currently applying this rapid method along with human-specific and universal Bacteroides measurements by quantitative PCR in two additional watersheds in Malibu and Ventura. We have detected human fecal pollution in the Ventura Marina and are currently continuing source tracking efforts.

Continued collaboration with the Southern California Coastal Water Research Project (SCCWRP) on an epidemiological study of adverse health impacts due to water and sand exposure.

This past summer, we analyzed FIB in sand at Doheny and Avalon beaches as part of the SCCWRP study. We also extracted and purified DNA, and are in the midst of analyzing DNA extracts for human-specific and universal *Bacteroides*. The study is ongoing and results are not yet available.

Selected Professional Presentations

Mika, K., G. Imamura, C. Change, C.M. Lee, J.A. Jay, Dynamics in Sand of Fecal Indicator Bacteria (FIB) and *Salmonella* From Contaminated Water, Runoff, and Sewage in an Urbanized Southern California Shoreline. American Geophysical Union Annual Meeting, San Francisco, CA, December, 2007.

Lee, C.M., W. Kaiser, J.A. Jay. Immunomagnetic separation and ATP quantification (IMS/ATP): Evaluating a method of rapidly detecting pathogen indicators in aquatic environments with respect to identifying hotspots of contamination. Gordon Research Conference. Environmental Sciences: Water, New Hampshire, June 22-26, 2008.

Collaborative Efforts

My lab has continued our very meaningful collaboration with Dr. Sharon Walker (UCR). We have received a second year of funding from UC Marine Council for a project stemming from our Center for Water Resources Grant. This year's UC Marine Council award is also in collaboration with Rich Ambrose (UCLA) and Trish Holden (UCSB). Our lab is also pursuing additional source identification funding in collaboration with Trish Holden, Ali Boehm (Stanford) and the Southern California Coastal Water Research Project (SCCWRP).

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