

## ABSTRACT

**Rationale.** Fecal contamination of recreational water poses direct health threats to beach users and cause economic losses due to frequent beach advisories and closures. Although the regulatory and research efforts have been traditionally focused on the water itself, recent studies have started to reveal the importance of beach sand to water biological quality. It is now widely observed that beach sand often harbors fecal indicators at concentration levels several orders of magnitude higher than the beach water. The goal of our study is to understand the relative importance of the environmental factors that can affect the survival of fecal indicators in Hawaiian beach sand.

**Methods and Results.** Stationary-stage cells of two common fecal indicators (i.e. *E. coli* and enterococci) were spiked into microcosms containing autoclave-sterilized sand. The microcosms were then inoculated with different community components of the sand extract to examine their effects on the die-off of fecal indicators. Our results showed that *E. coli* lost cultivability at a significantly faster pace in live beach sand than in autoclaved sterile sand, whereas *E. faecalis* showed a much prolonged survival in beach sand, indicating that biotic factors played more important roles than abiotic forces on the survival of *E. coli*. When different components of the indigenous sand microbial communities (i.e. bacteria, protozoa, and viruses) were examined for their effects on the survival of *E. coli*, the indigenous bacteria were shown to be responsible for the fastest disappearance of *E. coli* in sand from the Sand Island and Kailua Beaches.

## OBJECTIVES

- To determine the important environmental factors affecting the survival of fecal indicators in beach sand
- To identify indigenous bacterial antagonists contributing to the fast die-off of fecal indicators
- To develop technologies that enhance the indigenous antagonistic effects for the benefits of expedited recovery from fecal contaminations

## METHODS

### Experimental set-up

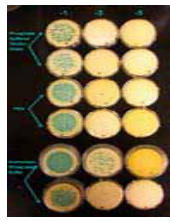
- Stationary-stage cells ( $OD_{600} > 1.2$ ) of *E. coli* and *E. faecalis* were spiked into the microcosms containing sterilized sand
- Different components of the indigenous sand microbial communities were amended
- Sand microcosms were incubated in dark and at room temperature (25°C)
- **Sampling and quantification**
  - 10 grams of sand were removed from each of the triplicate reactors and extracted by shaking with 20 ml of ammonium-phosphate buffer for 1 hour
  - Counting of *E. coli* and *E. faecalis* were conducted based on EPA methods 1603 (modified mTEC method) and 1106.1 (mE-EIA), respectively.

### Performance of extraction buffers

#### Sand Island Beach

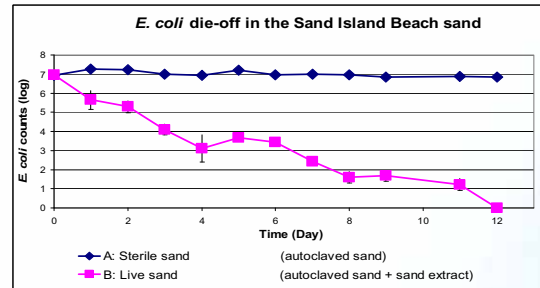


#### Kailua Beach

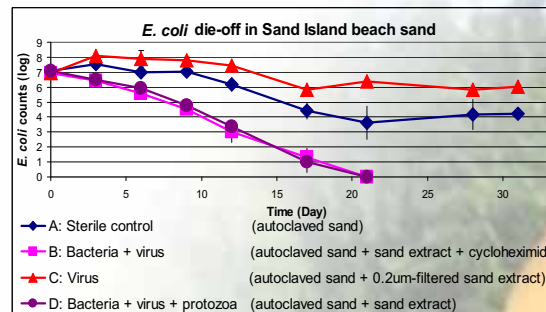


**Extraction buffer:** ammonium-phosphate buffer > PBS > phosphate-buffered water

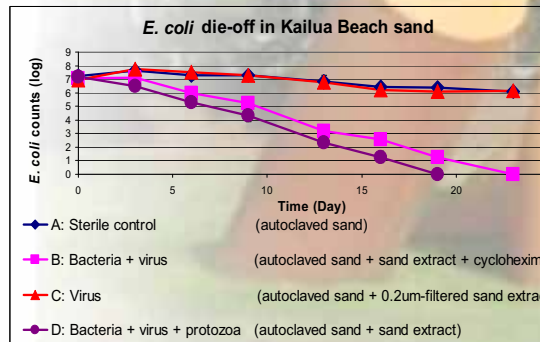
## RESULTS



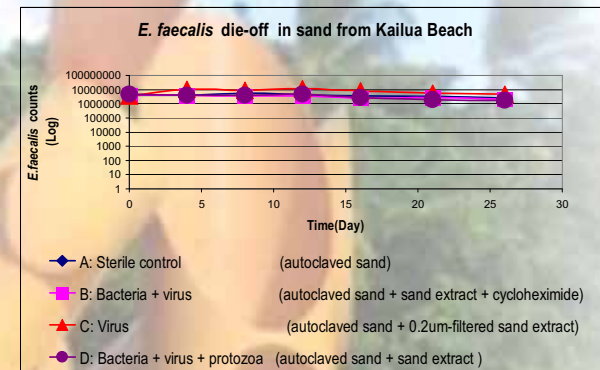
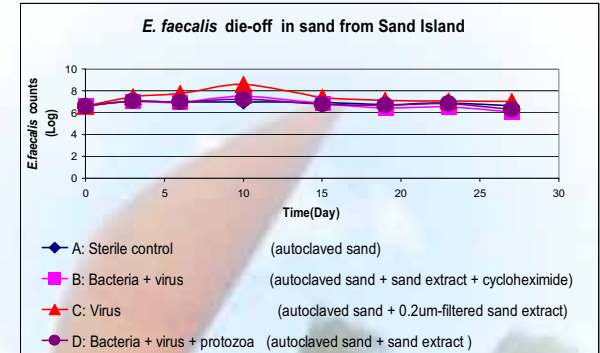
- The indigenous microbial communities played a more important role than the abiotic factors in the die-off of *E. coli* in sand from the Sand Island Beach.



- Protozoa did NOT contribute significantly to the disappearance of *E. coli* in sand from the Sand Island Beach
- When both protozoa and bacteria were removed by filtration, the die-off of *E. coli* in beach sand is the slowest, suggesting no active viral effects against *E. coli*.
- The indigenous **bacterial** community played a predominant role in the disappearance of *E. coli* in beach sand



- In sand from the Kailua Beach, the die-off of *E. coli* follows the outstanding pattern in the Sand Island Beach sand----the **indigenous bacteria played the most important role**.
- Viruses did not contribute significantly.
- However, protozoa exhibited a discernable level of contribution to the disappearance of *E. coli* in Kailua Beach sand.



- *E. faecalis* showed a much prolonged survival in beach sand than *E. coli* did.
- Indigenous microbial communities in beach sand did not affect the survival of *E. faecalis*

## CONCLUSIONS

- In beach sand, indigenous microbial communities, particularly bacterial antagonists, exert significant impacts on the survival of fecal indicator *E. coli*.
- *E. faecalis* subsists much longer than *E. coli* in beach sand, and did not appear to be affected by the indigenous microbial communities.

## FUTURE WORKS

- Identify the indigenous bacterial antagonists against fecal indicators *E. coli* and *E. faecalis* in Hawaiian beach sand.
- Identify the environmental parameters that can enhance the antagonistic effects.
- Develop beach sand remediation strategies based on the applicable parameters amenable to engineering interventions.

## ACKNOWLEDGEMENT

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