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Comparison of the occurrence and survival of fecal indicator bacteria in recreational sand between urban beach, playground and sandbox settings in Toronto, Ontario

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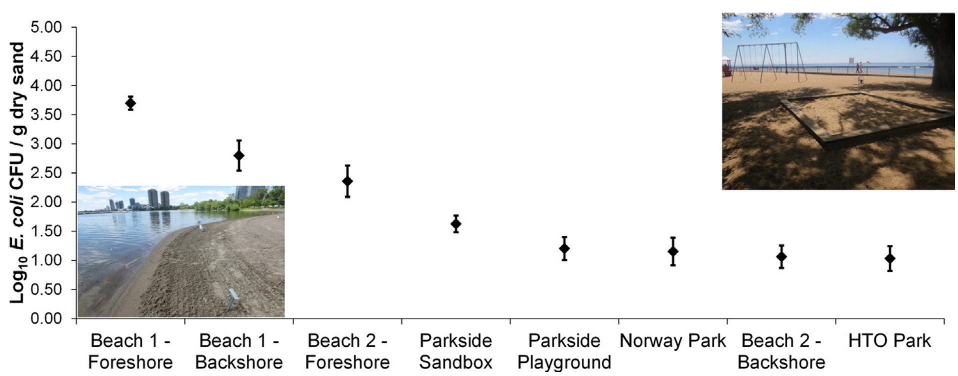
HIGHLIGHTS

- Consistently high FIB concentrations found in beach, sandbox and playground sands
- Foreshore beach sands had the highest levels of fecal contamination.
- Culture-based methods produced significantly different results in sands.
- In saturated sands, lower temperature increased *E. coli* survival over 28 days.
- In saturated sands, finer grain size increased *E. coli* survival over 21 days.

GRAPHICAL ABSTRACT



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ABSTRACT

While beach sands are increasingly being studied as a reservoir of fecal indicator bacteria (FIB), less is known about the occurrence of FIB in other recreational sands (i.e., sandboxes and playgrounds). In this study, different culture-based FIB enumeration techniques were compared and microbial source tracking assays were conducted on recreational sand samples from beaches, playgrounds and sandboxes around Toronto, ON. FIB were detected in every sand sample ($n = 104$) with concentrations not changing significantly over the five month sampling period. Concentrations of FIB and a gull-specific DNA marker were significantly higher in foreshore beach sands, and indicated these were a more significant reservoir of FIB contamination than sandbox or playground sands. Human- and dog-specific contamination markers were not detected. All culture-based FIB enumeration techniques were consistent in identifying the elevated FIB concentrations associated with foreshore beach sands. However, significant differences between differential agar media, IDEXX and Aquagenx Compartment Bag Test were observed, with DC media and Enterolert being the most sensitive methods to detect *Escherichia coli* and enterococci, respectively. To better understand the elevated occurrence of *E. coli* in foreshore sands, microcosm survival experiments were conducted at two different temperatures (15 °C and 28 °C) using non-sterile saturated foreshore beach sands collected from two urban freshwater beaches with different sand type (fine grain and sand-cobble). Microcosms were inoculated with a mixture of eight sand-derived *E. coli* strains and sampled

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over a 28-day period. *E. coli* levels were found to decline in all microcosms, although survival was significantly greater in the finer sand and at the cooler temperature (15 °C). These results indicate that FIB can be widespread in any type of recreational sand and, while *E. coli* can survive for many weeks, it is most likely to accumulate in cooler fine-grain sand as occurs below the foreshore sand surface.

1. Introduction

Beach sands, particularly foreshore beach sands, are known to harbor bacterial concentrations several orders of magnitude higher than those found in adjacent recreational waters (Alm et al., 2003; Byappanahalli et al., 2012; Gast et al., 2011; Whitman et al., 2014; Whitman and Nevers, 2003; Yamahara et al., 2007). While concentrations of fecal indicator bacteria (FIB) such as *Escherichia coli* and enterococci are routinely monitored in recreational waters in accordance with local or national guidelines (Health Canada, 2012; Ontario Minitry of Health, 1998; U.S. Environmental Protection Agency, 2012), no similar regulatory guidelines exist for beach sands. This is despite a growing number of studies that are finding an association between elevated concentrations of FIB in beach sands and the risk of contracting enteric disease (Heaney et al., 2012; Heaney et al., 2009; Whitman et al., 2009). In addition to the absence of regulatory guidelines for sands, sand-specific methods for FIB quantification are not well established. Presently, quantification requires creating a suspension of sampled sands, generally using a phosphate buffer solution, and using membrane filtration or most probable number methods originally designed for water quality assessment (Beversdorf et al., 2007; Boehm et al., 2009; Byappanahalli et al., 2006; Mika et al., 2009; Sampson et al., 2006; Whitman and Nevers, 2003). Emerging techniques for water quality assessment, such as the Aquagenx Compartment Bag Test (CBT), have not yet been applied to any type of recreational sands (Stauber et al., 2014). As the CBT has been designed to primarily assess potable water with *E. coli* concentrations less than 100 MPN/100 ml, this test might not perform optimally in sands which can have *E. coli* concentrations orders of magnitude higher.

In addition to beach sands, other recreational sands, such as playgrounds and sand boxes, may also harbor fecal contaminants, and have been associated with incidences of enteric disease (Doorduyn et al., 2006; Staff et al., 2012). Despite the potential health risk, the relative occurrence of FIB or source(s) of contamination across recreational sand settings, to our knowledge, has not previously been investigated. Other recreational sands generally do not interact with surrounding waters (surface waters or groundwater) that may contribute to the high bacterial populations associated with foreshore beach sands (Gast et al., 2011; Ge et al., 2012; Whitman et al., 2004). Nevertheless, recreational sands may still be subjected to fecal contamination events, primarily from wildlife, particularly birds, or from children playing in the sand. Following such a contamination event, these sands might similarly act as a reservoir for bacteria. Further, once introduced, bacteria may become particularly well-adapted, or “naturalized,” to recreational sands, as often occurs in beach sands, creating a persistent bacterial community (Ishii et al., 2007; Ishii and Sadowsky, 2008; Whitman et al., 2014).

While foreshore beach sands are a known reservoir for *E. coli* (Alm et al., 2003; Edge and Hill, 2007; Whitman and Nevers, 2003; Whitman et al., 2006), factors affecting the survival of *E. coli* in beach and other recreational sands are still not well understood. Although studies have examined the survival of *E. coli* in soils and sediments (Anderson et al., 2005; Badgley et al., 2010; Byappanahalli and Fujioka, 2004; Desmarais et al., 2002; Marino and Gannon, 1991; Topp et al., 2003), less is known regarding their survival in recreational sands. Most studies have focused on beach sands at marine beaches (Feng et al., 2010; Hartz et al., 2008; Lee et al., 2006; Mika et al., 2009; Yamahara et al., 2012), with only a few studies of *E. coli* survival at freshwater beaches, in particular those on the Great Lakes (Alm et al., 2006; Alm et al., 2014; Byappanahalli et al., 2006; Eichmiller et al., 2014). Among the most important factors thought to influence bacterial survival in sands are grain size and temperature (Beversdorf et al., 2007; Elliot et al., 1980; Sampson et al., 2006; Sessitsch et al., 2001). While some studies have investigated the survival and transport of *E. coli* in non-sterile sands (Engström et al., 2015; Foppen and Schijven, 2006), many studies have used soils (Elliot et al., 1980; Sessitsch et al., 2001) or have used sterilized sands only, which exclude biotic stressors such as predation (Alm et al., 2006; Hartz et al., 2008). Further, most studies investigating temperature effects for beach sands have used lab- or sewage/manure-derived bacterial strains rather than sand-derived, potentially “naturalized” strains (Beversdorf et al., 2007; Eichmiller et al., 2014).

To investigate the relative occurrence of FIB in recreational sands, FIB (*E. coli* and enterococci) were enumerated using multiple culture-based methods at two foreshore and two backshore sites at Sunnyside Beach, and nearby playground and sandbox sites in Toronto, ON, Canada. Microbial source tracking (MST) assays were also performed on these samples to assess fecal contamination from human, gull, and dog sources. In addition, a microcosm experiment was conducted to evaluate the influence of temperature and grain size on *E. coli* survival in recreational sands. For the microcosm experiments, eight environmentally-isolated, sand-derived *E. coli* strains were inoculated into non-sterilized beach sands from two Lake Ontario beaches with distinctively different sand characteristics at two temperatures (15 and 28 °C).

2. Materials and methods

2.1. Sampling and enumeration of recreational sands

2.1.1. Recreational sand site descriptions and sand collection

Recreational sands were collected at two foreshore and two backshore sites on Sunnyside Beach, as well as nearby playgrounds (Parkside, Norway Park, H_TO Park) and an outdoor sandbox (Parkside) along the waterfront in Toronto, ON. Sunnyside Beach is an urban beach that is sheltered behind a breakwall. Sampling was conducted at two transects. Each transect consisted of a foreshore sampling site ~1 m landward of the shoreline (wet sand) and a backshore site ~10 m landward of the shoreline (dry sand). One transect (designated SS1-F and SS1-B for the foreshore and backshore sites, respectively) has few beachgoers and is heavily impacted by Canada geese and gulls (site B in Edge et al. (2010)). The other transect (designated SS2-F and SS2-B) was located at the Sunnyside bathing pavilion and has a higher number of beach-goers and fewer water fowl (between site B and C in Edge et al. (2010)). Other recreational sand samples were collected from a sand playground area and an outdoor sandbox (about 5 m × 5 m) in Parkside Park which is located just landward of Sunnyside Beach. In addition, samples were collected from sand playground areas in Norway Park, located near Toronto Harbor, and H_TO Park located right on Toronto Harbor. Gulls and associated fecal droppings were regularly observed on foreshore sands at Sunnyside Beach, and occasionally in the vicinity of the other sand sampling sites. There were few bathers observed at Sunnyside Beach, while children were often observed playing in the playground and sandbox locations.

Sand samples were collected at each site weekly from June to September 2014, with an additional sampling event in late October. Two replicate composite samples were collected from each site. Each