



Foreshore beach sand as a reservoir and source of total phosphorus in Lake Ontario

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Many regions around the Great Lakes have been designated Areas of Concern as a result of consistent water quality problems from pollutants like phosphorus and Escherichia coli, which cause eutrophication, beach postings and Beneficial Use Impairments. While foreshore beach sand is a potential reservoir for E. coli, there is less understanding of whether it might also be a reservoir and source of phosphorus for adjacent beach waters. We measured levels of E. coli, total phosphorus and soluble reactive phosphorus at Sunnyside and Rouge Beaches in the Toronto and Region Area of Concern, and stormwater outfalls in the adjacent Humber and Rouge Rivers within their beachsheds. Additionally, we used microbial source tracking assays to detect human and gull fecal contamination. Soluble reactive phosphorus concentrations were highest in stormwater outfalls, with concentrations as high as $556 \mu\text{g l}^{-1}$ at an outfall in the Sunnyside beachshed, and $4780 \mu\text{g l}^{-1}$ at an outfall in the Rouge beachshed. In contrast, the highest total phosphorus concentrations were typically found in foreshore beach sand pore water and were more associated with gull fecal contamination. Beach sand total phosphorus levels were as high as $10,600 \mu\text{g l}^{-1}$ at Sunnyside Beach, although the highest total phosphorus concentration measured ($25,600 \mu\text{g l}^{-1}$) was in a Rouge River outfall. Concentrations of total phosphorus in outfalls were significantly correlated with concentrations of E. coli in both beachsheds and the human microbial source tracking marker in the Sunnyside beachshed outfalls. These results indicate that stormwater outfalls with sewage cross-contamination can deliver high concentrations of total phosphorus, soluble reactive phosphorus and fecal bacterial contamination to associated beachsheds. Further, similar to E. coli, foreshore beach sand can act as a reservoir of total phosphorus and a source for adjacent water bodies via wave action or groundwater discharge. High phosphorus inputs from beach sand could contribute localized changes to microbial communities and unique eutrophication effects along beach shorelines.

Keywords: E. coli, Area of Concern

Introduction

As a consequence of historic and on-going problems with water quality, many areas around

the Great Lakes have been designated as Areas of Concern (AOCs). The water quality problems and Beneficial Use Impairments (BUIs) in these AOCs result from pollutants like phosphorus causing

eutrophication, nuisance and harmful algal blooms, and *E. coli* causing beach postings. Within AOCs, fecal pollution sources such as wastewater treatment plant effluents and combined sewer overflows have long been recognized as legacy sewage contamination sources of phosphorus and *E. coli* to remediate (Marsalek and Ng, 1989). However, other sources such as stormwater and urban wildlife, such as waterfowl, can also be important sources to consider in remediation efforts.

Bird fecal droppings have been increasingly recognized as important sources of *E. coli* at urban AOC beaches (Edge and Hill, 2007; Lu et al., 2011; Staley and Edge, 2016a), and impacted beach sands can serve as a reservoir of *E. coli* to contaminate adjacent waters along beach shorelines. Bird fecal droppings can also contain high concentrations of nutrients such as phosphorus (Gagnon et al., 2013; Ganning and Wulff, 1969; Hahn et al., 2007, 2008; Otero et al., 2015; Telesford-Checkley et al., 2017). To date though, it is not well understood if bird fecal droppings contribute to high concentrations of phosphorus in beach sand, and if beach sands then serve as a reservoir of phosphorus, impairing water quality along beach shorelines.

Presently, most regulatory standards for recreational freshwater quality rely on concentrations of fecal indicator bacteria (FIB), such as *E. coli* (Health Canada, 2012; U. S. Environmental Protection Agency, 2012), however, the FIB paradigm is imperfect. FIB concentrations have been shown to persist and potentially grow in the absence of fecal contamination, particularly in beach sands, potentially out-living the pathogens they are used to predict (Alm et al., 2006; Whitman et al., 2014). Additionally, *E. coli* concentrations alone give no indication regarding the source(s) of contamination, which can hinder remediation efforts and lead to erroneous conclusions relating to public health risks (Field and Samadpour, 2007; Staley and Edge, 2016a). To combat this deficiency, microbial source tracking (MST) techniques targeting host-specific DNA markers have been used to identify the source(s) of fecal contamination in impacted watersheds (Hagedorn et al., 2011; Scott et al., 2002; U. S. Environmental Protection Agency, 2005).

The relationship between levels of phosphorus, including total phosphorus (TP) and soluble reactive phosphorus (SRP) and concentrations of

E. coli and MST is not well understood. Increased phosphorus concentrations may promote bacterial growth and survival in the absence of novel fecal contamination. Conversely, the presence of fecal contamination, which causes an increase in *E. coli* concentrations, may elevate overall phosphorus levels. The distribution of phosphorus at the sand-water interface, particularly in foreshore sand pore water, has also been under studied. Foreshore sand has been shown to act as a potential source and sink of *E. coli* for adjacent waters (Whitman et al., 2014) and may also act as a reservoir of TP or SRP.

In this study, TP and more biologically-available SRP were measured in stormwater outfall and beach sites throughout the Sunnyside and Rouge beachsheds, both systems within the Toronto and Region AOC (Toronto, ON). Additionally, *E. coli* were enumerated and MST assays were performed to quantify fecal contamination from humans and gulls. We hypothesized that correlations would be observed among *E. coli*, phosphorus and MST marker concentrations throughout the beachsheds, with sites experiencing greater levels of fecal contamination having higher concentrations of phosphorus.

Methodology

Site description and sample collection

This study was conducted in the Sunnyside beachshed, including associated stormwater outfalls and at transects along Sunnyside Beach at the mouth of the Humber River (Toronto, ON; Appendices 1 and 2 in the online [supplementary information](#)). The Humber River extends 100 km, and includes both rural (55%) and urban (45%) land use (Toronto and Region Conservation, 2015). Sunnyside Beach is an urban beach protected from Lake Ontario by a breakwall. Water samples from each site were collected weekly from May-August 2015. Similarly, specific sites within the Rouge beachshed, including stormwater outfalls and transects along Rouge Beach, where the Rouge River meets Lake Ontario, were sampled weekly between the months of June-September 2016 (Appendices 1 and 3 in the online [supplementary information](#)). The Rouge River watershed spans 336 km² and includes rural (40%), urban (35%), forest/wetland/meadow (24%), and waterbodies (1%) land use

(Toronto and Region Conservation, 2016). Sampling sites were grouped as outfall or beach sites for the purpose of this study.

From all outfall sites, grab samples were collected in sterilized 500 ml polypropylene bottles. Along Sunnyside Beach and Rouge Beach, three transects were sampled including interstitial sand pore water and at ankle- and chest-depth within the lake. Ankle- and chest-depth samples were collected in the same manner as outfall sites. To collect the sand pore water sample, a hole was dug down to the water table in the foreshore sand about one meter inland from the lake and a 250 ml polypropylene bottle was inserted into the hole to collect the water that accumulated (while minimizing sand collection). All samples were placed on ice and transported to the laboratory within six hours of collection.

E. coli enumeration and microbial source tracking

Water samples were filtered (0.45 μm pore size, 47 mm diameter nitrocellulose membranes) for *E. coli* enumeration over a range of dilutions (1–100 ml) according to standard membrane filtration methods (American Public Health Association, 1995). Filters were placed on differential coliform media, supplemented with cefsulodin, and incubated at 44.5°C for 22 h prior to enumeration. *E. coli* concentrations were reported as colony forming units (CFU) 100 ml⁻¹. An additional 300 ml sample (100 ml for pore water samples) was filtered as described above for DNA extraction using PowersoilTM DNA Isolation Kits (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to manufacturer's instructions. Quantitative polymerase chain reaction (qPCR) was performed on all extracted DNA samples for quantification of human- and gull-specific MST DNA targets. Reaction composition and conditions for these targets have been previously published (Green et al., 2014; Ryu et al., 2012; Staley and Edge, 2016a,b). Concentrations of MST markers were reported as copy number (CN) 100 ml⁻¹.

Quantification of total phosphorus and soluble reactive phosphorus

To quantify SRP, 125 ml of the water samples were filtered (0.45 μm pore size, 47 mm diameter

nitrocellulose membranes nitrocellulose) into 125 ml Boston round glass bottles (VWR, Mississauga ON, Canada). To quantify TP, 125 ml French square bottles (Systems Plus, Baden, ON, Canada) were prepared with 1 ml of 30% sulfuric acid and filled with water samples. Quantification of TP and SRP was performed by standard methods at Environment and Climate Change Canada's National Laboratory for Environmental Testing (Burlington, ON, Canada).

Statistical analysis

All values (*E. coli*, MST marker, TP and SRP concentrations) were log transformed prior to analysis. Analyses for the Sunnyside and Rouge beachsheds were conducted separately. To test for an effect of site type, Multiple Analysis of Variance (MANOVA) was conducted for both concentrations of TP and SRP, or the human and gull qPCR markers. ANOVA was performed to determine if there were significant differences in *E. coli* concentrations between outfalls, ankle-/chest-depth samples and pore water samples. Effects of shore normal location (ankle, chest or pore beach samples) were examined with MANOVA where the response variables were TP and SRP. Tukey's *post hoc* test was performed if a significant effect was detected. Nonparametric Spearman rank order correlations were used to assess relationships among concentrations of *E. coli*, qPCR, TP, and SRP. All analyses were performed in R version 3.3.1, and results were considered significant at the α -level of 0.05.

Results and discussion

All but one sample in the Sunnyside beachshed and three samples in the Rouge beachshed exceeded the Ontario water quality guidelines for total phosphorus of 30 $\mu\text{g l}^{-1}$ (Ontario Ministry of the Environment and Energy, 1994). MANOVA detected a significant difference between site types for TP and SRP concentrations in both the Sunnyside and Rouge beachsheds ($F_{2,20} = 14.18$, $P < 0.001$ and $F_{2,150} = 25.32$, $P < 0.001$, respectively). In the Sunnyside beachshed, post-hoc analysis revealed the significantly greater SRP concentrations in outfalls than at beach sites ($P < 0.001$). Similarly,

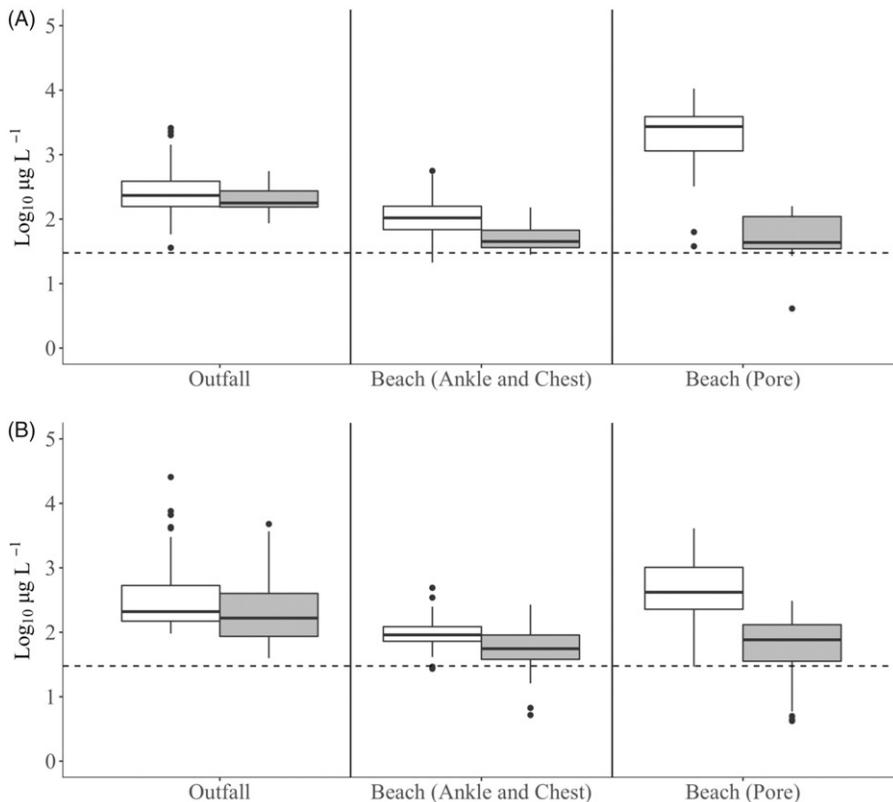


Figure 1. Box plots of TP and SRP for each site type for (a) the Sunnyside beachshed and (b) the Rouge beachshed. Box plots show the median *E. coli* concentration between the 25th and 75th percentiles; whiskers extend to the outermost data point within ± 1.5 this interquartile range. Closed circles depict outlier values. Clear box plots represent mean TP data, while grey box plots represent mean SRP data. The dashed horizontal line represents the Ontario water quality guideline for TP ($30 \mu\text{g l}^{-1}$).

SRP and TP concentrations were significantly higher in outfalls than beach sites in the Rouge beachshed ($P < 0.001$ and $= 0.003$, respectively; Figure 1), driven by extremely high concentrations in one outfall RR1 with a likely sewage cross-connection (TP maximum $= 25,600 \mu\text{g l}^{-1}$; SRP maximum $= 4780 \mu\text{g l}^{-1}$). When RR1 was excluded, MANOVA detected no significant difference among site types for TP or SRP in the Rouge beachshed. Elevated concentrations of both SRP and TP in outfalls are consistent with previous studies which have shown that storm drains are a likely source of SRP to adjacent watersheds and that sewage (which all outfalls in this study are affected by to some degree, based on the MST results) can be a major contributor of phosphorus to impacted rivers (Boehm et al., 2011; Jarvie et al., 2006).

Significant differences were also observed for *E. coli* and MST marker concentrations among

site types. ANOVA detected a significant difference between site types (outfall, ankle/chest-depth beach samples, and pore water samples) for *E. coli* concentrations in both the Sunnyside and Rouge beachsheds ($F_{2,245} = 49.73$, $P < 0.001$ and $F_{2,153} = 137.9$, $P < 0.001$, respectively). Pore samples had higher *E. coli* concentrations than outfalls and ankle- and chest-depth samples in the Sunnyside beachshed ($P < 0.001$ for both; Figure 2). However, in the Rouge beachshed, post-hoc analysis revealed that *E. coli* concentrations were significantly higher in outfalls than all beach sample types ($P = 0.004$ for pore samples, $P < 0.001$ for ankle- and chest-depth samples; Figure 2). Additionally, concordant significant differences were observed with regard to MST markers in both the Sunnyside and Rouge beachsheds ($F_{2,245} = 101.74$, $P < 0.001$ and $F_{2,153} = 318.10$, $P < 0.001$, respectively; Figure 1), with outfalls having significantly higher concentrations of the

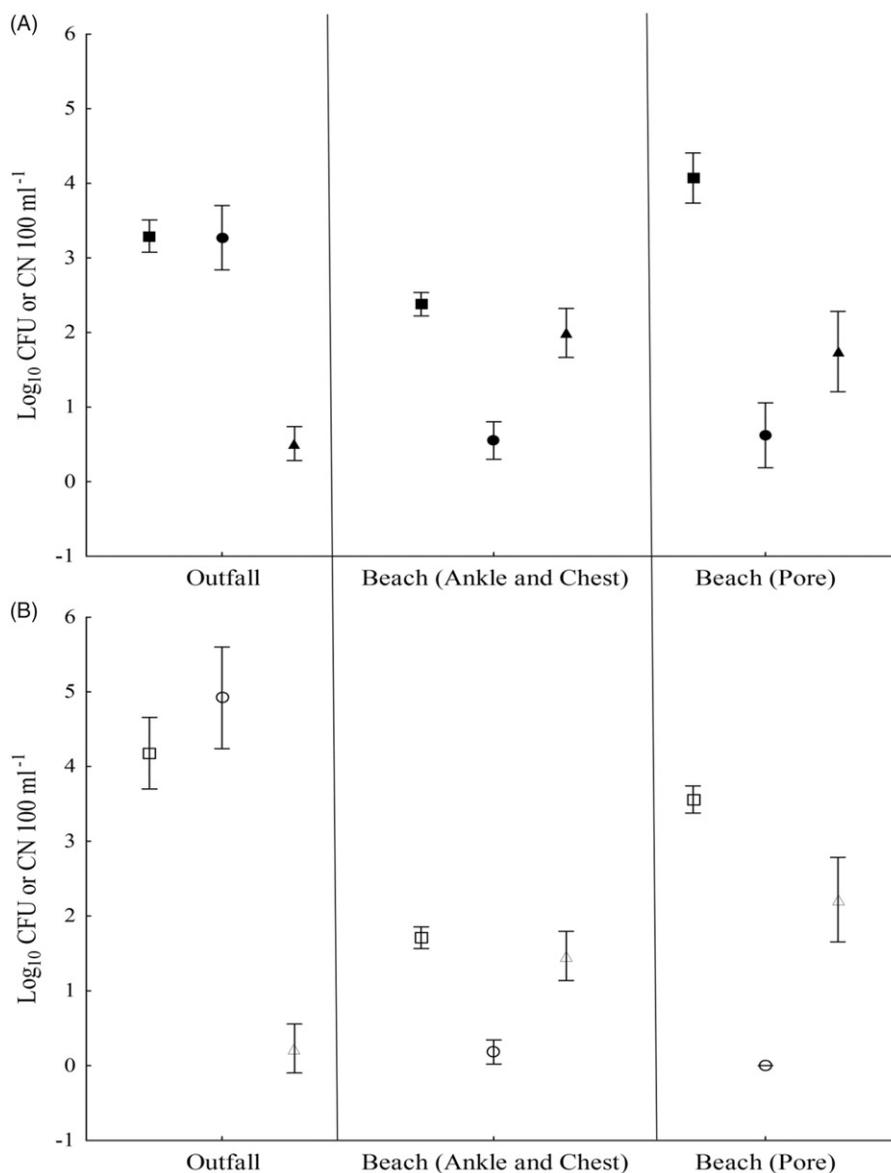


Figure 2. Mean concentrations of *E. coli* and the human and gull qPCR markers in (a) the Sunnyside beachshed and (b) the Rouge beachshed at each site type \pm SEM. Squares represent *E. coli*, circles represent the human marker, and triangles represent the gull marker.

human marker than beach sites ($P = < 0.001$ for both watersheds) and beach sites having significantly higher concentrations of the gull marker than outfall sites ($P < 0.001$ for both watersheds). Significant correlations between TP, *E. coli* and human marker concentrations were also observed in both beachsheds (Table 1). The elevated concentrations of TP, SRP, *E. coli* and the human MST marker at outfall sites, along with significant

correlations between these parameters, suggests that each outfall is impacted by sewage to some extent, resulting in not only an increase in *E. coli* concentrations, but also an influx of TP. This finding is consistent with previous studies in other watersheds which have observed correlations between *E. coli* and TP and have shown sewage to be a major contributor of phosphorus (Carrillo et al., 1985; Jarvie et al., 2006). While previous

Table 1. Spearman correlation coefficients between *E. coli* concentrations, gull and human DNA markers, and phosphorus assays for stormwater outfall and beach sites. ND indicates that a particular marker was never detected, while asterisks indicate a significant correlation ($P < 0.05$).

TP	<i>E. Coli</i>		TP		SRP	
	Sunnyside	Rouge	Sunnyside	Rouge	Sunnyside	Rouge
Outfall	0.25*	0.54*				
Beach (Ankle and Chest)	0.22*	0.15				
Beach (Pore)	0.18	.017				
SRP						
Outfall	−.034	0.70*	0.66*	0.81*		
Beach (Ankle and Chest)	−0.20	−0.09	0.80	0.72*		
Beach (Pore)	0.38	0.17	0.90*	0.04		
Human Marker						
Outfall	0.68*	0.61*	0.25*	0.31	0.58	0.35
Beach (Ankle and Chest)	0.12	0.39*	−0.14	−0.10	−0.26	−0.18
Beach (Pore)	0.00	ND	0.08	ND	0.41	ND
Gull Marker						
Outfall	0.10	−0.11	0.03	−0.09	ND	−0.2
Beach (Ankle and Chest)	0.35*	0.34*	0.07	−0.07	−0.77	−0.16
Beach (Pore)	0.29*	0.16	−0.16	0.09	0.46	0.20

studies have shown that outfalls would likely also contribute SRP (Boehm et al., 2011), which would be expected to correlate with *E. coli* and human marker concentrations, significant correlations were not observed in either the Sunnyside or Rouge beachshed. The relatively limited sample size with regard to SRP may have obscured this relationship in the present study.

With regard to beach samples, a significant difference was observed among pore water, ankle- and chest-depth samples at both Sunnyside Beach and Rouge Beach ($F_{4,240} = 22.61$, $P < 0.001$ and $F_{2,141} = 207.9$, $P < 0.001$, respectively) with TP concentrations being significantly higher in pore samples than ankle- or chest-depth samples (Figure 1). SRP concentrations did not significantly differ among shore normal locations. This suggested that foreshore beach sand was serving as a reservoir for TP, but not SRP, at both beaches. These results are similar to a previous study, which also found relatively higher levels of TP associated with the beach berm in Lake Michigan beaches (Cloutier et al., 2015). This phenomenon is similar to what is known for microorganisms at Great Lakes beaches such as *E. coli* (Alm et al., 2006; Whitman et al., 2014) and Aeromonads (Khan et al., 2009).

While high levels of TP in beach sand were associated with beach locations known to be heavily impacted by bird fecal droppings (Edge et al., 2010; Staley and Edge, 2016a), the associations between TP, *E. coli* and the gull DNA marker were not always clear. Among pore samples only, no significant correlations were observed at Rouge Beach. However, at Sunnyside Beach, significant correlations were found between concentrations of *E. coli* and the gull marker (Table 1). Elevated *E. coli* concentrations and significant correlations with the gull marker (with or without inclusion of pore samples) are consistent with predominantly gull fecal contamination along many beaches, as was observed for Sunnyside Beach in the previous year (Staley and Edge, 2016a). The presence of a large number of Canada geese and gulls at Sunnyside and Rouge Beach may contribute to the increased TP concentrations in the foreshore pore water samples, as waterfowl have been previously shown to be significant contributors of TP in the absence of other phosphorus inputs (Hahn et al., 2008; Manny et al., 1994; Unckless and Makarewicz, 2007). However, the lack of a significant correlation between concentrations of *E. coli* or the gull MST marker and TP may be

the result of differential decay, favoring a different rate of accumulation or loss of total phosphorus within the foreshore sand over the bathing season. MST markers have been shown to decay faster than culturable *E. coli* concentrations within foreshore pore water (Staley et al., 2015), with both bacterial species potentially decaying more rapidly than total phosphorus concentrations.

Conclusions

Total phosphorus concentrations were typically highest at our Toronto AOC study sites in foreshore beach sands impacted by bird fecal droppings. TP concentrations in beach sand pore water exceeded adjacent beach water concentrations at ankle and chest depth, and typically exceeded TP concentrations at stormwater outfalls with sewage cross-connections. Our results indicated that foreshore beach sands served as a reservoir of TP, but not SRP, over the bathing season. SRP was typically highest in stormwater outfalls impacted by human sewage cross-connections. While we hypothesized that both TP and SRP would be correlated to MST and *E. coli* concentrations, the only significant correlation was observed between concentrations of TP and the human marker in Sunnyside outfalls, suggesting that a direct relationship between phosphorus markers and human/gull fecal contamination may not always exist, possibly as a result of differential decay. However, as hypothesized, higher levels of SRP were found in the more sewage-impacted outfall sites, while higher levels of TP were found in the more gull contaminated pore water samples. Additional research is required to understand the significance of foreshore beach sand reservoirs of TP for contributing to localized changes to microbial communities and potentially unique eutrophication effects along beach shorelines.

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Supplemental material

Supplemental data for this article can be accessed on the [publisher's website](#).

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