Science of the Total Environment 630 (2018) 967–976

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Human *Bacteroides* and total coliforms as indicators of recent combined sewer overflows and rain events in urban creeks

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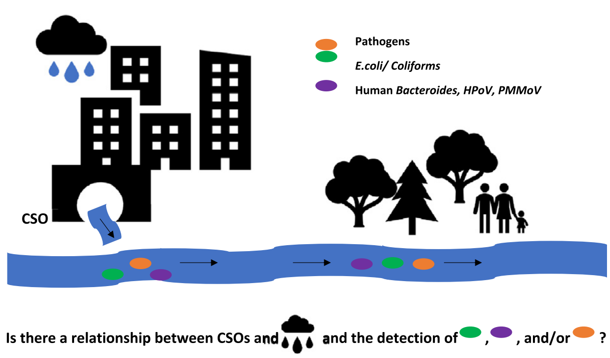
HIGHLIGHTS

- Measured relationships between fecal indicators, pathogens, CSOs, and rainfall
- Human *Bacteroides* and total coliforms correlated with CSOs and rainfall
- No indicators measured correlated with human pathogens
- Temporal relationships highlight the importance of survivability of organisms

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 November 2017
Received in revised form 8 February 2018
Accepted 9 February 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Fecal indicators
Combined sewer overflows (CSOs)
Enteric pathogens
Human *Bacteroides*
Stormwater
Rainfall

ABSTRACT

Combined sewer overflows (CSOs) are a known source of human fecal pollution and human pathogens in urban water bodies, which may present a significant public health threat. To monitor human fecal contamination in water, bacterial fecal indicator organisms (FIOs) are traditionally used. However, because FIOs are not specific to human sources and do not correlate with human pathogens, alternative fecal indicators detected using qPCR are becoming of interest to policymakers. For this reason, this study measured correlations between the number and duration of CSOs and mm of rainfall, concentrations of traditional FIOs and alternative indicators, and the presence of human pathogens in two urban creeks. Samples were collected May–July 2016 and analyzed for concentrations of FIOs (total coliforms and *E. coli*) using membrane filtration as well as for three alternative fecal indicators (human *Bacteroides* HF183 marker, human polyomavirus (HPoV), pepper mild mottle virus (PMMoV)) and nine human pathogens using qPCR. Four of the nine pathogens analyzed were detected at these sites including adenovirus, Enterohemorrhagic *E. coli*, norovirus, and *Salmonella*. Among all indicators studied, human *Bacteroides* and total coliforms were significantly correlated with recent CSO and rainfall events, while *E. coli*, PMMoV, and HPoV did not show consistent significant correlations. Further, human *Bacteroides* were a more specific indicator, while total coliforms were a more sensitive indicator of CSO and rainfall events. Results may have implications for the use and interpretation of these indicators in future policy or monitoring programs.

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https://doi.org/10.1016/j.scitotenv.2018.02.108
0048-9697/

1. Introduction

Combined sewer systems are a primary source of contamination of urban water bodies in the United States (Bryan Ellis and Yu, 1995). During rain events, these systems can overflow and release a mix of stormwater and wastewater into receiving waterways. As a result, these combined sewer overflows (CSOs) may introduce infectious pathogens that are found in human sewage into waterways used for drinking or recreation (Bryan Ellis and Yu, 1995; Ferguson et al., 1996; Rodríguez et al., 2012). Pathogens that have been identified in combined sewer outfalls or CSO-impacted waters include pathogenic bacteria such as *Campylobacter*, *E. coli*, and *Salmonella*, enteric viruses including adenovirus and norovirus (the most common cause of waterborne outbreaks), as well as protozoan parasites such as *Giardia* and *Cryptosporidium* (Eregno et al., 2016; ten Veldhuis et al., 2010; U.S. EPA, 2004). Recreation in CSO-impacted waters has been associated with an increased risk of gastrointestinal illness, which can adversely impact the health of certain groups including children (Cabelli et al., 1979; Wade et al., 2008) or low-income populations (Cabelli et al., 1979). In addition, pathogens found in CSO-impacted waters have been associated with waterborne disease outbreaks in the United States (Sinclair et al., 2009). For these reasons, CSOs are a potential threat to public and environmental health and may facilitate the spread of waterborne pathogens (Aslan et al., 2011).

Due to the expense and difficulty in isolating human pathogens from environmental samples, fecal indicator organisms (FIOs) are typically used to measure water quality and to create federal and state regulations for recreational water in the United States (United States Environmental Protection Agency (US EPA), 2004). Although epidemiologic studies have linked swimming-associated illness with high levels of FIOs (Wade et al., 2006, 2010), FIOs are not specific to human sources and therefore may not be indicative of contamination from human sewage (Boehm et al., 2009). FIOs may also originate from animal sources, including cats, dogs, and raccoons (Ram et al., 2007) or persist and grow in the environment (Solo-Gabriele et al., 2000), including in sediments and aquatic vegetation (Byappanahalli et al., 2003; Irvine and Pettibone, 1993). Further, it is well known that these indicators do not typically correlate with or are specific enough to predict the presence of pathogens (Bradshaw et al., 2016; Ferguson et al., 1996; Savichtcheva and Okabe, 2006; Wilkes et al., 2009).

Identifying contamination from human sources is important for protecting public health and setting environmental regulations (Field and Samadpour, 2007; Soller et al., 2010). For this reason, markers of human sewage detected using molecular methods have been of interest to both researchers and policy makers as potential alternative indicators of fecal pollution. Examples of these human sewage markers include the HF183 marker for human *Bacteroides* (Converse et al., 2009; Savichtcheva et al., 2007), human polyomavirus (HPoV) (Rachmadi et al., 2016), and pepper mild mottle virus (PMMoV) (Kuroda et al., 2015), all of which are found in high concentrations in human sewage and may be detected using qPCR. Despite the growing interest in the use of human sewage markers for measuring fecal contamination, there is a lack of research that examines the relationships between concentrations of these alternative indicators and the occurrence of CSOs and rainfall (Sauer et al., 2011; ten Veldhuis et al., 2010).

The purpose of this study was to better understand how concentrations of FIOs, human sewage markers, and human pathogens in urban creeks are related to recent CSOs and rainfall events. This study adds to existing literature by addressing a need to investigate relationships between alternative indicators including human *Bacteroides,* HPoV, PMMoV, and human pathogens including pathogenic bacteria and viruses in surface water (Field and Samadpour, 2007; Harwood et al., 2014; Wu et al., 2011). To our knowledge this is the only data set in the literature that has measured non-traditional fecal indicators (human *Bacteroides,* HPoV, PMMoV), total coliforms, *E. coli* and human pathogens simultaneously in an urban watershed while examining relationships with CSOs and rainfall. Further, this study addresses a need for improved understanding of how different types of traditional and alternative indicators of fecal pollution relate to CSOs and rainfall.

2. Methods

2.1. Study sites

Samples were collected from two sites along Cobbs and Tacony Creek in Philadelphia (Fig. 1). The creeks flow year-round and are located in city parks within residential sections of Philadelphia. Although the city parks provide some surrounding green space and tree coverage, Philadelphia as a whole has a large percentage of impervious surface coverage (about 54%). In addition, both sites are downstream of between 9 and 16 combined sewer outfalls which are monitored by the Philadelphia Water Department (Fig. 1). Because these sites are not downstream of any wastewater treatment plants (nearest plant is located 22 kms upstream on a water body that feeds into the Cobbs Creek sampling site) and are not thought to be significantly impacted by any industrial or agricultural centers, it is expected that CSOs and stormwater runoff are the primary sources of pollution for these sites. Further, urban creeks impacted by CSOs are also thought to be a common occurrence in the United States as the EPA estimates that over 746 communities in over 32 states have water bodies that are impacted by CSOs (US EPA, 2004).

Although recreation in Philadelphia's creeks is illegal, previous studies using hidden cameras have demonstrated that recreational activities, including wading, playing, and swimming occur in Philadelphia's creeks (Sunger et al., 2012). Study sites were chosen in areas where recreation is frequent using the data from Sunger et al. (2012), with an estimated 1–5 users engaged in these activities at the study sites per day during the May–September swimming season. In addition, during the present study, recreational activities were observed at both study sites.

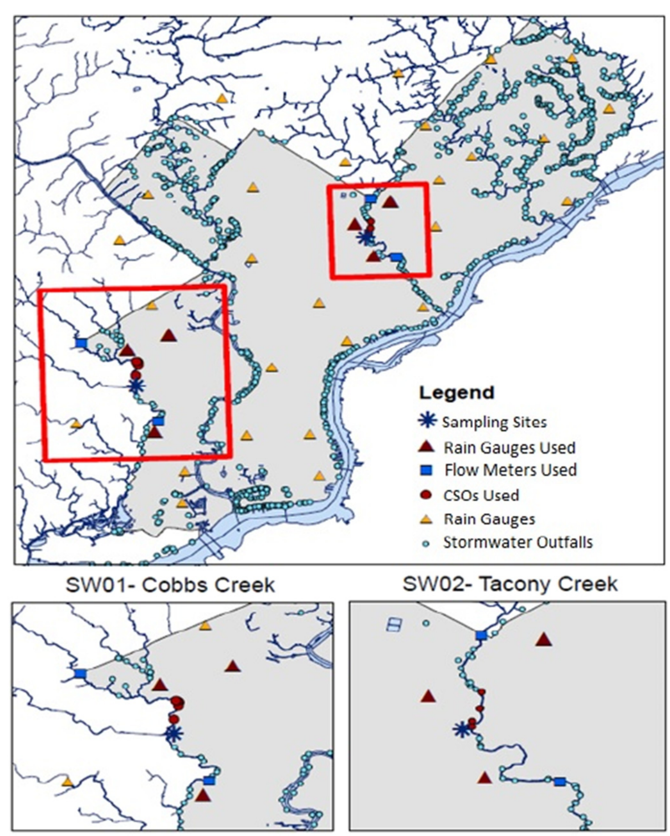

Fig. 1. Map of sampling sites, CSOs, and rain gauges used for analysis.