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Distribution and persistence of *Escherichia coli* and Enterococci in stream bed and bank sediments from two urban streams in Houston, TX

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HIGHLIGHTS

- Streambed and bank sediments were found to be a significant source of *E. coli* and enterococci bacteria to the water column.
- Viable *E. coli* and enterococci exist as deep as 60 cm in sediments.
- Sediments dominated by sand contained highest concentrations of fecal indicator bacteria.
- DNA fingerprinting analysis challenged the assumption that sediment resuspension only occurs in high flow conditions.
- Water quality goals may not be achievable due to an endless supply of fecal indicator bacteria from sediments.

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ABSTRACT

The purpose of this research was to determine if *Escherichia coli* and enterococci in streambed and bank sediments from two urban bayous, Buffalo Bayou and White Oak Bayou, in Houston, TX, USA are a significant source of the chronically high levels of these bacteria in the overlying water. The watersheds of the bayous lie within highly urbanized areas of Greater Houston and there is primary recreational contact with the public. Extensive sampling of the watersheds was conducted from 2008 to 2010. Both fecal indicator bacteria were found at $\geq 10^4$ MPN g dry wt.$^{-1}$ concentrations in the upper 1 cm of sediment cores with declines by orders of magnitude at 15 and 30 cm sediment horizons and in some cases 60 cm, but, nonetheless, indicating that they can remain viable even at depth. No interannual variation was observed. And, there was no correlation with percent organic matter, however there was moderate correlation ($R^2 = 0.12$; $p = 0.001$) of *E. coli* with sediment moisture. In sediments, most *E. coli* and enterococci in Buffalo Bayou (76%) and White Oak Bayou (87.5%) were associated with fine sand grains (60 to 250 μm). In the water column, *E. coli* was associated, in roughly equal percentages, with particle sizes <10, 10–25, 25–63, and ≥63 μm (21.9, 25.6, 30.4, and 32.9%, respectively). Enterococci were mostly attached to particle sizes in the ranges of 10–25 μm (36.0%) and 25–63 μm (31.1%) as well as ≥63 μm (37.7%) ($p = 0.0001$). Fingerprinting of *E. coli* isolates from both bayous with Rep-PCR and the BOX A1R primer was used to demonstrate translocation of sediments from the upper to lower watersheds.

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1. Introduction

Escherichia coli (EC) and enterococci (ENT) are the fecal indicator bacteria (FIB) recommended by the US Environmental Protection Agency (USEPA, 2012), the European Environment Agency (EEA, 2006) and the World Health Organization (WHO, 2001) to assess contamination of water bodies with fecal matter. Conventional sources include wastewater treatment plants, septic tank systems, animal manure, and storm water run-off (USEPA, 2012). Another source that is increasingly studied is bottom sediments.

Bottom sediments in aquatic systems are potentially large reservoirs of EC and ENT. Because of their tendency to attach to suspended sediments and flocs (Droppo and Ongley, 1994; Droppo et al., 2011), EC and ENT, and presumably other fecal pathogens, ultimately settle out to bottom sediments where they can survive and persist for months compared to only a few days in the water column (Anderson et al., 2005; Burton Jr. et al., 1987; Craig et al., 2004; Haller et al., 2009b). Both EC and ENT have been found at concentrations of 10^1 to 10^7 most probable number (MPN) or colony forming units (CFU) per g dry weight (GDW) in bottom sediments which are typically several fold higher than those in the overlying water column (Byappanahalli et al., 2012; Pachepsky and Shelton, 2011). Resuspension of bottom sediments allows EC and ENT to re-enter the water column either attached to sediments or free-living (Jamieson et al., 2005). This re-entry of EC and ENT can affect the results of water quality assessments.

We conducted a three-year study of EC and ENT in the streambed and bank sediments in two urban watersheds—Buffalo Bayou and White Oak Bayou located in the Greater Houston, TX (USA) Metropolitan Area. These watersheds have an almost 30 year documented history of chronically elevated FIB. In 1996 the main channels of the bayous were placed on the Texas Water Quality Inventory and Clean Water Act's Section 303d lists of Impaired Water Bodies (TCEQ, 2009). Fifteen of their tributaries were later added to the lists in 2002 and 2006. Both watersheds lie entirely within the most urbanized sectors of Houston and therefore present a high exposure potential of FIB and other pathogens to the public. Buffalo Bayou flows approximately 85 km from its upper watershed in west Houston to downtown where it widens to become the Houston Ship Channel and then discharges into Galveston Bay, located 40 km to the east. White Oak Bayou flows 37 km from its upper watershed in northwest Houston to its confluence with Buffalo Bayou in downtown Houston. More than 80% of the White Oak Bayou watershed is channelized with concrete or grass berms to convey storm water whereas most of Buffalo Bayou and its tributaries still have a natural streambed and riparian zone. Wastewater treatment plant (WWTP) effluents contribute 99% of the base flow in each bayou (TCEQ, 2009).

Despite self-reported regulatory compliance by the 126 wastewater treatment plants (WWTP) in the two watersheds (TCEQ, 2009), concentrations of EC have continued, even in dry weather, to exceed the USEPA's water quality standard for primary contact recreation (i.e. geometric mean of 126 MPN 100 ml^{-1}) and even secondary contact recreation (geometric mean of 630 MPN 100 ml^{-1}) by 10 to 100 fold indicating the existence of another source or sources. Excessive quantities of FIB in water and sediments have been linked to increased risk of pathogenic microorganism-induced illnesses to humans (Benham et al., 2006b; Donovan et al., 2008). Nonetheless, both bayous are fully accessible, without barriers, to the public and are frequently used for kayaking and canoeing, (i.e. secondary contact) and potentially for swimming (i.e. primary contact). There are several restoration projects and an interpretative nature center that involve the primary contact of school-aged children with bayou waters. Therein lies the conundrum because installing barriers on the bayous to restrict access is not an option to protect public health.

A Total Maximum Daily Loads (TMDL) study of all identifiable point sources and nonpoint sources concluded that the high EC loads were caused by illicit storm drain discharges during dry weather and storm water runoff during wet weather conditions (TCEQ, 2009). Resuspension of streambed sediments was also factored into the loading model, but only as a minor (<2%) source of EC and only for high flows occurring during rain events (Peterson et al., 2009). Our aim was to determine if the streambed and bank sediments in the bayous are a significant source of EC and ENT that could account for the chronically high levels found in the water column. We sampled by coring to determine if EC and ENT were depth limited or if sediments were a potentially endless source of these bacteria.

2. Experimental section

2.1. Site description and sampling

Houston, TX has a subtropical climate with average annual precipitation of ~127 cm and temperatures ranging from 18 °C in winter months to 35 °C in summer. The watersheds of Buffalo Bayou and White Oak Bayou have a combined drainage area of 1204 km^2. Each bayou has an average low or base flow of ~1.29 $m^3\ s^{-1}$ (TCEQ, 2009). From March to August in 2008, 2009, and 2010, sediment cores and water samples were collected at 15 sites within the Buffalo Bayou watershed (Fig. 1). Water samples were collected from 14 sites in White Oak Bayou watershed, however because it is lined with concrete in the lower reaches, sediment cores could only be collected at 7 sites located in the upper watershed. For continuity of data, sampling sites were the same as those used by the TMDL study (TCEQ, 2009). Samples were collected three to five times at most sites, and at some sites as many as 10 times, during the three year study period.

Water was collected approximately 1 m from the surface using an acid rinsed bucket and then transferred to sterile plastic jars. Because of variable stream heights, we arbitrarily collected sediment cores at the water line (WL) and 1 m above (AWL) and below (BWL) the water line using acid washed, clear, plexiglass core sleeves (9 cm diameter and 40 cm long) that were driven to a depth of ~35 cm and capped for upright transport back to the lab. At some sites 1 m below the water line (BWL), a core sleeve of ~70 cm was used to collect sediments at greater depth. At site BB8, in 2008, we also collected cores along a horizontal transect every 1 m starting midstream and extending to 5 m landward above the water line. Using sterile technique, sediment cores were extruded from core sleeves and subsampled at 1 cm, 15 cm, and 30 cm, and 60 cm horizons. The subsamples were then aliquotted for analysis of EC and ENT concentrations, moisture, and sediment grain size. Both sediment and water samples were stored at 4 °C for transport and were processed within 4 h of collection.

2.2. Determination of FIB concentrations

The IDEXX Colilert-18 and Enterolert with the Quantitray/2000 methods were used to estimate concentrations of EC and ENT, respectively, in water and sediments using most probable number (MPN) determination. Water samples (100 ml) were analyzed according to manufacturer instructions. For sediment samples, we followed the protocol described by (Byappanahalli et al., 2003) in which 5 g of fresh sediment was elutriated in a sterile, 50 ml conical tube by adding 35 ml of sterile distilled water and vortexing for 2 min. After standing for another 2 min, 10 ml of the elutriate was transferred to 90 ml of sterile, distilled water and then analyzed with both EC and ENT IDEXX methods. Positive (*E. coli* ATCC 11775) and negative (*Pseudomonas aeruginosa* ATCC 10145) controls were used for all sampling sets analyzed with Colilert 18. Similarly, positive and negative controls were used for Enterolert (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862, respectively). Concentrations of EC and ENT in sediments are expressed as MPN per g dry weight (GDW). Statistical analysis of data was conducted with STATA (version 13.1, Statacorp, College Station, TX) using a p value of <0.05 unless otherwise specified. For ANOVA Bartlett's test was conducted to confirm equal variances.