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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.

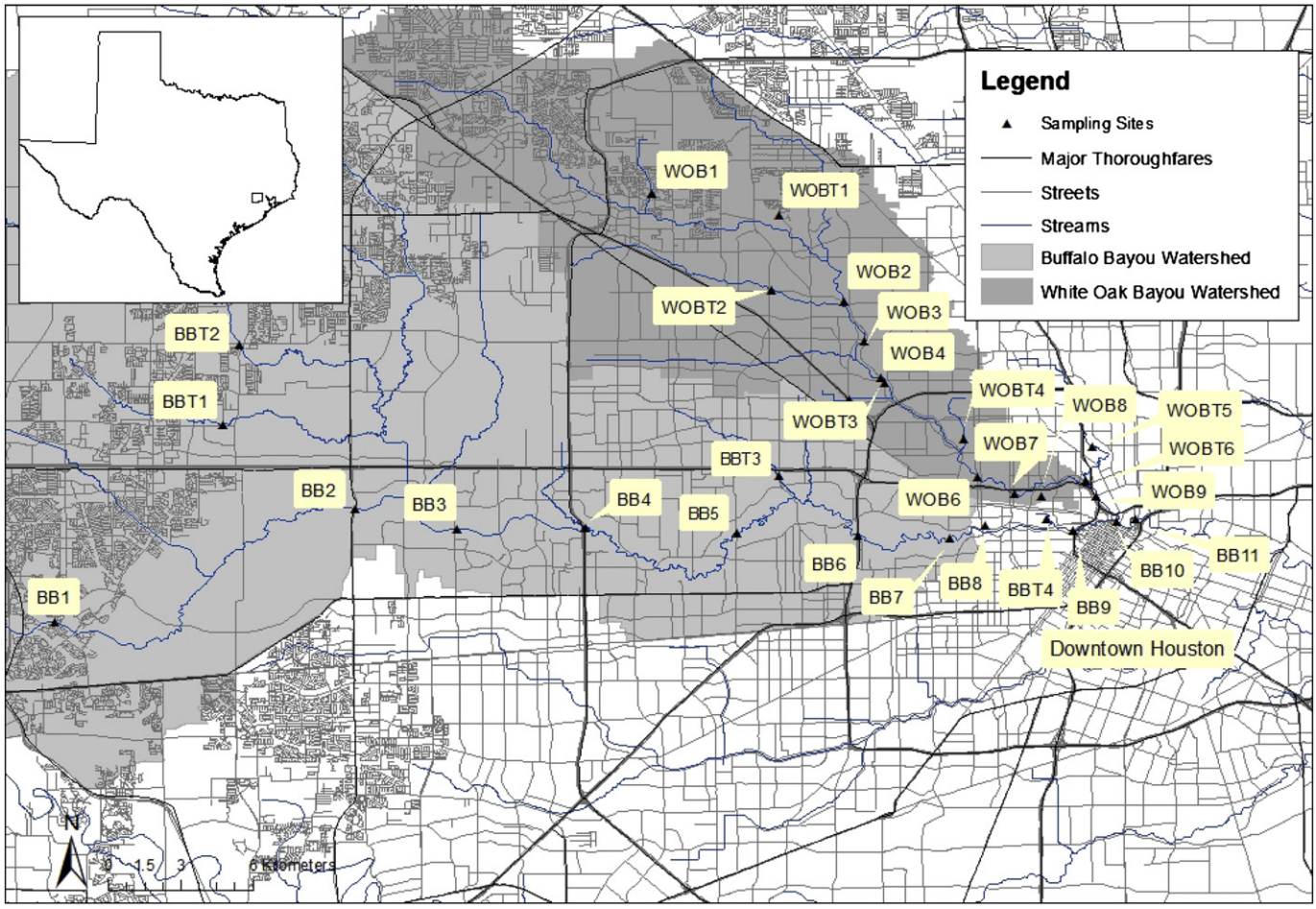


Fig. 1. Sampling sites in Buffalo Bayou and White Oak Bayou, Houston, TX. BB = Buffalo Bayou, BBT = Buffalo Bayou Tributary, WOB = White Oak Bayou, WOBT = White Oak Bayou Tributary.

2.3. Rep-PCR and BOX A1R primer fingerprinting

After IDEXX MPN estimations were completed, 500 μl from at least three 'positive' wells (i.e. fluorescing a blue color) in the Colilert 18 (i.e. EC) IDEXX trays were withdrawn using a sterile syringe + needle and then applied and spread onto selective and differential mTEC agar plates to confirm the presence of EC. The plates were incubated at 35 °C ± 0.5 °C for 2 ± 0.5 h to resuscitate injured or stressed bacteria, and then incubated at 44.5 °C ± 0.2 °C for 22 ± 2 h. Once typical red/magenta colonies were observed, they were transferred to EMB agar plates and incubated at 35 °C for 24 h. Positive colonies, recognized by a green sheen, were individually picked and transferred to sterile, 2 ml tubes containing Luria–Bertani broth and then incubated for another 24 h at 35 °C ± 0.5 °C. Thirty to fifty *E. coli* were isolated from each sample. In total, approximately 30,000 sediment strains and 6000 water strains were isolated. Bacterial cells were harvested by centrifugation and genomic DNA was extracted with 3% cetyltrimethylammonium bromide (CTAB) for 30 m followed by purification with 25:24:1 phenol:chloroform:isoamyl alcohol. DNA quantity and purity were determined spectrophotometrically using a NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE, USA).

Rep-PCR with the BOX A1R primer fingerprinting (Dombek et al., 2000) of EC environmental and control isolates (*E. coli* ATCC 11775) was conducted with a 20 μl PCR mixture containing 25–50 ng template DNA, PCR supermix (Promega Corp.) containing 1 × PCR buffer, 1 U *Taq* polymerase, 3 mM $MgCl_2$, and 400 μM each dNTP. The BOX A1R primer (5′-CTACGGCAAGGCGACGCTGACG-3′) was added at 1 μM final concentration. The thermocycling program had an initial incubation at 95 °C for 2 min, followed by 30 cycles of 94 °C for 3 s, 92 °C for 30 s, 50 °C for 1 min, and a final extension step at 65 °C for 8 min. The resulting amplified DNA bands from both environmental isolates and controls were visualized on 1% agarose gels, stained in a 3% ethidium bromide bath and digitally documented using a BioRad Gel Documentation System (BioRad, Hercules, CA). A size marker, Genescan-2500 (Applied Biosystems, Foster City, CA) containing 27 fragments of sizes 55 to 2500 bp was also loaded onto gels in 3 lanes (sides and center) to aid with the normalization of the gels. Digital gel images were imported into the Bionumerics software, version 6.01, (Bionumerics, Austin, TX) for lane identification, normalization, and band calling. DNA fingerprint similarities were calculated using the band-based Dice coefficient set at an optimization of 1.5% and tolerance of 1.7%. Dendrograms were generated using the unweighted pair group method with arithmetic means (UPGMA). More than 4000 BOX A1R fingerprints produced from individual EC isolates were included in the analyses but for the purpose of this manuscript, a representative subsample is presented. Isolates having genetic similarity of ≥92% were defined as being the same strain as determined by previous studies conducted by Ishii et al. (2006) and Johnson et al. (2004).

2.4. Sediment grain size and moisture determination

Grain size composition of sediments collected in 2008 was conducted with a Malvern Mastersizer 2000 Laser Particle Size Analyzer. Sub-samples from same sediment cores (i.e. AWL, WL, and BWL) and horizons (i.e. 1 cm, 15 cm, and 30 cm) used for bacterial analyses, were analyzed to determine percentage of clays (submicron), silt (2–63 μm),

sand (63 μm–2 mm), and gravel (2–63 mm). Sediment moisture content was calculated from the dry weight determination. Briefly, wet sediments (5 g) were weighed in preweighed glass beakers then dried at 105 °C for 48 h. After cooling in a dessicator, sediments, in the glass beakers, were weighed again. Percent moisture was calculated from the difference in weight of wet and dry sediments.

3. Results

3.1. Distribution of EC and ENT in sediments

Bacterial concentration data at each site, for all years, were grouped according to cores (AWL, WL, and BWL) to compare horizons (1, 15, 30, and 60 cm). The highest concentrations (10^4 MPN GDW^{-1} in Buffalo Bayou and 10^4 to 10^5 MPN GDW^{-1} in White Oak Bayou) of EC and ENT (Tables 1 and 2) were consistently found in the upper 1 cm of sediments. This was true for all cores whether collected at the water line (WL), 1 m above (AWL), or 1 m below the waterline (BWL). We also observed a significant order of magnitude or more reduction in EC and ENT concentrations from the top 1 cm (10^4 to 10^5) to the deeper 15, 30, and 60 cm horizons (10^3 to 10^2). No upstream to downstream differences in EC and ENT concentrations in sediments were observed. Proximity of sampling sites to wastewater treatment plant effluent discharge points was also compared but, again, no differences were observed in EC and ENT concentrations.

Since most samples were collected each year between April and August and had similar water temperatures, a one-way ANOVA was conducted to determine if there were 'year to year' temporal differences at each sampling site (Tables S1 and S2). EC and ENT concentrations collected in 2008, 2009, and 2010 were statistically the same at each site, indicating that the streambed and bank sediments are a persistent reservoir for these FIBs. The only exception occurred at Buffalo Bayou site BB11 in the BWL core where EC concentrations were higher in 2008 than in 2009 and 2010 (Table S1; $p = 0.04$).

Table 1
EC and ENT MPN GDW^{-1} in Buffalo Bayou sediment cores collected 1 m above the water line (AWL), at the water line (WL), and below the water line (BWL) at 0, 15, and 30 cm horizons. At some stations, an additional horizon at 60 cm was collected for the BWL cores.

EC

Core	Horizon (cm)	n	Mean	St. dev.	Between horizons p	Between cores p
AWL	0	71	2.5×10^4	5.9×10^3	0.0001	0.107
	15	71	2.5×10^3	1.4×10^3		
	30	71	1.3×10^3	1.1×10^3		
WL	0	73	1.8×10^4	3.9×10^3	0.00001	
	15	73	2.6×10^3	1.1×10^3		
	30	73	2.7×10^3	1.8×10^3		
BWL	0	69	1.4×10^4	2.8×10^3	0.0238	
	15	69	1.8×10^3	5.9×10^2		
	30	69	8.3×10^3	5.7×10^3		
	60	16	2.3×10^3	8.7×10^2		

ENT

Core	Horizon (cm)	n	Mean	St. dev.	Between horizons p	Between cores p
AWL	0	70	2.2×10^4	3.6×10^3	0.05	0.4564
	15	70	1.3×10^3	2.8×10^3		
	30	60	1.4×10^3	3.0×10^3		
WL	0	80	1.8×10^4	3.3×10^3	0.0006	
	15	78	7.8×10^3	2.2×10^3		
	30	73	8.5×10^3	2.1×10^3		
BWL	0	72	1.6×10^4	3.2×10^3	0.006	
	15	72	6.7×10^3	1.4×10^4		
	30	65	8.5×10^2	2.0×10^2		
	60	16	1.7×10^3	3.6×10^3		

Table 2
EC and ENT MPN GDW^{-1} in White Oak Bayou sediment cores collected 1 m above the water line (AWL), at the water line (WL), and 1 m below the water line (BWL) at 0, 15, and 30 cm horizons. At some stations, an additional horizon at 60 cm was collected for the BWL cores.

EC

Core	Horizon (cm)	n	Mean	St. dev.	Between horizons p	Between cores p
AWL	0	35	2.0×10^5	1.4×10^5	0.026	0.5352
	15	33	3.7×10^3	1.0×10^2		
	30	34	1.8×10^2	2.8×10^1		
WL	0	33	1.1×10^4	2.3×10^3	0.0001	
	15	34	1.7×10^3	7.2×10^2		
	30	32	5.5×10^2	2.5×10^2		
BWL	0	30	2.2×10^5	1.1×10^3	0.0472	
	15	31	1.6×10^3	4.0×10^3		
	30	30	2.8×10^3	9.2×10^2		
	60	15	1.7×10^2	1.6×10^2		

ENT

Core	Horizon (cm)	n	Mean	St. dev.	Between horizons p	Between cores p
AWL	0	33	3.0×10^4	3.8×10^3		
	15	35	1.8×10^3	3.3×10^3	0.0290	
	30	33	1.4×10^3	3.1×10^3		
WL	0	34	2.2×10^4	3.3×10^3		
	15	36	1.4×10^3	3.0×10^3	0.0019	0.1353
	30	34	5.1×10^3	1.2×10^3		
BWL	0	31	2.9×10^4	9.9×10^3		
	15	31	7.2×10^3	2.2×10^3	0.05	
	30	29	4.8×10^3	1.7×10^3		
	60	15	1.9×10^2	4.9×10^3		

3.2. Horizontal transect of streambed and bank

We also conducted a transect analysis of EC and ENT at site BB8 to examine distribution from midstream and onto the dry stream bank. Cores were collected every 1 m starting midstream (core 0) and landward onto the stream bank for 8 m. Highest EC (10^5 MPN GDW^{-1}) occurred midstream and was consistent in all horizons (i.e. 1, 15, and 30 cm) (Fig. 2A). In the top 1 cm, concentrations decreased gradually until 1 m above the waterline (core 4) where concentrations fell to 10^4 MPN GDW^{-1} and then decreased again to 10^3 MPN GDW^{-1} with increasing distance from the waterline ($R^2 = 0.71$, $p = 0.02$). Although not significant, in the 15 and 30 cm horizons, EC decreased to 10^3 MPN GDW^{-1} only 2 m (core 2) away from midstream and remained at this concentration as distance increased from midstream and finally decreased further to 10^1 MPN GDW^{-1} at core 8 located on the dry stream bank. Highest ENT (10^4 MPN GDW^{-1}) concentrations also occurred midstream in the top 1 cm (not significant) but fell to 10^3 MPN GDW^{-1} at 15 cm and 10^2 MPN GDW^{-1} at 30 cm (Fig. 2B).

3.3. Sediment moisture

In Buffalo Bayou, moisture content in cores collected 1 m above the water (AWL) line ranged from 8.5 to 19.4%, at the water line (WL) 9.1 to 21.2%, and below the water line (BWL) 9.2 to 36.4%. In White Oak Bayou, moisture in AWL cores ranged from 7.9 to 18.2%, WL cores 8.7 to 18.6%, and BWL cores 9.2 to 25.7%. EC concentrations were only weakly correlated with sediment moisture ($R^2 = 0.12$; $p = 0.001$) while for ENT there was no correlation.

We also examined sediment moisture at the transect site BB8. There was a weak correlation of EC and ENT with sediment moisture ($R^2 = 0.21$; $p = 0.001$ and $R^2 = 0.29$; $p = 0.001$, respectively). Cores were collected every 1 m starting midstream (core 0) and landward onto the stream bank for 8 m. Moisture (Fig. 2C) in all core horizons was approximately 25% of sediment weight in the midstream sediment

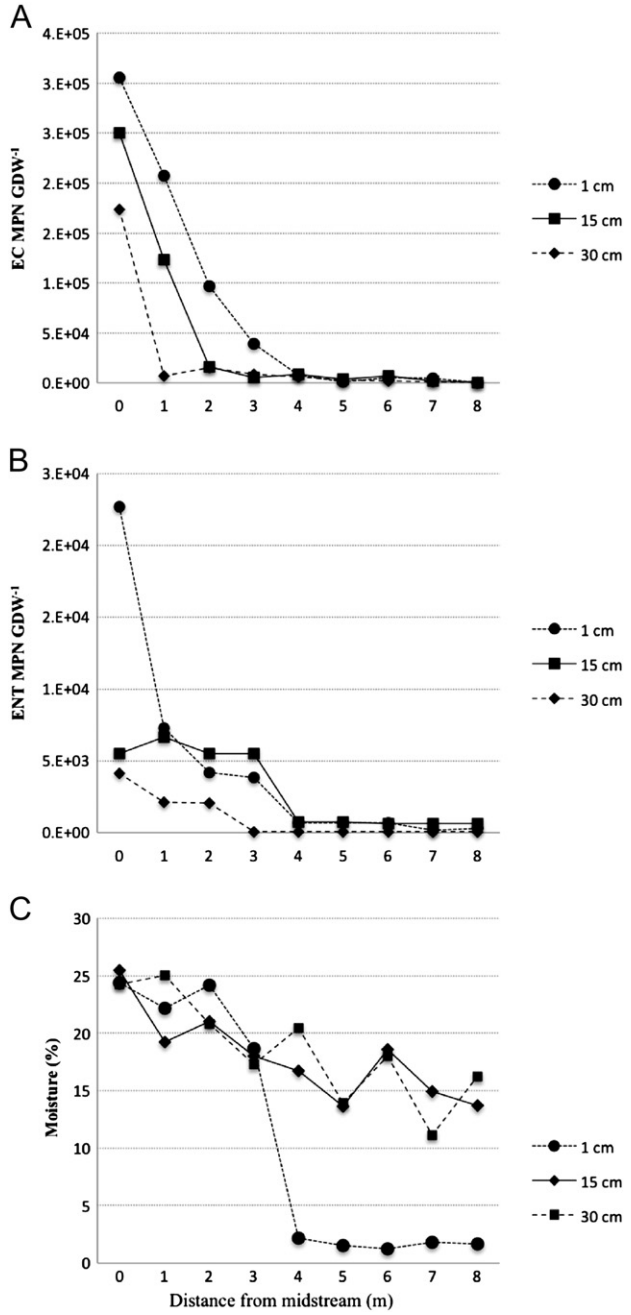


Fig. 2. Horizontal transect of sediment at site BB8 in Buffalo Bayou of EC (A) and ENT (B) (MPN GDW^{-1}) and percent moisture (C). Cores were collected every 1 m starting midstream (0) and progressing landward up onto the stream bank. Core 3 was collected at the water line and cores 4–8 were collected above the water line on the stream bank.

(0 m) but the upper 1 cm horizon rapidly decreased to <5% in cores collected from 4 to 8 m on the stream bank. In the deeper horizons (15 and 30 cm), percent moisture within the same cores (4 to 8 m) was variable ranging from <5 to 20%. Moisture content was highest in the deepest horizon (30 cm) for all transect cores. Highest EC and ENT concentrations were associated with sediments containing at least 2% moisture.

3.4. Sediment grain size vs. EC and ENT

Grain size composition of streambed and bank sediments in both bayous was examined to determine if EC or ENT were preferentially found in sediments having a particular grain size as reviewed by Pachebsky and Shelton (2011). Regardless of core (i.e. AWL, WL, or BWL) or horizon, most EC and ENT in Buffalo Bayou sediments (76%) and White Oak Bayou sediments (87.5%), were associated with fine sand grains (60 to 250 μm). This association is logical since Buffalo Bayou contained, on average, mostly sand (74%), with 4% clay, 16% silt, and 6% gravel (Table S3). White Oak Bayou contained more sand (84%) than Buffalo Bayou, but less clay (0.7%) and more silt (26%), however gravel content (5%) was similar. In general, sediments contained mostly sand followed by silt.

3.5. EC and ENT concentrations in water

EC and ENT ranged from 37 to 5000 MPN 100 ml^{-1} in Buffalo Bayou and 3.5 to 4800 MPN 100 ml^{-1} in White Oak Bayou (Fig. 3). There was no significant correlation between EC concentrations in the water column and sediments in either bayou (data not shown). However, there was a moderate correlation ($R^2 = 0.39$, $p = 0.002$) between ENT concentrations in water and sediment in Buffalo Bayou but not in White Oak Bayou.

We conducted a size fractionation analysis of total suspended solids (TSS) in water collected at Buffalo Bayou site BB8 (Table 3) and found roughly equal percentages of the total EC concentration attached to particle sizes <10, 10–25, 25–63, and ≥63 μm (21.9, 25.6, 30.4, and 32.9%, respectively). ENT 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%) (one-way ANOVA; $p = 0.0001$). Based upon TSS size distribution, 78% of EC and 80% of ENT were associated with fine suspended solids (FSS).

3.6. BOX A1R fingerprinting of EC

Like Wu et al. (2009) we used DNA fingerprinting to demonstrate resuspension and translocation of sediment EC isolates in a watershed. However, rather than ribotyping, we used the whole genomic fingerprinting technique of rep-PCR with the BOX A1R primer. The similarity dendrogram of BOX A1R fingerprints was dereplicated for figure S1, however we analyzed more than 4000 EC strains isolated from water and sediments (AWL, WL, and BWL) at tributary sites BBT3 and BBT2 and main stem sites BB1, BB3, BB4, BB6, BB8, BB9, and BB11 in Buffalo Bayou. Fig. 4 is an example of a fingerprint dendrogram for Buffalo Bayou isolates. Isolates having ≥92% fingerprint similarity were considered to be the same strain. Frequently, fingerprints of water (labeled as 'H2O') and sediment (labeled as 'Sediment') isolates from tributary or main stem sites located in the upper watershed were the same as those found in bed and bank sediments at sites located downstream indicating translocation of sediments from upstream to downstream. For example, water isolates from tributary site BBT2 were the same as sediment isolates from downstream site BB4. And, fingerprints of sediment isolates from site BB3 were the same as those found in sediments from downstream sites BB6 and BB11. Some water isolates were also the same as those found in sediments collected at the same site indicating resuspension. In total, 72% of EC isolates from the water column had ≥92% similar BOX A1R fingerprint similarity to EC isolates from the streambed and bank (30% of these had 100% similarity). Similarities of water isolates with sediment isolates were not limited to the top 1 cm. In several cases, water isolates were the same (i.e. 100% similar) as isolates from deeper sediment horizons (e.g. 15, 30, and 60 cm). At site BB8, isolates from size-fractionated water samples (<10, 10–25, 25–63, and >63 μm) frequently had 100% similar fingerprints to isolates from sediment samples throughout the watershed.

Since the lower watershed of White Oak Bayou is lined with concrete, we could only compare EC isolates from water at downstream sites WOB4, WOB3, WOB7, and BB11 (below the confluence of the bayous) to those from sediments in upper watershed tributaries WOBT2 and WOBT4. Again, the similarity dendrogram was dereplicated for figure S2. Like Buffalo Bayou, many water isolates had the same

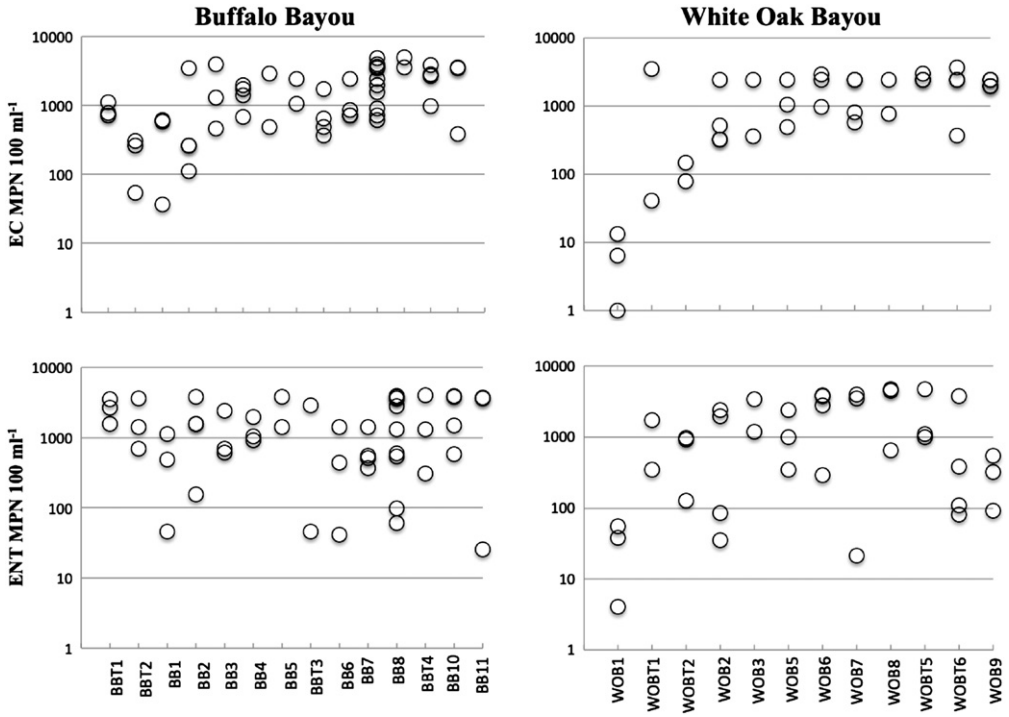


Fig. 3. EC and ENT concentrations (MPN 100ml^{-1}) in water in Buffalo Bayou and White Oak Bayou.

fingerprints (i.e. ≥ 92% similarity) as sediment isolates from upstream tributaries indicating sediment resuspension in the upper watershed and translocation to the lower watershed. For example, water samples from sites WOB3, WOB7, and BB11 in the lower watershed were the same as those from sediment at site WOBT2 located on a tributary in the upper watershed. Isolates from WOBT4 and WOB3 sediments were 92 to 100% identical to isolates from BB11 sediments. And, isolates from water and sediments collected at the same sites were frequently the same indicating resuspension of streambed sediments. In total, 36% of water isolates had ≥92% Box A1R fingerprint similarity to sediment isolates.

4. Discussion

In this study, we determined that streambed and bank sediments are significant sources of EC that could contribute to the chronically elevated levels in Buffalo Bayou and White Oak Bayou.

The highest concentrations of EC and ENT were found in the upper 1 cm of sediments. These results are in agreement with those from Alm et al. (2003), Haller et al. (2009), and Ferguson et al. (1996) who observed steep decreases in concentrations of FIB with depth. These decreases have been attributed to die off or inactivation (Pachepsky and Shelton, 2011). For EC, 90% die off has been observed in as few as 7 days to as long as 85 days depending upon the environment i.e. freshwater vs. marine, soil vs. sediment and physical parameters such as moisture and temperature (Anderson et al., 2005; Benham et al., 2006a; Davies et al., 1995; Garzio-Hadzick et al., 2010; Haller et al., 2009a; Van Donsel and Geldreich, 1971). We can estimate the die off time for EC and ENT in the bayous using Chick's Law:lnC = ln C_0 - μtwhere C and C_0 are initial and ending concentrations, respectively; μ is the inactivation rate; and t is time and using an inactivation rate constant of 0.03 for EC determined by Anderson et al. (2005) for freshwater sediment mesocosms inoculated with EC and ENT from wastewater. Therefore, a 90% die off of EC at 15 cm takes 77 days, and another 90% die off from 15 to 30 cm an additional 77 days so that the decrease in EC concentration at the top 1 cm of 10^4 MPN GDW^{-1} to 10^2 MPN GDW^{-1} at 30 cm takes about 154 days (i.e. 99% die off). For ENT, the same calculation using the inactivation rate constant of 0.21 (Anderson et al., 2005) results in a 99% die off in approximately 22 days. According to our calculations, survival of EC in bayou sediments is 7× longer than that of ENT. These results are in good agreement with Anderson et al. (2005) who determined that fecal coliforms (i.e. EC) had longer survival rates than ENT in fresh water.

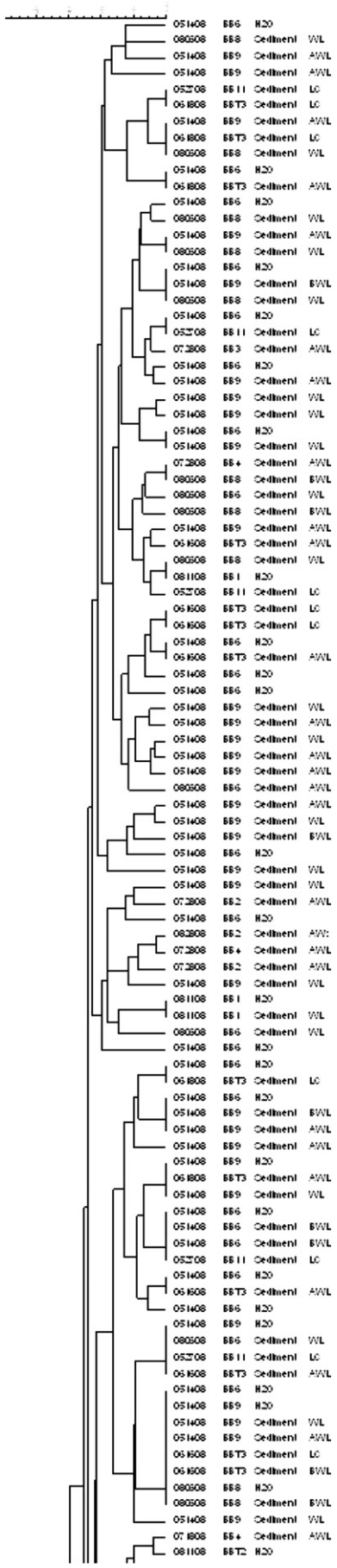
We only sampled during spring and summer months and so we could not determine if there were winter versus summer temporal variations as observed by others (Byappanahalli et al., 2003). We did measure lower water and sediment temperatures in March–April (range 15 to 19 °C) than in May–Aug (range 25 to 28.3 °C) (see Duan et al., 2014) but the differences were apparently not extreme enough to influence bacterial concentrations. Goyal et al. (1977) who examined fecal coliforms in sediments of Houston Area canal communities also found no change in concentration based upon temperatures of similar ranges. Other researchers have reported wet versus dry season alterations in sediment concentrations (Buckley et al., 1998; Crabill et al., 1999). The Houston area does not have distinct wet and dry periods or seasonal 'flushing' of sediments. And, the fact that almost 100% of the water in both bayous is from WWTP discharges means that there is flow throughout the year. We also found no differences in EC and ENT concentrations from 2008 to 2010 (Tables S1 and S2), at least during the months of March to August, indicating that bayou sediments are a long-term reservoir for these indicator bacteria.

The balance between sediment grain size or texture, moisture, and organic matter appears to be key for higher survival rates of EC and

Table 3
EC and ENT associated with different suspended sediment size fractions.

Fraction	EC		ENT	
	MPN/100 ml	%	MPN/100 ml	%
Unfiltered water	$8.2 \times 10^3 \pm 4.6 \times 10^2$	–	$6.1 \times 10^3 \pm 3.7 = \times 10^2$	–
<10 μm	$1.8 \times 10^3 \pm 6.8 \times 10^2$	21.9	$8.3 \times 10^2 \pm 1.0 \times 10^1$*	13.6
10–25 μm	$2.1 \times 10^3 \pm 5.5 \times 10^2$	25.6	$2.2 \times 10^3 \pm 1.9 \times 10^2$	36.0
25–63 μm	$2.5 \times 10^3 \pm 6.3 \times 10^2$	30.4	$1.9 \times 10^3 \pm 7.8 \times 10^2$	31.1
≥63 μm	$2.7 \times 10^3 \pm 6.8 \times 10^2$	32.9	$2.3 \times 10^3 \pm 3.1 \times 10^2$	37.7

* $p = 0.0001$.



other FIB in sediments. Sediments having higher fractions of cohesive silts and fine sands tend to have higher concentrations of EC and ENT (LaLiberte and Grimes, 1982; Piorkowski et al., 2014; Jamieson et al., 2005). LaLiberte and Grimes (1982) compared EC survival/concentration at two sites in a navigation lake on the Mississippi River that has the same soil type with high sand and clay content as our study sites and allows for a good comparison to our data. They found an order of magnitude or higher concentrations of EC (10^3 to 10^4 CFU GDW^{-1}) in sites with higher percent sand (71%), lower clay (26.5%) and lower silt (2.3%) even though moisture content was lower, 20.8% compared to 56.9% in a higher silt (39.5%) and clay (37.7%) and lower sand (22%) sediments. We also found that most EC and ENT in Buffalo Bayou and White Oak Bayou clustered within sediment grain diameter range of 60 to 250 μm, i.e. fine sand. Others have reported higher EC in silts and clays than in sand (Atwill et al., 2007; Niewolak, 1998). The fact that our samples are from cores rather than shallow grabs may also explain this difference. Nonetheless, there is a general trend that finer grained sediments harbor higher concentrations of EC and other FIB than coarse grained sediments owing to greater surface area of the smaller sediment particles as habitat for bacteria but also to greater straining or retention of bacteria during high flows (Benham et al., 2006a; Bradford et al., 2006).

No correlation between moisture and sediment grain size or moisture and EC or ENT concentration was found in the cores regardless of AWL, WL, or BWL or core horizon. However, our transect at study site BB8 in Buffalo Bayou spanning from midstream to 7 m above the water line showed that EC and ENT concentrations decreased 500-fold in the top 1 cm when moisture fell to 2% or less. Similar but less rapid, decreases in EC and ENT concentrations occurred at 15 and 30 cm even though the moisture content remained above 10% indicating that other factors are influencing bacterial concentration, like organic matter. Our transect samples demonstrate that the stream bank sediments, as much as up 1 m above the waterline, are still a potential major source for EC and ENT in Buffalo Bayou. Especially since the stream bank tends to break away and fall into the bayou along multiple reaches.

The amount of organic matter also influences abundance or survival of EC and ENT in sediments (Gerba and McLeod, 1976; Desmarais et al., 2002; Haller et al., 2009a; Lee et al., 2006). Garzio-Hadzick et al. (2010) showed that EC in sediments containing higher percent organic matter had slower die off rates than sediments having the same grain size distribution but lower organic matter. Duan et al. (2014) reported total organic carbon (TOC) in sediments in the same samples used by this study. There was no difference between TOC in AWL, WL, and BWL cores. Averaged carbon content in Buffalo Bayou sediments ranged from 0.1 to 5.1 wt.% TOC and in White Oak Bayou from 0.2 to 4.1 wt.% TOC. We also found no correlation between EC or ENT concentrations and TOC in the bayou sediments (not shown). Duan et al. (2014) found that both bayous have high dissolved organic carbon (DOC) concentrations (~602 μmol l^{-1}) as well relatively high particulate organic carbon (POC) concentrations. The generally high concentrations of organic matter in sediments (Duan et al., 2014) represent a consistent source of substrate that sustains bacteria in the sediments and potentially the water column. Haller et al. (2009) showed that EC and ENT in sediments are capable of utilizing interstitial DOC from WWTPs for prolonged survival.

FIB concentrations in the upper 1 cm of sediments were an order of magnitude or greater than in the water (ranging from 10^1 to 10^3 MPN 100 ml^{-1}) but like many studies reviewed by Pachepsky and Shelton (2011) we found no correlation between EC in sediments and the overlying water column and only a moderate correlation for ENT during base or even high flow conditions in Buffalo Bayou despite the natural stream bed. This is curious since there was a moderate

Fig. 4. Example of Dice-UPGMA dendrogram of Box AIR-DNA fingerprints of EC isolated from Buffalo Bayou water (H2O) and sediments collected above the water line (AWL), at the water line (WL), and below the water line (BWL).

correlation between the FSS and CSS fractions of TSS and stream flow (Duan et al., 2014) and both FIBs were found to be associated with FSS.

It may be the case that EC and ENT in the water column are translocated from resuspended sediments in the upper watershed to the lower watershed which could explain the lack of correlation. Our size fractionation study of TSS collected at site BB8 determined that EC were mostly associated with all fractions of FSS rather than with CSS indicating that these bacteria are primarily transported via fine particulates in the bayous. ENT were mostly associated with FSS >25 μm.

The most convincing data for sediment and FIB resuspension and translocation are the BOX A1R fingerprints of EC isolated from water which had ≥92% similarity with those from sediments collected throughout Buffalo Bayou and in the upper watershed of White Oak Bayou that did not have concrete lining. In many instances, the distances between upper watershed sediment sampling sites and lower watershed water sampling sites were greater than 70 km. Stream flows, including base flows, in both bayous at the time of sampling were often above the minimum threshold of 0.3 $m^3\ s^{-1}$ for fine sediment resuspension determined by Jamieson et al. (2005). There is a general assumption that if in stream FIB loads arise from resuspension, this can only occur during high flow conditions (Characklis et al., 2005; Jamieson et al., 2005; Peterson et al., 2009; Wu et al., 2009). The weak or lack of correlations between FIB in the water column and underlying sediments during base flows by this and other studies (Pachepsky and Shelton, 2011) tend to support this assumption, however our fingerprints indicate otherwise. The shallow depths at which most water samples are collected may be responsible for this disparity since during low or base flow conditions any resuspended sediments would be carried just a few cm above the stream bed. In the case of our TSS size fractionation experiment at station BB8 in Buffalo Bayou, samples were collected during intermediate flow conditions (6.4 $m^3\ s^{-1}$) at a water depth of ~1 m from the surface. EC isolated from size fractionated TSS also had BOX A1R fingerprints with ≥92% similarity to fingerprints from isolates from upper watershed sediments as well as the sampling site.

The TMDL study (TCEQ , 2009) of the bayous used a BLEST model (Peterson et al., 2009) that took into account the resuspension of sediment EC. Sediment samples were collected in the upper watershed, which, according to our study, had lowest EC concentrations therefore the daily load contribution was underestimated by the model. Moreover, the BLEST model only considered resuspension during high flow conditions. Although we found no correlation between EC concentrations in the sediment and overlying water, our BOX A1R fingerprinting indicates that even during base flow some resuspension is indeed occurring. According to the TMDL study for Buffalo Bayou and White Oak Bayou (TCEQ , 2009), the combined EC load from Buffalo Bayou and White Oak Bayou during base flow is approximately 10^{15} MPN day^{-1}. A rough calculation of the number of EC in the Buffalo Bayou streambed, alone, from the upper watershed to downtown Houston is 1.54×10^{13} MPN. This estimate is based upon 35 miles (=5,632,704 cm) of open stream, average 30 ft wide (=914.4 cm) and the average concentration of EC cells in the top 1 cm of sediments (10^4 MPN GDW^{-1}), and converting grams to cm (~1 cm^3) is 1.54×10^{14} cells. Assuming only the top 1 cm of sediment is resuspended in the water column, the contribution of EC from Buffalo Bayou sediment could contribute up to 90% of the daily load. This estimate assumes an endless supply of sediment bacteria which is reasonable since we determined a long survival time, 154 days for EC in the top 1 cm as well as viable EC and ENT as deep as 60 cm. Interestingly, EC and ENT concentrations in sediments from tributaries without upstream WWTPs were just as high as those with WWTPs. This suggests the possible existence of naturalized or 'background' EC and ENT potentially capable of replication in extraenteric habitats (Brennan et al., 2010; Derry and Attwater, 2014; Desmarais et al., 2002; Gordon et al., 2002; Ishii et al., 2006; Moriarty et al., 2008; Solo-Gabriele et al., 2000).

5. Conclusions

Fecal pathogens are the leading cause for impairment of 191,228 miles of rivers and streams in the U.S. (USEPA, 2012) and in the State of Texas, between 1992 and 2012, >65% of the surface water impairments, including coastal estuaries, was due to EC or ENT. TMDLs for most of these impairments do not consider streambed and bank sediments as a source. Our study demonstrates, unequivocally, the numeric importance of EC and ENT in sediments of the Buffalo Bayou and White Oak Bayou watersheds that can account for as much as 90% of the observed daily loads. These results may hold true for all the 72 Greater Houston area TMDLs as well as other surface water impairments in Texas. This finding also has far reaching implications regarding the inability of watersheds across the US and in other countries to meet water quality standards.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.09.071.

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