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Distribution and disinfection of bacterial loadings associated with particulate matter fractions transported in urban wet weather flows

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ABSTRACT

Urban runoff is a resource for reuse water. However, runoff transports indicator and pathogenic organisms which are mobilized from sources of fecal contamination. These organisms are entrained with particulate matter (PM) that can serve as a mobile substrate for these organisms. Within a framework of additional treatment for reuse of treated runoff which requires the management of PM inventories in unit operations and drainage systems there is a need to characterize organism distributions on PM and the disinfection potential thereof. This study quantifies total coliform, *Escherichia coli*, fecal streptococcus, and enterococcus generated from 25 runoff events. With the ubiquity and hetero-dispersivity of PM in urban runoff this study examines organism distributions for suspended, settleable and sediment PM fractions differentiated based on PM size and transport functionality. Hypochlorite is applied in batch to elaborate inactivation of PM-associated organisms for each PM fraction. Results indicate that urban runoff bacterial loadings of indicator organisms exceed U.S. wastewater reuse, recreational contact, and Australian runoff reuse criteria as comparative metrics. All monitored events exceeded the Australian runoff reuse criteria for *E. coli* in non-potable residential and unrestricted access systems. In PM-differentiated events, bacteriological mobilization primarily occurred in the suspended PM fraction. However, sediment PM shielded PM-associated coliforms at all hypochlorite doses, whereas suspended and settleable PM fractions provide less shielding resulting in higher inactivation by hypochlorite.

1. Introduction

On a global basis the conservation of water resources and promulgation of water reuse are increasingly central themes of sustainability. Future urban water demands can be addressed by reducing water consumption, recycling and reusing water discharges while simultaneously moving toward hydrologic restoration. However rainfall–runoff relationships modified by the urban interface have significantly altered the hydrologic cycle due to anthropogenic conditions including traffic, impervious pavement and hydraulically-efficient runoff conveyance systems. Runoff generated at the urban interface transports particulate matter (PM), microbial, chemical and nutrient loadings and can impair receiving waters (Heaney and Huber, 1984; House et al., 1993). Rainfall–runoff relationships modified by urban interfaces also alter natural hydrologic pathways with increases in peak flow and volume and a commensurate increase in transported

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constituent load (Marselek et al., 1993). Recognizing that hydrologic restoration is difficult to achieve in highly developed areas, runoff volume can be recycled through integrated management approaches, for example indirectly through green infrastructure systems such as permeable pavement or more directly through reuse. Implementation of hydrologic restoration through integrated management utilizing rainfall-runoff reuse is becoming more commonplace, however, Hatt et al. (2006) report that the current research base is inadequate to support immediate implementation of urban rainfall-runoff reuse. As such, additional research regarding appropriate decentralized treatment for urban reuse is warranted. As urban rainfall-runoff transports heterodisperse PM gradations (Kim and Sansalone, 2010), the urban environs are sources of fecal contamination and many urban treatment and conveyance systems function as unmaintained PM repositories providing favorable microbial habitat, identification of PM-associated microbial distributions is of value. Such distributions permit the selection of appropriate unit operations for PM separation to reduce runoff PM as a chemical and microbial vector while also illustrating the potential for PM-shielding from disinfection. Separation of PM before disinfection reduces disinfection demand and enhances microbial disinfection potential in runoff in order to minimize risks associated with reuse. However, PM separation by such systems that are not maintained also results in PM repositories functioning as microbial habitat.

The urban habitat represents a reservoir for potential bacteriological zoonotic agents. Fukushima et al. (1989) document the waterborne transmission of a *Yersinia* sp. to children from urban animals. Glaser et al. (2000) identify *Salmonella* sp., *Campylobacter* sp., *Leptospira* sp., as bacterial etiologic agents of zoonotic infection in urban settings due to domesticated and non-domesticated animals, rodents, reptiles, and birds. The vectors of zoonotic infection are varied and include direct and indirect contact exposure as well as waterborne transmission. The diarrheal disease burden in the US from these organisms is significant, with Samuel et al. (2004) specifying *Campylobacter* sp. as the primary bacterial agent. Mobilization and transmission of bacteriological zoonotic agents by wet weather events are not well studied in the context of urban treatment/reuse systems and there is the requisite need for ensuring the public health with reuse.

Indicator organisms are commonly utilized as surrogate indices of pathogenic organisms in receiving waters through epidemiologic correlations of illness and the establishment of ambient water chemistry criteria to lower public health risks (EPA, 1984). While bacteriological indicator organisms do not specifically signify the presence of pathogens, indicators point to potential fecal contamination and microbial mobilization, and are useful in disinfection process evaluation (Maier et al., 2009). When utilized appropriately, indicators are a bacteriological metric for water. Jin et al. (2004) document indicator organism loadings by urban runoff vectors into Lake Pontchartrain and subsequent recreational water closings to minimize public health risks. Characklis et al. (2005) illustrate that indicator organisms in urban runoff exist as planktonic and PM-associated organisms. Krometis et al. (2007) document no significant variation in intra-event partitioning of indicator organisms in runoff, but a significant variation in intra-event microbial loading rates. Finally, in examining runoff for reuse He et al. (2008) illustrate that a retention basin can potentially produce reuse water of acceptable microbial levels during dry weather periods but for wet weather flows will mobilize significant microbial loadings in excess of public reuse guidelines in Alberta, Canada.

While there are many disinfection processes available, chlorination has been in use for over a century and is the most common form of disinfection in practice today for drinking water (Hrudey and Hrudey, 2004) and wastewater (Leong et al., 2008). Chlorination has been identified as an appropriate disinfection process for combined sewer overflows (CSOs) in urban areas (EPA, 2003) and for wastewater reuse (FAC 62-600.440). In comparison, investigation of the microbial distribution on PM fractions and the efficacy of chlorination for PM fractions are more recent. Urban rainfall-runoff is a complex matrix of dissolved and heterodisperse PM fractions (Sansalone and Kim, 2008; Kim and Sansalone, 2010). With respect to the impact of PM on disinfection, LeChevallier et al. (1981) documented the shielding effect of PM, using turbidity as a surrogate for total PM, on the disinfection of ambient surface water in Oregon. Illustrating the shielding effect of organic PM in wastewater Berman et al. (1988) also demonstrate that the kinetics of chlorine permeation into smaller organic PM is more rapid than larger PM. Dietrich et al. (2003) extend these findings and model the wastewater intra-particle transport of free chlorine with a radial diffusion model. Winward et al. (2008) illustrated the application of chlorination for gray water reuse. Through this study, contributions to the existing knowledge focusing on wastewater and CSOs PM-shielding are expanded to urban runoff. The study targets the bacteriological water quality and treatment of urban runoff from a municipal separate storm sewer system (MS4) for utilization as potential source of water in a decentralized urban reuse scheme that does not include treatment at a traditional wastewater treatment plant. Specifically, given the granulometry, organic content and nutrient distribution for source area urban runoff PM fractions (Berretta and Sansalone, 2011; Dickenson and Sansalone, 2009), the present study examines indicator organism association with PM fractions and the efficacy of disinfection for these fractions. Specifically it is hypothesized that organisms do not distribute uniformly across the PM fractions. Furthermore it is hypothesized that disinfection efficacy is not equal for each PM fraction.

2. Methodology

2.1. *Urban catchment*

The urban interface translating rainfall to runoff in this study is an urban watershed in Gainesville, Florida as illustrated in Fig. 1. The watershed is 13,000 m^2 of surface parking of which an area of approximately 500 m^2 catchment is delineated, instrumented and monitored for this study. The catchment surface area is 75% conventional asphalt and 25% raised vegetated islands with trees and delineated by vertical concrete curbs. Measured average daily traffic loading is 530

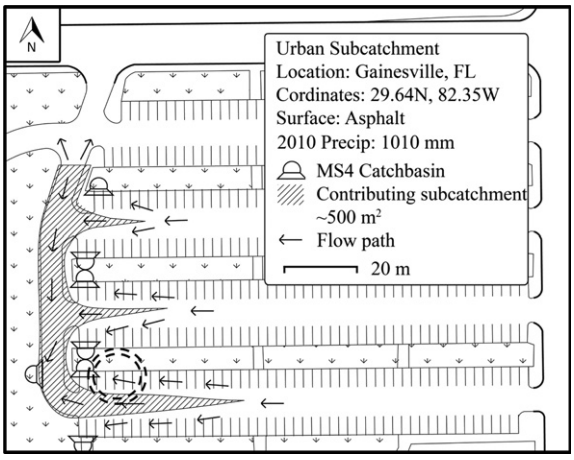

Fig. 1 – Schematic diagram of the instrumented 13,000 m² carpark field site and the contributing 500 m² sub-catchment to the modified catch basin (indicated by the double concentric hashed circles). N–S slope is 1–2% (typical) and E–W slope is 2–3% (typical).

vehicles across the catchment. Physical (PM), chemical (nutrients, metals and organic matter) and microbial loading sources are from anthropogenic (tire, vehicular and pavement abrasion and urban litter) and also biogenic sources (leaf litter, grass clippings, insects, and small urban animals and bird feces). The catchment drains by sheet and gutter flow to a catch basin modified to allow all flow to be diverted for full cross-sectional flow manual sampling during a monitored runoff event. Rainfall and flow are measured real-time with a tipping bucket rain gage and a calibrated Parshall flume combined with an ultrasonic flow depth transducer and data logger. Runoff is sampled at volumetrically-spaced intervals in replicate and each set of replicates is composited to construct paired event-based replicates for an event. Twenty-five events from 28 May 2010 to 20 April 2011 are monitored for source area runoff, PM and bacterial loadings. Four of these events are utilized for disinfection batch reactor analysis, and three events are monitored for bacterial partitioning as summarized in Table 1.

Table 1 – Batch reactor experimental matrix for the sediment, settleable, and suspended PM fractions showing HOCl dose, and event date. Two replicate reactors are utilized for each experiment to ensure reproducibility. x represents batch reactor initial concentration for the event.

Batch reactor experimental matrix			
HOCl (mg/L)	15	30	45
Time (min)	Contact time (mg min/L)		
0	0	0	0
1	15	30	45
5	75	150	225
10	150	300	450
20	300	600	900
40	600	1200	1800
120	1800	3600	5400
480	7200	14,400	21,600
	Event date		
21-Aug-2010	x	x	x
27-Sep-2010[a]		x	
4-Nov-2010[a]			x
16-Nov-2010[a]	x		

a Event utilized for microbial partitioning.

2.2. PM fractionation

Hydrology and PM indices for the monitoring phase of this study are presented in Table 2 for the events utilized in disinfection batch reactor analysis. Table 2 demonstrates that the sampled events represent varied hydrologic characteristics for the catchment and that the results are not unique to a specific event or characteristic hydrologic loading for the catchment, but instead that the events are characteristic of the catchment under varied rainfall loadings. Approximately 100 L of raw runoff is sampled for each event and within 30 min transferred to the laboratory for analysis. Prior to each event, runoff from a previous event loading the same catchment is collected and filtered (0.45 μm filter). This filtered runoff served as the aqueous fraction for re-suspending PM fractions. The aqueous fraction is autoclaved at 121 °C for 1 h to render the runoff matrix biologically inert. The runoff matrix is then stored at 4 °C. In this study the "runoff matrix" refers to the autoclaved aqueous runoff fraction utilized for PM re-suspension. PM is fractionated by wet sieving and microfiltration of sediment PM (4750–75 μm; Kim and Sansalone, 2008), settleable PM (75–25 μm; Kim and Sansalone, 2008), and suspended PM (25–0.45 μm; Kim and Sansalone, 2008) from the sampled 100 L volume. Sediment and settleable PM fractions are immediately re-suspended in 1 L of runoff matrix as PM concentrate and the filtrate from the 25 μm sieve (#500) is utilized as the suspended fraction. Event-based replicated composite samples are enumerated for total coliform, *Escherichia coli*, fecal streptococcus, and enterococcus organisms associated with each PM fraction. Batch reactors elaborate the hypochlorite inactivation for total coliform associated with each PM fraction from the runoff events. Samples are analyzed immediately or maintained at 4 °C and analyzed within 6 h.

2.3. Bacteriological enumeration

Bacteriological enumeration of the organisms utilizes the multiple tube fermentation, most probable number (MPN) method. This method is selected due to its applicability to turbid waters (APHA et al., 1995). For the inter-storm event bacterial monitoring, total coliforms and *E. coli* are enumerated according to Standard Method 9221B and fecal streptococcus and enterococcus organisms according to 9230B (APHA et al., 1995). The lauryl triptose broth in Method 9221B is amended with 4-methylumbelliferyl-β-D-glucuronide (LTB-MUG), to additionally detect *E. coli* by fluorescence under a 366 nm UV light (Feng and Hartman, 1982). For

Table 2 – Summary of hydrologic and event mean concentration indices and ranges for captured events (10 samples each event).

Event (2010)	PDH (h)	Rainfall duration (min)	Total runoff volume (L)	Q_{med} (L/s)	Q_p (L/s)	TDS (mg/L)	PM fractions (mg/L)		
							Suspended	Settleable	Sediment
7-Aug[a]	24	48	2623	1.01	4.3	17.0 (12–65)	13.1 (3–50)	32.2 (8–99)	222.5 (6–21414)
21-Aug[b]	83	31	299	0.03	1.5	42.1 (41–47)	2.2 (0.5–4)	36.8 (6–192)	301.1 (18–3295)
27-Sep	10	388	3842	0.01	10.9	26.1 (15–90)	44.5 (16–190)	50.0 (1–289)	874.1 (2–6035)
4-Nov	910	43	996	0.13	3.5	50.6 (38–105)	93.6 (15–319)	51.5 (4–225)	486.6 (5–18145)
16-Nov	286	34	307	0.01	1.8	85.5 (67–168)	123.2 (30–247)	137.8 (4–340)	332.2 (24–3208)

PDH is previous dry hours; Q_{med} is the median runoff flow rate; and Q_p is the peak runoff flow rate. TDS and PM fractions are volume weighted mean with sample range in parenthesis.
a The event utilized for runoff matrix collection.
b Baseline event.

Method 9221B, reference microbiological controls were simultaneously processed for quality assurance including *E. coli* (ATCC: 25922, positive lactose fermentation, positive UV fluorescence), *Enterobacter aerogenes* (ATCC: 13048, positive lactose fermentation, negative UV fluorescence), *Enterobacter faecalis* (ATCC: 29212, negative lactose fermentation, negative UV fluorescence), and a non-inoculated blank. For Method 9230B, fecal streptococcus organisms and enterococcus organisms are simultaneously detected by turbidity in azide dextrose broth (ADB) and esculin hydrolysis and differentiated by the Sherman Criteria of growth of enterococcus organisms in 6.5% NaCl brain heart infusion (BHI) broth. Reference microbiological controls were simultaneously processed for quality assurance including *E. coli* (ATCC: 25922, negative turbidity in ADB, negative esculin hydrolysis, negative turbidity in 6.5% NaCl brain heart infusion (BHI) broth), *E. faecalis* (ATCC: 29212, positive ADB turbidity, positive esculin hydrolysis, positive BHI turbidity), and a non-inoculated blank. For both bacteriological enumeration schemes, MPNs are calculated according to Standard Method 9221C (APHA et al., 1995). MPN tube dilutions had a detection limit of 2 MPN/100 ml for chlorinated reactors.

2.4. Batch reactors

Table 1 outlines the batch reactor experimental matrix. From the runoff events, a "baseline" storm on 21 August 2010 is utilized and for this event reactors are initialized in two replicate sets for the three PM fractions at sodium hypochlorite doses of 15, 30 and 45 mg/L for a total of eighteen reactors. Three additional storms are sampled on 27 Sept. 2010, 4 Nov. 2010, and 16 Nov. 2010 and initialized in two replicate reactors across the three PM fractions at a single sodium hypochlorite dose of 30, 45, and 15 mg/L, respectively, for an additional eighteen reactors. For the analysis, additional rainfall-runoff is collected on 7 Aug. 2010 and 5 Sept. 2010, filtered through 0.45 μm filters and autoclaved at 121 °C for 60 min. This runoff matrix is utilized to reconstitute the separated PM for the reactor studies.

PM is immediately fractionated from the runoff by a sterile wet-sieve procedure. Runoff is drained by gravity through sterilized 4750, 75, and 25 μm sieves. PM remaining on the 4750 μm sieve is defined separately as gross solids (Rushton et al., 2007) and is dried and characterized, but not included in reactor experiments. PM remaining on the 75 and 25 μm sieves are reconstituted in 1 L of the runoff matrix as sediment and settleable fractions, respectively, and stored at 4 °C until reactor initialization. PM passing through the 25 μm sieve into a sterile container is considered suspended material and is stored at 4 °C until reactor initialization.

The batch reactors illustrated in Fig. 2 are 2 L nominal glass jars with a 4 cm stir-rod that are prepared as chlorine demand free (APHA et al., 1995; Method 4500CL-G) and autoclaved at 121 °C for 20 min. Sediment and settleable PM reactors are filled with 150 ml of PM fraction concentrate and 1650 ml of runoff matrix and suspended PM reactors are filled with 1800 ml of 25 μm sieved filtrate. At time zero, batch reactors are brought to 25 °C ± 2 °C, initial water chemistry measurements of pH, temperature, and conductivity are recorded, a 100 ml aliquot of sample is removed for total coliform bacteriological analysis and the reactor is dosed with a standardized sodium hypochlorite solution according to the dosing schedule. The total coliform bacterial classification is utilized as a process indicator for hypochlorite disinfection in

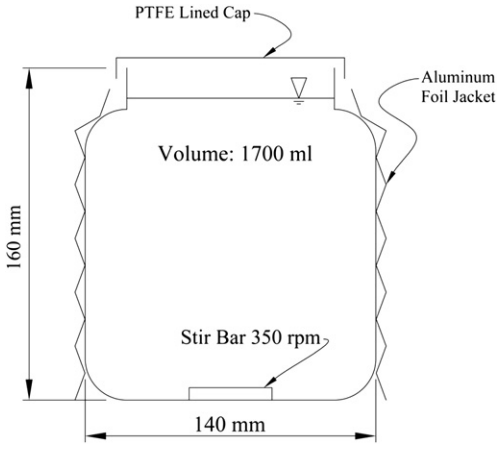

Fig. 2 – Continuously stirred batch reactor (CSBR) schematic. The CSBR is comprised of a chlorine demand free glass vessel containing a 40 × 6 mm polytetrafluoroethylene (PTFE) coated stir bar with a PTFE lined closure and outer walls lined with an aluminum foil jacket to prevent UV decomposition of free chlorine.

rainfall runoff. At each time interval in Table 1, water chemistry measurements are made, a 100 ml aliquot is removed for bacteriological analysis, and two replicate aliquots are removed to analyze the free chorine residual. Sterile syringes and pipette tips are used at each time interval and electrodes are sterilized before immersion in the reactor. Reactors are sealed with lined lids that are tightly secured on the reactors. Aliquots utilized for bacteriological analysis are processed in a sterile blender at 22,000 rpm to dissociate PM-associated organisms from PM (Borst and Selvakumar, 2003) prior to total coliform enumeration. A laboratory blank of sterile DI water is also processed through the blender and bacteriologically enumerated to ensure no cross contamination from the blending procedure. In addition to total coliform enumeration for the events on 27 Sept. 2010, 4 Nov. 2010, and 16 Nov. 2010, the time zero measurements for each batch reactor are enumerated for *E. coli*, fecal streptococcus, and enterococcus to determine the partitioning of each organism to the suspended, settleable, and sediment PM fractions. Following the experiment, batch reactors are analyzed for total PM measured with a suspended sediment concentration (SSC; ASTM, 2002) method and volatile suspended sediment concentration (VSSC; ASTM, 2002) and particle size distribution (PSD; ISO, 2009) to characterize the PM loading and validate the wet-sieve fractionation procedure. PM granulometry of each reactor is reported in Table 3.

Residual free chlorine is analyzed utilizing a DR2800 (Hach) spectrophotometer measuring absorbance of N,N-diethyl-p-phenylenediamine (DPD, Hach Chemical) at 530 nm. Replicate 2 ml (5:1 dilution), 5 ml (2:1), or 10 ml (1:1) aliquots,

Table 3 – Batch reactor particle granulometry.

Event (2010)	Fraction	HOCl (mg/L)	PM (mg/L)	d_{10}[a] (μm)	d_{50}[b] (μm)	d_{90}[c] (μm)	$d_{[4,3]}$[d] (μm)	Ct[e] (mg min/L)	C_8[f] (mg/L)
Baseline event	Suspended	15	50.6	2.1	10.1	29.8	13.7	20.6	0.0
21 August 2010	Suspended	15	24.5	1.6	7.7	29.0	16.0	41.9	0.0
	Suspended	30	25.9	1.7	6.9	39.0	15.4	1478.9	0.1
	Suspended	30	105.5	4.1	15.1	42.4	23.5	836.1	0.0
	Suspended	45	47.9	2.2	9.3	24.4	19.8	3665.0	1.6
	Suspended	45	22.1	1.9	8.2	27.2	14.2	4030.1	3.1
	Settleable	15	188.6	14.9	44.3	90.7	49.3	960.6	0.1
	Settleable	15	176.3	14.5	39.5	91.0	49.1	1353.0	0.1
	Settleable	30	139.2	12.9	42.8	69.9	48.2	6246.8	6.8
	Settleable	30	206.8	14.5	44.2	93.0	49.9	5410.2	4.4
	Settleable	45	147.9	13.4	43.1	89.7	48.2	11,320.0	14.7
	Settleable	45	197.8	14.0	43.6	90.7	51.7	10,637.6	15.4
	Sediment	15	284.8	53.8	244.9	958.3	390.4	1140.0	0.0
	Sediment	15	787.6	62.4	323.5	427.0	480.2	514.5	0.0
	Sediment	30	–	–	–	–	–	–	–
	Sediment	30	419.8	72.6	268.0	1059.9	438.1	2083.5	0.1
	Sediment	45	211.4	39.3	193.9	948.5	366.1	6361.8	1.4
	Sediment	45	391.6	66.3	310.8	1120.4	467.1	5703.0	3.8
Verification event	Suspended	30	36.8	2.2	9.5	25.1	12.0	7089.5	10.3
27 Sept. 2010	Suspended	30	30.2	2.3	9.9	28.0	15.7	6879.1	9.7
	Settleable	30	187.0	13.0	39.2	83.2	44.4	8962.1	13.7
	Settleable	30	149.4	11.8	37.7	82.1	45.8	8862.2	13.4
	Sediment	30	330.9	41.8	389.1	1165.7	501.9	5347.8	2.1
	Sediment	30	285.1	50.8	393.7	1288.6	561.8	5623.4	2.9
Verification event	Suspended	45	32.9	2.1	9.4	29.6	14.0	4505.5	2.5
4 Nov. 2010	Suspended	45	39.6	2.2	10.0	28.5	14.9	4566.5	2.4
	Settleable	45	83.1	8.4	35.0	61.9	41.6	16,033.3	27.3
	Settleable	45	75.7	8.1	35.8	100.5	53.2	13,151.8	14.1
	Sediment	45	330.4	54.0	203.5	887.7	347.0	7002.2	1.4
	Sediment	45	353.8	38.4	189.3	842.5	334.9	5339.2	0.4
Verification event	Suspended	15	89.3	1.8	8.7	27.3	15.8	80.6	0.2
16 Nov. 2010	Suspended	15	98.0	1.9	9.8	30.0	14.5	81.4	0.1
	Settleable	15	67.3	5.9	27.8	50.3	33.2	2693.2	2.5
	Settleable	15	67.5	7.7	40.6	245.0	88.7	2274.3	1.6
	Sediment	15	272.0	73.3	207.9	670.7	302.6	891.4	0.1
	Sediment	15	232.2	75.9	218.4	769.9	344.0	685.8	0.3

a Characteristic particle size of 10% finer by volume.
b Characteristic particle size of 50% finer by volume.
c Characteristic particle size of 90% finer by volume.
d De Brouckere volumetric mean: $\sum v_i d_i$ – which is analogous to the number mean volume size.
e Total *Ct* value for reactor at 8 h.
f Residual free chlorine concentration at 8 h.

depending on anticipated residual chlorine, are sampled from batch reactors and diluted to 10 ml with chlorine demand free water (Barnstead Nanopure). A custom absorbance curve is obtained for the DPD reagent for the analysis range and the measurement has an accuracy of ±0.1 mg/L for a 1:1 dilution ratio.

Microbiological controls and laboratory blanks are utilized for microbiological growth media, test sterility of the process, and ensure appropriate media response to reference organisms. Testing of blender blanks demonstrated no cross contamination between samples for each analyzed event. In addition, the media's response to reference organisms is utilized as comparators for UV fluorescence and turbidity where appropriate.

3. Results

3.1. Catchment monitoring

Fig. 3 summarizes the event-based results from the monitoring study of bacterial densities for indicator organisms from the 25 monitored runoff events. Results indicate that

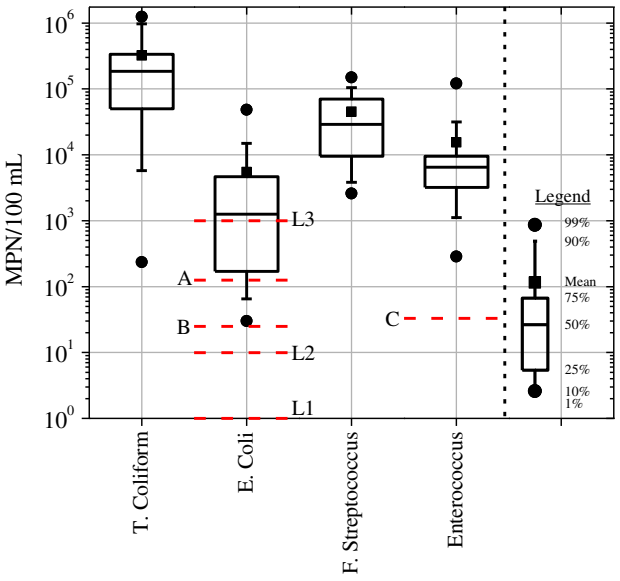

Fig. 3 – Event mean most probable number per 100 ml boxplot for twenty-five wet weather events on a small urban watershed in north central Florida. Comparative EPA regulatory guidance for freshwater recreational ambient bacteriological density is shown as (A), 126 MPN/100 ml (geometric mean) for *E. coli*. Comparative regulatory guidance in Florida for unrestricted urban reuse for wastewater effluent is shown as (B), 25 MPN/100 ml (single sample) for fecal coliforms. Comparative regulatory guidance for brackish/saltwater recreational use is shown as (C), 35 MPN/100 ml for enterococcus organisms. Comparative Australian regulatory guidelines for urban runoff reuse are: (L1), < 1 MPN/100 ml for non-potable residential reuse; (L2), < 10 MPN/100 ml for reuse in areas with unrestricted access; and (L3), < 1000 MPN/100 ml for reuse in areas with restricted access.

total coliform organisms are ubiquitous in this urban watershed and have a median density in excess of 10^5 organisms per 100 ml. *E. coli* densities range from 30 MPN/100 ml to greater than 10^4 MPN/100 ml with a median density of 1300 MPN/100 ml. From these storms, *E. coli* represented <1%–21% of total coliform organisms present. The fecal streptococci densities range from 3000 MPN/100 ml to 10^5 MPN/100 ml with a median of 5×10^4 MPN/100 ml and enterococcus densities range from 10^3 to 10^5 MPN/100 ml with a median value of 10^4 MPN/100 ml.

3.2. Batch reactors

The normalized log removal (NLR) of coliform organisms exposed to hypochlorite inactivation in batch reactors is presented in composite form in Fig. 4A. Results are normalized to allow comparison of disparate initial bacterial densities across fractions and events and illustrate shielding relative to complete inactivation (NLR of 1.0). Normalization is the log removal observed over the maximum detectable log removal for the reactor. A plateau at an NLR value of less than 1.0 as time increases indicates PM shielding of particle-associated coliforms (PAC). Suspended and settleable fractions did not exhibit significant shielding throughout the duration of the experiment rapidly achieving 90% of full log removal within 10 min of reaction time and within experimental resolution of the detection limit by the end of the experiment. However, the sediment PM fraction illustrated a treatment plateau at 10 min. Fig. 4B illustrates the normalized variability of the coliform enumeration across all events and PM fractions. Results indicate increasing inactivation variability across events with increasing PM size demonstrating increasing shielding capacity and increasing difficulty in representative enumeration for large diameter PM.

Table 3 presents batch reactor PM loading, granulometry, and free chlorine residual and integrated chlorine concentration over time (*Ct*) on a reactor basis. PM granulometric indices illustrate that the De Brouckere volumetric mean of each reactor is within the appropriate range for the PM fraction and the characteristic particle sizes delimit the separation of the fractions from the heterodisperse, mobilized PSD. Sediment and suspended PM fractions typically show 80–90% of reactor mass within the specified range and the settleable fractions approximately 60% due to sieving on both upper and lower bounds. Free chlorine residual at 8 h ranged from 0.0 mg/L for reactors with high chlorine demand and low chlorine dose to 27.3 mg/L for reactors with a high initial chlorine dose. *Ct* values at 8 h are the exposure of coliforms to chlorine over the duration of the experiment. *Ct* values range from 20.6 mg min/L to over 10,000 mg min/L.

3.3. Indicator organism distribution

On a PM gravimetric basis Fig. 5 summarizes the density of indicator organisms for runoff PM fractions. The density for each enumerated organism is highest for the suspended fraction with the median densities of the settleable and sediment fractions 1–2 orders of magnitude less than the corresponding suspended fraction for each enumerated organism. Total coliform organisms exhibit the greatest

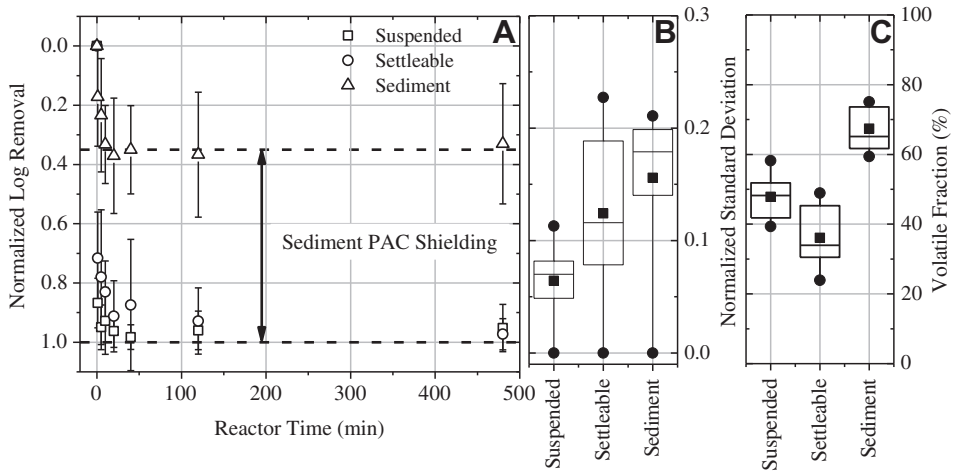

Fig. 4 – Composite normalized log removal of particle associated coliforms (PAC) for four monitored events ($n = 12$ per data point) at hypochlorite doses of 15, 30, and 45 mg/L. Shielding and measurement variability contradistinguishes inactivation across the particulate fractions with increasing particle diameters demonstrating intensifying shielding (A) and measurement variability (B). Sediment PM also exhibits a higher particle volatile fraction (C) than the suspended or settleable fractions potentially acting as a hypochlorite sink and contributing to the shielding phenomena. The sediment PM fraction exerts significant shielding regardless of applied hypochlorite dose.

bacteriological density across all PM fractions. Results illustrate low *E. coli* density of less than 25 MPN/mg of PM for the sediment fractions noting that the sediment fraction of PM provides the highest gravimetric contribution per particle as compared to settleable and suspended PM. Fig. 6 summarizes the percentage of mobilized organisms associated with each PM fraction for the events utilized in the reactor testing. In general, the largest percentages of monitored bacterial organisms normalized to PM mass are associated with the suspended PM fraction, which represents both planktonic organisms and organisms associated with PM less than 25 μm noting that sediment PM dominates PM mass.

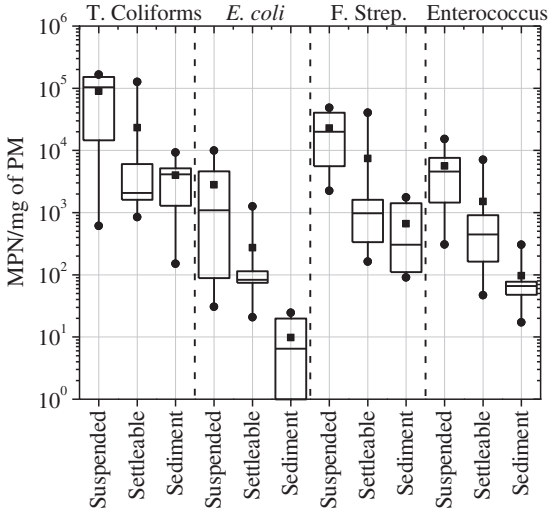

Fig. 5 – Distribution of PM-associated organisms to suspended, settleable, and sediment PM fractions. For each organism, the suspended PM fraction contains the highest bacterial density followed by the settleable and sediment fractions. In particular, the sediment PM fraction, which exhibits the greatest organism shielding potential contains the lowest density of the indicator *E. coli* (< 25 MPN/mg PM) and enterococcus (< 300 MPN/mg PM) organisms.

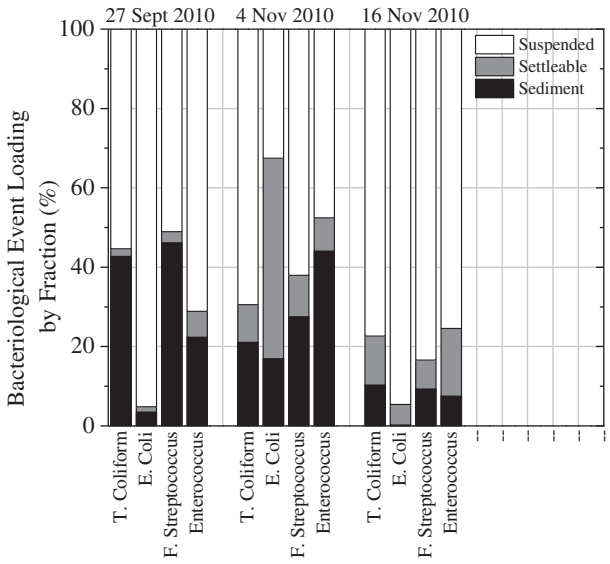

Fig. 6 – Distribution of event-based bacteriological loading by PM fraction. In general, urban source area bacterial loading is most mobilized in the suspended fraction, and least mobilized in the settleable fraction. Result indicates that filtration and/or microbial inactivation are necessary to limit source area microbial mobilization.

4. Discussion

Epidemiological studies of disease due to contact exposure of the types experienced in reuse applications with organisms transported by runoff acting as the etiological agents do not currently exist. Potential zoonotic pathogens on the urban watershed remains a poorly defined public health risk for urban water treatment and reuse systems when bacteriological management is not ensured by treatment, regular maintenance of unit operations or urban surface and drainage appurtenance maintenance. In general, the primary potential zoonosis from exposure to bacterial agents in an urban setting is acute gastroenteritis, with particular effect to the young or immune-compromised individuals. The state of Florida has only basic water chemistry criteria for the restricted and unrestricted reuse of reuse water. However, numeric bacteriological criteria in the regulations are specifically for the reuse water from domestic wastewater treatment facilities and do not apply to reuse of urban runoff. For utilization of urban runoff reuse in Florida, the implementer is required to demonstrate that the reuse application will not impair water chemistry criteria at or near the site of application and is required to demonstrate that the reuse application of urban runoff does not impair the public health or welfare (personal communication, Eric Livingston, Florida Department of Environmental Protection, FDEP). However, it is useful in the absence of specific, numeric regulatory guidance regarding the bacteriological level of urban runoff to consult the regulations regarding water chemistry criteria for recreational waters and wastewater reuse for restricted and unrestricted urban reuse (He et al., 2008). The U.S. Environmental Protection Agency (EPA) and many state agencies, including FDEP, issue water chemistry criteria for recreational waters, waters utilized as feed waters to drinking water treatment plants, and reuse/reclaimed waters. The USEPA bacteriological water quality criteria (1984) for freshwater recreational water use are, as geometric means, 126 MPN/100 ml for *E. coli* and 33 MPN/100 ml for enterococci and for brackish/saltwater recreational water use is 35 MPN/100 ml for enterococci; noting that the USEPA does not recommend the use of *E. coli* as an indicator organism in brackish/saltwaters. As illustrated in Fig. 3, urban runoff exceeds *E. coli* bacteriological USEPA recreational contact water quality criteria in 19 of 25 events and enterococcus criteria in 25 of 25 events. Thus, for recreational use, urban runoff can impair the bacteriological quality of the receiving water body. Dilution ratio and organism die off also impact whether the recreational water will meet public health water criteria. Australia has implemented runoff reuse criteria for *E. coli* on a tiered scale as: <1 MPN/100 ml for non-potable residential reuse, <10 MPN/100 ml for reuse in areas with unrestricted access, and <1000 MPN/100 ml for reuse in areas with restricted access (Dept. of Environment and Conservation NSW, 2006). Fig. 3 demonstrates that on this urban catchment, each event monitored exceeds both unrestricted and non-potable residential reuse criteria and the median of the events exceeds the restricted access application. Thus, a level of treatment is requisite for reuse of this urban source water to ensure public health.

As a primary source, sampled at the entrance to an MS4, there are no identified anthropogenic fecal influences. There are no nearby septic systems and no potential cross-connections due to the location of sampling. Thus, mobilized indicator organisms on this urban watershed are posited to be from small rodents, mammals, reptiles, birds and insects. From these, zoonotic pathogens may exist on an urban watershed and their environmental mobilization by wet weather flows is not yet well defined. As wet weather events are shown to mobilize waterborne pathogens present in a watershed (Hrudey and Hrudey, 2004), bacteriological disinfection confers a degree of public health and safety for runoff treatment and reuse systems. In addition some MS4s suffer from cross contamination from sanitary sewer systems, furthering considerations for disinfection where the integrity of the stormwater and sanitary conveyance system and cross-connections have not been ascertained.

Reactor results illustrate PM fraction shielding for PM-associated organisms. The greater shielding provided by the sediment PM fraction may be attributed to several factors. A primary driver of shielding is large PM size which represents a physical barrier to disinfectant penetration. In addition, the volatile fraction of PM is a surrogate indicator of the organic content. During the monitored events the volatile fraction (VF) of the PM for the sediment was ranged from 59 to 79%, whereas the VF of the suspended and settleable PM fractions was in the range of 24–58%. Thus, the organic material can potentially represent a competing chlorine demand diminishing disinfection efficacy otherwise targeting PM-associated organisms. In this study the VF was used as a surrogate or index for organic content. The two most common methods to determine organic content of soils (in this study PM) are oxidation of organic matter with H_2O_2 and loss on ignition; which is the VF utilized in this study. Oxidation with H_2O_2 is generally incomplete and varies from soil to soil while loss of ignition provides quantitative oxidation of organic matter if the substrate is not dominated by carbonate minerals which should otherwise be separated by pretreatment with reagents of HF and HCL acid (ASA and SSSA, 1982). Finally, the constituents of the suspended and settleable PM fractions contain more siliceous constituents (Kim and Sansalone, 2008) with less available pore structure than sediment PM. The surficial soils of this watershed, transported from the raised vegetated islands are siliceous fine sands to sandy silts by gradation and observation. Fig. 7 illustrates the porosity of the sediment fraction as compared to settleable and suspended fractions based on mercury porosimetry. The median pore diameter is approximately 70 μm for the sediment PM sample, 20 μm for the settleable PM sample and 15 μm for the suspended PM sample. These results indicate that the similarity of the smaller diameter internal pore structure of the suspended and settleable PM as compared to the sediment PM. This smaller diameter may produce an internal pore structure that is less available as habitat to PM-associated organisms, resulting in a largely surficial orientation for organisms on these fractions and making the finer PM fractions more amenable to disinfection by chlorination. The scanning electron micrograph images of the surface also illustrate the surficial porosity difference between the sediment and the settleable and suspended PM. These results

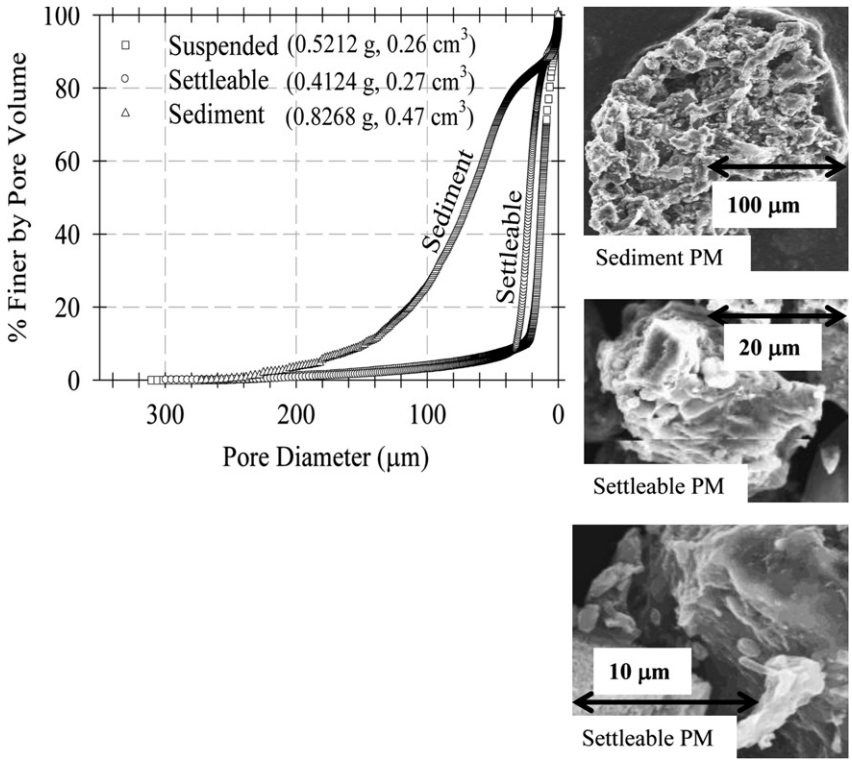

Fig. 7 – Mercury porosimetry results for each PM fraction illustrating the significantly larger pore size distribution of sediment as compared to settleable and suspended PM. Scanning electron micrographs images of suspended, settleable, and sediment PM is also illustrated. As compared to suspended and settleable PM, sediment PM contains a higher fraction of organic material as illustrated in Fig. 4 and significantly larger pore size and higher internal pore structure facilitating bacteriological shielding.

potentially explain the similarity of the rate of disinfection for the suspended and settleable fractions despite differences with respect to particle diameter and settling functionality.

Shielding of PM-associated organisms generates a deleterious effect for inactivation by chlorination of sediment PM. Residual chlorine presence and high Ct values at 8 h did not confer full bacteriological inactivation of sediment associated coliforms and as such, is not a recommended treatment option for inactivation of sediment PM. Sediment PM should be separated and managed before inactivation. However, chlorination at all levels studied is able to significantly inactivate PM-associated organisms in the suspended and settleable fractions even for reactors with high chlorine demand. Beyond granulometric size classes, PM fractions are separated into suspended, settleable, and sediment categories from a functional treatment perspective. Coarser sediment PM rapidly separates by gravitational quiescent settling as provided by preliminary unit operations such as hydrodynamic separators for urban runoff. Separation and management of the sediment fraction to reduce contact between sediment PM inventories and runoff intended for reuse would be a requisite step before disinfection by chlorine in an urban rainfall-runoff reuse scheme. Settleable PM settles in 60 min in an Imhoff cone, similar to 1 h of primary quiescent clarification and suspended PM remains suspended in the supernatant after 60 min. Thus, these categorical definitions indicate the applicability to treatment of urban rainfall-runoff by either primary gravitational settling or for the suspended fraction by secondary filtration. Relating these PM functional classes to particle size ranges is imperative to the quantification analysis of PM fate required in many studies. This correlation also enables extrapolations of these PM ranges to the viability of treatment mechanisms. Implementation of urban runoff reuse requires volumetric attenuation of flow and quantifiable clarification as part of conditioning runoff to optimize disinfectant dosage. Providing increasing PM clarification can be readily facilitated and quantified, in particular for the sediment PM exuding the largest shielding capability. In addition, the use of engineered media filters can further reduce the effects of PM-associated shielding allowing an optimized disinfection dose.

The use of chlorine as a disinfectant is a potential treatment option to ensure the public safety from exposure to bacterial organisms in urban reuse water. An issue that needs to be addressed is the potential for disinfectant by-product (DBP) formation, degradation and fate in a reuse application whether utilizing runoff or the widespread use of chlorinated wastewater reuse for urban irrigation. Thus, further research in batch and pilot studies is warranted to investigate the potential formation of DBPs in runoff, for example as compared to the formation of DBPs from wastewater reuse for urban irrigation, as well as to explore in depth the financial and potential ecological cost of utilizing chlorine as a disinfectant in such systems.

5. Conclusions

Results of the distribution of bacterial loadings on particulate matter (PM) fractions and disinfection of these PM fractions were conducted for loadings from an urban watershed comprised of 75% conventional (impervious) asphalt and 25% raised vegetated islands pavement in North Florida subject to 25 monitored rainfall–runoff events of highly variable rainfall, runoff and washoff of PM and bacterial loadings. The results generate the following conclusions.

- On an event basis, runoff exceeds *E. coli* bacteriological USEPA recreational contact water criteria in 19 of 25 events and exceeds enterococcus criteria in 25 of 25 events.
- On an event basis, all 25 events exceed comparative Australian unrestricted and non-potable residential runoff reuse criteria and the median of the events exceeds guidance for restricted access application.
- PM is a size heterodisperse mobile substrate for transport of bacterial loads and the granulometry of PM (size, pore characteristics and organic content as examined herein) impacts the potential for bacterial inactivation.
- Inactivation of PM-associated bacteria was effective for the suspended and settleable fractions in the applied hypochlorite doses (15–45 mg/L of HOCl), but that sediment PM-associated coliforms are shielded by the host PM even at hypochlorite doses up to 45 mg/L.
- PM is a mobile substrate for bacterial and chemical loadings and the current practice of unmaintained best management practices and drainage appurtenances provide both a temporary sink and source for PM, chemical and bacterial loads. Conveyance units or unit operation systems designed for PM separation require regular cleaning and management of separated PM in order that such systems do not function as microbial habitat and PM sources through scour. Such PM management before disinfection, whether by hypochlorite or other disinfection processes, will reduce microbial loadings, disinfection requirements and effluent loads of PM.
- Beyond regular operation and maintenance practices for treatment systems loaded by urban rainfall-runoff, disinfection of treated and reclaimed urban rainfall-runoff is required to ensure public health.

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