San Diego River Contamination Study:
Increasing Preparedness in the San Diego River Watershed for Potential Contamination

Final Report

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EXECUTIVE SUMMARY

Introduction

Distinguishing anthropogenic fecal pollutant inputs to streams is currently one of the most pressing environmental challenges for urban water managers. Although stormwater enters urban waterways from municipal separate storm sewer systems (MS4s) rather than from combined sewer systems (CSSs), streams and rivers in Southern California often exceed water quality targets for fecal indicator bacteria, and human pathogens have been detected during wet weather conditions. Pathogenic microorganisms and other pollutants may be introduced to streams from both surface and subsurface sources, and the relative importance of different sources is not yet well understood. In this study, microbial source tracking (MST) markers and chemical markers were measured in water and soils to evaluate pollutant inputs to the San Diego River and its tributaries from untreated wastewater and fecal waste associated with homeless encampments. The study was conducted from January 2018 to March 2019 and focused on the two hydrologic years following the Hepatitis A outbreak in San Diego, which included cases among the unsheltered population in San Diego. The MST and chemical markers used can help distinguish human-associated fecal pollution from general fecal pollution (which can also originate from other warm-blooded animals).

Dry weather contaminant inputs

Water samples collected during dry weather conditions at locations directly upstream and downstream of three active homeless encampments along the San Diego River and two of its tributaries had greater downstream concentrations of fecal indicator bacteria, *Escherischia coli* and fecal enterococci, but these bacteria may also originate from non-human fecal sources, such as pets and birds. By contrast, a human-associated microbial indicator of fecal pollution, the HF183 marker of Bacteroides, was only detected sporadically, and the concentrations were too low to conclude if there was a significant difference between upstream and downstream samples. There was also no significant change in the concentrations of caffeine and sucralose, two chemical pollutants associated with human waste. Within the scope of this study, there was no evidence that homeless encampments are causing increases in the concentration of microbial pollutants in water during dry weather conditions.

On the other hand, soils sampled in the vicinity of open defecation sites during homeless encampment cleanups tested positively for *E. coli*, enterococci, and HF183. However, HF183 was not detected in the soils one month after cleanup. There is evidence that the soils (but not the adjacent waters) within a former encampment are contaminated with fecal pollution, and therefore, environmental workers should use personal protective equipment (e.g., gloves) when handling or working around soils at former encampment sites, especially soils located near sites with evidence of open defecation.

Wet weather contaminant inputs

Knowledge of the fate and persistence of biological and chemical markers from untreated wastewater exfiltrating from cracked and aging sewer infrastructure or discharging into

waterways from sanitary sewer overflows (SSO) is essential to evaluate contributions of human fecal material to the San Diego River and its tributaries. Untreated wastewater had substantially higher caffeine (approximately 200 µg/L on average) than sucralose (approximately 25 µg/L). A caffeine/sucralose ratio of greater than 2 was found to be indicative of wastewater inputs to surface waters. The MST markers, HF183 and pepper mild mottle virus (PMMoV), a virus found in pepper-based sauces consumed almost exclusively by humans, were also measured in this study. The bacterial marker, HF183, was more than an order of magnitude higher in wastewater (>10^6 copies/100 mL) than the viral marker, PMMoV (approximately 10^5 copies/100 mL).

Experiments were also conducted to evaluate the persistence of microbial pollutants in soils from simulated sewer exfiltration and SSO events during wet weather. For soils spiked in the laboratory with untreated wastewater, *E. coli* concentrations decreased exponentially from >10^7 MPN/100 mL on the first day of the experiment to ~100 MPN/100 mL, levels that were still measurable, even 4 months after spiking. The MST markers, HF183 and PMMoV, were also measured at concentrations of 100 copies/100 mL to 1000 copies/100 mL four months after spiking. PMMoV had greater persistence in dry soils than HF183.

To simulate storm conditions, wastewater-spiked soils were also flushed repeatedly on the day after spiking. After 20 consecutive flushes, wastewater-spiked soils remained a source of *E. coli*, enterococci, and HF183, but not PMMoV. PMMoV was flushed away from soils after the first two flushes, possibly due to its smaller size and greater mobility in soils. These results provide new information on the typical concentrations of MST markers in wastewater contaminated soils that may serve as a guide for future tracking of wastewater inputs during storm events.

To simulate the leaching of pollutants from open defecation sources into stormwater, runoff experiments were conducted using a runoff simulation device at sites adjacent to the river with human feces on the soil surface. These experiments revealed several important findings about open defecation. Concentrations of *E. coli* and enterococci remained unchanged after multiple flushes of human fecal material on soil surfaces; therefore, open defecation is likely to be a continuous source of fecal indicator bacteria over the length of a storm event. Also, the human microbial marker, PMMoV, and the chemical marker, sucralose, were measured in every runoff experiment and found to be persistent in human fecal material in the environment. However, both HF183 and caffeine, were either absent or present in very low (undetectable) concentrations in the runoff experiments, which may be due to the short lifetime of both HF183 and caffeine in the environment. Combined with earlier evidence of high concentrations of HF183 and caffeine in untreated wastewater, these runoff experiment results indicate that increases in caffeine and HF183 concentrations in surface water are likely an indication of untreated wastewater inputs rather than open defecation inputs.

Source tracking during storm events

Monitoring of pollutagraphs for the San Diego River and its tributaries during storm events from January 2018 to March 2019 revealed that *E. coli* and enterococci concentrations exceeded wet weather targets by several orders of magnitude, with the highest concentrations (> 105 MPN/100 mL) and loadings (>1013 MPN/d) observed during the two largest storm events. Samples were also analyzed for gene targets of a fecal indicator virus (coliphage PhiX174), human associated

chemical markers (caffeine and sucralose), human-associated biological markers (HF183 and PMMoV), pathogenic human viruses (Hepatitis A virus [HAV] and norovirus GI [NoVGI]), pathogenic bacteria (Campylobacter coli and Campylobacter jejuni), dissolved organic carbon, total dissolved nitrogen, nitrate, and phosphorus. Samples collected from the San Diego River during a major storm (February 2018), which had strong evidence of SSO influence, had no detectable levels of HAV or *C. jejuni*, but the human pathogen, *C. coli* was detected. Subsequent storms also did not have measurable concentrations of HAV or *C. jejuni*; however, the human pathogen NoVGI was detected in the San Diego River during the first rain event of the 2018 hydrologic year, following 150 antecedent dry days.

The most telling information regarding sources of contamination comes from pollutagraphs of HF183, caffeine, and sucralose, which showed caffeine/sucralose ratios greater than 2 and large increases in HF183 during and after the peaks of storm hydrographs. The trends in concentrations of MST and chemical markers, as well as other chemical parameters, over the course of each storm suggest that untreated wastewater, from sewer exfiltration or SSOs, is the main source of microbial contamination in the San Diego River during storm events. Corresponding chemical analyses indicated that soils become saturated over the course of most storms, potentially mobilizing contaminants from the subsurface, where untreated wastewater may have accumulated around sewer pipes or where sewers may actively be leaking wastewater to the surrounding environment. Evidence also showed that, during very low volume rain events, in which surface runoff is the dominant mechanism for flushing pollutants into the water column, HF183 was undetectable and *E. coli* and fecal enterococci concentrations were one to two orders of magnitude lower than during larger storms, yet NoVGI was detected. These results indicate that flushing of open defecation sources during surface runoff are likely to transport pathogenic microorganisms into the water column, but that this source may only represent a fraction of the microbial pollutant inputs that occur from untreated wastewater. Overall, untreated wastewater sources are likely responsible for the majority of elevated microbial pollutants detected in the San Diego River and its tributaries during storm events.

This study suggests that efforts to address contamination of the San Diego River and its tributaries and meet wet weather pollution targets should prioritize replacement of cracked or failing sewer infrastructure or containment of sanitary sewer overflows. Despite the potentially lesser contribution of open defecation sites to pollutant loadings in the San Diego River during storm events, waste associated with homeless encampments remains an important source of fecal pollution to soils. Provision of improved water supply, sanitation, and hygiene facilities is recommended for unsheltered individuals experiencing homelessness in the region.

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1 INTRODUCTION

In 2018, more than 35 million visitors attended San Diego's beaches and other recreational activities centered around the city's parks, rivers, greenbelts, and inland waters (San Diego Tourism Authority, 2019). Also, each year, individuals contribute thousands of hours volunteering with environmental organizations such as the San Diego River Park Foundation to clean up sections of the San Diego River, engaging in restoration efforts, removing trash from the riverbanks, and monitoring water quality. Last year, the San Diego River Park Foundation coordinated over 26,000 hours of service from volunteers (San Diego River Park Foundation, 2019). Fecal contamination in ocean water adjacent to the San Diego River has been strongly associated with increased rates of gastrointestinal illness, sinus infections, ear infections, and infected wounds amongst surfers after rain events (Arnold et al., 2017), and human-associated fecal contaminants and pathogens have been detected in the San Diego River during storm events (Schiff et al., 2016).

In June 2019, the Water Quality Control Board of the San Diego Region issued an investigative order to identify and quantify the sources and transport pathways of human fecal pollution to the San Diego River (San Diego Region Water Board, 2019), identifying possible sources to include sanitary sewer overflows, discharges and exfiltration from sewer collection systems and laterals, discharges from on-site wastewater treatment systems, illicit connections and discharges to stormwater infrastructure, and discharges from homeless encampments. However, the relative contributions of each of these sources has not yet been determined. In general, the relative contribution of non-point sources of fecal pollution to surface water quality in general is poorly understood (Ahmed et al., 2019). Even less is known about the relationship between homeless encampments and water quality. As such, the purpose of this project was to investigate the potential for a broad range of emerging microbial and chemical contaminants to be transmitted from non-point sources in the riparian zone of the San Diego River and its tributaries.

The overarching goal of this study was to evaluate different inputs of microbial pollution to the San Diego River during dry and wet weather (Figure 1-1) conditions. To address this goal, objectives included determining if there was a significant change in pollutant concentrations and loadings in water downstream of homeless encampments during dry weather (Objective 1), evaluating fecal contamination in surface water and soils during homeless encampment cleanups (Objectives 2 and 3), simulating pollutant inputs from sites of open defecation and wastewater spills (Objective 4), and determining temporal variability in pollutant sources in the San Diego River and its tributaries during storm events (Objective 5). Table 1-1 shows a summary of the total number of samples analyzed for this project under each objective.

Table 1-1. Summary of the number of samples and types of analyses completed.
(continued on next page)

Objective	Number of Samples[1]	Contaminants and Water Quality Parameters Analyzed[2]
1. Upstream/Downstream	40	• *E. coli* • Enterococci • PhiX174 coliphage • HF183 • pH • Electrical conductivity • Total dissolved solids • Dissolved oxygen • Dissolved organic carbon • Total dissolved nitrogen • Nitrate • Phosphate • Caffeine • Sucralose
2/3. Cleanup (water)	26 (water) 28 (soil)	• *E. coli* • Enterococci • PhiX174 coliphage • HF183 • pH[2] • Electrical conductivity[2] • Total dissolved solids[2] • Dissolved oxygen[2] • Dissolved organic carbon[2] • Total dissolved nitrogen[2] • Nitrate[2] • Phosphate[2] • Caffeine[2] • Sucralose[2]
4a. Loadings from Soils: Laboratory flushing experiment	31	• *E. coli* • Enterococci • HF183[3] • PMMoV[3] • Dissolved organic carbon • Total dissolved nitrogen
4b. Loadings from Soils: Simulated runoff experiment	39	• *E. coli* • Enterococci • HF183 • PMMoV • Caffeine (when possible) • Sucralose (when possible)

Table 1-1 (continued). Summary of the number of samples and types of analyses completed

Objective	Number of Samples[1]	Contaminants and Water Quality Parameters Analyzed[2]
5. Storm Sampling	104	• *E. coli*[4] • Enterococci[4] • PhiX174 coliphage[5] • HF183 • PMMoV[5] • Bacterial and viral human pathogens[5,6] • pH • Electrical conductivity • Total dissolved solids • Dissolved organic carbon • Total dissolved nitrogen • Nitrate • Phosphate • Caffeine[7] • Sucralose[8]

1 The number of samples analyzed for Objective 1 includes upstream and downstream samples collected on 8 different dates at two different sites, plus an additional third site with upstream and downstream samples collected on four different dates. The original proposal only required N = 8 upstream and downstream samples at two different sites. The number of samples for Objectives 2/3 include water samples collected on the day of cleanup and one week after, as well as soil samples collected on the day of cleanup, one week after, and one month after. The number of samples for Objective 4b includes 3 – 7 replicate flushes from 9 different experimental sites across four former homeless encampments. The samples for Objective 5 originated from a total of 5 different storm events spread across two hydrologic years, with 1 – 3 sampling sites per storm event (see Table 5-1).

2 Water samples only were analyzed for these parameters

3 As described in the proposal, only 23 samples were analyzed for HF183 and PMMoV

4 *E. coli* and enterococci were analyzed for all storms except for the January 2018 storm in Alvarado Creek.

5 PhiX174, PMMoV, and human pathogens were only analyzed for the storms in February 2018 and October 2018.

6 Human pathogens include *Campylobacter jejuni*, *Campylobacter coli*, Hepatitis A virus, and norovirus genotype I.

7 Caffeine concentrations were analyzed for all storms and all sites except for the March 2019 storm.

8 Sucralose concentrations were analyzed for all storms and all sites except for the November 2018 storm in Forester Creek and the March 2019 storm in the San Diego River.

Figure 1-1. Discharge and surface runoff in Alvarado Creek, San Diego, CA during a storm event. Photo credit: Federick Pinongcos.

2 DRY WEATHER CONTRIBUTIONS FROM HOMELESS ENCAMPMENTS

2.1 INTRODUCTION

San Diegans experiencing homelessness often seek shelter in river margins and canyons but lack adequate access to clean water and sanitation. Encampments, including tents and other informal shelters, are often found in the riparian zone of the San Diego River and within tunnels and culverts conveying flow from tributaries of the San Diego River, such as Forester Creek and Forester Channel. Encampments are most active during the dry season and become inactive or abandoned during the wet season, when lower temperatures or inclement weather occur, or during storm events, when conditions become unsafe due to flooding. Much research has focused on water quality during storm events, but human interactions with urban streams occur during dry periods as well. Research conducted by Flanigan and Welsh (2020) indicates that ~20% (11 out of 56 interviewed) of unsheltered San Diegans dwelling near the river or in canyons use river water for non-drinking purposes and ~2% (1 out of 56 interviewed) use river water for drinking. Given that lack of sanitation around homeless encampments may result in some fecal contamination reaching the San Diego River during dry weather, an important goal of this study was to evaluate the contribution of pollution from homeless encampments in the San Diego River or its tributaries during dry weather conditions. Specifically, we addressed the following research question:

Are concentrations of chemical and microbial pollutants immediately downstream of homeless encampments significantly greater than they are immediately upstream?

To answer this question, we collected water samples from the river directly upstream and downstream of homeless encampments and analyzed them for the following physical-chemical water quality parameters and microbial pollutants: phosphate, nitrate, total dissolved nitrogen (TDN), dissolved organic carbon (DOC), dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity, pH, *E. coli*, enterococci, a gene target from coliphage PhiX174 (a member of the somatic coliphage group), and the HF183 gene target (from *Bacteroides dorei*), a human-associated bacterial indicator of fecal pollution. Our hypothesis with this research objective was that the concentrations of pollutants would be greater downstream from homeless encampments than upstream. If found to be true with statistical significance, this would indicate that the homeless encampments were contributing pollution to the water bodies. A lack of significant findings would mean that the homeless encampments studied for Objective #1 either did not contribute pollution or they contributed very low levels of pollution that cannot be detected with the sample size chosen for this study.

2.2 MATERIALS AND METHODS

On eight different dates during dry weather conditions, grab samples were collected directly upstream and downstream of active homeless encampments at two different sites near tributaries of the San Diego River. The target number of paired samples per site (N = 8) was determined based on the desired ability to detect a significant difference of 0.5 $\log_{10}$ units of fecal indicators between upstream and downstream samples using a paired sample t-test with 95%

confidence and 80% power (see Appendix 10 in this project's Quality Assurance Project Plan (QAPP)). Samples were also collected at a third site, but only with four paired samples. Site 1 was located on a section of Alvarado Creek adjacent to San Diego State University; Site 2 was located in an unnamed channelized tributary of Forester Creek in the city of El Cajon (referred to here as Forester Channel; Figure 2-1); and Site 3 was on a section of the San Diego River south of Fashion Valley shopping center (Figure 2-2). These sites all had active homeless encampments on the day that the first samples were collected. The presence of individuals subsequently decreased at Sites 1 and 3 but persisted at Site 2 for the duration of sampling.

Figure 2-1. An active homeless encampment in the shadows of a bridge near the Wisconsin tunnel over Forester channel in El Cajon, California on July 1, 2019. Photo credit: Mireille Garcia.

Figure 2-2. Sampling locations in the San Diego River and two of its tributaries. GPS coordinates for Site 1: (upstream); (downstream); Site 2: (upstream), (downstream); Site 3: (upstream), (downstream).

2.2.1 Sample collection and *in situ* measurements

Samples were collected over the periods shown in Table 2-1 and in accordance with standard operating procedures for inland microbiological sample collection for the California Surface Water Ambient Monitoring Program (SWAMP). Sample container preparation guidelines are summarized in Table 1 of this project's QAPP. All samples were transferred to the laboratory in a cooler on ice.

An Accumet AP85 multiparameter field water quality meter with pH and conductivity probes was used to measure pH, water temperature, electrical conductivity, and total dissolved solids (TDS). A YSI Pro DO meter was used to measure dissolved oxygen *in situ*. Instruments were calibrated and checked as described in Table 3 of this project's QAPP. Streamgaging was performed during each sampling effort. The velocity-area method was used to estimate total discharge using a Hach digital velocity meter (FH950) and top-setting wading rod. The area and velocity were measured at multiple points along a cross-section so that no more than 10% of the streamflow was measured at a time. Streamflow is the product of velocity and each sub-section. Selected cross-sections were less than 4-feet deep, free of obstructions, and had minimal turbulence. Total

streamflow through the channel cross-section is the summation of streamflow for each sub-section. At areas with low flow (depth with less than 0.1 ft), the float method was used to calculate the velocity of water and estimate the flowrate of the channel. There is inherent error in streamgaging methods, especially in shallow depths. The FH950 has an accuracy of ± 2% of reading ± 0.05 ft/s (± 0.015 m/s) through the range of 0 - 10 ft/s (0 - 3.04 m/s). If float method was the only available option in the field, the estimated surface velocity will be larger due to the free surface of the water, increasing the estimated streamflow. At the Fashion Valley site (USGS station 11023000), 15-min streamflow data is available for the upstream and downstream sampling dates. These data are considered accurate to within ±8% of the reported flow (Flint et al., 2012). The pollutant loadings were then calculated as the product of the pollutant concentration and the estimated flow rate.

Table 2-1. Description of sites sampled upstream and downstream of homeless encampments.

Site	Name	Dates sampled	Upstream GPS coordinates[1]	Downstream GPS coordinates[1]	Distance from tents to stream	Description
1	Alvarado Creek at SDSU	Jun – Nov 2018	32°46'41.8"N, 117°03'48.7"W	32°46'43.9"N, 117°04'07.7"W	Within the 20 m riparian zone on the north side of the channel	2+ tents with makeshift barriers and inhabitants present
2	Forester Channel in El Cajon	Jun – Aug 2019	32°47'38.7"N, 116°57'25.9"W	32°48'04.1"N, 116°57'52.0"W	Tents and individuals located in tunnel at channel[2]	Multiple people entering and exiting tunnels, 1 tent visible near tunnel exit[3]
3	San Diego River at Fashion Valley	Oct – Nov 2018	32°45'59.0"N, 117°09'52.0"W	32°45'51.8"N, 117°10'12.2"W	Within the ~45 m riparian zones on north and south side of the channel	Clothes, cushions, blankets, present. Active at start, but became inactive.

[1] Datum: WGS84

[2] Channel width = 4.5 m

[3] Encampments inside of tunnel not visited due to safety considerations.

2.2.2 Quantification of microbial pollutants

All samples were analyzed within 24 hours of collection for fecal indicator bacteria *E. coli* and fecal enterococci using the Colilert and Enterolert methods, respectively, with the IDEXX Quanti-Tray 2000 system, as described in Standard Methods 9223B (APHA, 2012), following manufacturer's recommended protocols. Samples were diluted according to anticipated concentrations from past measurements. Colilert trays were incubated at 35°C for 18 h and Enterolert trays were incubated at 41°C for 24 h, and wells fluorescing blue under UV light after the incubation periods were counted as positive. The most probable number (MPN) and 95% confidence intervals were then calculated using maximum likelihood estimation (MLE).

Microorganisms present in samples were concentrated by vacuum filtration using membrane filters with a pore size of 0.45 µm. Filters were then placed inside bead-beating tubes with 600 µL of lysis buffer from the AllPrep PowerViral RNA/DNA Kit (Qiagen). After vortexing at high speed for 10 min with a bead-beating tube adapter (Qiagen), filter lysates were transferred to clean microcentrifuge tubes, and nucleic acids were extracted and purified following steps delineated in the manufacturer's protocol, using 50 µL of molecular grade water for the final elution of DNA.

Molecular targets were quantified using the Bio-Rad QX200 droplet digital polymerase chain reaction (ddPCR) system. Primers and probes were ordered based on previously published assays given in Table 5 of this project's QAPP. Final concentrations of primers were 900 nM, the final concentration of the probe was 250 nM, and the ddPCR Supermix for Probes (Bio-Rad cat. no. 186-3010) was used at a final concentration of 1X. A volume of 3 µL purified DNA was used for all samples. Molecular grade water was added as necessary to bring the final reaction volumes up to 20 µL. Polymerase chain reaction (PCR) mixtures were prepared in 0.2 µL PCR tubes or in 96 well PCR plates. Each reaction well was mixed thoroughly by pipetting the entire volume up and down 20 times. Droplets were generated in all samples using manual pipetting with eight-sample cartridges and droplet generator oil (Bio-Rad cat. no. 1863005), following the steps recommended by the manufacturer. Thermocycling conditions were as follows: 95°C for 10 min followed by 40 cycles of 94°C for 30 s, then 60°C for 1 min. After completion, samples were held at 98°C for 10 min, as recommended.

Quality assurance and quality control protocols for ddPCR are described in this project's QAPP, following recommendations set forth by Huggett et al. (2013). Briefly, a gBlock (Integrated DNA technologies) was used as a positive control. All gBlocks were resuspended following the manufacturer's recommendations, then stored at 1 ng/µL with 15 ng/µL yeast tRNA (gBiosciences) at -20°C until further use. Prior to each ddPCR run, gBlocks were thawed, then quantified by Qubit following steps described in the Qubit 1X dsDNA HS Assay protocol, and then serially diluted to concentrations between 0.1 and 1,000 copies per reaction. At least one no template control (NTC) and two positive controls were analyzed alongside samples for each PCR run. All PCRs were performed in duplicate and Inhibition controls were analyzed by spiking samples with a known concentration of gBlocks.

2.2.3 Quantification of physical-chemical constituents

Samples were filtered with pre-combusted (at 500°C for 2 hours) and pre-filtered (ultrapure water) 0.7 µm glass fiber filters. Nitrate and phosphate were quantified using Hach kits, following the manufacturer's standard operating procedures (Hach Methods 8039 and 8048). In brief, 10 mL of the filtered samples were added with powder pillows in a sample cell, and the absorbance was measured using the Hach DR900 system, set to the programs 355 N Nitrate HR PP (Nitrate) and 490 P React PV (Phosphate Reactive). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a high temperature combustion method with a Shimadzu TOC-L Total Organic Carbon Analyzer. Blanks of ultrapure water were run every 10 samples and 15% of the samples were run in duplicate. Other quality control measures are described in this project's QAPP.

Samples (400 mL) were extracted and analyzed for caffeine and sucralose concentrations following U.S. EPA Method 1694, using an Agilent 6460 Triple Quad LC/MS/MS system equipped with Agilent Jet Stream Technology. The triple quadrupole MS-MS system was operated in the multiple reaction monitoring (MRM) mode, using the precursor and product ion transitions of the target compounds and their isotopically labeled surrogates to detect the analytes of interest. Liquid chromatography mass spectrometry (LCMS) water was used as field blanks and lab blanks, and duplicates were run for every 10-20 samples. Caffeine concentrations were lower than 0.071 µg/L in three field blanks and undetectable in two. Sucralose concentrations were undetectable in four out of five field blanks and 0.001 in the fifth. Other quality control measures are described in this project's QAPP.

2.2.4 Statistical analysis

All data were analyzed using a one-tailed, paired sample t-test. The null hypothesis (H_o) was that the upstream and downstream concentrations were equal, or in other words, that the difference between downstream and upstream concentrations was equal to zero. The alternative hypothesis (H_A) was that downstream concentrations were greater than upstream concentrations, or in other words, the difference between downstream and upstream concentrations was greater than zero. This implies that we expect to see that the pollutants downstream of the encampment are present in greater concentrations, if our original hypothesis is true. The only exception was for pH and dissolved oxygen data, where we used a *two-tailed*, paired sample t-test. Statistical tests were done on the $\log_{10}$-transformed concentrations of all microbial pollutants. All statistical tests were performed in Microsoft Excel.

2.3 RESULTS AND DISCUSSION

2.3.1 Changes in concentrations of pollutants

The measured changes in the concentrations are shown in Figure 2-3. The vertical axis in the plots for microbial pollutants shows the $\log_{10}$ difference between downstream and upstream concentrations. A value of 1 thus indicates that the downstream concentration is ten times greater than the upstream concentration and a value of 2 indicates that the downstream concentration is 100 times greater than the upstream concentration. For *E. coli* and fecal enterococci, concentrations were mostly greater downstream than they were upstream, with the exception of enterococci at the Alvarado Creek and Forester Channel sites. The magnitude of the increase in the concentrations of fecal indicator bacteria was greater for Forester Channel and Fashion Valley sites than it was for Alvarado Creek, but the measured difference at Alvarado Creek was more consistent (as indicated by the lower standard deviations shown in Table 2-2).

The vertical axis in the plots for physical-chemical pollutants shows the percent change in the upstream and downstream concentrations. A negative value indicates that concentrations downstream of the encampment were greater than the upstream concentrations, a positive value indicates that concentrations upstream were greater than the downstream concentrations, and a value of 0% indicates no change in the concentrations between the upstream and downstream sampling points. In general, the concentrations either did not change much

between upstream and downstream samples, or the change was not significant and extremely variable from sample to sample (see Figure 2-3).

2.3.2 Fecal Indicator Bacteria

The mean (and standard deviations) of the $\log_{10}$-transformed *E. coli* concentrations ($\log_{10}$(MPN)/100 mL) were 1.72 (0.28) at the upstream Alvarado Creek site, 1.87 (0.32) at the downstream Alvarado Creek site, 2.88 (0.37) at the upstream Forester Channel site, 3.48 (0.62) at the downstream Forester Channel site, 1.90 (0.38) at the upstream Fashion Valley site, and 3.05 (0.26) at the downstream Fashion Valley site. The mean (and standard deviations) of the $\log_{10}$-transformed enterococci concentrations ($\log_{10}$(MPN)/100 mL) were 2.18 (0.29) at the upstream Alvarado Creek site, 2.14 (0.34) at the downstream Alvarado Creek site, 3.30 (0.50) at the upstream Forester Channel site, 3.54 (0.48) at the downstream Forester Channel site, 2.19 (0.18) at the upstream Fashion Valley site, and 2.86 (0.19) at the downstream Fashion Valley site.

The median difference between downstream and upstream *E. coli* concentrations was 0.12 $\log_{10}$ units at Alvarado Creek, 0.39 $\log_{10}$ units at Forester Channel, and 1.0 $\log_{10}$ units at Fashion Valley; the median difference between downstream and upstream fecal enterococci concentrations was negligible for Alvarado Creek and Forester Channel, and was equal to 0.62 $\log_{10}$ units at the Fashion Valley site. The differences were significant for *E. coli* at all three sites at an alpha level of 0.05, based on one-tailed, paired sample t-tests (Table 2-2). For fecal enterococci, the difference was only significant at Fashion Valley. These fecal indicator bacteria originate in the feces of warm-blooded animals, so they are used as fecal indicators. However, naturalized strains of *E. coli* have been reported to persist and even grow in natural soils and sediments, where they may later leach into groundwater or surface waters (Brennan et al., 2010; Byappanahalli et al., 2003; Ishii et al., 2006). Therefore, the increase in the concentration of *E. coli* by itself is not necessarily indicative of a pollution input from the homeless encampments, especially for the Alvarado Creek and Fashion Valley sites, which were located in non-channelized sections of the river. For the Forester Channel site however, the upstream and downstream locations were on opposite ends of the Wisconsin stormwater tunnel, which is entirely lined with concrete. The significant increase in *E. coli* concentrations at that site might indicate that there were some pollution inputs at that site, but not necessarily associated with human feces.

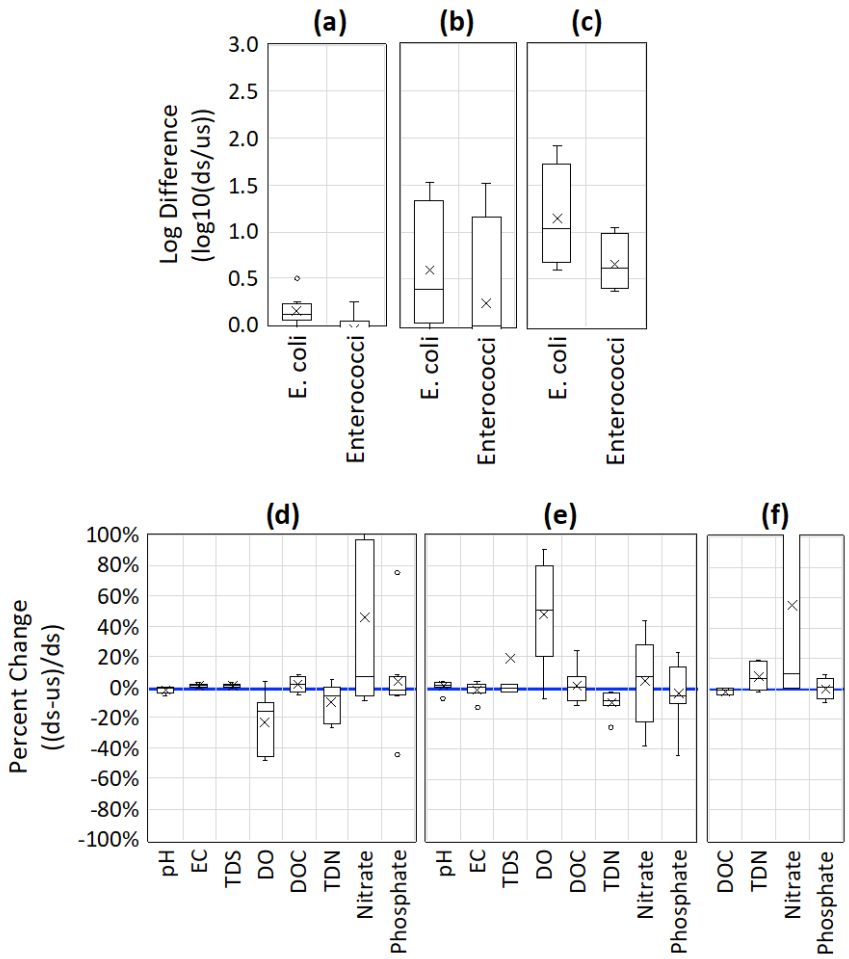

Figure 2-3. Log_{10} differences between concentrations of *E. coli* and fecal enterococci (a – c) and percent differences (d – f) in samples collected directly upstream (us) and directly downstream (ds) of homeless encampments during dry weather conditions: (a) and (d): Alvarado Creek near SDSU (N = 8); (b) and (e): Forester Channel at the Wisconsin tunnel (N = 8); and (c) and (f): the San Diego River at Fashion Valley (N = 4). Boxes show the interquartile range and the median, and whiskers show the minimum and maximum data points that are within 1.5 times the interquartile range. Plots also show mean values (×) and any outlier data points (◦) that are less than or greater than 1.5 times the interquartile range.

2.3.3 Human-Associated Bacteroides HF183

The human-associated bacterial fecal indicator HF183 was only sporadically detected at very low concentrations, prohibiting our ability to use the data to perform the statistical test. For example, with the exception of sporadic single positive droplets in only one of two replicates for three of the upstream samples, HF183 was not detected in any of the samples from Forester Channel. At Alvarado Creek, HF183 was only detected with one or two positive droplets in two upstream samples and two downstream samples, and at Fashion Valley it was only detected in a single upstream sample at a concentration of 553 GC per 100 mL (but it was not detected in the corresponding downstream sample). Therefore, there is sporadic evidence of human-associated

fecal pollution in the river and its tributaries during this dry period, but no evidence that the level of the human-associated fecal pollution increased as a result of the encampments.

Table 2-2. Mean and median differences, standard deviations, and results of one-tailed, paired sample t-tests comparing the log_{10}-transformed concentrations of *E. coli* and fecal enterococci in samples collected directly upstream and directly downstream of homeless encampments during dry weather conditions. Samples with significant differences are indicated in bold. Statistical results are not shown for the Fashion Valley site due to the small sample size (N = 4).

Site	Pollutant	Difference in the Concentrations[1]			Sample Size	p-value[3]
		Mean	Median	Std. Dev.[2]		
Alvarado Creek	***E. coli***	**1.4**	**1.3**	**0.16**	**N = 8**	**0.016***
	Fecal enterococci	~1	~1	0.16	N = 8	0.225
Forester Channel	***E. coli***	**3.9**	**2.5**	**0.65**	**N = 8**	**0.018***
	Fecal enterococci	1.7	~1	0.83	N = 8	0.221

[1] Values in this table should be read as follows: "Downstream concentrations were __ times as high as upstream concentrations." A value of ~1 indicates that upstream and downstream concentrations were approximately equal to each other.

[2] Standard deviation of the log_{10} difference between the concentrations in samples collected directly downstream (ds) and directly upstream (us) of homeless encampments.

[3] The p-value from a one-tailed, paired sample t-test of the log_{10}-transformed concentrations in samples collected directly upstream and downstream of homeless encampments. The null hypothesis is that upstream and downstream concentrations are equal; the alternative hypothesis is that downstream concentrations are greater.

* A value less than 0.05 indicates that the difference in concentrations is significant at the alpha = 0.05 level.

2.3.4 PhiX174 coliphage

The overall geometric mean concentration of PhiX174 coliphage at the Forester Channel site was 88 gc per 100 mL. There was no significant difference between upstream and downstream samples and the concentrations slightly decreased with respect to the sample collection date (Figure 2-4). PhiX174 is a virus that infects *E. coli*, but despite the significant differences noted in the concentrations of *E. coli* between upstream and downstream samples (Table 2-2), the same trend was not observed for PhiX174.

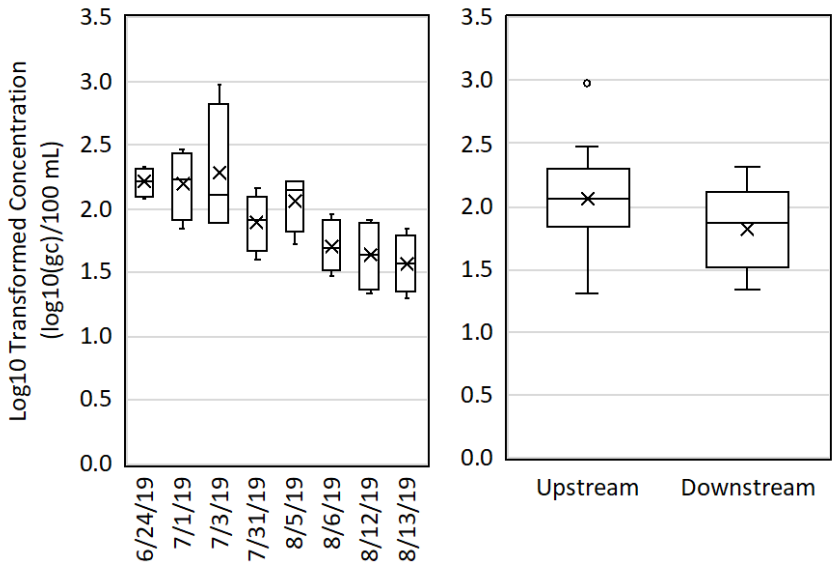

Figure 2-4. Box plots of PhiX174 concentrations at Forester Channel with respect to the date of sample collection and with respect to the sample location (upstream vs. downstream). Box plots for the left were generated using lab duplicates for duplicate samples (one upstream and one downstream). Box plots on the right were generated using lab duplicates for samples collected on all dates at the upstream or downstream location.

2.3.5 Caffeine and Sucralose

At the Alvarado Creek site, caffeine and sucralose concentrations were only measured on four of the eight sampling dates. At this site, the median caffeine concentrations were 0.036 ng/mL (upstream) and 0.044 ng/mL (downstream), and the median sucralose concentrations were 0.393 ng/mL (upstream) and 0.419 ng/mL (downstream). Caffeine concentrations increased by an average of 10% between upstream and downstream locations, but the difference was not significant. Sucralose concentrations remained nearly the same, with no significant difference (Table 2-3). At Forester Channel, caffeine and sucralose concentrations were measured on all eight dates, but the percent recovery from the internal standard for sucralose in one of the downstream samples was not within an acceptable range, so only seven samples were used to calculate the change in sucralose concentrations at that site. At this site, the median caffeine concentrations were 0.226 ng/mL (upstream) and 0.219 ng/mL (downstream), and the median sucralose concentrations were 0.580 ng/mL (upstream) and 0.509 ng/mL (downstream). Concentrations of caffeine and sucralose both increased by an average of 41%, but the differences were not significant at the 0.05 level. The median values were quite different—the median change in caffeine concentrations was -10% (i.e., concentrations were higher upstream), while the median change in sucralose concentrations was +27% (i.e., concentrations were higher downstream). Figure 2-5 contains a time series plot of the concentrations.

There are two sampling dates where the changes in concentrations were vastly different than they were on other dates. On 8/5/2019, there was an increase of >350% in the downstream concentrations of caffeine, and the next day, downstream caffeine concentrations were still almost twice as high as upstream concentrations. There were also large observed spikes in the

concentrations of *E. coli* and fecal enterococci on these two dates (Figure 2-5). Nevertheless, downstream of the encampment, caffeine/sucralose ratios were not greater than upstream (Figure 2-6) and were consistently below 1.0. Ratios above 2.0 are indicative of recent contamination by sewage. This result, combined with the fact that HF183 was also not detected in those samples, indicates that there is may have been some form of pollution input to the water during those days, but no strong evidence of human fecal pollution.

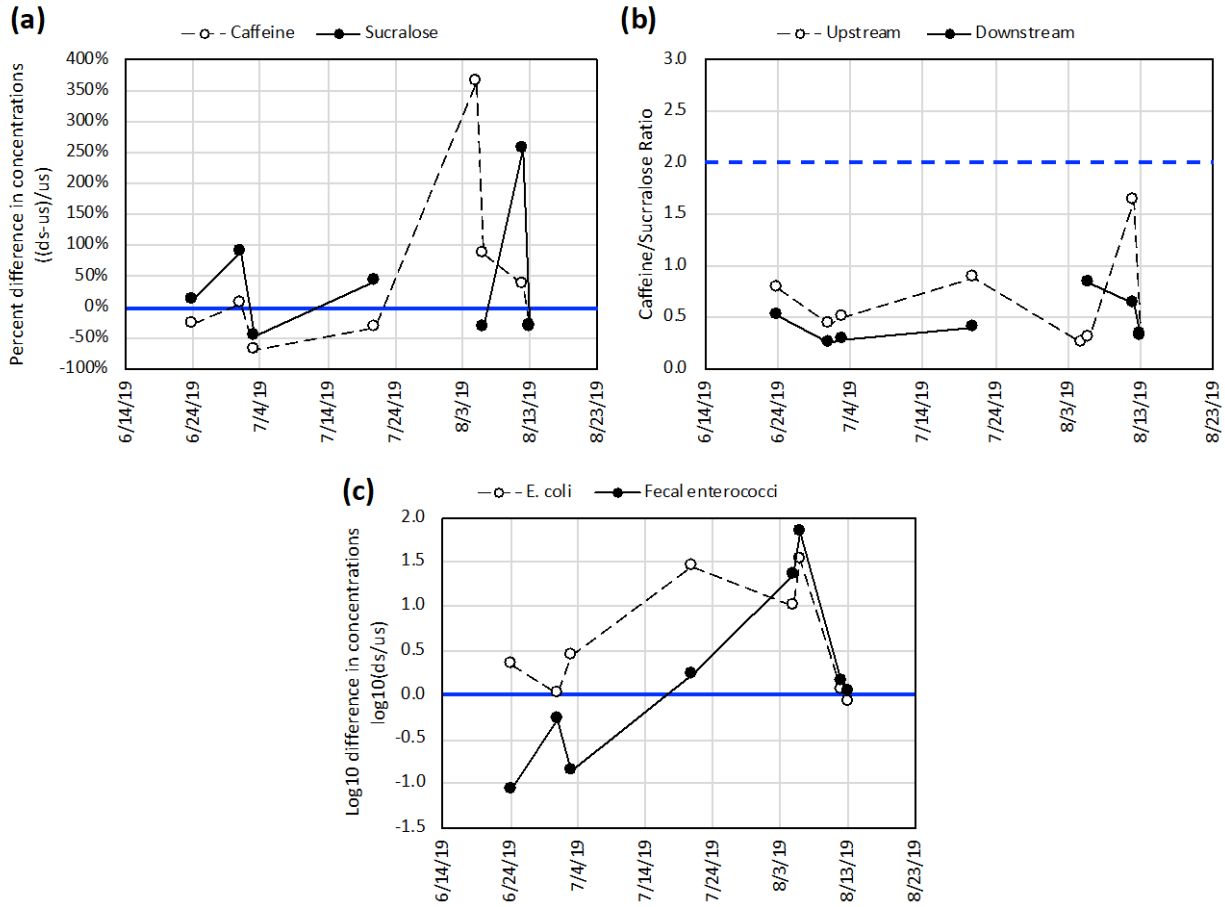

Figure 2-5. Time series plots showing: (a) the percent changes in the concentrations of caffeine and sucralose; (b) the change in the caffeine/sucralose ratios; and (c) the $\log_{10}$ differences in the concentrations of *E. coli* and fecal enterococci, at the Forester Channel site (negative values indicate concentrations were higher upstream than they were downstream; positive values indicate concentrations were higher downstream than they were upstream).

2.3.6 Physical Chemical Water Quality Constituents

Despite the significant increases in the concentrations of *E. coli*, the physical-chemical water quality data did not provide evidence that was supportive of an input of fecal pollution at the encampments. For example, at the Alvarado Creek and Forester Channel sites, total dissolved nitrogen concentrations were significantly lower downstream of the encampments than they

were upstream. Dissolved organic carbon concentrations increased only slightly but the increase was not significant. Nutrient levels also changed, but the changes were not significant. Dissolved oxygen concentrations significantly changed at these two sites, but in opposite directions—at the Alvarado Creek site, upstream samples had higher dissolved oxygen than downstream samples, concentrations of dissolved oxygen in Forester Channel were higher downstream than they were upstream of the encampment. It should be noted that the water was more stagnant at these sampling locations with lower observed dissolved oxygen levels.

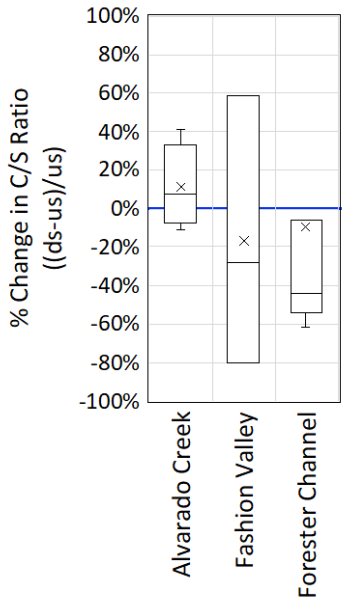

Figure 2-6. Percent changes in the ratio of caffeine/sucralose concentrations in the three sites (negative values indicate that the ratio was higher upstream than it was downstream; positive values indicate that the ratio was higher downstream than it was upstream). The changes observed in this ratio between upstream and downstream samples were not significant for any of the three sites.

2.3.7 Changes in pollutant loadings

The average flow rates measured during the sampling periods were 12.5 L/s for Alvarado Creek, 5.3 L/s for Forester Channel, and 23.0 L/s for the San Diego River at Fashion Valley. Flow rates measured upstream of homeless encampments were very similar to flow rates measured downstream, and flow rates did not vary greatly throughout the duration of the three sampling campaigns. The associated pollutant loadings are shown in Table 2-4. It is important to note that there is inherent error within the streamflow estimates, especially during low flow conditions, which can have significant implications for pollutant loadings. Forester Channel delivered higher loadings of *E. coli* and fecal enterococci than Alvarado Creek—in fact, the loadings per day from Forester Channel were approximately the same as the loadings per day measured at Fashion Valley (however, note that these sites were sampled in different years).

Table 2-3. Magnitude of mean and median differences and standard deviations and results of the paired sample t-tests comparing the concentrations of physical-chemical pollutants in samples collected directly upstream (us) and directly downstream (ds) of homeless encampments during dry weather conditions. Samples with significant differences are indicated in bold. Statistical results are not shown for the Fashion Valley site due to the small sample size (N = 4).

Site	Pollutant/Parameter	Difference in the Concentrations[1]			Sample Size	p-value[3]
		Mean	Median	Std. Dev.[2]		
Alvarado Creek	pH	-1.5%	-0.3%	2.5%	N = 8	0.133
	Electrical conductivity	**+1.2%**	**+1.1%**	**1.0%**	**N = 8**	**0.007***
	Total dissolved solids	**+1.3%**	**+1.5%**	**1.1%**	**N = 8**	**0.004***
	Dissolved oxygen	**-23%**	**-16%**	**20%**	**N = 8**	**0.017***
	Dissolved organic carbon	+1.8%	+1.8%	5.1%	N = 8	0.177
	Total dissolved nitrogen	**-9.6%**	**-5.4%**	**12%**	**N = 8**	**0.038***
	Nitrate	+46%	+7.4%	75%	N = 8	0.052
	Phosphate	+4.6%	-3.9%	36%	N = 8	0.464
	Caffeine	+11%	+1.4%	28%	N = 4	0.203
	Sucralose	+0.1%	+3.4%	13%	N = 4	0.299
Forester Channel	pH	+1.1%	+1.8%	3.6%	N = 8	0.477
	Electrical conductivity	-1.4%	0%	5.6%	N = 8	0.266
	Total dissolved solids	+20%[4]	-0.3%	62%[5]	N = 8	0.225
	Dissolved oxygen	**+48%**	**+51%**	**34%**	**N = 8**	**0.002***
	Dissolved organic carbon	+1.5%	+0.7%	12%	N = 8	0.441
	Total dissolved nitrogen	**-9.7%**	**-8.6%**	**7.6%**	**N = 8**	**0.002***
	Nitrate	+4.7%	+7.3%	29%	N = 8	0.396
	Phosphate	-3.3%	-5.7%	21%	N = 8	0.265
	Caffeine	+41%	-10%	139%	N = 8	0.251
	Sucralose	+41%	+27%	106%	N = 7	0.350

[1] Positive (+) values should be read as follows: "Downstream concentrations were ___% higher than upstream concentrations." For negative (–) values, take the absolute value and read: "Upstream concentrations were ___% higher than downstream concentrations". A value of ~0% indicates that concentrations were approximately equal.

[2] Standard deviation of the percent (%) difference between the concentrations in samples collected directly downstream (ds) and directly upstream (us) of homeless encampments.

[3] The p-value from a one-tailed, paired sample t-test of the concentrations in samples collected directly upstream and downstream of encampments. The null hypothesis is that upstream and downstream concentrations are equal; the alternative hypothesis is that downstream concentrations are greater (except for pH and dissolved oxygen, where the alternative hypothesis is that the concentrations are not equal, *i.e.* two-sided test).

[4] The high mean value is due to a single set of samples where the upstream concentration was very low and the downstream concentration was comparable to values measured on other dates. This data point is likely an outlier.

[5] If the outlier set of data points is omitted (see footnote #4), this standard deviation would be equal to 5.4%.

* A value less than 0.05 indicates that the difference in concentrations is significant at the alpha = 0.05 level.

Humans generate between 0.7 and 1.7 kg of excreta each day, including 50 – 87 g of total solids, 8 – 16 g of total nitrogen, and 1 – 4 g of total phosphorus (Feachem et al., 1983; Hansen and Tjell, 1979; Schouw et al., 2002). Nearly 90% of excreted nitrogen and approximately two-thirds of excreted phosphorus is in the urine, with the remainder originating from feces. Most nitrogen is excreted as urea, creatine, or ammonia and most phosphorus is excreted in an organic form, as nucleic acids. However, there are many other potential sources of nitrogen and phosphorus pollution to waterbodies during dry weather conditions, including natural and biological sources resulting from the decay of natural wildlife. Humans also generally excrete between 10^8 and 10^{11} MPN of *E. coli* and between 10^7 and 10^{10} MPN of fecal enterococci per day (Harwood et al., 2017), but there are also many other animal sources of these microbial pollutants, including dogs and ducks. Still, if excreta were a major source of pollution at the encampment sites, we would have also expected to see significant increases in the concentrations of total dissolved solids, dissolved organic carbon, and total dissolved nitrogen. If detergents or other soaps were used at the encampments, then we would expect to see an increase in the concentration of phosphate concentrations. However, this was not the case. Thus, based on the lack of detectable levels of HF183, the low caffeine/sucralose ratios observed, and the lack of consistent, significant changes in the measured concentrations of physical-chemical pollutants associated with feces, there is not enough evidence to suggest that the homeless encampments are contributing fecal pollution to these water bodies during dry weather conditions.

2.3.8 Limitations

It is important to note that there are several limitations to this study. First, our small sample size (N = 8 paired samples per site; fewer samples analyzed for some parameters) may have precluded our ability to detect a significant difference if the increase in pollution was very small. Due to the nature of random sampling and statistical analysis, it is possible that the concentrations of the pollutants analyzed were indeed greater downstream than they were upstream, but perhaps the magnitude of the difference was too small to be detected using our sample size of N = 8. We chose this sample size based on the desired ability to detect a 0.5-$\log_{10}$ (*i.e.*, 68%) increase in the concentration of microbial pollutants (see Appendix A) with four out of five odds (*i.e.*, a statistical power of 80%).

As was shown previously, there were far fewer encampments along the San Diego River in the 2018 hydrologic year compared to the previous year (124 in 2018, down from 188 in 2017). In general, active encampments were difficult to find in 2018 and 2019 along the main stem of the lower San Diego River. We observed not only fewer encampments but also fewer large encampments with multiple tent sites. Larger encampment sites would conceivably have had greater interactions with the San Diego River and its tributaries and possibly more measurable influences on water quality. Therefore, the lack of larger encampment sites is a limitation of this study. Nevertheless, the methods used here may be applied in other watersheds where larger encampments exist. It should be noted that in this type of field study, upstream and downstream monitoring efforts along tributaries or channels with lower flow (as we have done) are recommended due to their lower volumes, compared to the main stem of the river, where higher

the dilution effect of higher volumes would make it more difficult to discern significant anthropogenic inputs from homeless encampments.

In addition, while we did our best to select sites where the upstream and downstream sampling locations completely isolated the homeless encampments as the sole source of fecal pollution, there were still other potential pollution sources at all three sites. For example, sanitary sewer lines run alongside the river and creeks at all three sites, and we did not specifically measure sewer exfiltration. During dry weather conditions, groundwater is likely also a source of water to the Alvarado Creek and Fashion Valley sites, but we did not measure the concentrations of pollutants in groundwater samples. At the Forester Channel site, there are several piped stormwater inputs that enter the Wisconsin Tunnel between the upstream and downstream sites, but they do not drain a very large area. Nevertheless, we did not collect samples from each of these input pipes, as we were unable to access the interior of the tunnel for safety considerations.

Table 2-4. Flow rates and calculated loadings of microbial and physical-chemical pollutants in samples collected upstream (us) and downstream (ds) of homeless encampments during dry weather conditions.

Site	Flow Rate (L/s)		Pollutant	Mean Pollutant Loadings	
	Mean	Std. Dev.		Upstream	Downstream
Alvarado Creek*	55.5	39.6	*E. coli* (MPN/d)	3.8×10^{9}	5.1×10^{9}
			Fecal enterococci (MPN/d)	1.1×10^{10}	1.1×10^{10}
			Dissolved organic carbon (kg/d)	34.0	34.9
			Total dissolved nitrogen (kg/d)	4.0	3.7
			Nitrate (kg/d as N)	1.4	2.1
Forester Channel	5.3	2.3	*E. coli* (MPN/d)	4.2×10^{9}	3.2×10^{10}
			Fecal enterococci (MPN/d)	1.9×10^{10}	2.3×10^{10}
			Dissolved organic carbon (kg/d)	3.47	3.61
			Total dissolved nitrogen (kg/d)	2.14	2.17
			Nitrate (kg/d as N)	1.84	1.71
			Caffeine (mg/d)	123	151
			Sucralose (mg/d)	233	280
San Diego River at Fashion Valley	23.0	4.8	*E. coli* (MPN/d)	2.2×10^{9}	2.5×10^{10}
			Fecal enterococci (MPN/d)	3.4×10^{9}	1.6×10^{10}
			Dissolved organic carbon (kg/d)	28.4	27.9
			Total dissolved nitrogen (kg/d)	1.85	2.02
			Nitrate (kg/d as N)	0.411	0.612
			Caffeine (mg/d)	82.0	70.3
			Sucralose (mg/d)	852	659

*Caffeine and sucralose results in this dataset had high recoveries for several samples and are not reported at this time.

2.4 CONCLUSIONS AND RECOMMENDATIONS

Based on the results found within the scope of this study, we did not find strong evidence that homeless encampments are causing increases in the concentrations of water quality pollutants during dry weather conditions. Human-associated markers of fecal pollution were sporadically detected during dry weather conditions, but there is no indication that the homeless encampments assessed here were responsible for this pollution. There are many factors that may influence the potential for a homeless encampment to cause pollution to a waterbody during dry weather conditions, including the distance between the encampments and the waterway, the number of people living in the encampments, the distance of the sampling stations from the encampments, the number of sampling events, the time of day when samples are collected, and the sensitivity of analysis methods. The impact of all of these factors were not able to be fully addressed in the present study. Nevertheless, we recommend that lawmakers, policymakers, journalists, and others avoid making blanket statements implying that homeless encampments along waterways contribute fecal pollution to surface waters during dry weather conditions, unless new evidence emerges to indicate otherwise. Regardless of our inability to detect significant differences in the concentrations of fecal-associated pollutants from homeless encampments in this study, our group has still documented evidence of open defecation in the riparian zone of the San Diego River and its tributaries, and we recommend that improved water supply, sanitation, and hygiene facilities be provided for all individuals experiencing homelessness in California.

3 POLLUTANTS ASSOCIATED WITH HOMELESS ENCAMPMENT CLEANUP ACTIVITIES

3.1 INTRODUCTION

There is potential for a broad range of microbial and chemical contaminants to be transmitted from non-point sources to the water column and to persist in soils where they could continue to pose a concern to workers and volunteers conducting cleanup activities. Objectives #2 and #3 of the study sought to determine the presence of target microbial and chemical constituents in river water and in riverbank soils during and after the cleanup of a homeless encampment (Figure 3-1). Specifically, we addressed the following research questions:

- *What are the concentrations of pollutants immediately upstream, adjacent to, and downstream of a former homeless encampment during and after a cleanup event?*
- *What are the concentrations of pollutants in soils during and after the cleanup activities?*

To answer these questions, we coordinated with the San Diego River Park Foundation to collect water and soil samples during a cleanup event that took place on March 7, 2018, on the southern side of a section of the San Diego River in Mission Valley between Interstate 5, Interstate 8, and the Presidio Little League park. We returned to the same location a week after the cleanup, and again approximately one month after the cleanup, to collect more samples from the same locations. Samples were analyzed for the following constituents: *E. coli*, enterococci, and the human-associated bacterial indicator of fecal pollution, HF183 (from *Bacteroides dorei*), phosphate, nitrate, dissolved organic carbon DOC, total dissolved nitrogen (TDN), total dissolved solids (TDS), electrical conductivity and pH. Our research evaluated whether the concentrations of pollutants would be greater downstream from homeless encampment cleanup sites than upstream and whether the concentrations of pollutants downstream and in riverbank soils would decrease with respect to the number of days following the cleanup event.

3.2 MATERIALS AND METHODS

The location of the cleanup site and the locations of the water and soil sampling sites are shown in Figure 3-2. This cleanup site was chosen based on the San Diego River Park Foundation's schedule of cleanup activities at the time of the project and given the fact that there were sites within this area with evidence of open defecation, close to the river. Approximately one week prior to the cleanup event, the San Diego Police approached anyone present on the site to provide outreach services and notify them to vacate the site by the following week in order to avoid an arrest. On the day of the cleanup, there were no individuals present on site, and with the exception of one tent that appeared to be actively in use, all other campsites, belongings, and materials appeared to have been abandoned. Several sites with evidence of open defecation were identified. These sites were characterized by the presence of visible fecal material on the ground, surrounded by soiled toilet paper or napkins (Figure 3-1). The project team chose two such sites that were closest to the river's edge for soil sampling and analysis (Figure 3-2).

Figure 3-1. Volunteers from the San Diego River Park Foundation pick up trash near a site with evidence of open defecation (Site 1, left); evidence of open defecation near the San Diego River consists of soiled napkins or toilet paper located in close proximity with fecal material (Site 2, left). Photo credit: Matthew E. Verbyla.

Figure 3-2. Sampling locations at the San Diego River cleanup site in Mission Valley. GPS coordinates: 32°45'44.4"N 117°11'40.2"W (upstream water sample); 32°45'42.2"N 117°11'59.0"W (adjacent water

sample); 32°45'41.7"N 117°12'04.7"W (downstream water sample); 32°45'40.2"N 117°12'03.6"W (soil and fecal samples from site #1), 32°45'41.7"N 117°11'53.6"W (soil and fecal samples from site #2). Datum: WGS84.

3.2.1 Sample collection and in situ measurements

Samples were collected on three different dates following a riverbank cleanup event performed by the San Diego River Park Foundation. The cleanup occurred on March 7, 2018, starting in the early morning and lasting through until around noon. The first set of samples (water and soil) was collected at the same time as the cleanup event was happening (i.e., t = 0 days). The second set of samples (water and soil) was collected on March 15, 2018 (i.e., t = 8 days after cleanup), and the third set of samples (soil only, plus runoff experiments) was collected on April 12, 2018 (i.e., t = 36 days after cleanup). Between three and six soil samples were collected at two different sites with evidence of open defecation. The individual soil samples were collected at distances of approximately 30 cm surrounding the apparent human stools in opposing directions, on all different sides of the open defecation site. One approach for soil sampling is to collect composite soils from different sub-sites in a larger overall area. However, in order to capture some of the sample to sample variability, we used a different strategy for this exploratory study. Soil samples collected on different dates were collected in the same general vicinity of the sites, but they were individual samples—that is, they were collected in the same vicinity, but not in the exact same spots as the samples collected on the prior dates. All soil samples were collected at depths of 0 – 2 cm by scraping the surface with an autoclaved scoop and then transferring the soil to a 50-mL centrifuge tube (Falcon), using sterile techniques. One fecal sample was also collected from each of the two sites on the first sampling date only. Water samples were collected upstream of the cleanup site, adjacent to the cleanup site, and downstream of the cleanup site, in accordance with standard operating procedures for inland microbiological sample collection for the California Surface Water Ambient Monitoring Program (SWAMP) (see Appendix A for field data sheets). Sample container preparation guidelines are summarized in Table 1 of this project's Quality Assurance Project Plan (QAPP). All samples were transferred to the laboratory in a cooler on ice.

An Accumet AP85 portable multiparameter field water quality meter with pH and conductivity probes was used to measure pH, water temperature, electrical conductivity, and total dissolved solids (TDS). A YSI Pro DO meter was used to measure dissolved oxygen *in situ*. Instruments were calibrated and checked as described in this project's QAPP. Stream gaging was performed during each sampling effort. The velocity-area method was used to estimate total discharge using a Hach digital meter (FH950) and top-setting wading rod. The area and velocity were measured at multiple points along a cross-section so that no more than 10% of the streamflow was measured at a time. Streamflow is the product of velocity and each sub-section. Selected cross-sections were less than 4-feet deep, free of obstructions, and had minimal turbulence. Total streamflow through the channel cross-section is the summation of streamflow for each sub-section. There is inherent error in streamgaging methods, especially in shallow depths. The FH950 has an accuracy of ± 2% of reading ± 0.05 ft/s (± 0.015 m/s) through the range of 0 - 10 ft/s (0 - 3.04 m/s). Pollutant loadings were then calculated as the product of the pollutant concentration and the estimated flow rate.

3.2.2 Quantification of microbial pollutants

All samples were analyzed within 24 hours of collection for fecal indicator bacteria *E. coli* and fecal enterococci using the Colilert and Enterolert methods, respectively, with the IDEXX Quanti-Tray 2000 system, as described in Standard Methods 9223B (APHA, 2012), following manufacturer's recommended protocols. Samples were diluted according to anticipated concentrations from past measurements. Colilert trays were incubated at 35°C for 18 h and Enterolert trays were incubated at 41°C for 24 h, and wells fluorescing blue under UV light after the incubation periods were counted as positive. The most probable number (MPN) and 95% confidence intervals were then calculated using maximum likelihood estimation (MLE).

Microbial DNA from soil and fecal samples (~0.25 g) was extracted using the Qiagen PowerSoil kit, according to the manufacturer's instructions. Microorganisms present in water samples were concentrated by vacuum filtration using membrane filters with a pore size of 0.45 µm. Filters were then placed inside bead-beating tubes with 600 µL of lysis buffer from the AllPrep PowerViral RNA/DNA Kit (Qiagen). After vortexing at high speed for 10 min with a bead-beating tube adapter (Qiagen), filter lysates were transferred to clean microcentrifuge tubes, and nucleic acids were extracted and purified following steps delineated in the manufacturer's protocol. A volume of 50 µL of molecular grade water was used for the final elution of DNA from all samples.

Molecular targets were quantified using the Bio-Rad QX200 droplet digital polymerase chain reaction (ddPCR) system. Primers and probes were ordered based on previously published assays as described in Table 5 of this project's QAPP. Final concentrations of primers were 900 nM, the final concentration of the probe was 250 nM, and the ddPCR Supermix for Probes (Bio-Rad cat. no. 186-3010) was used at a final concentration of 1X. A volume of 3 µL purified DNA was used for all samples. Molecular grade water was added as necessary to bring the final reaction volumes up to 20 µL. Polymerase chain reaction (PCR) mixtures were prepared in 0.2 µL PCR tubes or in 96 well PCR plates. Each reaction well was mixed thoroughly by pipetting the entire volume up and down 20 times. Droplets were generated in all samples using manual pipetting with eight-sample cartridges and droplet generator oil (Bio-Rad cat. no. 1863005), following the steps recommended by the manufacturer. Thermocycling conditions were as follows: 95°C for 10 min followed by 40 cycles of 94°C for 30 s, then 60°C for 1 min. After completion, samples were held at 98°C for 10 min, as recommended.

Quality assurance and quality control protocols for ddPCR are described in the QAPP, following recommendations set forth by Huggett et al. (2013). Briefly, a gBlock (Integrated DNA technologies) was used as a positive control. All gBlocks were resuspended following the manufacturer's recommendations, then stored at 1 ng/µL with 15 ng/µL yeast tRNA (gBiosciences) at -20°C until further use. Prior to each ddPCR run, gBlocks were thawed, then quantified by Qubit following steps described in the Qubit 1X dsDNA HS Assay protocol, and then serially diluted to concentrations between 0.1 and 1,000 copies per reaction. At least one no template control (NTC) and two positive controls were analyzed alongside samples for each PCR run. All PCRs were performed in duplicate and Inhibition controls were analyzed by spiking samples with a known concentration of gBlocks.

3.2.3 Quantification of physical-chemical constituents

Samples were filtered with pre-combusted (at 500°C for 2 hours) and pre-filtered (ultrapure water) 0.7 µm glass fiber filters. Nitrate and phosphate were quantified using Hach kits, following the manufacturer's standard operating procedures (Hach Methods 8039 and 8048). In brief, 10 mL of the filtered samples were added with powder pillows in a sample cell, and the absorbance was measured using the Hach DR900 system, set to the programs 355 N Nitrate HR PP (Nitrate) and 490 P React PV (Phosphate Reactive). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a high temperature combustion method with a Shimadzu TOC-L Total Organic Carbon Analyzer. Blanks of ultrapure water were run every 10 samples and 15% of the samples were run in duplicate. Other quality control measures are described in this project's QAPP.

Samples (400 mL) were extracted and analyzed for caffeine and sucralose following U.S. EPA Method 1694, using an Agilent 6460 Triple Quad LC/MS/MS system equipped with Agilent Jet Stream Technology. The triple quadrupole MS-MS system was operated in the multiple reaction monitoring (MRM) mode, using the precursor and product ion transitions of the target compounds and their isotopically labeled surrogates to detect the analytes of interest.

3.2.4 Statistical analysis

For water and soil samples, descriptive statistics of parameter values are reported. The sample size for this objective was too small to assess whether or not any differences observed were significant. However, the purpose of this study was not to conduct a hypothesis test, but rather to gather exploratory data set. Data analyses and plots were done on the $\log_{10}$-transformed concentrations of all microbial pollutants, and all summary statistics were calculated in Microsoft Excel.

3.3 RESULTS AND DISCUSSION

3.3.1 Concentrations of pollutants in the river

The first question we addressed was to determine the concentrations of pollutants in the river water at locations immediately upstream, adjacent to, and downstream of a former homeless encampment during and after a cleanup event. Figure 3-3 shows box plots with the concentrations of fecal indicator bacteria upstream, adjacent, and downstream of the cleanup site, and on the day of cleanup (0 days) and approximately one week after the cleanup event (8 days). The box plots in the panels shown on the left sides of Figure 3-4 incorporate data from both sampling dates (day 0 and day 8), and the plots shown in the panels on the right sides of Figure 3-4 incorporate data from all three locations (upstream, downstream, and adjacent).

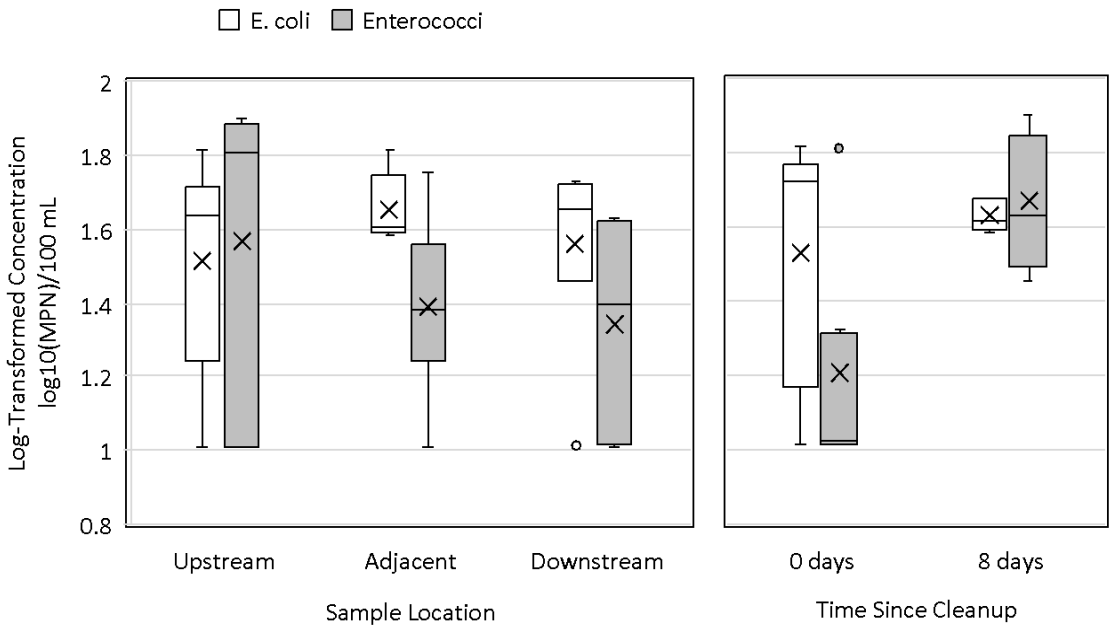

Figure 3-3. Log_{10} concentrations of *E. coli* and fecal enterococci in samples collected upstream and downstream of the San Diego River cleanup site at Mission Valley, on the day of the cleanup and 8 days after the cleanup. Boxes show the interquartile range and the median, and whiskers show the minimum and maximum data points that are within 1.5 times the interquartile range. Plots also show mean values (×) and any outlier data points (◦) that are less than or greater than 1.5 times the interquartile range. Box plots are based on triplicate samples collected upstream, adjacent to, and downstream of the cleanup site on the day of the cleanup (0 days) and approximately one week after the cleanup (8 days).

Figure 3-4 shows box plots with the concentrations of caffeine and sucralose, and the corresponding caffeine/sucralose ratios for the different sample dates and sample locations. The box plots in the panels shown on the left sides of Figure 3-4 incorporate data from both sampling dates (day 0 and day 8), and the plots shown in the panels on the right sides of Figure 3-4 incorporate data from all three locations (upstream, downstream, and adjacent). For all samples, sucralose concentrations were significantly greater than caffeine concentrations, and the caffeine/sucralose ratios were consistently below 0.3. This is not consistent with fresh sewage inputs, which generally show caffeine/sucralose ratios that exceed 2.0, as demonstrated in Chapter 5. As expected, it is unlikely that the fecal contamination observed on the soils is impacting the river in a way that can be measured during dry conditions. This is not to say that contamination will not occur during a rain event. Chapter 5 of this report addresses the questions related to contamination of the river during storm events.

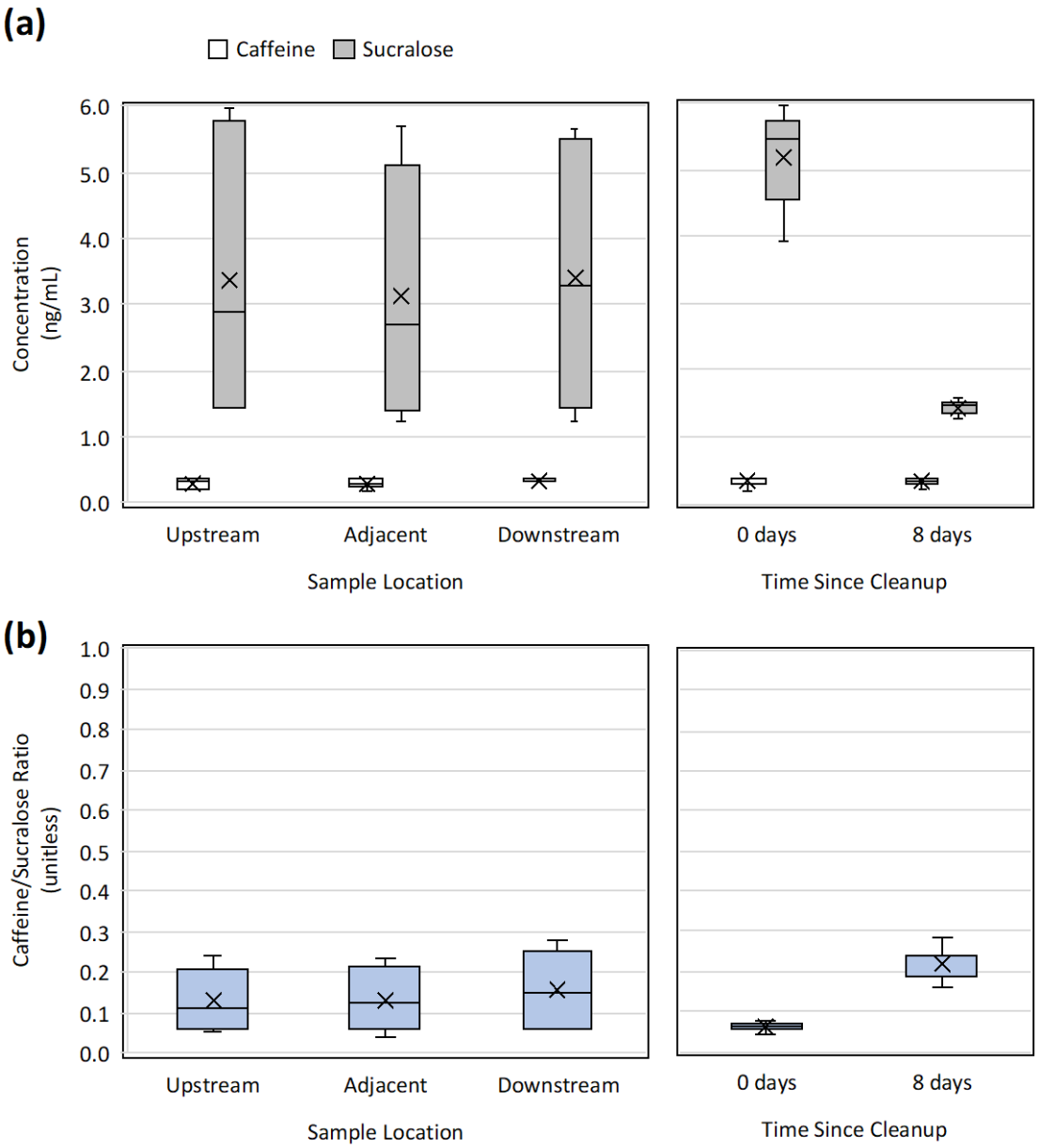

Figure 3-4. Box plots showing (a) the concentrations of caffeine and sucralose and (b) the caffeine/sucralose ratios in samples collected upstream and downstream of the San Diego River cleanup site at Mission Valley, on the day of the cleanup and 8 days after the cleanup. Plots show the mean (×), median (horizontal line), the interquartile range, and whiskers indicating minimum and maximum data points within 1.5 times the interquartile range. Box plots are based on triplicate samples collected upstream, adjacent to, and downstream of the cleanup site on the day of the cleanup (0 days) and approximately one week after the cleanup (8 days).

The average flow rate measured in the river adjacent to the cleanup site at Mission Valley during the sampling period was 370 L/s. The associated pollutant loadings are shown in Table 3-1.

Overall, the bacterial loads are lower than we reported for the San Diego River on a different date (Table 2-4). Dissolved organic carbon, total dissolved nitrogen, and nitrate are all higher than what we previously found for the San Diego River at Fashion Valley, sampled on a different date (Table 2-4).

Table 3-1. Measured flow rates and calculated loadings of microbial and physical-chemical pollutants in samples collected upstream (us) and downstream (ds) of the homeless encampment cleanup site during dry weather conditions.

Pollutant	Mean Pollutant Loadings
E. coli (MPN/d)	1.6×10^5
Fecal enterococci (MPN/d)	1.3×10^5
Dissolved organic carbon (kg/d)	311
Total dissolved nitrogen (kg/d)	22.5
Nitrate (kg/d as N)	13.2
Phosphate (kg/d as P)	7.5
Caffeine (g/d)	10.1
Sucralose (g/d)	105

3.3.2 Concentrations of pollutants in the soils

The second question addressed in this study was regarding the concentrations of pollutants in soils during and after the cleanup activities. Figure 3-5 shows the concentrations of fecal indicator bacteria in soil samples collected from the two different sites. There was very large variation from sample to sample, even though the samples were collected within a diameter of less than one meter. As shown on Figure 3-5, some of the samples were above the limit of quantification and others were below the limit of detection. Given the small sample size and the number of samples that yielded concentrations outside of the range of quantifiable values, it is not possible to quantify the decrease in the concentrations over the 36-day period following the cleanup event with statistical significance. However, in both sites on the day of the cleanup, 10 / 10 sites (100%) tested positive for *E. coli* and enterococci, and most of the samples had concentrations above 10^3 MPN/g for both microbial groups. After 36 days, only 3 / 6 samples (50%) tested positive for *E. coli* and 4 / 6 (67%) tested positive for fecal enterococci. The other samples were below the limit of detection (<0.4 MPN/g). Thus, microbial decay likely occurred during this period of time, but we were unable to quantify the extent of that decay with certainty.

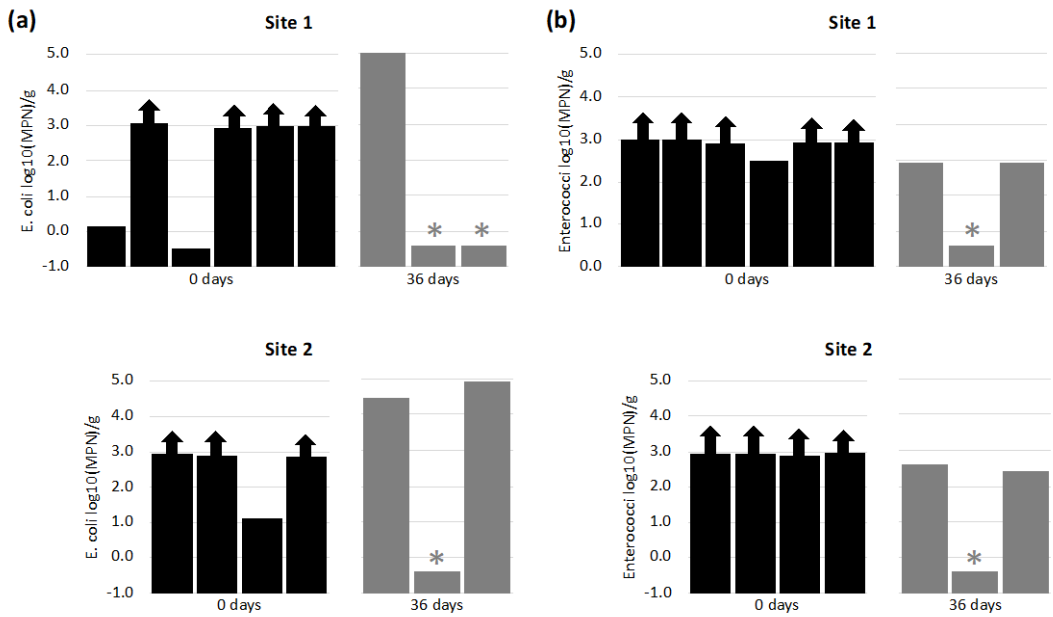

Figure 3-5. Magnitude of (a) *E. coli* and (b) fecal enterococci concentrations in soil samples collected at two different sites with evidence of open defecation, on the day of the cleanup event, and 36 days after the cleanup event.

The HF183 marker was not detected in either of the fecal samples on the day of the cleanup. However, on the day of the cleanup event, it was present in at least two of the six soil samples from Site 1, and in at least two of the four soil samples from Site 2. Concentrations of HF183 in the soil samples that tested positive could not be quantified due to problems with inhibition. Eight days after the cleanup event, HF183 was still detected in at least one of the four samples from Site 2 but was not detected in any of the samples from Site 1. HF183 was not detected in any of the soil samples at either of the two sites on the 36th day after the cleanup event. The conclusion from these analyses is that the soils were contaminated with human fecal bacteria on the day of the cleanup, and that contamination could still be detected a week after the cleanup, but after a month, the concentrations appeared to be lower, potentially below detectable levels.

3.3.3 Limitations

There are several important limitations to this study. First, because this was an objective-driven exploratory study, we did not design an experiment that lent itself to hypothesis testing. Second, we did not homogenize our soil samples, and as a result we were unable to calculate accurate estimates of the mean concentrations of certain constituents on the different sampling dates due to the fact that the sample concentrations were either too low or too high, and they were outside our range of detection (e.g., for fecal indicator bacteria). Third, we did not analyze control soil samples for comparison. Despite the limitations described above, this exploratory study still led to some important conclusions. For example, we think it is unlikely for there to be water contamination due to a site cleanup unless the cleanup crews were directly depositing material into the river, or cleaning tools right next to the river. There is not an obvious way for water

sampling to tell us that much in this case, so perhaps future studies could go without collecting water samples, and instead concentrate efforts in a hypothesis-driven study involving a larger soil sampling campaign. More resources and support would be needed to really do this properly. In soil ecology research, multiple samples of equal mass are collected and composited and homogenized—that way, true field replicates can be analyzed. This also helps deal with heterogeneity in soil contamination. Sites should be well marked (i.e., flagged) so that it is possible to navigate back to exactly the same site, and this was difficult with the sites we were looking at for this project because they were open to the public and anything left behind may not be found on a future date.

3.4 CONCLUSIONS AND RECOMMENDATIONS

We completed an exploratory study of the contamination of water and soil at a former homeless encampment during and after an environmental cleanup effort. The results show that soils near sites with evidence of open defecation are contaminated, but that the contamination in soil decreases over time. As such, environmental workers should use personal protective equipment (e.g., gloves) when handling or working around soils at former encampment sites, especially soils located near sites with evidence of open defecation. In this study, we documented evidence of open defecation in the riparian zone of the San Diego River at a former homeless encampment site, with evidence of the contamination of nearby soils, but not water in the nearby river. We recommend that improved water supply, sanitation, and hygiene facilities be provided for all individuals experiencing homelessness in California.

4 OPEN DEFECATION AND UNTREATED WASTEWATER SOURCES

4.1 INTRODUCTION

Both open defecation runoff and input of untreated wastewater derived from a range of sources (sanitary sewer overflows, sewer exfiltration, illicit connections) can result in contamination of surface waters. Sanitary sewer overflows release tens of thousands of gallons of untreated wastewater to rivers, creeks, stormwater conveyance, and soils. In the 2018 hydrologic year, over 150,000 gallons of sewage reached surface waters in San Diego, and these are only the reported flows (Gibson et al., 2018). Sewer mains run along much of the San Diego River and its tributaries, but both mains and laterals are susceptible to cracks and failures as the sewer infrastructure ages.

In terms of waste associated with homeless encampments, geospatial surveys conducted by the San Diego River Park Foundation (SDRPF) during their trash assessments counted 31 encampment sites with 39 tents adjacent to the San Diego River in 2017 and 26 encampments with 16 tents in 2018. SDRPF also conducted surveys of latrine sites with evidence of anal cleansing material or feces and found 16 such sites in the 2017 survey and 13 sites in 2018. Latrine sites were also located in close proximity to the San Diego River, with 50% found within approximately 60 m (200 ft) of the river margin (Figure 4-1).

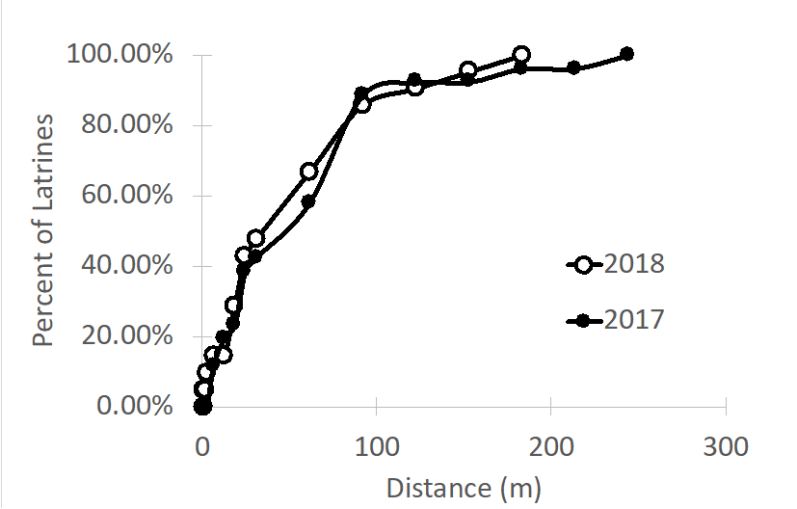

Figure 4-1. Percent of latrines as a function of distance from the river in 2017 and 2018.

Due to the need for a better understanding of the persistence of pollutants from untreated wastewater and open defecation sources, this research focused on measuring the concentrations of fecal indicator bacteria and other constituents in rainwater that flushes through wastewater-contaminated soils and feces-contaminated soils. Specifically, we addressed the following research questions associated with two objectives:

1. Determine the concentrations and loadings of general and human-associated microbial pollutants of fecal contamination in rainwater flushed through soils contaminated with sewage.
    a. How do those concentrations change with respect to the number of flushes?
    b. How do those concentrations change with respect to the number of days since contamination and for how long after a contamination event are the pollutants still present at detectable levels?
2. Determine the concentrations and loadings of human-associated microbial and chemical pollutants in simulated stormwater runoff from soil in homeless encampments with evidence of open defecation.
    a. How do those concentrations change with respect to the number of flushes?
    b. How do those concentrations vary from site to site and between different microbial groups?

To answer the first question, we collected uncontaminated soil from the bank of a tributary to the San Diego River and spiked it with untreated sewage to simulate sewer exfiltration or a sanitary sewer overflow (SSO) event. We then flushed the soil multiple times with synthetic rainwater, after waiting for different numbers of days, and analyzed the flushed water for the following physical-chemical water quality parameters and microbial pollutants: total dissolved nitrogen (TDN), dissolved organic carbon (DOC), *E. coli*, enterococci, the HF183 gene target (from *Bacteroides dorei*), and a gene target from pepper mild mottle virus (PMMoV).

4.2 MATERIALS AND METHODS

4.2.1 Microbial leaching in rainwater flushed from soils contaminated by sewer exfiltration

For this experiment, riverbank soil was first collected from a location approximately 10 m away from Alvarado Creek, which is a tributary of the San Diego River (32°46'40.4"N, 117°03'59.6"W). The area was selected based on the lack of fecal matter, trash, or other waste materials in the vicinity, and based on its proximity to the creek. A small area of soil was cleared out using a sterilized scooper, then the top 5 cm of the soil were scraped off to make sure that no pebbles were collected and that the soil was not contaminated. Approximately 4 kg of soil was collected from an area of 1860.5 cm^2, put into a sterilized bag, and brought back to the laboratory. The soil was homogenized, then its organic matter content was determined to be 6.9% based on the loss-on-ignition method. Briefly, after homogenization, the soil was placed in a drying oven at 105°C for 24 hours to remove moisture, then the percent loss on ignition was determined in triplicate in a muffle furnace at 550°C for 2 hours. The soil's composition was found to be 10.4% gravel, 76.8% sand, and 12.8% fine sediment based on a sieve analysis.

Untreated wastewater was collected from the San Elijo Water Reclamation Facility in Cardiff, CA. Within several hours of collection, samples of untreated wastewater were analyzed for the presence of *E. coli*, enterococci, HF183, and PMMoV. The untreated wastewater collected was analyzed for FIB, DOC, and TDN. Concentrations of *E. coli*, enterococci, HF183, and PMMoV in the untreated wastewater were 2.2×10^7 MPN/100 mL, 3.0×10^6 MPN/100 mL, 5.9×10^6 gc/100 mL, and 3.4×10^5 gc/100 mL, respectively; DOC and TDN concentrations in the untreated wastewater

were 38 and 75 mg/L, respectively. Synthetic rainwater was formulated by adding by adding $CaCl_2$, $Ca(NO_3)_2$, and Na_2SO_4 to Milli-Q water, to increase the concentrations of Cl^-, NO_3^-, and SO_4^{2-} ions until the specific conductance was approximately equal to 18 µS/cm (Smith et al., 2002), based on ion concentrations of actual rainwater collected in San Diego in 2018. The synthetic rainwater was then autoclaved and stored until further use.

Approximately 10 mg of the dried, homogenized soil was distributed into individual 50 mL sterilized 50 mL centrifuge tubes. Within 24 h of wastewater collection, 5 mL aliquots of wastewater were pipetted into each of the centrifuge tubes (Figure 4-2). Previous trials were done to evaluate that 5 mL would be sufficient to completely saturate the soil with the untreated wastewater. The centrifuge tubes were then lightly capped and placed inside a Class-II biosafety cabinet at ambient temperature. On the first day after spiking soils with wastewater, tubes were removed in duplicate and flushed once by adding 45 mL of sterile synthetic rainwater to the centrifuge tube. The tubes were turned twice using an end-over-end motion, then set aside to settle for 10 min. Supernatant was removed from each replicate tube by careful pipetting. After this first flush, additional consecutive flushes of 45 mL each were performed for a total of 20 flushes ("flushing experiment;" Figure 4-2a). The decanted supernatant from the first, second, fifth, tenth, and twentieth flushes were preserved for chemical and microbial analyses. Additional tubes were loosely capped and left in the biosafety cabinet to be flushed at later dates. Duplicate spiked sample tubes were flushed once with sterile synthetic rainwater after 14, 28, 60, and 121 days, and the supernatants were collected for analysis ("decay experiment;" Figure 4-2b).

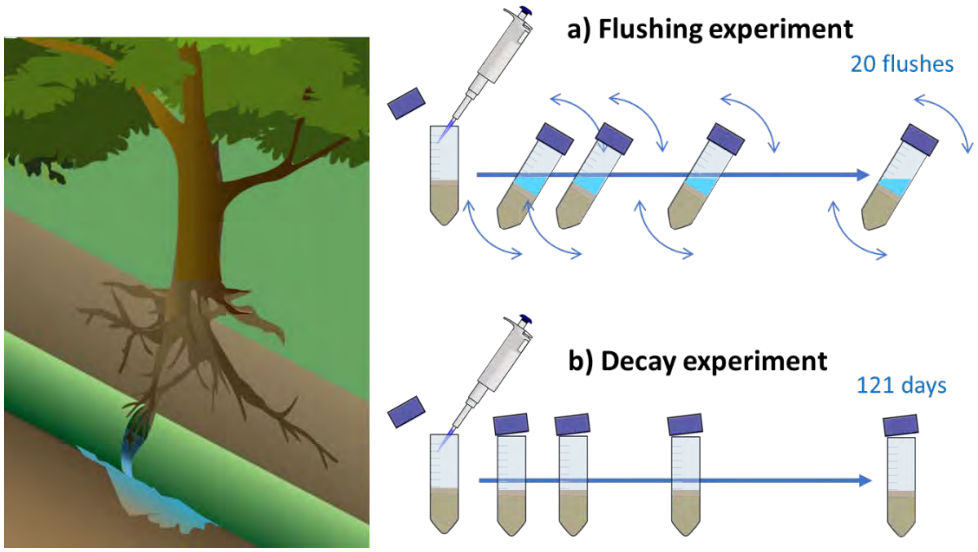

Figure 4-2. Experimental simulations of a) surface runoff or interflow flushing soils contaminated with untreated wastewater and b) persistence of microbial and chemical constituents in soils contaminated with wastewater.

4.2.2 Microbial leaching in stormwater runoff from soil contaminated by open defecation

Sites within transient encampments (Figure 4-3) with evidence of open defecation were identified during river clean-up walks with the San Diego River Park Foundation, using the following criteria: presence of apparent human fecal matter in the vicinity of used toilet paper or

napkins; proximity to the river and to an access trail or a recently-inhabited encampment site. At several sites, the fecal matter was also located near or adjacent to an object that could have been used as a seat, such as a fallen log, providing further evidence that it was likely human in origin. According to the US Soil Survey (websoilsurvey.sc.egov.usda.gov), soils at the experimental sites were classified as Riverwash (Rm), Tujunga sand (TuB), and Friant rocky fine sandy loam (FxE), which matched our onsite observations of mostly sandy soils at the surface.

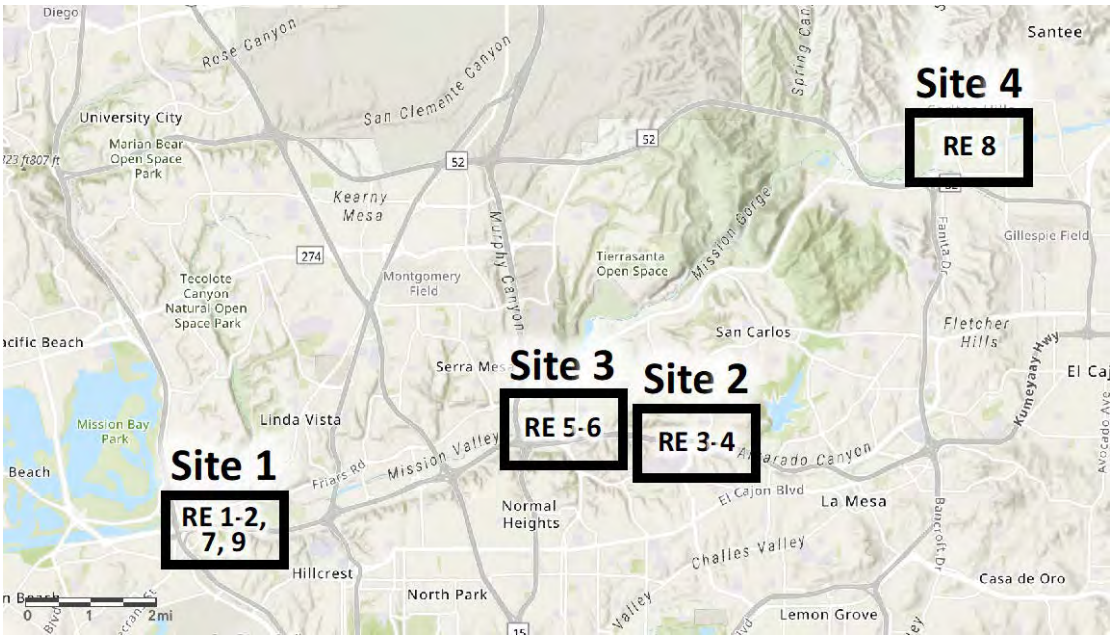

Figure 4-3. Site locations for the nine stormwater runoff experiments (RE) conducted at recently abandoned homeless encampments located near the San Diego River and one of its tributaries.

The experiments were performed using a custom-designed device that distributed the water to simulate sheet flow (Figure 4-4). At most sites, the experiments were performed *in situ*, without disturbing the fecal material. However, in a few sites, the fecal material was located in areas that were difficult to access, so the fecal material was transferred using sterile techniques to a nearby area with a flatter slope within a few feet of the original site. The area around the fecal material was then demarcated by placing a sheet of metal (8 cm × 25 cm) on either side of the area of interest, oriented in the direction of water flow (*i.e.* uphill to downhill). A third curved sheet of metal (10 cm × 25 cm with a radius of 10 cm) was then placed on the uphill end of the area of interest to be used as a pouring surface. Using gloved hands, a UV-sterilized plastic bag was secured with tape to the inside of a collection pan, which was placed at the downhill end of the demarcated area with the opening facing the inside (Figure 4-4). Then, 700 mL of synthetic rainwater was poured over the 625 cm^2 delimited area at a consistent rate (140 mL/s) into the curved metal sheet on the uphill side, to create the "first flush." Using the Rational Method (Kuichling 1889; Thompson 2006), this would be equivalent to the runoff rate anticipated during a 1-year, 24-hour storm with a drainage area of 1,500 m^2 with unimproved land use type (C = 0.2).

Supporting Calculations:

$$I = \frac{Q}{CA} = \frac{(140\ \mathrm{mL/s})(10^{-6}\ \mathrm{m^3/mL})(3600\ \mathrm{s/h})}{(0.2)(1500\ \mathrm{m^2})}(39.37\ \mathrm{in/m}) = 0.066\ \mathrm{in/h}$$

According to the NOAA Precipitation Frequency Data Server (https://hdsc.nws.noaa.gov/hdsc/pfds/), at 32.7611°N, 117.2001°W, a 1-year, 24-hour storm has an intensity of 0.06 in/h.

The process was repeated a total of 3 to 15 times (Table 4-1), to understand if the concentration of microbial and chemical pollutants changed with subsequent flushes. After completing each runoff simulation, the plastic bag containing the sample was detached from the collection pan and its contents was poured into an autoclaved sample collection bottle, which was transferred in the dark on ice to the laboratory. Sample container preparation guidelines are summarized in Table 1 of this project's Quality Assurance Project Plan (QAPP). All samples were transferred to the laboratory in a cooler on ice.

Qualitative descriptions of the fecal material were recorded for each experiment (Table 4-1). The physical appearance of the fecal material was characterized using the Bristol scale. A four-point Likert scale was used to rate the apparent moisture (moist, somewhat moist, somewhat dry, dry) and the apparent age (very fresh, fresh, somewhat fresh, not fresh). The number of pieces of fecal material was also recorded, as was the apparent color of the fecal material.

Figure 4-4. Photos showing typical evidence of open defecation and the setup for the simulated sheet flow runoff experiments.

Table 4-1. Set-up and design of the simulated stormwater runoff experiments and qualitative characteristics of the fecal material at each site

Experiment No.	Date	Site Location	Description of Fecal Material	Experimental Conditions
1	4/12/18	Site 1 (Mission Valley)	*Appearance:* Type 3 (Bristol) *Consistency:* Dry; Not fresh *Color:* Dark brown *Dispersal:* Several pieces	Five consecutive flushes Flushes 1, 2, 3, 5 were collected
2	4/12/18	Site 1 (Mission Valley)	*Appearance:* Type 2 (Bristol) *Consistency:* Dry; Not fresh *Color:* Dark brown *Dispersal:* Several pieces	Four consecutive flushes All flushes were collected
3	6/21/18	Site 2 (Alvarado Creek)	*Appearance:* Type 3 (Bristol) *Consistency:* Somewhat dry; Fresh *Color:* Dark brown *Dispersal:* One piece	15 consecutive flushes Every other flush was collected
4	7/24/18	Site 2 (Alvarado Creek)	*Appearance:* Type 1 (Bristol) *Consistency:* Dry; Not fresh *Color:* Very dark brown/black *Dispersal:* Many small pieces	15 consecutive flushes Every other flush was collected
5	6/21/19	Site 3 (Home Depot at Fairmont)	*Appearance:* Type 3 (Bristol) *Consistency:* Somewhat dry; Fresh *Color:* Dark brown *Dispersal:* One piece	Three consecutive flushes All flushes were collected
6	6/28/19	Site 3 (Home Depot at Fairmont)	*Appearance:* Type 4 (Bristol) *Consistency:* Moist; Fresh *Color:* Light brown *Dispersal:* One piece	Three consecutive flushes All flushes were collected
7	8/31/19	Site 1 (Mission Valley)	*Appearance:* Type 5 (Bristol) *Consistency:* Moist; Very fresh *Color:* Brown	Three consecutive flushes All flushes were collected

			Dispersal: Spread around	
8	9/7/19	Site 4 (Mast Park)	*Appearance:* Type 2 (Bristol) *Consistency:* Dry; Somewhat fresh *Color:* Very dark brown *Dispersal:* One piece	Three consecutive flushes All flushes were collected
9	10/2/19	Site 1 (Mission Valley)	*Appearance:* Type 3 (Bristol) Somewhat moist; Fresh *Color:* Brown *Dispersal:* Several pieces	Three consecutive flushes All flushes were collected

4.2.3 Quantification of microbial pollutants

All samples were analyzed within 24 hours of collection for fecal indicator bacteria *E. coli* and fecal enterococci using the Colilert and Enterolert methods, respectively, with the IDEXX Quanti-Tray 2000 system, as described in Standard Methods 9223B (APHA, 2012), following manufacturer's recommended protocols. Colilert trays were incubated at 35°C for 18 h and Enterolert trays were incubated at 41°C for 24 h, and wells fluorescing blue under UV light after the incubation periods were counted as positive. The most probable number (MPN) and 95% confidence intervals were then calculated using maximum likelihood estimation (MLE).

Bacteria and viruses in samples were concentrated using a modified version of the adsorption-elution method (Symonds et al., 2014; Verbyla et al., 2016). Briefly, water samples (30 mL – 250 mL) were adjusted to pH 3.0 – 3.5 with 1 M acetic acid, then vacuum-filtered through 0.45-µm, 47-mm mixed cellulose ester filters (HAWP type, Millipore). Filters were immediately placed into bead-beating tubes (lysis matrix E, MP Biomedical) with 600 µL of lysis buffer from the AllPrep PowerViral RNA/DNA Kit (Qiagen) and 10% β-mercaptoethanol, then stored at -80°C. Within one month of sample collection, the bead-beating tubes were thawed, then vortexed at high speed for 10 min with a bead-beating tube adapter (Qiagen). Filter lysates were then transferred to clean microcentrifuge tubes, and nucleic acids were extracted and purified using the AllPrep PowerViral RNA/DNA Kit, following the manufacturer's recommended protocol, and using 50 µL of molecular grade water for the final elution step. Extracted and purified DNA/RNA was stored at -80°C until further processing. Within 90 days of sample collection, samples reverse transcribed using the SuperScript IV First-Strand cDNA Synthesis System (Invitrogen), following the manufacturer's recommended protocol. Briefly, 8 µL of sample RNA was added to a 13 µL reaction mixture with dNTPs at a final concentration of 0.5 mM, and random hexamers at a final concentration of 2.5 ng/µL. After a 5-min incubation at 65°C, the reaction mixture was placed on ice for 1 min for annealing. Then, a reaction mixture with 4 µL of 5× SSIV buffer, 1 µL of 100 mM dithiothreitol, 1 µL of ribonuclease inhibitor, and 1 µL of SuperScript IV Reverse Transcriptase was added to the annealed RNA. After a 10 min incubation at 50°C, the enzymes were inactivated by incubating at 80°C for 10 min, then 1 µL of *E. coli* RNase H was added and samples were

incubated at 37°C for 20 min to remove leftover RNA. All cDNA was stored at -20°C until further analysis, and the remaining DNA from nucleic acid extraction was also moved to the -20°C freezer.

Molecular targets were quantified using the Bio-Rad QX200 droplet digital polymerase chain reaction (ddPCR) system. Primers and probes were ordered based on previously published assays given in this project's QAPP. Final concentrations of primers were 900 nM, the final concentration of the probe was 250 nM, and the ddPCR Supermix for Probes (Bio-Rad cat. no. 186-3010) was used at a final concentration of 1X. A volume of 2 – 5 µL purified DNA or cDNA was used in all reactions. Molecular grade water was added as necessary to bring the final reaction volumes up to 20 µL. Polymerase chain reaction (PCR) mixtures were prepared in 0.2 µL PCR tubes or in 96 well PCR plates. Each reaction well was mixed thoroughly by pipetting the entire volume up and down 20 times. Droplets were generated in all samples using manual pipetting with eight-sample cartridges and droplet generator oil (Bio-Rad cat. no. 1863005), following the steps recommended by the manufacturer. Thermocycling conditions were as follows: 95°C for 10 min followed by 40 cycles of 94°C for 30 s, then 60°C for 1 min. After completion, samples were held at 98°C for 10 min, as recommended.

Quality assurance and quality control protocols for ddPCR are described in the QAPP, following recommendations set forth by Huggett et al. (2013). Briefly, custom gBlocks (Integrated DNA technologies) were used as positive controls. All gBlocks were resuspended following the manufacturer's recommendations, then stored at 1 ng/µL with 15 ng/µL yeast tRNA (gBiosciences) at -20°C until further use. Prior to each ddPCR run, gBlocks were thawed, quantified by Qubit following steps described in the Qubit 1X dsDNA HS Assay protocol, and then serially diluted to concentrations between 0.1 and 1,000 copies per reaction. At least one no template control (NTC) and two positive controls were analyzed alongside samples for each PCR run. All PCRs were performed in duplicate and inhibition controls were analyzed by spiking samples with a known concentration of gBlocks.

4.2.4 Quantification of physical-chemical constituents

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured at a 1:20 dilution, prepared in amber bottles, using the high temperature combustion method with a Shimadzu TOC-L Total Organic Carbon Analyzer with a chemiluminescent nitrogen detector, which reports DOC concentrations as non-purgeable organic carbon. Standards for DOC ranging from 0.10 to 20 mg/L were prepared using a stock solution of potassium hydrogen phthalate. Standards for TDN ranging from 0.10 to 10 mg/L were prepared using a stock solution of potassium nitrate. DOC and TDN standards were loaded to the autosampler tray first, followed by samples from the suspected lowest concentration to the suspected highest concentration. Standards (covering the range of sample concentrations) were run as unknown samples and a calibration curve equation, developed using known and measured concentrations, was used to correct raw data of all samples. Two vials of ultrapure water were run as unknown samples in the beginning of the analysis, every five samples, and at the end of the analysis, to wash the sampling needle and tubing of the TOC analyzer to avoid carryover from previous samples. Approximately 5% of all samples were run in triplicate, and the standard deviations of these triplicate analyses were checked to make sure they were within 10% of the mean concentrations.

Samples (400 mL) were extracted and analyzed for caffeine and sucralose concentrations following U.S. EPA Method 1694, using an Agilent 6460 Triple Quad LC/MS/MS system equipped with Agilent Jet Stream Technology. The triple quadrupole MS-MS system was operated in the multiple reaction monitoring (MRM) mode, using the precursor and product ion transitions of the target compounds and their isotopically labeled surrogates to detect the analytes of interest. Liquid chromatography mass spectrometry (LCMS) water was used as field blanks and lab blanks, and duplicates were run for every 10-20 samples. Caffeine concentrations were lower than 0.071 µg/L in three field blanks and undetectable in two. Sucralose concentrations were undetectable in four out of five field blanks and 0.001 in the fifth. Other quality control measures are described in this project's QAPP.

4.3 RESULTS AND DISCUSSION

4.3.1 Microbial concentrations in water flushed from soils contaminated by sewer exfiltration

The measured changes in the concentrations with respect to each subsequent flush and with respect to the number of days since contamination are shown in Figure 4-5. The concentrations in the sewage used to spike the soils were 2.2×10^7 MPN/100 mL for *E. coli* and 3.0×10^6 MPN/100 mL for enterococci, and 5 mL of sewage was spiked into the soil, which means that a total of 1.1×10^6 MPN *E. coli* and 1.5×10^5 MPN enterococci were added to the soil on average. The geometric mean initial concentrations of fecal indicator bacteria in the water from the first flush, one day after soils were spiked with sewage, were 5.5×10^7 MPN/100 mL and 3.4×10^6 MPN/100 mL for *E. coli* and enterococci, respectively. Based on these values, the loadings of *E. coli* and enterococci flushed away from the soil were greater than the loadings originally added. Given that *E. coli* and enterococci were not detected in the original soil sample, it is possible that microbial growth may have occurred overnight. Subsequent flushes yielded substantially lower loadings of *E. coli* and enterococci, which tailed off by the 20th flush.

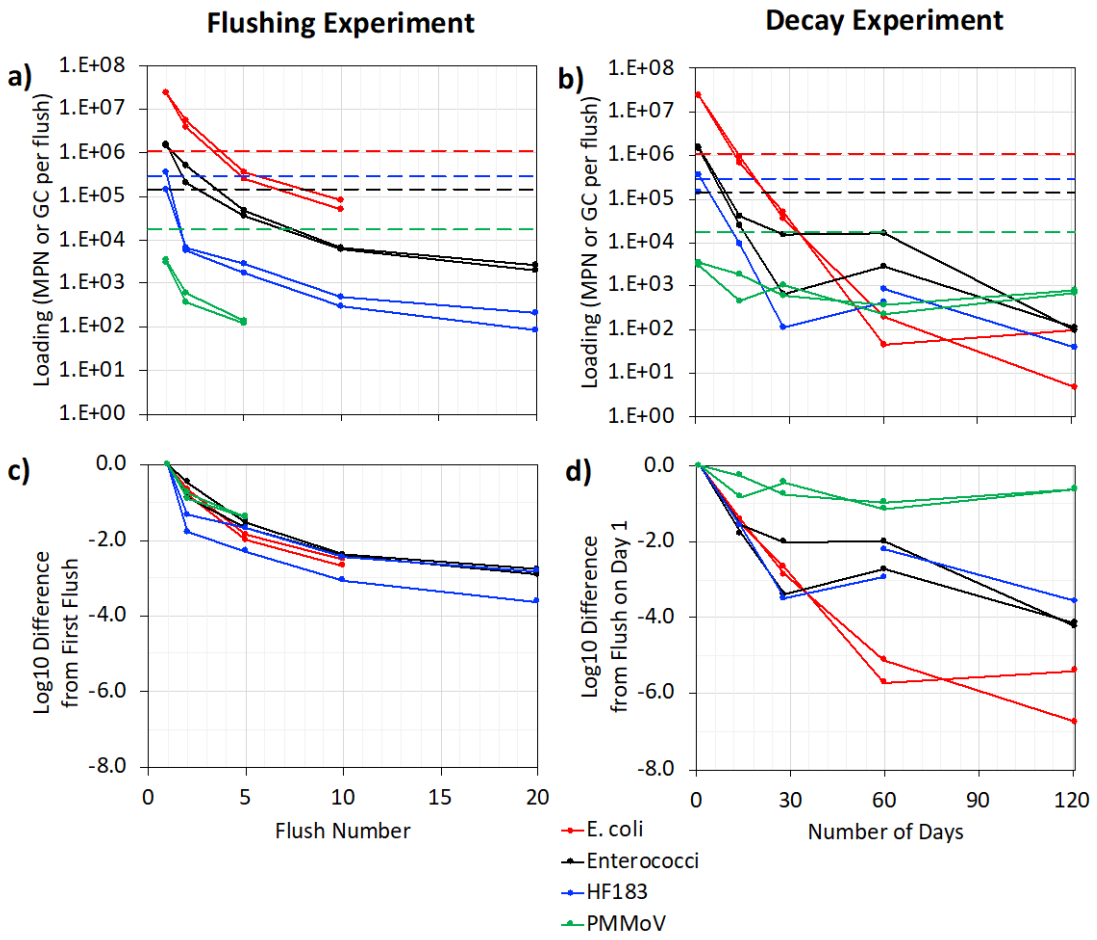

Figure 4-5. Survival curves showing the loadings (a and b) and $\log_{10}$ differences in the loadings (c and d) for *E. coli*, fecal enterococci, HF183, and PMMoV for synthetic rainwater flushed through soils contaminated with sewage with respect to the number of consecutive flushes (a and c) and with respect to the number of days since the soils were contaminated (b and d); dashed lines show the loadings from the sewage spiked into the soil on day 0. Data from samples analyzed for *E. coli* after the 20th flush are not shown due to the fact that the holding time for this analysis was exceeded and sample concentrations appeared to be outliers due to probable microbial growth during the extended holding time. Data from PMMoV in flushing experiment at 10 flushes and 20 flushes were non-detects. One of the duplicate samples from decay experiment after 28 days and after 121 days were non-detects for HF183.

Concentrations of HF183 and PMMoV in the sewage used to spike the soils were 5.9×10^6 gc/100 mL and 3.4×10^5 gc/100 mL, respectively. Given that the soils were each spiked with 5 mL of sewage, the total numbers of HF183 and PMMoV added to the soils were approximately 2.9×10^5 and 1.7×10^4 gene copies, respectively. The geometric mean initial concentrations of HF183 and PMMoV in the water from the first flush, one day after soils were spiked with sewage, were 5.0×10^5 MPN/100 mL and 7.0×10^3 MPN/100 mL, respectively. Based on these values, the loadings of HF183 and PMMoV flushed away from the soil were less than the loadings originally added. Subsequent flushes yielded substantially lower loadings of HF183 and PMMoV, which tailed off with a similar pattern to *E. coli* and enterococci (Figure 4-5c), although PMMoV was not

detected after the 10th flush, likely due to the lower concentration of PMMoV in the sewage used to spike the soils.

With respect to the number of days after contamination until the soils were flushed, there were great differences in the loadings for the different microbial groups, indicating differences in the persistence of these microbes or the related gene targets in contaminated soils. For instance, the loading of *E. coli* was highest in the flush that occurred one day after contamination (its concentration in sewage was also the highest). However, in the flush performed four months after contamination, the loading of *E. coli* in the flushed water was the lowest, indicating that it likely decayed faster than the rest of the microorganisms. This is consistent with earlier studies evaluating FIB decay in microcosms of coastal sediments inoculated with the bacteria, which showed almost complete decay of *E. coli* (6- to 7-log removal) compared to only 1- to 2-log removal of enterococci (Craig et al., 2002). On the contrary, PMMoV had the lowest loadings in flush water on the first day but had the highest loading in the flush water after four months. The decay rate for *E. coli* was higher than the decay rates for HF183 and enterococci, which were higher than the decay rate for PMMoV. It is important to note that PMMoV and HF183 were measured using ddPCR while *E. coli* and enterococci were measured using culture-based methods, so the gene targets detected for HF183 and PMMoV did not necessarily come from viable microorganisms.

The decay rates appeared to follow first order kinetics for the first month or two, with possible subsequent decelerating decay rates (tailing effect). Assuming first order decay for the first month only, the pseudo-first order decay rate coefficients (calculated on a natural log basis) were 0.103 $days^{-1}$ for *E. coli*, 0.100 $days^{-1}$ for enterococci, 0.121 $days^{-1}$ for HF183, and 0.022 $days^{-1}$ for PMMoV. This equates to T_{90} values of ~10 days and T_{99} values of ~20 days for *E. coli*, enterococci and HF183 (T_{90} and T_{99} values are the times required for 90% and 99% reduction, respectively). The T_{90} and T_{99} values for PMMoV could not be interpolated from the data, but both are expected to be >120 days based on the data. These are slower decay rates than what has previously been reported in the literature. For instance, in a study of the persistence of *E. coli* and HF183 in river water with 10 g/L of river sediments, Dick et al. (2010) reported T_{99} values of 2.80 days for *E. coli* and 1.88 days for HF183. Liang et al. (2012) reported 99% decay of the HF183 marker in 2.7 days in river water at ambient temperature in a greenhouse at 15°C (under natural light exposure), and Hamza et al. (2011) reported reductions of 1.1 log_{10} at 25°C and by 0.5 log_{10} at 4°C for the concentrations of PMMoV in river water after 21 days (under dark conditions). One possible explanation for the lower decay rate coefficients observed in our study compared to what has been reported in the literature is that most previous studies of the persistence of these microbial indicators have been performed in water matrices, while ours was performed in soil. Also, some of the previous studies left samples exposed to sunlight, which can increase the inactivation rate for bacteria and viruses, especially in a water matrix where sunlight interacts with dissolved compounds to form reactive intermediates (Nelson et al., 2018). Although, falcon tubes were loosely capped to allow oxygen in the headspace, anoxic and/or anaerobic conditions likely existed in the soil porewaters and may have influenced decay rates. In general, decay rates of *E. coli* and enterococcus are typically slower in sediments of smaller size and with greater organic matter content and faster in the water column (Craig et al., 2002). Finally, and perhaps most importantly, we measured concentrations of microorganisms flushed away from soils after a

single flush, which does not account for microorganisms that are still viable but may remain adhered or adsorbed to the soils. Despite the observed differences in decay rates, previous studies have shown that the PMMoV marker to be much more persistent than HF183 and fecal indicator bacteria, which is consistent with our findings.

4.3.2 Microbial concentrations in stormwater runoff from soil contaminated by open defecation

The concentrations of *E. coli*, enterococci, HF183, and PMMoV for each flush of simulated stormwater runoff for all experiments is shown in Figures 4-6 and 4-7, and the overall geometric mean concentrations are shown in Table 4-2. *E. coli*, enterococci, and PMMoV were detected in the simulated runoff from all flushes, but HF183 was only detected in one out of the nine experiments. *E. coli* was always at higher concentrations than PMMoV, and with the exception of Experiment 7, *E. coli* was also higher in concentration than enterococci. Coincidentally, the fecal matter present on the site for Experiment 7 appeared to be the freshest, and it was also the only experiment where HF183 was detected.

Results from the two-way ANOVA showed that the concentrations in the simulated sheetflow runoff differed significantly for the different microorganisms ($p < 0.001$) and for the different experiments ($p = 0.0014$). This could be due to natural variation in the concentrations of fecal microorganisms in human feces (Harwood et al., 2017), and the likelihood that the fecal matter at each experimental site had been present in the field for different amounts of time, enabling different levels of decay of the microbial pollutants. Nevertheless, the qualitative observations made of the fecal material at each site (Table 4-2) did not significantly correlate with the concentrations of microbial pollutants in the runoff.

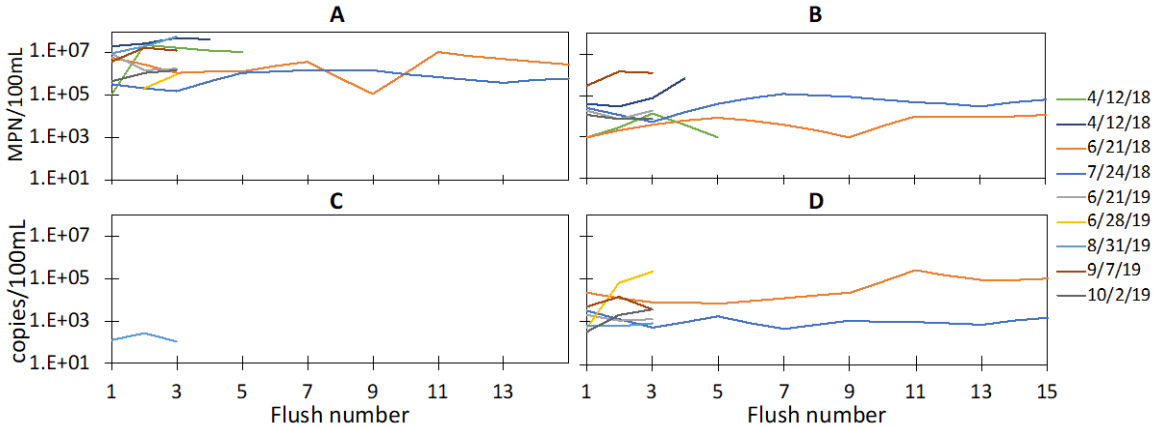

Figure 4-6. Concentrations of A) *E. coli*, B) enterococci, C) HF183, and D) PMMoV in simulated runoff experiments on soil with evidence of open defecation, with respect to the flush number.

Table 4-2. Microbial concentrations in stormwater runoff from sites with open defecation

Experiment No.	Qualitative Descriptions of Fecal Material	Sample Size[b]	Geometric Mean Concentrations per 100 mL

			E. coli	Enterococci	HF183	PMMoV
1	*Appearance:* Type 3 (Bristol) *Consistency:* Dry; Not fresh *Color:* Dark brown *Dispersal:* Several pieces	N = 4	4.4×10^{6}	2.6×10^{3}	$<1.6\times10^{3}$	N/A[c]
2	*Appearance:* Type 2 (Bristol) *Consistency:* Dry; Not fresh *Color:* Dark brown *Dispersal:* Several pieces	N = 4	3.2×10^{7}	8.6×10^{4}	$<1.3\times10^{3}$	N/A[c]
3	*Appearance:* Type 3 (Bristol) *Consistency:* Somewhat dry; Fresh *Color:* Dark brown *Dispersal:* One piece	N = 8	2.2×10^{6}	4.6×10^{3}	$<6.4\times10^{2}$	3.0×10^{4}
4	*Appearance:* Type 1 (Bristol) *Consistency:* Dry; Not fresh *Color:* Very dark brown/black *Dispersal:* Many small pieces	N = 8	6.0×10^{5}	3.1×10^{4}	$<8.7\times10^{2}$	1.1×10^{3}
5	*Appearance:* Type 3 (Bristol) *Consistency:* Somewhat dry; Fresh *Color:* Dark brown *Dispersal:* One piece	N = 3	2.8×10^{6}	1.4×10^{4}	$<6.1\times10^{2}$	1.5×10^{3}
6	*Appearance:* Type 4 (Bristol) *Consistency:* Moist; Fresh *Color:* Light brown *Dispersal:* One piece	N = 3	4.2×10^{5}	N/A[c]	$<5.0\times10^{2}$	2.1×10^{4}

7	*Appearance:* Type 5 (Bristol) *Consistency:* Moist; Very fresh *Color:* Brown *Dispersal:* Spread around	N = 3	2.2×10^{7}	$>2.4\times10^{8}$	1.6×10^{2}	6.6×10^{2}
8	*Appearance:* Type 2 (Bristol) *Consistency:* Dry; Somewhat fresh *Color:* Very dark brown *Dispersal:* One piece	N = 3	8.8×10^{6}	7.8×10^{5}	$<4.8\times10^{2}$	6.4×10^{3}
9	*Appearance:* Type 3 (Bristol) Somewhat moist; Fresh *Color:* Brown *Dispersal:* Several pieces	N = 3	8.6×10^{5}	8.6×10^{3}	$<3.5\times10^{2}$	1.4×10^{3}
Overall Geometric Mean Concentrations[a]			**3.2×10^{6}**	**2.2×10^{4}**	**$<5.9\times10^{2}$**	**3.5×10^{3}**

[a] Geometric mean of the geometric means for each experiment
[b] Number of flushes analyzed
[c] These samples were not analyzed for this parameter

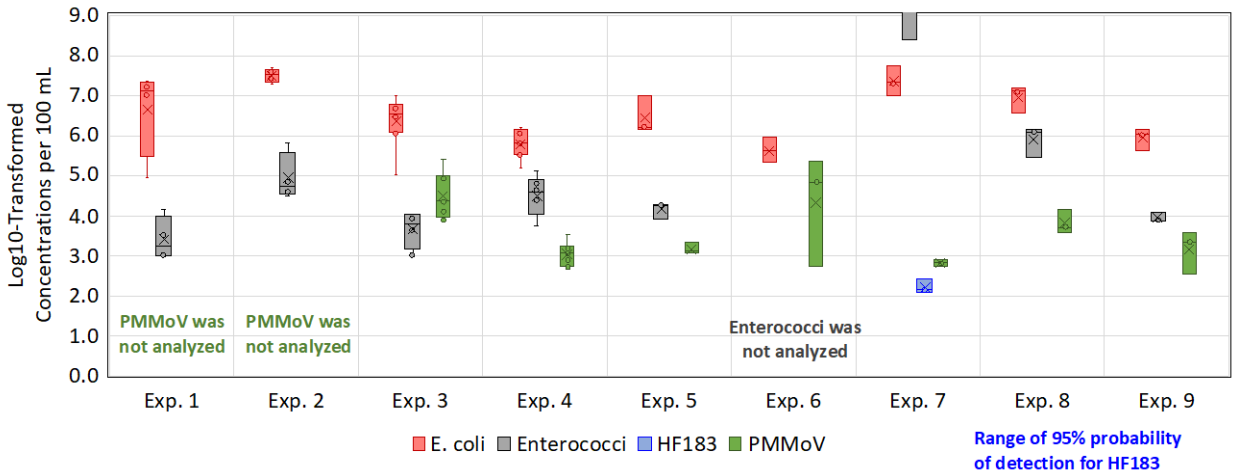

Figure 4-7. Concentrations of *E. coli*, enterococci, HF183, and PMMoV in simulated runoff experiments on soil with evidence of open defecation.

Results from the linear regression analysis performed on data from the two experiments that used a total of 15 flushes showed that microbial concentrations in the sheetflow runoff did not

significantly change with respect to the number of consecutive flushes (Figure 4-6), with the exception of PMMoV concentrations in stormwater runoff from Experiment 5, conducted on June 21, 2018, which increased in concentration slightly, but significantly ($p = 0.03$), with each consecutive flush. Nevertheless, the conclusion from this experiment is that microbial pollutants are consistently leached in the first flush of sheet flow runoff, which could end up contaminating the river. The microbial loadings delivered for each open defecation event will be different for different microbial pollutants and will likely depend on the amount of time the fecal matter has been out in the environment and the persistence of those microbial pollutants in fecal matter in the environment. The nine experiments performed in this study were performed with stormwater runoff delivered at a flow rate of 140 mL/s, which is consistent with the peak runoff rate from riverbank land during a 1-year, 24-hour storm with a drainage area of 1,500 m^2 (which is the approximate drainage area of some of the sites where the experiments were conducted). For this flow rate and based on the data from the experiments from this study, the loadings from sheet flow runoff based on the overall geometric mean concentrations would be ~4×10^6 MPN/s for *E. coli*, ~3×10^4 MPN/s for enterococci, ~5×10^3 copies/s for PMMoV, and as high as 8×10^2 copies/s for HF183.

The absence of HF183 in stormwater runoff samples from 8 out of 9 of the experiments could be explained by several factors. First, *Bacteroides* spp. bacteria likely decay faster than PMMoV in human feces, based on their relative decay rates in freshwater (Dick et al., 2010; Hamza et al., 2011; Liang et al., 2012). Also, PMMoV has been found to have significant positive correlations with HF183 in coastal waters exposed to point and non-point fecal pollution, but not in coastal waters exposed only to stormwater runoff, where PMMoV was present more often and in greater concentrations than HF183 (Hughes et al., 2017; Symonds et al., 2018, 2016). If the fecal matter had been out in the environment for a while prior to the experiments, it is possible that the *Bacteroides* spp. carrying the HF183 gene targets had decayed to concentrations that were too low to detect in stormwater runoff. Another possible explanation is that the fecal matter in all but one of the experiments did not contain the HF183 marker. Fecal samples from Experiments 1 and 2 were analyzed and HF183 was not detected in either of them (LOD = 2,136 copies/wet g). While HF183 has been found to be ubiquitous in sewage, it is not always detected in the fecal samples of every individual human (Harwood et al. 2018). For example, Haugland et al. (2010) and Layton et al. (2013) detected HF183 in stool samples from 100% of 16 and 20 healthy individuals, respectively, but van de Werfhorst et al. (2011) only detected HF183 in stool samples from five out of eight healthy individuals from California. Outside of the United States, Nshimyimana et al. (2017) detected HF183 in stool samples from 21 of 35 individuals from Singapore, and Odagiri et al. (2015) detected it in stool samples from only 5 out of 30 individuals from India, suggesting that diet may be a factor that affects the presence or absence of this microbial marker. To our knowledge, this is the first study of the presence/absence of HF183 in fecal samples from individuals experiencing unsheltered homelessness, and the first study of microbial indicator concentrations in stormwater runoff from soil contaminated by open defecation.

4.3.3 Caffeine and Sucralose

Caffeine and sucralose were analyzed in composite samples from runoff experiments 3, 4, 8, and 9. For the samples from experiments 3 and 4, internal standards for the recovery of sucralose were too high, so those results were not used. Using all other data, the average caffeine and sucralose concentrations in the simulated stormwater runoff were 0.335 ng/mL and 6.53 ng/mL, respectively.

Caffeine/sucralose ratios were below 0.05 for all stormwater runoff samples analyzed. This is much lower than the ratios we found in untreated sewage at the influent of the San Elijo Water Reclamation Facility in San Diego County, which had caffeine concentrations of 206 µg/L and sucralose concentrations that were up to an order of magnitude lower (see Chapter 5). While caffeine and sucralose are both associated with human activities (e.g., consumption of caffeinated beverages and Splenda-sweetened food and beverages), they have contrasting levels of biodegradability. Sucralose is more persistent in the environment than caffeine (Yang et al., 2017), which could explain the lower caffeine/sucralose ratios observed, especially if the fecal matter had been deposited a while prior to the experiments. Another possible explanation for the low caffeine/sucralose ratios is that approximately 78% of sucralose consumed by humans is excreted in feces, with only a small percentage excreted in urine (Roberts et al. 2000), while caffeine is the opposite, with only 5% excreted in feces (Callahan et al. 1982). If most of the urine volume infiltrated at the site of excretion or if the individuals practicing open defecation were urinating in different locations, then the preferential presence of sucralose in feces could explain the lower caffeine/sucralose ratios.

Interestingly, the ratios found in simulated stormwater runoff from this experiment were similar to the ratios we detected during dry weather flow in the San Diego River, which were ~0.10 (see Chapter 5). It should be noted that these ratios are only based on runoff experiments 8 and 9, since there were issues with the internal recovery of sucralose for experiments 3 and 4, and there was not sufficient volume of sample from the other experiments to complete the caffeine and sucralose analysis. Still, these ratios are much lower than what has been previously reported for raw sewage. Caffeine concentrations of 113 µg/L and sucralose concentrations of 4 µg/L have been previously reported in untreated wastewater from San Diego County (Batikian, 2018), which is a caffeine/sucralose ratio of ~28, higher than the caffeine/sucralose measured in untreated wastewater in the current study, which was greater than 9.0. Ratios greater than 2.0 have been suggested for surface waters receiving untreated wastewater from a combined sewer overflow event (Cantwell et al., 2018). In this study, we also use caffeine/sucralose of 2.0 to indicate untreated wastewater contributions. Local groundwater samples collected from the Sweetwater Authority had no detectable concentrations of caffeine or sucralose.

4.3.4 Limitations

Although the field and laboratory experimental work performed reveal important information about microbial and chemical markers, there are several limitations to this study. First, it should be noted that the probe used for the HF183 assay is different than the probe published recently in the EPA Standard Method (US EPA, 2019), and inter-laboratory studies indicated that the probe

used in this research may have lower sensitivity than the EPA probe (data not shown). Given different diets of different individuals and diverse characteristics of open defecation, an increased sample size for the simulated stormwater runoff experiments may have helped discern trends, such as the influence of aging of fecal material in the open environment on the persistence of HF183 and caffeine. In the flushing and decay experiments, the limited sample volume (due to 50 mL falcon tubes used) restricted the number of analyses that could be performed. If experimental work had been conducted in containers or columns of at least 500 mL volume, then it would have been possible to measure caffeine and sucralose concentrations. Also, column type experiments could help us better understand the mobility of the microorganisms from the soils under interflow conditions (compared to our end-over-end shaking/mixing). Finally, the high concentrations of caffeine in wastewater and sucralose in some of the fecal material stormwater runoff samples led to recoveries that exceeded our quality control guidelines, indicating that samples should have been diluted prior to extraction for caffeine and sucralose.

4.4 CONCLUSIONS AND RECOMMENDATIONS

The results from this study indicated that wastewater-spiked soils continued to leach detectable concentrations of *E. coli*, enterococci, HF183, and PMMoV, even after 4 months, and that runoff water from soils contaminated by open defecation leached consistent concentrations of *E. coli*, enterococci, and PMMoV after multiple flushes. Interestingly, caffeine and HF183 were found in high concentrations in untreated wastewater but were almost undetectable in simulated stormwater runoff from soil with open defecation. Sucralose on the other hand, was detected at consistently higher concentrations than caffeine in the stormwater runoff.

This study demonstrates the utility of using multiple chemical and microbial source tracking markers with differing levels of persistence to potentially characterize different sources of human fecal pollution in the environment. We recommend that policy-makers consider the application of multiple source tracking markers for future studies designed to identify and quantify sources of human fecal pollution.

5 CONTRIBUTIONS TO RIVER POLLUTION DURING STORM EVENTS

5.1 INTRODUCTION

As described in the previous section, both untreated wastewater discharges and sheetflow flushing open defecation sites may result in contamination of surface waters during storm events. Objective 5 of the project was focused on tracking concentrations and loadings of pathogens, human-associated microbial source tracking markers, and anthropogenic chemical tracers during storm events with the goal of gaining greater insights into the sources of anthropogenic pollution to the San Diego River. Our research addressed several key questions:

1. What is the temporal variability in concentrations of anthropogenic pollutants in the San Diego River during storm events?
2. How do patterns in anthropogenic pollution differ between the San Diego River and its tributaries?
3. How do the sources of contamination differ over the storm hydrograph in the San Diego River?
4. How do pollutant loadings differ among storms of different magnitude?

To answer these questions, we collected high-frequency samples (1 – 3 hour intervals) and analyzed them for the following physical-chemical water quality parameters and microbial pollutants: phosphate, nitrate, total dissolved nitrogen (TDN), dissolved organic carbon (DOC), dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity, pH, *E. coli*, enterococci, HF183, coliphage PhiX174, pepper mild mottle virus (PMMoV), hepatitis A virus (HAV), norovirus GI (NoVGI), *Campylobacter coli*, and *Campylobacter jejuni*.

5.2 MATERIALS AND METHODS

The San Diego River Watershed, which drains to the Pacific Ocean, is approximately 434 square miles and can be subdivided into four distinct hydrological areas with unique geological and environmental features. These subdivisions are: Lower San Diego, San Vicente, El Capitan, and Boulder Creek. Objective 5 activities were concentrated in the Lower San Diego. On five different storm events between January 2018 and March 2019 (Figure 5-1), samples were collected via autosampler from the San Diego River at Fashion Valley (Figure 5-2). Storm samples were also collected from two sites at Alvarado Creek and one site at Forester Creek, which are both tributaries of the San Diego River, located in the Lower San Diego River watershed near La Mesa (Figure 5-2). The Fashion Valley (FV) sampling site is located downstream, draining a large part of the watershed (1,111 square kilometers), but far enough east of the river mouth to avoid interferences from tidal flows. The time of concentration (Tc) is an estimate of the time for water to travel from the hydrologically connected and furthest point of the watershed to the outlet and can be estimated based on watershed area, length, and slope. The time of concentration to the Fashion Valley sampling site is approximately 26 hours. Alvarado Creek sites were centrally located along the creek adjacent to San Diego State University at a pedestrian bridge near Alvarado Road and Alvarado Court and at the culvert on the eastern end of the College Avenue, the contributing watershed area is approximately 28.7 square kilometers with a Tc of 4 hours.

The Forester Creek site is near the intersection of Prospect Ave, in an unnamed channelized tributary of Forester Creek (referred to here as Forester Channel) with the city of El Cajon in its watershed (Figure 5-2). Forester Creek has a contributing area of about 59 square kilometers and a Tc of 6 hours. The tributary sites were chosen mainly for their smaller contributing watershed area and more rapid time of travel of surface runoff. The choice of Forester Creek was also influenced by our aim to provide monitoring for an under-served region in San Diego (El Cajon).

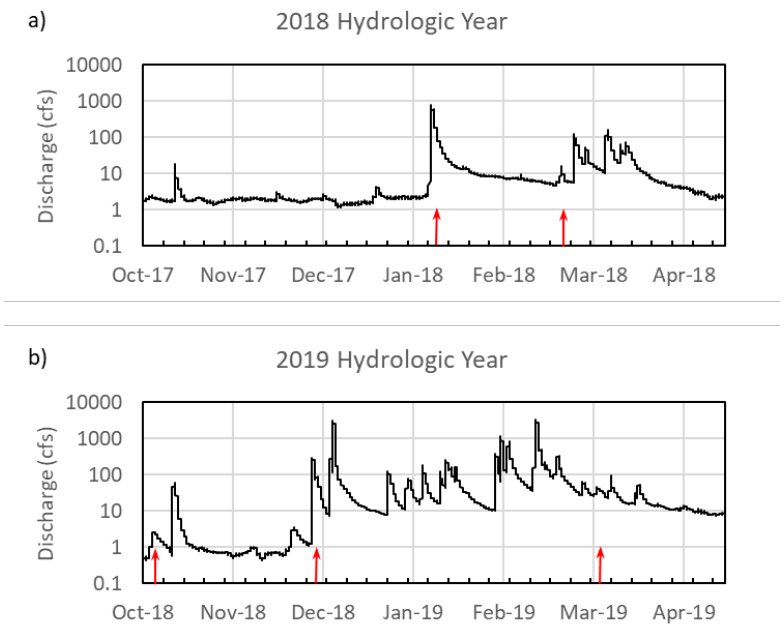

Figure 5-1. Discharge measurements at USGS Gage #11023000, San Diego River at Fashion Valley for the a) 2018 and b) 2019 hydrologic years. Sampling dates are shown with red arrows.

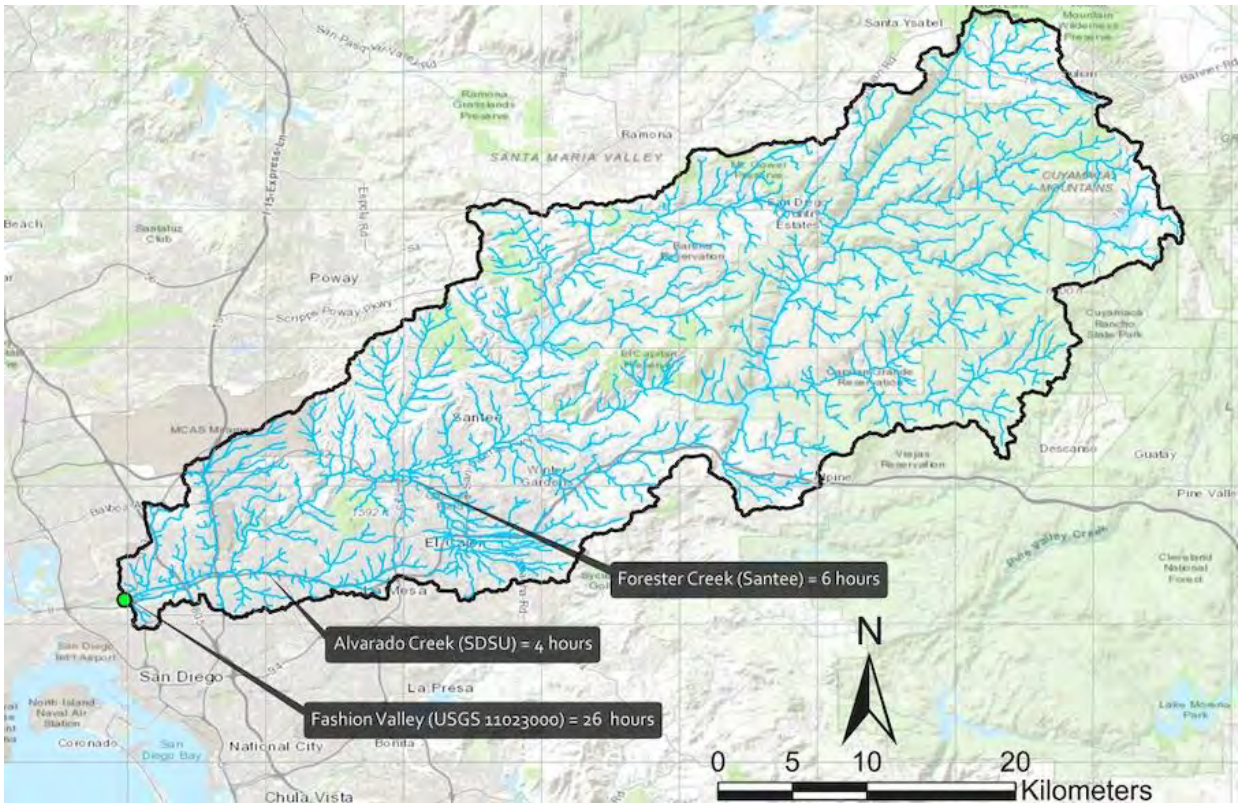

Figure 5-2. San Diego River watershed delineated to the most downstream storm sampling location at the Fashion Valley USGS streamflow gage (green circle). Alvarado Creek and Forester Creek are also shown. A stream network is highlighted and the time of concentration based on the Bransby and Williams method is noted.

The watershed areas of the two tributaries, Alvarado Creek and Forester Creek (Table 5-1), represent approximately 2.5% and 5.2% of the San Diego River watershed area, respectively. The first storms sampled in the 2018 and 2019 hydrologic year had the highest number of antecedent dry days. The first storm event of the 2018 hydrologic year (8-10 January 2018) followed an extended dry period with no rain since May of the previous year and only a 0.18 cm rain event occurring in December 2017. The peak discharge of the January 2018 storm event was > 630 cfs (18 m^3/s) making it the largest storm sampled in this study (Figure 5-1), which can be referred to as a "first flood" event. The first storm of the 2019 wet season (4 October 2018) also followed an extended dry period. The October 2018 storm did not make a significant change in the hydrograph of the river (raising the hydrograph only up to 0.0750 m^3/s) (Figure 5-1; note the log scale on the y-axis); therefore, we refer to it as a "first flush" event. Although there had been additional rain events during October and November, the 28 - 30 November 2018 storm event was the first major storm event of the 2019 hydrologic year reaching peak discharge > 100 cfs (3 m^3/s) and was the first flood event for that year.

Table 5-1. Sampling sites, sampling intervals, and watershed and storm characteristics.

Hydrologic Year	Date	Antecedent dry days*	Sampling site	Sampling interval (h)
2018	8-10 January (first flood)	244	San Diego River at Fashion Valley	3
			Alvarado Creek Pedestrian Bridge (ACB)	3
			Alvarado Creek Culvert (ACC)	2
	26-28 February	45	San Diego River at Fashion Valley	3
			Alvarado Creek Pedestrian Bridge (ACB)	3
2019	4 October (first flush)	150	San Diego River at Fashion Valley	1
	28-30 November (first flood)	5	San Diego River at Fashion Valley	1
			Forester Creek	2
	10-11 March	6	San Diego River at Fashion Valley	1

*Number of dry days preceding the event; ie., with negligible precipitation (<0.25 cm).

5.2.1 Storm sampling

For all samples collected at the San Diego River, a Teledyne ISCO 6712 Full-Size Portable Autosampler, loaded with sterilized (autoclaved at 121°C for 30 minutes) 1-liter plastic bottles, was installed. For Alvarado creek storm sampling, a Teledyne ISCO 6712 Full-Size Portable Autosampler was installed at the Pedestrian Bridge site and a Teledyne ISCO 3700 Full-Size Portable Autosampler was installed at the Culvert site. At Forester Creek, a Teledyne ISCO 6700 Full-Size Portable Autosampler was installed.

Baseline samples or “pre rain” grab samples were collected either on the same day the autosamplers were installed or the day prior to the storm event. “Post rain” samples were collected after the rain event, before uninstalling the autosamplers and when the discharge had returned to baseflow levels. Samples were delivered on ice to WIRLab for chemical analyses and Safe WaTER lab for microbiology analyses.

Sampling intervals for the 2018 storms were 3 h for most sites (Table 5-1). For the 2019 hydrologic year, the research team strived to collect higher resolution temporal data and intervals were set at 1 – 2 h. Samples were prepared in accordance with standard operating procedures for inland microbiological sample collection for the California Surface Water Ambient Monitoring Program (SWAMP). Sample container preparation guidelines are summarized in Table 1 of this project’s

Quality Assurance Project Plan (QAPP). All samples were transferred to the laboratory in a cooler on ice.

Conductivity, pH, and total dissolved solids (TDS) were measured using an Accumet AP85 Portable pH/Conductivity meter upon delivery of samples to the laboratory. Turbidity was measured using HACH DR900 absorbance method (at 520 nm absorption) and are reported in formazin attenuation units (FAU). The conversion of FAU to nephelometric turbidity units (NTU) is given by the equation: NTU = 0.7802(FAU) - 5.5607. Water temperature and dissolved oxygen (DO) concentrations must be measured at the time of sample collection, which was not possible due to safety limitations. Therefore, those parameters were not measured for this objective.

5.2.2 Quantification of microbial constituents

All samples were analyzed within 48 hours of collection for fecal indicator bacteria *E. coli* and fecal enterococci using the Colilert and Enterolert methods, respectively, with the IDEXX Quanti-Tray 2000 system, as described in Standard Methods 9223B (APHA, 2012), following manufacturer's recommended protocols. Samples were diluted according to anticipated concentrations from past measurements. Colilert trays were incubated at 35°C for 18 h and Enterolert trays were incubated at 41°C for 24 h, and wells fluorescing blue under UV light after the incubation periods were counted as positive. The most probable number (MPN) and 95% confidence intervals were then calculated using maximum likelihood estimation (MLE).

Microorganisms present in samples were concentrated by vacuum filtration using membrane filters with a pore size of 0.45 µm. Filters were then placed inside bead-beating tubes with 600 µL of lysis buffer from the AllPrep PowerViral RNA/DNA Kit (Qiagen). After vortexing at high speed for 10 min with a bead-beating tube adapter (Qiagen), filter lysates were transferred to clean microcentrifuge tubes, and nucleic acids were extracted and purified following steps delineated in the manufacturer's protocol, using 50 µL of molecular grade water for the final elution of DNA.

Molecular targets were quantified using the Bio-Rad QX200 droplet digital polymerase chain reaction (ddPCR) system. Primers and probes were ordered based on previously published assays. Final concentrations of primers were 900 nM, the final concentration of the probe was 250 nM, and the ddPCR Supermix for Probes (Bio-Rad cat. no. 186-3010) was used at a final concentration of 1X. A volume of 3 µL purified DNA was used for all samples. Molecular grade water was added as necessary to bring the final reaction volumes up to 20 µL. Polymerase chain reaction (PCR) mixtures were prepared in 0.2 µL PCR tubes or in 96 well PCR plates. Each reaction well was mixed thoroughly by pipetting the entire volume up and down 20 times. Droplets were generated in all samples using manual pipetting with eight-sample cartridges and droplet generator oil (Bio-Rad cat. no. 1863005), following the steps recommended by the manufacturer. Thermocycling conditions were as follows: 95°C for 10 min followed by 40 cycles of 94°C for 30 s, then 60°C for 1 min. After completion, samples were held at 98°C for 10 min, as recommended.

Quality assurance and quality control protocols for ddPCR are described in the QAPP, following recommendations set forth by Huggett et al. (2013). Briefly, gBlocks (Integrated DNA technologies) with the target sequences were used as positive controls. All gBlocks were resuspended following the manufacturer's recommendations, then stored at 1 ng/µL with 15

ng/µL yeast tRNA (gBiosciences) at -20°C until further use. Prior to each ddPCR run, gBlocks were thawed, then quantified by Qubit following steps described in the Qubit 1X dsDNA HS Assay protocol, and then serially diluted to concentrations between 0.1 and 1,000 copies per reaction. At least one no template control (NTC) and two positive controls were analyzed alongside samples for each PCR run. All PCRs were performed in duplicate and Inhibition controls were analyzed by spiking samples with a known concentration of gBlocks.

5.2.3 Quantification of chemical constituents

Chloride and bromide concentrations were measured on filtered samples using a Dionex 7 Ion Chromatograph. Blanks of ultrapure water were run every 10 samples and 10% of samples were analyzed in duplicate. Anion ratios have been used to trace the origin of water due to the unique Cl/Br ratios of different water types (Alcalá & Custodio, 2008), including groundwater (Cartwright et al, 2006). The Cl/Br mass ratio was calculated as the quotient of chloride and bromide mass concentrations. Nitrate and phosphate concentrations were quantified using Hach Methods 8039 and 8048 on a Hach DR900 colorimeter. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a high temperature combustion method with a Shimadzu TOC-L Total Organic Carbon Analyzer. Blanks of ultrapure water were run every 10 samples and 15% of the samples were run in duplicate. Other quality control measures are described in this project's QAPP.

Samples (400 mL) were extracted and analyzed for caffeine and sucralose concentrations following U.S. EPA Method 1694, using an Agilent 6460 Triple Quad LC/MS/MS system equipped with Agilent Jet Stream Technology. The triple quadrupole MS-MS system was operated in the multiple reaction monitoring (MRM) mode, using the precursor and product ion transitions of the target compounds and their isotopically labeled surrogates to detect the analytes of interest. Liquid chromatography mass spectrometry (LCMS) water was used as field blanks and lab blanks, and duplicates were run for every 10-20 samples. Caffeine concentrations were lower than 0.071 µg/L in three field blanks and undetectable in two. Sucralose concentrations were undetectable in four out of five field blanks and 0.001 in the fifth. Other quality control measures are described in this project's QAPP.

5.2.4 Discharge and constituent loading determinations

Stream discharge was measured at USGS Gage # 11023000 at the San Diego River at Fashion Valley. Discharge values in m^3/s were calculated from pressure transducer data at the Pedestrian Bridge and Culvert sites at Alvarado Creek and at the Forester Creek site using the velocity-area method to estimate streamflow and develop a rating curve for continuous values. Pollutant loadings were calculated at each sampling time as the product of the pollutant concentration and the flow rate at that time. Cumulative loadings for each storm were calculated as the sum of all products of constituent loading and respective time interval.

5.3 RESULTS AND DISCUSSION

5.3.1 Hydrology of the San Diego River during storm events

The conceptual models in Figure 5-3 illustrate four hydrologic phases underway during storm events in the San Diego River and its tributaries. Pre-storm conditions in panel 1 show a gaining reach fed by brackish groundwater. In Alvarado Creek, baseflow during dry weather has chloride concentrations of almost 650 mg/L (Figure 5-3) and electrical conductivity values > 2.0 mS/cm, which are representative of "brackish" waters and similar to the conductivity of groundwater collected by the USGS at the SDAQ (Qualcomm site), which ranges from 1.38 to 4.19 mS/cm. Conductivity values in the San Diego River during baseflow were also characteristic of brackish waters (> 2.0 mS/cm and as high as 4.43 mS/cm during the period of this study; Figure 5-4). During a storm event, rainfall is carried across the landscape as sheetflow (panel 2), which may transport pollutants from the soil surface into the channel. As channels fill with lower conductivity rainwater, there is mixing of the saline surface water, resulting in a dilution effect and decrease in conductivity (Figure 5-4). Over time, soils may also become saturated with infiltrating rainwater. Interflow occurs when rainfall infiltrates, saturates the vadose zone, and flows laterally through the subsurface into channels and other drainage networks (panel 3). Whereas sheetflow is likely to introduce surface contaminants, such as waste on the soil surface or in trash receptacles, into the surface water, interflow acts to transport contaminants that have accumulated in the subsurface, such as pollutants in vadose zone soils around cracked and leaking sanitary sewers. After precipitation ceases, conductivity rises as groundwater-fed baseflow supplies water to the channel (panel 4).

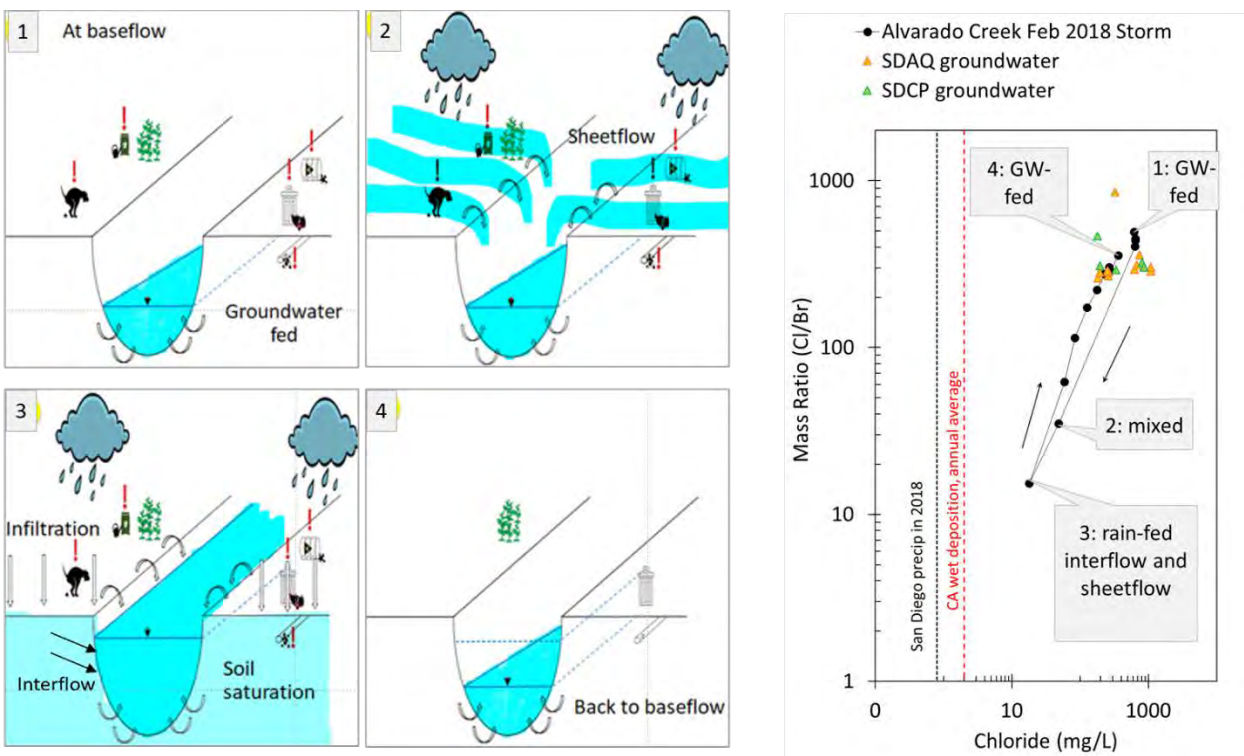

Figure 5-3. Conceptual model showing groundwater-surface water interactions during four phases of a storm event. Right panel shows chloride concentration and Cl/Br ratio for Alvarado creek water samples (black circles) from the Feb 2018 storm event. Orange and green triangles

indicate the Cl/Br signatures of USGS groundwater wells (SDAQ and SDCP, respectively). Illustration credit: F. Pinongcos.

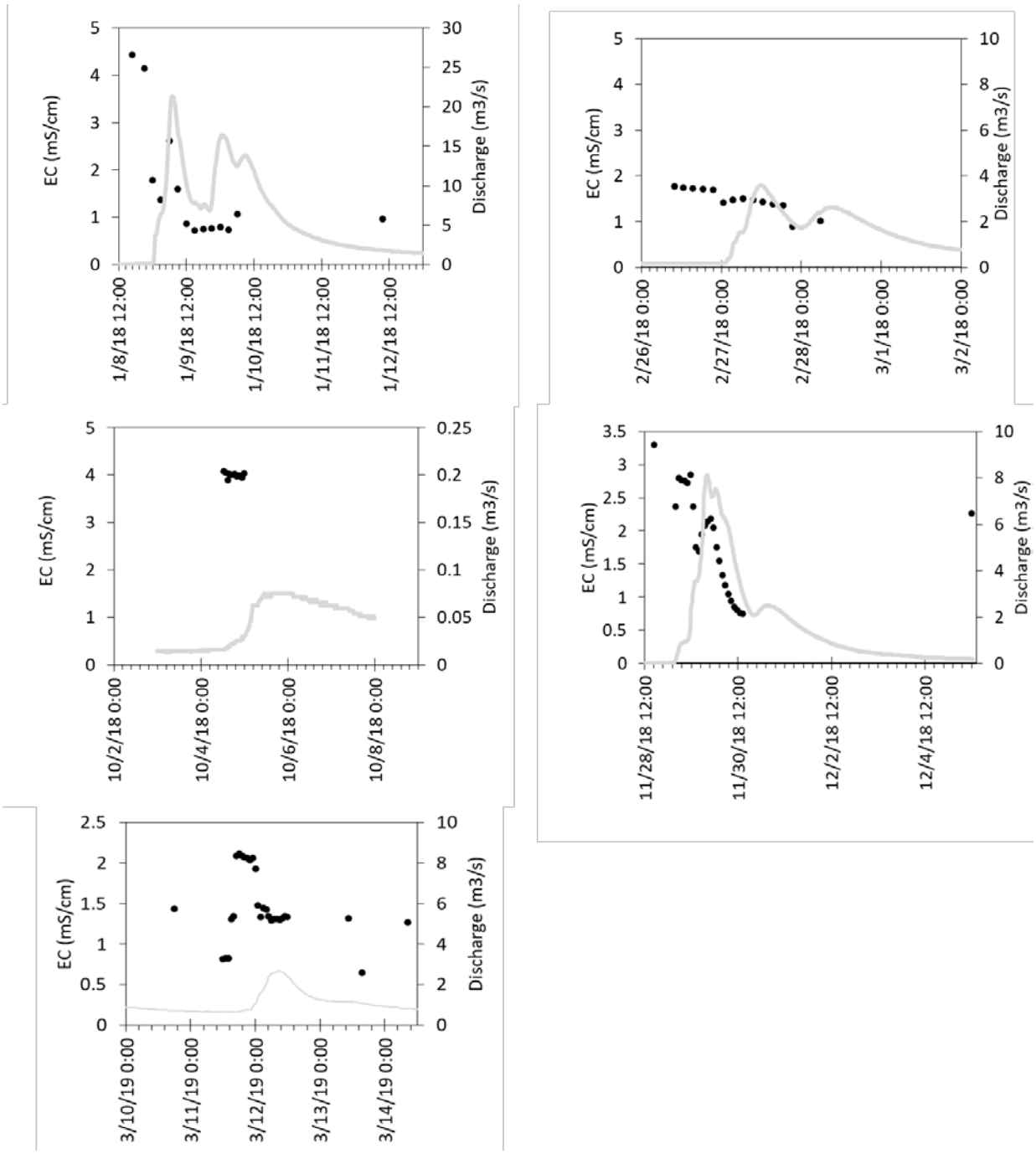

Figure 5-4. Electrical conductivity measured during each storm event in 2018 and 2019 in the San Diego River at Fashion Valley.

The chloride/bromide ratio is a metric that has been used to evaluate water sources in diverse environments. As with conductivity, chloride and bromide both decrease as stormwater becomes increasingly dominated by rainwater. The relationship of the Cl/Br mass ratio and the chloride

concentration (Figure 5-3) yields a hysteresis curve in which the signature of water returns to its historical, pre-storm value over the course of a storm event. To illustrate this phenomenon, the right panel of Figure 5-3 shows the Cl/Br and Cl concentration hysteresis curve for the February 2018 storm event in Alvarado Creek. Signatures in phase 1 were similar to those of shallow groundwater, as recorded by the USGS for groundwater collected from wells at their SDAQ (Lat 32°46'41.13", long 117°07'15.08") and SDCP (Lat 32°44'15.94", long 117°04'20.06"). In phase 2, there is mixing of rain-fed sheetflow, transporting water from the entire San Diego River watershed, with groundwater-fed water that previously dominated the San Diego River. In phase 3, chloride concentration values shifted to approach the California wet deposition end member concentration of ~2.0 mg/L, which is expected of rain-dominated sheetflow and interflow. It is during interflow that contaminants from belowground sewer infrastructure are likely to be mobilized. The return to higher conductivity values at the end of most storm events (Figure 5-4) supports the return to groundwater-fed conditions. It should be noted that, in some cases, samples were not collected early enough prior to the storm or long enough after a storm event to be able to capture this effect for each of the five storms (Figure 5-4).

Ongoing work by our group is pursuing hydrographic separation of runoff and baseflow (as in Margulis, 2017a) and an end-member mixing analysis using information on pollutant mass loadings and organic matter optical properties to tease apart pollutant contributions from the subsurface and soil surface. A deep understanding of the hydrologic and biogeochemical conditions of each watershed through evaluation of multiple lines of evidence is needed to distinguish major sources of microbial pollution.

5.3.2 Microbial pollution in the San Diego River during storm events

Figures 5-5 to 5-10 show the pollutagraphs for fecal indicator bacteria, *E. coli* and enterococci, and bacterial markers, HF183 and PMMoV, as well as chemical markers, caffeine and sucralose, during storm events sampled in 2018 and 2019 in the San Diego River. For most storms, FIB concentrations peaked initially during the rising limb of the hydrograph, decreased slightly at peak discharge, and peaked again on the falling limb. For all storm events with peak discharge > 2 m^3/s, FIB concentrations exceeded wet weather target concentrations of 400 MPN/100 mL for fecal coliforms and 104 MPN/100 mL for enterococci in the Lower San Diego River as specified in the region's Basin Plan (San Diego Water Board, 2016) by one to two orders of magnitude.

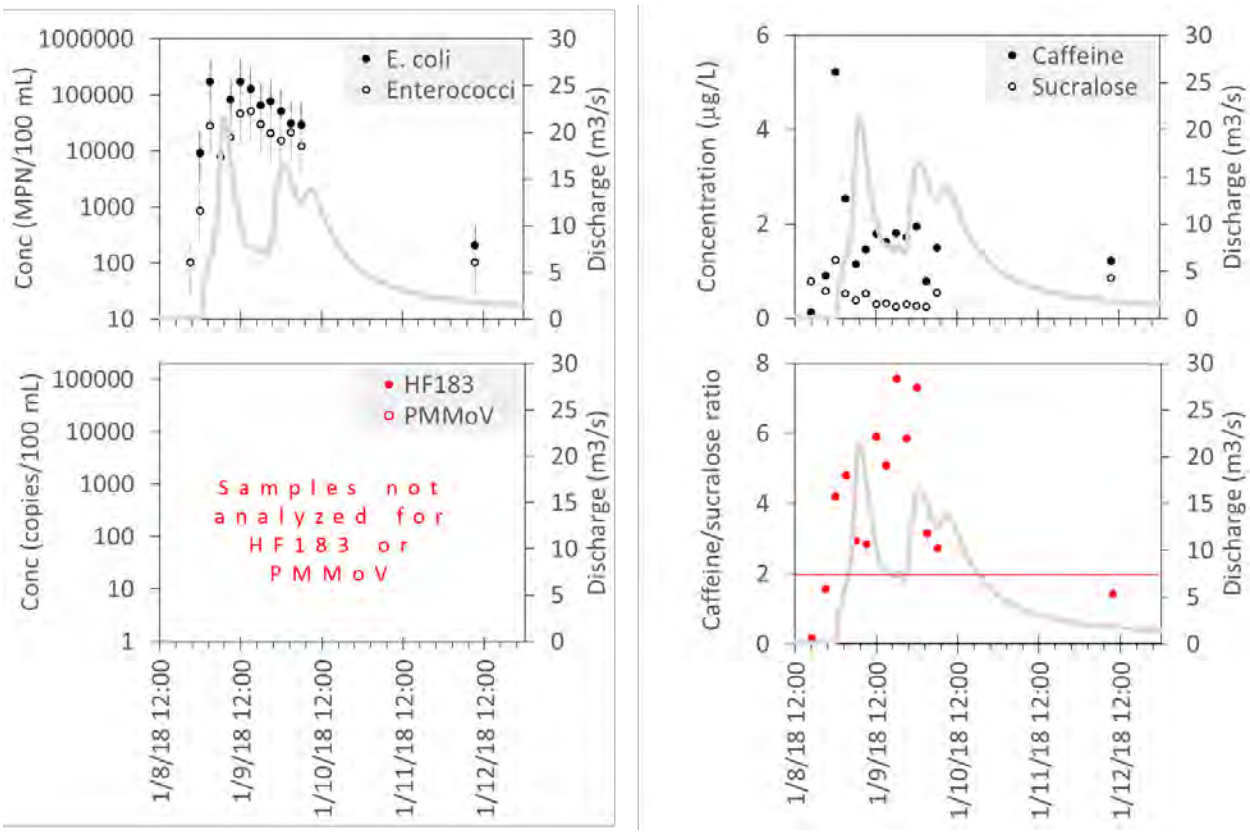

Figure 5-5. Pollutagraphs of *E. coli*, enterococci, and chemical markers (caffeine and sucralose), and the caffeine/sucralose ratio for the January 2018 storm event in the San Diego River at Fashion Valley. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Red horizontal line indicates caffeine/sucralose ratio of 2, above which sources are more likely to stem from untreated wastewater.

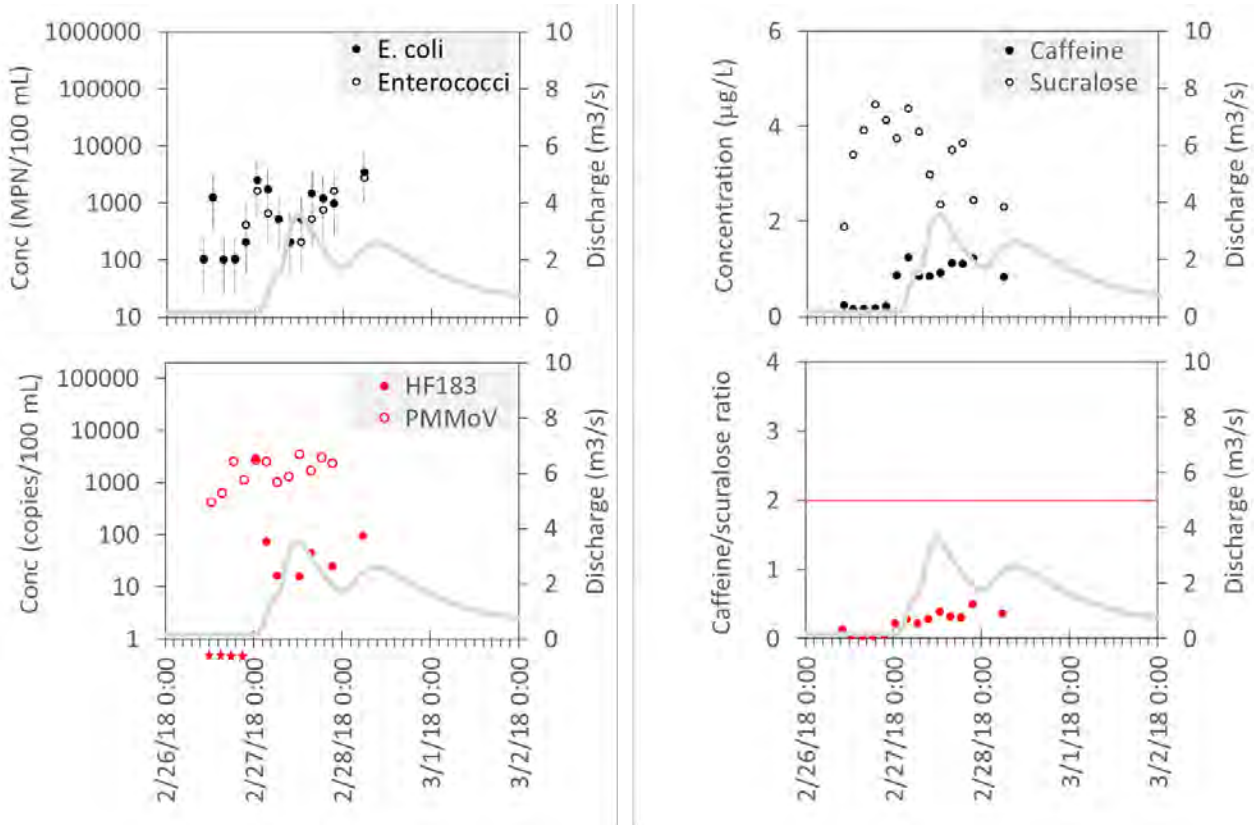

Figure 5-6. Pollutagraphs of microbial markers (*E. coli*, enterococci, HF183, and PMMoV), chemical markers (caffeine and sucralose), and the caffeine/sucralose ratio for the February 2018 storm event in the San Diego River at Fashion Valley. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Red stars indicate HF183 values below detection limits. Red horizontal line indicates caffeine/sucralose ratio of 2, above which sources are more likely to stem from untreated wastewater.

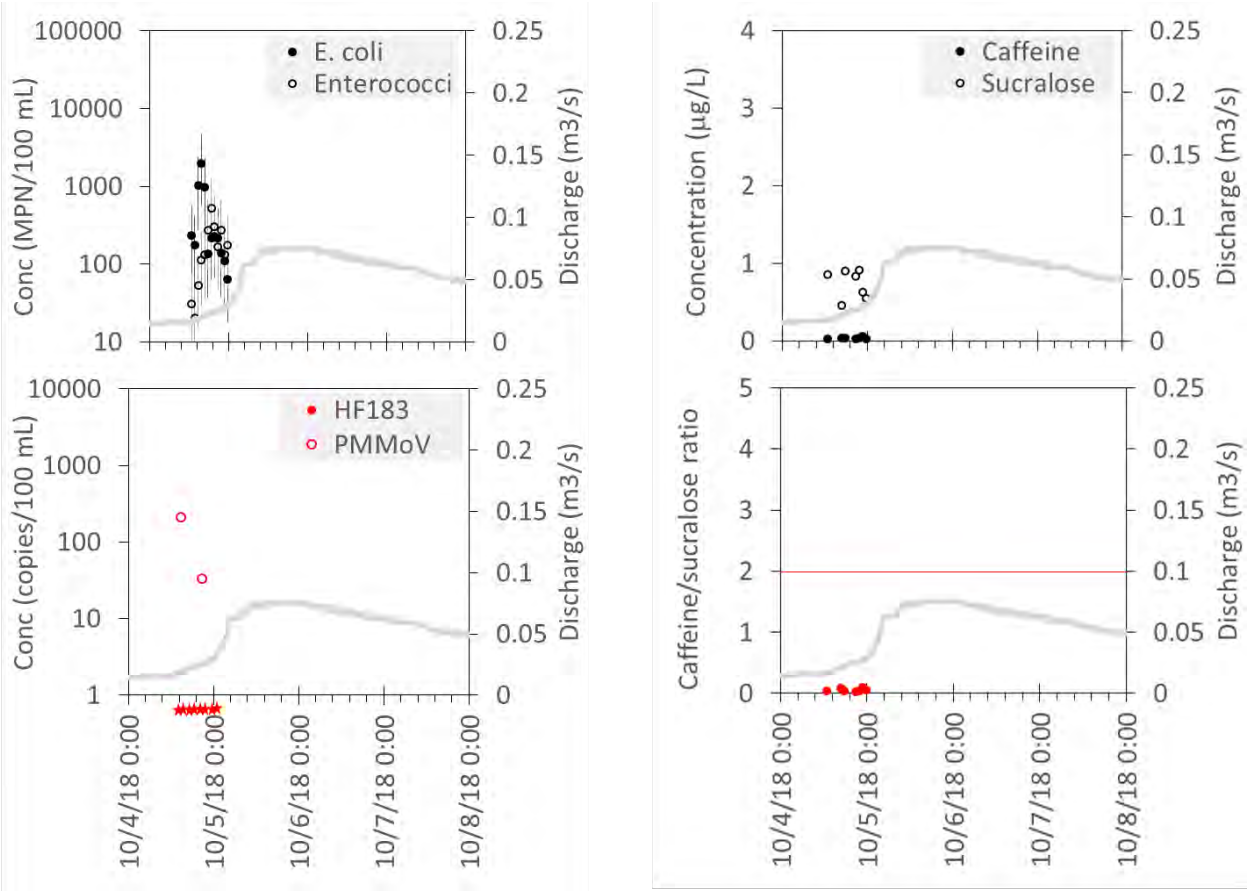

Figure 5-7. Pollutagraphs of microbial markers (*E. coli*, enterococci, HF183, and PMMoV), chemical markers (caffeine and sucralose), and the caffeine/sucralose ratio for the October 2018 storm event in the San Diego River at Fashion Valley. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Red stars indicate HF183 values below detection limits. Red horizontal line indicates caffeine/sucralose ratio of 2, above which sources are more likely to stem from untreated wastewater.

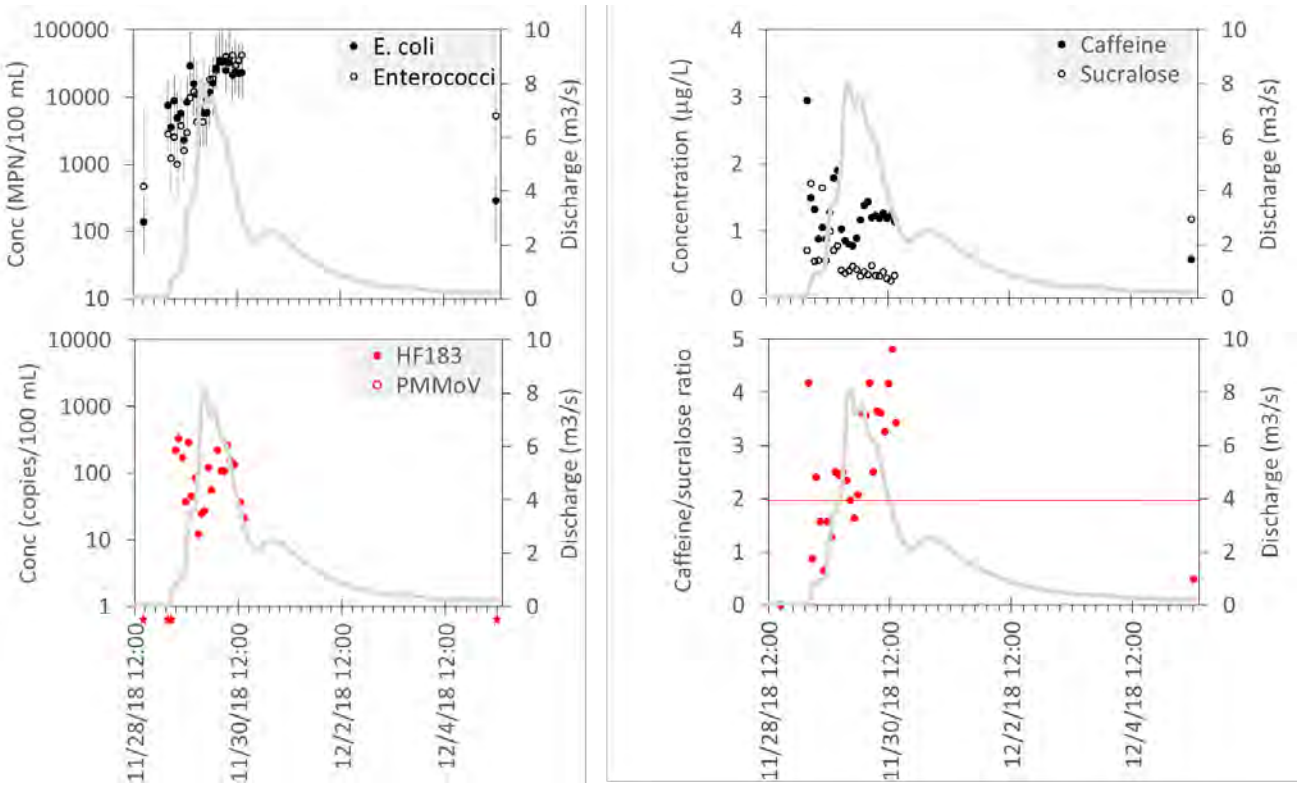

Figure 5-8. Pollutagraphs of microbial markers (*E. coli*, enterococci, HF183, and PMMoV), chemical markers (caffeine and sucralose), and the caffeine/sucralose ratio for the November 2018 storm event in the San Diego River at Fashion Valley. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Red stars indicate HF183 values below detection limits. Red horizontal line indicates caffeine/sucralose ratio of 2, above which sources are more likely to stem from untreated wastewater.

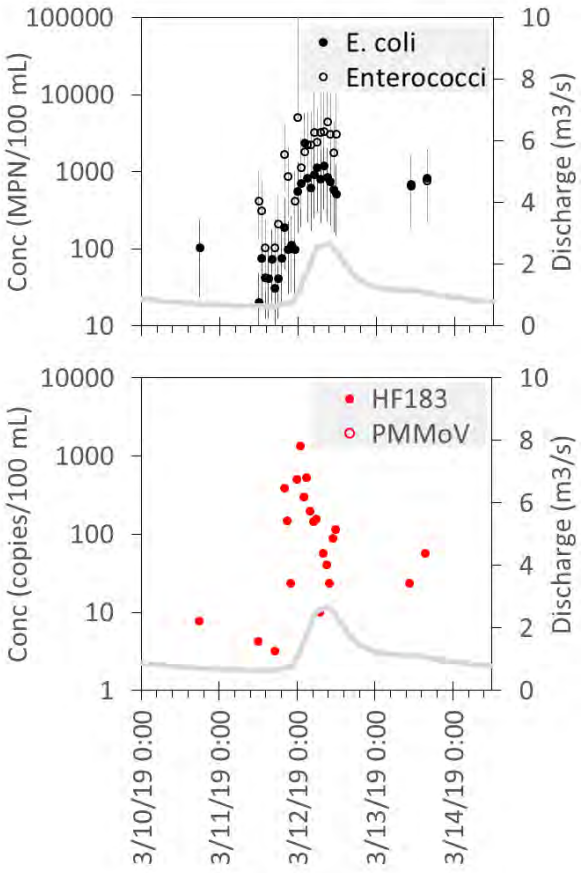

Figure 5-9. Pollutagraphs of microbial markers (*E. coli*, enterococci, and HF183) for the March 2019 storm event in the San Diego River at Fashion Valley. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Caffeine and sucralose concentrations were not measured for this storm.

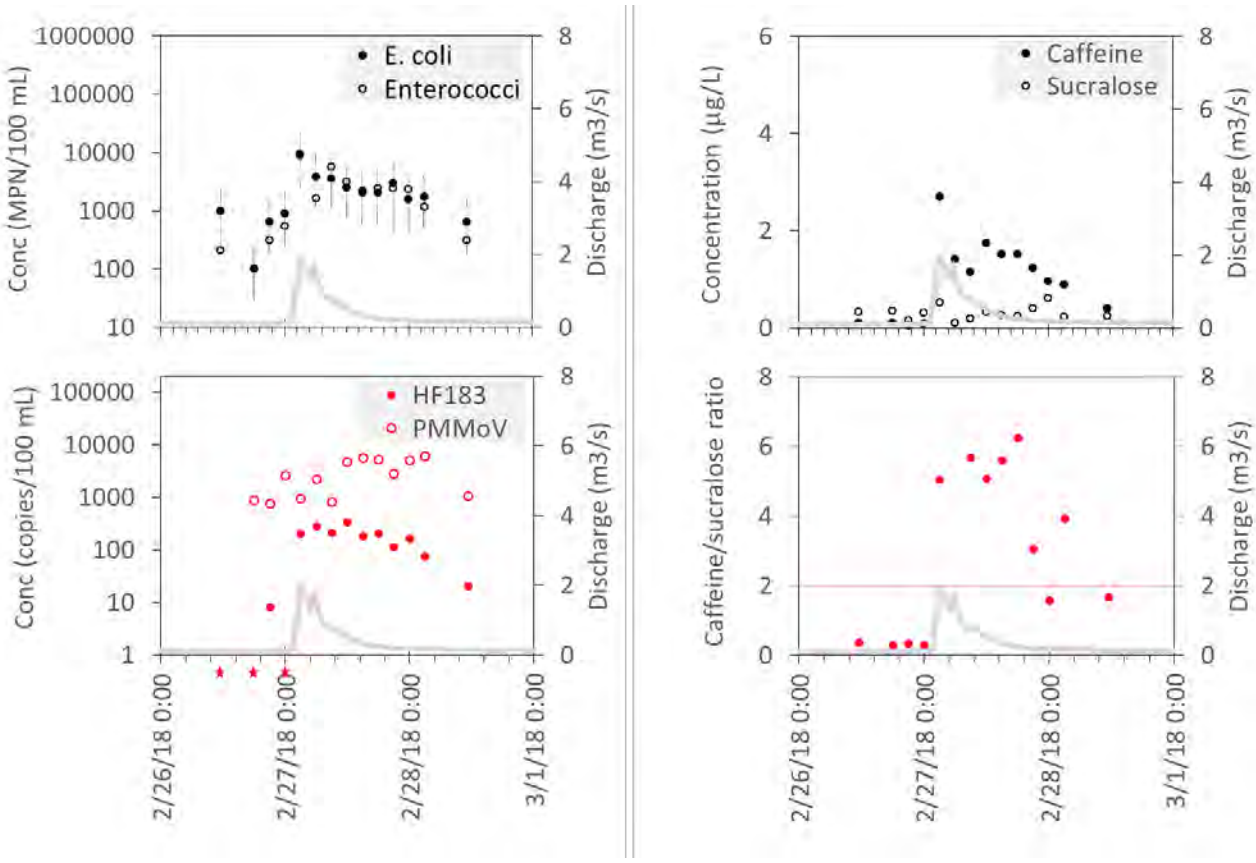

Figure 5-10. Pollutagraphs of microbial markers (*E. coli*, enterococci, HF183, and PMMoV), chemical markers (caffeine and sucralose), and the caffeine/sucralose ratio for the February 2018 storm event in Alvarado Creek at the Pedestrian Bridge site. Error bars show upper and lower limits of detection for *E. coli* and enterococci. Gray line represents discharge. Red stars indicate HF183 values below detection limits. Red horizontal line indicates caffeine/sucralose ratio of 2, above which sources are more likely to stem from untreated wastewater.

Bacterial markers, HF183 and PMMoV, which are both indicators of human fecal contamination, were detected for most storms. Although temporal patterns were difficult to discern due to the limits of detection (which varied depending on volume filtered, but were generally between 200 and 500 gene copies/100 mL for HF183 and between 300 and 1000 gene copies/100 mL for PMMoV), HF183 increased from values below the limits of detection (red stars in Figures 5-6, 5-8, and 5-10) to values that reach 10^2 to 10^3 gene copies/100 mL. The smallest volume storm (October 2018; Figure 5-7) had undetectable levels of HF183, although PMMoV, which is more persistent in the environment, was present. Given the much greater concentrations of HF183 in wastewater than in runoff from open defecation sites (Chapter 4), the pollutagraphs above suggest that the February 2018, November 2018, and March 2019 storms were influenced by untreated wastewater, whereas the October 2018 storm was not. Interestingly, during baseflow conditions, HF183 was detected at low concentrations or undetectable, whereas PMMoV was often detected (Chapters 2 and 3).

Results from higher resolution sampling in the 2019 hydrologic year showed the rapid response of FIB concentrations to changes in discharge, with concentrations sometimes increasing an order of magnitude within the one-hour sampling interval (Figure 5-7 to 5-9). In all cases, FIB concentrations peaked during the rising limb of the hydrograph or just before peak discharge. Samples were only successfully retrieved for the initial part of the October 2018 storm. However, for the November 2018 and March 2019 storms, results from samples collected after peak discharge indicate that FIB concentrations tended to increase further and remained high for hours to days after the peak flood.

Additional microbial markers including viral pathogens (HAV and NoVGI), bacterial pathogens (*C. coli* and *C. jejuni*), and a viral fecal indicator (coliphage PhiX174) were analyzed for two different storm events. The 26-28 February 2018 storm was not the first major flushing event of the 2018 hydrologic year, but it was one of the few storm events that followed the Hepatitis A outbreak in San Diego. It is worth noting that no detectable HAV was observed in the San Diego River or Alvarado Creek for this storm event. HAV was also not above the detection limit in the first storm event of the 2019 hydrologic year, on October 4, 2018, but NoVGI was detected at concentrations up to 863 gene copies/100 mL (Table 5-2). NoVGI was not detected in either of the two sampling sites for the 26-28 February storm event, and *C. jejuni* was not detected in any of the storm events. PhiX174, a virus that infects *E. coli*, was also detected in Alvarado Creek.

Table 5-2. Concentration ranges* or presence/absence of bacteria and viruses measured during two storm events in the San Diego River and its tributaries.

Hydrologic Year	Date	Sampling site	HAV	Norovirus GI	PhiX174 coliphage	*C. jejuni*	*C. coli*
			gc/100 mL	gc/100 mL	gc/100 mL	gc/100 mL	gc/100 mL
2018	26-28 February	San Diego River at Fashion Valley	ND	ND	14 – 573	ND	ND
		Alvarado Creek Pedestrian Bridge (ACB)	ND	ND	21 – 326	ND	ND – 105
2019	4 October	San Diego River at Fashion Valley	ND	ND – 863	11 – 602	ND	ND

* Concentrations of FIB, HF183 and PMMoV are given in Figures 5-6, 5-7, and 5-10. gc/100 mL = gene copies/100 mL. ND = not detected.

5.3.3 Microbial pollution in tributaries of the San Diego River during storm events

Both *E. coli* and enterococci were detected in the two tributaries of the San Diego River, Alvarado Creek (Figure 5-10) and Forester Creek. PhiX174, a virus that infects *E. coli*, was also detected in Alvarado Creek. *Campylobacter coli* was the only human pathogen detected in Alvarado Creek during the February 2018 storm event, and it was only detected sporadically and at low concentrations (Table 5-2).

Pollutagraphs, compiled for Alvarado Creek (Figure 5-10) for the 26-28 February storm event and Forester Creek for the 28-30 November 2018 storm event (not shown due to insufficient discharge data), further show higher FIB concentrations in the tributaries than in the main stem of the San Diego River. Despite having ~one-third of the discharge of the San Diego River, Alvarado Creek water samples had FIB, PMMoV, and HF183 concentrations that were as high as or higher than the San Diego River for the same February 2018 storm (Figures 5-6 and 5-10). Volume-weighted mean concentrations of *E. coli* and enterococci for both tributaries were higher than in the San Diego River during the February 2018 and November 2018 storm events (Table 5-3). It is interesting to note that during dry weather flow the opposite pattern arose; FIB and DOC concentrations were substantially higher in the San Diego River than in its tributary. During the larger volume November 2018 storm, DOC concentrations were also substantially higher in Forester Creek than in the San Diego River. By contrast, TDS concentrations were almost 7-fold lower in Forester Creek (Table 5-3), reflecting the greater contribution of rainfed runoff (low in TDS) to the channel. The diluted microbial pollutant concentrations in Fashion Valley can also be attributed to the greater contributing watershed area (1,111 square kilometers) and Tc (26 hours), which may equate to larger volumes of water from ~42.7 square kilometers per hour. Meanwhile, the elevated pollutant concentrations in Alvarado and Forester Creek can be attributed to the smaller drainage areas and faster time of concentrations (4 and 6 hours, respectively). This may result in lower volumes of water from smaller areas per time (~7.7-10.4 square kilometers per hour).

Table 5-3. Volume weighted mean concentrations of FIB and chemical constituents measured during two storm events in the San Diego River and its tributaries.

Date	Sampling site	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	DOC (mg/L)	TDN (mg/L)	TDS (mg/L)
26-28 February 2018	San Diego River at Fashion Valley	1.59E+03	1.18E+03	11.2	1.12	345
	Alvarado Creek Pedestrian Bridge	4.61E+03	4.50E+03	12.0	1.79	217
28-30 November 2018	San Diego River at Fashion Valley	1.58E+04	1.85E+04	14.9	1.89	866
	Forester Creek	7.13E+04	7.52E+04	21.3	1.90	132

Table 5-4. Baseline values (during dry weather) of FIB and chemical constituents in the San Diego River and its tributaries.

Date	Sampling site	Days after last rain	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	DOC (mg/L)	TDN (mg/L)	TDS (mg/L)
6/21/2018	Alvarado Creek Pedestrian Bridge	96	48	48.4	6.78	0.29	1683.33
6/29/2018		104	30.8	104	6.36	0.16	990.67
7/24/2018		129	57.4	109	8.48	0.52	981.00
9/21/2018		188	33.3	132	5.83	0.62	906.33
9/27/2018		194	26.6	155	6.30	0.91	960.00
10/12/2018		0	89	263	8.43	0.92	976.67
10/19/2018		7	185	397	6.97	0.88	743.33
10/31/2018		19	42.5	244	6.65	1.18	927.00
10/20/2018	San Diego River at Fashion Valley	8	596	865	13.15	0.82	NA
10/26/2018		14	2260	1160	NA	NA	NA
11/2/2018		21	787	411	13.71	1.18	NA
11/10/2018		29	1470	648	14.30	1.05	NA

5.3.4 Chemical markers of anthropogenic pollution in the San Diego River

Caffeine and sucralose are chemical markers of anthropogenic pollution due to their unique association with human activities (eg., consumption of caffeinated beverages and Splenda-sweetened food and beverages) and lack of natural sources within the San Diego River watershed. Both chemicals have been detected in soil and water environments due to anthropogenic contamination. Yet their contrasting biodegradability means that their concentrations vary depending on source, as shown in samples collected for this study from surface water and groundwater in San Diego and untreated wastewater and open defecation pollution sources (Table 5-5). Concentrations of sucralose, which is less biodegradable and more persistent in the environment than caffeine, tend to be an order of magnitude higher in dry weather baseflow in the San Diego River than caffeine concentrations (Table 5-5). Similarly, sterile water flushes of open defecation sites (summarized in Chapter 4) have higher sucralose concentrations than caffeine concentrations, likely because microorganisms in the soil preferentially degrade caffeine compared to sucralose. Also, 78% of sucralose consumed by humans is excreted in feces, with only a small percentage excreted in urine (Roberts et al. 2000), while caffeine is the opposite, with only 5% excreted in feces (Callahan et al. 1982).

For this study, we calculated caffeine/sucralose ratios for all potential sources of fecal contamination in the San Diego River watershed (Table 5-5). The caffeine/sucralose ratio of water flushing fecal material was very low and similar to the caffeine/sucralose of dry weather flow in the San Diego River (~ 0.10). By contrast, wastewater samples collected from untreated influent of the San Elijo Water Reclamation Facility in San Diego County had an order of magnitude higher concentration of caffeine (at 206 µg/L) than sucralose. Caffeine concentrations, ranging from 30 µg/L (Subedi and Kannan, 2014) to upwards of 200 µg/L, and sucralose concentrations in the

range of 17 to 46 µg/L, have also been reported in other studies (Batikian, 2018; Subedi and Kannan, 2014). The caffeine/sucralose for untreated wastewater in the current study was > 9.0 (Table 5-5), and a ratio of > 2.0 has been suggested for surface waters receiving untreated wastewater from a combined sewer overflow event (Cantwell et al., 2018). In this study, we also use caffeine/sucralose > 2.0 to indicate untreated wastewater contributions. Groundwater samples collected from the Sweetwater Authority had no detectable concentrations of caffeine or sucralose (Table 5-5).

Table 5-5. Caffeine and sucralose concentrations in different source areas.

Water source	Caffeine (µg/L)	Sucralose (µg/L)	Caffeine/sucralose ratio
San Diego River during baseflow	0.32 ± 0.06 (n = 18)	3.3 ± 2.0 (n = 18)	0.14 ± 0.09 (n = 18)
Flushes of open defecation sites[a]	0.46 ± 0.38 (n = 15)	6.7 ± 0.65 [d] (n = 3)	0.10 ± 0.11 (n = 3)
Untreated wastewater	206 [b] ± 10.4 (n = 4)	23 [c] ± 0.4 (n = 4)	9.1 ± 0.56 (n = 4)
Groundwater	0.0 ± 0.0 (n = 2)	0.0 ± 0.0 (n = 2)	-

[a] Concentrations based on 700 mL of sterile synthetic rainwater applied as sheetflow over the stool sites.
[b] Value exceeded the calibration curve. Samples should be re-run for accuracy.
[c] Recoveries exceeded 120%. Samples should be re-run for accuracy.
[d] Sucralose concentrations in 12 out of 15 open defecation flush samples were greater than 20 µg/L, but those samples had recoveries >120% due to the highly concentrated samples and cannot be reported. Nevertheless, it is informative to report that sucralose concentrations in all 15 samples were far greater than caffeine concentrations.

During storm events in the San Diego River and its tributaries, caffeine concentrations typically increased from the low baseflow levels to > 1 µg/L and were as high as 3 µg/L - 5 µg/L during the larger January 2018 and November 2018 storm events (Figures 5-5 and 5-8). By contrast, sucralose concentrations generally ranged from 0.1 µg/L to 1.5 µg/L, and were higher in pre-storm samples, decreasing during peak discharge. The resulting caffeine/sucralose ratios were low, mostly during the initial hours of the storm event, and typically exceeded the 2.0 threshold during the rising limb of the hydrograph and at peak discharge and stayed high well into the falling limb of the hydrograph. Low initial caffeine/sucralose ratios at the onset of each storm may reflect flushing and transport via sheetflow of human fecal material. Other inputs of caffeine may occur during a storm event due to other caffeine- and sucralose-containing pollutants (discarded beverages, food waste, etc.) that may have been present on soil surfaces; however, those sources could not be evaluated under the scope of this project.

5.3.5 Evidence of different sources of San Diego River contamination

Untreated wastewater. One of the most important findings of this study is that the highest FIB concentrations occurred when caffeine concentrations were elevated compared to sucralose and when HF183 concentrations were high (Figures 5-5, and 5-8 to 5-10). Therefore, during peak discharge, when FIB and HF183 concentrations were at their maximum, and during the falling limb of the hydrograph, when FIB and HF183 concentrations remained high, caffeine/sucralose ratios were high, mostly > 2.0. Results in Table 5-5 indicate that the high caffeine/sucralose ratio is most likely derived from the introduction of recent, untreated wastewater to the environment. In runoff at open defecation sites, the caffeine/sucralose ratio was consistently low. Other sources, such as discarded beverages or foods could not be evaluated, and may also contribute both chemicals to surface waters. Only two samples, collected during the November 2018 storm, had caffeine/sucralose ratios > 2.0 at the onset of the storm event, and these two data points may reflect sheetflow transporting fresh caffeine inputs, possibly from discarded beverages or foods. For all other samples, the increase in caffeine/sucralose to > 2.0 during or just prior to peak discharge and caffeine/sucralose values that remained elevated after peak discharge strongly suggest that recent untreated wastewater, likely derived from SSOs or sewer exfiltration, is the main source of caffeine, FIB, and other fecal pollutants during and after peak discharge. In addition, the high HF183 concentrations found in untreated wastewater and low or undetectable HF 183 that occurred in our runoff experiments, in which we flushed open defecation sites with synthetic rainwater (Chapter 4), provide strong support for untreated wastewater as the main source of microbial contamination of the San Diego River and its tributaries during storm events.

The highest bacterial loadings (mass/time), calculated as the product of constituent concentration (mass/volume) and discharge (volume/time), also occurred during and after peak discharge. Therefore, on a mass loading basis, the greatest FIB introduction to the San Diego River occurs when soils become saturated and interflow becomes a dominant hydrologic process (Figures 5-3 and 5-11), flushing subsurface sources of contamination.

Sanitary sewer overflow. The February 2018 storm event was the only event with uncharacteristically high sucralose concentrations, reaching > 4 µg/L. These high values were only detected in San Diego River samples (Figure 5-6) and not in Alvarado Creek samples collected on the same day, which had higher caffeine than sucralose (Figure 5-10). It is likely that the anomalous values in the San Diego River samples were due to a major sewage pipe failure that discharged > 52,000 L of untreated wastewater into a drainage area just upstream of the San Diego River confluence with Alvarado Creek (and approximately 2 miles (3.2 kilometers) downstream of the Alvarado Creek sampling site) on the evening of 15 February 2018 (SSO event 844870). Any sewage still remaining in stagnant pools or soils around the spill site likely contributed both caffeine and sucralose to the channel. If sucralose concentrations were as high in the sewage spill as they are in untreated wastewater (~20 µg/L; Table 5-5), mixing of spill volumes with water already in the channel, along with low degradation rates for sucralose, could result in the observed concentrations, which exceeded ~4 µg/L well before the rising limb of the hydrograph (Figure 5-6). Caffeine would have also had very high concentrations at the time of the SSO event, but, due to its higher biodegradability, would have rapidly degraded over the time period between the SSO event and the storm, which explains why caffeine concentrations were in the range observed for other storms (< 1.0 at storm onset), but sucralose was not.

Surface runoff. The October 2018 rain event, which produced very low discharge, barely reaching 0.1 m^3/s, and did not shift the caffeine/sucralose ratio to values greater than 2, may be the only event of this study that was dominated mainly by surface runoff. Although *E. coli* and enterococci concentrations in several of the water samples exceeded wet weather target concentrations of 400 MPN/100 mL for fecal coliforms and 104 MPN/100 mL for enterococci (San Diego Water Board, 2016), these FIB concentrations were two orders of magnitude lower than in other storm events. The low caffeine/sucralose ratio of water samples collected during this storm (Figure 5-7) and high values of PMMoV (which in general is more persistent than bacterial markers and other enteric viruses (Sassi et al. 2018; Symonds et al. 2018)), are consistent with results of sheetflow experiments flushing open defecation sites (Chapter 4). Also, low caffeine and FIB concentrations and undetectable HF183 concentrations suggest that wastewater was not the main source of microbial pollution during this storm. Instead, these multiple lines of evidence are consistent with the idea that open defecation and other sources of human and animal fecal material on the soil surface, where mainly the more persistent human markers (ie., PMMoV and sucralose) would be found, were introduced to the San Diego River via sheetflow. This event was also the first flush of the hydrologic year and the only storm event for which water samples had detectable concentrations of the viral pathogen, NoVGI. Therefore, although small first flush events mobilizing surface runoff may not result in substantial exceedance of benchmarks, their importance for introducing pathogens into the water column should be recognized.

5.3.6 Changes in constituent loadings

Loadings for bacterial and chemical constituents in the San Diego River during each of the five storm events are shown in Tables 5-6 and 5-7, respectively, and *E. coli* and enterococci loadings are shown in Figure 5-11. Most peak FIB loadings seemed to occur over a window of time, usually within 24 hours, before or after the peak of the hydrograph (Figure 5-11). For the largest storms (January 2018 and November 2018), *E. coli* and enterococci loadings, on the order of 10^{15} MPN/storm and x 10^{14} MPN/storm, respectively, begin to approach allowable TMDLs of 6.51 x 10^{16} MPN/year for total coliforms and 6.59 x 10^{15} MPN/year for enterococci (Chapter 7 of the Basin Plan; Water Board, 2016).

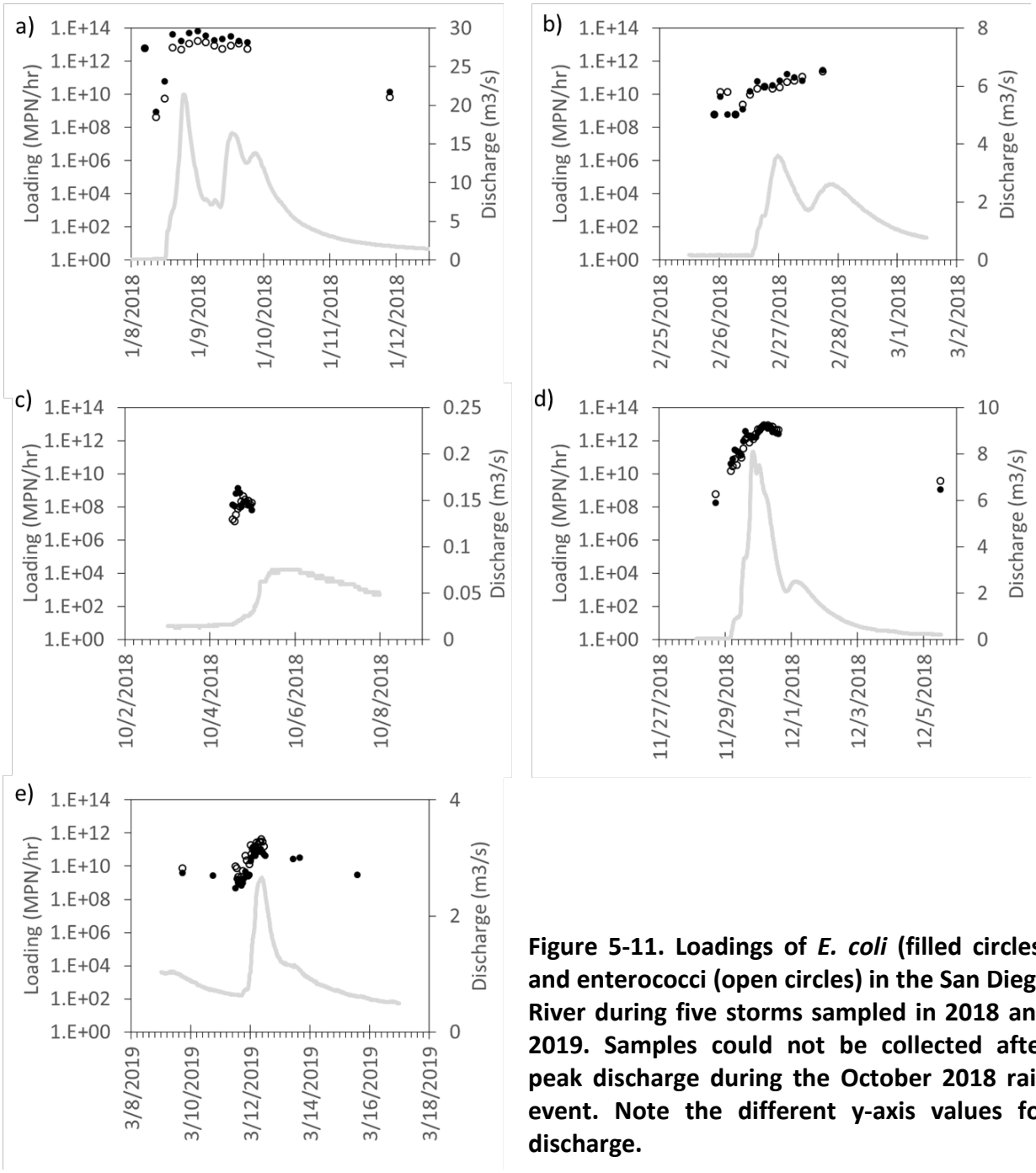

Figure 5-11. Loadings of *E. coli* (filled circles) and enterococci (open circles) in the San Diego River during five storms sampled in 2018 and 2019. Samples could not be collected after peak discharge during the October 2018 rain event. Note the different y-axis values for discharge.

Table 5-6. Bacterial loadings for each storm in the San Diego River.

Peak discharge (m^3/s)	Cumulative volume (m^3/storm)	Cumulative rain intensity (cm)	Constituent	Cumulative loading per storm event (MPN)	Daily loading (MPN/d)
8-10 January 2018					
21.38	18.1×10^5	5.28	*E. coli*	9.30×10^{14}	5.02×10^{14}
			Total coliform	5.45×10^{16}	2.90×10^{16}
			Fecal enterococci	2.91×10^{14}	1.61×10^{14}
26-28 February 2018					
3.60	2.57×10^5	1.42	*E. coli*	4.10×10^{12}	1.45×10^{12}
			Total coliform	4.05×10^{14}	1.70×10^{14}
			Fecal enterococci	3.09×10^{12}	1.06×10^{12}
4 October 2018					
0.08	9.67×10^2	0.10	*E. coli*	3.99×10^9	7.98×10^9
			Total coliform	1.11×10^{12}	2.22×10^{12}
			Fecal enterococci	1.88×10^9	3.76×10^9
28-30 November 2018					
8.10	6.56×10^5	3.45	*E. coli*	1.04×10^{14}	6.35×10^{13}
			Total coliform	9.56×10^{15}	5.80×10^{15}
			Fecal enterococci	1.17×10^{14}	7.17×10^{13}
11-13 March 2019					
2.67	4.63×10^5	0.51	*E. coli*	1.88×10^{12}	7.73×10^{11}
			Total coliform	7.39×10^{13}	2.04×10^{13}
			Fecal enterococci	4.22×10^{12}	2.39×10^{12}

Table 5-7. Loadings of chemical constituents for each storm in the San Diego River.

Peak discharge (m^3/s)	Cumulative volume (m^3/storm)	Cumulative rain intensity (cm)	Constituent	Cumulative loading per storm event (kg)	Daily loading (kg/d)
8-10 January 2018					
21.38	18.1×10^5	5.28	Total dissolved solids	1.08×10^6	4.31×10^5
			Dissolved organic carbon	2.71×10^4	1.05×10^4
			Total dissolved nitrogen	3.25×10^3	1.36×10^3
			Nitrate	2.35×10^3	9.58×10^2

Peak discharge (m^3/s)	Cumulative volume (m^3/storm)	Cumulative rain intensity (cm)	Constituent	Cumulative loading per storm event (kg)	Daily loading (kg/d)
			Phosphate	9.42×10^2	3.55×10^2
			Caffeine	2.56	1.07
			Sucralose	1.09	0.27
26-28 February 2018					
3.60	2.57×10^5	1.42	Total dissolved solids	1.68×10^5	8.40×10^4
			Dissolved organic carbon	2.91×10^3	1.37×10^3
			Total dissolved nitrogen	3.00×10^2	1.33×10^2
			Nitrate	1.06×10^2	39.6
			Phosphate	56.9	22.3
			Caffeine	0.24	0.12
			Sucralose	0.76	0.37
4 October 2018					
0.08	9.67×10^2	0.10	Total dissolved solids	1.93×10^3	3.87×10^3
			Dissolved organic carbon	4.67	9.34
			Total dissolved nitrogen	0.34	0.68
			Nitrate*	NC	NC
			Phosphate	0.26	0.52
			Caffeine	2.3×10^{-5}	4.6×10^{-5}
			Sucralose	4.3×10^{-4}	8.7×10^{-4}
28-30 November 2018					
8.10	6.56×10^5	3.45	Total dissolved solids	5.73×10^5	2.89×10^5
			Dissolved organic carbon	9.79×10^3	5.28×10^3
			Total dissolved nitrogen	1.24×10^3	6.68×10^2
			Nitrate	3.51×10^2	1.94×10^2
			Phosphate	3.54×10^2	1.87×10^2
			Caffeine	0.72	0.40
			Sucralose	0.36	0.16
11-13 March 2019					
2.67	4.63×10^5	0.51	Total dissolved solids	2.58×10^5	8.11×10^4

Peak discharge (m^3/s)	Cumulative volume (m^3/storm)	Cumulative rain intensity (cm)	Constituent	Cumulative loading per storm event (kg)	Daily loading (kg/d)
			Dissolved organic carbon	5.02×10^3	1.18×10^3
			Total dissolved nitrogen	1.30×10^2	5.26×10^2
			Nitrate	2.11×10^2	38.4
			Phosphate	1.29×10^2	28.4
			Caffeine*	NC	NC
			Sucralose*	NC	NC

* Loadings could not be calculated (NC) due to low numbers of samples extracted or insufficient sample volumes.

For all constituents, the larger the storm and the greater the total water volume, the greater the pollutant loading (Figure 5-12). Indeed, the flux of constituents (TDS, DOC, TDN, and *E. coli*) during the two largest "first flood" storms (January 2018 and November 2018) were several orders of magnitude greater than in smaller-sized storms, such as the October 2018 "first flush" storm event. Extended antecedent dry conditions prior to the first major storm (Table 5-1), during which fecal and other pollution can accumulate in the subsurface or on the soil surface, likely also played a role in the higher pollutant loadings of the first flush and first flood events. However, to evaluate the influence of antecedent dry days, at least one additional storm event, equivalent in magnitude to the first flood, should be sampled later in the same hydrologic year. In our study, storms occurring later in the rainy season did have much lower pollutant loadings, but those were also smaller volume storm events. Therefore, it is not possible to discern whether the lower pollutant loadings resulted from the smaller storms producing less interflow and runoff or due to contaminant source areas being flushed of pollutants by preceding storms.

Interestingly, the relationships between cumulative loadings per storm and either peak discharge or total stormwater volume were linear for most constituents. These results suggest that measures of peak discharge or total storm volume could be used in a predictive capacity to estimate different pollutant loadings of chemical constituents in future storms. By contrast, both *E. coli* (Figure 5-12) and enterococci data were best fit with exponential functions. These linear vs. exponential curves may reflect innate differences between chemicals and bacteria; that source areas for chemicals can be exhausted, whereas surfaces with bacterial pollution continue to be a source, even after hours or days of contact with water. Findings in Chapter 4 indicate that even after multiple flushes of wastewater-spiked soils, concentrations of fecal indicator bacteria were still measurable.

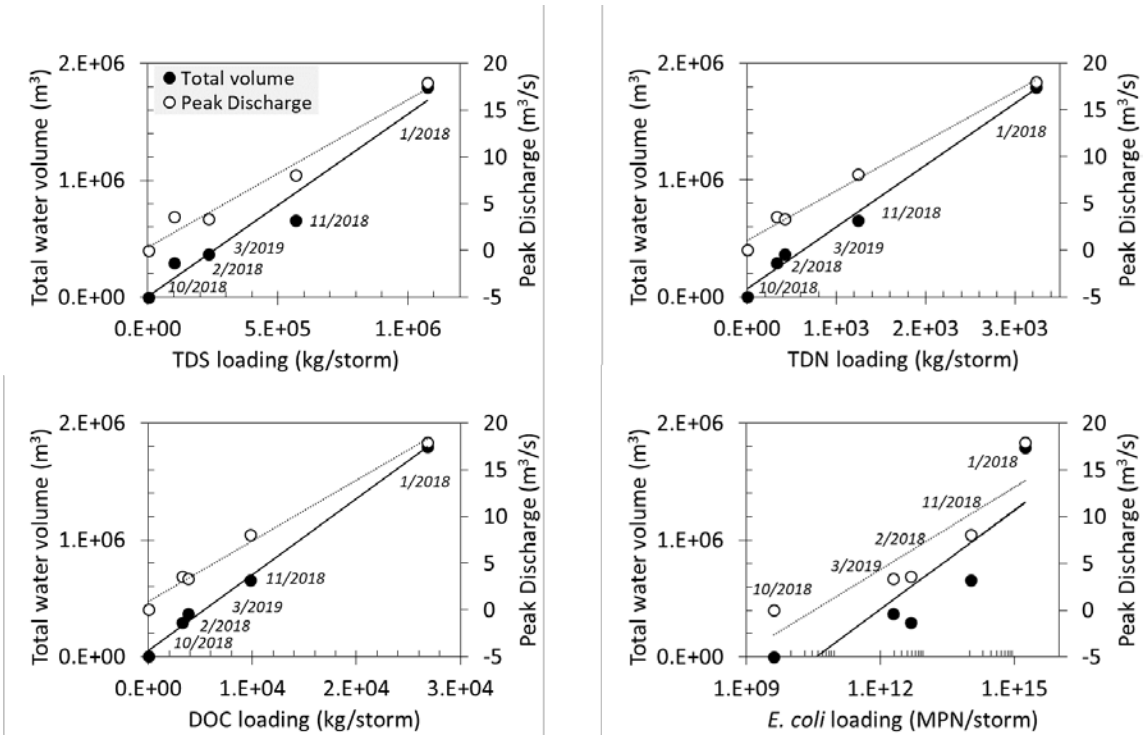

Figure 5-12. Relationships between total water volumes per storm event and peak discharges of five different storm events in the San Diego River and cumulative loadings of TDS, TDN, DOC, and *E. coli* per storm event. Linear relationships were found for TDS, TDN, and DOC loadings. *E. coli* data are plot on a natural log-scale and the relationships with total volume and peak discharge are exponential.

5.3.7 Limitations

The unpredictable nature of storm events poses a challenge to storm sampling and capturing peak concentrations and loadings of microbiological and chemical constituents, which do not necessarily reach peaks at the same time as stage or discharge. In addition, despite having sampled the San Diego River during 5 storm events and additional tributaries during some of the storms, comparisons among storms of different magnitude are difficult due to the wide changes in hydrologic conditions that occur during storms. For example, during small storms, such as the October 2018 storm, conductivity values barely changed, ranging from 3.9 mS/cm to 4.1 mS/cm, indicating that rain-fed runoff did not replace or substantially add to the groundwater-fed, brackish water already in the channel. During larger storm events, the conductivity and Cl/Br ratios of water in the channels change substantially (and more so in tributaries than in the main stem). Under such conditions, both sheetflow and interflow are underway and larger areas of the watershed can contribute water and pollutants to a stream. Although it was not a specific goal of this study, in order to better evaluate the influence of antecedent dry days and accumulation of waste that may occur over dry periods, it is important to capture the first flood event (as we

did) as well as a subsequent event of the same magnitude. Sampling storm events is riddled with many challenges, including ensuring safety, finding personnel available for analyses, often at inopportune times, and ensuring successful autosampler installation and operation. Also, committing resources to sampling a storm event is an important decision, and many considerations go into deciding which rain event to sample, especially when there is no certainty that another rain event will follow. Therefore, there will always be challenges and limitations for storm sampling and experience with the process is recommended.

Ultimately, future work will seek to distinguish different pollution sources using models that build on the multiple lines of evidence from this study and data on biological and chemical signatures of different pollution "end members." Although modeling was outside of the scope of this study, it is important to recognize assumptions and revisit them. For example, it is likely that groundwater or interflow does not transport waste exclusively from the subsurface – it is possible that fecal inputs from the soil surface could infiltrate into the vadose zone and then be transported to a stream via groundwater flow. If feces are deposited directly into a stream or physically moved into the stream by the action of runoff, then the fecal material, as an autochthonous source from within the stream, must also be considered. Although these scenarios must be considered in modeling efforts, the low caffeine/sucralose ratio of fecal material on the soil surface is not consistent with the high caffeine/sucralose ratios encountered in the river and creeks of this study, which point instead to untreated wastewater. In addition, the low or undetectable HF183 concentrations associated with open defecation sites are also not consistent with the elevated HF183 concentrations observed during most storm events. Therefore, the overall conclusion that open defecation sources of microbial pollution are minor compared to untreated wastewater sources would not change, but models would help constrain the relative contributions from these two important end member environments.

This study could be further improved with direct sampling from sanitary sewer lines that run adjacent to or cross the river and creeks (San Diego River, Alvarado Creek, Forester Creek). It is also advisable to collect soils from around known sites of sewer exfiltration (perhaps at sites with ongoing pipe replacement) and evaluate biological and chemical marker concentrations in samples from those environments.

5.4 CONCLUSIONS AND RECOMMENDATIONS

This study provides evidence that fecal contaminants of human origin were introduced to the San Diego River and its tributaries during each storm event. The results further indicate that the dominant sources of human fecal contamination were likely to change over the course of a storm event. Over the course of this study, there was evidence of pollution from untreated wastewater via sewer exfiltration and sanitary sewer overflows as well as potential contributions from open defecation sites. During peak discharge, which is also when the greatest mass flux (loading) of pollutants occurs, and during the falling limb of the hydrograph, caffeine and sucralose results suggest that there must be a recent source of human contamination, such as untreated wastewater, rather than waste that has been left in the environment for long periods of time. The greatest source of human contamination during peak discharge is therefore likely untreated wastewater, such as from sewer exfiltration or SSO events. Human fecal contamination found in

open defecation sites on the soil surface may provide a continuous, diffuse source of FIB and other pollutants over the course of storm events, but its contributions during wet weather are still not well constrained and merit further study. We recommend that replacement of cracked or failing sewer infrastructure or containment of SSOs be prioritized in efforts to meet wet weather pollution targets. Despite the potentially lesser contribution of open defecation sites to pollutant loadings in the San Diego River during storm events, waste associated with homeless encampments remains a source of fecal pollution. We recommend that improved water supply, sanitation, and hygiene facilities be provided for all individuals experiencing homelessness in California.

In terms of monitoring future pollution in the San Diego River and other urban waterways with potential sewage infrastructure concerns, it is recommended that both caffeine and sucralose be monitored due to their advantage of distinguishing fecal material on open surfaces from recently discharged, untreated wastewater. Monitoring of microbial markers, HF183 and PMMoV, which are more representative of human fecal sources than *E. coli* and enterococci, is also recommended. Attention should also be paid to large storm events, which are likely to continue to represent the largest flux of pollutants to the San Diego River. Use of rapid, in-situ sensors or other methods for tracking wastewater inputs in real time should also be considered as early warning systems for rapid response to sewage pollution events.

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