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Characterizing the chemical and microbial fingerprint of unsheltered homelessness in an urban watershed

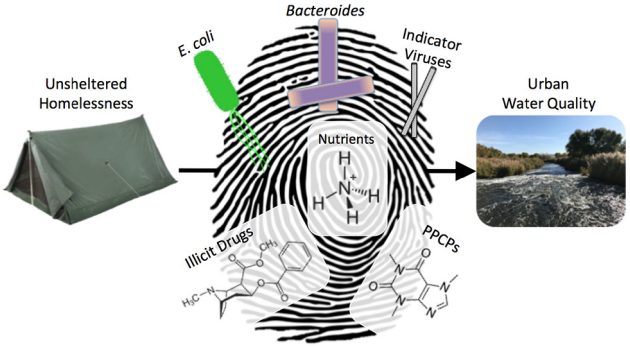
Daniel Gerrity [a,b,*], Katerina Papp [a], Eric Dickenson [a], Meena Ejjada [b], Erica Marti [b], Oscar Quinones [a], Mayra Sarria [b], Kyle Thompson [a,c], Rebecca A. Trenholm [a]

[a] Applied Research and Development Center, Southern Nevada Water Authority, P.O. Box 99954, Las Vegas, NV 89193, United States
[b] Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas, 4505 S. Maryland Parkway, P.O. Box 454015, Las Vegas, NV 89154-4015, United States
[c] Carollo Engineers, 8911 N. Capital of Texas Hwy, Austin, TX 78759, United States

HIGHLIGHTS

- Homeless density was linked to occurrence of key fecal indicators.
- Key illicit drug indicators include heroin, acetylmorphine, amphetamine, cocaine.
- Key trace organics (TOrCs) include acetaminophen, caffeine, ibuprofen, naproxen.
- Key microbial indicators include HF183, crAssphage, pepper mild mottle virus.
- Urban water quality impacts unsheltered individuals and the broader community.

GRAPHICAL ABSTRACT



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ABSTRACT

Unsheltered homelessness is rapidly becoming a critical issue in many cities worldwide. The worsening situation not only highlights the socioeconomic plight, but it also raises awareness of ancillary issues such as the potential implications for urban water quality. The objective of this study was to simultaneously leverage diverse source tracking tools to develop a chemical and microbial fingerprint describing the relative contribution of direct human inputs into Las Vegas' tributary washes. By evaluating a wide range of urban water matrices using general water quality parameters, fecal indicator bacteria (FIB), human-associated microbial markers [e.g., HF183, crAssphage, and pepper mild mottle virus (PMMoV)], 16S rRNA gene sequencing data, and concentrations of 52 anthropogenic trace organic compounds (TOrCs), this study was able to differentiate principal sources of these constituents, including contributions from unsheltered homelessness. For example, HF183 (31% vs. 0%), crAssphage (61% vs. 5%), and PMMoV (72% vs. 55%) were more frequently detected in tributary washes with higher homeless census counts vs. 'control' tributary washes. Illicit drugs or their metabolites (e.g., heroin, acetylmorphine, amphetamine, and cocaine) and select TOrCs (e.g., acetaminophen, caffeine, ibuprofen, and naproxen) were also detected more frequently and at higher concentrations in the more anthropogenically-impacted washes. These data can be used to raise awareness of the shared interests between the broader community and those who are experiencing homelessness, notably the importance of protecting environmental health and water quality. Ultimately, this may lead to more rapid adoption of proven strategies for achieving functional zero homelessness, or at least additional resources for unsheltered individuals.

* Corresponding author at: Applied Research and Development Center, Southern Nevada Water Authority, P.O. Box 99954, Las Vegas, NV 89193, United States.
E-mail address: Daniel.Gerrity@snwa.com (D. Gerrity).

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1. Introduction

Unsheltered homelessness is rapidly becoming a critical issue in many cities worldwide. In the United States, 580,000 people experienced homelessness on any given night in 2020 (HUD, 2020), and approximately 39% of those individuals were considered unsheltered. That equates to 226,000 people residing nightly "in a place not meant for human habitation, such as cars, parks, sidewalks, [or] abandoned buildings" (HUD, 2004). In many cities, unsheltered homeless individuals congregate in encampments located in or near riverbeds and flood control infrastructure. Beyond the socioeconomic plight, these encampments also have environmental and public health consequences—directly on unsheltered individuals and indirectly on the broader community—due to improper waste disposal and inadequate access to sanitation facilities (DeVuono-Powell, 2013). For example, in January 2018, Orange County (California) dismantled encampments along the Santa Ana River, displacing 700 individuals and requiring the removal of hundreds of tons of debris, thousands of pounds of human waste, and nearly 14,000 hypodermic needles (Walker, 2018).

Issues related to unsheltered homelessness are also evident in Southern Nevada. In 2020, Las Vegas had a point-in-time count of 5283 homeless individuals—66% of whom were unsheltered despite 41% of shelter beds being unoccupied on the night of the census (Help Hope Home, 2020). At the state level, Nevada actually had the second-highest unsheltered percentage in the U.S. (HUD, 2021). The 2020 point-in-time count identified 192 unsheltered individuals living in the flood control tunnels below the Las Vegas Strip (Help Hope Home, 2020), where illicit drug use, human waste, and debris are well documented (Newberg, 2020). Recently, the issue was exacerbated by the passing of local ordinances criminalizing unsheltered homelessness and by reductions in shelter capacity caused by the COVID-19 pandemic (Lyle, 2020).

Homeless individuals are particularly vulnerable to adverse health outcomes due to a combination of harsh or crowded living conditions, limited medical care, and inadequate hygiene and sanitation (Buechler et al., 2020). In 2019, the Southern Nevada Health District found that homeless individuals accounted for 80% of the hepatitis A cases confirmed during a prolonged outbreak (AP, 2019). Soon after, Vo et al. (2022) used wastewater surveillance to link COVID-19 infections within a Southern Nevada homeless shelter to a highly infectious variant of concern. Outside of Nevada, gastrointestinal infections linked to *Shigella*, *Cryptosporidium*, and norovirus have been documented among unsheltered homeless individuals (Brownstone, 2021) and hurricane evacuees in an emergency shelter (Yee et al., 2007). Beyond the spread of infectious disease, unsheltered homeless encampments in or around flood control infrastructure can result in serious injury or loss of life during flash flooding.

Considering the proximity of some unsheltered homeless encampments to urban waterways, there is potential for bidirectional transmission of chemical and microbial contaminants, including direct exposure of unsheltered individuals to etiologic agents. Accumulation of bulk litter results in the release of macro- and microplastics to the environment, which have been identified as potential transport vectors for trace organic compounds (TOrCs) and microorganisms (Galafassi et al., 2019; Teuten et al., 2009). Heavy precipitation is also a critical risk factor for diarrheal diseases among homeless individuals (Hines et al., 2018), either by exposing people to contaminated stormwater or by inducing behavioral changes (e.g., causing people to congregate in crowded shelters). Based on typical patterns of human waste production (Rose et al., 2015), unsheltered homelessness in Las Vegas (~3500 individuals nightly) could theoretically result in accumulation of ~160 metric tons of feces and 1800 m^3 of urine annually, with the potential for sudden mobilization of contaminants during storm events. This is a significant concern because one of Lake Mead's major tributaries is the Las Vegas Wash, which consists of ~90% disinfected wastewater effluent (7×10^5 m^3/day) but also all urban runoff, stormwater, and emerging groundwater from Southern Nevada. Lake Mead is the largest reservoir on the Colorado River system; a drinking water supply for 40 million people in Nevada, Arizona, California, and Mexico; and a recreational destination for 10 million people annually (Milly and Dunne, 2020).

Fecal contamination is often the principal concern for encampments along waterways. Historically, the most common indicators of fecal contamination include general water quality parameters and fecal indicator bacteria (FIB), but the global stormwater literature now encompasses a wide range of highly sensitive and source-specific markers (Ahmed et al., 2019). The toolbox includes persistent wastewater-derived TOrCs, such as the artificial sweetener sucralose (Oppenheimer et al., 2011); human-specific *Bacteroides*, including the HumM2/HumM3 (Shanks et al., 2009) and HF183 markers (Layton et al., 2013; Molina et al., 2014); and fecal indicator viruses, namely pepper mild mottle virus (Rosario et al., 2009; Symonds et al., 2018) and crAssphage (Stachler et al., 2018). In addition, genetic markers can be used to quantify overall *Bacteroidales* abundance or contributions specifically from animals (Boehm et al., 2013; Schriewer et al., 2013). Previous microbial source tracking (MST) studies identified canine (Ervin et al., 2014) and wild animal contamination (Jiang et al., 2007) as major sources of FIB to coastal waterways. Some studies observed only sporadic detection of human-specific markers (Li et al., 2019), while others found them to be ubiquitous (Cao et al., 2017). However, specific sources of human contamination [e.g., homeless encampments in Verbyla et al., 2021] have rarely been identified or definitively linked to FIB occurrence.

The objective of this study was to develop a chemical and microbial fingerprint capable of highlighting the relative contribution of direct human inputs into Las Vegas' tributary washes, particularly in the context of unsheltered homelessness (Fig. 1). A previous study eliminated septic tanks as the primary source of FIB in the Las Vegas Wash watershed and instead attributed the contamination to "indigenous wildlife, direct human waste, and urban sources that contribute through surface runoff" (Dano, 2003). The current study builds upon that prior effort by integrating culture-based methods for FIB; TOrC concentrations, including pharmaceuticals and personal care products (PCPPs), illicit drugs, and major metabolites; molecular methods for animal- and human-specific markers; and 16S rRNA gene sequencing for characterizing microbial community structure. Ultimately, this comprehensive dataset may help distinguish principal sources of contamination in other waterways, quantify contaminant loads specifically linked to unsheltered homelessness, and eventually characterize public health risks (Crank et al., 2019), both for individuals close to

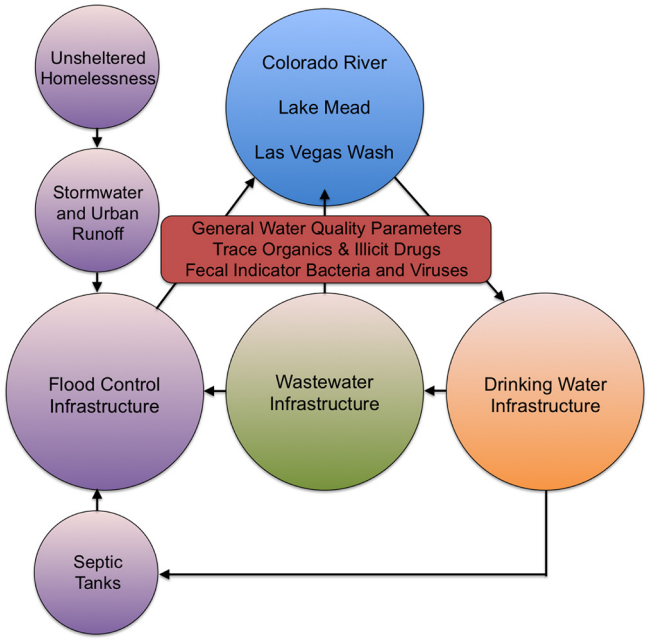

Fig. 1. Conceptual diagram of potential contaminants, sources, and interdependent infrastructure within the Southern Nevada watershed.

urban waterways and for the broader community due to the potential for local source water contamination. Highlighting the impacts of unsheltered homelessness on the broader community may help expedite wider adoption of proven strategies for achieving functional zero homelessness, including focusing on subpopulations (e.g., veterans experiencing homelessness) or alternative housing models (e.g., 'housing first'). Or at a minimum, this could help justify increased distribution of resources (e.g., trash and biowaste bags) and access to sanitation facilities (e.g., staffed restrooms and portable showers) in areas known to have high levels of unsheltered homelessness.

2. Methodology

2.1. Description of study sites and sample collection

Samples were collected from 20 locations throughout the Las Vegas Valley watershed (Fig. 2), with each site classified based on geographic location (i.e., Northern vs. Central vs. Southern tributary washes) and composition (i.e., tributary wash vs. wastewater effluent vs. effluent-impacted surface water vs. source water). Based on homeless census counts (King, 2019), the Northern and Central washes (sites 1–6) were hypothesized to be most impacted by unsheltered homelessness, particularly in comparison to the Southern 'control' washes (sites 16–18). During dry-weather conditions, the tributary washes (sites 1–6 and 16–18) carry base flows of ~1 × 10^4 m^3/day; hydrographs for sites with active streamflow gages are summarized in Fig. S1 (USGS, 2021). The tributary washes discharge into the Las Vegas Wash (sites 8–9, 12, 14–15), which is primarily composed of treated effluent from four wastewater treatment plants (WWTPs) that range in flow from 7 × 10^4 to 4 × 10^5 m^3/day (sites 7, 10–11, 13; treatment trains are summarized in Table S1). The Las Vegas Wash ultimately discharges into Las Vegas Bay (site 15) and then the Boulder Basin area of Lake Mead, which includes Boulder Beach (site 19) and Southern Nevada's drinking water intakes (site 20). Based on historical sucralose monitoring and hydrodynamic modeling, the drinking water intakes are estimated to contain ~1% treated wastewater effluent (Hannoun et al., 2021; Thompson and Dickenson, 2021).

The study consisted of seven sample events between October 2019 and April 2021: four during dry-weather conditions (10/1/2019, 11/12/2019, 10/13/2020, 4/27/2021) and three in close temporal proximity to rainfall events (3/10/2020, 1/25/2021, 3/16/2021). Notably, the study spanned the early portion of the COVID-19 pandemic, implementation of the aforementioned ordinances criminalizing unsheltered homelessness, and Southern Nevada's longest period without measurable rainfall (240 days spanning 4/20/2020–12/17/2020). During dry weather events, all 20 sites were sampled for general water quality and microbiological analyses, but only a subset of the sites were sampled for trace organic analyses: a Central tributary wash (site 5), a Southern tributary wash (site 18), all four WWTP discharges (sites 7, 10–11, 13), and two effluent-impacted confluences (sites 9 and 14). During wet-weather events, samples were collected at only three representative locations—a Central tributary wash (site 1 or 2), a Southern tributary wash (site 18), and an effluent-impacted site (site 9 or 14)—but multiple, independent samples were

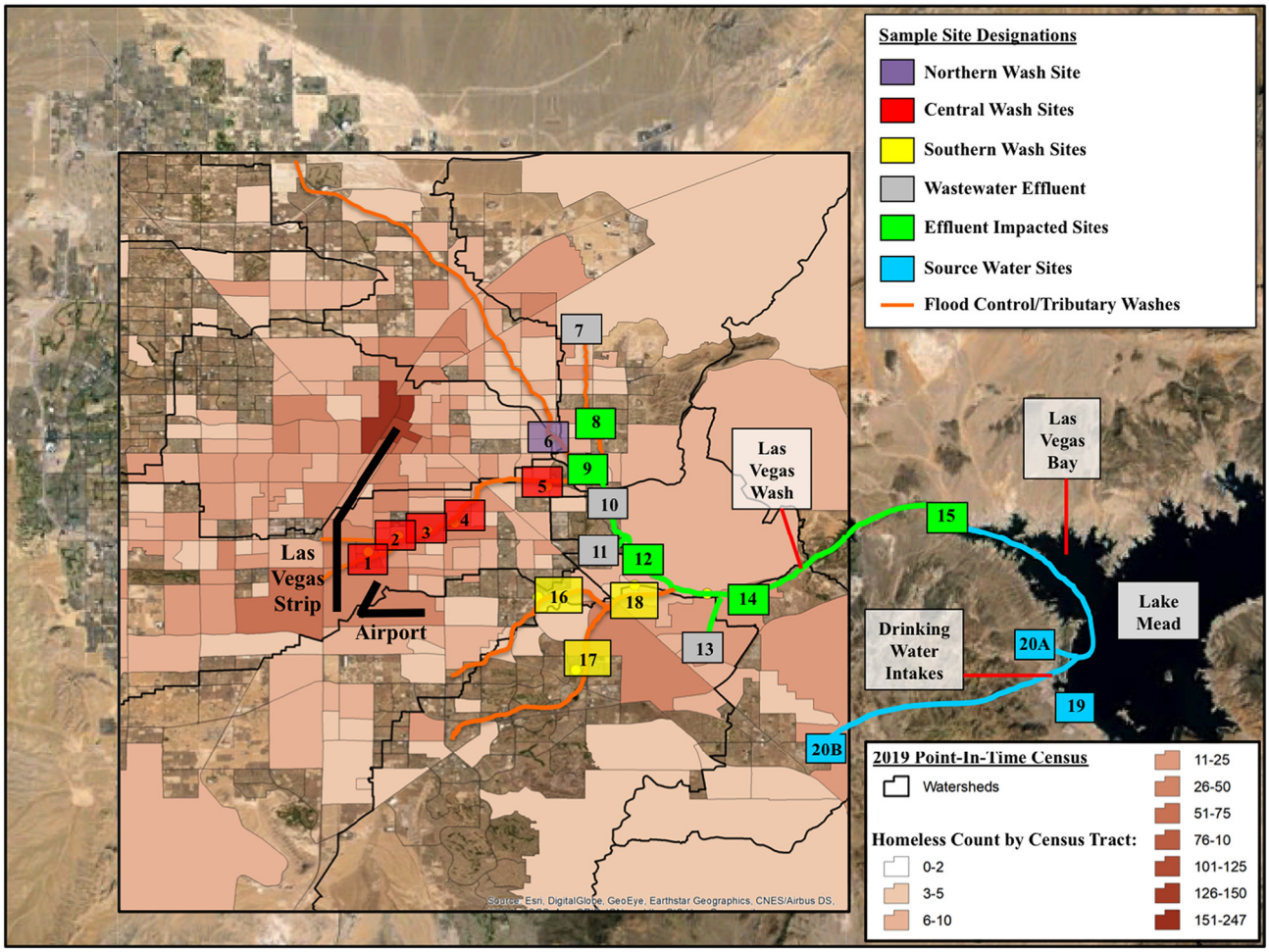

Fig. 2. Map of Southern Nevada showing sampling locations (with color-coded characterizations), watershed boundaries, and 2019 point-in-time homeless counts by census tract. Based on homeless census data, the Northern and Central wash sites were initially hypothesized to be more impacted by unsheltered homelessness than the Southern wash sites.

collected over approximately 4 h to capture temporal variability and first flush or dilution effects. After retrospective flow analysis, it was found that sampling generally occurred just before the onset of peak stormwater flows (Fig. S2), but stormwater was successfully captured at site 1 on 3/10/2020. Text S1 provides additional sampling details, including a study design summary (Table S1) and a discussion of field and laboratory blanks (no contamination observed during the study).

2.2. General water quality and culture-based fecal indicator bacteria

Chemical oxygen demand (COD) (Hach Method 8000), ammonia (Hach Method 10023), nitrite (Hach Method 10207), nitrate (Hach Method 10206), total nitrogen (Hach Method 10071), reactive phosphorus (Hach Method 10209), and total phosphorus (Hach Method 10210) were quantified at least in duplicate using a DR5000 spectrophotometer (Hach, Loveland, CO). Total dissolved solids (TDS) concentrations were measured with a Hach Pocket Pro TDS Tester, and turbidity was measured with a Hach 2100 N laboratory turbidimeter. Total organic carbon (TOC) was measured as non-purgeable organic carbon according to Standard Method 5310B using a Shimadzu TOC-V_{csh} (Kyoto, Japan) after acidification to pH < 2 with 2 N hydrochloric acid. UV_{254} absorbance was measured using an Aqualog spectrofluorometer (Horiba, Edison, NJ) after laboratory filtration with 0.7-μm syringe filters (GD/X, Whatman, Pittsburgh, PA). Total coliform (TC), *E. coli* (EC), and enterococci (ENT) were enumerated with Colilert and Enterolert, respectively, according to manufacturer's instructions (IDEXX, Westbrook, ME). FIB samples were collected in bottles supplemented with sodium thiosulfate, analyzed neat and diluted 1:200 in autoclaved tap water, and quantified with the Quanti-Tray/2000 platform. Autoclaved tap water was also used as a negative control (no contamination observed).

2.3. Trace organic compounds, illicit drugs, and metabolites

A suite of 30 TOrCs (Table S2) and 22 illicit drugs and metabolites (Table S4) was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with direct injection of aqueous samples or injection of methanol sample extracts following automated solid phase extraction (ASPE). All methods employed a CTC Autosampler (CTC Analytics, Zwingen, Switzerland) and an Agilent 1260 LC Binary Pump (Palo Alto, CA, USA). TOrC analysis was performed with SCIEX API 4000-series mass spectrometers (Redwood City, CA) using optimization processes and isotope dilution methods previously reported (Vanderford and Snyder, 2006). Data were collected in multiple reaction monitoring (MRM) mode for electrospray ionization (ESI) negative or positive compounds and their isotopically labeled analogs. For the illicit drugs and metabolites, samples were analyzed by direct injection, and analytes were monitored with positive ESI in MRM mode on a SCIEX 6500 QTRAP mass spectrometer (Redwood City, CA, USA). Additional details for all target compounds, including method reporting limits (MRLs) and MRM transitions, are included in Text S1.

2.4. Molecular markers for microbial source tracking

Ten-liter samples were concentrated through a two-step process consisting of (1) hollow fiber ultrafiltration (HFUF) (REXEED-25S, 30 kDa, Asahi Kasei Medical Co., Japan) and (2) Centricon centrifugal ultrafiltration (Centricon Plus-70, 30 kDa, Millipore Sigma, Burlington, MA, USA), based on an adaptation of Hill et al. (2005) and Papp et al. (2020). Using this approach, Hill et al. (2007) observed high recoveries (>70%) for a wide range of microbiological targets, including MS2, ϕX174, and *Enterococcus faecalis*. Additional sample processing details, including equivalent sample volumes (ESVs), are summarized in Text S1.

DNA and RNA were co-extracted from 350 μL of each Centricon pellet using a PureLink™ Viral RNA/DNA Mini Kit (ThermoFisher Scientific, Waltham, MA) according to manufacturer's instructions. Extracts were then purified with the QIAquick PCR Purification Kit (Qiagen, Germantown, MD, USA), and inhibition was assessed using an internal PCR control (QuantiFast Pathogen PCR + IC Kit, Qiagen) according to manufacturer's instructions (see Text S1 for additional details). Negative controls with Milli-Q water were included in all extractions. Complementary DNA (cDNA) was synthesized using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) and the conditions described in Text S1.

The purified nucleic acids were tested for the 8 molecular targets summarized in Table 1 (Caporaso et al., 2010; Hamza et al., 2011; Haugland et al., 2010; Kildare et al., 2007; Korajkic et al., 2020; Parada et al., 2016; Ryu et al., 2013, 2014; Stachler et al., 2018). Reactions were run in triplicate with a final volume of 10 μL: 1 μL of DNA or cDNA template, 5.0 μL of 2× iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 3.7 μL of sterile water, and 0.15 μL of each primer (Table 1) at a final concentration of 0.3 μM. Primers and gBlock gene standards were purchased from Integrated DNA Technologies (IDT, Skokie, IL, USA). All assays were run on a CFX96 or CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories) set to 95 °C for 30 s or 2 min for initial denaturation of cDNA or DNA, respectively, and then 40 cycles of denaturation at 95 °C for 5 s and annealing and extension at assay-specific temperatures for 30 s (Table 1). To account for probe

Table 1
qPCR assay summary. The 16S rRNA gene primers (515F and 806R) were also used for amplicon-based sequencing and characterization of microbial community structure. The limits of quantification (LoQs) represent averages of statistically-determined, assay-specific copy numbers divided by sample-specific equivalent sample volumes (ESVs). Additional details are provided in Text S1.

Assay	Target	Primer name	Primer sequence (5′ → 3′)	Annealing temp. (°C)	Reference	Average LoQ (log_{10} gc/L)
16S rRNA	16S rRNA gene (V4)	515F	GTGYCAGCMGCCGCGGTAA	50	Parada et al. (2016); Caporaso et al. (2010)	4.30
	Bacterial abundance	806R	GGACTACHVGGGTWTCTAAT			
GenBac3	*Bacteroides* genus	GenBactF3	GGGGTTCTGAGAGGAAGGT	65	Korajkic et al. (2020)	3.06
	General marker	GenBactR4	CCGTCATCCTTCACGCTACT			
HF183	*Bacteroides* genus	HF183_F	ATCATGAGTTCACATGTCCG	60	Haugland et al. (2010); Korajkic et al. (2020)	2.84
	Human-specific marker	BFDRev	CGTAGGAGTTTGGACCGTGT			
BacCan	*Bacteroides* genus	BacCan-545f1	GGAGCGCAGACGGGTTTT	65	Kildare et al. (2007)	3.17
	Canine-specific marker	BacUni-690r2	AATCGGAGTTCCTCGTGATATCTA			
Entero16S	16S rRNA gene	FaecalF	CGCTTCTTTCCTCCCGAGT	61	Ryu et al. (2013); Ryu et al. (2014)	3.17
	Enterococcus faecalis	FaecalR	GCCATGCGGCATAAACTG			
Camp2	*Campylobacter* genus	CampF2	CACGTGCTACAATGGCATAT	53	Ryu et al. (2014)	3.50
	Non-specific marker	CampR2	GGCTTCATGCTCTCGAGTT			
crAssphage	*Bacteroides* phage	CP_064_F	TGTATAGATGCTGCTGCAACTGTACTC	60	Stachler et al. (2018)	3.10
	φcrAssphage	CP_064_R	CGTTGTTTTCATCTTTATCTTGTCCAT			
PMMoV	Pepper mild mottle virus	PMMoV_F	GTGGCAGCAAAGGTAATGGT	55	Hamza et al. (2011)	4.44
	PMMoV	PMMoV_R	ATTTGCTTCGGTAGGCCTCT			

omission and the change to SYBR-based chemistry, the original assays from the literature were optimized by performing temperature gradient tests. Melt curves were also added to evaluate assay specificity. Changes to assay parameters and resulting assay efficiencies are summarized in Tables S6 and S7 (average assay efficiency was 94.8 ± 2.7%). Standard curves and associated melting temperature profiles are provided in Fig. S3. Limits of detection (LoDs) and quantification (LoQs) are described in Text S1, and average LoQs are summarized for each assay in Table 1 and for all samples in Table S8.

2.5. 16S rRNA gene sequencing for characterizing microbial community structure

16S rRNA gene sequencing was performed on approximately 100 representative DNA extracts to develop a robust dataset comparing the various sample locations (including field blanks) across all sample events. Frozen nucleic acid extracts were shipped to RTLGenomics (Lubbock, TX) where samples were amplified using degenerate primers targeting the V4 region of the 16S rRNA gene (Table 1). Paired-end sequences were generated with a MiSeq sequencer (Illumina, San Diego, CA) using 2 × 300 bp sequencing chemistry with a target of 10 k reads per sample. Additional details related to sequencing, data processing, taxonomy assignment, and the development of principal coordinate analyses (PCoAs) are available in RTL (2021) and Text S1. The raw sequencing data provided by RTLGenomics were deposited into NCBI's Sequence Read Archive (SRA) under BioProject PRJNA771263.

To objectively identify differentially abundant taxa, relative abundances in one sample type (e.g., wastewater effluent) versus another (e.g., Northern/Central washes) were plotted, and 'scores' were calculated using the following equation:

$$\text{Differential Abundance Score} = \left(\frac{|\text{Log } X_i - \text{Log } Y_i|}{\sqrt{2}}\right)^2 \times \left(\frac{1}{\text{Log } X_i}\right) \times \left(\frac{1}{\text{Log } Y_i}\right) \quad (1)$$

where, X_i = relative abundance of taxon *i* in sample type 1

Y_i = relative abundance of taxon *i* in sample type 2.

The first part of the equation squares the perpendicular distance from a perfect 1:1 correlation (i.e., a taxon that is equally abundant in both sample types), thereby weighting the deviation between sample types. The second and third terms then provide weighting for taxa with higher relative abundances in general.

2.6. Statistical analyses

For culture and molecular microbiological data, concentrations were log-transformed and analyzed using SPSS Statistics 28 (IBM, Armonk, NY, USA). Unless otherwise noted, left-censored data (i.e., <LoQ or <MRL) were excluded from reported average concentrations so it is important to simultaneously consider frequency of detection and average concentration when interpreting results. Statistics included Pearson correlations, Levene's test for homogeneity of variances, Welch's *t*-test, and the Games-Howell post-hoc test.

3. Results and discussion

3.1. General water quality and fecal indicator bacteria

Average TDS concentrations were expectedly lower for the source water sites (660 mg/L) but elevated in the Northern/Central washes, the WWTP discharges, and the effluent-impacted surface water (ranging from 1131 to 1877 mg/L) (Table S11). High TDS concentrations in the Southern washes (3708 mg/L) were indicative of high conductivity shallow groundwater influence (Dano, 2003). Average turbidity was similar across all site classifications (~2 NTU), with the exception of the Northern/Central washes where average turbidity ranged from ~3 NTU (site 1 excluded) to ~6 NTU (site 1 included) (Table S11). The first rainfall event resulted in mobilization of particles, sediment, and debris, which resulted in an overall average turbidity of 21 NTU for site 1. The Northern/Central washes, WWTP discharges, and effluent-impacted surface waters exhibited the highest levels of TOC (4.5–6.7 mg-C/L), COD (19–24 mg/L), and UV_{254} absorbance (0.080–0.109 cm^{-1}), while the Southern washes and source water sites were considerably lower (1.5–3.0 mg-C/L, 7–10 mg/L, and 0.028–0.051 cm^{-1}, respectively) (Table S11). Again, the first rainfall event resulted in elevated site 1 averages for TOC (11.5 ± 12.6 mg-C/L), COD (57 ± 50 mg/L), and UV_{254} absorbance (0.210 ± 0.231 cm^{-1}). These general water quality data provided an initial indication that the Northern/Central washes were likely impacted by anthropogenic contamination, particularly due to similarities with the effluent-impacted samples, while the Southern washes were influenced by shallow groundwater with high TDS but low levels of bulk organic matter.

Nitrogen and phosphorus results were mixed with no straightforward trends based on site classification (Table S11). For example, an upset in the biological wastewater treatment process at site 13 resulted in an effluent ammonia concentration of 8.5 mg-N/L on 11/12/2019, which inflated the overall average for this site (discussed later in the context of TOrC attenuation). Otherwise, the WWTP discharge data generally indicated complete nitrification and partial denitrification (i.e., low ammonia and moderate nitrate concentrations). Also, chloramine addition for quagga mussel control at one of the drinking water intakes likely explains the elevated ammonia concentration for site 20. When directly comparing the tributary washes, average ammonia (0.067 vs. 0.014 mg-N/L) and nitrite concentrations (0.046 vs. 0.016 mg-N/L) were higher in the Northern/Central washes, but nitrate was higher in the Southern washes (2.9 vs. 4.7 mg-N/L). This elevated nitrate concentration may have influenced microbial community structure, or vice versa (discussed later). Reactive and total phosphorus were about 2 to 3-fold higher in the Northern/Central washes than in the Southern washes.

FIB were ubiquitous in the Northern, Central, and Southern washes (except for site 17 in the south) and along the effluent-impacted Las Vegas Wash (Table 2). Concentrations were considerably lower or non-detect in the disinfected WWTP discharges and source waters. The Welch ANOVA indicated that TC, EC, and ENT concentrations exhibited statistically significant differences between the various site characterizations ($p < 0.001$). Importantly, the Central washes exhibited significantly higher concentrations for all FIB in comparison with the Southern washes ($p_{TC} = 0.015$, $p_{EC} < 0.001$, $p_{ENT} < 0.001$), effluent-impacted Las Vegas Wash ($p < 0.001$ for all), disinfected WWTP discharges ($p < 0.001$ for all), and source waters ($p_{TC} = 0.008$, $p_{EC} < 0.001$, $p_{ENT} < 0.001$). However, the Northern and Central washes were not significantly different from each other for any of the FIB ($p_{TC} = 0.999$, $p_{EC} = 0.081$, $p_{ENT} = 0.667$), hence these washes were combined for subsequent water quality comparisons.

FIB concentrations in the effluent-impacted Las Vegas Wash were considerably higher than those observed in the disinfected WWTP discharges, thereby suggesting that the tributary washes contributed a majority of the FIB loading. In fact, the Northern/Central washes contributed average relative loadings of 59%, 80%, and 59% for TC, EC, and ENT, respectively, across the dry-weather sample events. As noted earlier, Dano (2003) eliminated septic tanks as the primary source of FIB along the Northern/Central corridor and hypothesized that direct human inputs might be a significant source of the observed contamination. The WWTP discharges contributed 26%, 16%, and 18% for TC, EC, and ENT, respectively, which was due to relatively low FIB concentrations but high flow rates. Finally, the Southern washes contributed only 15%, 4%, and 23%, respectively. A complete mass balance was not possible without additional sampling/experiments to characterize the effects of environmental growth/die-off and additional downstream inputs from the surrounding environment.

Table 2
Culture-based concentrations of fecal indicator bacteria (log_{10} MPN/100 mL) for the 20 sample locations. All data represent averages ±1 standard deviation, with the number of sample events for each site indicated by N. A concentration of 0.5 MPN/100 mL (−0.3 log_{10} MPN/100 mL) was substituted for all left-censored data.

Classification	Sample location	Total coliform	*E. coli*	Enterococci
Northern	Average (*N* = 4)	4.35 ± 0.66	2.40 ± 0.43	3.49 ± 0.26
Central	1 (N = 8)	4.37 ± 0.62	3.21 ± 0.90	4.05 ± 1.01
	2 (N = 12)	4.48 ± 0.33	3.43 ± 0.49	3.70 ± 0.56
	3 (*N* = 4)	4.50 ± 0.86	3.32 ± 0.74	3.99 ± 0.96
	4 (*N* = 4)	4.61 ± 0.62	3.68 ± 0.57	3.86 ± 0.80
	5 (N = 4)	4.46 ± 0.83	2.76 ± 0.39	3.18 ± 0.67
	Average (N = 32)	4.47 ± 0.55	3.31 ± 0.66	3.77 ± 0.78
Southern	16 (*N* = 4)	4.56 ± 0.57	1.77 ± 0.36	2.93 ± 0.50
	17 (N = 4)	1.62 ± 0.95	−0.10 ± 0.40	0.20 ± 0.66
	18 (*N* = 12)	3.13 ± 1.59	1.38 ± 0.94	2.33 ± 0.96
	Average (*N* = 20)	3.11 ± 1.60	1.16 ± 1.00	2.02 ± 1.25
WWTP effluent	7 (*N* = 4)	−0.23 ± 0.15	−0.30 ± 0.00	−0.30 ± 0.00
	10 (N = 4)	3.10 ± 0.42	−0.23 ± 0.15	1.67 ± 0.52
	11 (N = 4)	2.31 ± 0.33	0.37 ± 0.47	0.25 ± 0.63
	13 (N = 4)	2.14 ± 0.36	0.58 ± 0.73	1.89 ± 0.50
	Average (*N* = 16)	1.83 ± 1.32	0.11 ± 0.56	0.88 ± 1.05
Effluent impacted	8 (N = 4)	4.01 ± 1.11	1.91 ± 0.53	3.36 ± 0.75
	9 (N = 12)	3.62 ± 0.84	1.90 ± 0.85	2.53 ± 0.98
	12 (N = 4)	4.10 ± 0.29	1.91 ± 0.34	3.27 ± 0.41
	14 (*N* = 8)	3.23 ± 0.52	1.80 ± 0.42	2.42 ± 0.83
	15 (N = 4)	3.95 ± 0.14	1.48 ± 0.81	2.26 ± 0.83
	Average (*N* = 32)	3.67 ± 0.74	1.82 ± 0.65	2.67 ± 0.89
Source water	19 (N = 4)	2.28 ± 1.26	0.42 ± 1.25	0.62 ± 1.23
	20 (N = 4)	0.47 ± 1.54	−0.30 ± 0.00	0.41 ± 1.43
	Average (*N* = 8)	1.38 ± 1.63	0.06 ± 0.90	0.19 ± 1.03

3.2. Trace organic compounds (TOrCs), illicit drugs, and metabolites

The discharge of treated wastewater effluent is a major pathway for the introduction of TOrCs into environmental waters and some drinking water supplies (Nguyen et al., 2018). Occurrence of these compounds can be traced to the feces and urine of individuals on medication; through consumer, recreational, or even illegal use; and through disposal of unused quantities. The concentrations of these compounds vary widely (i.e., low ng/L to nearly 1 mg/L) depending on frequency of use, typical prescription or recreational dosing, human metabolism, and biophysicochemical stability. Although not specifically designed for this purpose, WWTPs are effective in reducing the concentrations of many TOrCs, specifically during secondary biological wastewater treatment (Achermann et al., 2018; Gerrity et al., 2013), but numerous compounds remain detectable in treated effluent. Therefore, some TOrCs, including artificial sweeteners, anti-convulsants, and even some illicit drugs (Gerrity et al., 2011a, 2011b; Oppenheimer et al., 2011), serve as valuable indicators of anthropogenic contamination. Past indicator and source tracking studies have primarily focused on municipal wastewater systems so it is unclear whether these same compounds are representative of contamination originating from homeless encampments, considering the presumably different consumption patterns, access to medically-supervised use, and degree of natural or engineered water treatment.

The target compound list for this study included 52 TOrCs [including pharmaceuticals and personal care products (PPCPs), illicit drugs, metabolites, and industrial compounds] that were used to differentiate sites based on levels of anthropogenic contamination and natural or engineered treatment. We were specifically interested in contrasting the WWTP discharges, the Central washes, and the Southern washes. Therefore, target compound concentrations (Tables S12-S18) were grouped based on these site classifications to identify indicator compounds that could differentiate sources of contamination based on their abundance or frequency of detection. This was accomplished by providing a visualization of 'log_{10} fold changes' (or log_{10}FC) to compare average concentrations between sites (Figs. 3 and S4). Most target compounds were detected at reportable concentrations in at least one sample, except for ampicillin, lisinopril (excluded after sample event 2), 3,4-methylenedioxyamphetamine (MDA), delta-9-tetrahydrocannabinol (THC), and triclocarban.

The data supported the initial hypothesis that the WWTP discharges and Central washes exhibited higher levels of anthropogenic activity than the Southern washes. For example, amphetamine was the only compound detected at a higher relative concentration in the Southern wash vs. the WWTP discharges, and this resulted from only a single sample during the study. Only a few compounds, including carbamazepine and its metabolite carbamazepine epoxide, carisoprodol, meprobamate, primidone, sucralose, and sulfamethoxazole, were detected at higher relative concentrations or with greater frequency in the Southern vs. Central washes. These particular compounds are notoriously persistent and as a result serve as classical indicators of wastewater influence, including from WWTP discharges (Gerrity et al., 2011a, 2011b; Oppenheimer et al., 2011) and septic tanks (Schaider et al., 2016). This TOrC profile, coupled with higher TDS concentrations and a higher nitrate:ammonia ratio (i.e., some degree of biological conversion), suggests that the Southern washes may actually be influenced by septic tank leachate mixing with high salinity shallow groundwater (Fig. S5), as there are no WWTP discharges in this area. This contrasts with the earlier Dano (2003) study that eliminated septic tanks as a major source of contamination, but it is possible that septic tanks might contribute more mobile contaminants (e.g., TOrCs) but not necessarily FIB.

On the other hand, TOrC profiling within the Central washes supported the main conclusion from Dano (2003), namely that direct human inputs were a principle source of contamination (Tables S12-S18 and Fig. 3). For example, relative to the WWTP discharges, the Central wash profile was dominated by drugs of abuse and their metabolites, including methamphetamine and amphetamine; cocaine, benzoylecgonine, ecgonine methyl ester, and norcocaine; and heroin, acetylmorphine, and morphine. Moreover, heroin and acetylmorphine were detected multiple times and only in the Central washes. Opioids often associated with prescribed, legal medical use (e.g., codeine, hydrocodone, norfentanyl, oxycodone, and tramadol) or treatment of drug addiction (e.g., methadone and its principal metabolite EDDP) were nearly ubiquitous in the WWTP discharges (except for site 10), but they were generally absent from the Central washes, suggesting more controlled distribution and use were driving the municipal wastewater concentrations. Compounds that are ubiquitously consumed but significantly attenuated during wastewater treatment [e.g., acetaminophen and caffeine (Rosal et al., 2010)] also dominated in the Central washes, further supporting the direct input theory. Several other common compounds, including naproxen, ibuprofen, sucralose, sulfamethoxazole, and the flame retardant TCEP, were frequently detected in the Central washes but at lower average concentrations relative to the WWTP discharges. Therefore, the Central washes demonstrated similarities with treated wastewater in terms of the detection of commonly used TOrCs, but there were also distinguishing features, notably the detection of drugs of abuse and relevant metabolites that suggest more direct human contamination (e.g., from unsheltered individuals).

Despite the limited number of samples from site 1 during the first rainfall event, there were still significant correlations between general water quality parameters and TOrC concentrations, specifically in the context of stormwater mobilization. For example, higher levels of TOC and COD were correlated with higher levels of ammonia, ibuprofen, and caffeine ($p < 0.05$), and higher levels of EC and/or ENT were correlated with higher levels of benzoylecgonine and sucralose ($p < 0.05$). Unfortunately, there is no streamflow gage located at site 1 specifically so it was not possible to directly correlate these parameters with flow. However, increases in TOC, COD, and FIB may be suitable surrogates for stormwater flow, thereby illustrating how rainfall events can lead to the mobilization of anthropogenic contamination in the Las Vegas Valley watershed.

Beyond their role in elucidating sources of contamination, the TOrC data also highlighted their value as treatment process indicators. For

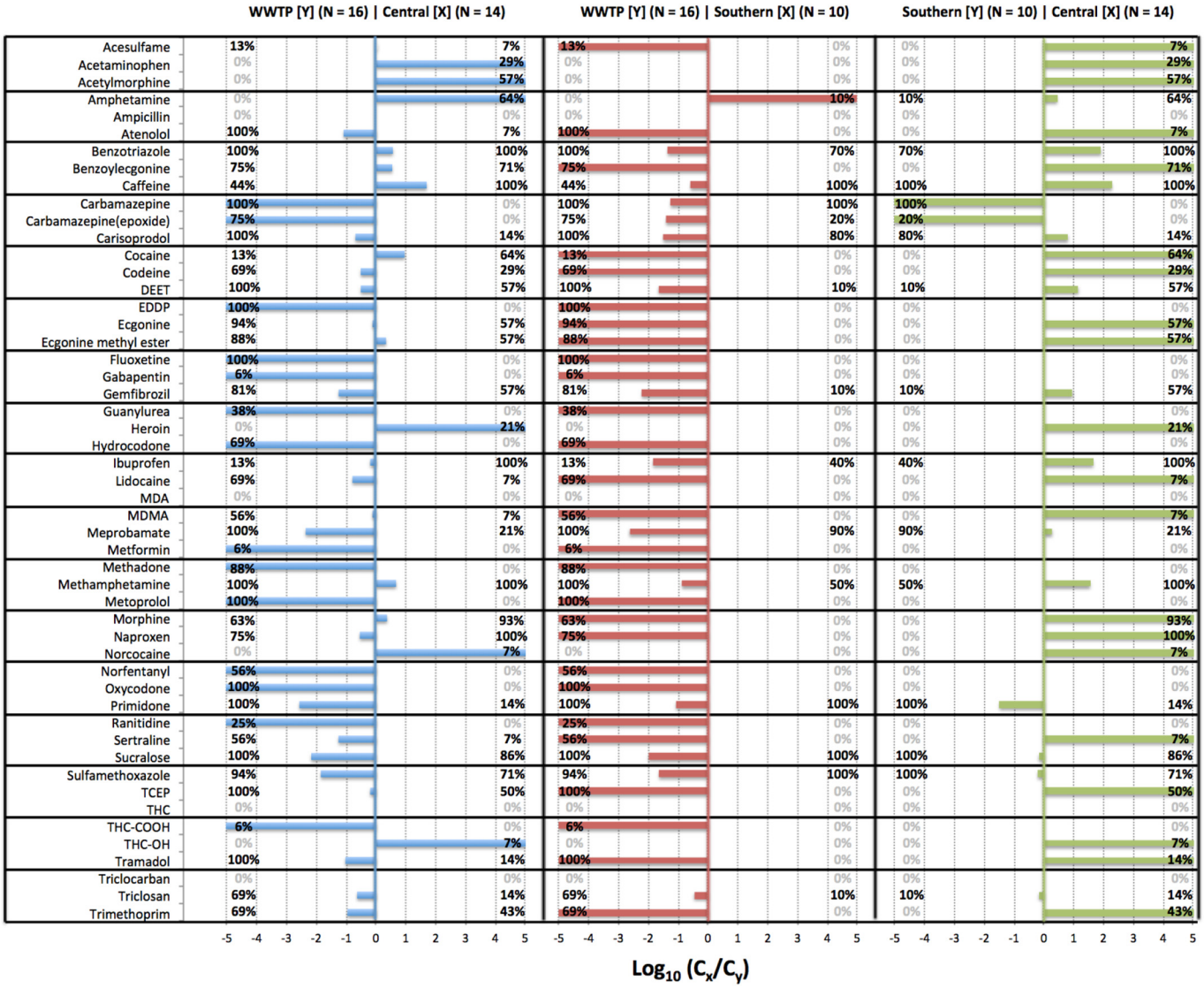

Fig. 3. Log_{10} fold changes (or $log_{10}FC$) for trace organics, illicit drugs, and metabolites. **(Left)** Wastewater treatment plant (WWTP) effluent vs. Central washes, **(Middle)** WWTP effluent vs. Southern washes, and **(Right)** Southern washes vs. Central washes. Bars indicate the direction and magnitude of site dominance for each compound. $Log_{10}FC$ values were determined from average concentrations across all samples for a given site classification. Left-censored data (i.e., <MRL) were excluded from the calculation, but the degree of bias is indicated by the frequencies of detection (percentages) across the indicated number of samples (N). $Log_{10}FC$ values of −5 or 5 indicate that only one site for the comparison in question had a sample detected at a reportable concentration.

example, an upset in the secondary biological treatment process at site 13 hindered nitrification during sample event 2. This was obviously reflected in the effluent ammonia concentration of 8.7 mg-N/L (<MDL in the other three sample events), but it was also reflected in the effluent concentrations of the highly biodegradable indicator TOrCs (Table S13). Caffeine (270 ng/L), ibuprofen (850 ng/L), and naproxen (2200 ng/L) concentrations were all elevated during sample event 2 but were < 100 ng/L in the other three sample events, with ibuprofen <20 ng/L. This sample event also resulted in the lone detections of gabapentin and metformin. This supports the conclusion that certain compounds can be useful indicators of human contamination when coupled with reduced (i.e., operational upset) or nonexistent (i.e., direct human input) treatment. The earlier $log_{10}FC$ comparison identified caffeine—but not ibuprofen and naproxen—as being differentially abundant in the Central washes. However, ibuprofen and naproxen were disproportionately impacted by this operational upset, artificially elevating the average WWTP discharge concentrations. Therefore, in the absence of an operational upset, all of these commonly used, biodegradable compounds would be highlighted in the $log_{10}FC$ comparison as being differentially abundant in the Central washes (Fig. S4). Nevertheless, they were still detected more frequently in the Central washes, highlighting their value as indicators of direct human inputs. With respect to the illicit drugs, the operational upset resulted in an elevated concentration of methamphetamine (1900 ng/L vs. <100 ng/L in all other WWTP discharges in the study), which is one of the more persistent illicit drugs (Gerrity et al., 2011a, 2011b), and even resulted in the lone detection of THC-COOH at 230 ng/L (<MRL in all other samples in the study). The effects of the operational upset were also detected immediately downstream in the Las Vegas Wash (site 14) in terms of elevated methamphetamine and naproxen concentrations.

3.3. Molecular markers for microbial source tracking

Following use of the QIAquick PCR Purification Kit for all samples, inhibition was observed in only one of 59 DNA extracts spiked with the internal PCR control (IPC) (Table S10). Coincidentally, that was also one of only two samples that had no amplification for any of the qPCR assays

after purification (one sample each from effluent-impacted site 15 and source water site 19). Those two samples were subsequently omitted from the study. The fact that the IPC highlighted the problematic sample from Site 15 indicates that successful amplification of the 16S rRNA gene assay served as a reasonable surrogate for assessing inhibition in the purified sample extracts. This provides confidence in the remaining samples for which the IPC control was not assessed.

BacCan (i.e., Canine-specific *Bacteroides*) and *E. faecalis* were either <LoQ or non-detect in all samples and were omitted from subsequent analyses. The molecular *E. faecalis* data seemingly conflict with the culture-based Enterolert data that indicated relatively high concentrations of enterococci in most samples. This may be partially due to inadequate sensitivity of the qPCR assay, considering that the average LoQ for *E. faecalis* across all samples was ~3.2 $\log_{10}$ gc/L (or 2.2 $\log_{10}$ gc/100 mL), but the Enterolert data consistently exceeded this concentration at multiple sites (Table 2). A more likely explanation is that the Enterolert method encompasses a wider range of species (*faecalis*, *faecium*, *avium*, *gallinarum*, *casseliflavis*, and *durans*) that were not captured by the *faecalis*-specific qPCR assay.

The 16S rRNA gene marker was the most abundant at all sites (7–8 $\log_{10}$ gc/L; 100% detection frequency) followed by PMMoV (4–6 $\log_{10}$ gc/L; 43–77% detection frequency), *Campylobacter* (4–5 $\log_{10}$ gc/L; 81–100% detection frequency), crAssphage (4 $\log_{10}$ gc/L; 5–61% detection frequency), and general (3–5 $\log_{10}$ gc/L; 43–78% detection frequency) and human-specific *Bacteroides* (3 $\log_{10}$ gc/L; 0–31% detection frequency) (Fig. 4). Interestingly, the 16S rRNA gene was most abundant at the source water sites, but the source water sites generally exhibited the lowest concentrations and/or detection frequencies for the other markers. The fact that there were any detections of HF183, PMMoV, and crAssphage at the source water sites might be linked to the fact that Southern Nevada's source water is ~1% treated wastewater. HF183 and PMMoV were comparably low in the Southern washes.

For the non-human-specific markers (16S rRNA gene, GenBac3, Camp2), the Northern/Central washes, Southern washes, and effluent-impacted sites exhibited similarly high concentrations and detection frequencies (Fig. 4). But with respect to the more human-associated markers, HF183 was never detected in the Southern washes, crAssphage was detected only once in the Southern washes, and PMMoV concentrations were significantly lower than in the Northern/Central washes and effluent-impacted samples ($p < 0.001$). These data suggest that the high levels of *Bacteroides* and *Campylobacter* in the Southern washes were originating from animal sources. The *Campylobacter* target is not specific to any particular source, but it is possible that wild birds may have been a significant contributing factor (Ryu et al., 2014). PMMoV, which is known to be a persistent molecular marker (Papp et al., 2020), was detected in 55% of Southern wash samples, which might be another indicator of minor septic tank influences.

Concentrations for all molecular markers in the Northern/Central washes and effluent-impacted samples were statistically similar ($p > 0.05$). While human markers are expected in effluent-impacted samples, the aforementioned TOrC profiles coupled with high concentrations and detection frequencies of HF183, crAssphage, and PMMoV provide evidence of *direct* human inputs in the Northern/Central washes. In fact, the Northern/Central washes exhibited the highest detection frequencies for HF183 (31%) and crAssphage (61%) and the second highest for PMMoV (72%).

It is important to highlight that the average concentrations presented here are based only on samples that were >LoQ—and in some cases only a single sample. This censored data approach certainly skews the averages higher for sites with low detection frequency (e.g., the Southern washes) so it is important that these averages not be used directly in quantitative microbial risk assessments. However, by simultaneously considering the frequencies of detection and average concentrations, these results can still be useful in characterizing the microbial fingerprint of specific sources (e.g., unsheltered homelessness).

3.4. 16S rRNA gene sequencing for characterizing microbial community structure

The sequencing data proved to be useful for general characterization of microbial community structure based on site classification but not

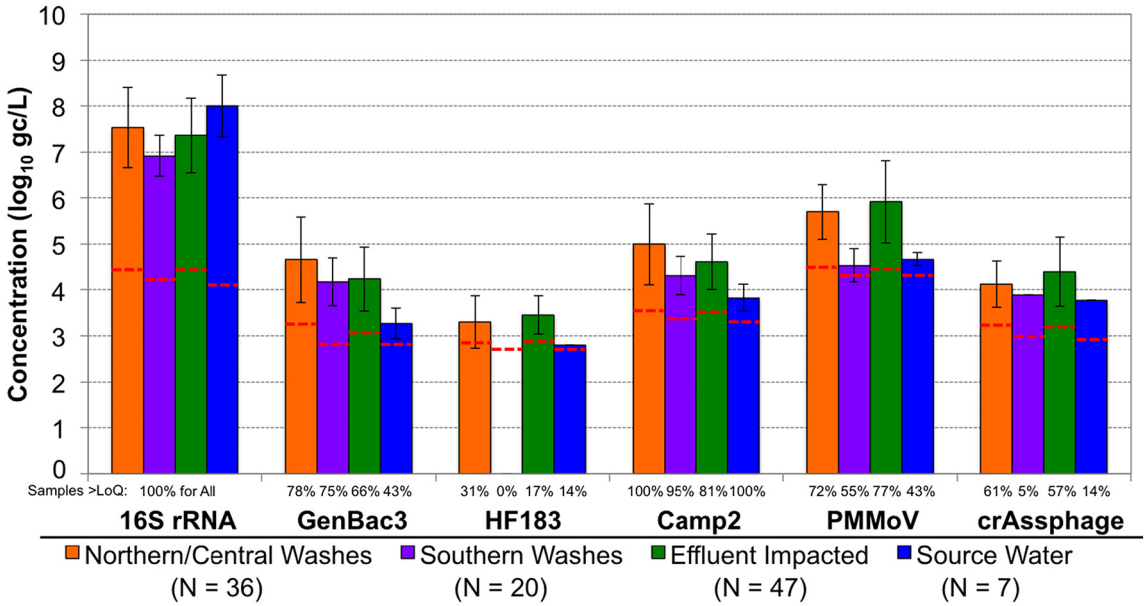

Fig. 4. Gene copy concentrations ($\log_{10}$ gc/L) for overall bacterial abundance (16S rRNA gene), general *Bacteroides* (GenBac3), human-specific *Bacteroides* (HF183), *Campylobacter* (Camp2), pepper mild mottle virus (PMMoV), and crAssphage grouped by site classification: Northern/Central washes (Sites 1–6), Southern washes (Sites 16–18), effluent-impacted sites (Sites 7–15), and source water sites (Sites 19–20). Concentrations represent averages (±1 standard deviation) across all samples for a given site classification; values <LoQ were excluded from the calculation. Percentages listed below each bar indicate the frequency of detection (i.e., >LoQ) relative to the total number of samples (N). Dashed red lines indicate average LoQs (see Table S8), which varied by assay (see Table S6) and sample due to differences in equivalent sample volume (see Table S5).

necessarily for developing a microbial fingerprint for anthropogenic contamination or unsheltered homelessness. With respect to beta diversity, PCoAs exhibited loose clustering for the Northern/Central washes and many of the effluent-impacted samples (Fig. S6). The Southern washes sometimes clustered with the Northern/Central washes and effluent-impacted samples, particularly for the abundance-weighted PCoAs, but they also demonstrated some dissimilarity as well. In particular, site 17 in the south actually clustered more closely with the field blanks and/or source water samples, which was perhaps linked to its low bulk organic matter content and shallow groundwater influence. Another notable feature of the PCoAs was the clustering of WWTP discharges based on treatment. The chlorinated WWTP discharges (sites 7 and 10) clustered with the field blanks, source waters, and/or site 17 for the weighted/non-phylogenetic (i.e., Bray-Curtis) and presence/absence (i.e., Jaccard) PCoAs. The UV-disinfected WWTP discharges (sites 11 and 13) generally clustered with the Northern/Central washes and wastewater-impacted surface water. This suggests that typical UV doses for disinfection are effective for microbial inactivation, but the resulting genome damage may not be distinguishable with high-level genomics tools.

The phylum-level taxa barplot (Fig. S7) supported many of the visual observations from the PCoAs. For example, the phylum composition at Site 17 exhibited clear dissimilarity from the other Southern wash sites, with lower levels of Bacteroidetes and higher levels of Thaumarchaeota, Omnitrophicaeota, and Nanoarchaeota. Among wastewater-impacted sites, the WWTP discharge from site 7 exhibited noticeably higher relative abundance of Epsilonbacteria and Firmicutes. As expected, the source water sites and negative controls exhibited unique profiles relative to the more impaired locations, and the negative controls were dominated by many of the 'contaminating genera' noted in other published studies (Salter et al., 2014).

To provide a more objective analysis, relative abundance data were plotted based on site classification, and the aforementioned scoring system (Eq. (1)) was used to identify differentially abundant taxa (Fig. 5 and Table S19). A direct comparison of the Northern/Central vs. Southern washes revealed that the Southern wash samples contained a larger number of differentially abundant taxa (Table S19). Many of those taxa were linked to nitrogen cycling (e.g., Nitrospirae and Nitrospinae at the phylum level), which was perhaps related to the higher levels of nitrate detected in the Southern washes. In that same comparison, the Northern/Central washes were dominated by Proteobacteria at the phylum level and *Flectobacillus*, *Pseudarcicella*, Sporichthyaceae, and Burkholderiaceae at lower taxonomic levels. In a direct comparison of the Northern/Central washes and WWTP discharges, the differentially abundant taxa in the washes included Bacteroidetes and Actinobacteria at the phylum level and 13 of the top 20-scoring taxa at the genus level (Table S19). Bacteroidetes, Proteobacteria, and Actinobacteria comprise a large percentage of the human gut microbiota (Rinninella et al., 2019), potentially offering evidence of fecal contamination. However, even Bacteroidetes is known to be ubiquitous in the environment (Thomas et al., 2011), thus it is not possible to attribute their differential abundance in the Northern/Central washes to fecal contamination and direct human inputs specifically.

Considering its significance as a fecal indicator, it was also important to evaluate differential abundance of the genus *Bacteroides*. In a

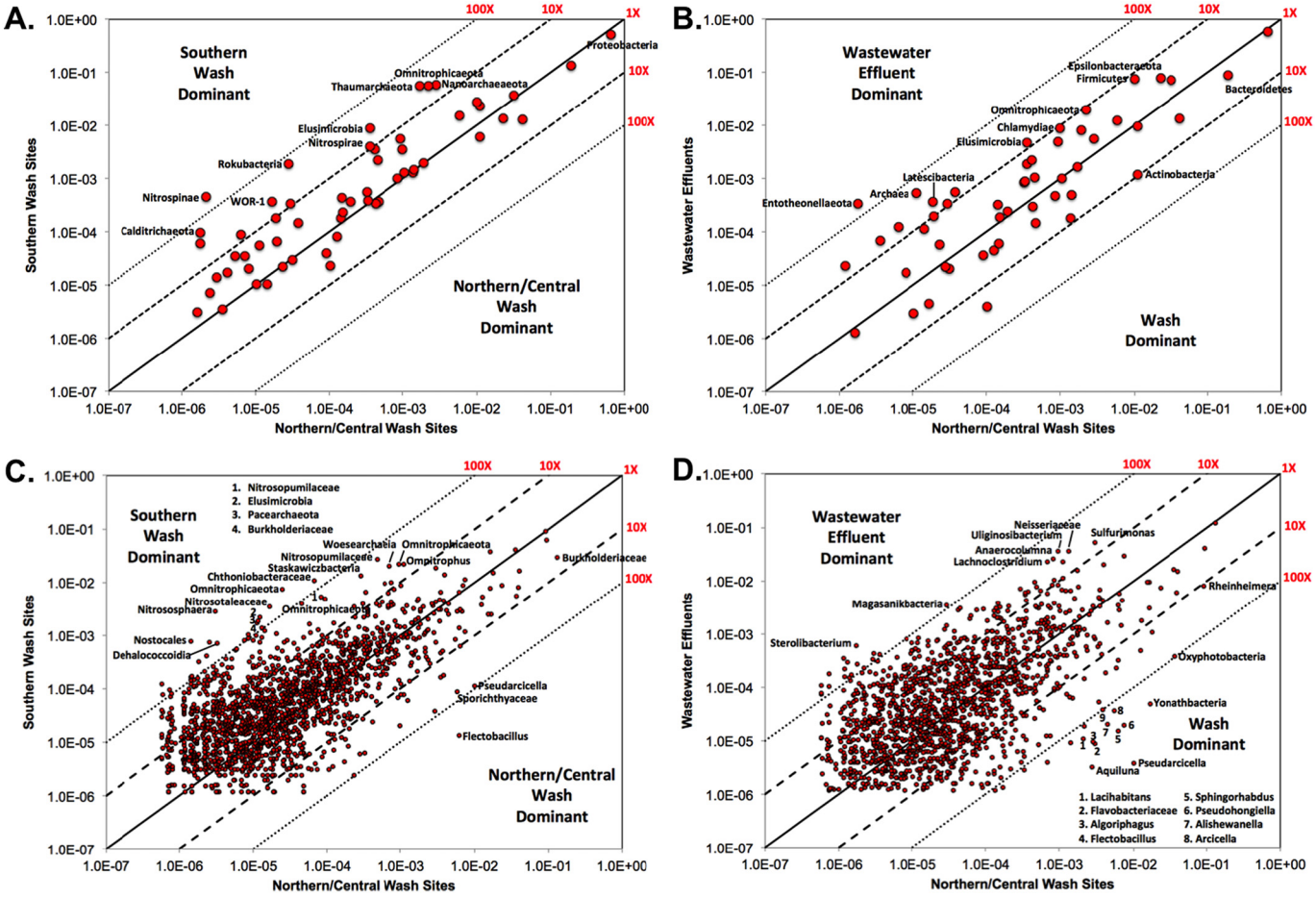

Fig. 5. Comparison of taxon-specific relative abundances by sample location (**left:** Southern washes vs. Northern/Central washes and **right:** wastewater effluent vs. Northern/Central washes) at the **(top)** phylum-level and **(bottom)** genus-level. The taxa labels refer to the top 10 phyla and top 20 genera identified by the differential abundance scoring system (see Eq. (1)). A summary of the taxa and scores is provided in Table S19.

scored comparison of Northern/Central washes vs. WWTP discharges, *Bacteroides* ranked 48th out of 3400 genus-level OTUs, with a relative abundance of 0.63% in the washes (100% hit frequency) and 0.03% in the discharges (75 % hit frequency). *Bacteroides* is known to be outcompeted in secondary biological wastewater treatment systems (Gerrity and Neyestani, 2019), which likely explains its low relative prevalence in the discharges. *Bacteroides* was also abundant in the Southern washes (0.64 % relative abundance and 75 % hit frequency), but it had a low differential abundance score in the Northern/Central vs. Southern wash comparison due to their similar relative abundances. The *Bacteroides* sequencing data support the earlier GenBac3 concentrations (Fig. 4), but similar to the qPCR assay, the lack of host specificity makes it impossible to differentiate human vs. animal sources, hence the importance of incorporating qPCR assays targeting source-specific markers such as HF183.

4. Conclusions

Unsheltered homelessness is a severe issue facing major cities throughout the world, and the COVID-19 pandemic only exacerbated its symptoms and underlying drivers, particularly in the U.S. However, the pandemic also raised awareness of this socioeconomic issue, potentially offering an opportunity to allocate resources and adopt innovative solutions with recent track records of success. Allocating more resources to this issue would simultaneously alleviate some of the environmental impacts associated with unsheltered homelessness, namely pollution of urban waterways. To this end, the main objective of this study was to leverage a suite of source tracking techniques to highlight the impacts of unsheltered homelessness on urban water quality.

This study revealed there are certain chemical and microbiological markers that are valuable in detecting anthropogenic contamination, but distinguishing the fingerprints of specific sources (e.g., unsheltered homelessness vs. septic tanks vs. wastewater effluent discharges) requires a holistic approach encompassing a range of targets. Using point-in-time counts as a proxy for unsheltered homelessness, this study highlighted an anthropogenic fingerprint consisting of fecal indicator bacteria and viruses (e.g., PMMoV and crAssphage), human-specific bacterial markers (e.g., HF183), and certain TOrCs (e.g., acetaminophen, caffeine, ibuprofen, and naproxen), illicit drugs (e.g., heroin), and their metabolites (e.g., acetylmorphine). Particularly for the TOrCs, it is important to differentiate compounds based on their use class, susceptibility to treatment, and relative concentrations to increase confidence in source determinations. Assessing microbial community structure can identify differentially abundant taxa that may be linked to fecal contamination, but 16S rRNA gene sequencing may not provide adequate resolution or specificity to inform source tracking efforts.

Future studies can use this information as a starting point to quantify associated public health risks from direct exposure to contaminated urban water (e.g., unsheltered individuals) or indirect exposure through downstream contamination of recreational or drinking water supplies. The desired outcome from this effort would be raising awareness of the shared interest of the broader community and those who are experiencing homelessness as a means of justifying additional resources to combat this pervasive challenge.

CRediT authorship contribution statement

Daniel Gerrity: Conceptualization, Funding acquisition, Project administration, Supervision, Formal analysis, Writing – original draft. **Katerina Papp:** Methodology, Data curation, Writing – review & editing. **Eric Dickenson:** Funding acquisition, Supervision, Writing – review & editing. **Meena Ejjada:** Data curation. **Erica Marti:** Funding acquisition, Supervision. **Oscar Quinones:** Methodology, Data curation, Writing – original draft. **Mayra Sarria:** Methodology, Data curation. **Kyle Thompson:** Methodology, Writing – review & editing. **Rebecca A. Trenholm:** Methodology, Data curation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156714.

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