Wastewater Compounds in Urban Shallow Groundwater Wells Correspond to Exfiltration
Probabilities of Nearby Sewers

Do Gyun Lee¹, Patrick R. Roehrdanz¹, Marina Feraud¹, Jared Ervin¹, Tarun Anumol², Ai Jia²,
Minkyu Park², Carlos Tamez³, Erving W. Morelius³, Jorge L. Gardea-Torresdey³, John Izbicki⁴,
Jay C. Means¹, Shane A. Snyder², Patricia A. Holden^{1*}

¹ Bren School of Environmental Science & Management and Earth Research Institute, University
of California, Santa Barbara, California 93106, United States

² Department of Chemical and Environmental Engineering, University of Arizona, Tucson,
Arizona 85721, United States

³ Department of Chemistry and Environmental Science & Engineering PhD Program, The
University of Texas at El Paso, El Paso, TX

⁴ U.S. Geological Survey, California Water Science Center, 4165 Spruance Road, San Diego, CA
92123, USA

*Corresponding author: Patricia A. Holden

*E-mail: holden@bren.ucsb.edu; phone: +1 805 893 3195; fax: +1 805 893 7612.

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Abstract

Wastewater compounds are frequently detected in urban shallow groundwater. Sources include sewage or reclaimed wastewater, but origins are often unknown. In a prior study, wastewater compounds were quantified in waters sampled from shallow groundwater wells in a small coastal California city. Here, we resampled those wells and expanded sample analyses to include sewage- or reclaimed water-specific indicators, i.e. pharmaceutical and personal care product chemicals or disinfection byproducts. Also, we developed a geographic information system (GIS)-based model of sanitary sewer exfiltration probability—combining a published pipe leakage model accounting for sewer pipe size, age, materials of construction, with interpolated depths to groundwater—to determine if sewer system attributes relate to wastewater compounds in urban shallow groundwater. Across the wells, groundwater samples contained varying wastewater compounds, including acesulfame, sucralose, bisphenol A, 4-*tert*-octylphenol, estrone and perfluorobutanesulfonic acid (PFBS). Fecal indicator bacterial concentrations and toxicological bioactivities were less than known benchmarks. However, the reclaimed water in this study was positive for all bioactivity tested. Excluding one well intruded by seawater, the similarity to sewage of groundwater, based on multiple indicators, increased with increasing sanitary sewer exfiltration probability (modeled from infrastructure within ca. 300 m of each well). In the absence of direct exfiltration or defect measurements, sewer exfiltration probabilities modeled from the collection system’s physical data can indicate potential locations where urban shallow groundwater is contaminated by sewage.

Keywords: GIS; sanitary sewer exfiltration; compounds of emerging concern; wastewater indicators; fluorescence spectroscopy

1. Introduction

Urban groundwater demands are increasing as populations grow, development increases, and climates change (Taylor et al. 2013). However, shallow groundwater is impacted by humans (Lerner 2002, Reilly et al. 1994, Schirmer et al. 2013), and shallow groundwater pollution, through hydrologic migration into subsurface faults or fractures, may threaten deep aquifers (Borchardt et al. 2007). Further, shallow groundwater can exchange with (Rivett et al. 2011, Westbrook et al. 2005) and pollute (Schwarzenbach et al. 2006) surface waters. Given the potential for shallow groundwater to impact urban water resources, urban shallow groundwater contaminants and their sources should be understood.

Leaking underground storage tanks, sanitary sewer overflows, chemically-treated gardens, and landfill leachates can contaminate urban shallow groundwater (Kuroda and Fukushi 2008, Lerner and Tellam 1992, Musolff et al. 2010a). Leaking sanitary sewers are another source of contamination (Wolf et al. 2012), since many aged sanitary sewer systems are defective (ASCE 2013). With estimates of sanitary sewer exfiltration rates ranging from 1-13.1% of dry weather collection system flow (Rueedi et al. 2009, Rutsch et al. 2008, Wolf et al. 2012), sanitary sewer exfiltration could contaminate shallow groundwater with nutrients and pathogens, but also with compounds of emerging concern (CECs) (Wolf et al. 2006, Wolf et al. 2012). CECs, including pharmaceutical and personal care product chemicals (PPCPs), hormones or other endocrine disrupting compounds (EDCs), and disinfection byproducts (DBPs), are detected in urban water environments (Benotti et al. 2009, Ellis 2006, Kolpin et al. 2002, Musolff et al. 2010b, Scheurer et al. 2009). CECs may also enter groundwater via land-applied reclaimed water, since many CECs are incompletely removed during conventional wastewater treatment (Du et al. 2014). Thus, the presence of wastewater compounds in urban shallow groundwater could signal

contamination from either leaking sewers or reclaimed water. To discriminate between these, specific evidence of untreated sewage contamination is required, and further understanding the probability of sewers leaking could also be informative.

The overall objective of this study was to advance understanding of the origins of wastewater contamination of urban shallow groundwater in relationship to sanitary sewer infrastructure. Specifically we sought to determine how sewer system attributes relate to wastewater compounds in urban shallow groundwater. The focus was a small central California coastal city where prior research showed that leaking sanitary sewers contaminated dry weather storm drain flows and surface waters (Cao et al. 2011, Holden et al. 2011, Sercu et al. 2011, Sercu et al. 2009) and that wastewater compounds were in shallow groundwater (Izbicki et al. 2009). However, whether exfiltrating sanitary sewers impacted shallow groundwater remained unknown. To address this, we investigated sewage-specific compounds in shallow groundwater at previously sampled wells (Izbicki et al. 2009) and then related our sample analysis results to probable sanitary sewer integrity failure estimates and proximity to groundwater. This addressed the main objective of discovering evidence that collection system physical attributes could influence sanitary sewer exfiltration into shallow groundwater. In a prior examination of potential exfiltration across the US, western states were deemed more susceptible, but regional or local studies were recommended (Selvakumar et al. 2004). Results herein indicate that sewage contamination of urban shallow groundwater could be explained by characteristics of the wastewater collection system.

2. Materials and methods

2.1. Study area and field sampling

The study area surrounds five shallow groundwater wells (Figure 1) in a small city (population 89,000; area 108 km²; total sewer line length approx. 470 km) on California's central coast. Municipal wastewater is conveyed in separate sanitary sewers to a wastewater treatment plant (WWTP), and secondary effluent was partly filtered through anthracite beds for reclamation, then chlorinated for disinfection before irrigating public lands (parks and median strips). The sanitary sewers range from 5 to 126 years old (Figure S1); their materials of construction include vitrified clay pipe (76.6% of system length), polyvinyl chloride (21.9%) with minor sections of high density polyethylene, reinforced concrete, and cast iron.

Waters previously sampled from the study wells (Figure 1) showed evidence of wastewater contamination (Izbicki et al. 2009). The wells are accessible, distant from known hydrocarbon or solvent groundwater plumes, have suitable recharge flow rates, and are screened at <8 m depth. Well GW9, the furthest inland, is in a residential area where sanitary sewer ages are up to 90 years (Figure S1a). The other wells (GW2, GW3, and GW4) are within a land use gradient from mixed-age residential zone to a commercial beachfront, where sanitary sewer ages vary (Figure S1b). GW11 is near an estuarine lagoon at a creek mouth, adjacent to a submerged interceptor main (Figure 1; Figure S1b).

Discrete groundwater samples were collected August 5-7, 2013. The weather conditions were dry; there had been no rainfall for 60 days. An untreated sewage sample (SEW) from the WWTP served as one positive control; the other positive control was a reclaimed water sample (RECL) from an irrigation distribution line near GW11 (Figure 1). One deep groundwater

sample (DEEP), from a municipal supply well extracting water from an aquifer beneath a confining layer, was the negative control. Groundwater samples were collected with a peristaltic pump using low-yield well sampling procedures (Kaminski 2006, New Jersey Department of Environmental Protection 2003, Nielsen and Nielsen 2006, U.S. EPA 2010), as per the Supplementary Information (SI).

2.2. Matrix interference assessment

The reliability of the analytical measurements of organic compounds was assessed through a matrix interference test, which first involved making three dilutions of SEW into G3 groundwater: 0.5SEW (50% V:V), 0.1SEW (10 % V:V) and 0.01SEW (1% V:V). These samples were analyzed identically to other samples. Matrix interference for each compound was evaluated by comparing the measured concentration to the concentration expected from a linear regression of SEW dilution series concentrations. Compounds for which the measured values significantly deviated from the expected values were excluded from further analysis. The matrix interference samples were also used to assess the cumulative toxicological effects of SEW upon dilution into groundwater.

2.3. Sample and data analyses

Samples were analyzed for PPCPs, hormones, volatile organic compounds (VOCs), fecal indicator bacteria (FIB), metals, and stable isotope ratios of water. Analytes included regulated surface or drinking water chemicals and previously reported groundwater contaminants. Methods analysis details (Tables S1 and S2) and the selected analytes (Tables S3 – S7) are described in the SI. Briefly, organic compounds including PPCPs, VOCs, and wastewater

indicator compounds were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Anumol and Snyder 2015) or gas chromatography-mass spectrometry (GC-MS). Microbial fecal indicators were measured by defined substrate culture methods and quantitative polymerase chain reaction (qPCR) assays. Metals were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES) or -mass spectrometry (ICP-MS). Overall, 249 analytes or water quality characteristics were measured (Table S1).

To assess the characteristics of dissolved organic matter (DOM), fluorescence spectroscopy of DOM (*f*DOM) was performed at a 600 nm/min scan rate over excitation and emission wavelengths 200-450 nm and 250-580 nm respectively using a Varian Cary Eclipse Fluorimeter (Varian Scientific Instruments, Mulgrave, Australia). Parallel Factor Analysis (PARAFAC) modeling of the processed excitation-emission matrices (EEMs) was used to reduce the data from complex DOM fluorophore mixtures to descriptive mathematical components for comparing DOM signatures across samples. EEM processing and PARAFAC modeling were conducted in MATLAB using the DOMFluor toolbox v1.6 and drEEM toolbox (Murphy et al. 2013).

In vitro bioassays were utilized to assess the cumulative mixture effects of compounds that exert bioactivity in each sample (Table S6) (Escher et al. 2013, Snyder 2014). Samples extracted using Oasis HLB solid-phase concentration cartridges (SPE) (Waters, Milford, MA) were tested with the following four bioassays: (1) GR (glucocorticoid receptor) activity (GeneBLAzer®GR DA Assay, Life Tech, Australia), (2) ER α (estrogen receptor) activity (GeneBLAzer®ER alpha DA Assay, Life Tech, Australia), (3) umuC genotoxicity with and without rat liver enzyme S9 for metabolic activation (Xenometrix, Switzerland), (4) aryl hydrocarbon receptor (AhR) expression activity using the rat hepato-carcinoma H4IIE-luc cell

line (Jarosova et al. 2012, Sanderson et al. 1996). Concentrations causing 10% of maximum effect (EC₁₀ for GR, ER α , and AhR) or an induction ratio (IR) of 1.5 (EC_{IR1.5} for umuC) were used as the thresholds for positive responses (Escher et al. 2013, Macova et al. 2011). Bioassay results were reported as a toxic equivalent concentration (TEQ) relative to a reference compound response, or as a relative enrichment factor if the TEQ could not be obtained.

2.4. GIS-based model of exfiltration probability

An *a priori* model, using spatially explicit attributes in the GIS database of the city sanitary sewers, was developed for interpreting water quality relative to the physical collection system characteristics. Attributes of individual pipe sections were extracted from the GIS database and entered into a published binary logistic regression equation to calculate the probability of a defect occurring within each pipe section. The equation that was adopted incorporates coefficients (where positive increases, and negative decreases, calculated probabilities) for pipe depth (-0.016; range 2 to 25 feet), age (0.022; range 5 to 126 years), size (-0.031; range 2 to 42 inch diameter), slope (0.073; range 0 to 19.1%), and length (0.004; range 2 to 2389 feet). Dummy variables for pipe material (vitrified clay: reference material; brick: -2.233; concrete: -0.624; clay tile: -1.171; reinforced concrete: -1.319) and road class (under street: 0.235; not under street: -0.139) were included (Salman and Salem 2012).

To prioritize pipes where exfiltration would be the dominant mode of interaction with groundwater and for which the retention time of sewage-associated compounds in the unsaturated zone would be short, only pipes within 3m above an interpolated groundwater elevation surface were considered. This subset comprises 32.6% of the entire city sewer network.

For any location in the city that was included (i.e. meeting the sewer depth criterion of <3 m above the groundwater surface), the total exfiltration likelihood was determined by summing the pipe defect probability-weighted lengths for all pipe sections within a 300 m radius circle around the location. Thus, the exfiltration score of an individual location is a function of the total length of pipes positioned above the water table, multiplied by the failure probability calculated for each section, within a 300 m radius.

The decision to only calculate the failure probabilities for pipes that were within 3 m above the water table was based on two factors: the tendency for submerged pipes to infiltrate, and published attenuation reports for sewage chemicals exfiltrating in the vadose zone. For example, while some compounds (e.g. acesulfame, carbamazepine) persist during migration through the vadose zone, many (e.g. atenolol, naproxen, sucralose, caffeine) can attenuate in 1 to 4 m of soil (Alidina et al. 2014, Laws et al. 2011, Seiler et al. 1999, Van Stempvoort et al. 2011). Therefore, the <3 m cutoff was chosen to represent the dual considerations of where sewers are more likely to be exfiltrating (i.e. not submerged in groundwater) and areas where a suite of wastewater associated analytes are more likely to be detectable in groundwater (i.e. due to persistence through the vadose zone).

Groundwater elevation was interpolated for the entire city with co-kriging of water table measurements over the period 2000-2013 from 1,017 individual wells having 15,671 unique measurements (U.S. Geological Survey 2013), using digital elevation data (Gesch et al. 2002, Gesch 2007) as per a previous method (Chung and Rogers 2012). The median standard deviation of water level measurements in individual study wells was 0.26 m, and 97% of wells had a standard deviation of water level less than 0.9 m.

Spatial analysis was conducted in ArcGIS version 10.1 with Spatial Analyst and Geostatistical Analyst extensions (ESRI 2012).

2.5 Statistical analyses

A multivariate statistical approach was used to assess the similarity between groundwater quality at each well (except GW11) and SEW. Multivariate similarity among samples (SEW, 0.5SEW, 0.1SEW, 0.01SEW, GW2, GW3, GW4 and GW9) was measured through Bray-Curtis distance. Each variable (analytes indicated by (†) in Table S8 and $f\text{DOM}$ (C3-F_{max})) was standardized to 1.0 variance to ensure equal contribution of variables, and then re-centered so that the minimum value of each variable was 0.0 to remove negative values. Analyte concentrations that were below detection limits were assigned a value of zero.

3. Results and discussion

3.1 Analytical sample analysis results

3.1.1. Field and bulk parameters, stable isotope ratios of water, radioactivity, metals, inorganics and nutrients

The concentrations of TOC and nutrients including ammonia, nitrite, nitrate, and phosphate in sewage (SEW) (Table S8) were typical of untreated municipal wastewater (Metcalf and Eddy 2013). Alkalinity, chloride, and sulfate were higher than typical, reflecting local water supply characteristics (data not shown). Stable isotope ratios of water ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) in reclaimed water (RECL) were similar to SEW, suggesting conservation through wastewater treatment (Table S8). Stable isotope ratios of water in GW11 were similar to RECL and SEW, but distinct from the other wells (Table S8 and S9). Specific conductivity and ion concentrations

(Cl, Br, K, Na) indicate that GW11 is impacted by 25-31% seawater (Table S10; Figure S5). Although hardness and several inorganic (e.g. Mn, Mg, and Ca) concentrations in GW9 appear similar to GW11, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$, and Cl, Br, K and Na concentrations suggest that GW9 is similar to GW2–GW4. Gross beta activity was highest for GW11 and next highest for GW9 (Table S9).

The groundwater trace element concentrations were higher than in SEW (Table S8 and S9). Six (As, B, Ba, Fe, Mn, Sr) exceeded benchmarks for drinking water standards (U.S. EPA 2009) in at least one shallow groundwater well. As these trace elements derive from the Monterey formation of coastal California (Piper and Isaacs 1995), the exceedances reflect local geochemistry. Deep aquifers in the Santa Barbara basin generally show exceedances for As, B, Fe, and Mn (Davis et al. 2013).

Interestingly, the nitrate concentration in GW9 exceeded that in SEW by a factor of ca. 18 (Tables S8 vs. S9). The positive redox potential value (151 mV) and the persistence of TCE, a highly oxidized chemical, in GW9 indicated that this well is under oxidizing conditions, which could stabilize nitrate in GW9 relative to other wells.

3.1.2. Organic indicator compounds

Of the 178 organic compounds measured, 74 were quantified in SEW, 47 were quantified in RECL, and one (perchlorate) was quantified in deep groundwater (DEEP) (Table 1). Most compound concentrations in SEW were comparable to those for wastewater influent in other studies (Table S11), supporting the use of SEW to assess sewage equivalent concentrations in groundwater samples. Sixty-seven, 58, and 37 organic compounds were detected in 0.5SEW, 0.1SEW, and 0.01SEW, respectively (Table 1). To evaluate potential interferences including

background contamination of the groundwater matrix, analyte concentrations for each sewage dilution sample were compared to the concentrations predicted from a linear dilution of SEW. Most (79%; 56 out of 71) detectable organic analytes were linearly related to the expected concentrations ($R^2 > 0.8$; Figure S2), indicating minimal matrix interferences.

Five, four, and zero to two organic compounds were detected in GW11, GW9, and the remaining wells, respectively (Table 1). Acesulfame, previously proposed as a sewage tracer because of its ubiquity and environmental persistence (Buerge et al. 2009, Robertson et al. 2013, Tran et al. 2014), was detected in GW11 and GW9 (Table 2). Based on the acesulfame concentration of 33,000 ng L⁻¹ in SEW (Table S8), the sewage equivalent concentrations in GW11 and GW9 were 0.7% and 0.1%, respectively (Table 2). BPA was quantified in GW11, GW9, and GW3 at sewage equivalent concentrations of 1.7%, 0.3%, and 0.1% (Table 2), respectively, based on the measured concentration of 400 ng L⁻¹ in SEW (Table S8). Estrone was quantified in GW11 and GW4 at 0.4% and 0.1% sewage equivalent concentrations (SEW concentration = 210 ng L⁻¹), respectively (Table 2; Table S8). Sucralose was only detected in GW11 and, based on its concentration in SEW (51.5 ng L⁻¹, Table S8), was at a 0.4% sewage equivalent concentration (Table 2). Notably, compounds that were sewage tracers in other studies (e.g. carbamazepine, caffeine, iopromide, iohexol, and iopamidol) (Buerge et al. 2003, Kuroda et al. 2012, Ruzicka et al. 2011, Sidhu et al. 2013, Wolf et al. 2006) were not detected in the groundwater samples (Table S9), but were detected in SEW (Table S8) at concentrations comparable to other studies (Table S11).

Three industrial organic compounds were quantified in the groundwater samples: trichloroethylene (TCE) and perfluorobutanesulfonic acid (PFBS) in GW9, and perchlorate in DEEP (Table 2 and Table S9). Detection of a perfluorinated compound (PFC) such as PFBS in

GW9 (36.3 ng L⁻¹; Table S9) may indicate sewage contamination (SEW=4460 ng L⁻¹, Table S8), as reported by Kuroda et al. (2012). The perchlorate content in DEEP (210 ng L⁻¹) is likely naturally occurring rather than from sewage, since the SEW concentration (160 ng L⁻¹) was lower than DEEP (Table S8) and perchlorate concentrations in regional groundwater were 240-3900 ng L⁻¹ (Davis et al. 2013). TCE was detected in GW9, but not quantifiable in SEW. Likewise, one detergent compound, 4-*tert*-octylphenol, was detected in GW3 (Table S9), but also was not quantifiable in SEW (Table S8).

The numerous organic compounds in RECL (n=47; Table 1) suggest that this reclaimed water could have been a source of wastewater compounds to shallow groundwater. Many organic compounds remain after conventional secondary wastewater treatment and disinfection, such as acesulfame, carbamazepine, iopamidol and sucralose (Buerge et al. 2009, Scheurer et al. 2009). In contrast, other organic compounds such as caffeine are almost completely removed during secondary wastewater treatment (Sui et al. 2010, Thomas and Foster 2005). This is consistent with the low caffeine concentration in RECL (68 ng L⁻¹) relative to SEW (186,000 ng L⁻¹, Table S8). RECL also contained several disinfection byproducts, including the known carcinogen *N*-nitrosodimethylamine (NDMA; 440 ng L⁻¹), at an elevated concentration relative to SEW (5.7 ng L⁻¹, Table S8).

3.1.3. Bacterial cell counts and fecal indicators

Total cell concentrations in SEW were typical of municipal wastewater (Manti et al. 2008), and the sewage dilution samples scaled according to the dilution factor (Table S8). RECL cell counts were on the order of 1% SEW (Table S8) and DEEP counts were 0.1% of SEW (Table S8). Total cell concentrations in GW samples were similar to RECL and to 1% SEW

(Table S8 and S9), and also similar to other reports for shallow urban groundwater (Pedersen et al. 2008).

The concentrations of culturable fecal indicator bacteria (FIB, total coliform, *E. coli* and *Enterococcus*, Table S8) in SEW were typical of municipal wastewater (Metcalf and Eddy 2013) and similar to previously reported concentrations for this WWTP influent (Van De Werfhorst et al. 2014). There were no culturable FIB in either RECL or DEEP (Table S8). The total coliform and *E. coli* concentrations in the sewage dilution samples scaled with the dilution factor, as did the *Enterococcus* concentrations for 0.1SEW and 0.01SEW (Table S8); for no apparent reason, the 0.5SEW *Enterococcus* concentration did not scale according to the SEW dilution (Table S8). Culturable FIB were either not detected or detected at low concentrations in the groundwater samples (Table S9). Total coliforms were quantifiable for three wells (GW9, GW2 and GW3) at several orders of magnitude below California's recreational water benchmark of 10,000 MPN 100mL⁻¹ (California Department of Health Services 2000). Culturable *Enterococcus* was only detected at the minimum level (1 MPN 100mL⁻¹) in GW9. In GW9, where several wastewater indicator organics were detected (Table 2), low FIB concentrations were observed (Table S9). If introduced with exfiltrating sewage, FIB may not persist since oxidizing conditions (Table S9) do not favor FIB survival (Liao et al. 2007); other sewage-derived toxins could also inhibit FIB survival.

The qPCR-based EnterolA gene marker concentration in SEW was similar to a prior report (Van De Werfhorst et al. 2014). The 0.5SEW concentration did not scale according to the dilutions, while the 0.1SEW and 0.01SEW concentrations did. No DNA amplification inhibition was observed during qPCR, but the high sewage concentration in 0.5SEW could lower DNA extraction efficiency (Lemarchand et al. 2005). EnterolA was detected in RECL (Table S8) and

in four of the five shallow groundwater wells, with GW11 containing the most (Table S9). However, since *Enterococcus* spp. may be introduced into shallow groundwater by non-human fecal sources such as birds or mammals (Layton et al. 2010, Sinton et al. 1993), the EnterolA gene marker is not a definitive indicator of sewage contamination. A more specific indicator of human fecal matter, the *Bacteroides* gene marker HF183 (Litton et al. 2010, Van De Werfhorst et al. 2011), was not detected in the shallow groundwater samples (Table 2; Table S9). This could be explained if fecal bacteria attenuate, e.g. related to environmental conditions within either the vadose zone or groundwater (Fontes et al. 1991, John and Rose 2005). HF183 was quantifiable in SEW at a concentration similar to previously reported values (Sercu et al. 2011, Van De Werfhorst et al. 2011). Surprisingly, although reclaimed water was not dechlorinated, the EnterolA and HF183 concentrations were very high in RECL (Table S8). Persistence of DNA from dead bacteria, or of viable but non-culturable bacteria, could explain (Laplace et al. 1997, Young et al. 2007). Taken together, the GW samples harbored bacteria, some of which were culturable FIB (albeit nonspecific, i.e. total coliform); more direct evidence of wastewater was the amplifiable EnterolA in most (except GW9) samples (Table S9).

3.1.4. Fluorescence spectroscopy of DOM

A three-component PARAFAC model was developed for *f*DOM data normalized to correct for differences in overall fluorescence intensity (Figure S4). To assess wastewater contamination, the peak fluorescence value ($F_{\max}$) for the component identified as protein-like and potentially associated with anthropogenic sewage loading (C3) ($\lambda_{\text{Ex}}/\lambda_{\text{Em}}$: 275/350 nm) (Murphy et al. 2011, Yang et al. 2014) was extracted in the PARAFAC model.

The highest $F_{\max}$ for C3 was observed in RECL, followed by GW9 and GW11. The $F_{\max}$ value obtained for RECL and GW9 placed the C3 content at between 3-10% of SEW, based on the linear dilutions of SEW into GW3 groundwater (where GW3 $F_{\max}$ C3 sewage equivalent concentration was <0.01%; Table S12). The $F_{\max}$ value obtained for GW11 estimated a sewage loading of 0.7-1%, whereas the estimated sewage loading in the other shallow groundwater wells was <0.3% (Table S12).

3.1.5. *In vitro* bioassays

Both SEW and 0.5SEW exhibited either marker bioactivity or general cytotoxicity of cultured cells for all four toxicological bioassay endpoints (Table 3). The presence of bioactivities for 0.1SEW and nearly absent bioactivities for 0.01SEW indicate that the sewage loading in the five shallow groundwater samples would be below the threshold reference levels for the four bioassay endpoints that were evaluated. This is consistent with the estimates by chemical and microbiological analyses. The observed estrogenicity levels in 0.1SEW and 0.01SEW (Table 3) are consistent with the dilution factors, suggesting that this bioassay is sensitive enough to assess the range of 1 to 10% sewage equivalent for this particular source (SEW).

RECL exhibited potential bioactivity for all four biological endpoints (Table 3). Both GR activity and AhR activity were observed above levels of concern for drinking water in RECL (Brand et al. 2013, The Ontario Ministry of the Environment 1990), despite the lack of analytical detection of glucocorticogenic compounds (triamcinolone, prednisone, hydrocortisone, and dexamethasone) or AhR-inducing compounds (PAHs) (Macova et al. 2010, Nagy et al. 2002). This indicates that compounds responsible for eliciting the observed GR and AhR responses

were either not analyzed or occurred at concentrations below the analytical detection limits. The genotoxicity result for RECL suggests causation by unidentified DBPs (Neale et al. 2012) or other potent genotoxic chemicals that were not analyzed. The presence of estrone along with detergent degradation products (e.g., 4-octylphenol, 4-nonylphenol and 4-nonylphenol diethoxylate) (Petit et al. 1997, White et al. 1994) are potential contributors to the observed estrogenicity in RECL.

Among the groundwater samples, estrogenic activity was observed in GW2 (Table 3) at a higher E2-EQ than either 0.01SEW or RECL, but lower than 0.1SEW (10% SEW). However, none of the target estrogenic compounds were detected in GW2, suggesting that the observed estrogenicity resulted from mixture effects of other unknown estrogenic compounds that were present but below analytical detection limits, and/or that were not measured. The absence of induced bioactivity in the other four shallow groundwater samples (Table 3) is consistent with the <1% level of sewage loading estimated through other indicators (Table 2).

In comparison to existing benchmarks, the bioassay response in groundwater impacted by 10% sewage (0.1SEW: 3.2 ng L⁻¹ E2-EQ) approximated the estrogenicity guideline for drinking water (3.8 ng L⁻¹ E2-EQ) (Brand et al. 2013), but exceeded concentrations causing chronic fish reproductive toxicity (2 ng L⁻¹ E2-EQ) (Anderson et al. 2012) (Table 3). Although genotoxicity does not have a comparative benchmark, the bioassay results indicated that 0.1SEW would exhibit genotoxicity. The observed AhR response in 0.5SEW exceeds the guideline for drinking water (15 pg L⁻¹ TCDD-EQ) (The Ontario Ministry of the Environment 1990), but the AhR response in 0.1SEW is below the guideline.

3.2. GIS model of exfiltration probability

The model of exfiltration probability was applied over the local spatial extent of sewer infrastructure, resulting in a grid of approximately 9 km × 6 km with 15 m resolution. The median citywide exfiltration score was 1716 (IQR = 552-3655). The five selected groundwater sampling locations spanned a range from 1265 (42%-ile) to 4882 (86%-ile) with four of five locations representing sites in the top half of the exfiltration score distribution (Table 1). A higher score for an individual well indicates a greater density of sewer pipes with greater likelihood of exfiltration within the 300 m search radius (Table 1).

GW9, located furthest inland and within a residential neighborhood, had the highest likelihood of contamination from sewer infrastructure (exfiltration score = 4882, Table 1). GW9 is surrounded by older sewers (length weighted average age of 71.07 years), with a range of diameters, where all are within 3 m above the water table (Figure S1a.). GW11, which is positioned near newer pipes (average age 44.5 years) including a large interceptor main that is modeled to be submerged (infiltration dominant), had the lowest exfiltration score among the sites (1265, Table 1). The other wells (GW2, GW3 and GW4) demonstrated a gradient of exfiltration scores, i.e. ranging between 2257 and 3057 (Table 1). Of these, GW2, which is mostly near submerged pipes (Figure S1b) had the lowest score (Table 1); GW4 which is near large submerged and also non-submerged pipes had the next to lowest score (Table 1), and GW3 which is in the vicinity of mostly non-submerged smaller pipes (Figure S1b) had the highest exfiltration probability (Table 1).

The most important factors contributing to the exfiltration model scores were pipe age and position relative to the interpolated water table. As only pipes modeled to be <3 m above the water table are included in this model, uncertainties or errors in the interpolated groundwater

elevation may erroneously include or exclude pipes. Also, local complexity in the hydrogeology or short time scale fluctuations in the groundwater elevation may not be completely captured through the interpolation algorithm. However, since standard errors of the interpolated water table depth are <0.3 m for the majority of the study area, the impacts of such errors on predictions are likely limited.

Although the sampled wells offer a range of exfiltration scores, other regions in the city generated the top 10% of exfiltration scores based on the GIS model (Figure 1). Expanded sampling, i.e. from areas associated with the upper end of exfiltration scores, would be required to further test and validate the model.

3.3. Relationships among measured analytes and predicted GIS-based exfiltration probabilities

Several constituents of the shallow groundwater samples (Table 1) are recognized as sewage contamination indicators (Reinstorf et al. 2008, Van De Werfhorst et al. 2011, Wolf et al. 2012, Yang et al. 2014). Across the potential indicators of sewage contamination (Table 1) and the measured concentrations of organic sewage-associated compounds (Table S9), GW11 exhibited the greatest evidence of contamination, followed by GW9. Since GW11 is impacted by approximately 25-31% seawater (Table S10), sewage-associated compounds present in GW11 are possibly conveyed from near-shore coastal waters through hydraulic interaction with the lagoon, which may be impacted by urban drains (Izbicki et al. 2009, Sercu et al. 2011, Sercu et al. 2009). GW11 is also positioned immediately down gradient of a large interceptor main joint that connects a 36 in diameter PVC pipe to a 33 in diameter VCP pipe. The measured groundwater elevation indicates that these pipes are likely always submerged and therefore not sources of wastewater contamination.

Excluding GW11 due to its coastal influence, the presence of relatively few organic compounds at low sewage equivalent concentrations ($<0.1\%$) in wells GW2, GW3 and GW4 suggests moderate impacts from leaking sewers at those locations. The exfiltration scores for these four wells correlate, albeit non-significantly, with Bray-Curtis similarity to SEW ($R^2 = 0.86$; $n = 4$; $p = 0.072$), with GW9 having a greater similarity to SEW (Fig. 2). The exfiltration scores for these four wells are also correlated, again non-significantly, with C3- $F_{\max}$ of the $fDOM$ component associated with potential sewage loading ($R^2 = 0.75$; $n = 4$; $p = 0.13$) (Fig.2).

However, the C3 $F_{\max}$ is significantly correlated with the Bray-Curtis similarity to SEW ($r_p = 0.961$; $n = 8$; $p < 0.001$) (Table 1). Also, BPA and acesulfame concentrations (Table 1) are significantly correlated with the Bray-Curtis similarity to SEW ($r_p = 0.985$; $n = 6$; $p < 0.001$ and $r_p = 0.987$; $n = 6$; $p < 0.001$, respectively). The HF183 gene copy number was correlated, albeit not significantly, ($r_p = 0.701$; $n = 6$; $p = 0.053$) to the Bray-Curtis similarity to SEW. The values of the stable isotope ratio of oxygen ($\delta^{18}O$) (Table 1) show an inverse and significant correlation with the Bray-Curtis similarity to SEW ($r_p = -0.974$; $n = 8$; $p < 0.001$), suggesting that $\delta^{18}O$ may provide useful evidence of sewage-associated contamination in areas where water supply and shallow groundwater have different sources and isotopic compositions, as demonstrated before (Izbicki et al. 2012). The major local water supply is a relatively isotopically enriched inland reservoir, resulting in a $\delta^{18}O$ gradient that could parse sewage-associated contamination of groundwater. This gradient of $\delta^{18}O$ is apparent in the analytical results where SEW and RECL showed $\delta^{18}O$ values of -3.9 and -3.8, respectively, whereas groundwater samples including DEEP showed $\delta^{18}O$ values from -6.1 to -6.8 (Table 1).

Taken together, the results suggest that targeted analyses (e.g., acesulfame, stable isotopes of water and $fDOM$), with a GIS model of exfiltration probability, could be useful for

assessing groundwater impacts from exfiltrating sewers. Quantifying the magnitude of contamination from leaking sewers using microbial fecal indicators alone may be difficult due to their variable concentrations, multiple possible microbial sources in addition to sewage, and low persistence as compared to conservative organic tracers (Ballesté and Blanch 2010, Guérineau et al. 2014, Liang et al. 2012). However, measurements of persistent organic indicators (e.g., acesulfame), along with compounds showing pseudo-persistence due to higher loading rates coupled with intrinsic persistence (e.g. caffeine) and also microbial fecal indicators, can provide complementary evidence of contamination that encompasses both acute sewage loading and chronic contamination.

4. Conclusions

This study revealed specific indicators of sewage in urban shallow groundwater and suggested, based on a model of local collection system characteristics, that exfiltrating sewers could be sources. The following additional conclusions may be drawn:

- Sewage-associated compounds in urban shallow groundwater were detectable at levels that were less than 1% of a sewage equivalent concentration.
- The study wells' Bray-Curtis similarities to SEW correlated with nearby collection systems' likelihood of exfiltration ($R^2 = 0.86$; $n = 4$; $p = 0.072$), based on pipe age and other physical characteristics.
- Fluorescence DOM analysis effectively, albeit semi-quantitatively, indicated sewage-associated contamination in urban groundwater.
- No significant bioactivity related to known benchmarks was observed for urban groundwaters sampled in this study. However, estrogenicity and genotoxicity could be exerted in urban groundwater at 10% of sewage equivalent loading.

The reported measurements are for single samples collected at selected groundwater wells, benchmarked to discrete samples from positive and negative controls. Concentrations of sewage-associated contaminants may vary in space and time, due to discharge or environmental variations. Additional research is needed, i.e. using expanded spatial and temporal sampling, to further test the approaches herein, and to provide a more complete understanding of urban shallow groundwater contamination by exfiltrating sewers.

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Table 1. Evidence of wastewater contamination across selected potential indicators. The Pearson coefficient (r_p) compares each indicator to Bray-Curtis similarity to SEW. SEW = untreated sewage; 0.01SEW, 0.1SEW, and 0.5SEW = matrix interference test samples; RECL = reclaimed water; GWx = shallow groundwater well x; DEEP = deep groundwater negative control.

Site	Bray-Curtis similarity to SEW	Number of organic compounds	Specific conductivity ($\mu\text{S cm}^{-1}$)	$\delta^{18}\text{O}$	HF183 (gene copies 100 mL^{-1})	BPA (ng L^{-1})	Acesulfame (ng L^{-1})	C3-F _{max}	Exfiltration score [%-tile]
SEW	1	74	2138	-3.9	2.3×10^7	400	33000	4.77	--
0.5SEW	0.621	67	--	-5.4	5.1×10^7	201	15510	4.03	--
0.1SEW	0.244	58	--	-6.2	3.0×10^7	17.9	2970	2.58	--
0.01SEW	0.056	37	--	-6.6	3.7×10^6	2.3	267	0.57	--
RECL	--	47	1345	-3.8	5.6×10^4	ND	16830	1.22	--
GW11	--	5	14530	-4	ND	6.98	235	0.63	1265 [42]
GW9	0.076	4	2500	-6.1	ND	0.99	30.6	1.05	4882 [86]
GW3	0.03	2	1718	-6.7	ND	0.31	ND	0.37	3057 [69]
GW4	0.023	1	1394	-6.6	ND	ND	ND	0.19	2658 [63]
GW2	0.034	0	1746	-6.7	ND	ND	ND	0.49	2257 [58]
DEEP	--	1	1069	-6.8	ND	ND	ND	--	--
r_p		0.842	0.368	-0.974	0.701	0.985	0.987	0.961	0.928
p		0.008	0.37	<0.001	0.053	<0.001	<0.001	<0.001	0.072

ND: not detected.

--: not applicable.

Table 2. Organic compounds detected in groundwater wells, and sewage equivalent concentration (%) for each compound. Sewage equivalents were calculated by dividing the organic compound concentration in the groundwater sample (Table S9) by the concentration in SEW (Table S8) and multiplying the result by 100. GWx = shallow groundwater well x; DEEP = deep groundwater negative control.

Well	Organic compound	Measured concentration (ng L ⁻¹)	Sewage equivalent concentration (%)
GW11	BPA*	6.98	1.7
	acesulfame	235	0.7
	sucralose	182	0.4
	estrone	0.8	0.4
	<i>p</i> -cresol	10	0.015
GW9	PFBS*	36.3	0.8
	BPA*	0.99	0.3
	acesulfame	30.6	0.1
	TCE*	1200	-- ¹
GW3	BPA*	0.31	0.1
	4- <i>tert</i> -octylphenol	M ²	
GW4	estrone	0.26	0.1
GW2	none detected	NA	NA
DEEP	perchlorate	210	131

*BPA: bisphenol-A; PFBS: perfluorobutanesulfonic acid; TCE: trichloroethylene.

¹ No quantifiable concentration in SEW.

² “M” represents that the chemical was present, but not quantifiable.

NA = not applicable, because no compounds were detected in this sample.

Table 3. Summary of the bioassay results. Values are expressed as average equivalent concentrations, except for the umuC assay results which are expressed as 1/REF. Detection limits of bioassays were calculated based on maximum REF tested (cytotoxic REFs were excluded) in each assay (Macova et al. 2010). SEW = untreated sewage; RECL = reclaimed water; GWx = shallow groundwater well x; DEEP = deep groundwater negative control; 0.01SEW, 0.1SEW, and 0.5SEW = matrix interference test samples.

Site	ER activity	GR activity	AhR activity	Genotoxicity	
				umuC- S9	umuC+S9
	E2-EQ (ng L ⁻¹)	Dex-EQ (ng L ⁻¹)	TCDD-EQ (pg L ⁻¹)	1/EC _{IR1.5} (1/REF)	1/EC _{IR1.5} (1/REF)
Drinking Water †TEQ benchmark	3.8 ^a	21 ^b	15 ^c	—	—
SEW	*	<400	<105	0.38	0.4
0.5SEW	*	<200	87.5	0.24	0.19
0.1SEW	3.2	<50	<11	0.06	0.05
0.01SEW	0.38	<25	<5	<0.01	<0.01
RECL	0.17	51.6	15.7	0.03	0.04
GW11	<0.15	<25	<5	<0.01	<0.01
GW9	<0.15	<25	<5	<0.01	<0.01
GW2	0.45	<25	<5	<0.01	<0.01
GW3	<0.15	<25	<5	<0.01	<0.01
GW4	<0.15	<25	<5	<0.01	<0.01
DEEP	<0.15	<25	<5	<0.01	<0.01

† TEQ: toxic equivalent concentration.

Drinking water TEQ benchmark: ^{a,b} Brand et al. 2013; ^c The Ontario Ministry of the Environment 1990.

“—”: not applicable, since there is no comparative benchmark.

* Cytotoxicity was observed at all REFs tested.

Figure 1. Sample locations and aggregated probability of exfiltration (exfiltration score, Table 1). Sewer mains <3 m, and >3 m above the groundwater elevation are shown as purple and red lines, respectively. Pipes that are below the water table are shown in blue. The thickness of the lines indicates the size of the pipes (range from 6 to 42 inches). Red-green color ramp is the aggregate probability of exfiltration (red = high; green = low). Locations of the shallow groundwater wells (GW2, GW3, GW4, GW9 and GW11) are indicated by $\oplus$, and locations of positive controls (untreated sewage (SEW) and reclaimed water (RECL)) are indicated by $\blacktriangle$. The negative control of deep groundwater (DEEP) was collected from a separate groundwater basin and is not shown on this map.

Figure 2. Comparison of (a) GIS-based exfiltration score against Bray-Curtis similarity to SEW ($R^2 = 0.86$; $n = 4$; $p = 0.072$) and (b) GIS-based exfiltration score against PARAFAC C3-F_{max} ($R^2 = 0.75$; $n = 4$; $p = 0.13$).

Graphic Abstract