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Spatial and Temporal Variability of Micropollutants Within a Wastewater Catchment System

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Abstract

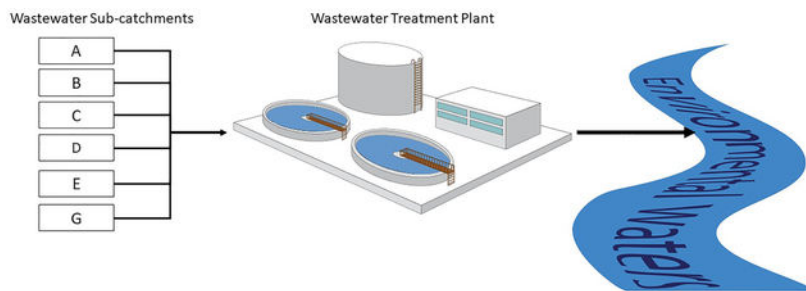
Treated wastewater effluent is a major contributor to concentrations of many anthropogenic chemicals in the environment. Examining patterns of these compounds measured from different catchment areas comprising the influent to a wastewater treatment plant, across many months, may reveal patterns in compound sources and seasonality helpful to management efforts. This study considers a wastewater catchment system that was sampled at six sub-catchment sites plus the treatment plant influent and effluent at seven time points spanning nine months. Wastewater samples were analyzed with LC-QTOF-MS using positive electrospray ionization and GC-QTOF-MS using negative chemical ionization and electron ionization. MS data were screened against spectral libraries to identify micropollutants. As expected, multiple classes of chemicals were represented, including pharmaceuticals, plasticizers, personal care products, and flame retardants. Patterns in the compounds seen at different sampling sites and dates reflect the varying uses and down-the-drain routes that influence micropollutant loading in sewer systems. Patterns in examined compounds revealed little spatial variation, and greater temporal variation. For example, the greatest loads of DEET were found to occur in the summer months. Additionally, groups of compounds exhibited strong correlation with each other, which could be indicative of similar down-the-drain routes (such as a group intercorrelated chemicals that are components of cleaning products) or the influence of similar physicochemical processes within the sewer system. This study contributes to the understanding of dynamics of micropollutants in sewer systems.

Graphical Abstract

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Conflict of interest

The authors declare no conflicts of interest.



Environmental Significance Statement

Wastewater treatment plants are faced with an increasingly diverse array of micropollutants that most were not specifically designed to treat. Few previous studies have measured contaminants at sites upstream of the treatment plant influent to examine differences in space and time, which could contribute to more effective management solutions.

Introduction

While the utility and benefits of many anthropogenic chemicals are undeniable, it is also a fact that these compounds have infiltrated the natural environment [1], to the detriment of environmental and human health. One source of chemical pollution in the environment is from treated wastewater, as not all micropollutants are effectively removed during treatment. Consequently, numerous pharmaceuticals, personal care products, flame retardants, plasticizers, and pesticides that find their way down the drain are emitted from wastewater treatment plants [2]–[5].

An example of a single, highly-studied compound can be found in fipronil, a common spot-on flea and tick treatment for pets, which was found to be dislodged during pet bathing up to 28 days post-application [6]. Furthermore, fipronil and its degradates were found to disperse throughout the home presumably via dust and dander [7], which may lead to down-the-drain transport via laundering or showering. Especially in drier climates such as California, where the influence of wastewater effluent on receiving surface waters is largest, these contaminants can have significant effects on aquatic life [8].

Application of broad-scope screening methods such as suspect and nontarget analysis of high-resolution mass spectrometry (HRMS) data can be valuable in environmental monitoring, where changing consumer use patterns and the introduction of new compounds may mean that conventional targeted screening fails to capture compounds of toxic concern. HRMS-based screening has been used to isolate “fingerprints” of human activity, indicating whether a waterway is primarily influenced by agricultural, industrial, or municipal wastewater pollution [9], [10]. Many studies have also trained these tools on understanding removal of compounds across treatment processes [11], [12], which may help to improve the treatment of wastewater before it enters the environment [13]–[15].

However, another potential application of these methods is to examine specific portions of the wastewater collection system to isolate the sources of these pollutants. Understanding

patterns of contaminant loads in time and space can provide key information to support cost-effective management practices such as source control or pretreatment. Few studies have examined the patterns in contaminants within the wastewater catchment system [16]. In this study, wastewater treatment plant (WWTP) influent and effluent plus sites within the catchment system were sampled seven times over a 9-month period. These samples were screened against a library of water contaminants to find spectral matches, which were later confirmed with analytical standards or structure elucidation. Comparison of contaminant patterns between sampling months and sampling sites revealed the complexity of the system.

Materials and Methods

Sampling

Fig. S1 is a schematic of the connections within the sewer system: trunkline sites A, B, C, D, E, F, and G all connect separately to the influent of the WWTP. Trunkline site F has been omitted from further analysis because it was sampled for fewer months than the other sites. All samples (excluding site F) were collected monthly with 15-minute sampling intervals as 24-hour time-weighted composite samples using a combination of ISCO Model 2910, Hach Sigma 900 Max, and Sigma SD900 autosamplers, except for the June 2016 samples which were collected at 30-minute intervals. In July and September, three additional “specialty” sites were sampled to capture wastewater from anticipated contributors of pesticides to wastewater: a laundromat, a pet groomer, and a pest control operation (PCO). These sewer laterals were sampled in such a way that the wastewater would be from the single targeted source.

Sample Preparation

Wastewater was transported on ice to the lab, stored at 4°C until extraction, and extracted within 24 hours of collection. Samples (200 mL of raw wastewater or 1 L of treated effluent) were filtered through a GF/F filter (0.45µm) which was extracted separately. Filtered samples were spiked with of a stable isotope labeled surrogate solution (Table S2), and passed over an Oasis HLB cartridge (Waters, Massachusetts, USA), then eluted ethyl acetate followed by methanol, which were collected separately. Jars were rinsed with 3:1 hexane: acetone, which was combined with the ethyl acetate eluate and concentrated to 1 mL. Methanol eluate was also concentrated to 1 mL. Filters were air-dried and then extracted in a sonicating bath with 1:1 hexane/acetone, then concentrated to 1 mL. One LC extract for each sample was created from 0.5 mL of the ethyl acetate, methanol, and hexane-acetone extracts, then concentrated to 0.2 mL and reconstituted to 1 mL with ultrapure water and spiked with the stable isotope labeled internal standard mix. Additionally, a GC extract was made from 0.5 mL of the ethyl acetate and hexane-acetone extracts, which was concentrated to 0.2 mL dibromooctafluorobiphenyl (DBOBF). Extracts were stored at -20°C until data acquisition.

It is possible that the filtration and nitrogen blowdown steps may have led to the loss of volatile compounds in the extracts. Most nontarget methods will not be able to recover all possible compounds, especially as this method was initially developed for targeted quantification of pesticides that would not be considered volatile [17]: of the original target pesticides the highest vapor pressure being 3.5e-05 mm Hg for prallethrin at 20°C¹. Of the

suspect-annotated compounds, D-limonene had the highest vapor pressure: 1.43 mm Hg at 20°C².

Suspect Screening

Data acquisition has been previously reported [17] and is summarized in the Supporting Information.

To support subsequent processing in MS-DIAL, raw data files from GC- and LC-QTOF-MS were converted from the vendor format to the analysis base file format (Reifycs Analysis Base File Converter v. 4.0.0). All data were then deconvoluted and aligned using MS-DIAL (v. 3.90). Tentative identifications of aligned features were established by searching against the NIST17 database for GC-EI data, an NCI-specific pesticide database for GC-NCI, and a combined library that included the Agilent Water Contaminants, Pesticides, and Forensic Toxicology libraries for LC-ESI+. Parameter selection and workflow performance evaluation are described in detail in the Supporting Information (Table S2 for MS-DIAL alignment parameters; Table S6 for detection performance of LC targets in standards and spikes; Table S7 for detection performance of GC targets in standards and spikes). For GC suspect screening, the use of retention-index dictionaries added an additional level of confidence, and additional identification steps were not taken.

LC-ESI+ data was also screened using the *Find by Formula* workflow in *MassHunter Qualitative Analysis* against the same library used in MS-DIAL, where a “qualified” identification required mass error less than 15 ppm, an intensity greater than 1000 counts, confirmation with at least one coeluting fragment ion (with a coelution score > 80%), and an overall match score of >80% (weighted score of accurate mass, isotopic spacing and isotopic abundance). The use of 15 ppm mass error rather than the conventional 10 ppm was to act as a broader filter, and additional confirmatory steps resulted in reported suspects ultimately falling within a mass error of 10 ppm. As in the MS-DIAL alignment, $[M + H]^+$, $[M + Na]^+$, and $[M + NH_4]^+$ adducts were searched. Qualified library hits were compared against library identifications made by MS-DIAL as separate lines of evidence. For each compound that was found by the *Qualitative Analysis* software, which does not perform alignment, there was a range of retention times (RT) and mass-to-charge ratios (m/z) if it was detected in multiple samples. The median, 25th and 75th percentiles of RT and m/z were used to compare against MS-DIAL aligned features (as each feature is represented by an average RT and m/z). Some compounds identified in *Qualitative Analysis* had quite large interquartile ranges (IQR) of RT, large enough for these ions to be considered different features in the MS-DIAL alignment. Compounds that had a RT IQR greater than 0.7 minutes and were detected in fewer than five samples were omitted from the dataset. Those with RT IQR greater than 0.7 minutes but five or more detections were divided into separate features based on RT. The filtered MS-DIAL alignment was searched for an aligned feature that was within 0.3 minutes of the median RT and ± 10 ppm mass error (using median m/z) for each compound from the *Qualitative Analysis* set. Additionally, a Pearson correlation coefficient was computed using the peak height in samples as reported by *Qualitative Analysis* and

¹ <https://pubchem.ncbi.nlm.nih.gov/compound/prallethrin#section=Vapor-Pressure>

² <https://pubchem.ncbi.nlm.nih.gov/compound/d-limonene#section=Vapor-Pressure>

MS-DIAL and was used to filter out features that did not show a significant correlation in sample abundance. Features were manually inspected in the MS-DIAL software to assess peak shape and to determine if there were any library hits that were below the score threshold, which indicated that MS-DIAL's deconvolution had identified some qualifying fragments. Suspect identified compounds were selected for targeted MS/MS experiments based on peak height and detection frequency in wastewater samples (rather than spikes, standards, or blanks), compound class, and if the laboratory had a standard on-hand.

Quantification of Suspect Compounds

Compounds identified as probable structures by library spectrum match in LC samples that were on-hand as standards were included in a 13-point calibration curve ranging from 0.1 ng/mL to 1000 ng/mL. The calibration curve was run with the LC extracts so that RT and coeluting fragments could be used to confirm the identifications. Limit of detection was defined in a given batch as the lowest calibration level in which the molecular ion peak appeared, whereas the limit of quantification was the lowest level in which a Gaussian peak with clear fragment ions appeared. Handling of samples with levels below the limits of detection and quantification is described below in computation of mass loads. Data analysis was carried out in *MassHunter Quantitative Analysis* software (v. 10.1), using the internal standard that was closest in retention time. Internal standards that had been added to the extracts were originally used for target pesticide quantification (listed in Table S3). The volumes of the extracts had changed since the original processing, due to reinjection for multiple analyses, and as such, we did not want to add any additional spikes (such as matrix spikes), since the concentration would be unknown. Finally, concentrations were multiplied by the concentration factor for the samples: 1 mL extract/L sample for effluent and 5 mL extract/L sample for influent and trunkline samples, respectively.

Structure Confirmation

For library spectrum-matched LC compounds not currently in the lab inventory, targeted MS/MS experiments were used to gain further insight on the structure. Samples containing high abundances of these compounds were run with a list of precursors to isolate (exact masses and retention times) where collision cell voltages again cycled through 0, 10, and 40 eV. Instrumental parameters are included in Table S1. Again, the data were deconvoluted using MS-DIAL (parameters in Table S2), and results were exported to both MS-FINDER (v. 3.24) and SIRIUS CSI:FingerID for identification using *in silico* fragmentation approaches [18], [19]. Additionally, at the end of each targeted MS/MS run, a mix of RTI compounds was run in *All Ions* mode for input into an RTI prediction platform (<http://rti.chem.uoa.gr/>) [20], which was then used to evaluate the top-ranked structure options for a suspect using experimental RT.

Computation of Mass Loads

Quantified concentrations in wastewater extracts for confirmed structures was both left- and right- censored, due to relative responses below the limit of quantification as well as above the highest calibration point. The function `ros()`, from R package NADA (ver. 1.6–1.1) was used [21] to impute non-detect values, both if the peak was below the limit of detection or quantification. For points exceeding the calibration curve range, the highest calibration point

was used. Censored values in mass load per capita box-and-whisker plots are differentiated by shape from un-censored values. To calculate the mass load (mg/d*person), routine measurements of ammonia-nitrogen (NH₄-N) were used to estimate population, assuming a population equivalent of 8.1 g of NH₄-N per person per day [22].

Statistical Analysis for Significant Difference

To determine if mass loads per capita were statistically significantly different between sampling months and sites, a pairwise Wilcoxon rank-sum test with a Benjamini-Hochberg correction was implemented for compounds with 50% un-censored data points. For annotated GC compounds, the raw peak height was divided by the peak height of an internal standard, 4,4'-dibromooctafluorobiphenyl (DBOFB), to obtain a scaled peak height. This was used in a similar pairwise Wilcoxon rank-sum test. A similar approach was used for the three annotated LC features, using instead the peak height of the internal standard, simazine-d10, for scaling.

Compound Correlation Test

Some compounds were observed to follow similar monthly or site-wise patterns. The function `rcorr()` from package `Hmisc` (v. 4.7-0) [23] was used to obtain a correlation matrix for the concentrations of quantified LC compounds and scaled peak heights of annotated GC and LC features at the six trunkline sites plus influent and effluent. The correlation matrix consisted of correlation coefficients and p-values of the Spearman rank-order correlation of each compound against the other. A Spearman correlation was used rather than Pearson because relationships between compounds were not necessarily expected to be linear. Finally, p-values were adjusted for multiple tests to control the false discovery rate using `p.adjust()` with method "fdr".

Results and Discussion:

Quantification of micropollutants

In the LC data, there were 63 library hits corresponding between MS-DIAL and Qualitative Analysis. Fourteen of these were available in the lab inventory as standards and were quantified. Additionally, the fungicide diethofencarb was identified by suspect screening, but when compared against an analytical standard, was not confirmed. This might be due perhaps to degradation of the already infrequently detected peaks in the original sample analysis (2016–2017), although others have found that a peak originally thought to be diethofencarb was in fact metoprolol acid, a metabolite of metoprolol and atenolol [24]. Additionally, a feature annotated as 8-hydroxyquinoline and one as benzotriazole were detected with moderate frequency (approximately 40% of samples) but were not confirmed when compared against standards. Benzotriazole is commonly detected in wastewater, owing to its application as a corrosion inhibitor in dishwashing machines [25], [26]. Table S8 presents the list of suspect hits, the frequency of feature detection, use class, and level of identification confidence (if confirmed) [27]. Table 1 summarizes the detection frequency of the compounds that were confirmed with analytical standards.

Among the list of compounds that were tentatively identified via suspect screening, many of the common use-classes of chemicals detected in wastewater were included, with the notable exception of any sweeteners. This absence could be due to the exclusion of electrospray negative ionization (ESI-) data, as others have detected common sweeteners in environmental water and wastewater using ESI- [28], [29]. Other food-related compounds including caffeine, caffeine metabolites, and piperine were suspect-hits, with caffeine then being confirmed at a confidence level 1, and piperine at 2a. In previous studies of wastewater treatment plants and surface water, sweeteners are detected with a ubiquity to even lend the artificial sweetener, acesulfame, the proposed role as indicator of anthropogenic load on surface waters [28].

Compounds detected at frequencies greater than 50% and at concentrations on the order of micrograms per liter of wastewater represent a range of use classes and potential down-the-drain routes. This group included caffeine, DEET, bis(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), iohexol, oleamide, and tris(2-butoxyethyl) phosphate (TBEP). This is reflective of the high frequency of use of these compounds among the population, or in the case of iohexol, the dosage at which it is administered. Iodinated x-ray contrast media (ICM), such as iohexol, are consumed in large dosages, with one hospital studied by Weissbrodt et al. (2009) reporting iohexol as the ICM with the largest amount consumed per day. DEP and DEHP, although both phthalates, have different uses and thus may have different pathways to the wastewater system: DEP is used as a solvent and in multiple kinds of personal care products, whereas DEHP is primarily employed in soft plastics such as toys, food containers, and food packaging [31]. TBEP (also abbreviated as TBOEP) is a organophosphate ester flame retardant, a class that has seen an increase in use with the phase-out of brominated flame retardants [32]. A study of the San Francisco Bay, to which the WWTP of this study discharges, found a 100% detection frequency of TBEP in bay water samples [33]. TBEP and DEHP can enter the wastewater system via laundering of fabrics, which have been found to accumulate TBEP and similar semi-volatile compounds from air and dust [34]. Oleamide is a polymer lubricant used in plastics such as food and medicine containers, and has also been found to leach out of these plastics [35]. Sewer sampling that occurred at the sites of a groomer, laundry, and pest control operation (PCO) found a similar subset of compounds that were detected with high frequency, as detailed in Table 2.

Compounds that were quantified less frequently or at lower concentrations were all pharmaceuticals or metabolites of pharmaceuticals (Table 3). Multiple classes of pharmaceuticals were represented: two antibiotics, sulfamethoxazole, and trimethoprim; one antihistamine, fexofenadine; a beta-blocker, metoprolol; an anti-seizure medication, carbamazepine; and the metabolite of an opioid painkiller, o-desmethyltramadol. Additionally, valsartan, an anti-hypertensive, was detected most frequently of the quantified pharmaceuticals. Valsartan and o-desmethyltramadol were the only pharmaceuticals detected at the specialty sites, reflecting the relatively small catchment served by these sampling locations. The higher frequency of detection of these compounds in WWTP effluent samples is most likely due to the higher sample volume processed and less-complex matrix. All pharmaceuticals quantified here have been documented in other studies of wastewater and surface water. A report of seven different WWTP in the San Francisco Bay area found

valsartan to be among the top ten highest concentration pharmaceuticals in influent samples, with valsartan, metoprolol, carbamazepine, and sulfamethoxazole included in the top ten for effluent samples [36].

Detection frequency of annotated compounds

Of the suspect hits in the LC dataset, that were not quantified, 23 were selected for targeted MS/MS experiments due to peak height, sample detection frequency, and use class. However, only 3 structures were confirmed with tMS/MS experiments. These compounds were acetaminophen, mycophenolic acid, and piperine. In trunkline, influent, and effluent samples, corresponding MS-DIAL aligned features had detection frequencies of 57%, 54%, and 25%, respectively, where a detection was counted as a peak with height greater than 3000 counts. A study of seven different Bay Area WWTP found acetaminophen among the top-ten pharmaceutical compounds in WWTP influent [36]. Mycophenolic acid is an immunosuppressant drug developed for prevention of organ transplant rejection [37] and is also used in cancer treatment [38]. One study found mycophenolic acid in 6 out of 6 WWTP effluents [39]. Piperine is an alkaloid present in black pepper, is excreted in the feces, and thus has been found to be ubiquitous in wastewater [40].

The aligned GC-EI dataset returned 1,470 annotated features. The subset considered herein was created by choosing a mix of use-classes and variable patterns within sampling dates and sites. Similarly, the GC-NCI aligned dataset yielded 48 annotated features, most of which were pesticides, largely those covered by targeted analysis in Budd et al. [17]. Again, MS-DIAL aligned peaks with heights greater than 3000 counts were counted as detections, and the detection frequencies of a subset of suspect-identified compounds is included in Table S9. Of these, the most frequently detected compound in the trunkline, influent, and effluent samples was oxybenzone, a UV filter. Equally ubiquitous was 3-methyl phenol, a human urinary metabolite of toluene.

Comparison of LC compound loads across months and sites

Concentrations of quantified compounds were converted to mass loads per capita to gain a better understanding of contributions from different sections of the wastewater catchment system and throughout the year. Table 4 details the comparisons found to be significantly different ($p < 0.05$) using a Wilcoxon rank sum test. One interesting pattern that emerged was the significantly lower loads in the months of May and June for caffeine, DEET, DEHP, DEP, Iohexol, and TBEP. Alternatively, oleamide and valsartan displayed fewer significant differences in monthly loads. In the case of caffeine, this difference in mass loads is unexpected, since it is typically assumed that caffeine consumption remains broadly consistent throughout the year (Figure 1). Although caffeine has been proposed as a proxy for population, it is also susceptible to biodegradation by biofilms within the sewer system, a process which can vary depending on a number of variables [41]. Bis(2-ethylhexyl) phthalate and tris(2-butoxyethyl) phosphate have a similar pattern to caffeine, with May and June both lower than the other five months. The load per person of tris(2-butoxyethyl) phosphate measured here is greater than that reported by O'Brien et al. [32], which was a cumulative organophosphate flame retardant load of $0.8 - 2.6 \text{ mg person}^{-1} \text{ day}^{-1}$, with

TBEP making up the greatest share; the quantifiable loads in this study varied from 7 $\mu\text{g person}^{-1} \text{ day}^{-1}$ to as high as 3.7 $\text{mg person}^{-1} \text{ day}^{-1}$.

For the X-ray contrast agent, iohexol, per capita loads in May and June are only significantly lower than the August load (Figure 1). The sampling site C had significantly higher loads than site E (Figure 1).

However, in the case of DEET, the summer months of July, August, and September show significantly higher loads than all other months, which may correspond with times of mosquito prevalence (Figure 1). Diethyl phthalate follows a similar pattern to DEET (Figure 1): highest in July through September, with January and November greater than June and May. This similar pattern may be linked to the similar applications of products containing the two compounds.

Because the samples were collected as time-weighted composites, rather than flow-weighted composites, there is an associated uncertainty with the load patterns presented here [42]. Ort et al. [43] show that a pulse of anthropogenic gadolinium (an x-ray contrast agent) can occur over the span of 15 to 20 minutes at the influent of a WWTP; such pulses may be narrower upstream in the system at the sub-sewershed sampling sites. Samples were collected as 24-hour composites, with a sampling frequency of 15 minutes. Additionally, greater flows occurred in January, despite the fact that the system normally receives negligible inflows from non-sanitary sources [17].

Comparison of GC annotated compounds by month and site

For compounds annotated from the GC-EI and NCI datasets, absolute peak heights were scaled by the height of DBOFB internal standard in the sample before comparison. 2-Naphthalenol is a human xenobiotic metabolite (Phase 1) of naphthalene; the most likely routes human of exposure are through fuels, moth repellents, and cigarette smoke [44]. Phenol, 3-methyl (also known as m-cresol), is a urinary metabolite and used as a biomarker for human exposure to toluene, which may be found in fuels as well [45]. Presumably the general population could be exposed to naphthalene and toluene from vehicle exhaust. These two compounds show different patterns by month, with higher levels of 2-naphthalenol in May and June, and a majority of non-detects in later months (Figure 2). 3-methylphenol was detected consistently across the months, although January and November are significantly lower than May, June, August, and September.

2-Propanol, 1-chloro-, phosphate (3:1) (TCPP) is a chlorinated organophosphate flame retardant that is now frequently used in flexible polyurethane foam products and can be found in house dust [46]. The most probable down-the-drain route for this compound would be from laundry water [34], [47]. Compound abundance in September is significantly higher than in May, July, November, and January (Figure 2). January is also significantly lower than June and August, and November lower than May, June, and August.

Correlations between micropollutants

As noted above, correlations among compounds were examined to search for common usage and/or fate processes across different chemicals and implications of these correlations are

discussed here. Cholestan-3-ol, (3.β.,5.β.)³ is a breakdown product of cholesterol by gut bacteria, and has been used as an indicator of fecal contamination of environmental waters [48]. The scaled peak abundance is higher in November and January with all other sampling months having detections below the 3000-count cut-off. Shown in Figure 3, this compound was found to be positively correlated with seven other compounds including two plant related steroids (stigmastanol and stigmasta-5,24(28)-dien-3-ol) and guaifenesin, an expectorant found in cough medicines. Allopregnane-3α,20α-diol, an endogenous metabolite of progesterone, was positively correlated with another fecal indicator, 3-methylindole (skatole). Significant positive correlations were also observed among many pairs of pharmaceuticals. These included correlations between the antibiotics sulfamethoxazole and trimethoprim, which are used together to treat, for example, pneumonia in HIV/AIDS patients, and both were correlated with the anti-hypertensive medication valsartan. Mycophenolic acid, another antibiotic was correlated with three other pharmaceuticals (acetaminophen, iohexol, and trimethoprim). Another group of intercorrelated compounds with the potential for common sources were components of common cleaning products including 5-heptyldihydro-2(3H)-furanone, D-limonene, m-cresol and p-cresol.

Most correlations were observed between compounds measured with the same instrument. This could be due in part to the molecular properties of compounds that make them amenable to GC versus LC. For example, compounds that tend to sorb to suspended solids in the sewer system may vary in the same ways, depending on the TSS present. However, performing a Spearman's correlation with TSS and all annotated compounds in the GC data revealed that only hexadecamethyl heptasiloxane and 3-methyl phenol had a corrected p-value < 0.05, with $\rho = 0.50$ and 0.48 , respectively. However, given the differences in physicochemical properties between LC- and GC- amenable compounds, this separation is not surprising. There are many parameters within the sewer system that might influence these groups of chemicals differently [49].

Conclusions

There have been many studies addressing the wide range of contaminants that find their way from the built environment, down the drain, and for some, into environmental waters. The dynamics of these compounds within the sewer system have been found in other studies to vary with physical, chemical, and biological parameters, not even considering the many anthropogenic processes that could drive variation. For these reasons, considering sub-sewershed contaminant patterns becomes even more complex. Ultimately, for the compounds considered here, which are found in a diverse range of product categories, distinct geographical usage patterns (i.e., systematic variation by trunkline sampling site) were not observed. This may be because the sub-sewershed sites are quite similar in land-use patterns. Instead, it seems that seasonal variation in product use or within-sewer processes seems to be primarily responsible for the overall system variation. However, there

³Other names used in literature include 5β-coprostanol, 5β-cholestan-3β-ol. Cholestan-3-ol, (3.β.,5.β.) is the name used in the NIST spectral library.

were many chemical features that went unidentified or undiscussed because the scope of chemicals within a sewer is so great.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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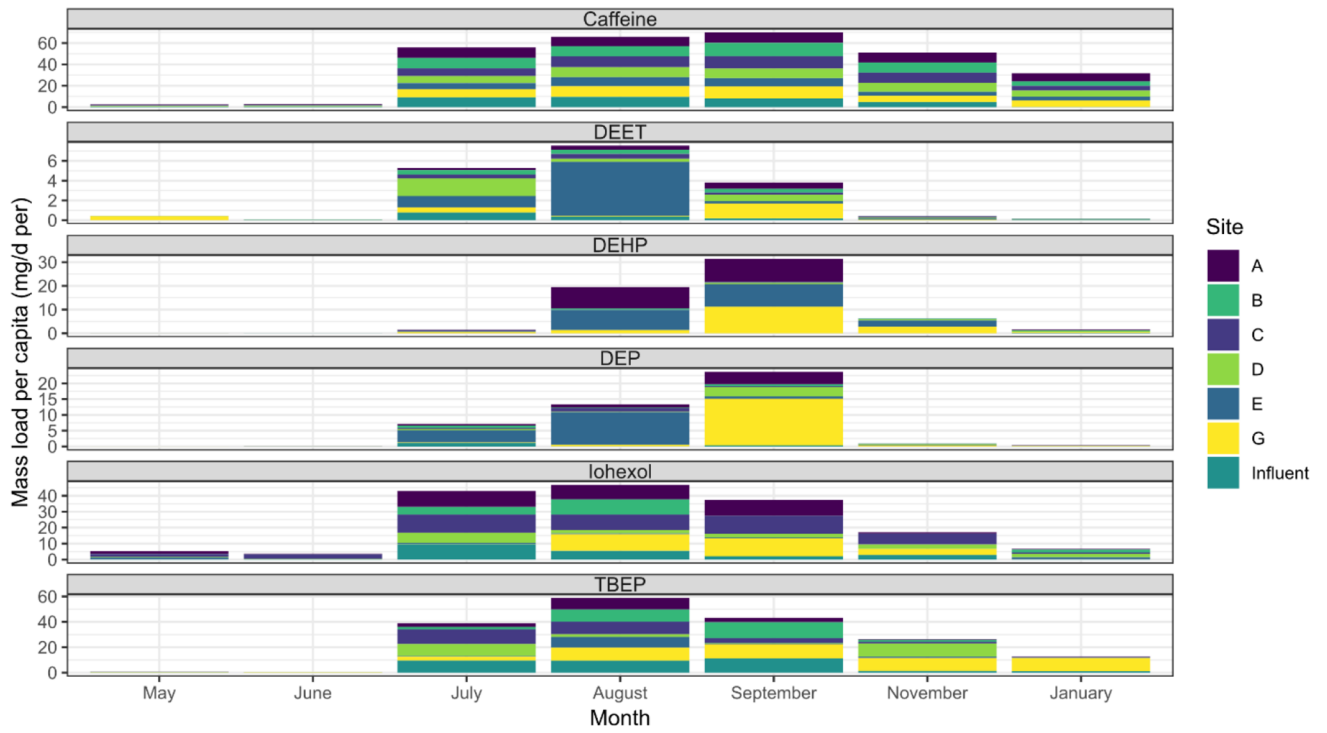


Figure 1. Mass load per capita of caffeine, DEET, DEHP, DEP, iohexol, and TBEP in each site, arranged by month.

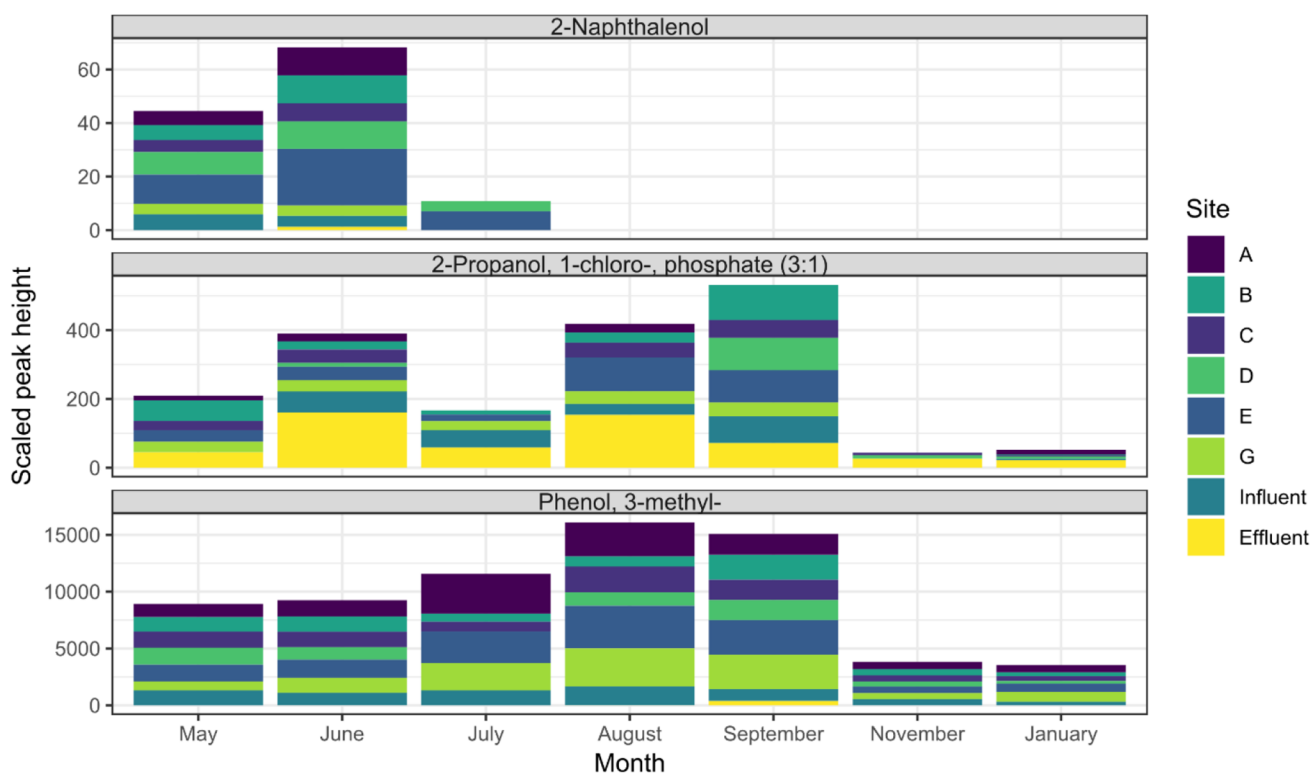


Figure 2. Scaled peak height of three GC-suspect annotated compounds including two human biomarkers for exposure to xenobiotics, 2-naphthalenol and phenol, 3-methyl- (m-cresol).

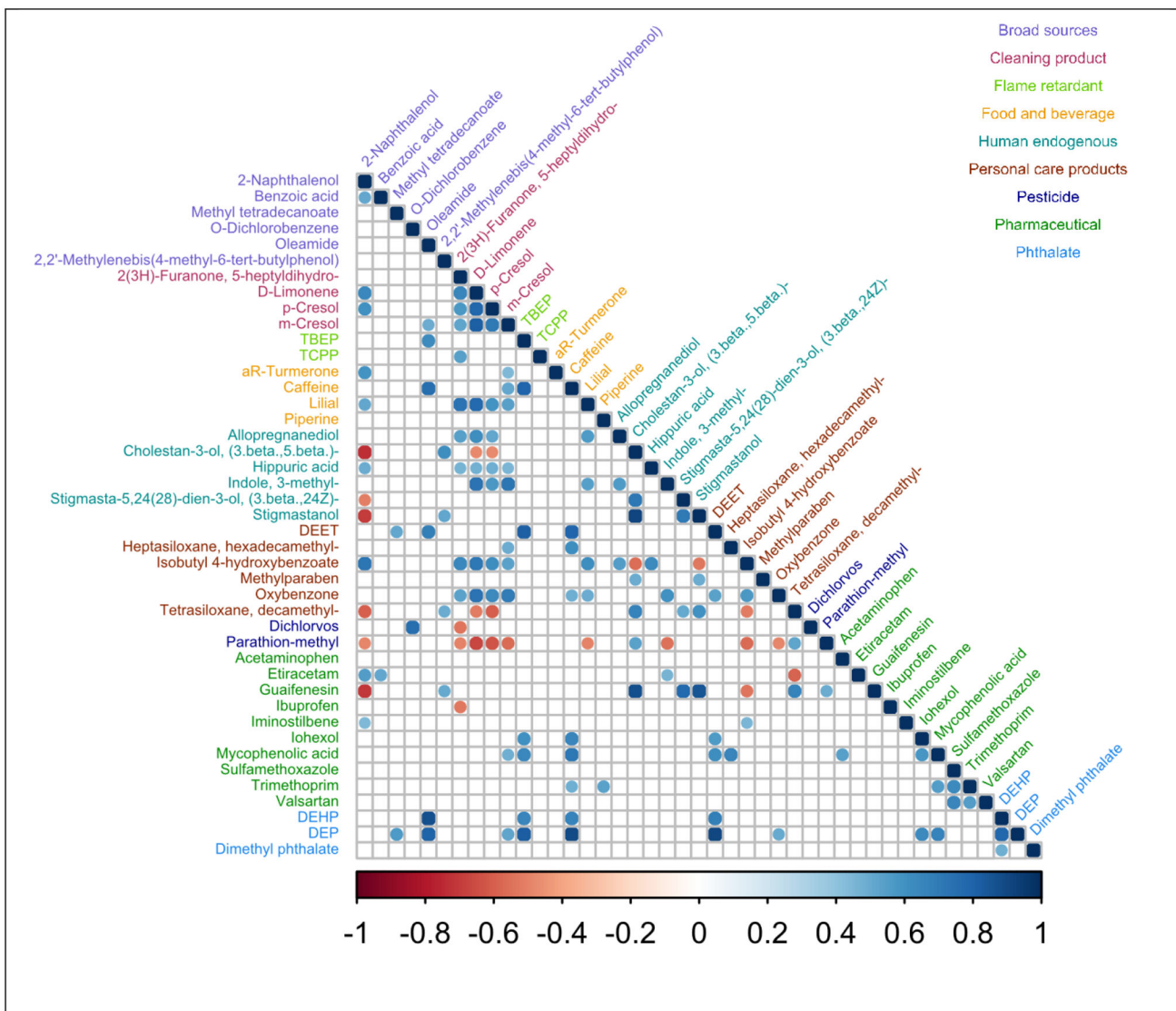


Figure 3. Correlation matrix showing statistically significant correlations between micropollutants identified in the sewer system. Compound names are colored according to general use, as shown by the key in the upper right corner.

Table 1:

Detection frequency in trunkline, influent, and effluent samples of quantified micropollutants

	Detection frequency		Detection frequency	
Caffeine	87.5%	Metoprolol	7.14%	
Carbamazepine	1.79%	O-Desmethyltramadol	5.36%	
DEET	92.9%	Oleamide	89.3%	
DEHP	58.9%	Sulfamethoxazole	44.6%	
DEP	75%	Tris(2-butoxyethyl) phosphate (TBEP)	92.9%	
Fexofenadine	12.5%	Trimethoprim	14.3%	
Iohexol	85.8%	Valsartan	58.9%	

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Table 2:

Detection frequency of quantifiable micropollutants in specialty sampling sites

Compound	Groomer (n = 4)	Laundry (n = 4)	PCO (n = 2)
Caffeine	100%	100%	100%
DEET	ND *	100%	100%
DEHP	25%	100%	100%
DEP	100%	100%	100%
Iohexol	ND	75%	ND
O-Desmethyltramadol	50%	ND	ND
Oleamide	25%	ND	100%
TBEP	100%	100%	100%
Valsartan	ND	ND	50%

* Not detected

Table 3:

Concentrations (ng/L) of infrequently detected* quantifiable compounds

Month	Site	Sulfamethoxazole	Fexofenadine	Trimethoprim	Metoprolol	Carbamazepine	O-Desmethyltramadol
May	C	43.8					
May	Effluent	6.54	59.6				
June	A	29.0					
June	C	25.3					
June	D	29.0					
June	Effluent	6.62	52.8				
July	C	51.6		102			
July	D	32.1					
July	Influent	26.4					
July	Effluent	22.5	196		23.1		
Aug	C	35.7					
Aug	D	25.4					
Aug	Influent	25.5		68.1			
Aug	Effluent	79.3	> Cal	21.7	174	63.3	54.2
Sep	A	40.9		70.0			
Sep	G						156
Sep	Effluent	19.2	365	12.3	74.5		32.7
Nov	A	43.5		60.3			
Nov	B		138				
Nov	C	132.4					
Nov	D	33.6					
Nov	E	28.4					
Nov	Influent	31.8					
Nov	Effluent	15.5	118		42.3		
Jan	B	27.3		66.1			
Jan	E	181.5		415			
Jan	Effluent	5.37					

* Only samples with at least one of the infrequently detected compounds are included in this table

Table 4:

Per capita load (mg person⁻¹ day⁻¹) comparisons found to be statistically significant using a Wilcoxon rank-sum test with a Benjamini-Hochberg test for multiple comparisons ($p < 0.05$)

Caffeine	May, Jun, Jan < Jul, Aug, Sep	May, Jun < Nov, Jan
DEET	May, Jun, Nov, Jan < Jul, Aug, Sep	May, Jun < Nov, Jan
DEHP	May, Jun < Jul, Aug, Sep, Nov, Jan	
DEP	May < Jun, Jul, Aug, Sep, Jan, Nov Nov, Jan < Jul, Aug, Sept	Jun < Jul, Aug, Sep, Nov, Jan
Iohexol	E < C	May, Jun < Aug
Oleamide	Inf < A, B, C, E, G	May < Sep, Jan
TBEP	May, Jun, Jan < Jul, Aug, Sep	May, Jun < Nov, Jan

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