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Correlation of Trace Contaminants to Wastewater Management Practices in Small Watersheds

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Abstract

Ten low-order streams draining headwater catchments within the East Fork Little Miami Watershed were evaluated throughout one year for the presence of six steroidal hormones, the antibiotic sulfamethoxazole, the antimicrobials triclosan and triclocarban, and the artificial sweetener sucralose. The wastewater management practices in the catchments included septic systems, sanitary sewers, a combination of both, and a parkland with no treatment systems. The concentrations and detection frequencies of sucralose showed a significant positive correlation with the septic density in each catchment. A similar relationship was found for sulfamethoxazole. Both sucralose and sulfamethoxazole are hydrophilic and unlikely to be removed effectively by sorption during septic treatment. The concentrations and detection frequencies of the antimicrobials were also positively correlated with septic density. The presence of the antimicrobials in the streams indicates that although they are hydrophobic, removal during septic treatment was incomplete. The target analytes that correlated with septic density were also detected in stream samples collected below a wastewater treatment plant located within the same watershed. The steroidal hormone, estrone, was the most frequently detected analyte at all sites. However, the estrone concentrations and detection frequencies did not correlate with the septic density due to multiple non-point sources.

Environmental Impact

Onsite wastewater disposal systems, or septic systems, provide nearly half of residential wastewater treatment in rural and suburban areas in the U.S. However, few studies have evaluated the impact of septic systems on streams. In the present study, ten streams draining headwater catchments were evaluated for the presence of ten organic contaminants throughout one year. The concentrations and detection frequencies of four xenobiotic compounds, sucralose, sulfamethoxazole, triclosan and triclocarban, and septic density in the individual catchments were significantly correlated, indicating incomplete removal by septic treatment. In contrast, concentrations and detection frequencies of the natural steroidal hormones evaluated were not correlated with septic density. These results establish a link between septic systems and the occurrence of four xenobiotic contaminants in headwaters.

Introduction

Numerous organic contaminants, including endocrine disrupting compounds (EDCs), pharmaceuticals, and personal care products, have been identified in surface waters in the U.S.[1-3] The majority of previous studies on the occurrence of trace organic contaminants in the aquatic environment have focused on water bodies, such as rivers and lakes, known to be impacted by wastewater treatment plant effluents.[1,4,5] The inputs to rivers and other water bodies with large drainage basins are often too numerous to establish linkages between contaminants and their sources, especially non-point sources. In contrast, the sampling of low-order streams draining headwater catchments has a greater possibility of identifying diffuse or non-point sources of contamination, such as septic systems and agricultural operations. In the U.S., approximately 20% of all households have onsite wastewater disposal systems, also known as septic systems.[6] These systems represent a larger portion of residential wastewater management in rural (50%) and suburban (47%) areas.[6] Most of the limited number of studies evaluating the impact of septic systems on water resources have focused on groundwater contamination.[7-12] The present study evaluated the occurrence of selected organic contaminants in ten low-order streams within the East Fork Little Miami Watershed located in Southwestern Ohio. Headwater catchments were selected to represent the use of septic systems, sanitary sewers, or a combination of both for wastewater management (Figure 1). One catchment area, a forested parkland, had no wastewater management systems present.

The organic contaminants determined in this study included six steroidal hormones, the antibiotic sulfamethoxazole, two antimicrobials found in personal care products, and the artificial sweetener sucralose. Although these trace organic contaminants are often present at concentrations below 1 µg/L in surface waters [1,3,5,13,14], concerns over known and potential ecological and human health risks associated with chronic exposure to these compounds have been expressed in the scientific literature, the popular media and by regulatory agencies. The Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency (EPA), has included several naturally occurring and synthetic estrogens (EDCs) on the current Contaminant Candidate List (CCL3) for possible regulatory action,[15] due to their documented adverse effects in the environment,[16,17] and in laboratory studies conducted at environmentally relevant concentrations.[18,19] The U.S. Food and Drug Administration (FDA) has recently issued a proposed rule that would require manufacturers to provide more substantial data to demonstrate the safety and effectiveness of antibacterial soaps, many of which contain triclosan and triclocarban.[20] Sulfamethoxazole at environmentally relevant concentrations has been shown to impact cell growth and denitrification in ground water.[21,22] The presence of sucralose in a water has been shown to indicate wastewater contamination.[23] Although the use of sucralose as a general purpose sweetener for foods has been approved by the U.S. FDA,[24] Wiklund et al. have suggested the possibility of ecological effects based on behavioral changes in crustaceans observed in laboratory studies.[25]

The surface waters were analyzed using two analytical methods, both including solid phase extraction and determination by ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS). The target analyte list included the steroid hormones 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, testosterone and progesterone; sulfamethoxazole; triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol); triclocarban (3,4,4'-trichlorocarbanilide); and sucralose.

Methods and Materials

Sampling sites

Surface water samples were collected from ten low-order streams within the East Fork Little Miami Watershed located in Southwestern Ohio. The headwater catchments selected for the study included Twin Bridges Stream crossing (TBS), Upper Hall Run (UHL), South Lucy Tributary (SLT), Shaylor Crossing (SHC), Upper Salt Run (USR), Grassy Fork Tributary (GRT), South Harsha Tributary (SHA), Heiserman Stream (HST), Owensville Tributary (OWT) and Newtonsville Stream (NWT) (Figure 1). Each sampling site was located at the headwater catchment outlet point where the low-order stream intersects the catchment boundary. The catchment areas ranged in size from 0.94 to 7.9 km^2 and varied with respect to the type(s) of wastewater management practices used (Table 1). The sites were categorized as predominantly served by septic systems or sanitary sewers based on the density of septic systems relative to the sewer line density within each catchment. No wastewater management systems were present in the TBS catchment, a forested parkland. Sites HST and USR have sandy clay loam as the predominant soil type. All other sites have sandy clay or clay loam as the predominant soil type. The catchment areas were obtained from the Soil and Water Assessment Tool (SWAT) model [26,27] being developed for the East Fork Little Miami River Watershed. Sanitary sewer pipe lengths and septic system counts were calculated from geographic information system (GIS) data layers obtained from the Clermont County Office of Environmental Quality (Batavia, OH).

The SWAT model was also used to estimate stream flows at the time grab samples for water chemistry were collected. The SWAT model uses the curve number approach to estimate rainfall-runoff relationships that also consider soil type, land use characterizations, and other geophysical properties of the small catchments. The SWAT model was calibrated and validated for hydrology based on two USGS gauging stations. Nexrad radar precipitation data (15 minute intervals) obtained from the National Weather Services' office in Wilmington, OH was used to drive the SWAT rainfall-runoff simulations. SWAT modelled flows were binned into high or low flow based on the median flow at each site, with flows above the median binned as high flow and those at or below the median flow considered low flow.

At the time of each collection event, temperature, specific conductivity, dissolved oxygen, pH, and turbidity were measured by placing a Yellow Springs Instruments (YSI, Inc) 6600 multi-probe sonde into the stream at the point of grab sampling. The sonde was left to equilibrate with the stream water column for 40 seconds, after which measurements were recorded at 20 second intervals for 2 minutes. Averages were computed for the 2 minute sampling interval for each parameter. Maximum, minimum, and median values for these parameters at each site are listed in Table S1 of the Electronic Supplementary Information.

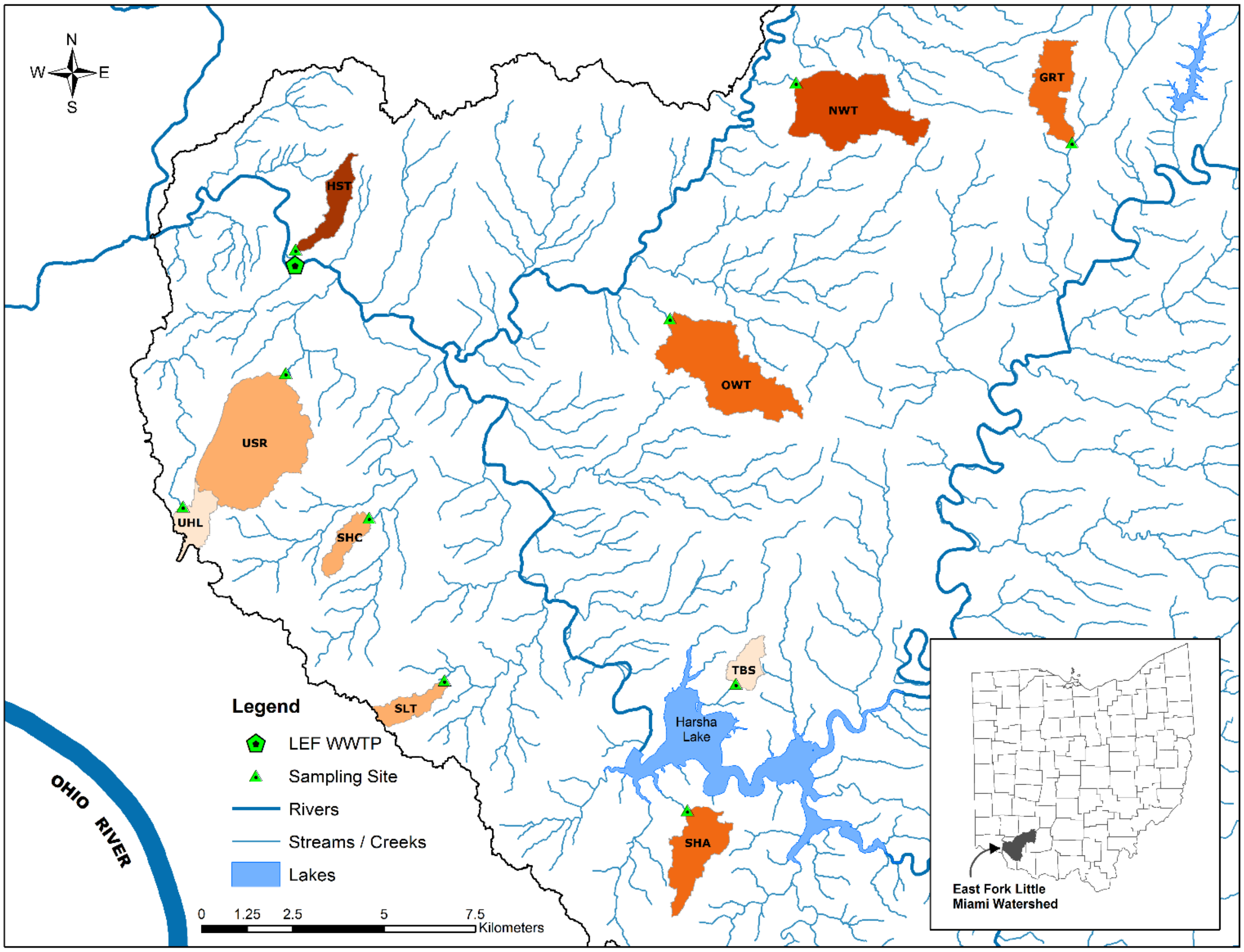

Figure 1. Map of the East Fork Little Miami Watershed and locations of 10 headwater catchments with sampling sites.

Table 1. Headwater Catchment Wastewater Management Practices

Site	Catchment Area (km^2)	Septic Density[a] ($\#/km^2$)	Sewer Density[b] (10^3 ft/km^2)	Predominant Wastewater Management Practice in Catchment
Twin Bridge Stream (TBS)	0.96	0.0	0.0	None
Upper Hall Run (UHL)	1.3	0.0	18	Sanitary Sewers
South Lucy Tributary (SLT)	1.0	2.9	23	Sanitary Sewers
Shaylor Crossing (SHC)	0.94	4.2	23	Sanitary Sewers
Upper Salt Run (USR)	7.9	4.2	17	Sanitary Sewers
Grassy Run Tributary (GRT)	2.3	19	0.0	Septic Systems
South Harsha Tributary (SHA)	2.6	31	1.1	Septic Systems
Heiserman Stream (HST)	1.4	86	1.7	Septic Systems
Owensville Tributary (OWT)	5.7	23	4.2	Septic Systems
Newtonsville (NWT)	5.2	43	0.0	Septic Systems

[a] Septic density equals the number of septic tank systems per km^2 in respective catchment area.
[b] Sewer line density equals the linear feet of pipe per km^2 in respective catchment area.

Sample collection and preservation

Samples were collected during a one year period from April 2013 to March 2014. Eighteen sampling events were conducted throughout the year, which included at least one sampling event in each month. For each event, grab samples from each of the ten streams were collected within one day. The use of grab sampling allowed for the evaluation of possible correlations between analytical results and changing stream conditions, such as flow. Two additional sampling events were conducted for which only sucralose and sulfamethoxazole determinations were made. At some sites, the total number of samples collected was lower due to road closures, but the number of samplings at each site was at least 15. For each sampling event, a field blank, duplicate and matrix spike samples were collected at one of the ten sampling sites. The site used for the field controls was rotated through the ten sites. Silanized glass bottles (Glenn Research Corp.) were used to collect the water samples. The water was immediately transferred to 600 mL silanized glass bottles containing 6 mg $CuSO_4 \cdot 5H_2O$ as a preservative. Personnel collecting samples wore gloves to avoid sample contamination. Samples were transported on ice, stored at 4°C, and extracted within 72 hours of collection.

Chemical analysis

Concentrations of target analytes were determined using UPLC/MS/MS. Sucralose and sulfamethoxazole were determined using automated on-line solid phase extraction (SPE) by Method 1. The remaining analytes were determined by Method 2, which included concentration by SPE followed by derivatization.

Reagents and standards

Analytical standards were prepared from neat estriol, estradiol, ethynylestradiol, estrone, testosterone and progesterone purchased from Steraloids (Newport, RI), and neat triclocarban purchased from Sigma-Aldrich (St Louis, MO) by creation of 1 mg/mL stock solutions in methanol (Optima grade, Fisher Scientific, Pittsburgh, PA). A certified standard of triclosan was purchased from Accustandard (New Haven, CT) and standards of sucralose and sulfamethoxazole were purchased from Absolute Standards (Hamden, CT). The stably labeled internal standards testosterone-d3, progesterone-d9, estriol-d3, triclosan-d3, bisphenol A-d8, and sulfamethoxazole-d4 were purchased from CDN Isotopes (Pointe-Claire, Canada); sucralose-d6 was purchased from Toronto Research Chemicals (Toronto, Canada); and 13C6-estradiol, 13C6-estrone, 13C2-ethynylestradiol, 13C6-triclocarban and the extraction surrogate bisphenol A-d16 were purchased from Cambridge Isotopes Laboratory (Tewksbury, MA). Acetone and hexane used in sample preparation were Optima grade (Fisher Scientific, PA). Reagent grade water was purified by MilliPore MilliQ using EDSPak carbon filtration as the final purification step. Dansyl chloride and $NaHCO_3$ were purchased from Sigma-Aldrich. Acetonitrile used in the UPLC separation was Chromasolve Plus grade (Sigma-Aldrich, MO), and $Na_2EDTA \cdot 2H_2O$ and the Puriss grade formic acid modifier were purchased from Fluka (Sigma-Aldrich, MO).

Method 1

Online concentration with determination by LC/MS/MS

Internal standards were added to a 10 mL aliquot of sample, which was filtered through a 0.2 micron GHP Acrodisc 13 mm syringe filter (Pall Corp. Ann Arbor, MI). The Thermo Scientific (Waltham, MA) Accela UPLC system included an Accela 600 loading and an Accela 1250 gradient pump. The injection volume of 1 mL was concentrated onto an Oasis® HLB 15 micron 2.1 X 20 mm concentrator column. The concentrator column was backflushed onto a Thermo Hypersil Gold 1.9 micron 2.1 X 50 mm analytical column, with separation by a water/acetonitrile gradient with formic acid used as a modifier. Identification of target analytes was performed using a Thermo Scientific Vantage Triple Stage Quadrupole (TSQ) operated in the selective reaction monitoring (SRM) mode. Identification of the target analytes was based on the presence of both quantitation and confirmation daughter ions. Quantitation was performed using internal standard calibration, with identification of the internal standards performed using a single transition. A minimum of five calibration standards were used. LC and MS parameters are detailed in the Electronic Supplementary Information.

Method 2

Solid phase extraction and derivatization:

Prior to extraction, Na_2EDTA (24 mg) was added to reduce the amount of copper present in the eluates. Bisphenol A-d16 (5 ng) was added as an extraction surrogate. Samples serving as positive controls were fortified with 1 ng of each target analyte. Samples were passed through glass fiber filters on top of 47 mm C18 Empore disks (3M, St. Paul, MN). Disks and filters were rinsed with 20 mL 20% (v:v) methanol in water then eluted with 10% acetone in methanol. The extract was spiked with a solution containing stably labeled analogues of each target analyte and concentrated to dryness.

Sensitivity was improved by reaction of the phenolic moiety with dansyl chloride. The residue was resuspended in 0.1 mL aqueous 0.1 M $NaHCO_3$, and 0.1 mg dansyl chloride in 0.1 mL acetone was added and allowed to react at 70°C for 5 minutes. As a cleanup step, the solution was extracted with three 0.5 mL portions of hexane. The organic layers were combined, concentrated to dryness and stored at -20°C until analysis.

Determination by LC/MS/MS

Concentrates were resuspended in 50:50 methanol:water. The analytes were separated by UPLC using a Restek Pinnacle DB Biphenyl 1.9 micron 2.1 X 100 mm analytical column and a water/methanol/acetonitrile gradient with formic acid used as a modifier. Analysis was performed using the instrumentation described above in SRM mode, with two transitions required for identification, and calibration using internal standards. Experimental details are provided in the Electronic Supplementary Information.

Quality assurance

Each sampling event included a field blank, duplicate and matrix spike samples from one collection site. A reagent blank and lab fortified reagent water (positive control) were included in each extraction batch. Acceptable recovery was 50-150% for all fortified controls. Acceptable recovery for the extraction surrogate was also 50-150%. All samples determined by method 1 were within quality control limits. Of the 173 samples determined by method 2, six failed quality criteria due to low surrogate recovery. One reagent water blank was found to have testosterone above the detection limit, and all testosterone data from that sampling day was excluded. In addition, three samples had broad co-eluting components that interfered with detection of testosterone. Data failing quality assurance criteria were excluded from statistical evaluation. The Lowest Concentration Minimum Reporting Limits (LCMRL)[28] and detection limits (DL) for each analyte are shown in Table 2. All concentrations above the DL were included in statistical evaluation of the data. For all duplicate samples with concentrations above the LCMRL, the relative percent difference were < 50%.

Statistical analysis

In order to evaluate possible relationships between the target analytes and septic density, two simple but telling indicators are nonparametric measures of association between septic density and detection frequency and between septic density and concentration. Spearman's rho (r_s) was used to compute nonparametric correlations and the associated p-values. Spearman's rho for variables X and Y is the Pearson correlation between the ranks of the X values and the ranks of the Y values. The rank transformation applied to variables X and Y transforms any monotone relationship (either increasing or decreasing but not both) between X and Y into a linear relationship. Since the Pearson correlation coefficient is a very effective statistic to detect and measure the strength of linear relationships, Spearman's rho is an effective tool to detect and measure the strength of general monotone relationships. It is a very useful tool for exploratory data analysis when there is no reason to believe that the relationships between variables should necessarily be linear, that the data are normally distributed, or that the variances are homogeneous.

When computing Spearman's rho all nondetects are treated as ties which are lower than any reported value. Thus the question of substitution values for nondetects does not enter into the data analysis. Also, since the rank of the largest of *n* values is always *n*, regardless of how large the actual value is, Spearman's rho is relatively robust against outliers.

In computing Spearman's correlation between septic density and detection frequency, the detection frequencies are aggregates (number of detections/number of measurements) by site. Each value of Spearman's rho is based on ten pairs of data, one for each site. In computing Spearman's correlation between septic density and concentration, each value of Spearman's rho is based on all measurements for the corresponding analyte.

Results and Discussion

Occurrence of target analytes in stream samples

Summary results for all the low-order stream samples analyzed in this study are presented in Table 2. The most frequently detected analyte was the steroid hormone estrone (82%), with the other naturally occurring steroidal hormones detected less frequently and at lower concentrations. The high frequency of estrone, relative to estradiol and estriol, the other major natural estrogens, may be due to the biodegradation of estradiol to estrone in surface waters.[29] Progesterone was detected in 50% of samples, but only 3% of samples were found to have progesterone concentrations above the LCMRL of 0.66 ng/L. Testosterone was detected in 34% of samples. The synthetic estrogen, ethynylestradiol, was the only analyte not detected at least once in this study. Although the current study analyzed grab samples from small streams that would be expected to be highly variable due to localized influences, it is notable that the values found in the current study are similar to those previously reported in a U.S. treatment plant study in which nineteen source waters were evaluated.[3]

Sulfamethoxazole was detected in 21% of the stream samples. This is consistent with the 19% detection frequency reported by Kolpin et al.,[2] even though their study targeted streams considered susceptible to contamination from various wastewater sources. In contrast, Benotti et al.[3] reported a detection frequency of 89% for drinking water sources. This discrepancy is likely due, at least in part, to a lower reporting level for sulfamethoxazole, approximately 35 times lower than the detection limit in this study. In addition to lower detection limits, the high detection frequencies reported for sulfamethoxazole by Benotti et al. may also reflect the cumulative impact of episodic use within large drainage basins, relative to the small watersheds sampled in the present study.

The analyte detected in the highest concentrations was the non-nutritive sweetener sucralose. Sucralose is widely used in the U.S., with an estimated daily intake of 0.1-2.0 mg/kg body weight[30] and passes through the body nearly unchanged.[13] The maximum concentration detected in this study, 7600 ng/L, was within the range (120-10,000 ng/L) reported by Oppenheimer et al.[23] for drinking water sources with known upstream point source wastewater discharges. In the same study, sucralose was not detected in source waters without wastewater discharges.

The antimicrobials, triclosan and triclocarban were frequently found together, which is consistent with the findings reported by Halden and Paull.[31] In samples with concentrations of triclocarban above the LCMRL, triclosan was always detected. Of the 21 samples in which triclosan was above the LCMRL, 20 had detectable triclocarban. Triclosan was detected in 56% of samples while triclocarban was detected in 32%. The greater frequency of detection of triclosan may be due, at least in part, to its lower detection limit. The triclosan concentration range and detection frequency are again comparable to those found in source waters by Benotti et al.[3] Kolpin et al.[2] also reported a similar detection frequency for triclosan (58%), even though their reporting limit (50 ng/L) was considerably higher than that of the present study. Their triclosan concentration range was much higher, possibly because their study targeted streams susceptible to contamination by wastewaters.

Table 2. Analytical Results

Target analyte	CAS	N	DL (ng/L)	Detection Frequency (%)	LCMRL (ng/L)	Frequency >LCMRL %	Median (ng/L)	Maximum (ng/L)
Sucralose	56038-13-2	193	70	49	162	44	bdl[a]	7600
Sulfamethoxazole	723-46-6	193	8.6	21	14	16	bdl	540
Testosterone	58-22-0	154	.044	34	.092	18	bdl	1.9
Progesterone	57-83-0	167	.11	50	.66	3.0	0.11	1.1
Estriol	50-27-1	167	.46	1.2	1.38	0.6	bdl	2.1
Estradiol	50-28-2	167	.072	8.3	.26	0.6	bdl	0.45
Ethynylestradiol	57-63-6	167	.064	0	.14	0	bdl	Bdl
Estrone	53-16-7	167	.064	82	.28	45	0.23	4.1
Triclosan	3380-34-5	167	.11	56	1.28	12	0.18	22
Triclocarban	101-20-2	167	.32	32	1.48	9	bdl	94

[a] below detection limit

Correlation of xenobiotics to septic density

Figure 2 shows the concentrations and detection frequencies of sucralose, sulfamethoxazole, the antimicrobials, triclosan and triclocarban, and estrone at each of the individual collection sites, graphed in order of increasing number of septic systems in the catchment area. Concentrations and detection frequencies for stream samples collected approximately 1.5 to 2 meters below the outfall of the Lower East Fork Wastewater Treatment Plant are also included. The concentrations and detection frequencies of the four xenobiotic analytes generally increased with increasing number of septic systems (Figure 2A, 2B and 2C). In contrast, there appears to be no relationship between the number or density of septic systems and the concentrations and detection frequencies of estrone, suggesting additional sources of the hormone (Figure 2D).

The sucralose concentrations and the septic density in the individual catchments have a strong and highly significant positive correlation, with an r_s value of 0.79 and p value < 0.001 (Figure 2A). The positive correlation between sucralose detection frequencies and the septic density is also strong and significant ($r_s = 0.90$ and $p < 0.001$). In the present study, sucralose was never detected in the TBS catchment, a forested parkland, without any wastewater management systems. In catchments predominantly served by sanitary sewers, and with less than 5 septic systems, the detection frequency was 6% or less. One of these sites, UHL, had a single sample with a sucralose concentration of 160 ng/L, following excavation around a sewer line near the collection site. The largest catchment area, USR, is predominantly served by sanitary sewers, but the presence of 33 septic systems could account for the detection frequency of 56% and a median sucralose concentration of 140 ng/L. In the five catchment areas predominantly served by septic systems, the average sucralose detection frequency was 82%, with a median concentration of 475 ng/L.

Sucralose has been suggested as an indicator of domestic wastewater impact on waters in the U.S.[14,23,32] In the present study, the results indicate that sucralose is an indicator of septic system impacts on headwaters. This is due to the low sucralose removal rates of 6 - 24% by various types of septic systems.[33] Septic systems mainly rely on the processes of settling, adsorption and anaerobic and aerobic biodegradation to remove contaminants from domestic wastes.[34] Sucralose has been reported to be resistant to anaerobic and aerobic biodegradation,[35,36] and its removal by adsorption is limited due to its hydrophilicity with a K_{ow} of 0.3.[37]

The correlation between sulfamethoxazole and the septic density in each catchment is similar to that of sucralose (Figure 2B). Both the concentrations and detection frequencies of sulfamethoxazole and the septic density are significantly correlated, with r_s values of 0.41 and 0.84, respectively, and p values of < 0.001 and 0.002, respectively. Similarly to sucralose, this correlation is believed to result from the persistence of sulfamethoxazole through septic system treatment. Sulfamethoxazole is highly water soluble,[38] and thus not likely to be removed by adsorption during septic system treatment, but can be biodegraded under aerobic conditions with sufficient reaction time.[38-40] Du et al.[33] reported sulfamethoxazole removal rates of between 8 and 48% by various types of septic systems. The incomplete removal of sulfamethoxazole by septic treatment has been reported to account for its presence in ground water[10] and in ponds fed by ground water.[41] However, in small watersheds sulfamethoxazole would not necessarily function as a marker for septic system impacts due to its low detection frequency related to its episodic use. Additionally, sulfamethoxazole is used in veterinary practice[42] and its presence could be due to domestic animals.

The concentrations and detection frequencies of the antimicrobials and the septic density in each catchment are also significantly correlated, with r_s values of 0.50 and 0.78, respectively, and p values of < 0.001 and 0.007, respectively (Figure 2C). However, there is an obvious deviation at site UHL. This site had the highest measured concentration, which was detected following initiation of excavation near the collection site. The high concentration of antimicrobials observed following excavation highlights the fact that many factors, in addition to septic systems, can greatly affect the concentrations of organic wastewater contaminants in small streams. Similarly, in a survey of streams in the Neuse River basin in North Carolina, a suspected sewer line leak had a greater effect on the detection frequencies of organic wastewater contaminants than the type of wastewater management practice used.[43]

Triclosan and triclocarban are hydrophobic, with a K_{ow} of 4.8 and 4.9 at pH 7, respectively,[31] and would be expected to be removed during septic treatment by adsorption. Removal of triclosan from septic tank effluent has been reported as greater than 97% in 60 cm of sandy loam subsoil.[44] The correlation between antimicrobial concentrations and detection frequencies and septic density observed in this study can be attributed to the low detection limits, 0.11 ng/L for triclosan and 0.32 ng/L for triclocarban, and the high usage rates. The per capita annual usages of triclosan and triclocarban are 1130 and 1030 mg/year, respectively,[31] and concentrations of triclosan in raw wastewater have been reported to be in the range of 10 μg/L.[45] Thus, a high production volume compound that is effectively removed during on-site treatment may be detected in small streams.

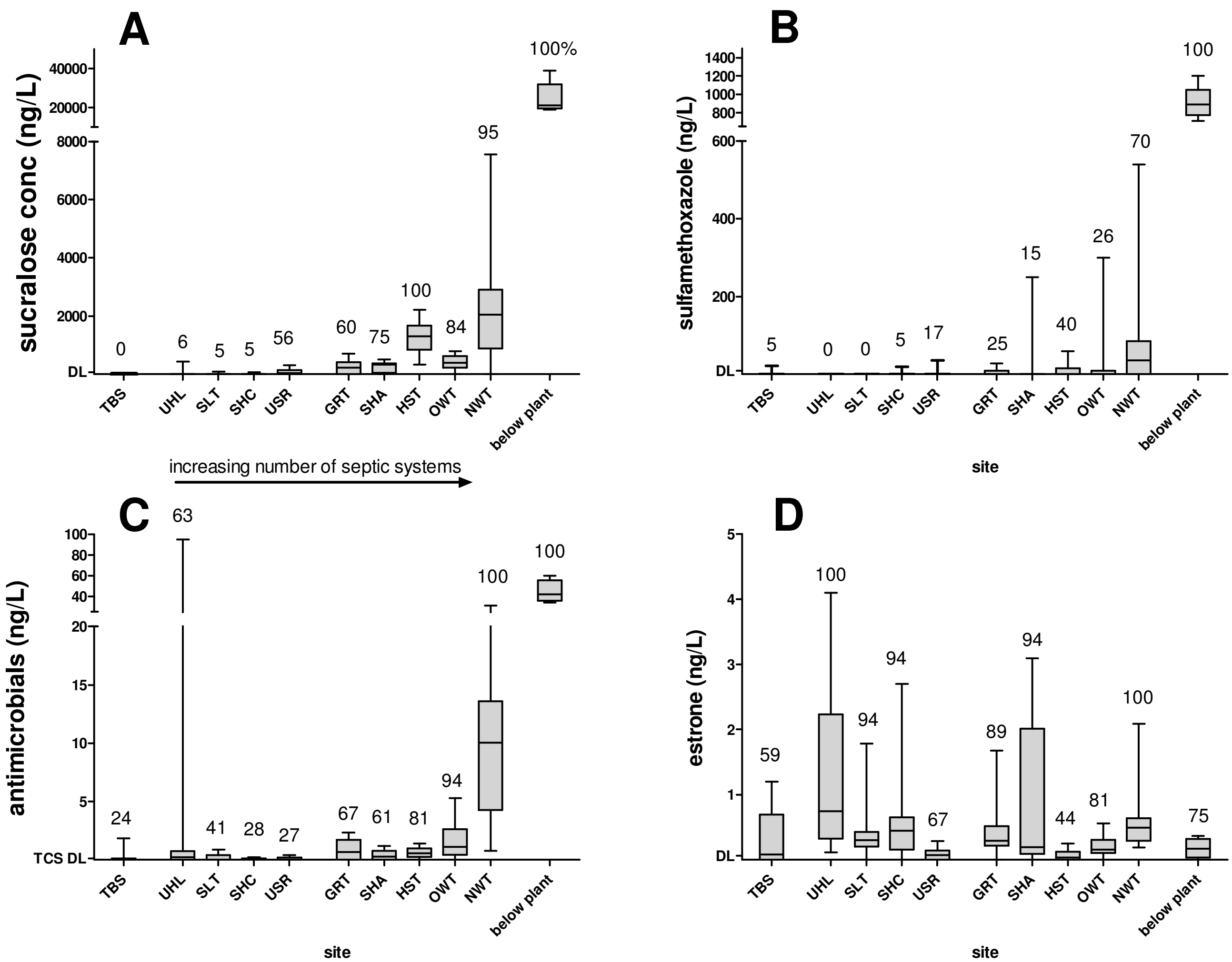

Figure 2. Detection frequencies and concentration ranges of selected analytes at individual collection sites. The box covers the 25th-75th percentile, with the median indicated by a line, and the whiskers indicate minimum and maximum. The number above the whiskers indicates detection frequency.

Absence of correlation between estrone and septic density

In contrast to the trend of increasing concentrations and detection frequencies associated with septic density for the xenobiotic compounds, estrone concentrations and detection frequencies have no apparent correlation with septic density. Estrone was detected in 59%, 96%, and 83% of samples from parkland, catchments with sewers, and catchments with septic systems, respectively. The lack of correlation of estrone to septic systems may be due to both effective removal by septic treatment and additional sources of estrone unrelated to septic systems.

The removal of estrogenic activity during septic treatment has been previously studied by Wilcox et al.[46] Their results showed that the use of advanced treatments, either aerobic treatment or sand filtration, reduced the levels of estrogenicity in the septic effluents by a factor of ten compared to traditional treatment. Thus, for removal of estrogenicity, the type of septic treatment used may be of greater importance than the total number of systems. The HST catchment, with 121 septic systems, has the lowest detection frequency of estrone, one of the compounds responsible for estrogenic activity in wastewaters. This low detection frequency may be due in part to the HST catchment having the highest percentage of systems with advanced treatment; two-thirds of the systems use aerobic digestion or sand filters.

The majority of sampling sites in this study were located in rural areas, consistent with the predominance of onsite wastewater management in five of the catchments. Domestic and wild animals are possible sources of estrone, and individual sites were investigated for evidence of contamination by animals. The two parkland collection sites, TBS and SHA, were near horse trails, and the estrone concentrations were highest at these sites during the summer when trail use would be greatest. GRT and OWT sites were adjacent to farms with domestic animals. The SHC catchment included a retention pond with a large population of geese. During excavation near the UHL site, the leakage of untreated sewage likely contributed to the presence of contaminants, including estrone, in the stream samples. Thus, the presence of multiple non-point sources of estrone prevented its attribution to septic systems.

Effects of streamflow, sewer overflows, wastewater reuse, season, soil composition, and septic system condition

In addition to the septic density within each catchment, the impact of streamflow, sewer overflows, wastewater reuse, season, soil composition, and septic system condition were considered. To determine the possible impact of streamflow on the correlations between the xenobiotic compounds and septic density, the modelled streamflow data were binned into high and low flow sampling events which were evaluated separately using Spearman's rho. The concentrations of the xenobiotic compounds and septic density were positively correlated during both high and low flow samplings (p values of <0.001). The correlations between the detection frequencies for the xenobiotics and septic density were also significant during high flow (p values of <0.005). During low flow samplings, the correlations between the detection frequencies for sucralose and sulfamethoxazole and septic density were significant (p values of <0.005). The correlation between the detection frequencies of the antimicrobials and septic density was also significant during low flow when site UHL, which was undergoing excavation only during low flow samplings, was excluded (p value <0.05).

There were two sampling events potentially impacted by overflows reported to the Ohio EPA. One reported overflow, occurring within the week prior to sampling, may have impacted SHC. The second overflow potentially impacted both SHC and USR. The single detection of sulfamethoxazole at SHC occurred following one of the reported overflows. With this exception, no other differences were observed between the samplings following overflows and the other sampling events. No indications of wastewater reuse were found in the headwater catchments evaluated in this study. None of the fields authorized by Ohio EPA for land application of biosolids were located near the catchments.[47] No golf courses, which may use reclaimed water for irrigation, drain into the study catchments. The only seasonal pattern observed was increased estrone in the summer at sites SHA and TBS, both in parkland used for horse riding. High organic content and permeability in soils would be expected to enhance adsorption of the antimicrobials and steroids limiting transport.[48,49] Of the 10 catchments evaluated, 8 have primarily clay soils with very low permeability, and HST and USR catchments have predominantly sandy clay loam with low permeability, providing limited adsorption capacity. Design and maintenance also impact septic system treatment effectiveness. Systems which incorporate advanced treatments, such as aerobic conditions and sand filtration, can result in greater removal of contaminants as previously discussed. Maintenance information was not readily available, except for site NWT. The septic systems in this catchment were determined to have a high failure rate (>30%) based on a survey conducted in 2011.[50] However, the correlations between the concentrations and detection frequencies of the xenobiotic compounds and septic density would remain significant, even if the data for the NWT site are excluded.

Comparison of headwaters to a wastewater impacted stream

To permit comparison between the headwaters and a wastewater impacted stream, a limited number of samples were collected below the outfall of the Lower East Fork Wastewater Treatment Plant (LEF WWTP) which processes the majority of wastewater from SHC, UHL, and USR. The LEF WWTP has a design flow of 9 MGD, uses an aerobic activated sludge process for secondary treatment with a sludge retention time of 18 days, and ultraviolet light disinfection. The contaminants found to correlate with septic density in small streams were detected in all samples collected below the plant at median concentrations above those observed in the headwater catchments as shown in Figure 2. In contrast, the detection frequency and median concentration of estrone below the WWTP were within the range of those observed for the headwaters. These findings are consistent with the similarity of treatment processes, including settling, adsorption and anaerobic and aerobic biodegradation, used in both on-site and centralized wastewater treatment.

Conclusions

The sampling of low-order streams draining headwater catchments allowed for the identification of on-site wastewater management systems as the primary source of the xenobiotic compounds evaluated in this study. The current study is unique due to the number of sites and samples evaluated and the low analyte detection limits, which contributed to the determination of the correlations between the xenobiotic compounds and the septic density within each catchment. Estrone, an estrogenic compound present in wastewaters, was frequently detected in the small streams in this study. However, the concentrations and detection frequencies of estrone could not be attributed to septic systems due to the presence of multiple non-point sources, including domestic and wild animals. The adverse effects of estrogenic compounds on aquatic organisms at low concentrations have been well documented,[16,17] but the potential for adverse effects on stream ecosystems due to the continual introduction of other wastewater contaminants is not completely understood.[51]

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Notes and references

Electronic Supplementary Information (ESI) available: 6 tables with detailed collection site information and LC/MS/MS acquisition parameters. See DOI: 10.1039/b000000x/

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