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Temporal variation of pharmaceuticals in an urban and agriculturally influenced stream

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ABSTRACT

Pharmaceuticals have become ubiquitous in the aquatic environment. Previous studies consistently demonstrate the prevalence of pharmaceuticals in freshwater but we do not yet know how concentrations vary over time within a given system. Two sites in central Indiana with varying land use in the surrounding watershed (suburban and agricultural) were sampled monthly for pharmaceutical concentrations and stream physiochemical parameters. Sediment samples were also collected at each sampling event for measurement of $\delta^{15}N$ natural abundance and sediment organic content. Across sites and sampling events, twelve pharmaceuticals were detected including acetaminophen, caffeine, carbamazepine, cotinine, N,N-diethyl-meta-toluamide (DEET), gemfibrozil, ibuprofen, sulfadimethoxine, sulfamethazine, sulfamethoxazole, triclosan, and trimethoprim. Sulfathiazole, lincomycin, and tylosin were not detected at either site at any time. The agriculturally-influenced site had comparable pharmaceutical concentrations to the urban-influenced site. In general, pharmaceutical concentrations increased during winter at both sites and decreased during spring and summer. Multiple regression analyses indicated that water column dissolved oxygen, the number of days since precipitation, and solar radiation influenced total pharmaceutical concentration in the urban-influenced site; whereas pH, chlorophyll *a* concentration, and total amount of rainfall in the previous 10 days influenced total pharmaceutical concentrations in the agriculturally-influenced site. Pharmaceutical concentrations were not correlated with sediment $\delta^{15}N$ across or within sites. However, sediment in the urban-influenced site had higher mean $\delta^{15}N$ signatures relative to sediment in the agriculturally-influenced site. These data indicate pharmaceuticals are persistent in aquatic ecosystems influenced by both agricultural and suburban activity. Pharmaceuticals are designed to have a physiological effect; therefore, it is likely that they may also influence aquatic organisms, potentially threatening freshwater ecosystem health.

1. Introduction

Human populations have been increasing by approximately 78 million people per year (Jones, 2000). In addition, by the year 2025 almost two thirds of the world's population will live in urbanized locations (Pimentel et al., 2007). In conjunction with an increasing human population, contamination of freshwater resources due to human activities will also likely increase (Pimentel et al., 2007). Emerging contaminants in freshwater ecosystems is expected to increase with greater human populations including trace organics, such as pharmaceuticals.

Pharmaceuticals have been recognized as an environmental threat since the 1970s when compounds were first detected in freshwater (Kummerer, 2004; Tabak and Bunch, 1970). Not only have pharmaceutical contaminants been detected in aquatic environments throughout the United States (e.g., Barnes et al., 2008; Focazio et al., 2008; Glassmeyer et al., 2005; Kolpin et al., 2002; Kolpin et al., 2004), but these contaminants have also been detected in freshwater around the world (e.g., Camacho-Muñoz et al., 2010; Daneshvar et al., 2010; Sim et al., 2010; Vieno et al., 2005). Despite the ubiquity of pharmaceuticals in freshwater ecosystems, there has been limited quantification of environmental transport and fate of these compounds.

Pharmaceuticals and personal care products (PPCPs) can enter freshwater ecosystems via multiple sources including human excretion, drug disposal, and agricultural runoff associated with therapeutic treatment of livestock (Jorgensen, 2000). Higher concentrations of pharmaceuticals in freshwater are generally associated with inputs from wastewater treatment effluent (Phillips et al., 2005; Walraven and Laane, 2008) but concentrations vary with secondary waste treatment processes (Bartelt-Hunt et al., 2009; Phillips et al., 2005). Further, although effluent is thought to be a primary source of pharmaceutical pollutants, Kolpin et al. (2002) sampled 139 streams across the U.S. not influenced by wastewater and found >80 organic waste contaminants from residential, industrial, and agricultural sources. Additionally, Barnes et al. (2008) sampled 47 groundwater sites suspected to be contaminated from animal and human waste but not receiving effluent and also detected multiple pharmaceutical compounds. It is clear that PPCPs have become pervasive in freshwater, yet factors related to their persistence are still largely unknown.

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Pharmaceutical frequency of detection and concentrations detected in freshwater differ among individual compounds. For example, acetaminophen, caffeine, ibuprofen, and cotinine have been found at maximum concentrations of 10 μg/L (Kolpin et al., 2002), 8 μg/L (Glassmeyer et al., 2005), 25.4 μg/L (Camacho-Muñoz et al., 2010), and 1.03 μg/L (Glassmeyer et al., 2005), respectively. However, acetaminophen (50% detection frequency) and ibuprofen (36%) are not detected as frequently in freshwaters relative to caffeine (82.6%) and cotinine (92.5%) (Daneshvar et al., 2010; Glassmeyer et al., 2005; Kolpin et al., 2004). Compounds with the greatest detection frequencies across studies include caffeine, carbamazepine, cotinine, DEET, and sulfamethoxazole (>70% samples collected contain concentrations above detection limits) whereas, gemfibrozil, sulfamethazine, and sulfadimethoxine have been infrequently detected (<5% samples) (Glassmeyer et al., 2005; Kolpin et al., 2002; Kolpin et al., 2004). Similarly, acetaminophen, carbamazepine, DEET, and ibuprofen are detected at concentrations an order of magnitude greater than sulfamethazine, sulfadimethoxine, and trimethoprim (Barnes et al., 2008; Camacho-Muñoz et al., 2010; Kolpin et al., 2002). The variability in detection frequency and concentrations measured is likely due to usage rates, surrounding inputs and land use, as well as individual chemical properties (e.g., carbamazepine is more persistent than gemfibrozil). Further, variability may also be due to the

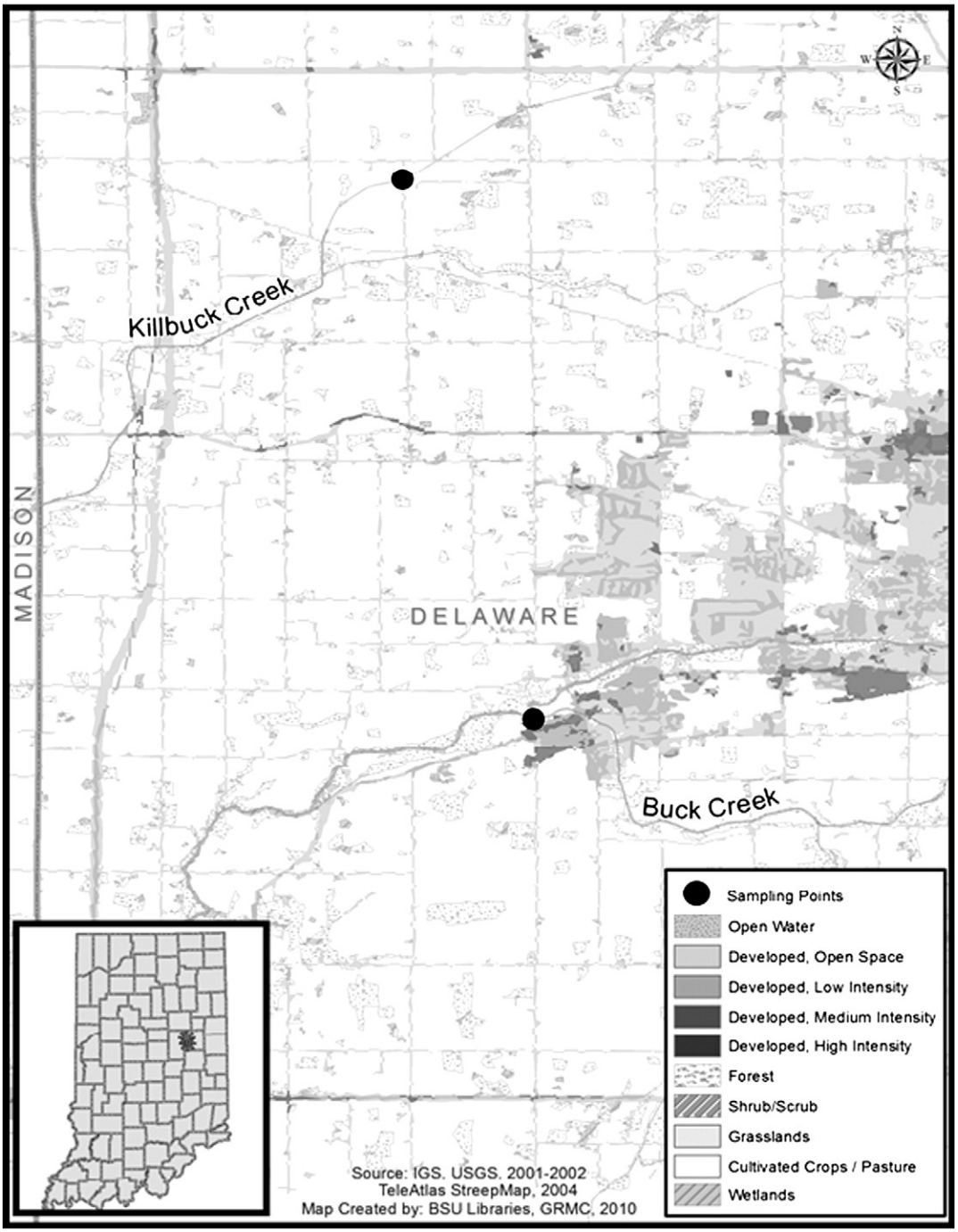


Fig. 1. Sampling locations in the Upper White River Watershed of central Indiana including an agricultural site (Buck) and an urban/suburban site (Killbuck). Surrounding land use denoted by shading.

Table 1
Study site characteristics. Values reported are means (ranges) of 24 sampling events. NO_3-N = dissolved nitrate; DO = dissolved oxygen in water column; PO_4-P = phosphate. * denotes parameters significantly higher in Buck Creek than Killbuck Creek ($p \leq 0.05$).

Study site	Land use	Discharge* (m^3/s)	pH	Temperature (°C)	DO* (mg/L)	NO_3-N (mgN/L)	PO_4-P (mgP/L)
Buck Creek	Suburban	2.4 (0.8–6.9)	7.8 (5.8–10.9)	10.8 (0.8–20.3)	15.1 (9.6–19.19.0)	3.5(0.01–15.3)	0.18 (0.001–1.1)
Killbuck Creek	Agricultural	0.43 (0.03–2.0)	7.1 (4.9–8.9)	11.7 (0.5–26.8)	11.8 (5.0–17.3)	4.7 (0.01–18.9)	0.28 (0.001–3.0)

timing of sample collection as temperature, and flow may influence abundance or persistence of pharmaceutical compounds. Few studies have quantified temporal variability within a given site to quantify the role of these influential factors (but see Daneshvar et al., 2010; Vieno et al., 2005).

The objective of this study was to quantify temporal variation in pharmaceutical concentrations in a suburban and agriculturally-influenced stream in central Indiana, as well as to identify environmental stream variables and sediment gradients related to the observed pharmaceutical concentrations. We hypothesized that pharmaceutical concentrations would be higher in the urban-influenced stream due to human waste contribution, as combined sewer overflows (CSO). We further hypothesized that both sites would have lower pharmaceutical concentrations in winter due to fewer overflow events and lower temperatures.

2. Material and methods

2.1. Study sites

Study streams were located in the Upper White River Watershed (UWRW) of central Indiana (Fig. 1). The UWRW encompasses a total of 174,830 acres with a gradient of both urban and agricultural land use. The two sites selected within the watershed (Buck and Killbuck Creek) were representative of differing land uses within corresponding sub-watersheds. Buck Creek is predominantly influenced by urban/suburban inputs whereas Killbuck Creek is influenced by agricultural inputs, specifically row-crop agriculture (Table 1). Buck Creek is located in the Buck Creek sub-watershed and drains 16,090 acres with silt loam soils compromising ~50% and loam comprising ~25% of the sub-watershed area. The present day land use within the sub-watershed is ~54% agriculture, primarily corn and soybeans; ~28.6% residential and greenspace, 11.6% commercial and transportation utilities, 2.9% industrial, and 0.4% government and institutional (White River Watershed Project, 2001). Killbuck Creek is located in the Killbuck/Mud Creek sub-watershed and drains 10,039 acres with silt loam soil compromising ~50% and silty clay loam comprising ~20% of the sub-watershed area. The present day land use in Killbuck Creek is 74.4% agricultural, 21.1% residential and greenspace, 4.1% commercial and transportation utilities, 0.4% industrial, and 0.4% government and institutional. *Escherichia coli* contamination has been found in Killbuck Creek due to septic system leakage (White River Watershed Project, 2001).

The Buck Creek sampling site was located ~9.7 km downstream of the last combined sewer overflow (CSO) in Muncie, Indiana and is influenced by a total of six CSO points upstream of the sampling site. Wastewater in the sub-watershed is treated via an aerobic activated sludge process unless discharged as raw sewage via CSO during high rainfall events. Killbuck Creek is influenced by residential septic tanks upstream of the sampling site. Due to the combined sewer overflow discharge solely present in Buck Creek, and septic tank leakage near Killbuck Creek, Buck Creek is considered the suburban site whereas Killbuck Creek is considered the agricultural site although other sources of waste may exist for both. Both sites are third order headwater streams with similar topography, geology and soils (White River Watershed Project, 2001).

2.2. Pharmaceutical collection

For this study, all compounds will be broadly defined as pharmaceuticals although certain compounds analyzed are considered medicinal drugs. Buck Creek and Killbuck Creek were sampled twice each month for a 12 month period (June 2009–May 2010). During the first sampling event each month, water samples for pharmaceutical analyses were collected during early to late morning in addition to measurement of physiochemical parameters and water column nutrient concentration measurements. The second sampling event each month consisted only of physiochemical parameter and nutrient concentration measurements. For the first sampling event each month, two filtered water samples (1000 mL; 150 mL) were collected and filtered on site using a 60 mm syringe connected to a syringe filter containing a 25 mm Whatman© glass fiber filter (GF/F; 0.7 μm pore size). Each water sample was collected from the middle of the water column in a well-mixed portion of the stream. The first water sample was filtered into a 1 L amber baked glass bottle containing the dechlorinating agent sodium thiosulfate as a preservative. After filtration, the water sample was placed on ice and shipped overnight to the State Hygienic Laboratory at the University of Iowa for analysis of 15 pharmaceutical analytes: acetaminophen, caffeine, carbamazepine, cotinine, DEET, gemfibrozil, ibuprofen, lincomycin, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfathiazole, triclosan, trimethoprim, and tylosin using a Micromass Quattro tandem quadruple system equipped with a Waters 2995 high performance liquid chromatography (HPLC) autosampler with an electrospray interface to generate ions from the HPLC column flow (Table 2). Pharmaceutical measurements were

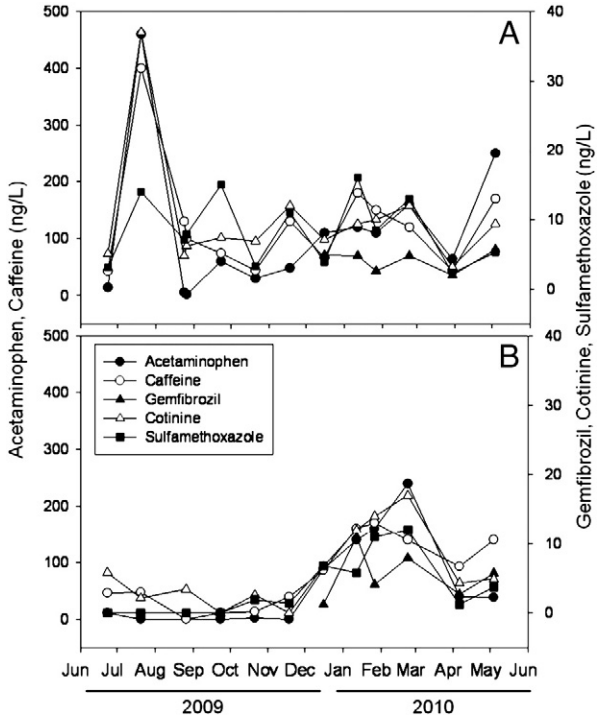
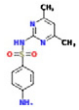
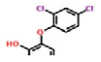
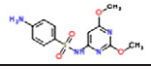


Fig. 2. Temporal patterns of pharmaceutical concentrations for high frequency compounds in A) Buck Creek and B) Killbuck Creek.

Table 2
Pharmaceutical frequency of detection (%) and concentration range for all analytes detected in Buck (N = 13) and Killbuck Creek (N = 12) combined. Analytes not detected: lincomycin, sulfathiazole, tylosin.

Compound	Chemical structure	Primary use	Frequency (%)	Ranges (ng/L)
Gemfibrozil		Lipid regulating agent	100	1.2–11
Caffeine		Stimulant	96	11–400
Cotinine		Nicotine by-product	92	2.1–37
Acetaminophen		Analgesic/fever reducer	84	1.6–460
Sulfamethoxazole		Human antibiotic	84	1.2–16
Ibuprofen		NSAID/pain reliever	68	1.8–42
DEET		Insect repellant	64	8–290
Carbamazepine		Anticonvulsant	28	1.1–2.7
Trimethoprim		Human Antibiotic	28	3–58
Sulfamethazine		Veterinary antibiotic	20	1.2–22
Triclosan		Antiseptic/ disinfectant	12	9.1–22
Sulfadimethoxine		Veterinary antibiotic	4	2.2

determined via a calibration curve with a quadratic curve fit using standards of ≥5 concentration levels including a range from 0.1 to 100 ng/mL. In addition, one laboratory blank was also analyzed for pharmaceuticals with each set of water samples each month in conjunction with QAQC assessments. If contamination was found, the reporting level was increased to 5 X the amount found in the blank. For most laboratory blanks, trace concentrations of caffeine and DEET were measured.

A total of 25 samples were analyzed for all pharmaceutical compounds except for gemfibrozil which was not included in the analyses until December 2009, yielding a total of 12 samples analyzed for gemfibrozil. All individuals assisting with pharmaceutical sample collection did not ingest or use any pharmaceuticals included in the analyte list for ≥24 h prior to collection and were required to wear neoprene gloves during water sample collection to prevent contamination.

2.3. Measurement of independent variables

The second water sample collected at all sampling events was filtered into a 150 ml acid washed Nalgene© bottle, placed on ice, returned to the laboratory, and immediately frozen for subsequent chemical analyses of anions and cations including: nitrate (NO_3-N), phosphate (PO_4-P), chloride (Cl^-), sulfate (SO_4^{2-}), bromide (Br^-), ammonium (NH_4-N), lithium (Li^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) using ion chromatography (DIONEX, ICS-3000). Stream physiochemical parameters were also measured at each sampling event using a Hydrolab© minisonde equipped with a Luminescent Dissolved Oxygen (LDO) sensor for measurement of water column dissolved oxygen (mg/L) as well as temperature (°C), pH, total dissolved solids (g/L), specific conductivity (mS/cm), and turbidity (NTU). The minisonde was placed in a well-mixed portion of the stream for measurements and allowed to equilibrate. Discharge (m^3/s) was estimated at each sampling event using a line transect with 5 equidistant points measured for depth and velocity using a Marsh-McBirney© flow meter. Chlorophyll *a* concentrations (μg/L) were measured using a hand-held Aquaflor© fluorometer. Precipitation data, the number of days since precipitation at the time of sampling, and the total amount of rainfall within 10 days of sampling, were provided from the National Weather Service (National Weather Service, 2010) using data for Muncie, Indiana and reported in millimeters. Solar irradiation measurements were measured in-stream using a Apogee© Basic Quantum meter Model BQM-S and reported as $\mu mol\, m^{-2}\, s^{-1}$. Combined sewer overflow data was provided by the town of Muncie, Indiana Waste Pollution Control Facility CSO Discharge Monitoring Reports.

2.4. Sediment collection

At each sampling event, three sediment samples were collected from the top 5 cm of the stream benthos at equidistant points across the reach. Sediment was transported to the laboratory and subsequently dried at 15.5 °C in a Model 30 GC Laboratory Oven. After drying, a sub-sample of all 3 sediment samples were combined and homogenized using a 2.38 mm USGS no. 5 sieve, crushed with a coffee grinder, and sent to the Marine Biological Laboratory (Woodshole, MA, USA) for analysis of $\delta^{15}N$ natural abundance and nitrogen content via mass spectrometry following combustion. The remaining sediment (≥3 g) was pre-weighed and placed in aluminum weighing boats, ashed in a Barnstead Thermolyne© FB 1400 muffle furnace for ≥2 h and then weighed to calculate percentage of organic matter content for each sediment sample.

2.5. Statistical analyses

Pharmaceutical concentrations were analyzed as both the concentration of individual pharmaceutical compounds as well as the total pharmaceutical concentration which was calculated as the sum of all pharmaceutical compounds detected. Bonferroni-corrected Pearson correlation coefficients were used to evaluate potential relationships between physiochemical parameters, sediment characteristics (i.e., sediment $\delta^{15}N$, % N, or % organic matter), precipitation measurements, and solar radiation with individual and total pharmaceutical concentrations both with sites combined (N = 25 sampling events) and within an individual site (Buck Creek N = 13; Killbuck Creek N = 12 sampling events). Bonferroni-corrected Pearson correlation statistics were also used to assess factors influencing sediment characteristics. All independent variables measured (N = 17) were included in correlation matrix analyses. Multiple regression with backward elimination analyses were used to develop predictive models describing factors influencing pharmaceutical concentrations both with sites combined (N = 25 sampling events) and within an individual site (Buck Creek N = 13; Killbuck Creek N = 12 sampling events). Independent variables included in multiple regression analyses were based on initial Pearson correlations and included discharge, temperature, pH, turbidity, water column dissolved oxygen, chlorophyll *a*, the number of days since precipitation at the time of sampling, the total amount of rainfall in the previous 10 days before the time of sampling, and solar irradiance. A

two sample *t*-test was used to identify differences in stream variables and total pharmaceutical concentrations among study sites. Pearson correlation coefficients were performed using SAS statistical software (SAS Institute® 9.2, 2002–2008 Cary, NC, U. S.). Multiple regression analyses and two sample t-tests were performed using Minitab 16 (Minitab® Inc. 2010, USA).

3. Results

3.1. Study site characteristics

Temperature, nitrate, and phosphate concentrations were similar between sites ($p>0.1$) (Table 1). However, mean pH (7.8) was greater in Buck Creek than Killbuck Creek (pH = 7.1) during the sampling period ($p=0.04$). Mean discharge (2.4 m^3/s), width (19 m), depth (0.22 m), and velocity (0.4 m/s) at Buck Creek was significantly greater relative to Killbuck Creek (0.43 m^3/s, 9.4 m, 0.3 m, 0.11 m/s, respectively; $p\leq0.04$) and exhibited more variation over time during the sampling period. In addition, mean water column dissolved oxygen concentrations were greater in Buck Creek (15.1 mg/L) than Killbuck Creek (11.8 mg/L; $p<0.01$).

3.2. Pharmaceutical frequency of detection and concentration range

Across all sampling events, twelve pharmaceuticals were detected including acetaminophen, caffeine, carbamazepine, cotinine, DEET, gemfibrozil, ibuprofen, sulfadimethoxine, sulfamethazine, sulfamethoxazole, triclosan, and trimethoprim (Table 2). Three compounds were not detected in any sample including lincomycin, sulfathiazole, and tylosin. Gemfibrozil (100%), caffeine (96%), cotinine (92%), acetaminophen (84%), and sulfamethoxazole (84%) exhibited the highest frequencies of detection, whereas triclosan and sulfadimethoxine were detected in <10% of the samples analyzed. The concentrations measured for detected compounds varied over an order of magnitude across sampling events for most pharmaceuticals including acetaminophen (1.6–460 ng/L), caffeine (11–400 ng/L), and DEET (8–290 ng/L); compounds measured that exhibited less variability in concentrations included gemfibrozil (1.2–11 ng/L), cotinine (2.1–37 ng/L), sulfamethoxazole (1.2–16 ng/L), ibuprofen (1.8–42 ng/L), carbamazepine (1.1–2.7 ng/L), trimethoprim (3–58 ng/L), sulfamethazine (1.2–22 ng/L), and triclosan (9.1–22 ng/L). Sulfadimethoxine was only detected once during sampling efforts (2.2 ng/L) (Table 2).

In Buck Creek, acetaminophen, caffeine, cotinine, ibuprofen, triclosan, and carbamazepine were highest in July whereas sulfamethoxazole, sulfamethazine, DEET, and trimethoprim were highest in January (Figs. 2, 3). In addition, in Buck Creek, gemfibrozil was highest in May. In Killbuck Creek, acetaminophen, cotinine, ibuprofen, and sulfamethoxazole were highest in the February sampling whereas caffeine, DEET, gemfibrozil, triclosan, and trimethoprim were highest in the January sampling (Figs. 2, 3). In Killbuck Creek, sulfamethoxazine was highest in the November sampling. Further, in Killbuck Creek, carbamazepine and sulfadimethoxine were only detected in the January sampling.

3.3. Factors influencing pharmaceutical abundance

Across sites, pharmaceutical compounds detected in >65% of samples (gemfibrozil, caffeine, cotinine, acetaminophen, sulfamethoxazole, ibuprofen) were consistently correlated with water column dissolved oxygen ($p\leq0.05$) (Fig. 4) except gemfibrozil which may be due to the fewer number of samples collected for this compound. In addition, cotinine, sulfamethoxazole, and DEET were negatively correlated with precipitation measurements ($p\leq0.06$) (Fig. 5). No other physiochemical

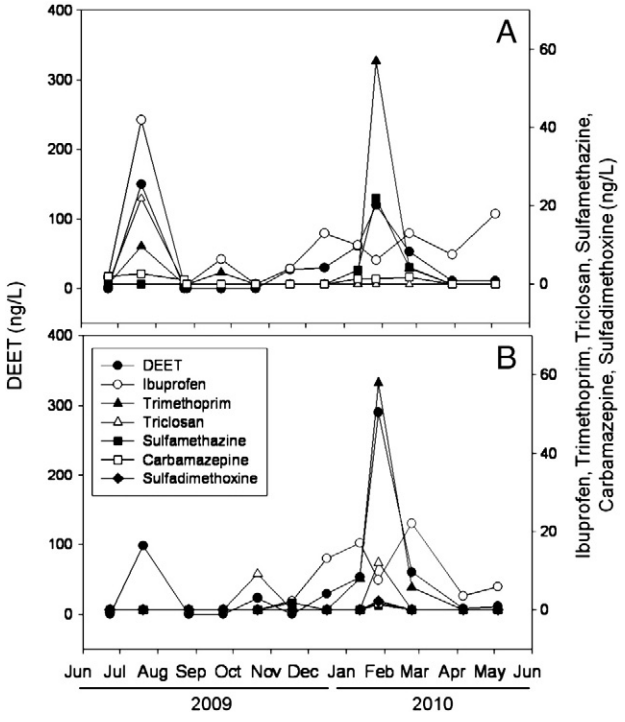


Fig. 3. Temporal patterns of pharmaceutical concentrations for low frequency compounds in A) Buck Creek and B) Killbuck Creek.

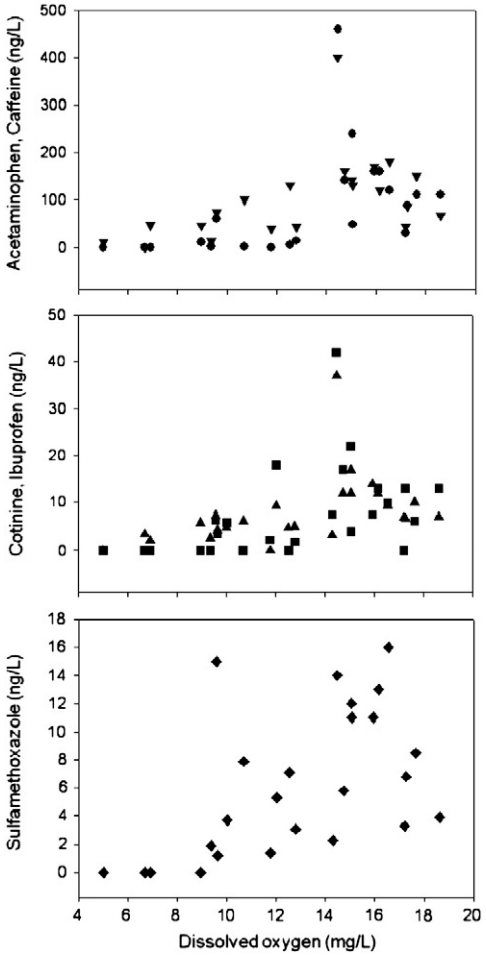


Fig. 4. Relationship between water column dissolved oxygen concentration and sulfamethoxazole, cotinine, acetaminophen, caffeine, and ibuprofen concentration across sites. Different symbols denote different pharmaceutical compounds: ● acetaminophen; ▼ caffeine; ▲ cotinine; ■ ibuprofen; ♦ sulfamethoxazole. See Table 3 for Pearson correlation statistics.

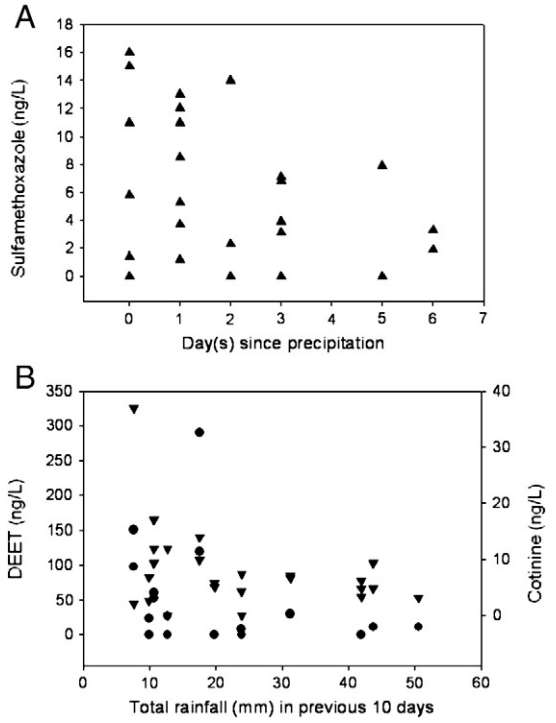


Fig. 5. Relationship between A) the number of days since precipitation and sulfamethoxazole concentration across sites; and, B) total rainfall in previous 10 days and DEET and cotinine concentration across sites. Different symbols denote different pharmaceutical compounds: ● DEET; ▼ cotinine; ▲ sulfamethoxazole. See Table 3 for Pearson correlation statistics.

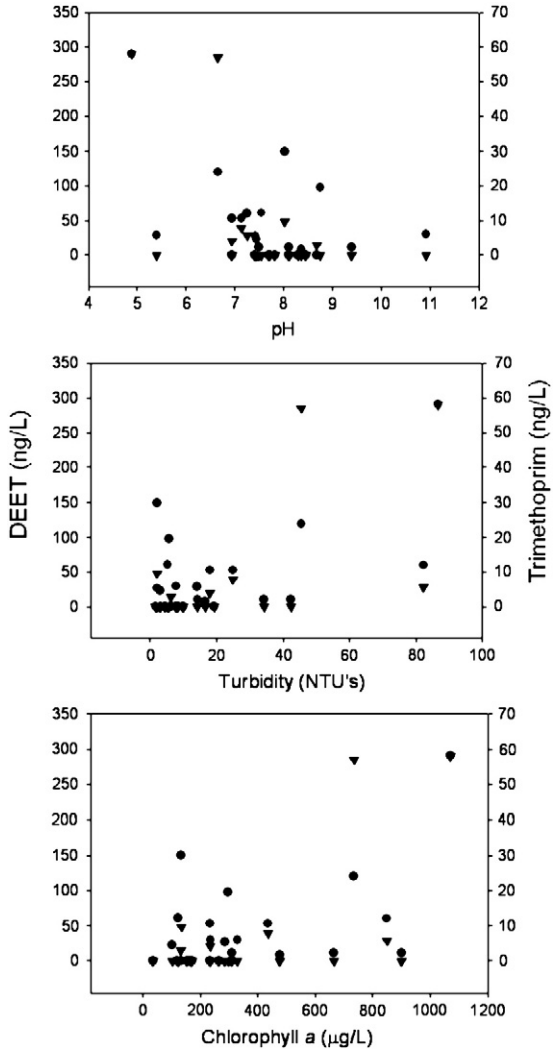


Fig. 6. Relationship between pH, turbidity, and chlorophyll *a* concentration and DEET and trimethoprim concentration across sites. Different symbols denote different pharmaceutical compounds: ● DEET; ▼ trimethoprim. See Table 3 for Pearson correlation statistics.

parameters measured were correlated with these high frequency compounds across sites.

Low frequency compounds (detected in <65% of sampling events) were not correlated with water column dissolved oxygen concentrations. Rather, DEET and trimethoprim were negatively correlated with pH ($p \leq 0.02$) and positively correlated with turbidity ($p \leq 0.01$) and chlorophyll *a* concentrations ($p \leq 0.01$) across sites (Fig. 6). Across sites, no pharmaceutical compounds were significantly correlated with any other stream variables measured (Table 3).

Within the suburban Buck Creek site, sulfamethoxazole was negatively correlated with days since precipitation at the time of sampling ($p = 0.02$). Within the agricultural Killbuck Creek site, caffeine, ibuprofen, and sulfamethoxazole were positively correlated with water column dissolved oxygen ($p \leq 0.02$). Caffeine and sulfamethoxazole were positively correlated with stream discharge ($p \leq 0.02$) at Killbuck Creek. Caffeine concentrations were also positively correlated with chlorophyll *a* concentrations at the Killbuck Creek (agricultural) site ($p < 0.01$). All high frequency compounds, excluding gemfibrozil, exhibited a negative correlation with water temperature ($p \leq 0.05$) within Killbuck Creek.

The sediment $\delta^{15}N$ content differed between sites ($p < 0.01$). Specifically, Buck Creek had significantly higher sediment $\delta^{15}N$ (mean = 7.13‰) relative to Killbuck Creek (mean = 68‰) ($p < 0.001$). In addition, Killbuck Creek had significantly higher N content in sediment (mean = 0.12% N) relative to Buck Creek (mean = 0.03% N) ($p < 0.01$) (Fig. 7). Within Killbuck Creek, $\delta^{15}N$ was negatively correlated with sulfamethazine ($p < 0.01$). Within Buck Creek, sediment $\delta^{15}N$ was negatively correlated with water column chloride concentration whereas sediment% OM was positively correlated with chloride concentrations ($\delta^{15}N$ $p = 0.03$;% OM $p = 0.01$). Sediment %N was also positively correlated with water column ammonium concentrations ($p = 0.04$) within Buck Creek (Table 3).

Across sites, sediment % N was negatively correlated with carbamazepine ($p = 0.05$). Sediment % N was also negatively correlated with stream width, mean velocity, discharge, water column dissolved oxygen, and chlorophyll *a* concentration ($p \leq 0.05$); and positively correlated with mean depth ($p = 0.04$) (Table 3). In contrast to pharmaceutical concentrations, sediment $\delta^{15}N$ was positively correlated with mean depth, mean velocity, and discharge across sites ($p \leq 0.02$), but negatively correlated with chloride ($p = 0.03$). Sediment organic matter (OM) content was negatively correlated with mean width ($p < 0.01$), and mean velocity ($p = 0.01$) and positively correlated with depth ($p = 0.02$). Measures of sediment quality were not significantly correlated with individual or total pharmaceutical concentrations when sites were combined ($p > 0.1$) (Table 3).

Independent variables measured but not significantly correlated to individual or total pharmaceutical concentrations across sites or within individual sites included total dissolved solids, nitrate, phosphate, solar irradiation, and CSO discharge in the previous 10 days before a sampling event ($p > 0.10$). Further, CSO discharge was not correlated with any measure of sediment quality (i.e., sediment $\delta^{15}N$, % N, or % organic matter; $p > 0.1$) (Table 3).

Multiple regression analyses indicated that water column dissolved oxygen controlled total pharmaceutical concentrations across sites (Table 4). When sites were analyzed independently, days since precipitation, dissolved oxygen concentrations, and solar radiation influenced total pharmaceutical concentrations in Buck Creek; whereas,

Table 3
Pearson correlation coefficient assessing relationships between pharmaceuticals in Buck and Killbuck Creek and physiochemical parameters. Bold values denote significant correlations ($p \leq 0.05$; $n = 25$; except gemfibrozil $n = 14$). Acronyms listed represent the following pharmaceuticals: ACE (acetaminophen), CAR (carbamazepine), CAF (caffeine), COT (cotinine), GEM (gemfibrozil), IBU (ibuprofen), SMX (sulfamethoxazole), SMZ (sulfamethazine), SDX (sulfadimethoxine), TRC (triclosan), TRP (trimethoprim), TP (total pharmaceuticals).

	ACE		CAR		CAF		COT		DEET		GEM		IBU	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Discharge (m^3/s)	0.27	0.2	0.21	0.31	0.13	0.55	0.07	0.73	0.01	0.98	**0.86**	**0.01**	0.2	0.33
Temperature °C	−0.32	0.12	−0.14	0.51	−0.24	0.26	−0.23	0.28	−0.36	0.08	−0.04	0.88	−0.33	0.11
pH	−0.03	0.88	−0.24	0.25	−0.12	0.57	−0.14	0.52	**−0.47**	**0.02**	**−0.79**	**0.01**	0.02	0.94
TDS (g/L)	0.15	0.46	0.003	0.99	0.18	0.39	0.24	0.25	−0.04	0.84	**0.59**	**0.04**	0.2	0.32
Dissolved oxygen (mg/L)	**0.45**	**0.02**	0.37	0.07	**0.42**	**0.04**	**0.44**	**0.03**	0.35	0.09	−0.49	0.08	**0.44**	**0.03**
Turbidity (NTUs)	0.28	0.18	0.11	0.61	0.18	0.38	0.21	0.32	**0.58**	**0.01**	**0.55**	**0.04**	0.2	0.34
Chlorophyll *a* (mg/L)	0.18	0.38	−0.01	0.99	0.19	0.38	0.1	0.64	**0.52**	**0.01**	0.17	0.57	0.14	0.52
Nitrate (mg/L)	0.2	0.36	0.11	0.61	0.05	0.84	0.1	0.64	0.16	0.45	0.13	0.65	0.25	0.24
Day(s) since precipitation	−0.28	0.17	−0.12	0.56	−0.34	0.1	−0.17	0.42	−0.23	0.27	**0.56**	**0.04**	−0.29	0.16
Total rainfall (mm)	−0.23	0.27	−0.3	0.14	−0.2	0.34	−0.35	0.08	−0.38	0.06	0.27	0.36	−0.2	0.34
Solar radiation (MJ/m^2)	−0.17	0.43	−0.1	0.62	−0.12	0.55	−0.15	0.48	−0.29	0.16	−0.37	0.47	−0.22	0.3
$\delta^{15}N$	0.15	0.46	−0.06	0.77	0.16	0.45	0.17	0.43	−0.25	0.23	−0.43	0.17	0.14	0.51
Sediment% N	−0.19	0.36	**−0.4**	**0.05**	−0.34	0.1	−0.22	0.28	−0.01	0.96	0.16	0.62	−0.14	0.51
Sediment% organic matter	−0.1	0.64	−0.34	0.1	−0.29	0.16	−0.16	0.44	−0.12	0.58	0.26	0.42	−0.05	0.83

	SMX		SMZ		SDX		TRC		TRP		TP	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Discharge (m^3/s)	0.17	0.43	0.33	0.1	−0.07	0.75	−0.14	0.51	0.15	0.49	0.18	0.39
Temperature °C	**−0.44**	**0.03**	−0.34	0.1	−0.27	0.18	0.01	0.98	**−0.4**	**0.05**	−0.36	0.07
pH	−0.26	0.21	−0.24	0.24	**−0.5**	**0.01**	−0.2	0.33	**−0.51**	**0.01**	−0.22	0.29
TDS (g/L)	0.29	0.16	−0.15	0.47	−0.09	0.66	0.02	0.92	−0.16	0.44	0.12	0.56
Dissolved oxygen (mg/L)	**0.53**	**0.01**	0.34	0.1	0.17	0.4	0.1	0.65	0.36	0.08	**0.48**	**0.01**
Turbidity (NTUs)	0.2	0.34	0.24	0.25	**0.61**	**0.01**	0.1	0.63	**0.64**	**0.01**	**0.39**	**0.05**
Chlorophyll *a* (mg/L)	0.07	0.73	0.27	0.2	**0.54**	**0.01**	0.05	0.82	**0.61**	**0.01**	0.33	0.11
Nitrate (mg/L)	0.09	0.66	−0.08	0.7	0.23	0.29	−0.03	0.9	0.14	0.53	0.16	0.45
Day(s) since precipitation	−0.38	0.06	−0.19	0.37	−0.11	0.6	0.1	0.62	−0.2	0.33	−0.32	0.11
Total rainfall (mm)	−0.28	0.18	−0.17	0.43	−0.08	0.69	−0.31	0.13	−0.19	0.36	−0.3	0.15
Solar radiation (MJ/m^2)	**−0.44**	**0.03**	−0.26	0.21	−0.16	0.45	0.05	0.82	−0.26	0.2	−0.23	0.27
$\delta^{15}N$	0.29	0.16	−0.05	0.81	−0.31	0.13	0.02	0.94	−0.21	0.32	0.05	0.82
Sediment% N	**−0.38**	**0.06**	−0.22	0.29	0.04	0.84	−0.01	0.96	−0.09	0.68	−0.22	0.29
Sediment% organic matter	−0.27	0.18	0.04	0.86	−0.16	0.44	−0.15	0.47	−0.07	0.75	−0.18	0.39

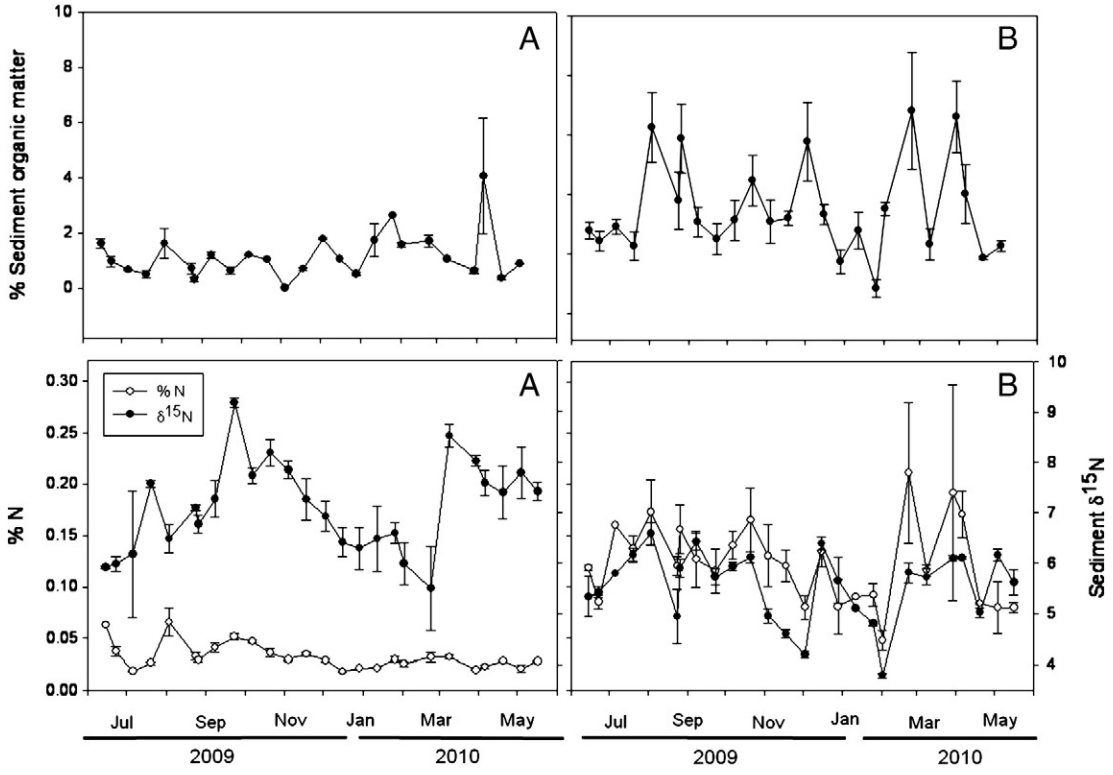


Fig. 7. Temporal patterns of sediment nitrogen content (as %N), isotopic natural abundance (as $\delta^{15}N$), and % sediment organic matter in A) Buck Creek, an agricultural stream; and B) Killbuck Creek, a suburban stream.

Table 4
Multiple linear regression equation and R^2 values for models developed across both sites (N = 25) and within each site (Buck Creek, N = 13; Killbuck Creek N = 12). Equations reference the following independent variables: DO = water column dissolved oxygen, days precipitation = days since precipitation at time of sampling, chlor A = chlorophyll *a* concentration, total rainfall = total amount of rainfall in previous 10 days prior to sampling. All models developed were significant at $p<0.05$.

Site	Regression equation	R^2
Buck Creek	= −1285 + 112 DO − 160 days precipitation + 41 solar irradiance	51.4
Killbuck Creek	= 694.8 − 79 pH + 0.52 chlor *a* − 167 total rainfall	93.6
Combined	= −156.9 + 33 DO	23.3

total rainfall in the previous 10 days, pH, and chlorophyll *a* concentrations were identified as factors influencing total pharmaceutical concentration in Killbuck Creek (Table 4).

4. Discussion

4.1. Range of pharmaceutical concentrations and frequency of detection

Pharmaceutical concentrations measured in this study were comparable to concentration ranges previously measured in U.S. streams and rivers (Camacho-Muñoz et al., 2010; Daneshvar et al., 2010; Glassmeyer et al., 2005; Kolpin et al., 2002; Kolpin et al., 2004; Vieno et al., 2005). However, concentrations measured in this study were lower than those previously measured in sewage treatment plant effluent (Glassmeyer et al., 2005; Godfrey et al., 2007) which may be due to dilution effects (Kolpin et al., 2004) (Table 5). In this study, pharmaceuticals found at highest concentrations detected were acetaminophen (460 ng/L), caffeine (400 ng/L), and DEET (290 ng/L); whereas, sulfadimethoxine (2.2 ng/L), triclosan (22 ng/L), and sulfamethazine (22 ng/L) were found in lower concentrations. Compounds detected most frequently in this study (i.e., gemfibrozil, caffeine, cotinine, acetaminophen, sulfamethoxazole, and ibuprofen) did not necessarily have the highest or most variable concentration ranges. Further, frequencies of detection for pharmaceuticals in this study are not consistent with previous studies for some compounds. For example, gemfibrozil has typically been found at lower frequencies (3.6%) (Kolpin et al., 2002) than observed in this study (100%, N = 12) (Table 5). In contrast, compounds not frequently detected in this study (carbamazepine, trimethoprim, and triclosan) have been more frequently detected in wastewater (carbamazepine 82.5%, triclosan 62.5%) (Glassmeyer et al., 2005) and some surface waters (Glassmeyer et al., 2005; Kolpin et al., 2004) suggesting pharmaceutical abundance in freshwaters is likely site-specific.

Variation in both detection frequency and concentrations measured across studies may indicate differential input, persistence, or sediment sorption characteristics. Pharmaceutical input into the aquatic ecosystem is a function of surrounding land use, usage rates, and wastewater treatment. In contrast, differential persistence and accumulation in sediment is predominantly a function of the properties of individual pharmaceutical compounds, sediment characteristics (i.e., sediment organic content), and degradation potential of the sediment microbial communities.

4.2. Spatial variation in pharmaceutical concentrations

All pharmaceutical compounds in this study were found at both sites with the exception of sulfadimethoxine which was found only once in Killbuck Creek. Due to wastewater effluent derived from a potentially larger contributing population, the suburban site was predicted to have higher pharmaceutical concentrations. However, contrary to this hypothesis, total pharmaceutical concentrations were not significantly different between the surburban and agriculturally influenced stream. Bunch and Bernot (2010) sampled ten sites within the UWRW and also consistently detected pharmaceuticals across a range of surrounding land uses although a negative correlation between urban land use in the sub-watershed and pharmaceutical concentrations was detected. These data suggest that the contributing population may be less predictive of pharmaceuticals in receiving waters than previously thought. Rather, wastewater treatment coupled with the contributing population may better predict pharmaceutical presence in receiving waters.

Studies measuring pharmaceuticals in the aquatic environment have primarily investigated urban sources such as sewage treatment plant effluent (Bartelt-Hunt et al., 2009; Glassmeyer et al., 2005; Phillips et al. 2008). However, advanced wastewater treatment (i.e., aerated activated sludge, trickling filter) associated with urban areas likely results in some removal of pharmaceuticals present before discharge to receiving waters (Phillips et al. 2008). Although the suburban site had a higher contributing population to wastewater effluent, wastewater was treated via activated sludge prior to discharge except during periods of combined sewer overflows (Fig. 8). Activated sludge as a secondary treatment has been documented to be an effective form of removal of some pharmaceuticals (Bartelt-Hunt et al., 2009; Buerge et al. 2003; Phillips et al. 2008); therefore, lower pharmaceutical concentrations are released into streams. In this study, the use of activated sludge treatment may account for lower pharmaceutical concentrations than expected due to higher removal efficiencies relative to the septic systems used in the agricultural site. Septic tanks commonly leak and result in large plumes of contaminated sewage (Carrara et al., 2008) potentially contributing to pharmaceuticals in receiving waters. Godfrey et al. (2007) sampled pharmaceuticals in septic tank effluent and groundwater below septic drainfields. Higher concentrations were generally seen in effluent, but certain pharmaceuticals (e.g., carbamazepine, sulfamethoxazole) persisted in groundwater (Godfrey et al., 2007). It has been suggested that increased persistence of pharmaceuticals from septic leakage may be influenced by oxidizing conditions and organic soil with high surface areas (Carrara et al., 2008). Although the surrounding landscape at the agricultural site consists of silt loam and silt clay loam, anoxic soil conditions may have allowed for compounds to persist.

Variable concentrations and detection frequencies across studies may also be due to differential sediment sorption. In this study, pharmaceuticals were measured only as dissolved compounds in the water column. However, some pharmaceuticals may persist in the environment sorbed

Table 5
Comparison of pharmaceutical concentrations detected in this study relative to previous studies investigating freshwater and wastewater effluent. Reported concentrations are median values. * = compound not included in study; ** = mean values reported; <DL = below detection limit.

Reference	Sample Type	Pharmaceutical concentration (μg/L)											
		Acetaminophen	%	Caffeine	%	DEET	%	Gemfibrozil	%	Carbamazepine	%	Triclosan	%
This Study	IN Streams	0.46	84	0.4	96	0.29	64	0.011	100	0.0027	28	0.022	12
Bunch and Bernot, 2010	IN Streams	0.109	50	0.057	100	*	*	*	*	*	*	*	*
Kolpin et al., 2002	U.S. Streams	0.11	24	0.081	62	0.06	74	0.048	4	*	*	0.14	58
Kolpin et al., 2004	Iowa Streams	0.67	21	0.05	74	0.064	3.6	<DL	0	0.091	26	0.046	3.3
Glassmeyer et al., 2005***	U.S. WWTP effluent	0.006	73	0.053	73	0.18	82	*	*	0.08	91	0.25	100
	Downstream from effluent	<DL	40	0.05	60	0.117	80	*	*	0.075	100	0.11	60

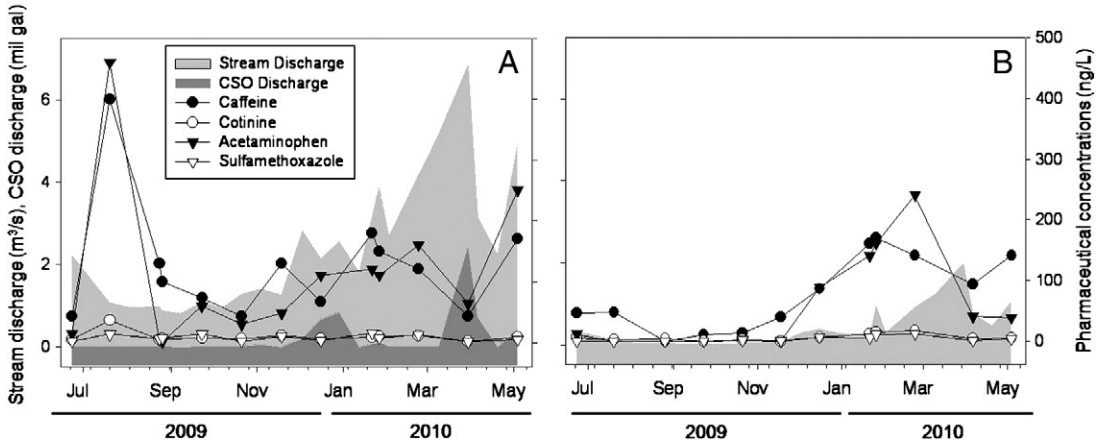


Fig. 8. Pharmaceutical concentrations, stream discharge and combined sewer overflow discharge (CSO) over the sampling period for A) Buck Creek, an agricultural stream; and, B) Killbuck Creek, an urban/suburban stream. See Table 1 for site descriptions. Pharmaceuticals plotted are high frequency compounds detected over the study period (Table 2).

to sediment and the rate of sorption is likely spatially and temporally variable depending on sediment type, pharmaceutical compound, and stream discharge. The ability of a compound to sorb to sediment is based on the organic carbon partitioning coefficients (K_{ow}), acid dissociation constant (pKa), and pH of the water (Jones et al., 2004; Lorphensri et al., 2007). Acetaminophen (log $K_{ow} = 0.46$), caffeine (log $K_{ow} = 0$), and sulfamethoxazole (log $K_{ow} = 0.89$) have low K_{ow} yielding minimal potential for sediment sorption relative to ibuprofen (log $K_{ow} = 3.5$), carbamazepine (log $K_{ow} = 2.45$), or triclosan (log $K_{ow} = 5.4$) potentially resulting in higher water column concentrations (Buerge et al. 2003; Jones et al., 2002; Radjenovic et al., 2008; Son et al., 2009). In this study, acetaminophen, caffeine, and sulfamethoxazole were more frequently found than ibuprofen, carbamazepine, and triclosan, consistent with reduced capability for sediment sorption. Although the K_{ow} is constant for a given pharmaceutical compound, water pH is variable yielding spatial and temporal variation in sorption potential. Further, during periods of high flow, pharmaceuticals may be transported via suspended particulates before settling. Cushing et al. (1993) suggested that surficial fine particulate organic matter is transported on average 4–8 km $days^{-1}$ indicating that particulate matter is quickly exported downstream. If pharmaceuticals are attached to particulate matter, they may be transported downstream further than unattached compounds.

Alternatively, comparable pharmaceutical concentrations between the agricultural and urban site could result from differential usage rates per person in the sub-watershed. Pharmaceutical usage rates are not available for comparison among watersheds but we assume they are not significantly different. In addition, pharmaceuticals detected in both streams were primarily human pharmaceuticals. Although only four of the pharmaceuticals included in the analyte list (i.e., sulfamethazine, sulfadimethoxine, tylosin, lincomycin) are used in veterinary or livestock operations, these compounds were either never detected in sampling efforts or had frequencies of ≤20% indicating that agricultural operations are not likely the primary source of pharmaceutical input.

Previous studies have shown that higher $\delta^{15}N$ values in sediment can result from anthropogenic sources such as urban activities, notably wastewater input (Vander Zanden et al., 2005) whereas lower $\delta^{15}N$ values in sediment result from agricultural practices such as fertilizer application (Fogg et al., 1998). Thus, $\delta^{15}N$ signatures of primary consumers have been linked to sewage derived from populated areas (Cabana and Rasmussen, 1996). However, in this study, sediment $\delta^{15}N$ did not correlate with CSO discharge in Buck Creek although the site did have significantly higher sediment $\delta^{15}N$ content relative to Killbuck Creek indicating higher input of human and animal waste (Fogg et al., 1998; Steffy and Kilham, 2004) (Fig. 7). Further, Killbuck Creek had significantly higher sediment %N than Buck Creek (Fig. 7) indicating nitrogen input from sources other than sewage are potentially being discharged into Killbuck Creek.

4.3. Temporal variation in pharmaceutical concentrations

In this study, acetaminophen, carbamazepine, caffeine, cotinine, ibuprofen, and triclosan concentrations were highest during the July sampling in Buck Creek (Figs. 2, 3). During July, stream discharge (1.06 m^3/s) and CSO discharge (0.02 million gallons) were relatively low compared to discharge over the study period. Kolpin et al. (2004) found that pharmaceutical concentrations were lower during high flow conditions likely due to dilution. Lower dilution may partially explain the high concentrations seen in July in the urban site although CSO input in the previous 10 days before the sampling event was low. However, stream discharge was not correlated with pharmaceutical concentrations across or within sites indicating dilution alone did not dictate the observed pharmaceutical concentrations. Both nitrate and ammonium concentrations were highest two weeks prior to the July pharmaceutical sampling (Fig. 9) indicating another waste source other than CSO discharge may have emptied into Buck Creek. Therefore, other sources of waste may exist that are contributing to pharmaceutical abundance in the water.

Within Killbuck Creek, acetaminophen, caffeine, and cotinine were highest during winter months (December, January, February) (Fig. 8). Further, excluding the July sampling event, pharmaceutical concentrations in Buck Creek were also highest during winter months. Higher concentrations of pharmaceuticals during winter have previously been documented in Finnish and Swedish streams (Daneshvar et al., 2010; Vieno et al., 2005) and likely a function of lower temperature and irradiance as well as higher input relative to spring, summer, and fall. Seasonal variation of pharmaceuticals present in streams has only recently been documented (Daneshvar et al., 2010; Vieno et al., 2005). Thus, these data are consistent with other recent studies indicating pharmaceutical concentrations in freshwater are higher during winter. More compounds were detected during winter months (i.e., December, January, February) and more frequently than at other times of the year.

Several mechanisms are likely responsible for higher pharmaceutical abundance during winter including temperature, irradiance and input. Decreased water temperature not only within the stream itself, but also within the sewage treatment plant, likely reduces biodegrading activity yielding higher concentrations of pharmaceuticals to be transported downstream (Vieno et al., 2005). Yuan et al. (2004) examined biodegradation of nonylphenol (NP) in river sediment and found that between 20 °C and 50 °C, higher temperatures yielded greater biodegradation of NP. Further, increased biodegradation rates associated with

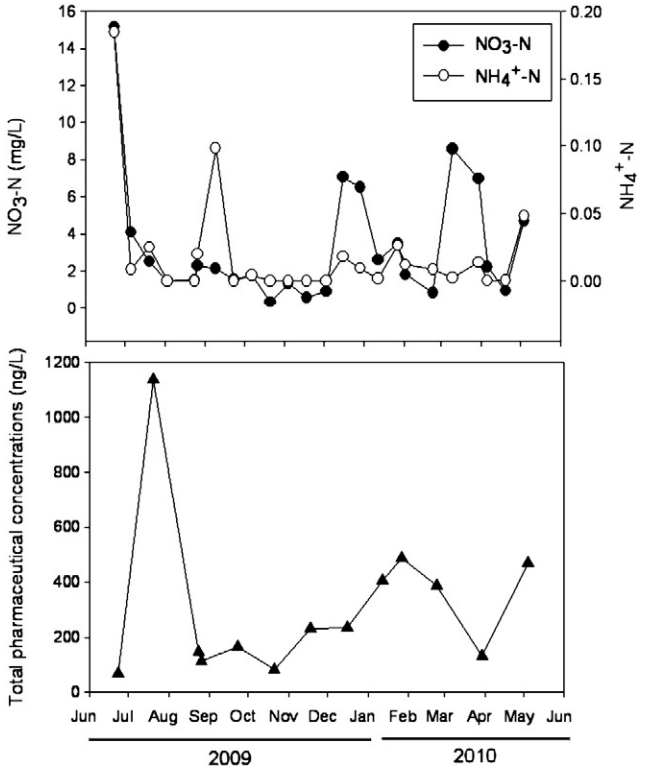


Fig. 9. Temporal patterns of nitrate (NO_3-N) and ammonium (NH_4-N) concentrations and total pharmaceutical concentrations in Buck Creek, a suburban stream.

higher temperatures may explain negative correlations identified between temperature and several of the high frequency compounds detected (i.e., caffeine, cotinine, acetaminophen, ibuprofen, sulfamethoxazole) in Killbuck Creek. Decreased solar irradiation in winter may also allow reduce abiotic degradation of pharmaceutical compounds (Daneshvar et al., 2010). During winter, snow and ice may reduce irradiation reaching surface water, in addition to shorter daylight periods, therefore reducing photodegradation of susceptible contaminants. Lam et al. (2004) found that certain pharmaceuticals (e.g., sulfamethoxazole) are transformed via photoreactions under sunlight whereas more recalcitrant compounds, such as carbamazepine, are more resistant to photodegradation. Thus, temporal variation in irradiance will likely only influence persistence of some pharmaceutical compounds.

4.4. Primary factors influencing pharmaceutical concentrations

Across sites, water column dissolved oxygen was positively correlated with concentrations of several individual pharmaceutical compounds (Fig. 4). In contrast to other studies, this may indicate that biota under low oxygen conditions may be better able to degrade frequently detected compounds (e.g., caffeine, cotinine, acetaminophen, sulfamethoxazole, ibuprofen) (Carrara et al., 2008). Within the agricultural site, dissolved oxygen correlated with only a few compounds (caffeine, ibuprofen, and sulfamethoxazole) and no relationships between dissolved oxygen and any pharmaceutical compounds were found within the suburban site. In addition, pH, turbidity, and chlorophyll *a* were significantly correlated with low frequency compounds (i.e., DEET and trimethoprim) (Fig. 6), but when examined across sites, no correlations were found. Different correlations identified across sites compared to within an individual site may suggest that only when sites are combined are strong predictors for pharmaceutical concentrations identified. Further, mechanistic explanations for persistence are likely more indicative within an individual site.

Discharge and precipitation have been proposed as a dominant predictor of pharmaceutical in freshwaters (Kolpin et al., 2004). However, our data indicate only weak relationships between pharmaceutical concentrations in streams and measures of water flow and precipitation (Table 3). Weakly significant correlations present between precipitation measurements and sulfamethoxazole, DEET, and cotinine (Fig. 5) are consistent with the concept of input and dilution playing a major part in the persistence of pharmaceutical contaminants. In older sewage infrastructures in the U.S., untreated sewage and storm water are released through sewers into waterways when the amount of influent exceeds a sewage plant carrying capacity (Benotti and Brownawell, 2007; Buerge et al., 2006; Musolff et al., 2010). Therefore, a major source of pharmaceuticals is likely through wastewater effluent. However, these weak associations found with precipitation measures and the high pharmaceutical concentrations measured in July (Fig. 8) suggest that there are other sources that contribute significantly to contaminants reaching the aquatic ecosystem.

5. Conclusions

Twelve pharmaceuticals were detected in two sites with differing land use in east central Indiana. More comprehensive studies are needed to develop a predictive understanding of pharmaceutical variation within aquatic ecosystems. Pharmaceuticals in freshwaters may not only alter biological processes, but also may enter human drinking water supplies leading to unintentional consumption of drug mixtures. Further investigation is essential to reduce anthropogenic contaminants from entering aquatic environments and assess regulatory need.

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