

PHARMACEUTICAL OCCURRENCE IN GROUNDWATER AND SURFACE WATERS
IN FORESTS LAND-APPLIED WITH MUNICIPAL WASTEWATER

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Abstract: The occurrence and fate of pharmaceutical and personal care products in the environment are of increasing public importance because of their ubiquitous nature and documented effects on wildlife, ecosystems, and potentially humans. One potential, yet undefined, source of entry of pharmaceuticals into the environment is via the land application of municipal wastewater onto permitted lands. The objective of the present study is to determine the extent to which pharmaceuticals are mitigated by or exported from managed tree plantations irrigated with municipal wastewater. A specific focus of the present study is the presence of pharmaceutical compounds in groundwater and surface water discharge. The study site is a municipality that land-applies secondary treated wastewater onto 930 hectares of a 2000-hectare managed hardwood and pine plantation. A suite of 33 pharmaceuticals and steroid hormones was targeted in the analysis, which consisted of monthly grab sampling of groundwater, surface water, and wastewater, followed by concentration and cleanup via solid phase extraction and separation, detection, and quantification via liquid chromatography coupled with tandem mass spectrometry. More than one-half of all compounds detected in irrigated wastewater were not present in groundwater and subsequent surface water. However, antibiotics, nonsteroidal anti-inflammatory drugs, caffeine, and other prescription and over-the-counter drugs remained in groundwater and were transported into surface water at concentrations up to 10 ng/L. These results provide important documentation for pharmaceutical fate and transport in forest systems irrigated with municipal wastewater, a previously undocumented source of environmental entry. *Environ Toxicol Chem* 2016;35:898–905. © 2015 SETAC

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INTRODUCTION

As human populations grow, the need for sustainable treatment and reuse of municipal wastewater becomes increasingly essential. Most municipal wastewater generated in the United States is treated through various levels of wastewater treatment plants (WWTPs), and the treated effluent is discharged into receiving waters, which can include streams, rivers, and lakes. Because of the unique chemical nature of many pharmaceuticals and personal care products (PPCPs), these frequently administered and consumed compounds can remain in wastewater throughout the treatment and discharge process and have been detected in ambient rivers and streams throughout the United States [1]. Once in the environment, PPCPs can result in detrimental effects on aquatic wildlife. Natural and synthetic estrogen compounds, including 17 β -estradiol, estrone, and 17 α -ethynylestradiol, have been reported to cause decreased fecundity in fathead minnows [2] and reduced testicular development in trout [3], among other negative reproductive effects in fish. Furthermore, inhibition of growth and development of secondary sexual characteristics in *Daphnia magna*, an important indicator species in aquatic ecosystems, was reported after exposure to a variety of endocrine-active chemicals [4]. Other commonly used compounds that are persistent in wastewater treatment effluent are nonsteroidal anti-inflammatory drugs. These compounds, including ibuprofen and naproxen, have been reported in the environment at concentrations of 300 ng/L in

surface waters [5], and they elicit negative reproductive effects on aquatic organisms at low, but potentially environmentally relevant, concentrations [6]. Furthermore, commonly used antidepressant drugs, once in the environment, can elicit behavioral changes in fish. Antibiotics in the environment lead to the development and propagation of antibiotic resistance [7–9] and have been frequently detected in surface waters and in wastewater lagoons at concentrations exceeding 100 ng/L. These concentrations have been reported to influence the proliferation of antibiotic-resistant bacteria in the environment and thus present potential public health concerns [1]. In addition to the effects of individual classes of chemical compounds on aquatic organisms, exposure to mixtures of chemicals likely leads to compounded and interactive effects on aquatic species and ecosystems. Because pharmaceutical compounds are often found at low levels and in mixtures from wastewater effluent, the potential additive, antagonistic, or synergistic effects of mixtures on aquatic ecosystems is important to understand. Luna et al. [10] reported that mixtures of 17 β -estradiol and the antidepressant fluoxetine decreased reproductive success of *D. magna* significantly more than either chemical compound alone.

An alternate solution to direct discharge of treated wastewater is the land application of municipal wastewater onto permitted lands [11]. In the United States, land application technologies were implemented in the 1970s with the introduction of the National Environmental Policy Act and amendments to the Federal Water Pollution Control Act in 1972 [12]. This technology involves the irrigation of municipal wastewater onto agricultural or forested lands; the treatment of wastewater may vary from no treatment to tertiary treatment before actual irrigation onto land. The state of North Carolina has 86 permitted sites for the land application of municipal wastewater. The study

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site of interest in the present study applies secondary treated wastewater onto 930 hectares of mixed hardwood and pine forests with a potential capacity to irrigate 2000 hectares of forest (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The existing forest and new bioenergy hardwood tree plantations comply with nutrient management requirements for groundwater [13].

Although these forest systems filter nutrients from wastewater to acceptable criteria levels before reaching surface water sources, the fate and transport of PPCPs from wastewater application to groundwaters and surface waters is unknown. Preliminary screening data indicated detectable amounts of the wastewater indicator and plasticizer bisphenol A in groundwater collected at the site via a nontargeted qualitative method. The evaluation of PPCP release from land-applied forest systems to aquatic ecosystems requires characterization of flow paths over time, because the volume of land-applied wastewater can be greater during dry conditions and lower during significant rainfall events. The hydrological characterization of the study site is the focus of a separate research publication and indicated that wastewater makes up an average of 24% of the mean daily discharge of the site (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The objective of the present study was to determine the presence of PPCPs in the land-applied system and provide preliminary data as to the extent to which PPCPs can be mitigated by or exported from managed forest systems irrigated with municipal wastewater.

METHODS

Site description and sampling

The wastewater land-application facility receives approximately 19 000 m³/d of municipal wastewater from a city with a population of 70 000 and a daily wastewater outflow of approximately 19 700 m³ [13]. Secondary treated wastewater is land-applied using 1-m irrigation risers after residence times of 7 d to 14 d in open reservoirs onto 930 hectares, as needed, of forested land. The land application facility has been in operation since 1998, when it was constructed in response to the failure of a traditional WWTP. Soils within the watershed containing the land-application site generally fall under the sandy loam or fine sand soil textural class and are well to moderately well drained. The soils at the groundwater sampling locations are specifically classified as Norfolk loamy sand. The watershed receives an annual average rainfall of 1300 mm. On average, 1200 mm wastewater is land-applied on a yearly basis on this site [13]. During the present study period, the watershed received 130 mm precipitation and 120 mm wastewater irrigation per month. The land-application process is designed such that there is no overland flow of wastewater, indicating that all of the applied wastewater is either retained in the soil vadose zone, is evapotranspired, or becomes groundwater.

Six groundwater wells and 2 surface water points were selected from the 2000-hectare wastewater land application site. Groundwater wells were selected where previous research indicated infiltration of wastewater by means of similar isotopic signatures ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) in the groundwater to that of wastewater (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The groundwater gradient labeled transect A (TA) connects a large upland area of irrigation (wells 2, 3, and 6) to a small tributary (TA SW) down gradient, providing the capability to analyze PPCP concentrations along a groundwater gradient (Figure 1). Transect B is groundwater unrelated to the gradient in TA, and the final

surface water sampling point (outlet) was included to determine PPCP concentrations in surface water leaving the facility's property (this sampling point was not sampled for August and September 2014).

Sampling occurred monthly from August 2014 to January 2015 and followed US Geological Survey field manual protocols for groundwater and surface water [14]. Monthly sampling was conducted to cover greater temporal variation, the lack of which is a common critique of environmental sampling and analysis of PPCPs [15]. For groundwater, wells were purged 3 volumes before collection of 1 L groundwater, using a stainless steel bailer, into precleaned and baked amber glass bottles. The bailer was rinsed with water and acetone between groundwater wells. One-liter depth-integrated and 1-L width-integrated surface water samples were collected by using amber glass liter bottles; wastewater in the irrigation system was collected from a central spigot between the holding reservoirs and the land application irrigation system. A total of 10 samples (6 groundwater samples, 2 surface water samples, 1 wastewater sample, and 1 field duplicate) were collected each sampling event. Samples were transported to the laboratory on ice and stored at 4 °C until extraction, within 10 d of sampling.

Target compounds

The 33 compounds targeted in the present study were selected according to their potential risk to aquatic organisms, or are commonly prescribed or consumed pharmaceuticals in the United States (Table 1). Chemical standards were purchased from Sigma Aldrich, and stable-isotopically labeled internal standards were purchased from Cambridge Isotope Laboratories.

Extraction and analysis

Extraction procedures were similar to those described by Cahill et al. [16], Klosterhaus et al. [17], and Phillips et al. [18] with minor amendments. All water samples were filtered through 2.7- μm Whatman glass fiber filters (Fisher Scientific) and spiked with surrogate internal (recovery) standards before extraction. Samples were extracted and concentrated using Oasis HLB solid phase extraction cartridges (Waters). Cartridges were preconditioned with methanol and water, and prefiltered samples were loaded through solid phase extraction cartridges under vacuum at 10 mL/min to 15 mL/min. The solid phase extraction cartridges were dried under vacuum for 15 min, rinsed with 2 mL water, and dried under vacuum for 15 min again before elution with 2–4-mL aliquots of methanol followed by 4 mL acidified methanol (0.1% trifluoroacetic acid). Eluents were evaporated to near dryness, rinsed and combined in methanol, and evaporated to dryness. Samples were reconstituted in a final volume of 250 μL composed of 200 μL 1:1 methanol:water and 50 μL of stable-isotopically labeled reference internal standard. Processed samples were then stored at -80°C until instrumental analysis. Immediately before analysis, samples were centrifuge-filtered using Pall Life Sciences Nanosep 3K Omega filters.

Two separation and analysis methods were used to separate, detect, and quantify target compounds (Supplemental Data, Tables S1 and S2). The basic/neutral targeted compounds were separated by reverse-phase liquid chromatography, using an Acquity UPLC BEH (Ethylene Bridged Hybrid) Shield RP18 (2.1 mm $\times$ 150 mm, 1.7 μm) column from Waters and analyzed on a Thermo Scientific TSQ Quantum Ultra triple-quadrupole mass spectrometer configured with a Waters Acquity UPLC separation system. The second method was used to target the

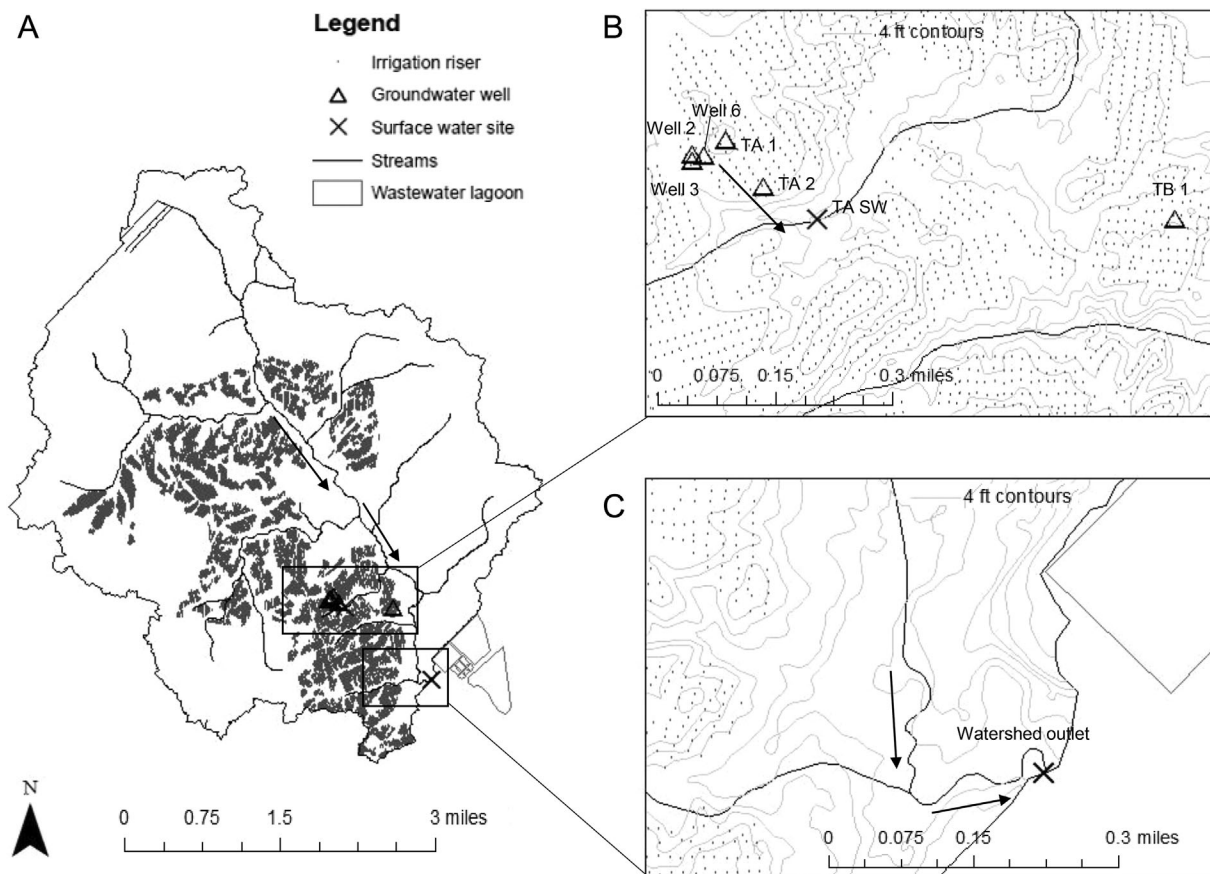


Figure 1. Map of the land-application area, targeted sampling locations, and associated watershed. (A) The watershed is represented, including streams, groundwater wells, surface water sites, wastewater irrigation risers, and the wastewater holding lagoons. Areas of sampling are zoomed in from the larger watershed image. (B) Groundwater flow direction connecting the upland groundwater wells (wells 2, 3, and 6) and transect A (TA; TA 1, TA 2, TA SW) is depicted. Transect A 1 and TA 2 are groundwater wells, and TA SW is a surface water discharge point at the bottom of the gradient. (C) The watershed outlet and stream flow directions are depicted (C). TA = transect A; TA SW = transect A surface water.

more acidic analytes, and separation occurred via an Acquity UPLC BEH C18 (2.1 mm $\times$ 100 mm, 1.7 μ m) column. Compound detection was achieved via electrospray ionization in positive mode for the first method and negative mode for the second. Target compounds were quantified using an internal standard method comprising 7 levels of calibration standards.

Quality assurance

Field duplicate samples were collected for every sampling event and analyzed in the same method. In addition, for each set of samples collected, 1 field blank and 1 standard spiked blank were extracted and analyzed in the same manner to determine

background contamination and ongoing method recoveries, respectively. Finally, all samples were spiked with 2 stable-isotopically labeled surrogate recovery standards before extraction. Method recoveries and relative standard deviations (SDs) of recoveries were within the range of other studies targeting a similar number of compounds from water and wastewater [17,19]. Average standard analyte recoveries for all compounds of interest were 67%, and average relative SD of recovery (%RSD) was 26% (Supplemental Data, Table S3). Two surrogate internal standards, [13 C] carbamazepine and [13 C] 17 β -estradiol, were added to every sample before extraction, and ongoing %RSDs of these were 18% and 50%, respectively. In field blanks only, %RSD was 4% ([13 C]

Table 1. Pharmaceuticals and personal care products (PPCPs) targeted in chemical analysis

Chemical analysis grouping ^a	PPCP
Steroid hormones	17- α ethynyl estradiol, 17- β estradiol, estrone, estriol, progesterone, testosterone
Antibiotics and antimicrobials	Sulfamethoxazole, lincomycin, sulfamethazine, tylosin, trimethoprim, triclosan, erythromycin
Nonsteroidal anti-inflammatory drugs	Naproxen, ibuprofen, acetaminophen, salicylic acid (aspirin metabolite)
Prescription and nonprescription drugs	Gemfibrozil, meprobamate, fluoxetine, diltiazem, paroxetine, valsartan, carbamazepine, atenolol, diphenhydramine, triamterene
Caffeine	Caffeine, paraxanthine (metabolite)
N,N-diethyl-meta-toluamide (DEET)	DEET
Nicotine metabolite	Cotinine
Plasticizer	Bisphenol-A (BPA)

^aSpecified groupings for chemical analysis based on general chemical class.

carbamazepine and [^{13}C] 17 β -estradiol). Only the compound N,N-diethyl-meta-toluamide (the insect repellent DEET) was detected in extracted field blanks above limits of detection. Field duplicates were used to determine the relative percentage difference of targeted compounds. Average relative percentage difference was 37% ($\pm 15\%$) across all PPCPs detected in duplicate samples throughout the sampling period. Method detection limits were compound specific. Most analyte method detection limits values were less than 1 ng/L, and no detection limit was higher than 15 ng/L.

RESULTS

PPCP detection and quantitation

Of the 33 pharmaceutical compounds targeted in analysis, on average 24 were detected in wastewater during each of the 6 sampling months, with 27 being the maximum number of compounds detected in any given month and 20 the minimum. No more than 12 PPCPs were detected in any groundwater well during any given sampling event, and no more than 10 PPCPs were detected in most groundwater samples (Figure 2). Excluding wastewater, the greatest number of compounds were detected in the watershed outlet surface water point. Detections of pharmaceuticals at the watershed outlet exceeded detections at individual groundwater locations throughout the site (Figure 2). With respect to compound-specific prevalence in groundwater and surface water, cotinine, caffeine, carbamazepine, and DEET were detected in more than 80% of all sampling locations (not including wastewater) for nearly every month of sampling (Figure 3). Other frequently observed compounds included sulfamethoxazole (40–70% of all sampling locations, depending on the month) and paraxanthine (40–100%). Estrone and progesterone were the only steroid hormones identified. Progesterone was present only in wastewater, and estrone was intermittently found in all water sample types. No significant patterns were observed in the frequency of detection of compounds by month of the year.

Pharmaceutical concentrations in groundwaters and surface waters were at least 1 order of magnitude lower than land-applied wastewater. The compounds with the highest average

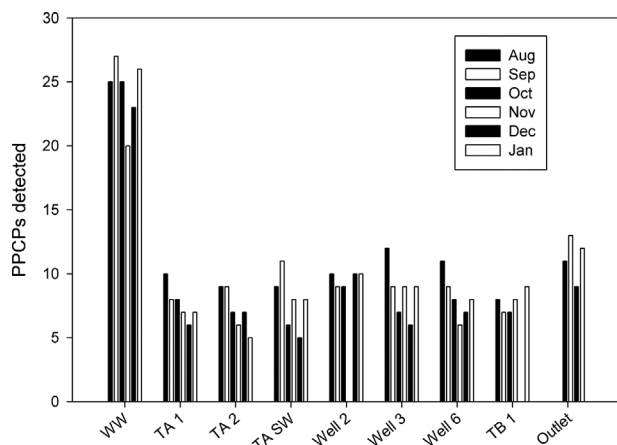


Figure 2. The total number of targeted pharmaceuticals and personal care products (PPCPs) detected in water samples by sampling month (August 2014–January 2015). Samples are wastewater (WW), transect A surface water (TA SW), and watershed outlet (Outlet). The remaining are groundwater wells. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TAs 1 and 2) flowing down from an upland area (wells 2, 3, and 6) to a surface water discharge point (TA SW). TB = transect B.

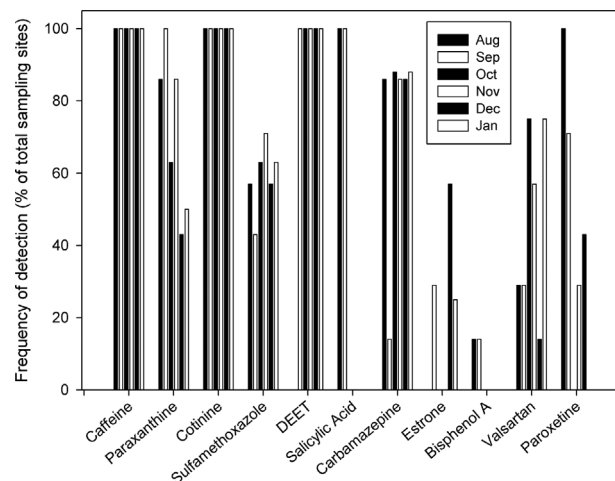


Figure 3. Frequency of detection of selected pharmaceuticals and personal care products (PPCPs) in groundwater and surface water samples by sampling month (August 2014–January 2015). The PPCPs along the x-axis are arranged in order of lower to higher octanol-water partition coefficient values, indicating which PPCPs would be more or less likely to persist in water or sorb to soil. Cotinine (a nicotine metabolite), caffeine, N,N-diethyl-meta-toluamide (DEET), and carbamazepine (an antiepileptic prescription drug) were some of the most frequently detected compounds throughout the sampling periods.

concentrations in irrigated wastewater were valsartan (1000 ng/L), sulfamethoxazole (500 ng/L), trimethoprim (900 ng/L), diphenhydramine (400 ng/L), and gemfibrozil (750 ng/L). Of these, only sulfamethoxazole was consistently present in groundwater, and average concentrations were less than 5 ng/L (Supplemental Data, Table S4). The highest concentrations of pharmaceuticals in groundwater were cotinine (mean = 9 ng/L), caffeine (12 ng/L), carbamazepine (5 ng/L), and sulfamethoxazole (4 ng/L; Figure 4). The PPCPs present at the highest average concentration in the watershed outlet surface water point were lincomycin (19 ng/L), cotinine (23 ng/L), DEET (29 ng/L), caffeine (8 ng/L), and trimethoprim (7 ng/L).

In an effort to simplify observations, the targeted pharmaceuticals were grouped according to chemical class (Table 1). This grouping was for observational purposes only and was not meant to indicate mechanistic similarities or additive effects, or to adhere to other groupings in the literature. Summed concentrations of pharmaceuticals for specific sampling points were analyzed to investigate the change in concentration and movement along a gradient to a surface water discharge point, and ultimately out of the watershed through the outlet (Figure 5). Antibiotics and antimicrobials and other prescription and nonprescription drugs make up the greatest summed concentrations in the land-applied wastewater. This pharmaceutical group was present all the way through a groundwater gradient to a surface water discharge point and in the watershed outlet, but caffeine and caffeine's major metabolite paraxanthine were present at similar concentrations throughout groundwater and surface water. Most chemical groups decreased throughout the gradient to the surface water discharge point; however, the watershed outlet surface water point contains markedly higher concentrations than the other surface water point sampled. Antibiotics, DEET, and antimicrobials made up the greatest fraction in the watershed outlet point.

DISCUSSION

Determining effective approaches of municipal wastewater treatment and disposal is critical to the future of societal and

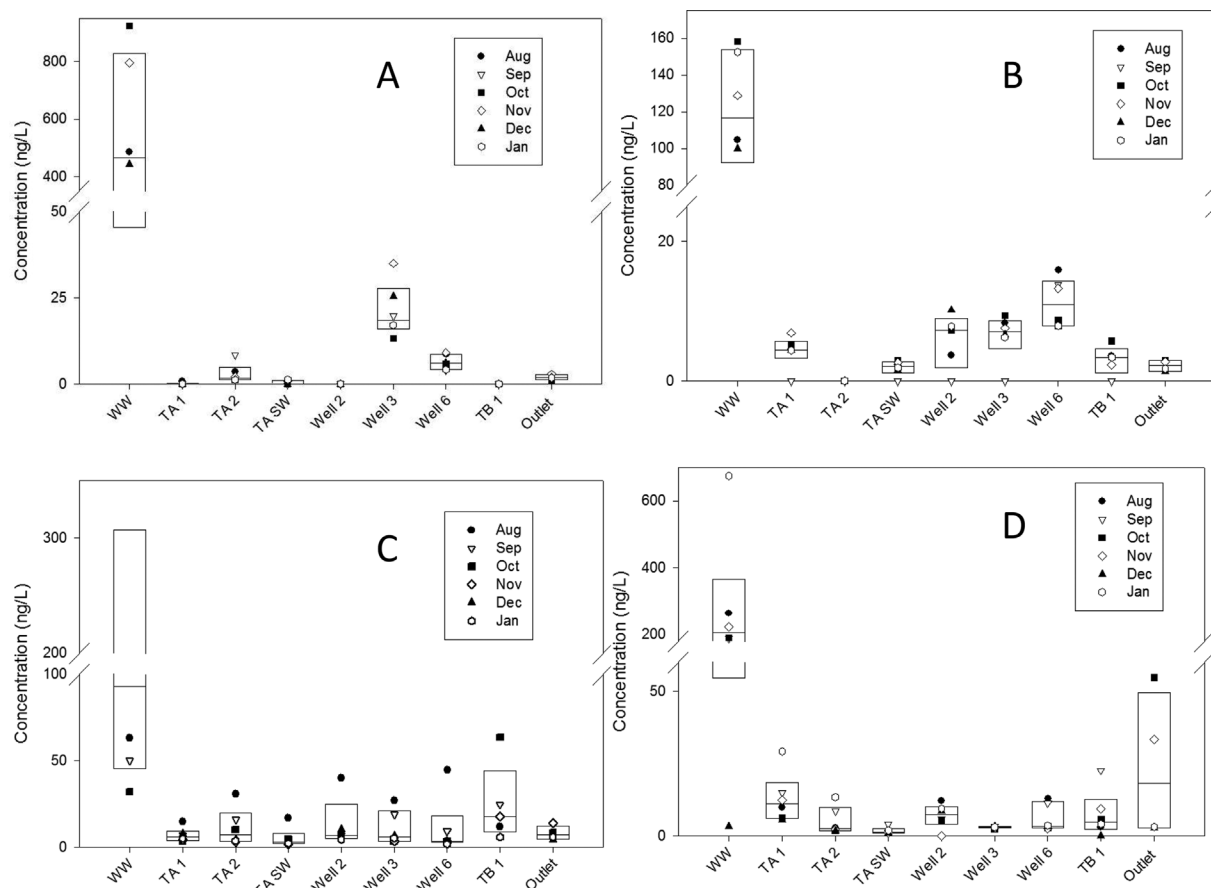


Figure 4. Boxplots depicting median, 25th, and 75th percentiles of pharmaceutical concentrations (ng/L) of selected pharmaceuticals from each of the 6 mo of sampling (August 2014–January 2015): (A) cotinine; (B) caffeine; (C) sulfamethoxazole; (D) carbamazepine. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TA 1 and 2) flowing down from an upland area (wells 2, 3, and 6) to a surface water discharge point (TA SW). TA SW = transect A surface water; WW = wastewater; TB = transect B.

environmental health. Therefore, determining how land application of minimally treated municipal wastewater may lead to environmental input of pharmaceuticals is crucial to the greater understanding of wastewater treatment options and development. Few studies in the literature have reported concentrations of pharmaceuticals in groundwater. Barnes et al. [20] conducted a study sampling groundwater from 18 states across the country and detected pharmaceuticals, including sulfamethoxazole, caffeine, ibuprofen, cotinine, and fluoxetine. The groundwaters sampled in that study were specifically selected to be of potential concern because of their location proximate to agriculture operations and cities; however, they were not directly related to wastewater or land application. Of the PPCPs observed in the Barnes et al. study [20], sulfamethoxazole was detected in the greatest percentage of groundwater sources and was observed at a similar frequency in the present study area. Caffeine and cotinine were detected in only 13% and 2% of groundwater samples, respectively, whereas these 2 compounds were ubiquitous in the present study area. Overall, the targeted PPCPs were more frequently detected in the present study than in the Barnes et al. study [20]; however, concentrations were not higher. In addition, only 1 such study currently in the literature examines groundwater in relation to wastewater land application. In that study, ibuprofen was not detected in any groundwater samples, and caffeine was detected only intermittently [21]. In addition, these compounds were not consistently detected in wastewater effluent, indicating that these results may vary substantially from the

present study, in which high concentrations of pharmaceuticals were present in the wastewater.

By examining a groundwater gradient, it was possible to determine which PPCPs were the most persistent from land application to groundwater transport and surface water export and thus the extent to which infiltration of wastewater occurs. As an additional indicator of wastewater infiltration on the site, chloride concentrations in groundwater directly down gradient of irrigation were significantly higher than those not receiving irrigation (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). Surface water data from the watershed outlet represent sample collections from October to January only, when water-use and transpiration of water by the forest system declines because of hardwood and other plant dormancy. Several targeted PPCPs, including caffeine, DEET, sulfamethoxazole, and cotinine, were detected throughout the subsurface pathway and in surface waters (Figure 5). Caffeine and DEET were observed at the highest concentrations throughout groundwater and into surface water, followed by the prescription and nonprescription drugs. These chemical compounds were also present in the watershed outlet surface water point; however, antibiotics and antimicrobials made up greater total concentrations than any other group in this surface water. Even though these chemical compounds were prevalent throughout a groundwater gradient, the concentrations are several orders of magnitude below that which is land-applied in wastewater, likely because of chemical sorption to soil and decomposition before reaching groundwater. However, despite

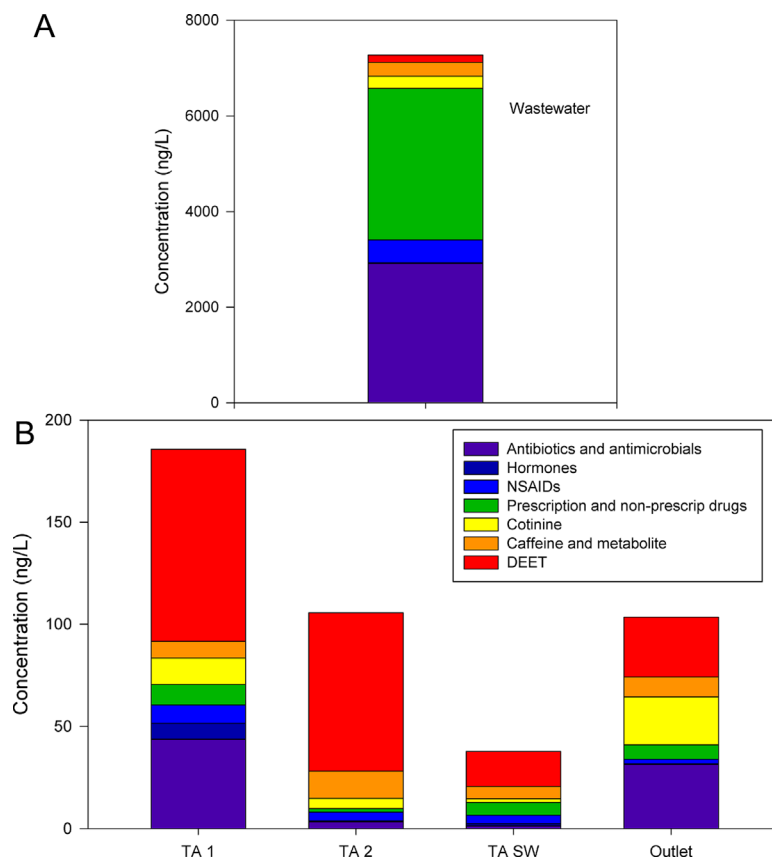


Figure 5. Six-month average concentrations of targeted pharmaceuticals, presented in summed groupings (Table 1): (A) wastewater concentrations. (B) 2 selected groundwater samples and 2 surface water points. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TA 1 and 2) flowing down from an upland area to a surface water tributary (TA SW). Outlet is the watershed outlet surface water point. Caffeine, N,N-diethyl-meta-toluamide (DEET), prescription and nonprescription drugs, and antibiotics and antimicrobials remain the most persistent throughout groundwater into surface water. NSAIDs = non-steroidal anti-inflammatory drugs.

reduction in concentration, these chemical compounds are persistent throughout groundwater and remain so into surface water. With the present data and temporal scope, we are unable to explicitly define the land-application facility as the sole source of PPCPs leaving the watershed via surface water at the outlet, because an upstream surface water point was not included throughout the present study period. However, after the present study period, 1 surface water sample was analyzed from upstream of the entire watershed and indicated little input into the system (7 PPCPs were present, and the highest concentration of any of these, DEET, was 10 ng/L; all others were less than 3 ng/L). A more extensive sampling regimen is underway to characterize PPCPs in surface waters entering and exiting the land application site.

To assess differences in overall environmental input and mitigation, the environmental input of PPCPs from wastewater land application was compared with WWTP effluent discharge into receiving waters. Several studies have quantified pharmaceuticals in WWTP effluent and receiving surface waters to determine environmental input from incomplete removal during the WWTP process [1,19,22]. In general, concentrations detected in surface waters proximate to or downstream from WWTPs in the literature were greater than those detected in groundwater at this forested land application site (Table 2). However, caffeine, sulfamethoxazole, and DEET were quantified in groundwater at similar concentrations to those reported in surface waters, albeit at the low end of these ranges. Similarly, most pharmaceuticals quantified in surface water in the present study were at concentrations below that which has been reported

in the literature. However, DEET and trimethoprim concentrations in the watershed outlet on the land-application site were similar to those reported in surface water sites throughout the country and world.

To more thoroughly assess the effectiveness of the forest system as a wastewater treatment technology, the percentage of decrease in concentration of target compounds was calculated. For those compounds detected in the watershed outlet, decreases

Table 2. Selected pharmaceutical concentrations in groundwater and surface water from the present study compared with literature values in surface water, presented in ng/L

Pharmaceutical	Present study		Literature values
	GW range	SW range	SW
Cotinine	3–13	1–23	20–50 ^a
Caffeine	6–25	5–7	105 ^b
Carbamazepine	0–11	2	25 ^a , 157 ^c
Sulfamethoxazole	0–21	0–2	60–150 ^a , 20 ^b , 1 ^c
DEET	6–150	6–80	22 ^b
Trimethoprim	0–2	0–20	10–150 ^a , 71 ^c
Ibuprofen	0–2	0–2	200 ^a , 28 ^b
Erythromycin	0	0	3.4 ^b
Estrone	0–20	0	3.6 ^b
Naproxen	0–12	0–6	11 ^a , 53 ^c

^aPresented as range of median concentrations [1].

^bPresented as mean concentration.

^cPresented as median concentration [19].

GW = groundwater; SW = surface water; DEET = N,N-diethyl-meta-toluamide.

in concentration between that in land-applied wastewater and the watershed outlet were largely greater than 98%, with few exceptions. Lincomycin (19 ng/L) and sulfamethoxazole (4 ng/L) concentrations were higher in the watershed outlet than in land-applied wastewater (9 ng/L and 1 ng/L, respectively); however, these concentrations were still lower than those previously reported in surface waters. Trimethoprim, present at 1 of the highest concentrations in land-applied wastewater, experienced a 99% decrease in concentration before reaching the watershed outlet point. And DEET, which was present at the highest concentration in the watershed outlet, was reduced by 81% compared with land-applied wastewater concentrations. This indicates that the forested system effectively removes significant percentages of PPCPs before reaching the watershed outlet. Changes in concentration of PPCPs along the groundwater gradient to the surface water discharge point were also determined. More than 50% of paraxanthine, caffeine, carbamazepine, and valsartan remained in the water between well TA 1 and surface water site TA SW (Figure 1). This indicates that these compounds persist in groundwater and are likely to remain in groundwater to the watershed outlet. However, 75% to 100% of cotinine, acetaminophen, triclosan, and DEET were removed between well TA 1 and surface water site TA SW, indicating that although these compounds infiltrate to groundwater, they are less persistent and degrade before reaching surface water. The concentrations of the vast majority of targeted PPCP compounds are substantially reduced from concentrations in land-applied wastewater before being exported off of the site in surface water.

When comparing PPCP concentrations at this forested land application system with traditional WWTP effluent, one must qualify several attributes. First, the location of surface water samples relative to a traditional WWTP effluent outfall is critical. Distance downstream from a discharge location can change PPCP concentrations by orders of magnitude. Second, the type of WWTP is critical, because certain WWTPs (traditional tertiary, advanced activated charcoal, and so forth) may remove larger percentages of pharmaceutical compounds before release into surface water. Although several pharmaceuticals were routinely detected in groundwater and surface water at this facility, which land applies secondary treated wastewater, most of these compounds were present at concentrations lower than that reported in surface waters downstream of traditional WWTPs which discharge tertiary-treated effluent.

In general, acute toxicity studies have reported concentrations at which 50% of a population exhibit a response (EC50) and concentrations lethal to 50% of a population (LC50) in the milligram per liter range, several orders of magnitude greater than the concentrations reported in the present study. For example, carbamazepine was acutely toxic to *D. magna* at 17.2 mg/L [23,24], and the mean concentration in groundwater and surface water in the present study was less than 25 ng/L, indicating no acute risk at the measured concentrations. Chronic effects are likely more appropriate to consider because of the continuous input of pharmaceutical compounds at low levels. Rarely are acute effects seen at environmentally relevant concentrations; however, low-level exposure over time can lead to subtle changes in aquatic organisms and populations. Nonsteroidal anti-inflammatory drugs were reported to affect reproduction in *Daphnia* at concentrations of 1.8 mg/L [25], and carbamazepine has been reported to elicit sublethal effects as low as 10 µg/L [26]. In the present study, even when added together, all nonsteroidal anti-inflammatory drugs total less than 50 ng/L, and carbamazepine never exceeded 20 ng/L in

groundwater or surface water. Another study examining changes in morphology and feeding in the cnidarian *Hydra attenuata* reported that sulfamethoxazole, trimethoprim, and caffeine all exhibited EC50 values greater than 100 mg/L, and gemfibrozil, ibuprofen, and carbamazepine exhibited EC50 values greater than 1 mg/L [27]. Concentrations of pharmaceuticals in land-applied wastewater are high enough to elicit chronic effects, and perhaps acute effects; however, pharmaceutical concentrations in groundwater and surface water at this land application facility were lower than the range of direct risk to aquatic organisms. The lone exception is the presence of antibiotics at concentrations sufficiently high to influence the proliferation and propagation of antibiotic resistance in aquatic environments. Risk associated with the development of antibiotic resistance is insufficiently addressed when considering WWTP effluent or land application, where continuous input of antibiotics occurs.

CONCLUSIONS

The present preliminary assessment found that PPCPs are present in secondary-treated municipal wastewater after residence times of 7 d to 14 d in open reservoirs before land-application onto a mixed pine-hardwood forest. Select pharmaceuticals were detected in groundwaters and surface waters on site and at the watershed outlet of the site, but concentrations were lower than PPCP concentrations in irrigated wastewater as well as concentrations reported elsewhere in surface waters downstream of conventional WWTPs. Research is ongoing to more fully address how land application systems function with regard to PPCP fate and transport.

Supplemental Data—Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3216.

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Data availability—Data are available on request by contacting A.D. McEachran (admceach@ncsu.edu) or E.G. Nichols (egnichol@ncsu.edu) at North Carolina State University.

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