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Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges

Angela L. Batt [a], Ian B. Bruce [b], Diana S. Aga [a,*]

[a] Department of Chemistry, The State University of New York at Buffalo, 608 Natural Sciences Complex, Buffalo, NY 14260-3000, USA
[b] Department of Geography, Buffalo State College, Buffalo, NY, USA

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This work investigates the extent of antibiotic concentrations in receiving waters from discharges of wastewater treatment plants.

Abstract

Effluents from three wastewater treatment plants with varying wastewater treatment technologies and design were analyzed for six antibiotics and caffeine on three sampling occasions. Sulfamethoxazole, trimethoprim, ciprofloxacin, tetracycline, and clindamycin were detected in the effluents at concentrations ranging from 0.090 to 6.0 μg/L. Caffeine was detected in all effluents at concentrations ranging from 0.19 to 9.9 μg/L. These findings indicate that several conventional wastewater management practices are not effective in the complete removal of antibiotics, and their discharges have a large potential to affect the aquatic environment. To evaluate the persistence of antibiotics coming from the wastewater discharges on the surrounding surface waters, samples were collected from the receiving streams at 10-, 20- and 100-m intervals. Ciprofloxacin, sulfamethoxazole, and clindamycin (0.043 to 0.076 μg/L) were found as far as 100 m from the discharge point, which indicates the persistence of these drugs in surface waters.

Keywords: Antibiotics; Wastewater; Surface water; Liquid chromatography/tandem mass spectrometry (LC/MS/MS)

1. Introduction

The occurrence of antibiotics in aquatic environments is of ecotoxicological concern because of potential ecosystem alteration. Prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could transfer the resistance genes to other bacterial species (Levy, 1997). Antibiotic residues have been detected in the final effluents of wastewater treatment plants (WWTPs) worldwide (Batt and Aga, 2005; Carballa et al., 2004; Costanzo et al., 2005; Miao et al., 2004). Contamination of surface waters by antibiotics and other pharmaceutical compounds has been reported in recent studies through discharge from domestic sewer systems (Metcalfe et al., 2003). The potential of these low antibiotic concentrations to promote antibiotic resistance has been substantiated by the detection of several antibiotic resistant bacteria and/or genes in municipal wastewater discharges (Schwartz et al., 2003; Volkmann et al., 2004), surface water (Schwartz et al., 2003), and drinking water (Schwartz et al., 2003).

Antibiotics used in human treatment have the potential to enter the environment by excretion or by disposal of surplus drugs into sewage systems, which are eventually released into the local aquatic surroundings from the effluent of WWTPs (Jorgensen and Halling-Sorensen, 2000). The removal of antibiotics from wastewater depends primarily on the secondary and advanced stages of the treatment processes, with many different processes being available for each stage of treatment. The primary treatment stage, where

* Corresponding author. Tel.: +1 716 645 6800x2226; fax: +1 716 645 6963.
E-mail addresses: abatt@hotmail.com (A.L. Batt), ianbbruce@gmail.com (I.B. Bruce), dianaaga@buffalo.edu (D.S. Aga).

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the solid contents of the waste are removed by screening and settling, is usually common to most WWTPs. However, the secondary treatment, which typically relies on a biological process to remove organic matter and/or other nutrients, can differ significantly between WWTPs. In some wastewater facilities effluent is also disinfected before it is released into surface water, typically by chlorination or ultraviolet radiation. In addition, advanced waste treatment processes can be applied to remove nitrogen, phosphorus, and other pollutants or particles (Crites and Tchobanoglous, 1998). Conventional WWTPs are not designed and operated to remove very low concentrations of contaminants, such as pharmaceuticals, and these compounds are consequently released into surface waters (Glassmeyer et al., 2005; Kolpin et al., 2002; Stumpf et al., 1999).

Laboratory studies have been conducted to assess the capability of various wastewater treatment technologies in the removal of antibiotics and other pharmaceuticals (Kim et al., 2005; Ternes et al., 2002, 2003, 2004a). A study by Adams et al. (2002) compared the efficiency of several water treatment processes in the removal of antibiotics, including five sulfonamides and trimethoprim, from surface and distilled water, and found that many of the currently used wastewater treatment techniques are not effective in removing these compounds. Another recent laboratory study by Perez et al. (2005) evaluated the biodegradability of three sulfonamides and trimethoprim at the different stages of sewage treatment. For the sulfonamides, degradation was most rapid in the activated sludge from a secondary aerobic treatment stage, although significant degradation required three or more days. Trimethoprim on the other hand did not show degradation under the same conditions. The only considerable degradation of trimethoprim was observed in the mixed liquor from a nitrification sludge process, which is currently only available in a few WWTPs in the United States.

The objectives of this study are to compare the concentrations of antibiotics in the effluent of three local WWTPs that employs different waste treatment processes, and to determine the distribution of antibiotics in the surface waters receiving discharges from the WWTPs to determine the susceptibility of a watershed to pharmaceutical contamination. The target compounds in this study are six antibiotics approved for human use in the United States and caffeine, which are listed in Table 1. These compounds were chosen for analysis because they are some of the most frequently detected human antibiotics in surface waters (Kolpin et al., 2002). Caffeine concentrations were monitored because it can be used as an anthropogenic marker of untreated domestic wastewater entering the aquatic environment (Buerge et al., 2003).

2. Materials and methods

2.1. Chemicals and reagents

Tetracycline (TC) was purchased from Fluka (St. Louis, MO, USA). Erythromycin (ERY), sulfamethoxazole (SMX), and caffeine (CAF) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trimethoprim (TRI) was purchased from ICN Biomedicals Inc. (Aurora, OH, USA), clindamycin (CLD) from MP Biomedicals (Aurora, OH, USA), and ciprofloxacin (CIP) was supplied by Bayer (Stillwell, KS, USA). Individual standard solutions at a concentration of 1 mg/mL were prepared in methanol, with the exception of ciprofloxacin which was prepared in methanol with 5% 0.1 M sodium hydroxide due to its limited solubility in 100% methanol. The standard solutions were stored at −40 °C for a maximum of 3 months. Working standard solutions were always prepared fresh on the day of analysis by dilution of stock solution with water. An isotopically labeled sulfonamide, $^{13}C_6$-sulfamethazine, was used as the internal standard and was purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

Acetonitrile (ACN) and methanol (MeOH) were purchased either from Burdick and Jackson (Muskegon, MI, USA) or Fisher Scientific (Fair Lawn, NJ, USA). Formic acid and *o*-phosphoric acid (H_3PO_4) were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Disodium ethylenediamine tetraacetate (Na_2EDTA) was purchased from EMD Chemicals (Darmstadt, Germany). Water was prepared using a NANOpure DIamond™ Ultrapure water system with a 0.2-μm filter (Barnstead International, Dubuque, IA, USA).

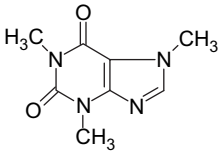
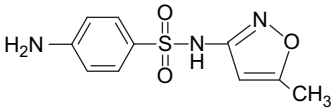
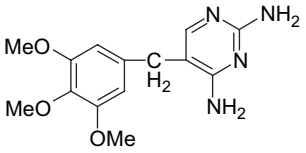
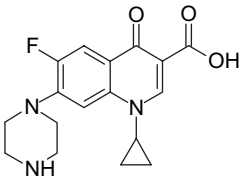
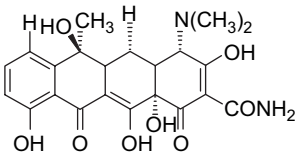
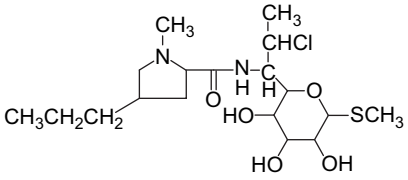
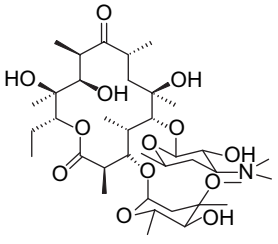
2.2. Description of the wastewater treatment plant processes

A summary of the various stages of wastewater treatment in the three WWTPs sampled is provided in Table 2. While all WWTPs included a primary treatment process, the secondary processes differ from each other and are variations of an aerobic biological treatment process. For instance, the Lackawana plant utilizes pure oxygen activated sludge, where high-purity oxygen is used instead of air (Crites and Tchobanoglous, 1998). The East Aurora plant uses extended aeration, which holds the sludge in an aeration tank for 24 h in contrast to the typical 6–8-h aeration time for activated sludge (Salvalto et al., 2003). The Holland plant uses rotating biological contactors, which contain a set of disks with a fixed film of microorganisms that are affixed to a shaft. The contactors are halfway submerged in the effluent, and the slow rotation of the shafts allows the microorganisms on the disks to alternate between the effluent (where the microorganisms adsorb or degrade organic matter) and the air (where microorganisms can consume oxygen) (Cheremisinoff, 2003). All three plants included an advanced treatment stage, which are used to remove colloidal particles or nutrients, such as nitrogen or phosphorus, to further improve water quality. Metal salts, such as salts of iron, aluminum, and calcium, are often added at this stage to induce precipitation/coagulation (Henze et al., 2002). The Lackawana WWTP utilizes aluminum sulfate, while the East Aurora plant has a ferrous chloride metal salt precipitation/coagulation. The WWTP in Holland employs a sand filter to remove colloidal particles. After secondary or advanced treatment, a disinfection method is also included to reduce bacterial populations and assist in odor control. The Lackawana WWTP disinfection method uses chlorination all year round while the East Aurora Plant uses chlorination seasonally (from the months of May through October). On the other hand, ultraviolet radiation, which is a less common disinfection method, is utilized seasonally (May through October) at the Holland WWTP.

2.3. Sample collection

Grab samples were collected in clean, baked 1-L amber glass bottles. Wastewater effluents were collected from three WWTPs in Erie County, New York (Lackawana, East Aurora, and Holland) on November 2, 2004, November 22, 2004 and February 8, 2005. The surface water surrounding the WWTP discharge was sampled at specified intervals (Table 3) from the outfall of two WWTPs located in East Aurora and Holland, New York on November 22, 2004, and February 8, 2005. Surface water samples were collected from the middle of the stream at an intermediate depth between the surface and stream bottom, with the distances being determined using the navigation feature of a Trimble Global Positioning System (GPS) device. Bottled samples were transported to the laboratory in coolers with ice immediately following collection. To each 1-L sample, 50 μL of the $^{13}C_6$-sulfamethazine was then added on the day of sampling and all samples were stored at 4 °C no longer than 1 day until extraction.

Table 1
Summary of the target analytes, MS/MS parameters, and method limits of detection (LOD)

Compound (Abbreviation) Class	Structure	Precursor ion (MH^+)	Product ions 1, 2	Collision energy (%)	Isolation width	LOD (μg/L)
Caffeine (CAF) Stimulant		195	138, 149	44	1.0	0.055
Sulfamethoxazole (SMX) Sulfonamide		254	188, 156	34	1.0	0.042
Trimethoprim (TRI) Used with sulfonamides		291	230, 258	44	1.0	0.049
Ciprofloxacin (CIP) Fluoroquinolone		332	288, 268	44	1.0	0.030
Tetracycline (TC) Tetracycline		445	410, 427	34	1.0	0.044
Clindamycin (CLD) Lincosamide		425	377, 126	40	1.0	0.060
Erythromycin (ERY) Macrolide		716[a]	522, 558	30	1.5	0.075

[a] Assessed as erythromycin-H_2O.

2.4. Sample preparation

Samples were extracted according to a previously reported solid phase extraction method (Batt and Aga, 2005) using 500 mg Oasis HLB™ cartridges (Waters, Milford, MA, USA). Ten milliliters of 5% (w/v) Na_2EDTA were added to each 1-L sample of wastewater or surface water as a chelating agent. The sample pH was adjusted using 40% *o*-phosphoric acid so that it was between 2.8 and 3.0. The cartridges were conditioned with 6 mL of ACN, followed by 6 mL of NANOpure™ water. The samples were passed through the cartridges at a rate of 3–5 mL/min using a Supelco vacuum manifold (Sigma–Aldrich, St. Louis, MO, USA). The cartridges were eluted twice using 4 mL of ACN. The volume of each eluate was reduced to 0.2 mL under a gentle stream of air at 40 °C. NANOpure water was added to each eluate to a final volume of 1 mL for liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis.

2.5. Sample analysis by LC/MS/MS

The LC/MS/MS method employed in this study was a modification of a previously reported method (Batt and Aga, 2005), except that the mass spectral acquisition method included a full MS/MS scan event only for the six target antibiotics and caffeine to provide a more sensitive and selective detection of the selected analytes. The sample extracts were analyzed using a LCQ Advantage™ ion trap mass spectrometer (IT-MS), which is equipped with an electrospray ionization source (ESI) and connected to a Surveyor LC system (Thermo Finnigan, San Jose, CA, USA). The column used was a BetaBasic-18 C_{18} column (100 × 2.1 mm internal diameter with 3 μm particle size) equipped with a UNIPHASE™ guard cartridge (10 × 2.1 mm internal diameter with 3 μm particle size), both purchased from Thermo Hypersil-Keystone (Bellefonte, PA, USA). The flow rate was 200 μL/min, the column oven temperature was 30 °C, and the full loop injection volume was 20 μL.

Table 2
Summary of the treatment processes and characteristics of three WWTPs in Erie County, NY

WWTP	Population served	Primary treatment	Secondary treatment	Advanced treatment	Disinfection method
Lackawana	12 000	Yes	Pure oxygen activated sludge	Aluminum sulfate	Chlorination
East Aurora	7700	Yes	Extended aeration (Schreiber process)	Ferrous chloride	Seasonal Chlorination (5/15–10/15)
Holland	1750	Yes	Rotating biological contactors	Sand filtration	Seasonal UV light (5/15–10/15)

The ESI-IT-MS was operated in positive ion mode. The capillary temperature was 235 °C and the spray voltage was 5.25 kV for all applications. Nitrogen was used as a sheath gas at a flow rate of 20 arbitrary units and helium gas was used to induce dissociation for the acquisition of MS/MS data. Individual tune files were created for each standard in continuous flow mode to determine the optimum capillary voltages, lens settings, collision energies, and fragment ions, which are listed in Table 1. The isolation width for all compounds was 1.0, with the exception of erythromycin-H_2O, which required an isolation width of 1.5. The separation was performed using a ternary gradient mobile phase consisting of ACN (A), MeOH (B), and water with 0.3% formic acid (C), using the gradient solvent program previously reported (Batt and Aga, 2005). Full scan and full scan MS/MS data were collected simultaneously. The acquisition was divided into three time segments, with full scan MS and full scan MS/MS events collected for each of the respective analytes.

2.6. Quantification

The method limit of detection (LOD) was determined using calibration curves prepared from spiked NANOpure water samples. Five hundred milliliter volumes of NANOpure water were spiked to contain 0.1, 0.25, 1.0 and 2.5 μg of the seven target analytes, with duplicate samples extracted at the higher concentrations (0.25, 1.0 and 2.5 μg) and four replicates extracted for the lowest concentration (0.1 μg). The calibration curves were plotted, and the LOD was defined as the concentration corresponding to signal at the *y*-intercept plus three times its standard deviation. The detection limits of the modified acquisition method were calculated to be between 0.030 and 0.075 μg/L (Table 1). Quantification of target analytes was based on external calibration curves, which were constructed from a plot of the peak area ratio of the analyte signal for the product ion of highest intensity (Product ion 1, see Table 1) to the $^{13}C_6$-sulfamethazine product ion of highest intensity signal versus concentration. The surface water collected 100 m upstream from the WWTPs served as field blanks. None of the field blanks showed detection of any of the analytes, indicating that there was no cross-contamination during sample collection and also suggesting that the method is not susceptible to false positives due to matrix effects. Non-detection of analytes in laboratory blanks also indicated no carryover from the SPE procedure or the chromatographic column. Duplicate control samples at a concentration of 0.5 μg/L prepared from spiked NANOpure water were extracted along with the wastewater and surface waters. The average percent recovery of the two control samples was used to adjust the reported analyte concentrations.

3. Results

Effluents from three WWTPs in Erie County (Western New York) were analyzed for the presence of the six target antibiotics and caffeine. The locations of the WWTPs relative to the Buffalo River Watershed are shown in Fig. 1. The results listed in Table 3 show that except for the erythromycin metabolite, all other target analytes were detected in the WWTP outfall and in the receiving streams. The most frequently detected compounds were ciprofloxacin (89%) and sulfamethoxazole (89%), which also had the highest concentrations in the effluents of all WWTPs sampled. Trimethoprim, which is used in combination with sulfamethoxazole, was also detected in all sampled locations, although at lower concentrations than the ciprofloxacin and sulfamethoxazole. Clindamycin and tetracycline were not present in quantifiable concentrations in the Lackawana and East Aurora effluents. In contrast, the tetracycline concentrations were well above the LOD in the Holland

Table 3
Summary of the analyte concentrations detected at the sampling locations in μg/L

WWTP	Location	Date	CAF	TRI	TC	CIP	SMX	CLD	Total antibiotics
Lackawana	Outfall at WWTP	2-Nov-04	2.0	0.10	n.d.	0.97	1.1	det.	2.17
	Outfall at WWTP	8-Feb-05	9.9	0.53	n.d.	0.36	0.70	n.d.	1.59
East Aurora	Outfall at WWTP	2-Nov-04	0.48	0.21	n.d.	0.091	0.37	n.d.	0.67
	Upstream 100 m	22-Nov-04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
	Outfall at WWTP	22-Nov-04	n.d.	det.	det.	0.36	0.66	det.	1.02
	Downstream 10 m	22-Nov-04	n.d.	det.	n.d.	0.36	0.45	det.	0.81
	Downstream 20 m	22-Nov-04	n.d.	det.	n.d.	0.19	0.2	det.	0.39
	Downstream 100 m	22-Nov-04	n.d.	det.	n.d.	0.076	0.065	det.	0.14
	Outfall at WWTP	8-Feb-05	0.20	0.09	n.d.	0.22	0.41	n.d.	0.72
	Downstream 10 m	8-Feb-05	0.20	0.08	n.d.	0.17	0.37	n.d.	0.62
Holland	Outfall at WWTP	2-Nov-04	0.24	0.16	0.56	0.34	1.3	n.d.	2.36
	Upstream 100 m	22-Nov-04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
	Outfall at WWTP	22-Nov-04	0.19	0.15	0.14	5.6	6.0	1.0	12.89
	Downstream 10 m	22-Nov-04	n.d.	det.	n.d.	0.28	0.19	0.14	0.61
	Downstream 20 m	22-Nov-04	n.d.	n.d.	n.d.	0.031	0.056	0.066	0.15
	Downstream 100 m	22-Nov-04	n.d.	det.	n.d.	det.	0.043	0.060	0.10
	Outfall at WWTP	8-Feb-05	0.47	0.51	0.32	0.76	1.34	0.092	3.02
	Downstream 10 m	8-Feb-05	0.31	det.	n.d.	0.09	0.08	n.d.	0.17

n.d., not detected; det., detected, but not quantified.

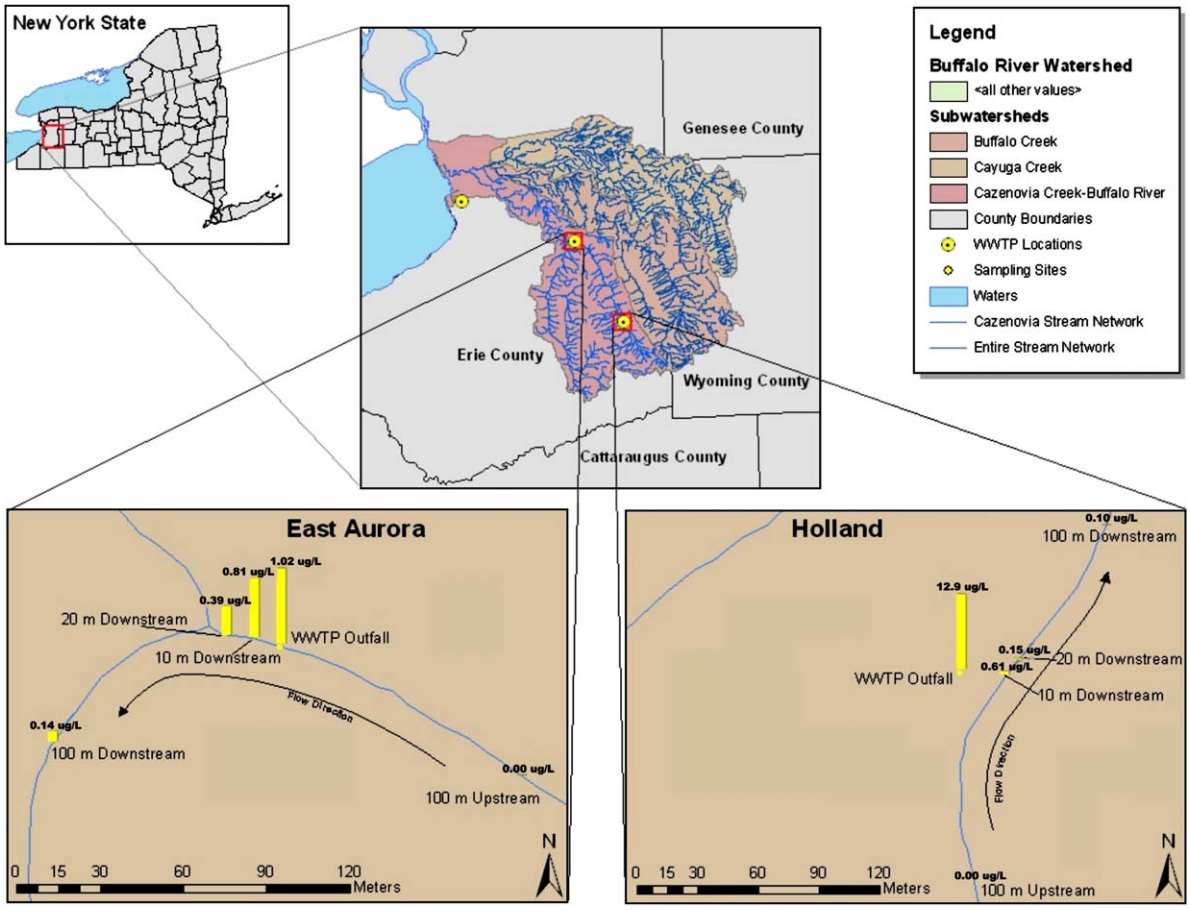


Fig. 1. A map of the Buffalo River Watershed located in Western New York, with the location of the three sampled WWTPs, the surface water sampling sites, and the detected total antibiotic concentrations indicated.

effluent on all three sampling dates. Caffeine was detected in seven of eight effluent samples (0.19–9.9 μg/L), and the concentrations detected are similar to those previously reported in wastewater effluent (Buerge et al., 2003). A sample MS/MS chromatogram of the six compounds detected in the Holland WWTP effluent is shown in Fig. 2.

Surface water samples were collected at 10, 20, and 100 m downstream from the East Aurora and Holland WWTP discharge points, similar to the sampling design shown in recent studies (Ashton et al., 2004; Glassmeyer et al., 2005), with the sampling locations and the measured total antibiotic concentrations indicated in Fig. 1. Surface water was not collected from the receiving stream near the Lackawana WWTP because it was not easily accessible for sampling. Samples collected 100 m upstream of each WWTP showed no trace of the target compounds, indicating that the streams were not impacted by other WWTP discharges or by other non-point sources. This also further validates that the LC/MS/MS method used is highly specific and does not give false positive results because it can differentiate between background and concentration signals at or near the LOD. During the November 22 sampling, 1.02 μg/L of total antibiotics were detected at the WWTP outfall of the East Aurora Plant. This concentration consisted of ciprofloxacin and sulfamethoxazole. Furthermore, sulfamethoxazole and ciprofloxacin were also still detected in surface water 100 m from the discharge point. The tetracycline, trimethoprim, and clindamycin were either not detected or not present above the LOD (Table 3). In Holland, 12.9 μg/L of total antibiotics was detected at the outfall, which was mostly composed of ciprofloxacin and sulfamethoxazole, but also contained tetracycline, trimethoprim, and clindamycin at quantifiable concentrations. Significant concentrations of sulfamethoxazole (0.043 μg/L) and clindamycin (0.060 μg/L) were present 100 m from the Holland WWTP outfall. It is interesting to note that antibiotics were still detected 100 m from the discharge point at both WWTPs, demonstrating that surface waters are vulnerable to antibiotic contamination from point sources. Similar results were found when the outfall and surface water 10 m from the discharge plant were resampled in February, also indicating a chronic, low level exposure of aquatic organisms to several antibiotics in the local surface waters.

4. Discussion

Although the antibiotics were still detectable in surface waters, the concentrations were much lower than those observed at the WWTP outfall, indicating that some processes,

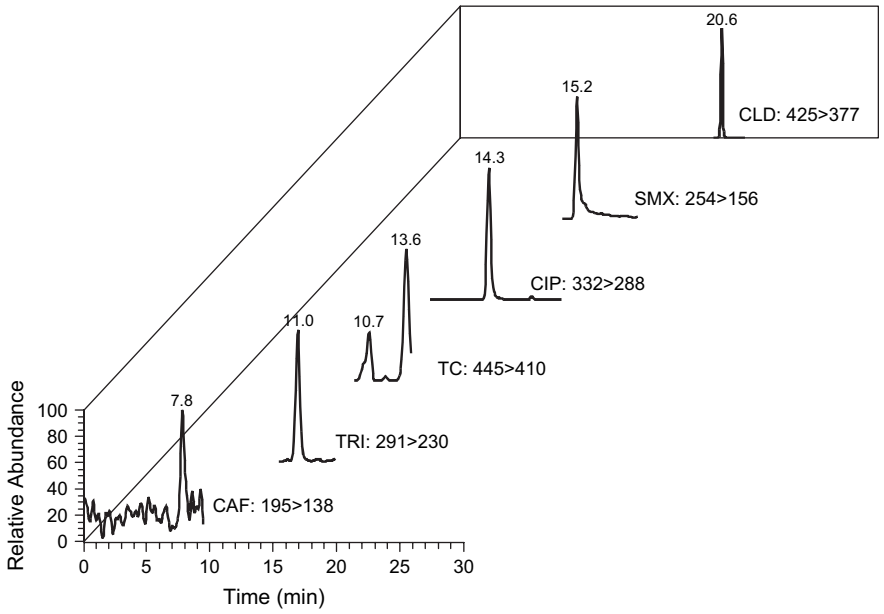


Fig. 2. Extracted ion base peak chromatograms of the six target analytes detected in the WWTP effluent collected from Holland, NY.

such as dilution, degradation, or sorption, are taking place during stream transport. Given that only water was analyzed in this study, the potential exposure of organisms to these compounds may be underestimated, since antibiotics have also been detected in streambed sediments (Loeffler and Ternes, 2003; Simon, 2005). The concentration of antibiotics in streambed sediments may become more important for antibiotics with higher sorption coefficients (K_ds), such as ciprofloxacin (417 mL/g) (Stuer-Lauridsen et al., 2000) and tetracycline (8400 mL/g) (Kim et al., 2005). On the other hand, for sulfamethoxazole and trimethoprim sorption is negligible (Perez et al., 2005). Bacteria resistant to the sulfamethoxazole/trimethoprim combination, trimethoprim, ciprofloxacin, tetracycline, and erythromycin have been previously detected in a WWTP with similar concentrations of antibiotics (Costanzo et al., 2005). Although determination of resistant bacteria is outside the scope of this study, the results from previous investigations suggest the potential presence of antibiotic resistant bacteria in the final effluents of the three WWTPs sampled.

From the results of this study, it is apparent that amount of antibiotics that are introduced into the receiving surface waters not only depend on the nature of each compound, but can also depend on the operation and design of the WWTP. In the secondary treatment process, antibiotics can either undergo biodegradation or sorption to the biosolids. Sulfonamides and trimethoprim are highly water-soluble and have been found to have negligible sorption to the sludge biomass (Perez et al., 2005). On the other hand, tetracyclines (Kim et al., 2005) and ciprofloxacin (Ternes et al., 2004b) have been found to sorb significantly to soils or sewage sludge. The large amount of microorganisms present in the sewage sludge promotes removal of organic matter via rapid biodegradation. The sulfonamide antimicrobials have been found to biodegrade in activated sludge (Carballa et al., 2004; Perez et al., 2005). Carballa et al. (2004) followed the behavior of selected pharmaceuticals throughout a sewage treatment plant, and found that sulfamethoxazole was degraded by about 67% in a conventional activated sludge process. Perez et al. (2005) observed significant biodegradation of four sulfonamides within three days of exposure to activated sludge (degraded to 26% or less); however, in most WWTPs antibiotics and other compounds are not exposed to the activated sludge long enough to allow this level of degradation.

Although the majority of biodegradation and sorption of antibiotics occur during the secondary process, advanced treatment and disinfection processes have also been found to play an important role in the removal of antibiotics from wastewater. While it was found that sulfonamides and trimethoprim are not removed through the precipitation/coagulation process (Adams et al., 2002), tetracyclines precipitate with Fe^{3+}, Mg^{2+}, and Ca^{2+} (Ternes et al., 2004b), and also form a strong complex with Al^{3+} (Tolls, 2001). Moreover, in the study by Adams et al. (2002), chlorination was found to remove 90% of five sulfonamide antibiotics in 28 min or less and 90% of trimethoprim in 40 min from surface water. In contrast, while chlorination was effective in the removal of these antibiotics from surface water, ultraviolet radiation at typical dosages of 254 nm did not degrade the sulfonamides or trimethoprim effectively. Although these antibiotics do absorb UV radiation, they could not compete with the amount of natural organic matter found in surface water (Adams et al., 2002).

Based on what is known in the literature, and the observed concentrations of antibiotics in the study, it can be inferred that the Lackawana WWTP, which utilizes a conventional activated sludge process in combination with aluminum salt coagulation, can have the majority of ciprofloxacin and tetracycline removed through sorption to the biomass during the secondary treatment (Kim et al., 2005; Ternes et al., 2004b). In addition, tetracyclines may also be precipitated in the metal salt coagulation process (Ternes et al., 2004b), which could explain their

non-detection in WWTP effluent. On the other hand, the partial removal of sulfamethoxazole in the WWTP could be attributed to biodegradation, as this antibiotic does not sorb to sludge and is more easily degradable (Perez et al., 2005). Lastly, it is likely that further degradation takes place during the chlorination disinfection process prior to releasing the effluent in surface water, as previously shown (Dodd et al., 2005; Renew and Huang, 2004). The extended aeration secondary treatment employed at the East Aurora WWTP has the potential to more effectively remove antibiotics through degradation or sorption due to an exposure to the biomass that is three to four times longer than that of the conventional activated sludge. The East Aurora Plant uses a metal salt precipitation/coagulation with ferrous chloride, which could also explain the non-detection of tetracycline in the effluent and downstream from this WWTP. Finally, the Holland WWTP uses rotating biological contactors as a secondary aerobic treatment, but without a metal salt coagulation/precipitation process. Therefore, it is not surprising that while tetracycline was not found at quantifiable concentrations at either of the other two sampling locations, it was detected in the effluent of Holland WWTPs during all three sampling dates. Although the Holland WWTP effluent was not using UV radiation to disinfect effluent during the time of sampling, it is not expected to change the concentrations of the antibiotics in the surface water because UV radiation has been found to have little impact on the removal of antibiotics from distilled and surface water (Adams et al., 2002). In fact, when the Holland WWTP was resampled in October 2005, no differences in concentrations of were observed before and after the UV radiation disinfection (data not shown), substantiating our hypothesis. Clindamycin was also detected in the effluent from two of the plants, but information regarding the behavior of this antibiotic in wastewater treatment processes is currently not available.

5. Conclusion

The effluent from three local wastewater treatment plants revealed concentrations of six of the seven test compounds on three sampling occasions, indicating that a chronic exposure to low levels of antibiotics exists as the result of incomplete elimination by current wastewater treatment processes. Although all sampling took place during the fall and winter seasons, it would be likely that some detection of these compounds would also take place during summer and spring conditions. Results from this study show that the sampled wastewater treatment plant processes are not effective in the complete removal of the target antibiotics. However, since only the effluent from each plant was sampled, it is not possible at this time to quantify the percent removal efficiency of antibiotics by the different processes employed in the plants. Currently, work is being conducted to investigate the removal of these recalcitrant antibiotics in WWTPs using various waste treatment processes. The ongoing study will provide invaluable information needed to improve the removal of antibiotics from recycled wastewaters in order to reduce potential risks associated with exposure of biota to these compounds in receiving waters.

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