PERSISTENT ORGANIC COMPOUNDS IN WASTEWATER: AZITHROMYCIN AND UROBILIN CONCENTRATIONS IN WASTEWATER TREATMENT PLANT SAMPLES FROM MURRAY, KENTUCKY, USA

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Abstract
The presence of pharmaceutical and personal care products (PPCPs) in the environment and their possible impact on wildlife and human health is an emerging area of research. In this study, we collected influent and effluent samples during summer, fall and winter months from a small wastewater treatment plant in Murray, Kentucky USA and analyzed for azithromycin and urobilin. Azithromycin concentrations ranged from 4.4 ng/L to 52.6 ng/L. Urobilin concentrations ranged from below detection limit (<1 ng/l) to 39,573 ng/L The highest concentrations were found in influent samples collected during the month of February 2007. In general, influents contained higher concentrations of the analytes than the effluents indicating that the compounds degraded during the wastewater treatment processes. To our knowledge this is the first report on the occurrence of azithromycin and urobilin in wastewater treatment plant samples from Murray, Kentucky, USA.

Introduction
Pharmaceutical and personal care products (PPCPs) residues have been detected in environmental samples including groundwater, surface water, and municipal wastewater[1-4]. Pharmaceutical drugs given to people as well as to domestic animals include antibiotics, hormones, pain relievers, tranquilizers, and chemotherapy chemicals given to cancer patients. Many drugs are designed to be persistent and lipophilic, so that they can retain their chemical structure long enough to do their therapeutic work. These drugs are excreted and distributed into the environment by flushing toilets as well as by spreading manure and sewage sludge onto soil[2]. These chemicals persist in the environment, enter the food chain, bioaccumulate, biomagnify, and cause harmful effects in wildlife and humans. Because of aquatic contamination by these chemicals, bacteria and other microbes in the aquatic environment can become more resistant to these chemicals. This results in the development of more antibiotic resistant and virulent pathogens in the environment. Therefore, the persistence of pharmaceutical chemicals in the environment has become a global problem. Azithromycin, a commonly used antibiotic and urobilin, a breakdown product of bilirubin in intestines were selected as target compounds for this study. Chemical structures of azithromycin and urobilin are shown in Figure 1 and 2. Contamination levels of these compounds were measured in water samples collected from a wastewater treatment plant located in Murray, Kentucky, USA. Murray is a small university town with a population of approximately 25,000 including 10,000 students. Murray wastewater treatment plant processes about 5.2 million gallons (19.8 million liters) wastewater per day. Source of wastewater for the plant is predominantly domestic along with small fraction from commercial business and industries. The objectives of the study was to (i) determine the levels of azithromycin and urobilin in influent and effluent samples collected from Murray Wastewater Treatment Plant, (ii) assess seasonal variation in concentrations of the analytes and (iii) elucidate removal and/or degradation of analytes during the wastewater treatment processes. Understanding current levels of persistent pharmaceuticals and other organic compounds is important in order to identify the source of the pollutants, ‘chemicals of concern’ in the region, and to prevent further contamination in order to protect wildlife and human health.

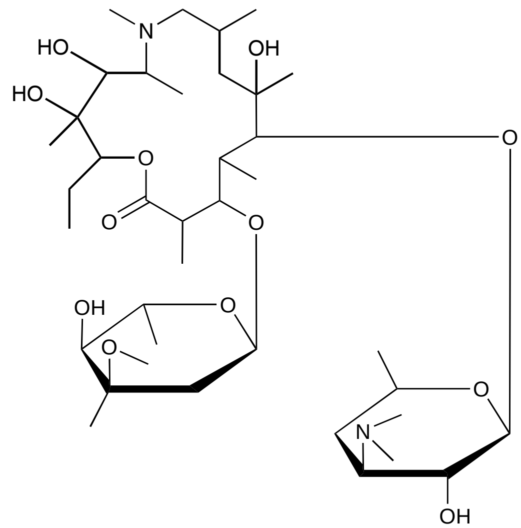

Figure 1. Structure of azithromycin

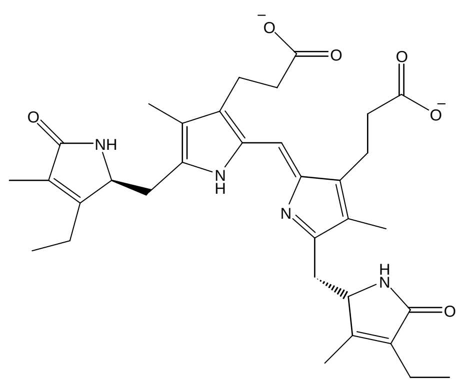

Figure 2. Structure of urobilin

Materials and Methods

Water samples were collected from the Murray Wastewater Treatment Plant (MWWTP) during the months of August, September, December 2006, February, and March 2007. Samples were collected using high density polyethylene (HDPE) bottles, stored in ice during sampling, and upon arrival at laboratory the samples were stored at -20°C until analysis. The samples were filtered using glass fiber filter (10µ pore size) and pH was lowered to 3 or less using 12 M HCl. SPE-HBL (Water Oasis SPE-HBL 6cc, 200 mg) cartridges were used for sample extraction. The cartridges were prepared by running 5 mL of methanol through each cartridge, followed by a further rinse with 10 mL of deionized water. Each sample filtrate, 100-200 mL, was passed through an SPE-HBL cartridge using vacuum suction at a constant flow of 3-4 mL/min. Polar compounds were eluted using 20-25 mL 1% acetic acid in methanol at a rate of approximately 3-4 mL/min. The eluate was then microconcentrated down to 1.0 mL or less using N_2 gas (< 25 degrees C). The samples were further concentrated using Zymark TurboVac II (21°C Bath tub, 4-8 psi) and then volume was made up to 0.5 mL using methanol. LC-MS analysis was performed in the Environmental Protection Agency National Exposure Research Laboratory, Las Vegas, NE. Varian Prostar model 410 auto sampler was used for sample injection. Mobile phase consisted of 0.5 % formic acid in 82% methanol/18% acetonitrile, 0.5% formic acid in DI water. Formic acid was present for proton transfer and soft ionization. Solvent Pump model was Varian Prostar: Solvent Delivery Model with 2 pumps (A&B, psi: 730). Mass Spectometer model was Varian 500-M-S IT Mass Spectrometer. Precursor ions 749.5 (M+H)+ and 591 (M+H-HCl)+ were used for detection of azithromycin and urobilin respectively.

Results and Discussion

Concentrations of azithromycin and urobilin in influent and effluent samples from Murray wastewater treatment plant samples were shown in Table 1. Azithromycin was detected in all samples analyzed. Highest concentration of azithromycin recorded was 52.6 ng/L in influent sample collected during February 2007. Effluent contained relatively less azithromycin than influent samples. Urobilin was detected in all influent samples. Concentrations of urobilin were several orders of magnitude higher than azithromycin residues (Table 1).

Table 1. Concentrations of azithromycin and urobilin (ng/L) in influent and effluent water samples collected from Murray Wastewater Treatment Plant samples during different months.

DATE OF SAMPLING	AZITHROMYCIN		UROBILIN	
	Influent ng/L	Effluent ng/L	Influent ng/L	Effluent ng/L
08/09/2006	18.0	7.2	6600	<1
09/22/2006	7.2	13.7	3520	<1
12/18/2006	37.7	23.4	6720	<1
02/09/2007	52.6	20.3	39570	37.1
03/09/2007	4.4	4.9	12740	4.4

A notable observation is that when azithromycin and urobilin concentrations were compared, azithromycin concentrations did not change greatly with influent and effluent samples, whereas urobilin concentration in effluents were several orders of magnitude lower than influent samples. This remarkable difference in concentrations shows that urobilin is degraded or lost during wastewater treatment processes, while azithromycin is relatively more persistent and wastewater treatment processes rarely affect the concentrations of azithromycin. Therefore, azithromycin will be transported more to the receiving waters from the wastewater treatment plants. Azithromycin is a macrolide, similar to erythromycin, as well as clarithromycin and roxithromycin. In a similar study conducted in Switzerland, concentrations of 131 ng/L for clarithromycin and 35 ng/L for roxithromycin were detected in effluent samples. The degraded form of erythromycin, which no longer showed antibiotic function, was also found[3]. As an antibiotic, azithromycin is useful for treating many infections of the respiratory system and skin. It is effective against pathogens such as *Haemophilus influenzae*, *Chlamydia pneumoniae*, *Streptococcus pyogenes*, *Mycoplasms pneumoniae*, and *Legionella* species. It is also useful against most *Streptococcus pneumoniae* and *Staphylococcus aureus* that are resistant to erythromycin[5]. By remaining in the environment, azithromycin resistant strains of pathogens are likely to occur through natural selection, causing azithromycin no longer to be effective in fighting infections of microbes for which it had been in the past. The results indicate that azithromycin can build up in the environment without degradation, causing need for further study and development of new, less environmentally persistent drugs and tracking the fate of currently used antibiotics.

Urobilin is formed from the break down of bilirubin in the intestines. A study conducted on human urobilin excretion in Scotland found healthy males under the age of 36 to excrete 1.19 mmol/kg in feces while women of the same age excreted 1.04 mmol/kg in feces[4]. Urobilin has been shown useful as a marker of human waste in water systems because it is found in human feces and urine[6] This study provides evidence that detectable levels of azithromycin and urobilin are found in Murray wastewater treatment plant samples. Variations in concentrations of the analytes during different seasons indicate variation in input of these chemicals during different months. Azithromycin is more persistent and not affected greatly by the wastewater treatment processes. Future monitoring studies with greater number of samples is needed in order to describe the distribution, mass loading, behavior and fate of these compounds in the environment.

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