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Funeral Homes and Slaughterhouses: Contributions of Emerging Contaminants to Municipal Wastewaters

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Abstract

Attention to emerging contaminants due to their appearance in receiving waterbodies has resulted in efforts to identify contributions from alternative sources, including those contributed from funeral homes and slaughterhouses. This paper describes results of a monitoring program to characterize the source magnitudes of emerging contaminants into municipal wastewater treatment plants (WWTPs), specifically from two funeral homes in two different cities, and a slaughterhouse, to identify source magnitudes of the selected beta-blockers, antidepressants, antibiotic, estrogens, acidic pharmaceuticals and basic/neutral pharmaceuticals in WWTPs. Acetaminophen, caffeine, ciprofloxacin and ibuprofen were detected in all the samples from funeral homes and slaughterhouse while 17a-ethinyl estradiol (EE2), citalopram, gemfibrizol, sulfamethoxazole, sulfapyridine, and venlafaxine were detected in samples at more than 50% frequency. There were differences in compounds detected 100% of time between funeral home-A and funeral home-B. In slaughterhouses, acetaminophen, atenolol, bisphenol A, caffeine, ciprofloxacin, citalopram, gemfibrizol and ibuprofen were detected in all the samples. When the contributions from funeral homes and slaughterhouse are compared to the total loading for each compound at the WWTPs, it was determined that funeral homes and the slaughterhouse contributed <3.5% of the mass loading. The loading percentages for all five groups for funeral homes-A and B and the slaughterhouse (were 0.14%, 0.70%, 3.2%, respectively, where the major contributions were from acetaminophen and sulfamethoxazole. The substantial presence of acidic and basic/neutral pharmaceuticals, especially acetaminophen from both funeral home-B and the slaughterhouse, and natural estrogens and industrial estrogens specially Estrone and Nonylphenol in the slaughterhouse are noted due to their major contributions towards the total load.

***Keywords**: Emerging contaminants; Municipal wastewater; Funeral homes; Slaughterhouse*

Introduction

Emerging contaminants such as pharmaceuticals and personal care products are receiving increased attention as researchers study their occurrence in the environment and, in turn, their effects on living organisms [1-3]. For example, beta-blockers, antidepressants and estrogen have been found in the tissues of fish [4-8]. However, little information is available regarding the contribution of pharmaceuticals and other emerging contaminants from the industrial, commercial, and institutional sectors [8]. In a search to find the point of entry for some emerging contaminants into the natural environment, scientists are routinely led to municipal wastewater treatment plant effluents.

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Since there are thousands of chemicals and substances available for industrial processes and consumer goods, and with additional chemicals being developed every day, the impacts of these chemicals are not necessarily known. The effects of many on human health and the environment, including water, are not established [9]. Additional chemicals that may emerge in increasing concentrations in the next several decades include those used for embalming processes and slaughterhouses, including by products.

Ruhoy and Daughton [10] estimated that 17.9 metric tons of medicine are disposed of annually in the U.S., to the sewage system from activities associated with deceased individuals (the major part of the drugs are in the deceased person's body, some of it in their saliva and urine which will be disposed by the funeral staff to sanitary sewers (although blood in some jurisdictions is a controlled waste).

With respect to slaughterhouses, several tranquilizers and beta-blockers are employed to relieve anxiety and stress of food-producing animals and hypnotic sedatives are commonly injected to calm the animals as they are transported to the slaughterhouse. Choi et al. [11] and Shao et al. [12] reported on occurrence and concentrations of pharmaceutical drugs in slaughterhouse wastewater, reflecting the drug usage in animal husbandry.

Brodin et al. [7] noted that antibiotics are used for both humans and animals to help curb antibiotic resistance and veterinarians administer them differently for animals. While individual animals can be treated with specific antibiotics, it is most common for antibiotics to be administered to groups via the animal feed or water. There are more than 90 million cattle, 5.3 million sheep's and lambs, 66 million hogs, 200 million turkeys and eight billion chickens on U.S. farms. The combined weight of livestock and poultry in the U.S. is roughly 3.5 times that of the combined weight of American men and women. A 1200 pound steer is equal to approximately six men, and hence, if a steer needs treatment for pneumonia, logic indicates it will require a larger dose than a person. Similarly, it is logical that the combined U.S. livestock and poultry herds and flocks will require more antibiotics by volume than the combined human population [7]. Some parts of these medications are retained in the bodies of the animals and are destined to slaughterhouses.

In order to understand the fate and transport pathways of these compounds within the overall wastewater system, this research focused on six classes of emerging contaminants monitored in discharges from funeral homes and a slaughterhouse, namely beta-blockers, antidepressants, antibiotics, natural estrogens and industrial estrogens, acidic pharmaceuticals and basic/neutral pharmaceuticals, through monitoring efforts from the wastewater effluents of two funeral homes and one slaughterhouse. Atenolol, metoprolol, propranolol and sotolol were analysed in the beta-blocker group. In the anti-depressants group, citalopram, desmethylcitalopram, venlafaxine and desmethyl-venlafaxine were analysed. In the antibiotics group, ciprofloxacin, sulfamethoxazole, sulfapyridine and trimethoprim were included. In the natural estrogens and industrial estrogens group, estrone, 17α-estradiol (E2) and 17α-ethinyl estradiol (EE2), nonylphenol, octylphenol and bisphenol A were analysed. Acetaminophen, gemfibrozil and ibuprofen were included in the acidic pharmaceuticals group while caffeine and carbamazepine were analysed in the basic/neutral pharmaceuticals group. In total, the concentrations to municipal wastewater of 22 compounds in wastewater effluents were investigated. Most of the 22 compounds are medications prescribed by medical professionals to treat a variety of conditions as discussed below.

Beta-blockers are a class of drugs which are designed to inhibit the action of beta-adrenergic receptors that are part of the central nervous system. Beta-blockers are known to have an antihypertensive effect and are used to treat individuals with high blood pressure. Propranolol, one of the most commonly-used beta-blockers, has revolutionized the treatment of angina. Van der Vring et al. reports that propranol hydrochloride is used for large animals [13,14]. The four beta-blockers evaluated in this study are atenolol, sotalol, metoprolol, and propranolol.

Antidepressants are a class of pharmaceuticals that affect neurotransmitters, the chemicals that nerves within the brain use to communicate with each other. Serotonin, dopamine and norepinephrine are examples of neurotransmitters. It is thought that an imbalance in these neurotransmitters is the cause of depression and may play a role in anxiety. Antidepressants are believed to work by inhibiting the release, or affecting the action of, neurotransmitters. O-desmethylvenlafaxine, velafaxine, citalopram, and desmethylcitalopram are the antidepressant compounds evaluated in this study. O-desmethylvenlafaxine, a major active metabolite of venlafaxine, also functions as a serotonin-norepinephrine reuptake inhibitor (SNRI). It is also synthetically produced (desvenlafaxine) and was approved by Health Canada in 2009 [15] for the treatment of depression. Venlafaxine is a SNRI prescribed for the treatment of depression, depression with associated symptoms of anxiety, generalized anxiety disorder, social anxiety disorder and adult panic disorder. Citalopram is a selective serotonin reuptake inhibitor (SSRI) prescribed for the management of depression as well as treating obsessive-compulsive disorder, panic disorder, premenstrual dysphoric disorder, anxiety disorder and post-traumatic stress disorder. Desmethylcitalopram, an active metabolite of citalopram, also functions as an SSRI.

Antibiotics are a class of drugs used to kill or slow the growth of bacteria. They are available by prescription from a doctor and are used to treat a variety of bacterial infections. Antibiotics are among the most commonly prescribed drugs used in human medicine and can be lifesaving drugs. Ciprofloxacin is a broad-spectrum antimicrobial carboxyfluoroquinoline. This medication is used to treat a variety of bacterial infections. Ciprofloxacin belongs to a class of drugs called quinolone antibiotics. It works by stopping the growth of bacteria. Sulfamethoxazole is used for bacterial infections such as urinary tract infections, bronchitis, and prostatitis and is effective against both gram negative and positive bacteria such as Listeria monocytogenes and *E. coli*. Sulfapyridine is a sulfonamide antibiotic. The sulfonamides are synthetic bacteriostatic antibiotics with a wide spectrum against most gram-positive and many gram-negative organisms. In some countries, this medicine is only approved for veterinary use and hence may be relevant to slaughterhouse wastewaters and is of considerable interest as sulfapyridine is high in slaughterhouse wastewaters. Trimethoprim is an antibiotic used mainly in the treatment of bladder infections and hence it may show up in the urine of the deceased persons in funeral homes. The use of trimethoprim and sulfonamide in animals is also reported with trimethoprim often combined with a sulfonamide to produce a synergic effect which is both bactericidal and has broad spectrum activity [16].

Estrogenic compounds are substances that interact with the human body's hormone system. Estrogens of pharmaceutical origin are prescribed for indications such as oral contraceptives, hormone replacement therapies, motor deficits associated with menopause, hypoestrogenism, and the management of some pre- and postmenopausal symptoms. Sources of estrogen include birth control pills and postmenopausal hormone treatments, as well as the estrogen that women produce naturally and excrete. Other drugs that have been found include caffeine (which, of course, comes from several other sources besides medications); carbamazepine, an anti-seizure drug; fibrates, which lower cholesterol levels; and some fragrance chemicals [17].

17α-estradiol is a steroidal weak estrogen and potent 5α-reductase inhibitor used topically in the treatment of androgenic alopecia (hair loss) in men and women. 17α-ethynylestradiol (EE2) is a synthetic hormone, which is a derivative of the natural hormone, estradiol (E2). EE2 is an orally bio-active estrogen, and is one of the most commonly used medications for humans as well as livestock and aquaculture activity.

Estradiol-17b, alone or in combinations with progesterone, testosterone or trenbolone acetate are given to animals to improve their rate of weight gain and feed efficiency. Administration is by subcutaneous implantation in the ear. The ear, along with any residual drug is discarded at slaughter. In cattle, 84% of the estradiol-17b is converted into the non-estrogenic metabolite estradiol-17a which is excreted by the liver [18]. Under the freedom of information summary, the medicine REVALOR-XS, Sponsored by Intervet Inc., it was revealed that REVALOR-XS Implant (pellets) for Cattle (Steers Fed in Confinement for Slaughter) consists of Trenbolone Acetate and Estradiol and it has a slow-release delivery system which increases rate of weight-gain and improves feed efficiency for up to 200 days in steers fed while in confinement for slaughter [19]. Henricks and Frank [20] reports that Estradiol-17a was detected in liver, kidney and kidney fat tissues of steers treated with estradiol-controlled release implants. EE2 has become a widespread problem in the environment due to its high resistance to the process of degradation and its tendency to (i) absorb organic matter, (ii) accumulate in sediment and (iii) concentrate in biota [21]. Bisphenol A (BPA) is an organic synthetic compound and produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins. Some of these products are used in slaughterhouses and funerals homes. In slaughterhouse, sources of contamination of BPA might be the cutting boards, the plastic films used to wrap charcuteries or meat during storage on the selling stall. Additionally, contamination can occur *via* the food handlers since some cosmetics are known to contain BPA [22].

Pharmaceuticals can be divided up into acidic, basic, or neutral compounds and can be used to treat pain, inflammation and a variety of other conditions. They range in prevalence from acetaminophen to caffeine, which is found in coffee, tea, and chocolate among other goods. Acetaminophen is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. Gemfibrozil is the generic name for an oral drug used to lower lipid levels. It belongs to a group of drugs known as fibrates. Gemfibrozil helps reduce cholesterol and triglycerides (fatty acids) in the blood. High levels of these types of fat in the blood are associated with an increased risk of atherosclerosis (clogged arteries). Gemfibrozil is used together with diet to treat very high cholesterol and triglyceride levels in people with pancreatitis. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). It works by reducing hormones that cause inflammation and pain in the body. Ibuprofen is used to reduce fever and treat pain or inflammation caused by many conditions such as headache, toothache, back pain, arthritis, menstrual cramps, or minor injury. Carbamazepine is used to treat seizures and nerve pain such as trigeminal neuralgia and diabetic neuropathy. It is used in schizophrenia along with other medications and as a second line agent in bipolar disorder. Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class. It is the world's most widely consumed psychoactive drug. Caffeine is a stimulant that speeds up the central nervous system. Caffeine occurs naturally in products such as coffee, tea, chocolate and cola soft drinks, and is added to a variety of prescription and over-the-counter medications, including cough, cold and pain remedies. Energy drinks may contain both naturally occurring and added caffeine.

Funeral homes

In 1995, the National Funeral Home Directors Association (NFDA) conducted a study to gather information regarding the origin, nature, quantity, and fate of wastewaters from funeral homes as discharged WWTPs (NFDA, 1995). The NFDA collected samples from five funeral homes; separate samples of the total embalming wastewater and samples of 24-hour domestic wastewater flow were collected and analyzed (because the embalming wastewater was kept separate from the sanitary flow, USEPA was able to use this approach to characterize the sanitary waste only). All five samples were analyzed in accordance with established USEPA protocols for the following groups of parameters: 44 volatile organic compounds, 82 acid/base/neutral organics and miscellaneous organics and reported that following constituents were detected: Chloroform, Dichlorobromomethane, Trichloroethylene, T-butyl alcohol, Phenol, Bis 2-ethylhexyl phthalate, Diethyl phthalate, Di-n-octyl phthalate, Formaldehyde, 1,4-dichlorobenzene and Acetone. The NFDA (1995) does not mention the concentrations of any of the emerging compounds as monitored in this research, although the report itself is a very comprehensive report. Many jurisdictions treat the disposal of body and embalming fluids to the municipal sewer system as an accepted discharge point, but increasingly, jurisdictions such as New Zealand, have classified it as 'Controlled Wastes' that is wastewater from funeral homes would become a Conditional discharge, rather than a Permitted one [23]. In Florida, funeral homes that do not practice embalming are not considered biomedical waste generators but reports that body fluids are those fluids which have the potential to harbor pathogens, such as human immunodeficiency virus and hepatitis B virus and include blood, blood products, lymph, semen, vaginal secretions, cerebrospinal, synovial, pleural, peritoneal, pericardial and amniotic fluids [24]. In instances where identification of the fluid cannot be made, it shall be considered to be a regulated body fluid. Body excretions such as feces and secretions such as nasal discharges, saliva, sputum, sweat, tears, urine, and vomitus are not considered biomedical waste unless visibly contaminated with blood [25].

In UK, all waste materials from the preparation room or embalming theatre are potentially infectious and are dealt with as hazardous waste taking into consideration the requirements of the Environmental Protection Act - The Controlled Waste Regulations and the Special Waste Regulations, and The Safe Management of Healthcare Waste: Health Technical Memorandum 07-01 (EHPU, 2009). In US, the waste that is generated from the embalming process is considered medical waste and must be dealt with according to state and federal laws [26].

Slaughterhouses

Cavenati et al. [27] reports that direct discharges of livestock or slaughterhouse industry effluents into the aquatic environment (directly drained out to rivers and lakes) can also be a significant source of these compounds in the environment. Soil Science Society of America [28] reported that between 9 and 13 million kg of antibiotics are used in livestock operations in the US annually. Shao reported the determination of 76 pharmaceutical drugs in slaughterhouse wastewater. A maximum concentration of 32 ng/L of metaprolol, a beta-blocker, was reported.

Several tranquilizers and alpha, beta-blockers have been employed to relieve anxiety and stress of food-producing animals. Several hypnotic-sedatives are even injected into pigs to calm them during transportation to the slaughterhouse. Therefore, slaughterhouse wastewater have been reported as potentially a main sources of pollution with veterinary drugs. While experts agree that we need to use antibiotics responsibly in both humans and animals to help curb antibiotic resistance, it’s also important to understand the differences between use of human and animal antibiotics. While both human and animal antibiotics are prescribed by medical professionals, the prescribing doctors and veterinarians administer them differently. Humans receive individual therapies for their specific ailment. While individual animals may be treated with specific antibiotics, it is most common for antibiotics to be administered to groups via the feed or water [7]. Veterinary pharmaceuticals are widely used as additives in food or water at intensive animal-feeding facilities to protect animal health, prevent economic loss, and promote animal growth. All these activities result in a significant release of pharmaceuticals into the environment and the pollution of the environment by organic micro-pollutants has been a topic of concern [29]. Slaughterhouse wastewater contains high levels of organics, pathogenic and non-pathogenic microorganisms, and detergents and disinfectants used for cleaning activities [30].

This research investigates the sources of beta-blockers, antidepressants, antibiotic, estrogens, acidic pharmaceuticals and basic/neutral pharmaceuticals, into the sewer system from monitoring results from the wastewater effluents of two funeral homes, and a slaughterhouse in order to determine the primary route of entry for these emerging contaminants into the wastewater treatment system. Monitoring results from two different municipalities were utilized and will be referred to as cities A and B.

Methods

Sampling

The sampling locations were carefully selected to obtain the best opportunity to characterize emerging contaminants discharged into municipal wastewater systems. Most of the samples were 24-h composites obtained using an auto-sampler; where this was not possible, such as for the funeral homes, grab samples were used. Samples were obtained every two months for four sampling events per location.

Sampling locations

Samples were taken from manholes directly downstream of funeral homes, a slaughterhouse, residential neighbourhoods and influents of wastewater treatment plants by municipal staff. Due to the batch process of embalming a body, grab samples were used for both funeral homes and coordinated with the funeral home staff, to ensure that the sample was taken during times when there was an embalming occurring. At each location, three liters of sample were collected and split into two amber glass bottles and one HDPE bottle before they were stored at $4^{o}C$ until analysis. Analytical work was completed at the Worsfold Water Quality Centre at Trent University. The following table TABLE 1 outlines the sampling locations within each city.

TABLE 1**. Sampling Locations.**

City	Sampling location
A	Funeral Home, Residential (712,575 inhabitants), Treatment Plant Influent (Inflow (293 ML/day)
B	Funeral Home, Slaughterhouse, Treatment Plant Influent (Inflow (87.5 ML/day))
C	Residential (800,000 inhabitants)

Five samples each were taken for funeral home A and slaughterhouse B. Four samples were obtained from funeral home B while from residential area A and residential area C, ten and two samples were obtained, respectively. Moreover, five samples were taken from influent to City-A wastewater treatment plant and three samples were taken from influent to City-B wastewater treatment plant.

Analysis

The samples were filtered, acidified, and then extracted using Waters Oasis MCX solid phase extraction cartridges. The extracts were subsequently preconditioned with acetone, methanol, and dilute sulphuric acid before being eluted from the cartridge with ammonium hydroxide in methanol. They were then evaporated to almost dryness and reconstituted in methanol.

The target compounds were analyzed by Micromass Quattro LC triple-quadrupole mass spectrometer. The target compounds were analyzed in positive ion mode. Multiple reaction monitoring (MRM) was employed for analyte quantisation. Chromatographic separation was conducted on a Waters model 2695 HPLC system with a Genesis C18 column (150 × 2.1 mm i.d. 4 µm). The mobile phase A and B consisted of acetonitrile and aqueous ammonium acetate. Prior to extraction, the samples were spiked with stable-isotope labeled standards. Concentrations of the analytes in the sample were determined by comparing the relative ratio of the response of the target analyte to the response of the labeled standard with external standards. The beta-blocker procedure followed the method described in Scheurer.

For Quality Analysis and Quality Control, a laboratory blank for each sample batch and at least one sample duplicate from each set from the same sampling location was conducted. As well, surrogate standards were added into each sample to monitor and correct for any potential loss during the sample analysis.

Results

At each sampling location, the concentrations of beta-blockers are characterized in the following graphs for each of the source locations. FIG.1-4 show the concentrations of each compound at the various source locations. The box plot (a.k.a. box and whisker diagram) is a standardized way of displaying the distribution of data based on the five number summary: minimum, first quartile, median, third quartile, and maximum. In the simplest box plot, the central rectangle spans the first quartile to the third quartile (the interquartile range or IQR). A segment inside the rectangle shows the median and "whiskers" above and below the box show the locations of the minimum and maximum.

Beta- Blockers

Atenolol was detected in wastewater of slaughterhouse 100% of time. The average concentrations in slaughterhouse discharge were 371 ng/L while the maximum concentration in wastewater was 1190 ng/L. Atenolol was detected in funeral home-A and funeral home-B 50% and 75% of time. Metoprolol was detected in wastewater of funeral home-A 75% of time while it was detected 100% of time in funeral home-B and 80% of time in slaughterhouse wastewater. The average concentrations in funeral home-B and the slaughterhouse were 1084 and 108 ng/L respectively. Propranolol was detected in wastewater of funeral home-A and slaughterhouse 75% and 40% of time while in funeral home-B with 100% detection rate, the average concentration in wastewater was 13 ng/L. Sotalol was detected in wastewater of funeral home-A, funeral home-B and slaughterhouse 100%, 75% and 80% of time respectively. The average concentrations in funeral home-A and slaughterhouse were 97 and 287 ng/L respectively.

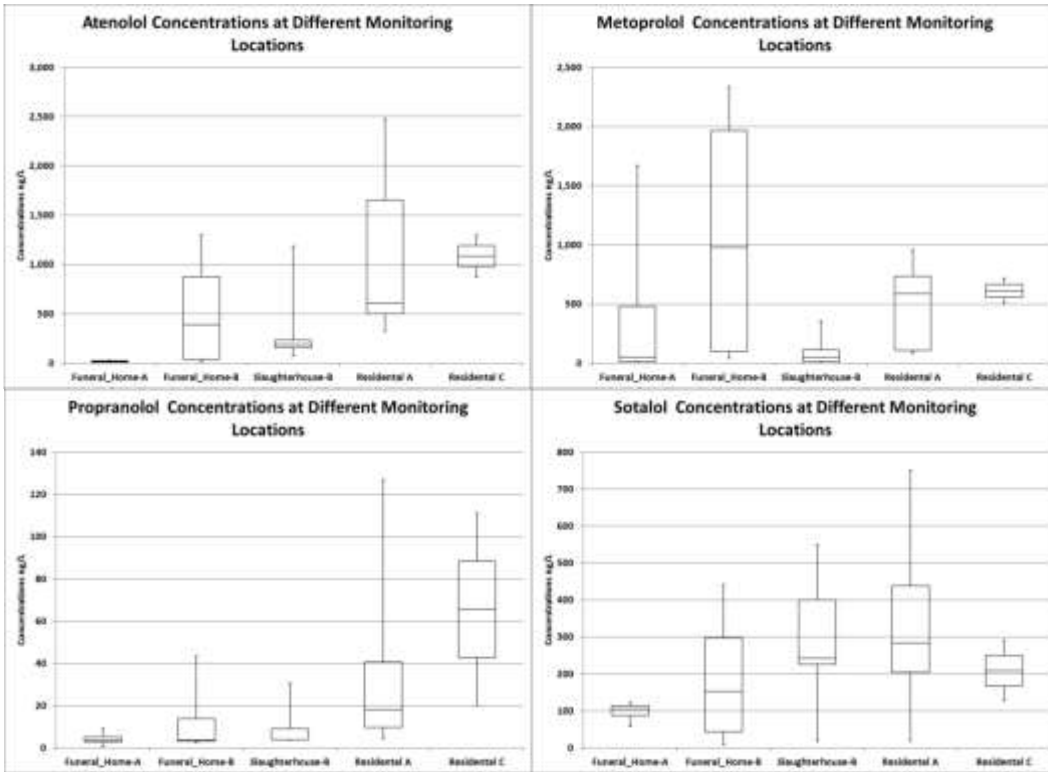

FIG.1. **Beta Blocker Concentrations at Different Sample Locations.**

Antidepressants

At each sampling location, the concentrations of antidepressants were characterized. The following graphs show the concentrations of each compound at the various source locations. Citalopram was detected 80%, 100% and 100% of the time in wastewater from funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 86, 759 and 182 ng/L, respectively, with the maximum being in funeral home-B wastewater with concentrations of 2382 ng/L. Desmethyl Citalopram was detected in wastewater of funeral home-A 60% of time while it was detected 100% of the time in funeral home-B and slaughterhouse. The average concentrations in funeral home-B and slaughterhouse were 535 and 217 ng/L respectively. Venlafaxine was detected 60%, 100% and 100% of the time in wastewater from funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 12, 56 and 208 ng/L respectively. O-Desmethyl Venlafaxine was not detected in wastewater of funeral home-A while it was detected 100% of time in funeral home-B and slaughterhouse. The average concentrations in funeral home-B and slaughterhouse were 128 and 602 ng/L respectively.

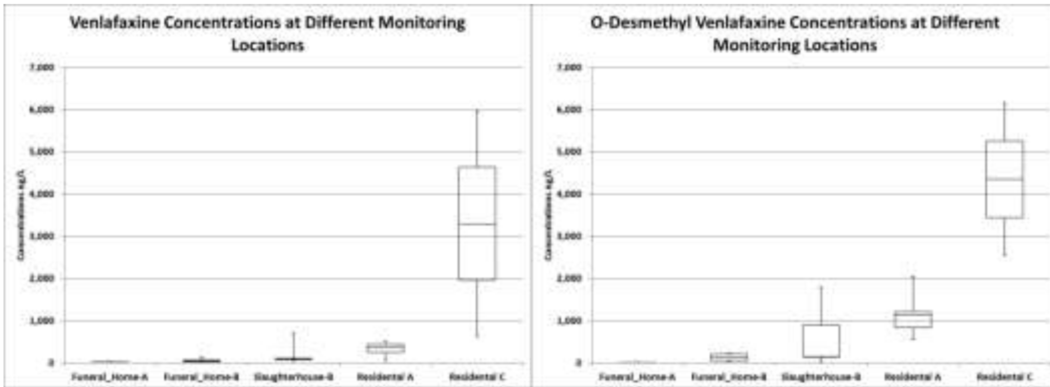

FIG. 2. **Venlafaxine and O-desmethyl venlafaxine Concentrations at Different Sample Locations.**

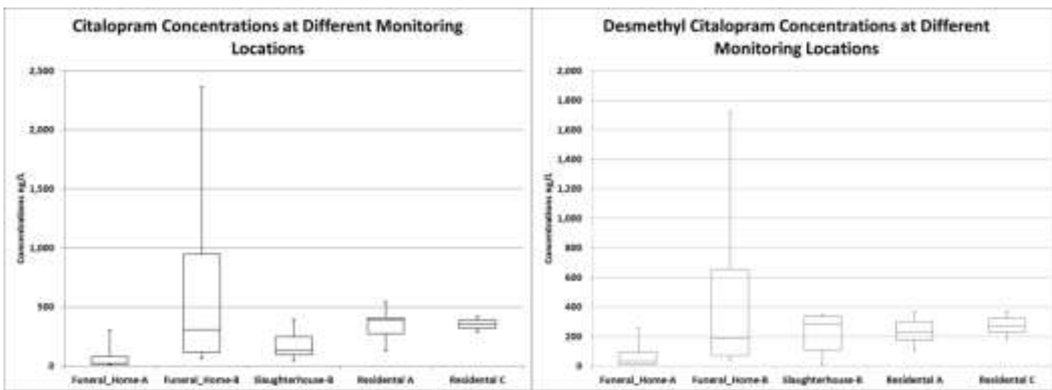

FIG. 3. **Citalopram and desmethyl citalopram Concentrations at Different Sample Locations.**

Antibiotics

At each sampling location, the concentrations of antibiotics are characterized. The following graphs show the concentrations of each compound at the various source locations. In the antibiotics group, ciprofloxacin, sulfamethoxazole, sulfapyridine, trimethoprim were included.

Ciprofloxacin was detected in all the samples. The maximum concentration of 30470 ng/L ciprofloxacin was observed in wastewater of funeral home-A. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 16041, 331 and 275 ng/L respectively. Sulfapyridine was detected 60%, 100% and 100% of the time in wastewater from funeral home-A, funeral home-B and slaughterhouse, respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 30, 329 and 215 ng/L respectively. The maximum being in funeral home-B wastewater with a concentration of 899 ng/L. Sulfamethoxazole was detected 60%, 100% and 80% of the time in wastewater from funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 31, 6408 and 189 ng/L respectively. The maximum being in funeral home-B wastewater with a concentration of 25240 ng/L. Trimethoprim was detected 100% of time in wastewater of funeral home-B with a maximum concentration of 2412 ng/L. Trimethoprim was also detected 20% and 40% of the time in wastewater from funeral home-A and slaughterhouse respectively. The average concentrations in wastewater from funeral home-A, funeral home-B and slaughterhouse were 13, 622 and 10 ng/L respectively.

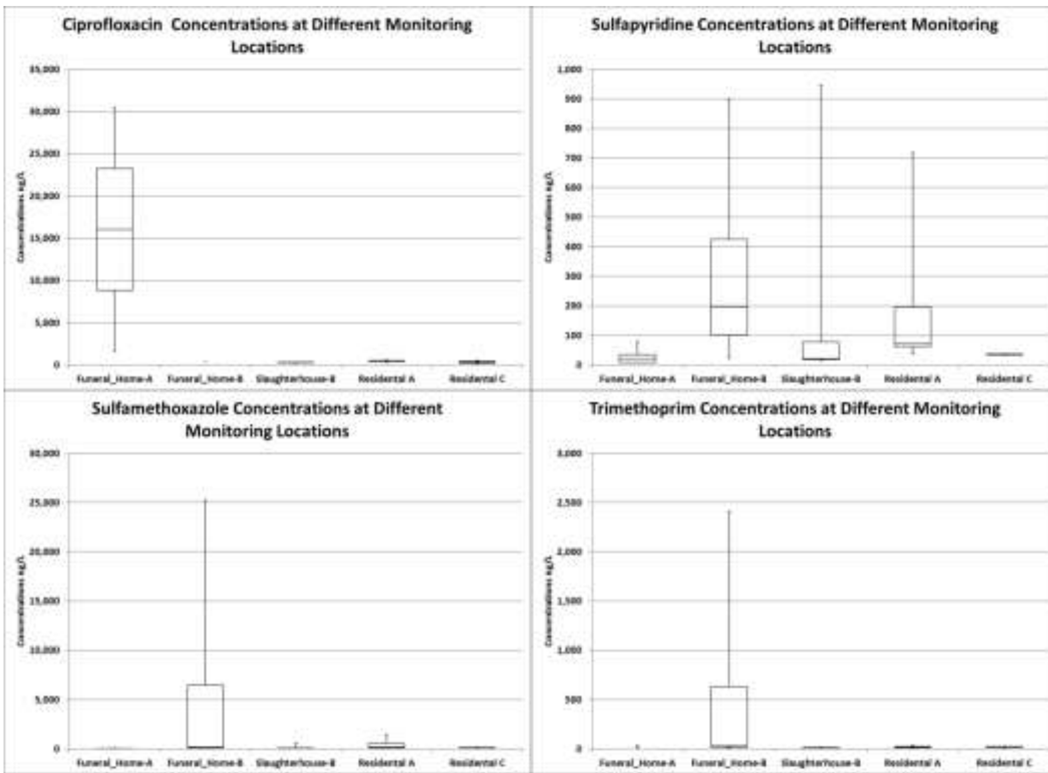

FIG.4. **Selected Antibiotics Concentrations at Different Sample Locations.**

Natural estrogens and industrial estrogens

At each sampling location, the concentrations of natural estrogen and industrial estrogen groups are characterized. The following graphs show the concentrations of each compound at the various source locations. The natural estrogens and industrial estrogens group, estrone, 17□-estradiol (E2) and 17□ -ethinyl estradiol (EE2), nonylphenol, octylphenol, bisphenol A were analysed. Estrone was detected 80%, 25% and 80% of the time in wastewater from funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 63, 17 and 52 ng/L respectively and the maximum being in funeral home-A with a concentration of 111 ng/L in wastewater. 17α-estradiol (E2) was detected 20%, 75% and 20% of the time in funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 19, 67 and 18 ng/L.

17α-ethinyl estradiol (EE2 was detected 80%, 75% and 80% of the time in funeral home-A, funeral home-B and slaughterhouse respectively. The average concentrations in funeral home-A, funeral home-B and slaughterhouse were 85, 80 and 51 ng/L. The maximum concentration in any of the samples from the three above mentioned sources was 224 ng/L being in funeral home-A (FIG. 5-8).

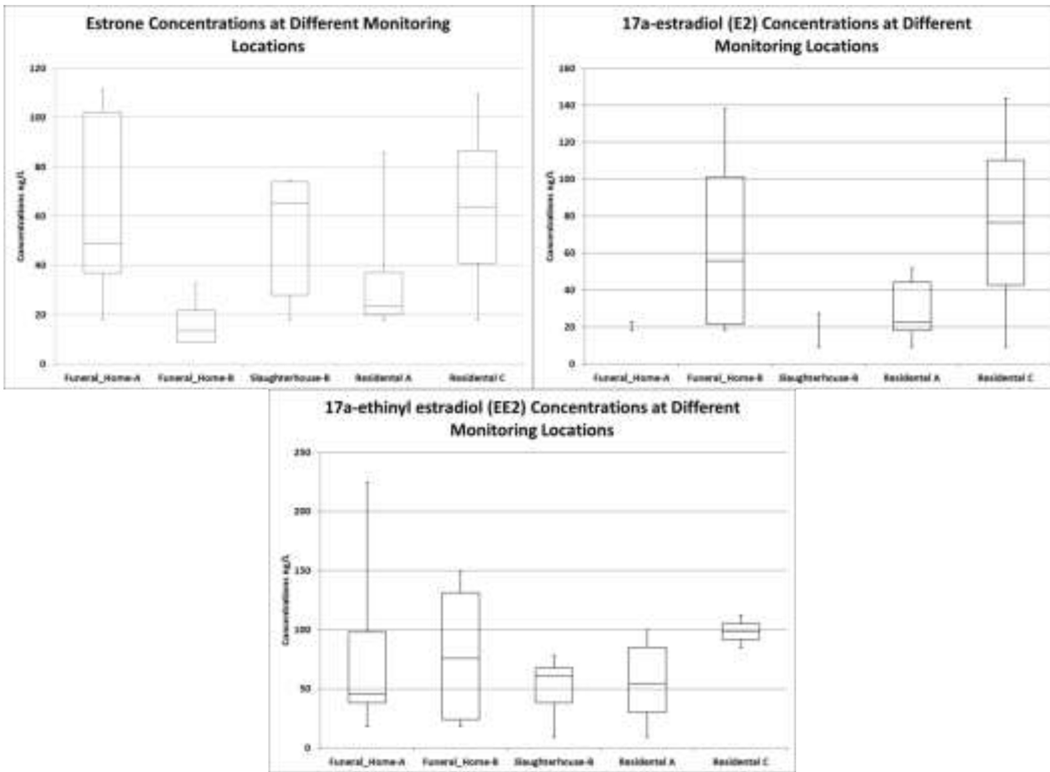

FIG.5. S**elected Natural Estrogens Concentrations at Different Sample Locations.**

Bisphenol-A was not detected in any of the samples from funeral home-A, but was detected in all of the samples from funeral home-B and slaughterhouse. The average concentrations were 317 ng/L and 224 ng/L in funeral home-B and slaughterhouse respectively. The maximum concentrations were 519 ng/L and 374 ng/L in funeral home-B and slaughterhouse respectively. Octylphenol was not detected in any of the samples from slaughterhouse while its detection rate in funeral home-A and funeral home-B were 60% and 50% respectively. The average concentrations in them were 37 and 26 ng/L. Nonylphenol was detected 20%, 25% and 20% of the times in in funeral home-A, funeral home-B and slaughterhouse respectively and the maximum concentration was in 238 ng/L in funeral home-B while the average concentrations 33, 83, 36 in in funeral home-A, funeral home-B and slaughterhouse respectively.

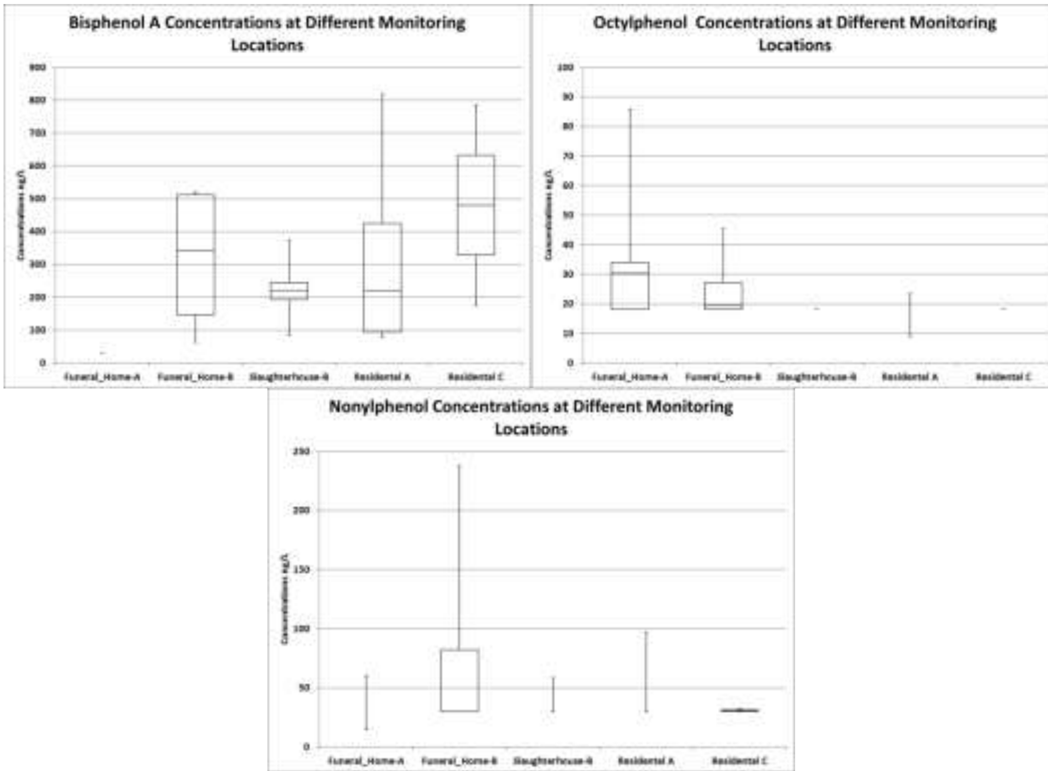

FIG.6. **Selected Industrial Estrogens Concentrations at Different Sample Locations.**

Acidic and basic/neutral pharmaceuticals

At each sampling location, the concentrations of acidic and basic/neutral pharmaceuticals group are characterized. The following graphs show the concentrations of each compound at the various source locations. Acetaminophen, gemfibrozil and ibuprofen were included in acidic pharmaceuticals group while caffeine and carbamazepine were analyzed in basic/neutral pharmaceuticals group. Caffeine was detected in all the samples from funeral home-A, funeral home-B and slaughterhouse. The average concentrations ranged from 7579 ng/L (in slaughterhouse) to 17,490 ng/L in funeral home-A with maximum concentration of 43790 ng/L being in funeral home-B. Carbamazepine was detected 20% of the samples in funeral home-A, it was not detected in any of four samples from funeral home-B whereas it was detected in two out of five samples in slaughterhouse. The maximum concentration in all these samples was 220 ng/L.

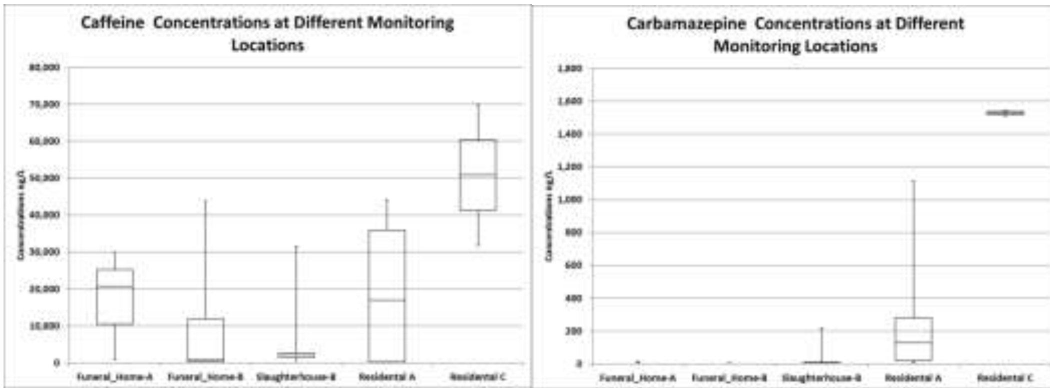

FIG.7. **Selected Acid Pharmaceuticals Concentrations at Different Sample Locations.**

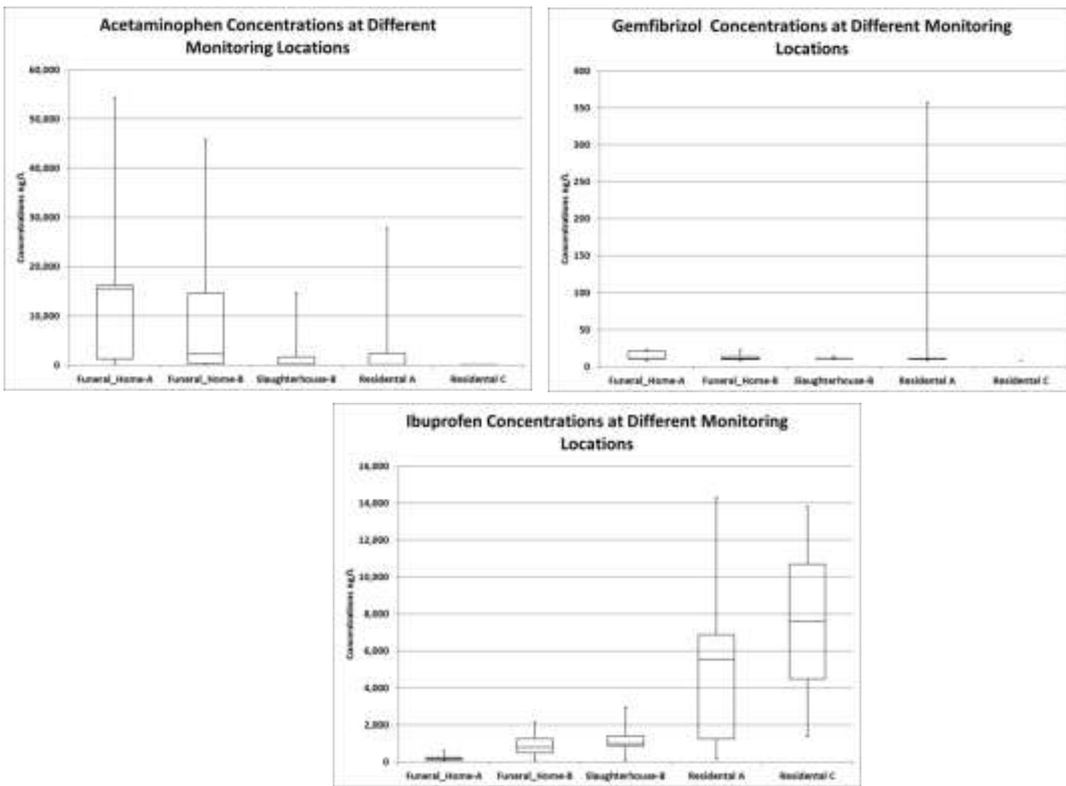

FIG.8. **Selected Basic/Neutral Pharmaceuticals Concentrations at Different Sample Locations.**

Acetaminophen was detected in all the samples from the three sources. The maximum concentration in wastewater of funeral home-A was 54310 ng/L. The average concentration of acetaminophen in funeral home-A, funeral home-B and slaughterhouse were 17550, 12651 and 3317 ng/L respectively. Gemfibrizol was detected in all the samples of slaughterhouse where as it was 80% and 75% of the time in funeral home-A and funeral home-B. The average concentration of gemfibrizol in funeral home-A, funeral home-B and slaughterhouse were 15, 13 and 11 ng/L respectively.

Ibuprofen was detected in all the samples from the three sources. The maximum concentration in wastewater of slaughterhouse was 2941 ng/L. The average concentration of ibuprofen in funeral home-A, funeral home-B and slaughterhouse were 239, 945 and 1261 ng/L respectively.

Discussion

Source concentrations

Acetaminophen, caffeine, ciprofloxacin and Ibuprofen were detected in all the samples. 17a-ethinyl estradiol (EE2), acetaminophen, caffeine, citalopram, gemfibrizol, ibuprofen, sulfamethoxazole, sulfapyridine, and venlafaxine were detected in all the samples more than 50% of time. In both funeral houses, the following compounds were detected 100% of time: acetaminophen, caffeine, ciprofloxacin, and ibuprofen. There were differences in the compounds detected 100% of time between funeral home-A and funeral home-B. In funeral home-A acetaminophen, caffeine, ciprofloxacin, ibuprofen and sotalol were detected in all the samples. While in funeral home-B acetaminophen, bisphenol a, caffeine, ciprofloxacin, citalopram, desmethyl citalopram, ibuprofen, metoprolol, o-desmethyl venlafaxine, propranolol, sulfamethoxazole, sulfapyridine, trimethoprim and venlafaxine were detected in all the samples. In slaughterhouse acetaminophen, atenolol, bisphenol a, caffeine, ciprofloxacin, citalopram, gemfibrizol, ibuprofen, o-desmethyl venlafaxine, sulfapyridine, and venlafaxine were detected in all the samples.

A comparison of average concentrations of selected beta-blockers, antidepressants, pharmaceuticals, antibiotics and estrogens was conducted with reference to concentrations in residential areas and are shown in FIG. 9-13. The average concentrations of atenolol were higher in the residential areas whereas metaprolol concentrations were higher in funeral homes. Propranolol and sotalol average concentrations were almost the same from all sources. The average concentrations of venalfaxine and o-desmethyl venlafaxine were higher in residential area C, while citalopram and desmethyl citalopram average concentrations were almost same from all sources.

The average concentrations of ciprofloxacin, sulfamethoxazole and trimethoprim were higher in funeral homes while other selected antibiotics average concentrations were almost same from all sources. The average concentrations of bisphenol-A were higher in residential while the average concentrations of other natural and industrial estrogens were almost the same from all sources.

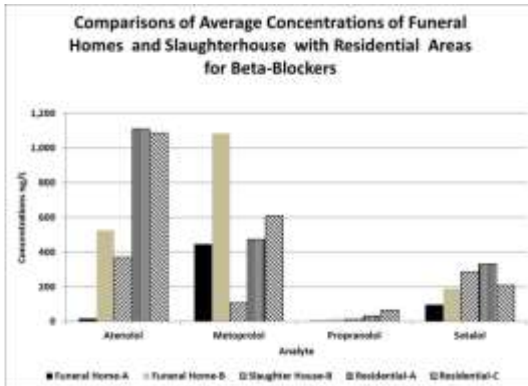

FIG.9. **Comparison of average concentrations of selected beta-blockers at Different Sample Locations.**

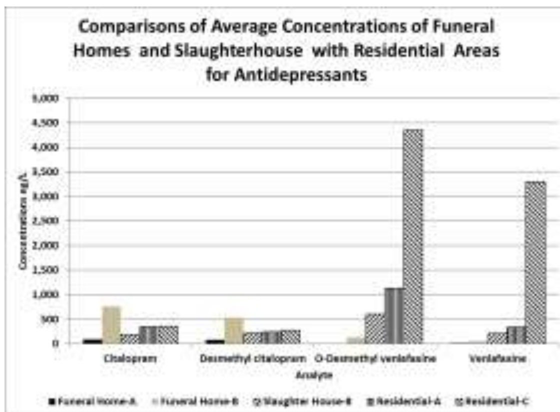

FIG.10. **Comparison of average concentrations of selected antidepressants at Different Sample Locations.**

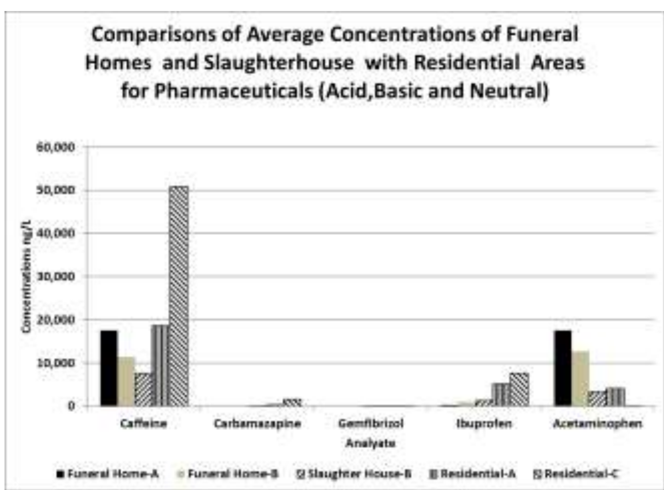

FIG.11. **Comparison of average concentrations of selected pharmaceuticals at Different Sample Locations.**

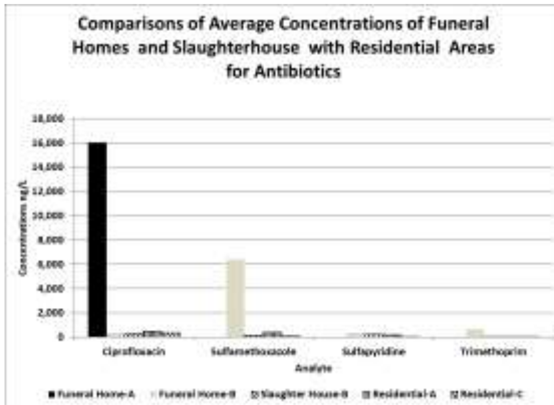

FIG.12. **Comparison of average concentrations of selected antibiotics at Different Sample Locations.**

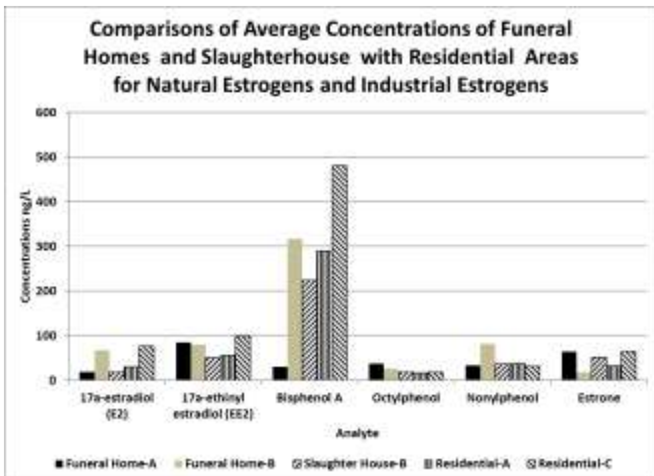

FIG.13. **Comparison of average concentrations of selected natural and industrial estrogens at Different Sample Locations.**

Analysis of contaminants loadings percentages of selected compounds from funeral home-A, funeral home-B and slaughterhouse to wastewater treatment plants

City-A treats an average of 293 ML of wastewater daily while city-B treats an average of 49.44 million liters of wastewater daily. The population of city-A is 712,575 and city-B is 140,410 and deaths were 5.2 people per 1000 people in 2009 as per StatsCan [29]. TABLE 2 from NFDA, [30] study provides an average flow rate of 2400 liter for one death so for the funeral home-A, it was assumed that 25000 liters of daily outflow was assumed while for funeral home-B an amount of 5000 liter of daily outflow was assumed based on the population of these cities. The slaughterhouse has reported that the outflow is around 52,250 liters per day [31]. Average load of each contaminant was determined and the percentages to total load of that contaminants in wastewater treatment plant was estimated.

TABLE 2. **Contaminants Loadings percentages of Selected Compounds from Funeral Home-A, Funeral Home-B and Slaughterhouse to Wastewater Treatment Plants.**

	Funeral Home-A	Funeral Home-B	Slaughterhouse
Variable	**% of Influent Load**	**% of Influent Load**	
Beta-Blockers			
Atenolol	0.000%	0.004%	0.030%
Metoprolol	0.012%	0.024%	0.024%
Propranolol	0.001%	0.003%	0.030%
Sotalol	0.002%	0.004%	0.069%
Total	0.015%	0.035%	0.153%
Antidepressants			
Citalopram	0.003%	0.016%	0.040%
Desmethyl Citalopram	0.003%	0.016%	0.067%
Venlafaxine	0.000%	0.001%	0.023%
O-Desmethyl Venlafaxine	0.000%	0.001%	0.028%
Total	0.006%	0.033%	0.158%
Acidic and Basic/Neutral Pharmaceuticals			
Caffeine	0.020%	0.127%	0.184%
Carbamazepine	0.000%	0.000%	0.019%
Acetaminophen	0.018%	3.959%	10.847%
Gemfibrizol	0.002%	0.002%	0.020%
Ibuprofen	0.001%	0.002%	0.025%
Total	0.041%	4.090%	11.096%
Antibiotics			
Ciprofloxacin	0.138%	0.003%	0.039%
Sulfamethoxazole	0.000%	1.240%	0.308%
Sulfapyridine	0.001%	0.015%	0.104%
Trimethoprim	0.003%	0.191%	0.032%
Total	0.142%	1.449%	0.483%
Natural Estrogens and Industrial Estrogens			
Estrone	0.010%	0.013%	0.405%
17a-estradiol (E2)	0.004%	0.008%	0.022%
17a-ethinyl estradiol (EE2)	0.012%	0.013%	0.089%
Bisphenol A	0.001%	0.013%	0.094%
Nonylphenol	0.009%	0.028%	0.126%
Octylphenol	0.018%	0.013%	0.099%
Total	0.055%	0.088%	0.835%
All	0.259%	5.695%	12.726%

The percentages of loads for beta-blockers towards wastewater treatment plants were 0.015% and 0.035% and for slaughterhouse, it was 0.153%. Antidepressants the percentages of loads towards wastewater treatment plants were 0.006% and 0.033% and for slaughterhouse, it was 0.158%. Acidic and basic/neutral pharmaceuticals the percentages of load towards wastewater treatment plants were 0.041% and 4.09% and for slaughterhouse, it was 11.096%. In funeral home-B acetaminophen contributed 3.96% of the load while in slaughterhouse, again, acetaminophen contributed 10.84% of the load. Antibiotics the percentages of loads towards wastewater treatment plants were 0.142% and 1.449% and for slaughterhouse, it was 0.4898%. In funeral home-B sulfamethoxazole contributed 1.24% of the load while in slaughterhouse, again, sulfamethoxazole contributed 0.308% of the load. Natural estrogens and industrial estrogens the percentages of loads towards wastewater treatment plants were 0.055% and 0.088% and for slaughterhouse, it was 0.835%. The loadings percentages for all the five groups for funeral home-A, funeral home-B and slaughterhouse were 0.259%, 5.695%, 12.726% respectively where the major contributions were from acetaminophen and sulfamethoxazole.

It was hypothesized that the percentages of loads for some of the chemical compounds were high because of the outliers in the data. USEPA ProUCL Ver 4.1 was used. The Dixon test was used to test for a single outlier in a univariate data set since the number of samples were around 5. This test is primarily used for small data sets (Dataplot limits the sample to be between 3 and 30). It can be used to test whether the minimum value is an outlier, the maximum value is an outlier, or either the minimum or maximum value is an outlier. Following outliers were identified. The concentrations of 43790 ng/l and 31540 ng/L of caffeine were identified as outliers in funeral home-B and slaughterhouse. For acetaminophen, the concentrations of 45870 ng/L and 14660 ng/L were identified as outliers in funeral home-B and slaughterhouse whereas for ciprofloxacin a concentration of 30,470 was identified as an outlier in funeral home-B. For sulfamethoxazole, concentrations of 102.7 ng/l, 25240 ng/l, 595.6 ng/l were identified as outliers for funeral home-A, funeral home-B and slaughterhouse, respectively. A concentration of 945.6 ng/L of sulfapyridine in slaughterhouse was identified as an outlier whereas a concentration of 2411 of trimethoprim in funeral home-B was identified as an outlier. All these data points were removed from the data and load analysis was done again and the results are presented in TABLE 3.

TABLE 3. **Contaminants Loadings percentages of Selected Compounds from Funeral Home-A, Funeral Home-B and Slaughterhouse to Wastewater Treatment Plants.**

	Funeral Home-A	**Funeral Home-B**	**Slaughterhouse**
Variable	**% of of Influent Load**	**% of of Influent Load**	
Beta-Blockers			
Atenolol	0.000%	0.004%	0.030%
Metoprolol	0.012%	0.024%	0.024%
Propranolol	0.001%	0.003%	0.030%
Sotalol	0.002%	0.004%	0.069%
Total	0.015%	0.035%	0.153%
Antidepressants			
Citalopram	0.003%	0.016%	0.040%
Desmethyl Citalopram	0.003%	0.016%	0.067%
Venlafaxine	0.000%	0.001%	0.023%
O-Desmethyl Venlafaxine	0.000%	0.001%	0.028%
Total	0.006%	0.033%	0.158%

Acidic and Basic/Neutral Pharmaceuticals			
Caffeine	0.020%	0.007%	0.184%
Carbamazapine	0.000%	0.000%	0.019%
Acetaminophen	0.018%	0.494%	1.591%
Gemfibrizol	0.002%	0.002%	0.020%
Ibuprofen	0.001%	0.002%	0.025%
Total	0.041%	0.505%	1.840%
Antibiotics			
Ciprofloxacin	0.014%	0.003%	0.039%
Sulfamethoxazole	0.000%	0.025%	0.084%
Sulfapyridine	0.001%	0.015%	0.016%
Trimethoprim	0.003%	0.008%	0.032%
Total	0.018%	0.051%	0.171%
Natural Estrogens and Industrial Estrogens			
Estrone	0.010%	0.013%	0.405%
17a-estradiol (E2)	0.004%	0.008%	0.022%
17a-ethinyl estradiol (EE2)	0.012%	0.013%	0.089%
Bisphenol A	0.001%	0.013%	0.094%
Nonylphenol	0.009%	0.010%	0.126%
Octylphenol	0.018%	0.013%	0.099%
Total	0.055%	0.070%	0.835%
All	0.135%	0.695%	3.158%

Acidic and basic/neutral pharmaceuticals the percentages of load towards wastewater treatment plants were 0.041%, 0.505 % and 1.84% funeral home-A, funeral home-B and slaughterhouse respectively. Funeral home-B acetaminophen contributed 0.49% of the load while in slaughterhouse, again, acetaminophen contributed 1.6% of the load. Antibiotics the percentages of loads towards wastewater treatment plants were 0.018% and 0.051% and for slaughterhouse, it was 0.171%. Natural estrogens and industrial estrogens the percentages of loads towards wastewater treatment plants were 0.055% and 0.070% and for slaughterhouse, it was 0.835%. The loadings percentages for all the five groups for funeral home-A, funeral home-B and slaughterhouse were 0.135%, 0.695%, 3.16% respectively where the major contributions were from acetaminophen and sulfamethoxazole [32,33].

Conclusion

The selected beta-blockers, antidepressants, antibiotic, estrogens, acidic pharmaceuticals and basic/neutral pharmaceuticals were detected in the effluents of funeral homes and slaughter house on their way into the sewer system. Acetaminophen, caffeine, ciprofloxacin and ibuprofen were detected in all the samples. In both funeral houses, the following compounds were detected 100% of time: acetaminophen, caffeine, ciprofloxacin, and ibuprofen. There were differences in compounds detected 100% of time between funeral homes-A and B. In funeral home-A Acetaminophen, caffeine, ciprofloxacin, ibuprofen and sotalol were detected in all the samples while in funeral home-B acetaminophen, bisphenol a, caffeine, ciprofloxacin, citalopram, desmethyl citalopram, ibuprofen, metoprolol, o-desmethyl venlafaxine, propranolol, sulfamethoxazole, sulfapyridine, trimethoprim and venlafaxine were detected in all the samples.

When the contribution due to funeral homes and slaughterhouse was compared to the total loading for each compound at the wastewater treatment influent, it was determined that funeral homes and slaughterhouse account for <3.5% of the mass loading. The loadings percentages for all the five groups for funeral home-A, funeral home-B and slaughterhouse were 0.135%, 0.695%, 3.16% respectively where the major contributions were from acetaminophen and sulfamethoxazole. The presence of acidic and basic/neutral pharmaceuticals specially acetaminophen from both funeral home-B and slaughterhouse and natural estrogens and industrial estrogens specially estrone and nonylphenol in slaughterhouse warrants some further studies because of their major contributions towards the total load.

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