International Journal of Advanced Research and Review

www.ijarr.in

SEASONAL VARIATIONS IN THE OCCURRENCE AND DISTRIBUTION OF PHARMACEUTICALS IN THE ODO-IYA ALARO RIVER, LAGOS STATE SOUTHWEST NIGERIA

Olatayo M. Ogunbanwo[a,b]

[a] School of Geography/water@leeds, University of Leeds, Leeds, West Yorkshire, LS2 9JT, UK
[b]Lagos State Polytechnic, School of Agriculture, Department of Fisheries Technology, Ecotoxicology Research Laboratory, Ikorodu, Lagos State, Southwest Nigeria
Corresponding Author E-mails: tayogunbanwo@yahoo.com & ogunbanwo.o@mylaspotech.edu.ng

ABSTRACT

The occurrence and seasonal variations of 37 pharmaceuticals belonging to 19 therapeutic classes in a sewage treatment plant (Alausa STP) and surface water in Lagos, Southwest Nigeria was investigated. Water samples were collected quarterly from April 2017 to January 2018. Among the targeted analytes, 26 compounds were detected. The ten most frequently detected compounds across the sampling locations were fexofenadine, carbamazepine, paracetamol, metformin, diazepam, cimetidine, codeine, sulfamethoxazole, atenolol and trimethoprim. The highest concentrations was observed for antibiotic and analgesic: sulfamethoxazole (129474 ngL^{-1}) had the highest maximum concentration followed by paracetamol (111374 ngL^{-1}). Over all, paracetamol had the highest mean concentration (18178 ngL^{-1}) while sulfamethoxazole had the second highest mean concentration (11160 ngL^{-1}). Cimetidine had the third highest maximum concentration of (95689 ngL^{-1}) and mean concentration (10458 ngL^{-1}). There were no specific spatial trends observed in this investigation and concentrations of pharmaceuticals in the study locations were high throughout the catchment revealing that there are potentially many contributing sites. So there were statistically significant differences between the different site categories (Pharmaceutical manufacturing sites (PME), Alausa STP site (SE), Semi- Urban and Urban sites (GLM: χ^2 (3) = 883.32, p <0.001). There are seasonal variations in the number of analytes detected in each sampling station. The mean and median concentrations of all the pharmaceuticals detected in the Odo-Iya Alaro River are extremely higher in the peak of the dry season than any other season. Hence, there were statistically significant differences between the dry season, peak of dry season, the wet season and peak of the wet season ((GLM: χ^2 (3) = 8.63), p<0.001). More pharmaceuticals were detected in the peak of the wet season (22) than the other seasons. 17 analytes were each found in the wet and dry seasons while 16 pharmaceuticals were detected at the peak of the dry season. Pharmaceuticals are indispensable to human health although their usage and discharge into the aquatic environment may lead to ecological problems and antibiotic resistance. This investigation showed that pharmaceutical pollution of the aquatic environment is a major challenge in Nigeria and management efforts are needed to address this issue.

Keywords: Pharmaceuticals; Rivers; Water quality; Pollution; Sewage

1.0 INTRODUCTION

Pharmaceutical presence in the aquatic environment was first detected in the 1970's (Tabak and Bunch, 1970; Norpoth et al., 1973). Numerous studies had been carried out on

occurrence of pharmaceuticals in the aquatic environment, but these have mostly been undertaken in Europe and North America (K'Oreje et al., 2012; Hughes et al., 2013). Fewer studies have been carried out in the developing countries of Africa, Asia, South America and the Middle East (Hughes et al., 2013; Madikizela et al., 2017). Some relatively high concentrations have, however, been found in countries such as China and India where treatment/regulation is less stringent than in the West. A similar situation exists in Africa, where high concentrations of pharmaceuticals are likely due to many regions suffering from little or no treatment of sewage before discharge to surface waters, particularly in rural areas and even in big cities. Even where sewage treatment plants (STP) exist many pharmaceuticals are not entirely removed by existing wastewater treatment methods (Gracia-Lor, 2010; Van Ginneken et al., 2017; Ortiz de Garcia et al., 2013).

Illegal discharge of raw sewage effluent into rivers by vacuum truck operators who collect sewage from residential homes may also be an issue in Africa. This activity is common in most countries in Africa where there is little or no legislation (Ogunbanwo, 2011). Even where there is legislation there is often little or no enforcement activity, leading to frequent discharges of untreated effluents into the aquatic environment (Ogunbanwo, 2011).

A particular concern arising from the pharmaceutical contamination of surface waters is where river waters are abstracted for portable uses (Lacey et al. 2008; Verlicchi et al., 2012b; Kay et al. 2017). In Western Europe, China, Canada, and the United States >30 pharmaceutical substances have been found in tap/drinking water (Tim Aur Der Beek et al., 2016). In France, traces of drugs have also been detected in bottled water (Bruchet et al., 2005) and, in many African countries, drinking water production mainly relies on surface water abstraction which may be contaminated. Surface water quality is a growing area of research in Africa, but there are significant gaps regarding the occurrence of pharmaceuticals in the aquatic environment. Scientists and their respective governments are beginning to realise the need for a cleaner environment in Africa. Evidence from industrialised nations on the occurrence of pharmaceuticals in the environment is encouraging scientists and their governments to study surface water quality more closely in Africa.

In the past six (6) years, publications on occurrence and fate of pharmaceuticals in Africa aquatic system have been on the increase, however, very limited information are available for Nigeria (only three publications to date). For example, Olarinmoye et al. (2016), using LC-MS/MS for quantification reported pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria, for the surface water, ibuprofen showed the highest concentrations up to 8.8 μg/L, while diclofenac was more abundant in sewage sludge with concentrations up to 1100 μg/kg dry weight. Olaitan et al. (2014) also reported the detection of pharmaceutical compounds in surface and groundwater samples collected from an irrigation canal and several wells in a pharmaceutical industrial area of Sango Ota, Ogun State, Nigeria. The average concentrations of the targeted pharmaceuticals such as diclofenac, chloroquine, paracetamol and ciprofloxacin were 17 μgL^{-1}, 5 μgL^{-1}, 3 μgL^{-1} and 1 $\mu g\ L^{-1}$, respectively. Inam et al., (2015) investigated the occurrence and risks posed by emerging organic pollutants (EOPs) in Ikpa river basin freshwater ecosystem in Niger-Delta, Nigeria between April and June 2013 (medium to heavy rainfall period). Seventeen compounds were detected at the ngL^{-1} levels: seven antibiotic drugs (acetamidophenol, chloramphenicol, ciprofloxacin, erythromycin, lincomycin HCl, roxythromycin, and sulfamethoxazole), three bactericides/antimicrobial agents (sulfathiazole, triclosan and triclocarban), an antiepileptic drug (carbamazepine), an analgesic drug (diclofenac sodium), a resin precursor (bisphenol A), a sunscreen product (oxybenzone), a hormone (equilin), an

insect repellent (DEET), and a stimulant (caffeine) in surface water samples from Ikpa River Basin as well as in the storm water from hospital dumpsite and municipal landfill leachate discharged into the freshwater body through run-offs. Low levels of maximum MEC were recorded for the commonly prescribed antibiotics: ciprofloxacin (2.3 ngL^{-1}), erythromycin (11.4 ngL^{-1}) and sulfamethoxazole (2.8 ngL^{-1}).

K'Oreje et al (2012) developed a new methodology involving both full-scan screening and selective target analysis to investigate the presence of 43 priority pharmaceutically active ingredients in the Nairobi River. Ten (10) human pharmaceutically active ingredients were found whose concentrations ranges from (low ngL^{-1} to high μgL^{-1}) Agunbiade and Moodley (2015) investigated the occurrence and distribution of eight acidic pharmaceuticals in South Africa and found that all were present in sediments, wastewater, and surface water samples. Wood et al. (2015) surveyed the occurrence of anti-retroviral compounds used for HIV treatment in South African surface waters and found average concentrations between 27 and 430 ngL^{-1}.

Madikizela et al. (2017) reviewed the status of pharmaceuticals in African (Kenya, South Africa and Tanzania) water bodies, finding that NSAIDs, antimicrobial and antimalarial compounds are the most common drugs in the aqueous environment and that concentrations in wastewater exceed the levels found in developed countries. K'Oreje et al. (2016) investigated the occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater in two cities in Kenya and found that antiretroviral drug-nevirapine and antibiotics were present in all the samples and more prevalent compared to Europe. K'Oreje et al. (2018) also studied the occurrence, fate and removal of pharmaceuticals, personal care products and pesticides in wastewater stabilization ponds and receiving rivers in the Nzoia basin of Kenya. Paraben concentration was up to 1 μgL^{-1}, antiretroviral and antibiotics were most prevalent measuring up to 100 μgL^{-1}, and low concentrations of pesticides was also detected. Kermia et al. (2016) investigated the presence of four (4) pharmaceutical active compounds belonging to the group of NSAIDs in the wastewater, surface water and drinking water of Algiers. The targeted compounds (ibuprofen, diclofenac, ketoprofen and naproxen) were all detected in wastewater influent/effluent with concentration ranging from 0.156 μgL^{-1} to 6.554 μgL^{-1} and surface water with concentrations of diclofenac and naproxen 0.073 μgL^{-1} and 0.228 μgL^{-1} respectively. The concentrations of ibuprofen and ketoprofen in drinking water was 0.142 μgL^{-1} and 0.111 μgL^{-1} respectively. Relic et al., (2017) studied the occurrence of two antiretroviral drugs, efavirenz and nevirapine in wastewater treatment works from Southern Gauteng, South Africa and found that efavirenz concentrations entering the WWTP ranged between 5.5 μgL^{-1} to 14 μgL^{-1} and nevirapine concentrations ranges between 0.092 μgL^{-1} and 0.473 μgL^{-1}. Ngumba et al. (2016) investigated the occurrence of three antibiotics (sulfamethoxazole, trimethoprim and ciprofloxacin) and three antiretroviral (lamivudine, nevirapine and zidovudine) drugs in Nairobi River Basin, Kenya. All the studied compounds were detected with sulfamethoxazole having the highest detection frequency of 97.5 % and ciprofloxacin had the lowest at 60 %. Abafe et al, (2018) investigated the use of LC-MS/MS to determine thirteen antiretroviral drugs used in the treatment and management of HIV in the influents and effluents from wastewater treatment plants in KwaZulu-Natal in South Africa. He found that only three compounds were completely removed in the wastewater treatment plants.

In Nigeria, analgesics, antibiotics, antacid, antihistamines, anticonvulsants, steroids, antimalarial and antihypertensive are among the most consumed classes of compounds and

are routinely purchased without a prescription (Odusanya, 2005). However, the statistics available on the usage of pharmaceuticals are not reliable because of the activities of unregistered pharmacies in some cities (e.g., Lagos) (Akande and Ologe, 2007; Oshikoya and Ojo, 2007; Nwolisa et al., 2006; Odusanya, 2005).

Here the first detailed/comprehensive study of pharmaceutical occurrence in Nigerian river is presented, covering more detected compounds than existing work in Nigeria and other African nations. The main objectives were: (i) to understand the extent to which 37 drugs belonging to different therapeutic classes are found in the river, (ii) to quantify spatial patterns of pharmaceutical contamination and, (iii) to determine seasonal dynamics of contamination.

2. METHODS

2.1 Study area and sample collection

2.1.1 Study compounds

Pharmaceuticals were selected to provide data for a range of different therapeutic classes (Table 3.1.1). Many of these compounds are high-use pharmaceuticals that have been found previously in rivers around the world (Hughes et al., 2013) thus enabling benchmarking of our new information from Lagos, Nigeria against studies undertaken worldwide.

Table 3.1.1: Study compounds, and physico-chemical properties, monitored in the Odo Iya Alaro river, Lagos Southwest Nigeria (Source: www.drugbank.ca)

Therapeutic Group	Compound	LogKow	pKa	Molecular Wgt (g ml^{-1})	Formula	Solubility (mg L^{-1})
Analgesic & Anti-inflammatory	Codeine	1.19	8.21- 10.60	299.37	$C_{18}H_{21}NO_3$	9000
	Hydrocodone	2.16	8.23	299.37	$C_{18}H_{21}NO_3$	n/a
	Paracetamol	0.46-0.49	9.38	151.17	$C_8H_9NO_2$	14000
	Tramadol	3.01	9.41	263.38	$C_{16}H_{25}NO_2$	630
Antacid	Cimetidine	0.40	6.80	252.34	$C_{10}H_{16}N_6S$	9380
	Ranitidine	0.27	8.08	314.40	$C_{13}H_{22}N_4O_3S$	24700
Antiallergic	Loratadine	5.20	5.00	382.89	$C_{22}H_{23}CIN_2O_2$	0.011
Antibiotics	Erythromycin	3.06	8.88-8.90	733.94	$C_{37}H_{67}NO_{13}$	2000
	Sulfamethoxazole	0.89	1.60-5.70	253.28	$C_{10}H_{11}N_3O_3S$	610
	Trimethoprim	0.91	7.12	290.32	$C_{14}H_{18}N_4O_3$	400
Anticonvulsant	Carbamazepine	2.45	13.90	236.27	$C_{15}H_{12}N_2O$	17.7
	Gabapentin	-1.10	3.68-10.70	171.24	$C_9H_{17}NO_2$	4490
Antidepressant	Amitriptyline	4.92	9.40-9.76	277.41	$C_{20}H_{23}N$	9.71
	Desvenlafaxine	2.72	10.11	263.38	$C_{16}H_{25}NO_2$	1400
	Diltiazem	2.7	8.06	414.52	$C_{22}H_{26}N_2O_4S$	465
	Oxazepam	2.24	10.90	286.72	$C_{15}H_{11}CIN_2O_2$	179
	Venlafaxine	3.20	10.09	277.41	$C_{17}H_{27}NO_2$	267
Antihistamine	Diphenhydramine	3.27	8.98	255.36	$C_{17}H_{21}NO$	3060
	Fexofenadine	2.81	4.28-8.76	501.67	$C_{32}H_{39}NO_4$	0.024
	Ketotifen	3.85	8.43	309.43	$C_{19}H_{19}NOS$	15.3
	Cetirizine	1.70-3.57	3.58-7.74	388.89	$C_{21}H_{25}CIN_2O_3$	65.8
Antidiabetic	Metformin	-2.64	12.40	165.63	$C_4H_{12}CIN_5$	n/a

	Sitagliptin	1.39	8.78	407.32	$C_{16}H_{15}F_6N_5O$	179.2
Antipsychotic	Diazepam	2.82	3.40	284.74	$C_{16}H_{13}CIN_2O$	50
	Temazepam	n.a.	-1.4-10.68	300.74	$C_{16}H_{13}CIN_2O$	164
Anti-malaria	Artemisinin	2.90	4.60	282.22	$C_{15}H_{22}O_5$	n/a
Antiarrhythmic	Lidocaine	2.26	8.01	234.34	$C_{14}H_{22}N_2O$	4100
Antiretroviral	Lamivudine	-9.54	-0.16-14.29	229.25	$C_8H_{11}N_3O_3S$	70000
Antiviral	Oseltamivir	0.95	7.70	312.41	$C_{16}H_{28}N_2O_4$	1600
Contraceptive	Norethisterone	2.97	-1.7-17.59	298.43	$C_{20}H_{26}O_2$	7.04
Beta Blocker	Atenolol	0.16	9.60	266.34	$C_{14}H_{22}N_2O_3$	13300
	Propranolol	-0.45	9.42	259.35	$C_{16}H_{21}NO_2$	61.7
SERM	Raloxifene	6.09	7.99-9.92	473.59	$C_{28}H_{27}NO_4S$	0.25
Diuretics	Triamterene	0.98	3.11-15.88	253.27	$C_{12}H_{11}N_7$	48.2
Calcium-Channel Blocker	Verapamil	3.83	8.92	454.61	$C_{27}H_{38}N_2O_4$	4.47
SSRIs	Sertraline	4.30	9.47	306.23	$C_{17}H_{17}CI_2N$	3.5
	Citalopram	1.39	9.50	324.40	$C_{20}H_{21}FN_{20}$	n/a

n.a. = Not Available
Wgt=Weight

3.1.2 Study catchment: Lagos State, Nigeria

Lagos State is a low-lying coastal region occupying 187 km of Nigeria's coastline. It is situated between latitudes 6° 22'N to 6° 42'N and longitudes 2° 42'E to 4°20'E. It is bounded in the north by Ogun state and in the east by Ondo state. It shares an international boundary of about 45 km with the Republic of Benin while the Atlantic Ocean constitutes approximately 180 km along the southern limit. The state covers an approximately 3,577 sq. km which represents 0.39 % of Nigeria's territorial land mass. Lagos drains two-thirds of South-west Nigeria and is characterized by wetlands and basin, five major upstream rivers from neighbouring states discharge into the Atlantic Ocean through the Lagos lagoon. The low-lying land and wet-lands occupy 78 % of the entire land mass of the state. 85 % of the state population resides in just 37 % of the state territorial land mass. It is the smallest state in Nigeria but has the largest population of 22 million people. It is one of the fastest growing cities in the world. 45 % of the Nigeria's skilled labour force is in Lagos. Lagos State is the commercial nerve centre of Nigeria. It harbours over 2,000 industrial complexes, 10,000 commercial ventures and 22 industrial estates. It has more than 100 both local and multinational pharmaceutical manufacturing industries located in the state. This rapid urbanisation and population increase leads to large number of pharmaceutical manufacturing industries in the state and hence large quantities of pharmaceutical wastes are generated which may leads to high level of water pollution. The state has ten Lagoons and many creeks, rivers, streams and drainage canals. The largest of all the lagoon system is the Lagos lagoon through which all others drain, and Lagos lagoon enters the Atlantic Ocean through the Lagos harbour. With this ever-increasing urban population vis-à-vis the scarcity of dry lands, many of the natural streams had been sand filled and converted due to proliferation of urban residential and industrial establishment.

The study was conducted at Odo-Iya Alaro River (Figure 3.1.2), the Odo-Iya Alaro River forms a sub-catchment of the Ogudu river, which discharges into the Lagos lagoon. The river is 15.8 km in length and flows through Ogba, Ikeja and Maryland which have a combined population of 2.5 million. The catchment contains a sewage treatment plant (STP), two major pharmaceutical manufacturing plants and many smaller ones located in the industrial estates of Ogba and Ikeja which discharge their effluents through drainage pipes and canals into the river. Some of these canals also pass through densely populated urban areas which discharges untreated domestic waste to them. Along the river are located mechanical workshops, illegal buildings and shanty structures with domestic waste discharged untreated into the river and in places like this the river flow is slow. Raw sewage may also enter the river due to emptying of vacuum trucks which collect untreated effluent in urban areas (Ogunbanwo, 2011).

Twenty-two (22) sampling stations along the river (Figure 3.1.2) were chosen based on accessibility and the possibility of sampling both receiving waters up and downstream of the effluents discharge points (Table 3.1.2). Alausa (STP) is one of the four STPs in the whole of Lagos State with a population of 22 million people. The treatment plant aerates the wastewater influent by stirring after which it undergoes sedimentation and chlorination before the final effluent is discharged into the receiving water. The treatment plant was designed to serve a population of 255,000 but there are indications that the plant is handling far more than its installed capacity (Engr Adepoju-plant manager Alausa STP, personal communication, 2nd August 2017). The plant has an inflow rate of 1000 m^3 day^{-1}, hydraulic retention time (HRT) of 18 hrs and sludge retention time (SRT) of 20 days, both domestic and municipal wastewater are being treated at Alausa (STP).

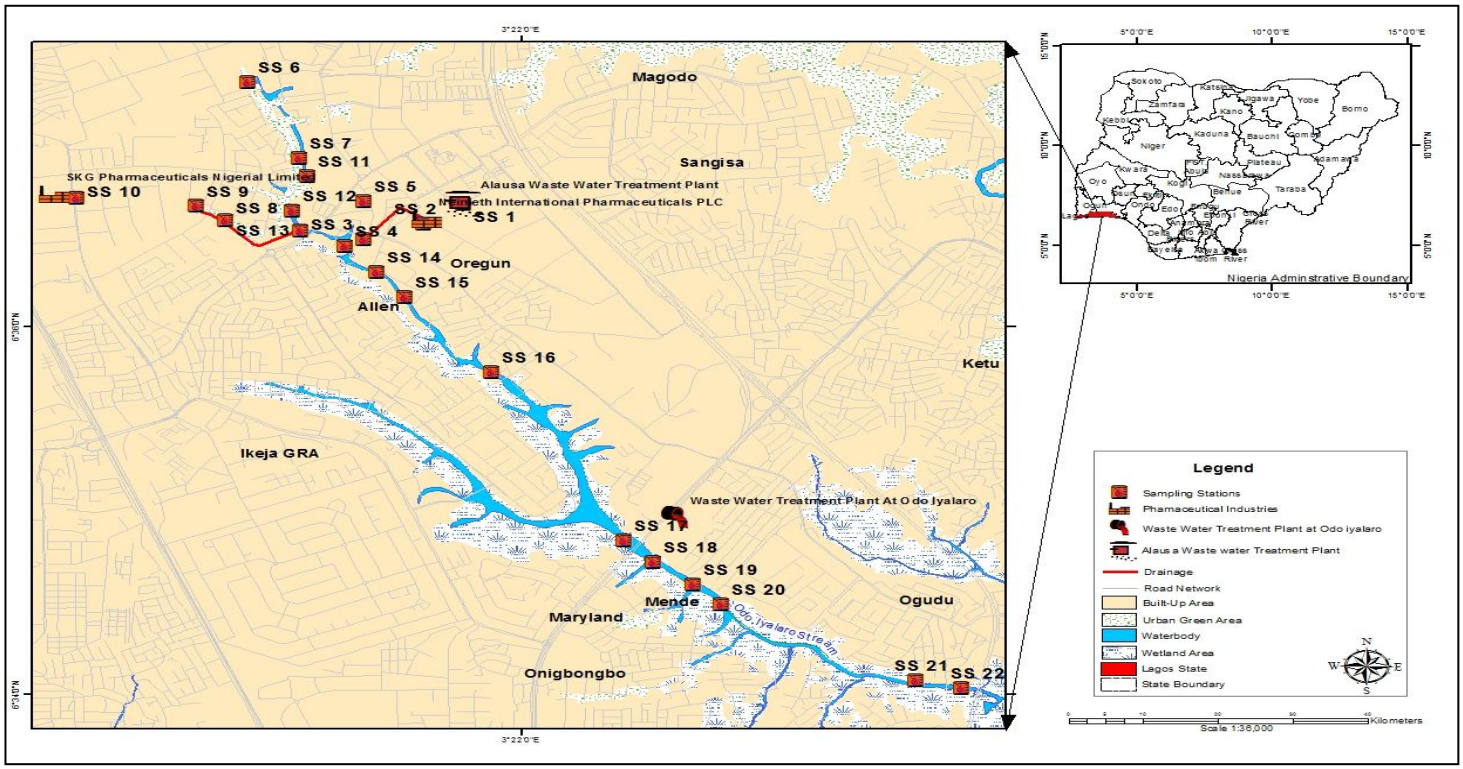

Figure 3.1.2: **Map of the Odo-Iya Alaro river showing the sampling stations (n=22) in Lagos State, Southwest Nigeria**

Table 3.1.2: Sampling stations, description of the stations, site categories and Global Positioning System (GPS) locations.

Sampling station	Site Category	Description of site	Longitude	Latitude
SS1	Sewage effluents (SE)	Alausa sewage treatment plant	3˚21' 46.4"	6˚36' 38.73"
SS2	Pharmaceutical effluents (PE)	Neimeth pharmaceuticals	3˚21' 36.2"	6˚36' 33.32"
SS3	Sewage effluents (SE)	Storm water /underground discharge from Alausa STP	3˚21' 20.1"	6˚36' 29.1"
SS4	Sewage effluents (SE)	Chamber inside Oregun sewage discharge point	3˚21' 19.4"	6˚36' 27.0"
SS5	Sewage effluents (SE)	Outlet of discharge at Oregun sewage discharge point	3˚21' 18.5"	6˚36' 23.0"
SS6	River (Semi Urban)	Surulere industrial estate road	3˚20' 46.4"	6˚37' 17.1"
SS7	River (Semi Urban)	Adekunle village	3˚21' 04.9"	6˚36' 55.2"
SS8	River (Urban)	Adeniyi Jones junction 1	3˚20' 41.3"	6˚36' 39.3"
SS9	River (Urban)	Adeniyi Jones junction 2	3˚20' 41.4"	6˚36' 39.3"
SS10	Pharmaceutical effluents (PE)	SKG pharmaceuticals	3˚20' 41.25"	6˚36' 56 .3"
SS11	River (Semi Urban)	Channel along the back of Coca cola bottling company. Mechanic village 1	3˚21' 08.2"	6˚38' 38.2"
SS12	River (Semi Urban)	Channel along the back of Coca cola bottling company. Mechanic village 2	3˚21' 02.8"	6˚36' 35.2"
SS13	River (Urban)	New Alade market	3˚21' 06.6"	6˚36' 29.6"
SS14	River (Urban)	Samplers collected before joining Oregun sewage discharge point	3˚21' 17.1"	6˚36' 24.2"
SS15	Sewage effluents (SE)	Channels along Oregun discharge point	3˚21' 18.5"	6˚36' 23.0"

SS16	River (Urban)	Opebi link road	3˚21'49.6"	6˚35'42.6"
SS17	River (Semi Urban)	Odo Iya-Alaro (under bridge)	3˚22'22.5"	6˚34'48.0"
SS18	River (Urban)	End of Olatunji street, Ojota	3˚22'32.7"	6˚34'38.4"
SS19	River (Urban)	End of Alhaji Amoo street, Ojota	3˚22'38.1"	6˚34'35.4"
SS20	River (Urban)	End of Victoria, street, Ojota	3˚22'39.2"	6˚34'33.6"
SS21	River (Urban)	Before Ogudu bridge	3˚23'38.1"	6˚34'03.2"
SS22	River (Semi Urban)	Ogudu bridge before joining Lagos Lagoon	3˚23'44.1"	6˚34'03.0"

3.1.3 Sample collection

At each sampling station, three 50 mL water samples were collected into amber vials with Teflon® lined caps (Fisher Scientific, UK) and then homogenised into a single 150 mL composite sample of which 20 mL was taken. Sampling was undertaken on a quarterly basis to incorporate both the wet (April and July 2017) and dry seasons (October 2017 and January 2018). Sampling vials were rinsed with 100% methanol once and deionised water three times to remove potential contamination before sampling. Samples were collected at the same time of day and in the same location, checked using a Global Positioning System (GPS).

3.1.4 Sample Preparation

A 10 mL aliquot of each composite sample was filtered on site at the points of collection using the procedure of Wilde et al. (2004, with updates through 2009) through a Whatman GFF (0.7 µm pore size) glass microfiber syringe filters into a 20 mL amber glass vial with a Teflon-lined screw cap. The filtered samples were frozen immediately on site with dry ice before shipping within 24 hrs to the University of York Centre of Excellence in Mass Spectrometry, York, United Kingdom for analysis. The samples arrived in York three days after shipment and were immediately thawed and analysed. In order to reduce potential degradation during shipment, filtering was conducted in the field to remove microbial and particulate content. Other studies have shown that longer periods of storage (up to 6 months) even at 4 °C caused no appreciable change in spiked concentrations (Hughes et al., 2013). The remaining samples were stored in the dark at -12°C in the Lagos State Environmental Protection Agency (LASEPA) laboratory.

3.1.5 Analytical procedure and method validation

Quantification was achieved using HPLC-MS/MS with a Thermo Scientific TSQ Endura Mass spectrometer coupled with an UltiMate 3000 liquid chromatograph. The method employed was adapted from Furlong et al. (2014) and validated for this purpose at the University of York Centre of Excellence in Mass Spectrometry (Burns et al., 2018).

Briefly, prior to starting the quantitative analysis, 500 µL of each water sample was diluted with 495 µL of HPLC-grade water and spiked with 5 µL of a mixture of internal standards (each at a concentration of 80 μgL^{-1}) in glass autosampler vials. The 50 % dilution was done in order to clean the samples and bring analytes concentrations to within the calibrated range. Where concentration was found to still exceed the calibrated range, further dilution and reanalysis was done. A random number generator was used to randomise the order in which samples were injected onto the HPLC-MS/MS.

Analysis was conducted by direct injection of 100 µL of respective samples onto a Phenomenex Eclipse Plus C18 chromatography column using a Phenomenex C18 (ODS, Octadecyl) 4 mm x 3 mm ID guard column. Mobile phase A was HPLC-grade water with 0.01 M formic acid and 0.01 M ammonium formate while mobile phase B was 100 % HPLC-grade methanol, flow rate of 0.45 mL min^{-1} was used with a gradient starting at 10 % B which then increased to 40 % at 5 min, 60 % at 10 min, 100 % at 15 min, and remaining 100 % B until 23 min then dropping to 10 % at 23 min prior to a re-equilibration. The autosampler temperature was kept at 4°C and the HPLC column compartment at 40°C. The collision gas was argon at a pressure of 2 mTorr.

Quantification was done with a 16-point calibration using deuterated internal standards (Burns et al., 2018) ranging from 1 to 32000 ngL^{-1}. Calibration r^2-values were consistently >0.95. Analytical limits of detection were calculated as described by Burns et al. (2018) and

ranged from 0.9 ngL^{-1} (carbamazepine) to 12.4 ngL^{-1} (gabapentin) (Table 3.1.5). Quality control (QC) measures were used throughout the analysis. Briefly, method blanks (n=6) were made with an identical collection procedure as the environmental samples except using HPLC-grade water. Concentrations of target pharmaceuticals were consistently below levels of analytical quantification in the method blanks. Additionally, QCs consisting of all target pharmaceuticals at a concentration of 80 ngL^{-1} were injected after every four samples followed by an instrumental blank consisting of pure HPLC-grade water. Analytical tolerance was consistently within ±15 % and the instrumental blanks did not contain detectable residues of the target analytes.

Table 3.1.5: Limits of detection (LOD) and quantification (LOQ) for selected pharmaceuticals analysed in this study (ngL^{-1}).

Pharmaceutical	LOD	LOQ
Amitriptyline	1.09	2.18
Atenolol	8.87	17.7
Carbamazepine	0.89	1.78
Cetirizine	1.87	3.74
Cimetidine	2.04	4.08
Citalopram	2.13	4.26
Codeine	2.61	7.84
Desvenlafaxine	2.15	4.3
Diazepam	1.38	2.76
Diltiazem	1.09	2.19
Diphenhydramine	1.17	2.34
Erythromycin	11.15	22.3
Fexofenadine	2.05	2.1
Gabapentin	12.39	37.16
Hydrocodone	1.02	2.04
Ketotifen	2.89	5.78
Lidocaine	1.36	2.76
Loratadine	5.03	10.06
Metformin	4.19	8.38
Noreistherone	7.25	14.51
Oseltamivir	6.67	13.33
Oxazepam	5.38	10.76
Paracetamol	7.08	14.16
Propranolol	6.49	12.98
Raloxifene	6.34	12.68
Ranitidine	6.23	12.46
Sertraline	9.14	18.28
Sitagliptin	7.06	14.11
Sulfamethoxazole	9.12	18.23
Temazepam	3.59	7.19
Tramadol	3.55	7.1
Triamterene	10.81	21.61
Trimethoprim	1.27	2.55
Venlafaxine	1.53	3.06
Verapamil	10.09	20.18

3.1.6 Data analysis

Data were organised using Excel (Microsoft, 2013) and residuals of the data were checked for normal distribution using the Shapiro-Wilk normality test and homogeneity of variance using the Bartlett test of homogeneity of variances. R (R Development Core Team, 2008) was used to analyse the data and ggplot 2 to create figures (Barplot, Box-and-Whisker). The barplot shows the distribution of the categorical variables. The box-and-whisker plots display a statistical summary of variables: median, quartiles, range and possibly extreme values (outliers). An outlier value is defined as a value that is smaller than the lower quartile (25 percentile) minus 1.5 times the interquartile range, or larger than the upper quartile (75 percentile) plus 1.5 times the interquartile range. Generalised linear model and Chi-square were used to find if there are differences between the sampling sites. Seasonal variations were analysed using one-way ANOVA where assumptions of normality and homogeneity were met followed by Tukey's post-hoc tests to determine if there is any variation in concentrations between the wet and the dry the seasons.

3.2 Results

3.2.1 Detection frequency

Out of the 37 targeted analytes 26 were detected at the Alausa STP (SE) (Figure 3.2.1A) and at the receiving river (Urban site category) (Figure 3.2.1B). 25 and 15 analytes were detected in receiving river (Semi-urban) and the pharmaceutical manufacturing effluents (PE) respectively. The ten most frequently detected compounds across the site categories were fexofenadine, carbamazepine, paracetamol, metformin, diazepam, cimetidine, codeine, sulfamethoxazole, atenolol and trimethoprim. Analytes not detected were venlafaxine (SE site category), triamterene (Urban site category), triamterene and venlafaxine (Semi-urban site category), gabapentin, hydrocodone, raloxifene, verapamil, diltiazem, oseltamivir, propranolol, sitagliptin, temazepam, triamterene, venlafaxine and tramadol (PE site category).

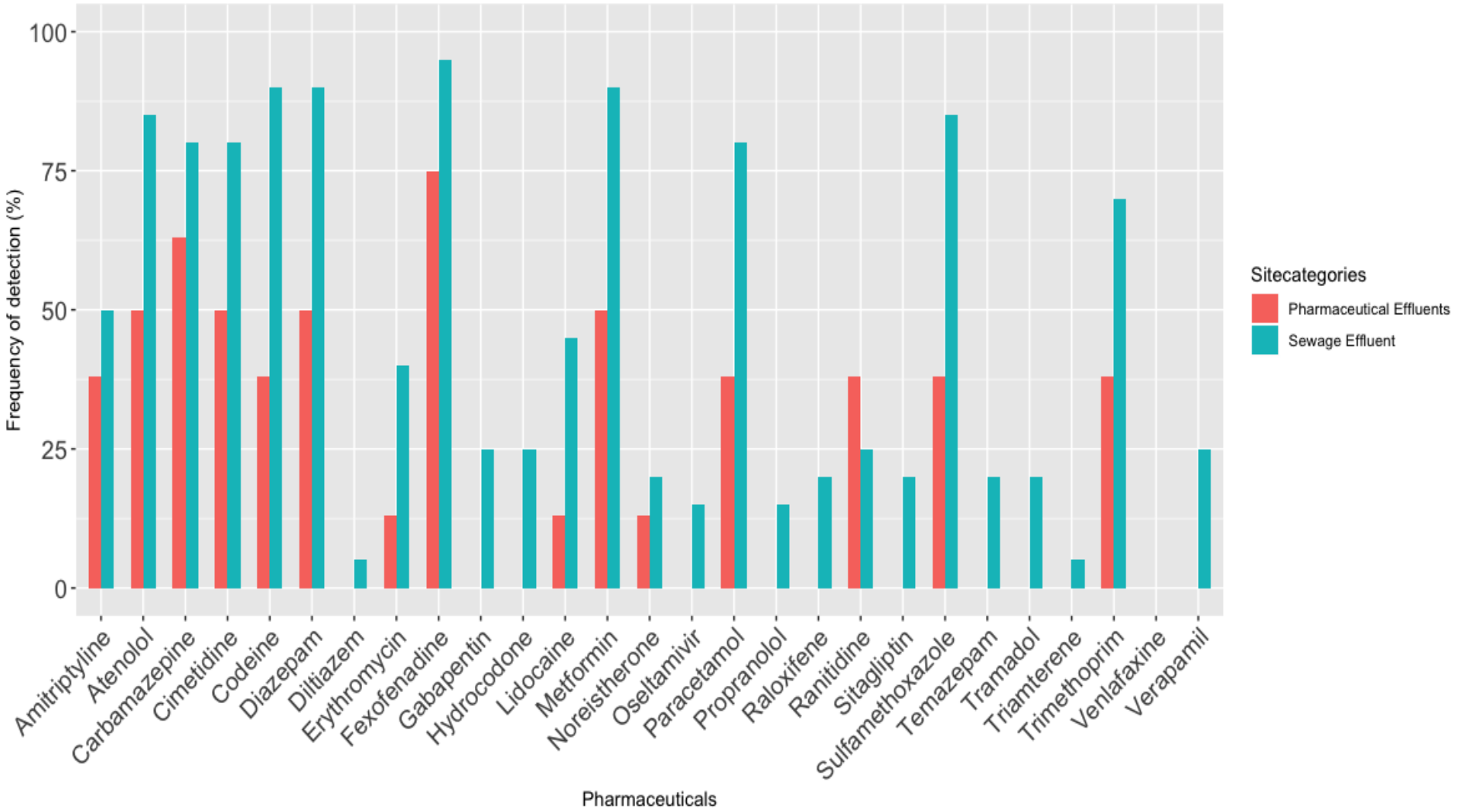

Figure 3.2.1A: Frequency of detection of pharmaceuticals in sewage effluents(SE) and pharmaceutical effluents (PE) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.

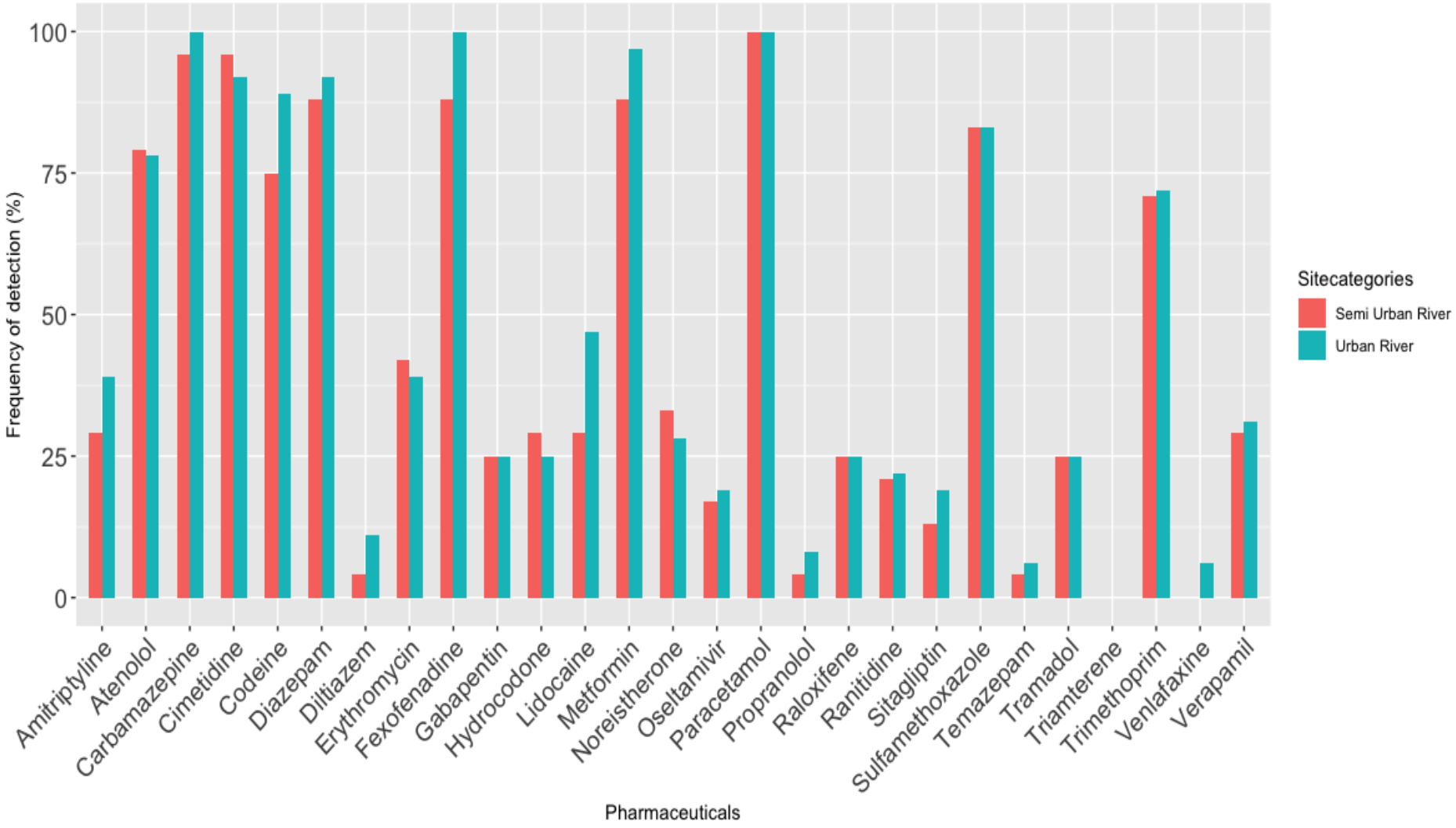

Figure 3.2.1B: Frequency of detection of pharmaceuticals in receiving river (Urban and Semi-urban site categories) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.

3.2.2 Mean and maximum concentrations

Peak concentrations were typically in the range of low micrograms per litre while mean concentrations were an order of magnitude lower (Figure 3.2.2). Antibiotic and analgesics were detected at the highest concentrations; sulfamethoxazole (129474 ngL^{-1}) had the highest maximum concentration followed by paracetamol (111374 ngL^{-1}). Over all, paracetamol had the highest mean concentration (18178 ngL^{-1}) while sulfamethoxazole had the second highest mean concentration (11160 ngL^{-1}). Cimetidine had the third highest maximum concentration of (95689 ngL^{-1}) and mean concentration (10458 ngL^{-1}). The maximum concentration of a further seven analytes (fexofenadine, carbamazepine, metformin, diazepam, atenolol, trimethoprim, and codeine) also exceeded 39000 ngL^{-1}. Mean concentrations for these substances were in the low micrograms per litre range; metformin (9690 ngL^{-1}), fexofenadine (8409 ngL^{-1}), carbamazepine (7705 ngL^{-1}), atenolol (3044 ngL^{-1}), diazepam (2551ngL^{-1}), trimethoprim (1874 ngL^{-1}), and codeine (1764 ngL^{-1}).

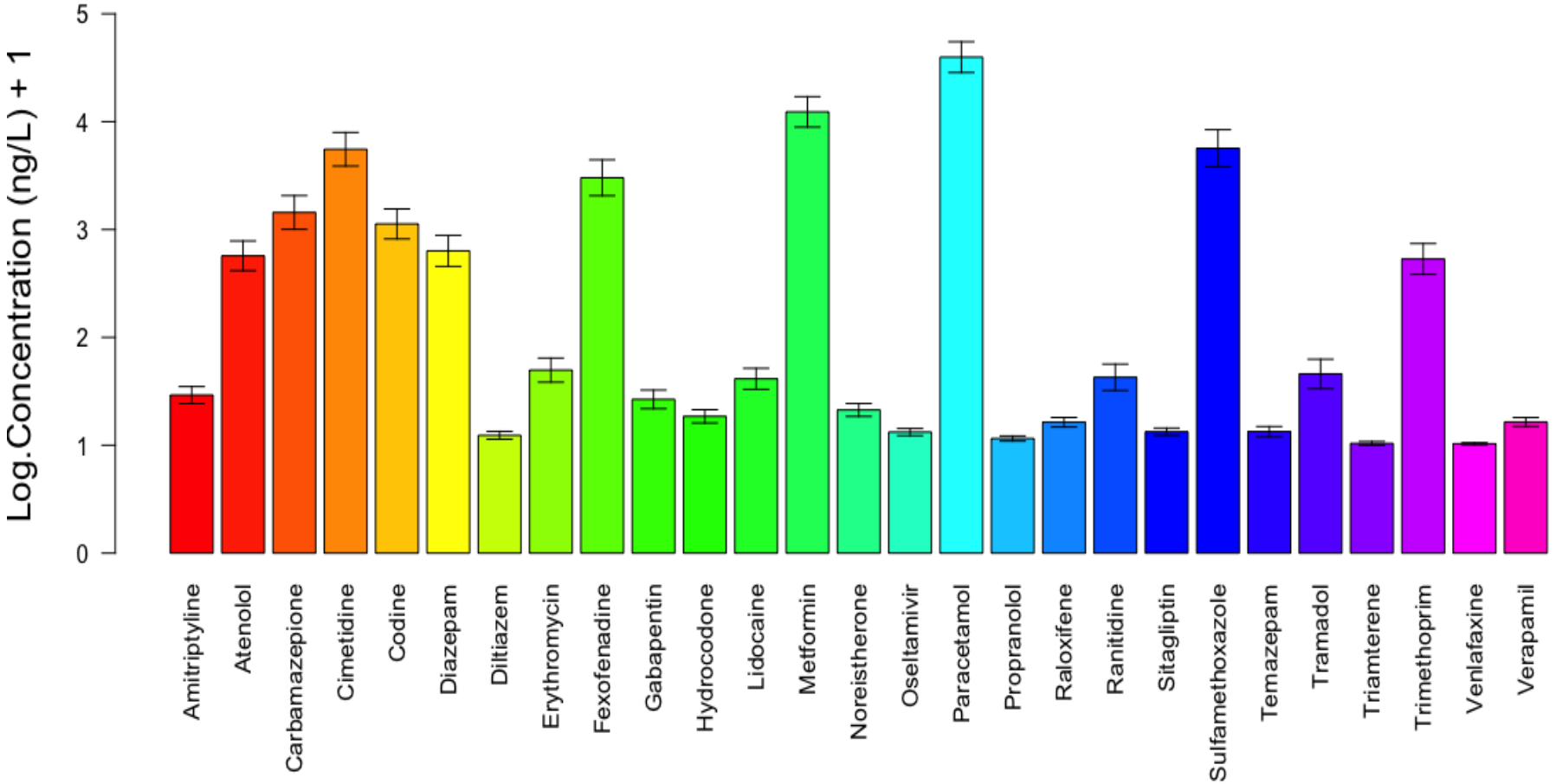

Figure 3.2.2: Mean concentrations (±SEM) of pharmaceuticals detected in the Odo Iya-Alaro river, Lagos, Southwest Nigeria.

3.2.3 Spatial distribution of pharmaceuticals in Odo Iya Alaro river

Pharmaceutical pollution was ubiquitous in the Odo Iya Alaro river with no obvious spatial patterns (Table 3.2.3). Although there are diverse sources of pharmaceuticals into the river such as STPs, pharmaceutical manufacturing facilities, urban waste collection areas and vacuum truck operators who collect sewage from residential apartments and discharged to water course without treatments. There were statistically significant differences between the different site categories (Pharmaceutical manufacturing sites (PME), Alausa STP site (SE), Semi- Urban and Urban sites) (Figure 3.2.3) (GLM: χ^2 (3) = 883.32, p <0.001).

There are variations in the number of analytes detected in each site categories. For instance, 15 pharmaceuticals were detected at the pharmaceutical manufacturing site (PME) and the list detected analyte was noreistherone with mean concentration of 2.24 ngL^{-1} and peak concentrations of 17.91 ngL^{-1}. 26 analytes were detected in Alausa STP site (SE) and Urban receiving river. The list detected was oseltamivir with mean and peak concentrations of 0.69 ngL^{-1} and 5.52 ngL^{-1} respectively for Alausa STP site (SE) and venlafaxine with mean and peak concentrations of 0.24 ngL^{-1} and 4.75 ngL^{-1} respectively was the list analytes detected in the urban site. 25 analytes were detected in semi-urban location with sitagliptin detected at mean concentration of 0.45 ngL^{-1} and maximum concentration of 4.25 ngL^{-1} respectively.

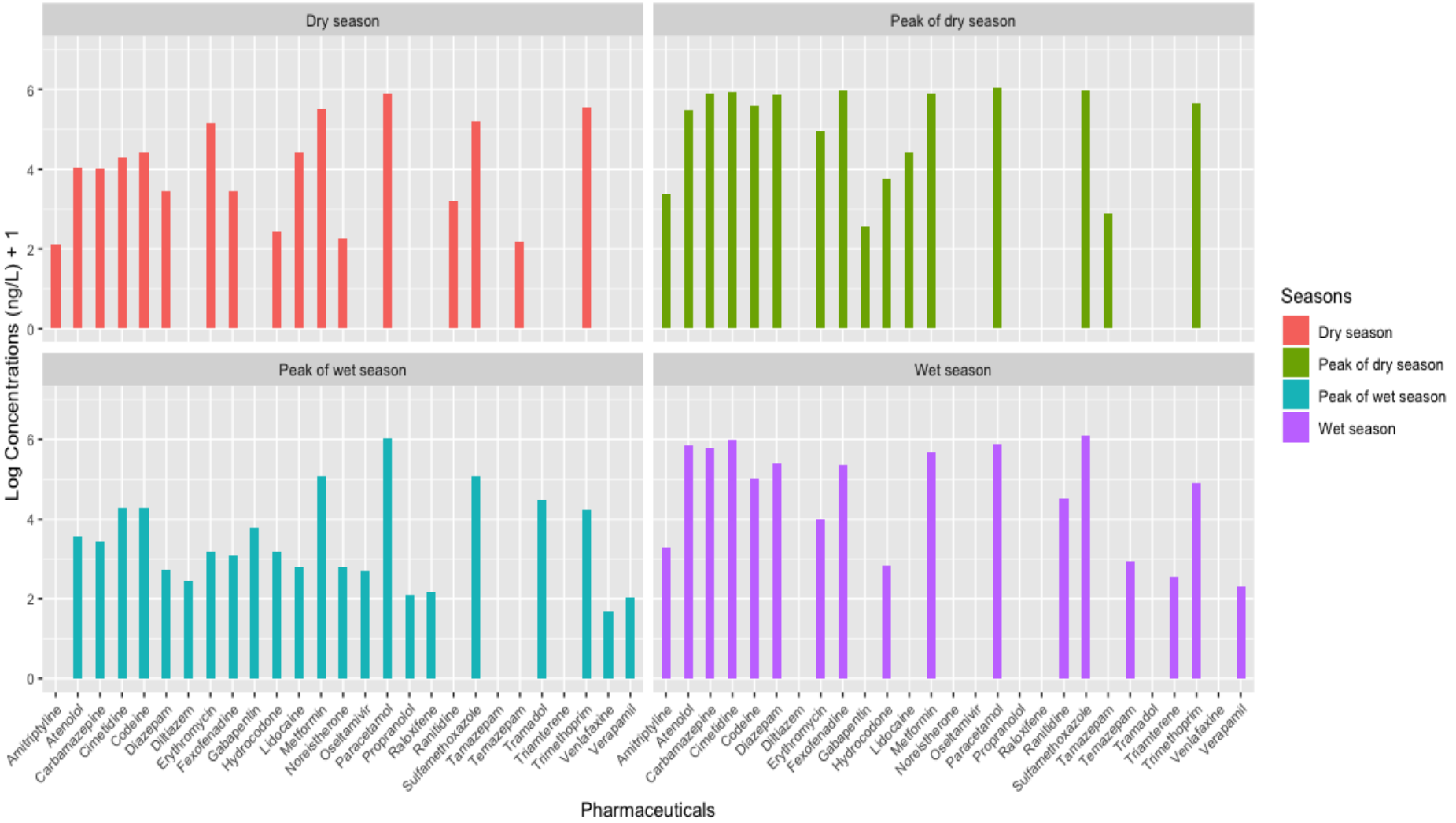

Figure 3.2.3: **Log concentrations (ngL^{-1}) + 1 of pharmaceuticals detected at different locations : Pharmaceutical Manufacturing Effluents (PME), Sewage Effluents (SE) and receiving river (Semi-Urban and Urban) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.**

Table 3.2.3: Mean concentrations in ngL-1 ± 1SD (n=4) of pharmaceuticals detected in the Odo Iya- Alaro river, Lagos, Southwest Nigeria.

Compound	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15	SS16	SS17	SS18	SS19	SS20	SS21	SS22
Amitriptyline	21 ± 21	40 ± 47	2 ± 5	26 ± 46	21 ± 26	6 ± 9	1 ± 1	13 ± 27	1 ± 1	1 ± 2	2 ± 5	0.00	1 ± 2	2 ± 3	2 ± 4	37 ± 70	1 ± 1	54 ± 100	64 ± 122	28 ± 48	48 ± 96	42 ± 83
Atenolol	526 ± 459	222 ± 430	7015 ± 125	7448 ± 143	8032 ± 152	7755 ± 153	5460 ± 108	7 ± 9	40 ± 55	31 ± 43	15 ± 22	3715 ± 721	1228 ± 215	7595 ± 147	99 ± 113	99 ± 133	66 ± 79	90 ± 110	91 ± 133	183 ± 247	17233 ± 344	25 ± 29
Carbamazepine	1348 ± 184	3763 ± 525	163 ± 187	182 ± 217	1058 ± 152	638 ± 126	1291 ± 257	1784 ± 211	1890 ± 374	721 ± 144	2004 ± 399	4003 ± 799	7212 ± 143	20717 ± 410	16080 ± 320	17039 ± 340	9725 ± 194	11865 ± 234	20240 ± 39(	18150 ± 349	15018 ± 174	14634 ± 292
Cimetidine	15563± 192	72 ± 145	11646 ± 217	7976 ± 151	24397± 475	3320 ± 458	1730 ± 235	34090 ± 405	3694 ± 513	117 ± 190	5104 ± 918	3611 ± 615	14298 ± 236	20760 ± 252	16967 ± 170	4155 ± 728	19221 ± 344	27392 ± 418	2010 ± 359	2235 ± 392	9298 ± 127	2436 ± 438
Codeine	6302 ± 994	2 ± 4	3651± 437	839 ± 850	11077± 189	68 ± 95	64 ± 79	810 ± 158	849 ± 129	692 ± 994	233± 395	44 ± 69	508 ± 696	2216 ± 215	1904 ± 251	1862 ± 329	141 ± 162	73 ± 121	1986 ± 177	4031 ± 737	768 ± 977	28 ± 44
Diazepam	1470 ± 291	25307 ± 35	115 ± 140	714 ± 116	4011 ± 779	337 ± 664	1095 ± 218	1807 ± 220	1156 ± 231	1084 ± 216	349 ± 665	1663 ± 329	1463 ± 289	2613 ± 493	2463 ±485	2320 ± 458	1981 ± 393	1227 ± 241	341 ± 645	531 ± 102	2653 ± 306	1435 ± 285
Erythromycin	46± 92	ND	52 ± 60	11 ± 17	50 ± 100	5 ± 11	5 ± 11	2 ± 4	ND	3777 ± 756	15 ± 30	601 ± 812	246 ± 477	184 ± 359	60 ± 73	19 ± 24	1 ± 2	10 ± 11	147 ± 286	14 ± 16	2 ± 4	2355 ± 468
Fexofenadine	11289 ± 21	15717 ± 20	8994 ± 172	23761 ± 465	10429 ± 151	3516 ± 615	3604 ± 648	7283 ± 936	6212 ± 116	7024 ± 134	5272 ± 990	3408 ± 608	3112 ± 562	3522 ± 597	7910 ± 991	11076 ± 211	12823 ± 241	7620 ± 141	7555 ± 986	5461 ± 104	8362 ± 106	11050 ± 133
Gabapentin	147 ± 294	ND	22 ± 45	31 ± 62	9 ± 18	9 ± 18	6 ± 13	5± 9	6 ± 12	ND	8 ± 16	5 ± 11	36 ± 71	33 ± 66	33 ± 66	29 ± 58	18 ± 37	17 ± 33	26 ± 53	34 ± 67	34 ± 67	35 ± 70
Hydrocodone	18 ± 34	ND	39 ± 79	2 ± 5	ND	140 ± 278	1 ± 1	55 ± 110	ND	ND	ND	1 ± 1	4 ± 8	2 ± 4	6 ± 13	4 ± 8	2 ± 3	3 ± 5	1 ± 3	8 ± 14	1 ± 2	4 ± 7
Lidocaine	13 ± 15	274 ± 548	39 ± 46	707 ± 138	16 ± 32	ND	1 ± 1	2 ± 4	2 ± 3	ND	688 ± 138	1 ± 1	8 ± 9	364 ± 696	40 ± 69	15 ± 20	12 ± 15	26 ± 39	21 ± 25	18 ± 23	25 ± 28	13 ± 25
Metformin	25726 ± 147	915 ± 166	18445 ± 214	12024 ± 111	15342 ± 302	564 ± 844	3528 ± 416	14304 ± 202	5676 ± 790	1903 ± 233	1677 ± 142	1888 ± 208	4188 ± 449	20696 ± 225	15198 ± 244	7801 ± 102	3928 ± 598	25855 ± 373	25854 ± 20(	6348 ± 106	1188 ± 115	130 ± 261
Noreisterone	3 ± 7	4 ± 8	4 ± 9	7 ± 14	ND	3 ± 7	8 ± 9	2 ± 4	8 ± 9	ND	8 ± 10	3 ± 6	5 ± 10	6 ± 11	16 ± 32	3 ± 7	4 ± 7	14 ± 29	2 ± 4	4 ± 8	5 ± 10	6 ± 11
Oseltamivir	1 ± 2	ND	1 ± 2	ND	ND	6 ± 12	ND	1 ± 2	ND	ND	12 ± 24	1 ± 1	1 ± 2	1 ± 2	1 ± 3	1 ± 2	ND	1 ± 2	1 ± 3	2 ± 4	ND	2 ± 3
Paracetamol	45649 ± 552	2773 ± 548	6366 ± 513	10563 ± 106	6624 ± 132	3481 ± 129	8209 ± 315	17899 ± 459	6896 ± 543	ND	5844 ± 978	5311 ± 179	22497 ± 380	26357 ± 143	15633 ± 126	19696 ± 519	7765 ± 357	16908 ± 110	48516 ± 35(	29362 ± 206	6029 ± 169	5286 ± 288
Propranolol	3 ± 6	ND	2 ± 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1 ± 2	1 ± 2	1 ± 2	2 ± 4	2 ± 4	ND	ND	ND	ND
Raloxifene	2 ± 5	ND	3 ± 6	2 ± 5	ND	3 ± 6	2 ± 4	2 ± 4	2 ± 4	ND	3 ± 6	3 ± 5	3 ± 5	3 ± 5	2 ± 4	3 ± 5	2 ± 4	2 ± 4	4 ± 8	3 ± 6	2 ± 4	3 ± 5
Ranitidine	168 ± 336	816 ± 163	110 ± 220	27 ± 54	61 ± 123	109 ± 219	74 ± 149	216± 432	26 ± 52	808 ± 151	155 ± 311	57 ± 113	182 ± 364	111 ± 221	66 ± 132	78 ± 155	49 ± 98	184 ± 368	173 ± 345	120 ± 241	ND	ND
Sitagliptin	8 ± 17	ND	8 ± 17	1 ± 2	ND	ND	1 ± 2	ND	ND	ND	ND	ND	1 ± 2	1 ± 2	1 ± 3	2 ± 3	1 ± 2	1 ± 2	1 ± 2	2 ± 4	1 ± 2	1 ± 2
Sulfamethoxazole	6469 ± 400	3632 ± 253	5899 ± 245	4739 ± 218	281 ± 533	165 ± 273	7432 ± 123	32545 ± 372	20479 ± 29(	ND	18937 ± 33(	13478 ± 244	13043 ± 248	33773 ± 638	8132 ± 147	8322 ± 152	26791 ± 448	3633 ± 417	13913 ± 11(	3574 ± 522	19766 ± 379	517 ± 754
Temazepam	41 ± 48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19 ± 37	ND	9 ± 13	4 ± 8	17 ± 35	ND	ND	ND	ND	ND
Tramadol	224 ± 449	ND	527 ± 105	348± 696	ND	117 ± 235	116 ± 233	341 ± 682	245 ± 490	ND	122 ± 243	119 ± 237	731 ± 146	354 ± 708	555 ± 111	452 ± 905	338 ± 676	318 ± 636	318 ± 636	339 ± 678	374 ± 747	355 ± 709
Triamterene	8 ± 17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	1165 ± 141	21479 ± 24(	675 ± 773	738± 108	23 ± 37	94 ± 93	78 ± 85	89 ± 106	324 ± 345	ND	110 ± 151	82 ± 95	468 ± 610	697 ± 976	302 ± 344	144 ± 133	124 ± 133	1006 ± 137	12348 ± 18(	1197 ± 174	52 ± 60	41 ± 48
Venlafaxine	ND	ND	ND	ND	ND	ND	ND	1 ± 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	1 ± 2	ND	ND	ND	ND
Verapamil	1 ± 2	ND	1 ± 2	2 ± 5	ND	1.67 ± 3.34	1 ± 2	1 ± 2	1 ± 3	ND	6 ± 10	1 ± 2	1 ± 2	7 ± 10	6 ± 10	7 ± 10	1 ± 3	1 ± 2	1 ± 3	2 ± 3	1 ± 2	2 ± 3

ND=Not detected.

3.2.4 Seasonal variations in pharmaceutical concentrations

There were statistically significant differences between the dry season, peak of dry season, the wet season and peak of the wet season ((GLM: χ^2 (3) = 8.63), $p<0.001$). More pharmaceuticals were detected in the peak of the wet season (22) than the other seasons. 17 analytes were each found in the wet and dry seasons while 16 pharmaceuticals were detected at the peak of the dry season.

Although more pharmaceuticals were detected in the peak of wet season, there was distinct variation in concentrations of many pharmaceuticals which generally higher at the peak of the dry season (concentration level) (Figure 3.2.4). Fexofenadine for example, an antihistamine, has the highest mean and median concentrations (28272 ngL^{-1} and 22318 ngL^{-1}) respectively in the peak of the dry season (Table 3.2.4) compared to all other compounds and other seasons. The mean and median concentrations of fexofenadine are more than 500 times higher than the concentration in dry, peak of the wet or wet seasons. Carbamazepine, a psychotic drug has the second highest mean concentration (25654 ngL^{-1}) in the peak of the dry season and followed closely by paracetamol (24616 ngL^{-1}) in the same season. The mean concentration of paracetamol in the peak of the dry season was almost 1.5 times higher than the peak of the wet season. The median concentration was 9228 $ngL^{-1}$for peak of dry season, 8930 ngL^{-1} for dry season, 8940 ngL^{-1} for peak of wet season and 7130 ngL^{-1} for wet season.
The mean and median concentrations of all the pharmaceuticals detected in the Odo-Iya Alaro river are extremely higher in the peak of the dry season than any other season except the following compounds that are either not detected in the peak of dry season or detected in low concentrations in other seasons: diltiazem 6 ngL^{-1} (peak of wet season), erythromycin 785 ngL^{-1}(dry season), gabapentin 97 ngL^{-1} (peak of the wet season), lidocaine 269 ngL^{-1} (dry season), noreistherone 18 ngL^{-1} (peak of wet season), oseltamivir 6 ngL^{-1} (peak of wet season), propranolol 2 ngL^{-1}(peak of wet season)(Table 3.2.4). Raloxifene was only detected in the peak of the wet season at a mean concentration of 9 ngL^{-1}. Other compounds detected at low mean concentrations are ranitidine (dry season and wet season), sitagliptin (peak of wet season), temazepam (not detected in peak of wet season only), tramadol (detected only in peak of wet season), triamterene (detected only in wet season), venlafaxine (detected only in peak of wet season) and verapamil (detected both in wet and peak of wet seasons).

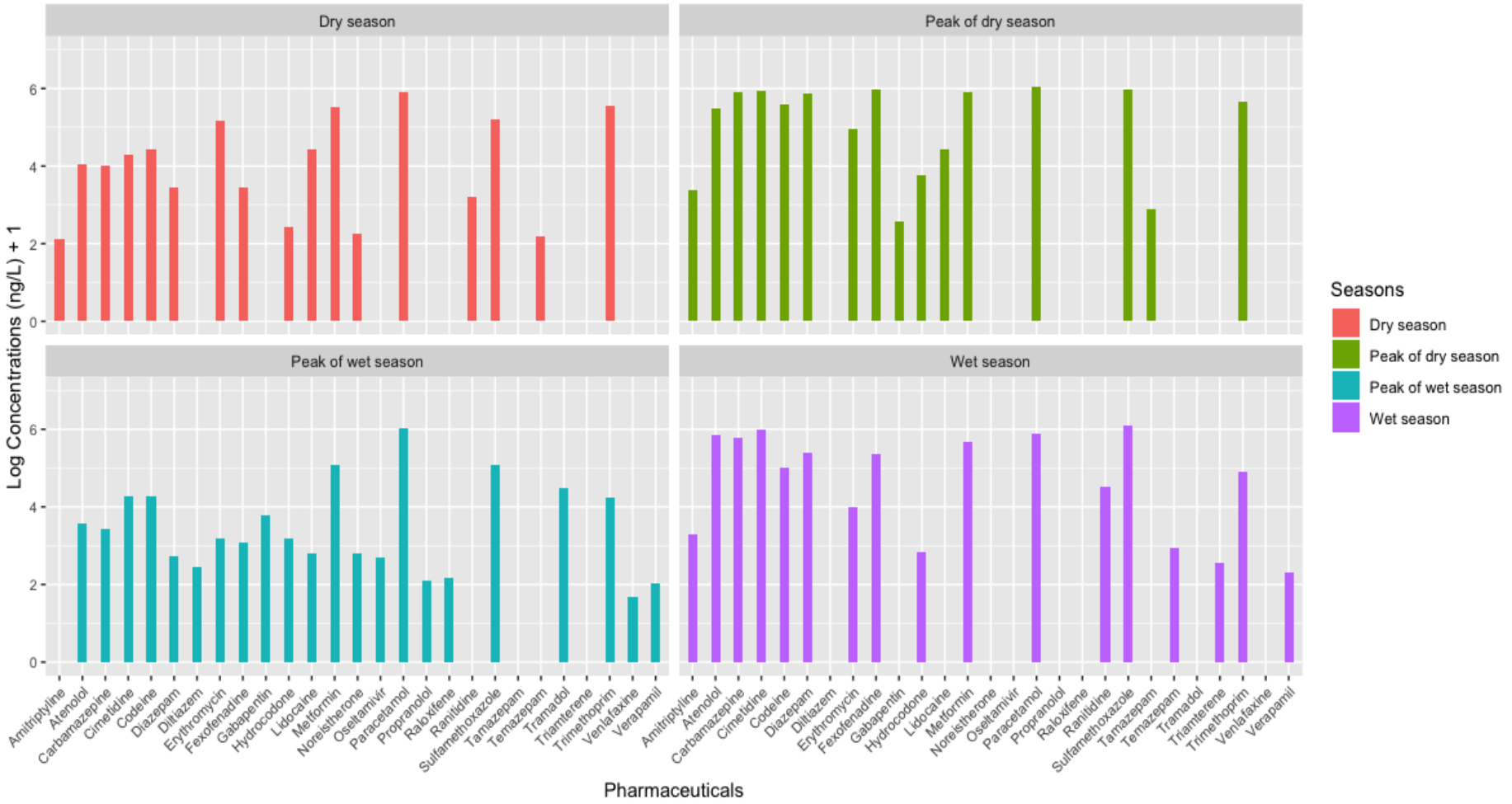

Figure 3.2. 4: Barplots displaying log concentrations (ngL^{-1})+1 of seasonal variation of pharmaceuticals detected at Odo Iya Alaro river, Lagos, Southwest Nigeria.

Table 3.2.4: Summary results (ngL^{-1}) for the seasonal variations in concentrations during the quarterly monitoring campaign at the Odo-Iya Alaro river. The concentration range, median and mean concentrations for dry and peak of dry seasons, wet and peak of wet seasons are reported.

Pharmaceuticals	Peak of dry season			Dry season			Peak of wet season			Wet season		
	Range	Median	Mean	Range	Median	Mean	Range	Median	Mean	Range	Median	Mean
Amitriptyline	n.d. - 248	0	42	n.d. - 13	0	1	n.d.	0	0	0.50 - 193	9	32
Atenolol	n.d. - 30768	0	8473	n.d. - 1077	40	115	n.d. - 371	41	63	n.d. - 68869	292	3527
Carbamazepine	n.d. - 82196	7749	25654	n.d. - 1016	32	128	n.d. - 272	39	58	1.78 - 58418	322	4984
Cimetidine	n.d. - 88681	10388	22739	4.08 - 2001	247	469	n.d. - 1895	353	468	n.d. - 95690	8547	18160
Codeine	n.d. - 39381	106	4505	n.d. - 2756	250	564	n.d. - 1872	58	150	n.d. - 9977	1261	1839
Diazepam	n.d. - 75031	4727	8292	n.d. - 275	8	34	n.d. - 55	8	10	6.39 - 25923	45	1871
Diltiazem	n.d.	0	0	n.d.	0	0	n.d. - 28	0	6	n.d.	0	0
Erythromycin	n.d. - 9373	0	505	n.d. -15110	22	785	n.d. - 149	6	19	n.d. - 962	0	74
Fexofenadine	11180 - 93448	22318	28272	12.85 - 286	26	47	n.d. - 119	5	11	757 - 22238	1553	5308
Gabapentin	n.d. - 37	0	2	n.d.	0	0	n.d. - 590	82	97	n.d.	0	0
Hydrocodone	n.d. - 559	0	35	n.d. - 28	0	1	n.d. - 160	5	13	n.d. - 70	0	3
Lidocaine	n.d. - 2751	0	125	n.d. - 2779	30	269	n.d. - 63	18	22	n.d.	0	0
Metformin	n.d. - 80967	15165	22525	n.d.-32917	842	4658	n.d.-12378	549	1574	n.d.- 49325	3138	10003
Noreistherone	n.d.	0	0	n.d. - 18	0	3	n.d. - 63	15	18	n.d.	0	0
Oseltamivir	n.d.	0	0	n.d.	0	0	n.d. - 48	3	6	n.d.	0	0
Paracetamol	n.d. - 111374	9228	24616	n.d.-78731	8930	18173	n.d.-105028	8940	15120	n.d. - 75415	7130	14804
Propranolol	n.d.	0	0	n.d.	0	0	n.d. - 13	0	2	n.d.	0	0
Raloxifene	n.d.	0	0	n.d.	0	0	n.d. - 15	10	9	n.d.	0	0
Ranitidine	n.d.	0	0	n.d. - 164	0	7	n.d.	0	0	n.d. - 3265	441	646
Sitagliptin	n.d.	0	0	n.d.	0	0	n.d. - 35	4	6	n.d.	0	0
Sulfamethoxazole	n.d. - 93359	5599	22204	n.d.-16022	718	2224	n.d. - 12396	983	1752	n.d.-129475	5741	18460
Temazepam	n.d. - 76	0	8	n.d. - 15	0	1	n.d.	0	0	n.d. - 89	0	7

Tramadol	n.d.	0	0	n.d.	0	0	n.d. - 2924	1312	1144	n.d.	0	0
Triamterene	n.d.	0	0	n.d.	0	0	n.d.	0	0	n.d. - 35	0	2
Trimethoprim	n.d. - 47025	0	3907	n.d.-37241	190	2176	n.d. - 1784	95	175	n.d. - 7820	437	1240
Venlafaxine	n.d.	0	0	n.d.	0	0	n.d. - 5	0	0	n.d.	0	0
Verapamil	n.d.	0	0	n.d.	0	0	n.d. - 11	5	5	n.d. - 21	0	4

n.d. = Not Detected

3.3 DISCUSSION

Pharmaceuticals are biologically active and pseudo-persistent, in the environment due to the continual input of wastewater effluent to rivers (Kay et al., 2017; Yamamoto et al., 2009). They, therefore, potentially pose a toxicological risk to non-target organisms (Boxall et al., 2002; Huang et al., 2012). The results presented in this work provide new information about the presence of pharmaceuticals in a Nigerian river, including frequency of occurrence, concentration ranges, spatial and temporal patterns and seasonal distribution. This work contributes significantly to the knowledge of pharmaceuticals in African rivers, which has wider relevance to developing countries worldwide.

3.3.1 Frequency of detection

The detection of 26 pharmaceuticals in the Odo Iya Alaro river has helped to confirm the presence of these substances in Nigerian watercourses including some that have not previously been observed in African rivers more widely. Pseudo persistence was observed, presumably due to continuous discharge of effluents to the river, similar to that found in other studies around the world (Burns et al., 2018; Hughes et al., 2013; Kay et al., 2017). Many of the substances found are the same as those in these other studies and certain substances are clearly used in great quantities around the world, including, for instance, fexofenadine, cimetidine, paracetamol, the sulphonamides and carbamazepine. Similarly, some substances appear to enter the aquatic environment in much lower amounts globally, e.g. propranolol. Furthermore, frequency of occurrence is higher in the receiving water than the STP and the pharmaceutical manufacturing effluents.

3.3.2 Mean and maximum concentrations

Overall, the mean (18178.27 ngL^{-1}) and maximum (129474.92 ngL^{-1}) concentrations in this study were 2-3 orders of magnitude higher than previously reported for Europe and the US (Tim Aus der Beek et al., 2016; Burns et al., 2018; Hughes et al., 2013; Madikizela et al., 2017; Verlicchi et al. 2012) but similar or an order of magnitude higher than those measured in China (Fatta-Kassinos et al., 2011) and India (Balakrishna et al., 2017). Some compounds found at particularly high concentrations were sulfamethoxazole and paracetamol which were prevalent at all seasons and locations in the catchment throughout the year. This indicates very frequent release of pharmaceuticals to rivers, a suggestion supported by other works (Andreozzi et al., 2003; Tim Aus Der Beek et al., 2016; Matongo et al., 2015a & 2015b; Ternes, 1998; Vieno et al., 2007). In South Africa, however, Agunbiade and Moodley (2014) reported surface water concentrations ranging from 500 to 30000 ngL^{-1} showing that concentrations may be lower in more developed regions of Africa. Concentrations in Kenya (Ngumba et al. 2017) were of the same order as the ones measured in this study and may be attributed to a range of factors including over-the-counter sales, differences in health issues, poorer removal efficiencies at STPs, unregulated discharges by pharmaceutical manufacturing companies, illegal disposal of sewage by vacuum trucks and climatic conditions. Without further study it is currently not possible to disentangle the range of factors potentially influencing pharmaceutical pollution of rivers.

3.3.3 Spatial distribution of pharmaceuticals

There were no specific spatial trends observed in this work and concentrations were high throughout the catchment revealing that there are potentially many contributing sites. Studies in Europe and the US have found that STP are the major source of pharmaceutical pollution (Hughes et al., 2013) but in the developing world it seems that there are a greater range of sources contributing to loads in rivers. These may include STPs, pharmaceutical manufacturing plants, urban waste collection areas and disposal of effluent by vacuum trucks.

Similarly, pharmaceutical production facilities in Hyderabad, India have been found to be a key source in this developing country (Balakrishna et al., 2017: Fick et al., 2009; Larsson et al., 2007).

3.2.4 Seasonal variations in pharmaceutical concentrations

A number of studies have previously proposed a range of reasons for variation on concentrations of pharmaceuticals in river across the year, including seasonal usage and changes in environmental conditions (e.g. temperature and river flow) (Kolpin et al., 2014; Tewari et al., 2013). Typically, concentrations are highest during low flow conditions when sewage effluent makes up a greater proportion of river flow. As for spatial patterns though, seasonal trends in the data were complex with some compounds being found at extremely high concentrations in the peak of the dry season and conversely, some compounds such as atenolol, carbamazepine, cimetidine, codeine, diazepam, fexofenadine, metformin, paracetamol, sulfamethoxazole and trimethoprim are equally high during the wet period (Table3.1.4). Seasonal usage is unlikely to explain this as many compounds would be used equally over the year to treat persistent illnesses, e.g. carbamazepine and metformin. It may be that the multiple sources of pharmaceuticals in the catchment results in this complex picture with some that are associated with continuous effluent discharges (e.g. from STPs and manufacturing facilities) being diluted in the wet season but other sources (e.g. urban waste sites) which see pollutants mobilised in periods of rainfall.

3.3 CONCLUSION

This is the most detailed study to date of pharmaceuticals in African rivers and has highlighted their occurrence at high concentrations. Concentrations in Nigerian rivers appear to be several orders of magnitude higher than those reported for Europe and the US and, in some cases, even higher than the few existing values produced for other developing countries (e.g. Africa, China and India). Spatial and temporal patterns were complex and probably affected by a greater range of sources contributing to pharmaceutical loads than in many existing studies. This pose a particular issue for understanding and managing pharmaceutical pollution in African rivers. The scenario presented here has a strong likelihood of being replicated in other major African cities as well as megacities in other developing nations globally, where pharmaceuticals are available over the counter and where wastewater discharges to rivers proceed untreated. A key implication for the global research agenda on pharmaceutical effects in surface waters (e.g. ecotoxicological effects, antibiotic resistance) is that studies of pharmaceuticals in the environment should focus more on developing countries where contamination of water is likely much more significant.

REFERENCES

Agunbiade, F.O., Moodley, B., 2014. Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. Environ. Monit. Assess. 44, 7273-7291.

Agunbiade,F.O., Moodley, B. (2016). Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi river, Kwazulu-Natal, South Africa. Environ Tox, Vol.35,No 1, pp. 36-46.

Akande, T. M., Ologo, M. O. (2007). Prescription Pattern at a Secondary Health Care Facility in Ilorin, Nigeria. Annals of African Medicine 6 (4): 186 – 189.

Andreozzi, R., Marotta, R., Paxéus, N., 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere.

Ashton, D.; Hilton, M.; Thomas, K. V. (2004). Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Sci. Total Environ. 333 (1-3), 167-184.

Balakrishna K., Rath A, Praveenkumarreddy Y., Guruge K, Subedi B.(2018). A review of the occurrence of pharmaceuticals and personal care products in Indian water. Bodies. Ecotox Environ Safety 137, 113- 120.

Boxall, A.B.A, Blackwell P., Cavallo, R., Kay, P., Tolls, J. (2002). The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol Lett. 131:19-28.

Bruchet A, Hochereau C, Picard C, Decottignies V, Rodrigues JM, Janex-Habibi ML. (2005). Analysis of drugs and personal care products in French source and drinking waters: The analytical challenge and examples of application. Water Sci Technol 52:53–61.

Burns, E.E., Thomas-Oates, J., Kolpin, D.W., Furlong, E.T. and Boxall, A., 2017. Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose?. Environmental Toxicology and Chemistry. 36(10), p. 2823-2832.

Ebele,A.J, Abdallah, M.A,, Harrad, S, (2017). Pharmaceuticals and personal care products (PPCPs) in freshwater aquatic environment. Emerging contaminants 3, 1-16.

Fatta-Kassinos, D., Meric, S., Nikolaou, A., (2011). Pharmaceutical residues in environmental waters and wastewater. Anal. Bioanal. Chem. 399, 251-275.

Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D.G.L. Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents, Environ. Sci. Technol. 44 (2010) 2661-2666.

Furlong, E.T., Noriega, M.C., Kanagy, C.J., Kanagy, L.K., Coffey, L.J. and Burkhardt, M.R., 2014. Determination of human-use pharmaceuticals in filtered water by direct aqueous injection: high-performance liquid chromatography/tandem mass spectrometry (No. 5-B10). US Geological Survey.

Gracia-Lor, E., Sancho, J.V., & Hernandez, F. (2010). Simultaneous determination of acidic, neutral and basic pharmaceuticals in urban wastewater by ultra high-pressure liquid chromatograpgy-tandem mass spectrometry. *Journal of Chromatorgraphy A, 1217, 622-632.*.

Hughes, S.R., Kay, P., Brown, L.E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol., 47, (2) 661-677.

Kay, P., Hughes, S.R., Ault, J. R., Ashcroft, A. E., Brown, L. E. (2017). Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. J. Envpol. 10 (087), 0269-7491.

K'Oreje, K.O., Vergeynst L., Ombaka D., De Wispelaere P., Okoth M.,Langenhove H., Demeestere K., (2016). Occurrence patterns of pharmaceutical residues in wastewater, surface water, and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149, 238-244.

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., & Tobin, J. (2008). An LCMS method for the determination of pharmaceutical compounds in wastewater treatment plant influent effluent samples. Talanta, 75, 1089-1097.

Larsson, E., al-Hamimi, S., Jonsson, J.A., (2014). Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography-mass spectrometry. Sci. Total Environ. 485e486, 300-308.

Madikizela, L.M., Tavengwaa, N.T., and Chimuka, L., (2017). Status of pharmaceuticals in African water bodies: Occurrence, removal and analytical methods.Environ Management 193 (2017) 211-220.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015a. Pharmaceutical residues in water and sediment of msunduzi river, KwaZulu-Natal, South Africa. Chemosphere 134, 133-140.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015b. Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. Environ. Sci. Pollut. Res. 22, 10298e10308.

Ngumba E., Gachanja A., Tuhkanen T., (2016a). The occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Science of the Total Environment, 539, 206-213

Ngumba, E., Kosunen, P., Gachanja, A., Tuhkanen, T., 2016b. A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC- MS/MS and matrix-matched standards. Anal. Methods 8, 6720-6729. https://doi.org/10.1039/C6AY01695B.

Nicholas J. Niemuth and Rebecca D. Klaner, (2015). Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish.Chemosphere, vol 135, 38-45.

Norpoth K, Nehrkorn A, Kirchner M, Holsen H, Teipel H (1973) Investigations on the problem of solubility and stability of steroid ovulation inhibitors in water, wastewater and activated sludge. Zbl Hyg I Abt Orig B 156: 500–511

Nwolisa C.E., Erinaugha E.U., Ofoleta S.I. (2006). Prescribing practices of doctors attending to under-fives in a children's outpatient clinic in Owerri, Nigeria. J. Tropical Pediatrics. 52(3): 197-200.

Odusanya O. O. (2005). Drug use indicators at a secondary health care facility in Lagos, Nigeria. J. Community Medicine Primary Health Care. 16(1):21-24

Ogunbanwo,O.M.A.(2011). Distribution and variation of macrobenthic invertebrates in Ikorodu axis of Lagos Lagoon. Unpublished manuscript, M.Sc thesis, University of Lagos, Lagos State, Southjwest Nigeria.

Olaitan,O.J.,Anyakora,C.,Bamiro,T.,Tella,A.T.(2014) Determination of pharmaceutical compounds in surface and underground water by solid phase extraction- liquid chromatography, J. Environ. Chem. Ecotoxicol. 6 (2014) 20-26.

Olarinmoye,O.,Bakare,A.,Ugwumba,O.,Hein,A.(2016)Quantificationof pharmaceutical residues in wastewater impacted surface water and sewage sludge from Lagos, Nigeria, J. Environ. Chem. Ecotoxicol. 8 (2016) 14e24.

Onesios, K.M., Yu, J.T., Bouwer, E.J., (2009). Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 20, 441–466.

Ort, C. Madikizela, L.M., Rieckermann, J.R., Joss, A., (2010). Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions Valid? A critical review. Environ. Sci. Technol. 44 (16), 6024-6035.

Oshikoya K. A., Ojo O. L. (2007), Medication errors in paediatric outpatient prescriptions of a teaching hospital in Nigeria. Nig Q. J. Hosp. Med. Apr-Jun. 17(2):74-8

Ternes, T. A. (1998). The occurrence of drugs in German sewage treatment plants and rivers. Waters Res. 32 (11), 3245-3260.

Tim Aus Der Beek, Frank-Andreas Weber, Axel Bergmann, Silke Hickmann, Ina Ebert, Arne Hain and Anette Kuster., 2016. Pharmaceuticals in the environment-Global occurrence and perspectives. Environ Toxi and Chem. 35 (4) 823-835.

Vieno, H., Harkki, H., Tuhkanen, T., Kronberg, L. Occurrence of pharmaceuticals in river

water and their elimination in a pilot-scale drinking water treatment plant, Environ. Sci. Technol. 41 (2007) 5077e5084

Waiser, M. J. Humphries, D.; Tumber, V.; Holm, J. (2011). Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wacana Creek, Saskatchewan, Canada Environ. Toxicol. Chem. 30 (2), 508-519.

Watkinson, A. J.; Murby, E. J.; Kolpin, D.W.; Costanzo, S. D. (2009). The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. Sci. Total Environ. 407 (8), 2711-2723.

Weigel, S.; Berger, U.; Jensen, E.; Kallenborn, R.; Thoresen, H.; Huhnerfuss, H. (2004). Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromso/Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56 (6), 583-592.

Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., 2004 with updates through 2009, Processing of water samples (version 2.2): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5, accessed July 2, 2012, at http://water.usgs.gov/owq/FieldManual/chapter5/html/Ch5_contents.html.

Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, Assempour N, Iynkkaran I, Liu Y, Maciejewski A, Gale N, Wilson A, Chin L, Cummings R, Le D, Pon A, Knox C, Wilson M. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2017 Nov 8. doi: 10.1093/nar/gkx1037. PubMed: 29126136

Wood, T.P., Basson, A.E., Duvenage, C., Rohwer, E.R., 2016. The chlorination behaviour and environmental fate of the antiretroviral drug nevirapine in South African surfacewater. Water Res. 104, 349-360. https://doi.org/10.1016/ J.WATRES.2016.08.038.

World Health Organization. (2012). Pharmaceuticals in drinking water. Geneva, Switzerland.

Yamamoto, H., Nakamura, Y. Morigushi, S., Nakamura, Y., Honda., Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., (2009). Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. Water Res. 43 (2). 351-362.