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High concentrations of pharmaceuticals in a Nigerian river catchment

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Abstract

Pharmaceutical contamination of the environment is recognized as a global problem although work has focused on Europe and North America to date and there remains a dearth of information for developing countries, including those in Africa. To address this data gap the occurrence of thirty-seven pharmaceuticals belonging to nineteen therapeutic classes was monitored in surface water and effluent in Lagos State, Southwest Nigeria. Samples were collected quarterly from twenty-two sites and twenty-six compounds were detected at least once, many in the micrograms per litre range. Maximum concentrations for those compounds detected ranged from 75 to 129 $\mu g\ L^{-1}$ and even mean concentrations for thirteen compounds were in the order of $\mu g\ L^{-1}$. These values are amongst the highest ever measured globally. Sewage effluent was more important than drug manufacturing waste in polluting rivers although there are likely to be numerous unregulated sources of effluent being discharged to rivers which require further study, including urban waste collection areas and vacuum trucks which collect effluent. Seasonal trends in the data were complex with some compounds being found at higher concentrations in the dry season and, conversely, others being greater during the wet period, this variation potentially relating to the variety of pollution sources in the catchment. Pharmaceuticals are indispensable to human health although their usage and discharge into the aquatic environment may lead to ecological problems and antibiotic resistance. The data presented in this paper indicate that pharmaceutical pollution of freshwaters is a serious issue in Nigeria and management efforts are needed to ameliorate this issue.

Keywords: Pharmaceuticals; emerging contaminants; rivers; water quality; pollution; sewage

1. Introduction

Pharmaceuticals were first detected in the environment in the 1970's (Tabak and Bunch, 1970; Norpoth et al., 1973) and since then numerous studies have quantified their occurrence in aquatic systems. The majority of these studies have been undertaken in Europe and North America (Hughes et al., 2013) whilst far fewer have been performed in low to medium income countries, including those in Africa, South America and the Middle East (Hughes et al., 2013). A small number of studies have been undertaken in Africa and detection frequencies have been high at between 60 and 100 % (Ngumba et al., 2016) with concentrations typically greater than those measured in the West (Fekadu et al., 2019). In Kenya concentrations of up to 167 µg L^{-1} were found in sewage effluent and surface waters (K'Oreje et al., 2012, 2016, 2018) whilst in South Africa pharmaceuticals have also been found to be ubiquitous in effluent and freshwaters at concentrations ranging from ng L^{-1} to µg L^{-1} (Agunbiade and Moodley, 2015; Gumbi et al., 2017). Such high concentrations may be due to a number of factors including high drug usage and poor regulation of this, the presence of numerous pharmaceutical manufacturing plants, and poorly developed sewage treatment facilities (Fekadu et al., 2019). In Nigeria, analgesics, antibiotics, antacids, antihistamines, anticonvulsants, steroids, antimalarials and antihypertensives are among the most consumed classes of drugs and are routinely purchased without a prescription (Odunsanya, 2005). However, the statistics available on the usage of pharmaceuticals are not reliable because of the activities of unregistered pharmacies in some cities such as Lagos (Akande and Ologe, 2007; Oshikoya and Ojo, 2007; Nwolisa et al., 2006; Odunsanya, 2005). The current paper aims to add to the limited occurrence data for pharamceuticals in African effluent and surface water and, therefore, improve our understanding of the global importance of pharamceutical pollution. We report the results of a monitoring campaign to understand the occurrence of thirty-seven pharmaceuticals in the Odo-Iya Alaro River catchment in Lagos, Nigeria, a

country where few pharmaceutical monitoring data are available. The main objectives were: (i) to understand the extent to which drugs belonging to different therapeutic classes are found in effluents and surface water, (ii) to quantify spatial and temporal patterns of pharmaceutical contamination and, (iii) to highlight particular compounds of environmental concern.

2. Methods

2.1. Substances monitored

The pharmaceuticals monitored (Table 1) were chosen based on their expected presence in surface waters (Burn et al., 2018), being high-use drugs that have been found previously in rivers around the world (Hughes et al., 2013).

2.2. Study catchment

The Odo-Iya Alaro River (Figure 1) forms a sub-catchment of the Ogudu river, which discharges into the Lagos lagoon, and flows through Ogba, Ikeja and Maryland which have a combined population of 2.5 million people. The catchment contains a sewage treatment plant (STP), three 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 pass through densely populated urban areas which discharge untreated domestic waste to them and raw sewage may also enter the river due to emptying of vacuum trucks which collect untreated effluent in urban areas (Ogunbanwo, 2011). Twenty-two sampling stations were located along the river based on accessibility and the potential to sample both receiving waters and effluents being discharged to them (Table S1).

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Table 1. Physico-chemical properties of the pharmaceutical compounds monitored in the Odo Iya Alaro river, Lagos, Nigeria (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
	Ceterizine	1.70-3.57	3.58-7.74	388.89	$C_{21}H_{25}CIN_2O3$	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	$C_{16}H_{13}CIN_2O_2$	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

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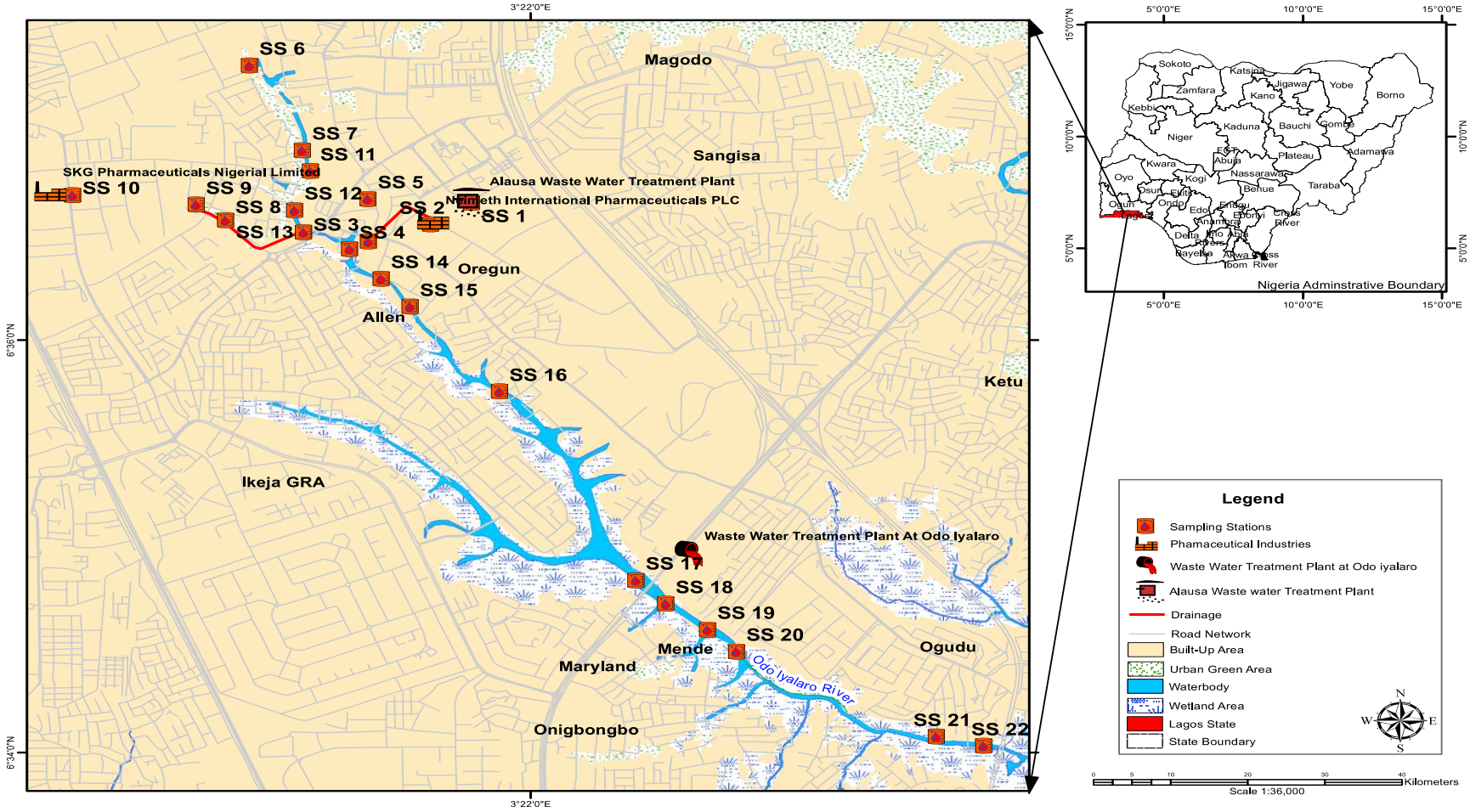


Figure 1. The Odo-Iya Alaro river in Lagos State, Southwest Nigeria and the twenty-two sampling stations used in the current study.

2.3. Sample collection

Effluent and surface water samples were collected on a quarterly basis to incorporate both the wet (April and July) and dry seasons (October and January). Amber glass sampling vessels 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 location, checked using a Global Positioning System (GPS). At each sampling site, three 50 mL water samples were collected and then homogenised into a single 150 mL composite sample. A 10 mL aliquot of each composite sample was then filtered on site through a Whatman GFF (0.7 µm pore size) glass microfiber syringe filter into a 20 mL amber glass vial with a Teflon-lined screw cap (Fisher Scientific, UK). Samples were frozen immediately with dry ice before shipping within 24 hrs to the UK for analysis. On arrival (three days), samples were thawed immediately and analysed.

2.4 Chemical analysis

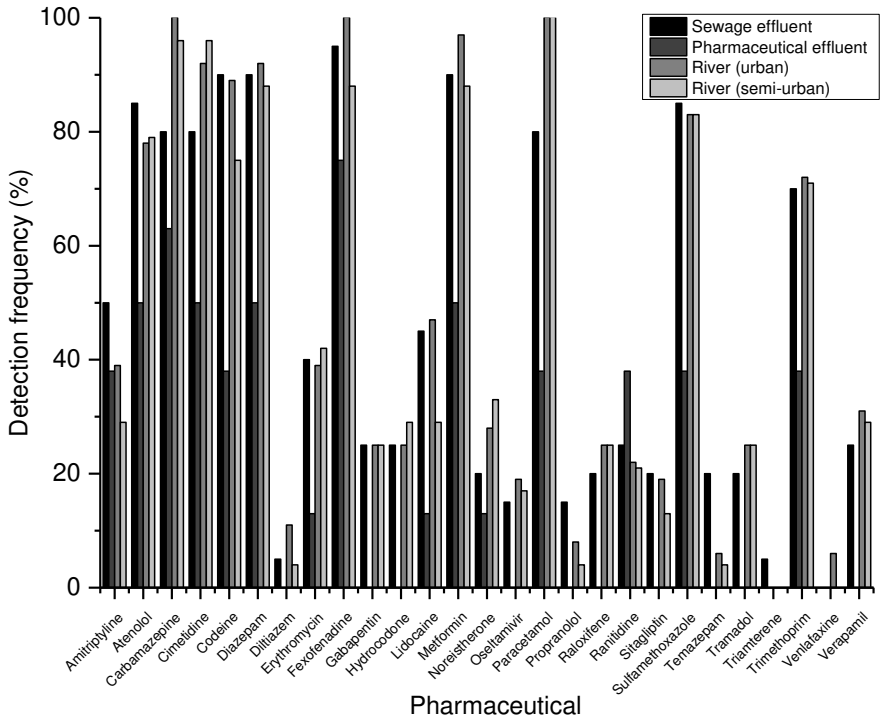
Quantification of pharmaceutical concentrations was achieved using HPLC-MS/MS with a Thermo Scientific TSQ Endura Mass spectrometer coupled with an UltiMate 3000 liquid chromatograph. The method used was adapted from Furlong et al. (2014) and further validated (Burns et al., 2018). Briefly, prior to 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 µg L^{-1}) in glass autosampler vials. The 50 % dilution was done in order to clean the samples and bring analyte concentrations to within the calibrated range. Where concentrations were found to still exceed the calibrated range further dilution and reanalysis was carried out. 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 each sample into 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. A 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 ng L^{-1} and 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 ng L^{-1} (carbamazepine) to 12.4 ng L^{-1} (gabapentin) (Table S2). 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 although using HPLC-grade water. Concentrations of the 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 4 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.

2.5 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; general linear model and Chi-square were used to find if there were differences between the sampling sites. Seasonal variations were analysed using one-way ANOVA where assumptions of normality and homogeneity were met followed by

149 Tukey's post-hoc tests to determine if there is any variation in concentrations between the
150 wet and the dry the seasons.
151
152 # 3. Results
153 ## 3.1 Detection frequencies
154 All of the study compounds were detected although the frequency of detection varied greatly
155 for different substances (Figure 2). Some, including carbamezapine, fexofenadine and
156 paracetamol, were present in sewage effluent and surface waters most of the time whereas
157 others, such as diltiazem, propranolol and venlafaxine, were rarely detected. Detection
158 frequencies in pharmaceutical manufacturing effluent were significantly lower than in
159 sewage effluent and river water (GLM: χ^2 (3) = 883.32, p <0.001).



160

161 **Figure 2**. Detection frequencies for pharamceuticals measured in sewage effluent,
162 pharmaceutical manufacturing waste and rivers (urban and semi-urban).
163

3.2 Pharmaceutical concentrations

Peak pharmaceutical concentrations were in the range of hundreds of micrograms per litre while mean concentrations were several orders of magnitude lower (Figure 3). The antibiotic sulfamethoxazole was detected at the highest concentration of 129.4 µg L^{-1} whilst paracetamol was also measured at 111.4 µg L^{-1}. Paracetamol and sulfamethoxazole also had the highest mean concentrations of 18.2 and 11.2 µg L^{-1} respectively. Many other compounds were found at concentrations only slightly lower than these including cimetidine, fexofenadine, carbamazepine, metformin, diazepam, atenolol, trimethoprim, and codeine (Table S3).

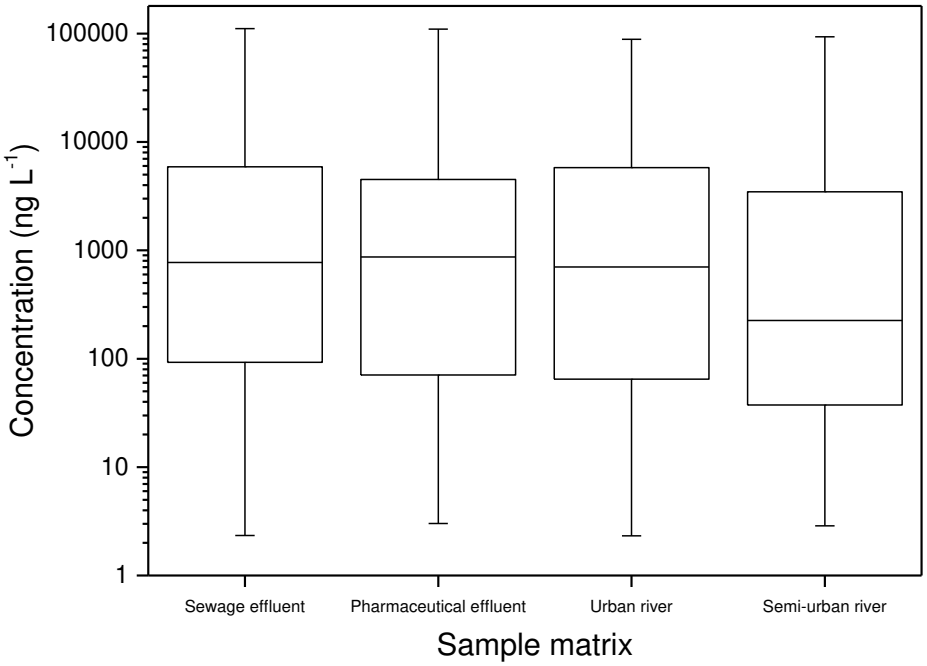


Figure 3. Pharmaceutical concentrations measured in the Odo Iya Alaro river catchment, Lagos, Nigeria. Boxes represent median and 25th and 75th percentiles whilst whiskers show minimum and maximum values.

There was a significant difference in pharmaceutical concentrations in the different matrices sampled (GLM: $\chi 2$ (3) = 883.32, p <0.001). When pharmaceuticals were detected in manufacturing effluent concentrations tended to be higher than in sewage effluent and river water. Drugs were diluted slightly after sewage effluent had entered urban rivers although concentrations were lower still in semi-urban reaches. Although detection frequencies were generally higher in the wet season, concentrations were often higher in the dry season (Figure 4).

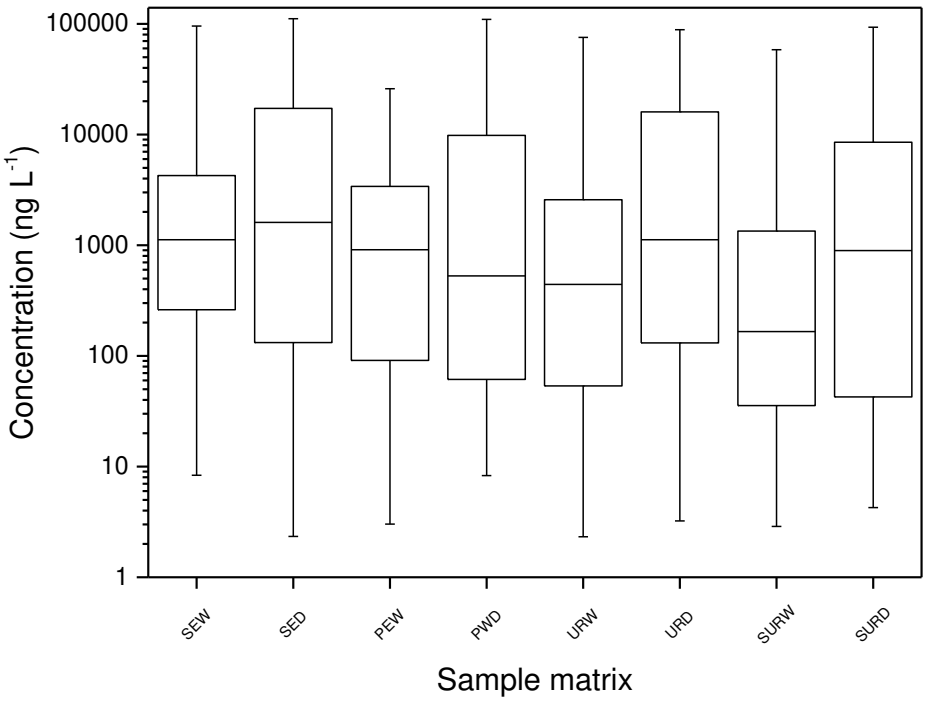


Figure 4. Comparison of pharmaceutical concentrations measured in the wet and dry seasons in different matrices in the Odo Iya Alaro river, Lagos, Nigeria. SEW = sewage effluent in wet season, SED = sewage effluent in dry season, PEW = pharmaceutical manufacturing effluent in wet season, PED = pharmaceutical manufacturing effluent in dry season, URW = urban river in wet season, URD = urban river in dry season, SURW = sub-urban river in wet season, SURD = sub-urban river in dry season. Boxes represent median and 25th and 75th percentiles whilst whiskers show minimum and maximum values.

4. Discussion

Pharmaceuticals are biologically active and pseudo-persistent in the environment due to the continual input of wastewater effluent to rivers (Hughes et al., 2013; Kay et al., 2017; Burns et al., 2018) and, therefore, potentially pose a toxicological risk to non-target organisms (Boxall et al., 2002; Huang et al., 2012). Monitoring has mainly taken place in Europe and the United States though and data are severely lacking for developing countries, such as those in Africa. This paper addresses this important research gap by presenting new information about the presence of pharmaceuticals in pharmaceutical manufacturing effluent, sewage effluent and surface water in Lagos, Nigeria, a country which has been studied very little.

The detection of twenty-six pharmaceuticals in the Odo Iya Alaro river confirmed the presence of these substances in Nigerian watercourses including some that have not previously been observed in African rivers more widely. These were from a wide range of therapeutic classes including anti-inflammatories, antidepressants and antihistamines, and were present at relatively high concentrations compared to some other drugs that were measured. Pseudo-persistence was observed, presumably due to continuous discharge of effluents to the river, similar to that found in other studies. One of the key findings of the current study is that pharmaceutical concentrations in the environment are often two to three orders of magnitude higher than typically reported in Europe and the US where most monitoring has been undertaken (Verlicchi et al. 2012; Hughes et al., 2013; Aus der Beek et al., 2016; Madikizela et al., 2017; Burns et al., 2018). Some of the highest pharmaceutical concentrations ever found in rivers globally have been reported in this paper, it not being uncommon to have measured levels in the tens of micrograms per litre range up to 129 $\mu g\ L^{-1}$. This is in-line with findings in Kenya ((K'Oreje et al., 2012, 2016, 2018). Particular compounds of concern in the Nigerian environment appear to be carbamezapine, cimetidine, fexofenadine, metformin, paracetamol and sulfamethoxazole. The presence of a range of

220 substances at such high concentrations may be attributed to a range of factors including over-
221 the-counter sales, differences in health issues, poorer removal efficiencies at wastewater
222 treatment plants, unregulated discharges by pharmaceutical manufacturing companies, and
223 illegal disposal of sewage by vacuum trucks. Other workers have proposed that these sources
224 are likely to be important in Africa (Fekadu et al., 2019) and further study is needed to
225 disentangle inputs from these various sources.

226 There were few spatial trends observed in pharmaceutical pollution in this study which
227 appears to be ubiquitous although the absence of many compounds in pharmaceutical
228 production effluent suggests that sewage effluent is the main source of pollution. Indeed, the
229 occurrence and concentrations of pharmaceuticals in wastewater treatment plant effluent and
230 surface water were very similar. This highlights that receiving waters have little capacity to
231 dilute effluent but also that further unregulated and unmonitored sources of effluent may be
232 discharging to the river, such as vacuum trucks collecting effluent in urban areas. Other work
233 in India has however proposed that pharmaceutical production facilities are a key source of
234 pharmaceutical pollution developing countries (Balakrishna et al., 2017).

235 Although season had an impact on pharmaceutical pollution a complex picture exists with
236 some compounds being found at extremely high concentrations in the dry season and,
237 conversely, others being relatively high during the wet season. Previous studies have
238 proposed a range of reasons for variation across the year, including seasonal usage and
239 changes in environmental conditions (e.g. temperature and river flow) (Tewari et al., 2013;
240 Kolpin et al., 2014; Fekadu et al., 2019). It may be that the multiple sources of
241 pharmaceuticals in the catchment results in the complexity of spatial patterns found in the
242 current study, with some continuous effluent discharges being diluted in the wet season but
243 other sources (e.g. urban waste sites) having pollutants mobilised in periods of rainfall.

5. Conclusion

This is the most detailed study to date of pharmaceutical pollution in African river catchments and it has highlighted their occurrence at some of the highest concentrations ever found globally. 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. Sewage effluent appears to be the key source of pollution although it is speculated that further investigation of unregulated sources is needed. Whilst also important, it appears that many compounds are not discharged from drug manufacturing plants. The complexity of temporal patterns across seasons is proposed to be due to a greater range of sources contributing to pharmaceutical loads than in many existing studies, which poses a particular research challenge 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 without regulation. A key implication for the global research agenda on pharmaceutical occurrence, fate and effects is that studies should focus more on developing countries where contamination of water is likely to be most significant.

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