JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
341

Elijah Ngumba

Occurrence and Control of Selected Antibiotics and Antiretroviral Drugs in Urban Hydrological Cycles

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa YAA303
helmikuun 16. päivänä 2018 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2018

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Cover photos: Tuula Tuhkanen and Elijah Ngumba

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-7344-5

URN:ISBN:978-951-39-7344-5
ISBN 978-951-39-7344-5 (PDF)

ISBN 978-951-39-7343-8 (print)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2018

ABSTRACT

Ngumba, Elijah
Occurrence and control of selected antibiotics and antiretroviral drugs in urban hydrological cycles
Jyväskylä: University of Jyväskylä, 2018, 71 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 341)
ISBN 978-951-39-7343-8 (print)
ISBN 978-951-39-7344-5 (PDF)
Yhteenveto: Antibioottien ja antiretroviraalisten lääkeaineiden esiintyminen ja kontrolli urbaanissa hydrologisessa kierrossa
Diss.

The occurrence of active pharmaceutical ingredients (APIs) including antibiotics and antiretroviral drugs in the environment has been identified as an environmental challenge in the last two decades. The main objective of this thesis was to determine the occurrence and control of selected antibiotics and antiretroviral drugs in the aquatic environment of three urban hydrological cycles of Nairobi-Kenya, Lusaka-Zambia and Jyväskylä-Finland. First, a multiresidue analytical method for trace determination of the analytes based on solid phase extraction and liquid chromatography tandem mass spectrometry was developed. In treated municipal wastewater, the maximum mean concentrations for individual pharmaceuticals were 537 ng l^{-1} in Jyväskylä, 55760 ng l^{-1} in Lusaka and 3940 ng l^{-1} in Nairobi. Similarly, high surface water concentrations of up to 13800 ng l^{-1} in Nairobi and 49700 ng l^{-1} in Lusaka compared with 54 ng l^{-1} in Jyväskylä were measured. The compounds were only sporadically present in Lusaka groundwater samples with concentration in ranging from below the limit of quantification to 880 ng l^{-1}. High individual pharmaceutical concentrations of up to 12.8 mg l^{-1} were measured in Lusaka source separated urine implying that source separation can be a significant barrier to environmental contamination. The high antibiotics and antiretroviral drugs concentrations in Nairobi and Lusaka was attributed to high disease prevalence especially HIV/AIDS, unregulated sale of antibiotics as well as inadequate or absence of waste collection and treatment facilities. Evaluation of post-treatment removal of three of the antibiotics and three of the antiretroviral drugs by UV photolysis and advanced oxidation processes (UV/Cl_2 and UV/H_2O_2) showed that the UV/H_2O_2 process required the lowest electrical energy to remove 90 % of the pharmaceuticals.

Keywords: Antibiotics; antiretroviral drugs; groundwater; post-treatment; source separated urine; surface water; wastewater.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four original publications, referred to in the text by roman numerals I–IV.

In papers I and IV, Ngumba Elijah (NE) planned and performed the experiments, interpreted the results and wrote the paper with inputs from Tuhkanen Tuula (TT) and Gachanja Anthony (GA). In paper II, NE planned the experiments, Kosunen Päivi (KP) did the preliminary experiments, NE interpreted the results and wrote the paper with inputs from TT, GA and KP. In paper III, NE planned the experiments, Maldonado Johanna (MJ) did the sampling and sample preparation, NE did the final sample analysis, interpreted the results and wrote the paper with inputs from TT, GA, MJ and James Nyirenda.

I. Ngumba E., Gachanja A. & Tuhkanen T. 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of the Total Environment* 539: 206–213.

II. Ngumba E., Kosunen P., Gachanja A. & Tuhkanen T. 2016. 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. *Analytical Methods* 8: 6720–6729.

III. Ngumba E., Maldonado J., Nyirenda J., Gachanja A. & Tuhkanen T. Occurrence of antibiotics and antiretroviral drugs in source separated urine, groundwater surface water and wastewater in the peri–urban area of Chunga in Lusaka, Zambia. (Submitted manuscript)

IV. Ngumba E., Gachanja A. & Tuhkanen T. Removal of selected antibiotics and antiretroviral drugs during post-treatment of municipal wastewater with UV, UV/chlorine and UV/hydrogen peroxide. (Manuscript)

1 INTRODUCTION

1.1 General introduction

The occurrence and fate of active pharmaceutical ingredients (APIs) in the environment have gained more attention in the last two decades (Daughton and Ternes 1999, Kümmerer 2009c). APIs are a relatively new unregulated environmental pollutants in the class of contaminants of emerging concern whose environmental fate and effects are not yet well understood (Segura *et al.* 2015). APIs generally refer to diverse group of chemicals used for human health or products used to enhance the growth or health of livestock. There are more than 4000 APIs currently in use such as antibiotics, antivirals, analgesics, anti-inflammatory, antiepileptic, beta-blockers, blood lipid regulators, contraceptives and cytostatic drugs amongst others (Monteiro and Boxall 2010). The advances in selective and sensitive analytical techniques have led to the detection of these compounds in various environmental compartments (Nikolaou *et al.* 2007). Since the late 1990's, a wide range of APIs have been detected mostly in wastewater, surface water and groundwater and less frequently in drinking water (Pérez and Barceló 2007, Kostopoulou and Nikolaou 2008). APIs enter the environment from various sources (Fig. 1) including wastewater treatment plants, industrial effluents, direct discharge of untreated domestic wastes and agricultural activities (Kümmerer 2008, 2009c).

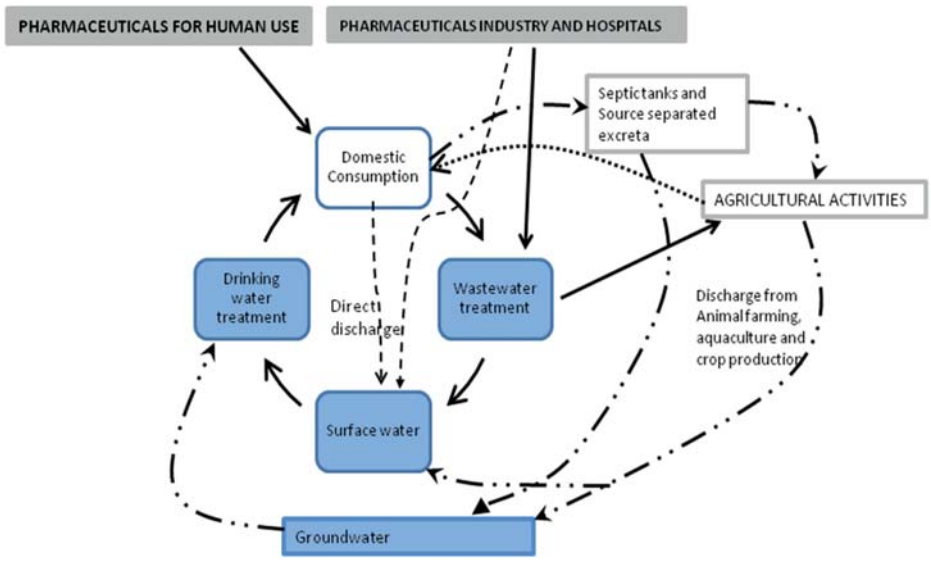

FIGURE 1 Sources of pharmaceuticals to the environment and their major pathways in the urban water cycle.

Domestic households discharge significant amounts of APIs into the environment by the excretion of consumed pharmaceuticals or direct disposal of unused/expired drugs (Dietrich *et al.* 2005). After ingestion, APIs are largely excreted as unchanged parent compounds, conjugates and metabolites primarily in urine and feces (Heberer 2002, McArdell *et al.* 2003, Kasprzyk-Hordern *et al.* 2008b, Al Aukidy *et al.* 2012). Some of the conjugates are known to cleave back to the parent molecules in WWTPs (Jelic *et al.* 2012). In addition, several studies have shown that a large number of APIs are not entirely eliminated during the conventional wastewater treatment process and may enter aquatic environment and drinking water supplies (Joss *et al.* 2008). The WWTP effluents are mostly discharged into surface waters, used for irrigation or recycled while the sewage sludge is used as soil amendments or dumped into landfills (Caliman and Gavrilescu 2009). As a result, municipal discharges are widely considered a major point source of APIs to the environment (Daughton and Ternes 1999, Kümmerer 2003, 2009a, Zhang and Li 2011, Celle-Jeanton *et al.* 2014, Kosma *et al.* 2014, Tang *et al.* 2015).

Pharmaceuticals are pseudo-persistent since they are continuously being discharged into the environment (Daughton 2003). They can affect the quality of water and are potential threat to human health and the ecosystem. Though there is no scientific evidence on the human health effect of the APIs at the detected concentration levels, precautionary measures need to be taken for the largely unknown long term effects (Joss *et al.* 2008, Li *et al.* 2014). Laboratory studies have shown that pharmaceuticals can have adverse effects such as mortality, impaired reproduction and stunted growth of aquatic organisms (Kim *et al.* 2007, Brausch *et al.* 2012). Estrogenic effects in fish and diclofenac

toxicity to vultures have been reported as some of the adverse effects of pharmaceuticals in real aquatic and terrestrial environments, respectively (Desbrow *et al.* 1998, Swan *et al.* 2006, Kümmerer 2010, Anthérieu *et al.* 2014). Of a greater concern is the presence of antibiotics in the environments since their occurrence presents one of the possible pathways of propagation of antimicrobial resistance (Kümmerer 2009b, Singer *et al.* 2016). It is only recently that the European Union (EU) added three pharmaceuticals among the pollutants in the watch list to be continuously monitored in surface waters in order to establish possible need for future prioritization and regulation (European Commission 2012). Since pharmaceuticals are released to environment as mixtures, long term cocktail effects are not yet known resulting to greater public health concerns (Jelic *et al.* 2012, Rivera-Utrilla *et al.* 2013). Most of the ecotoxicological studies of pharmaceuticals in the environment have been based on acute exposures, however the greatest concern emanates from the chronic exposure at low doses of pharmaceutical mixtures (Hughes *et al.* 2013).

The rate of production of pharmaceuticals and subsequent release into the environment is expected to continue rising for the next couple of decades because of several reasons. First, the standards of living continue to rise resulting to a wider global accessibility and usage of pharmaceuticals especially in the developing countries. Secondly, with the relatively higher life expectancy, the number of old people is globally increasing leading to extensive use of multiple medications. Thirdly, the availability of cheap generics due to increased number of expiring patents (Daughton 2003, Kümmerer 2010)

Presently, the majority of studies on the occurrence and fate of APIs in the environment have been done in high income countries in North America, Europe and parts of Asia with little or no information available from regions such as Russia, southern Asia, Africa, the Middle East, South America, and Eastern Europe (Hughes *et al.* 2013, Segura *et al.* 2015, Beek *et al.* 2016). In the African continent for example, out of 54 independent states, less than 10 countries have published data on occurrence of APIs in the environment despite there being relatively high prevalence of infectious diseases, over prescription, the availability cheap over-the-counter drugs as well as inadequate environmental barriers (Kaplan and Mathers 2011, Hughes *et al.* 2013, Miraji *et al.* 2016, Ebele *et al.* 2017, Madikizela *et al.* 2017). This can be majorly attributed to the inaccessibility of the expensive analytical equipment and expertise necessary to undertake studies of such kind. In addition, there are only a few studies globally that have been published on the occurrence of antiretroviral drugs in the environment.

1.2 Selected pharmaceuticals

The main focus of this study is on three antiretroviral drugs and seven antibiotics (Table 1). The selection was based on their high rate of consumption

and lack of data on the occurrence in urban hydrological cycles particularly in sub-Saharan Africa. The antiretroviral drugs together with some of the antibiotics are used in the management of HIV/AIDS. As of the year 2015, there were approximately 33 million people living with HIV and more than 80 % were in Sub-Saharan Africa (United Nations Programme on HIV/AIDS 2016). In addition, there has been dramatic growth in the consumption of anti-HIV drugs and as of 2016, some 18 million people worldwide were under antiretroviral therapy (Kaplan and Mathers 2011, World Health Organization 2016a, b)

The management of HIV/AIDS involves the use of multiple medications in an attempt to control viral load and other co-infections. The dosage will for example include three first-line antiretroviral therapy (ART) fixed dose combination of two nucleoside reverse-transcriptase inhibitors plus a non-nucleoside reverse-transcriptase inhibitor or an integrase inhibitor (World Health Organization 2016b). In addition to the antiretroviral drugs, the ART for most patients will include a fixed dose of co-trimoxazole (dose ratio of 5:1 sulfamethoxazole:trimethoprim) prophylaxis especially in regions where bacterial infections are prevalent (World Health Organization 2016b). Patients with other opportunistic infections take extra drugs depending on the nature of infection. For example, an adult person taking first line ART will daily ingest 600 mg of zidovudine, 300 mg of lamivudine, 400 mg of nevirapine, 800 mg of sulfamethoxazole and 160 mg of trimethoprim (World Health Organization 2006a, 2013). Based on the excretion rates listed in Table 1, the amount of APIs collectively released by this group of patients to the environment is significantly high especially in areas with high HIV prevalence. In addition, a large proportion of urban residents in areas with highest HIV prevalence live in the densely populated informal settlements that are not connected to the centralized WWTPs leading to large-scale contamination of surface water and groundwater (Wamukwamba and Share 2001, National Council for Population and Development 2013, African Population and Health Research Center 2014, Wang *et al*. 2014).

TABLE 1 Selected pharmaceuticals, pysico-chemical properties and the percentage of the pharmaceutical excreted as parent compound.

Compound	CAS NO.	Water solubility (mg l^{-1})[1]	pKa[1]	Excretion rate as unchanged compound (%)[2]
Sulfamethoxazole (SMX)	723-46-6	610	5.6, 1.83	15–25
Ciprofloxacin (CIP)	85721-33-1	13500	6.4, 8.2	80
Norfloxacin (NOR)	70458-96-7	1010	5.77, 8.68	60
Doxycycline (DOX)	564-25-0	630	7.75	70
Tetracycline (TET)	60-54-8	1330	8.24	80–90
Amoxicillin (AMO)	26787-78-0	958	3.23, 7.43	60–80
Trimethoprim (TMP)	738-70-5	400	7.2, 17.33	80–90
Zidovudine (ZDV)	30516-87-1	20100	9.7	15–20
Lamivudine (3TC)	134678-17-4	70000	4.3, 14.29	70
Nevirapine (NVP)	129618-40-2	0.7046	2.8	2.7

[1]Wishart *et al.* (2006, Babić *et al.* (2007), USEPA and SRC (2012)
[2]Harlass (1996), Riska *et al.* (1999), Jjemba (2006), Kumar *et al.* (2006), Radke *et al.* (2009), Kasprzyk-Hordern *et al.* (2009), Straub (2013)

1.3 Pharmaceuticals in WWTPs, surface water and groundwater

Most conventional municipal wastewater treatment plants are designed to remove relatively easily biodegradable organic macro-pollutant compounds normally in concentration of mg l^{-1} and microorganisms (Mulder *et al.* 2015). However, the concentration of APIs in most influent municipal wastewaters generally is in the range of ng l^{-1} – µg l^{-1} with most of them having physico-chemical properties that do not favour removal in the conventional WWTPs (Jjemba 2008). As a result, the municipal WWTPs have been widely identified as the primary source of human pharmaceuticals into the environment (Michael *et al.* 2013). Table 2 summarizes some published data from different regions for the selected antibiotics and antiretroviral drugs in municipal WWTPs, groundwater and surface waters. Difference in occurrence between different regions is generally reflective of the pharmaceutical consumption, per capita water, availability and effectiveness of wastewater collection and treatment facilities as well as socio-economic and environmental conditions (Segura *et al.* 2015, Madikizela *et al.* 2017). Significantly high concentrations of antibiotics in surface waters especially for sulfamethoxazole, ciprofloxacin, norfloxacin and trimethoprim were detected in Mozambique, South Africa, Ghana, Kenya and India (Fick *et al.* 2009, Agunbiade and Moodley 2014, 2016, Matongo *et al.* 2015a, b, Segura *et al.* 2015, K'oreje *et al.* 2016). Discharge from pharmaceutical company in India contributed massively towards the high concentration of ciprofloxacin of up to 2.5 mg l^{-1} and 14 µg l^{-1} in surface water and ground water, respectively (Fick *et al.* 2009).

Studies on the removal efficiency of the antibiotics and antiretroviral drugs in the WWTPs vary significantly based on the physico-chemical properties of the compound, plants treatment technology, hydraulic retention time, sludge retention time and the prevailing environmental conditions (Luo *et al.* 2014, Evgenidou *et al.* 2015). For example; the removal efficiency for lamivudine, nevirapine and zidovudine in Nairobi and Kisumu, Kenya was between 24–59 %, 11–49 % and >95 %, respectively and in other studies, the removal ranged between 4–88.9 % for sulfamethoxazole and < 0–81.6 % for trimethoprim (Luo *et al.* 2014); < 0–78 % for norfloxacin and < 0–73 % for tetracycline (Gulkowska *et al.* 2008); 49.7 % for amoxicillin (Mutiyar and Mittal 2014); 45–78 % for ciprofloxacin (Castiglioni *et al.* 2006) and 70 % for doxycycline (Lindberg *et al.* 2005).

TABLE 2 Literature concentrations of selected antibiotics and antiretroviral drugs in wastewater, surface water and groundwater from different countries.

		Concentration (ng l^{-1})				
Analyte	Country	Influent	Effluent	Surface water	Groundwater	Reference
SMX	Germany	na	na	<LOQ–114	<LOQ	Burke *et al.* (2016)
	Sweden	144–674	135–304	na	na	Lindberg *et al.* (2005)
	Spain				<LOQ–65	López-Serna *et al.* (2013)
	USA	na	na	na	<LOQ–113	Schaider et al. (2014)
	Kenya	10140–54830	2860–4090	0.11–49562	20–30	Segura *et al.* (2015) and K'oreje *et al.* (2016)
	USA			<LOQ–2	<LOQ–21	McEachran *et al.* (2016)
	South Africa	59280*	1600*	11–10568		Agunbiade and Moodley (2014), Matongo *et al.* (2015a, b) and Segura *et al.* (2015)
	Mozambique	na	na	511–53828	na	Segura *et al.* (2015)
	Ghana			<LOQ–9640		Segura *et al.* (2015)
	China	0.4–61.1	0.3–59.1	<LOQ–96	124.5	Peng *et al.* (2014), Tong *et al.* (2014), Dong *et al.* (2016) and Yao *et al.* (2017)
	Bolivia	1265.2**	1309.5**	12–218	47.8–251.5	Archundia *et al.* (2017)
CIP	Spain	na	na	na	<LOQ–443	López-Serna *et al.* (2013)
	India	na	na	<LOQ–2.5x106	44–14000	Fick et al. (2009)
	South Africa	27100*	14100*	14300*	na	Agunbiade and Moodley (2016)
	China	0.82–147	0.4–88.5	na	na	Dong *et al.* (2016)
	Finland	<LOQ–4230	<LOQ–130	<LOQ–36	na	Vieno *et al.* (2006, 2007a,b)
NOR	Spain	na	na	na	<LOQ–462	López-Serna *et al.* (2013)
	India			<LOQ–4700	<LOQ–31	Fick *et al.* (2009)
	China	na	0.3–527	<LOQ–277	<LOQ–96.9	Tong *et al.* (2014, Dong *et al.* (2016), Yao *et al.* (2017)
	Finland	<LOQ–960	<LOQ–110	<LOQ	na	Vieno *et al.* (2006, 2007b)
	Australia	<LOQ–2200	<LOQ–2500	<LOQ–1150	na	Watkinson *et al.* (2009)
	Sweden	66–174	<LOQ–37	na	na	Lindberg *et al.* (2005)
	France	na	na	<LOQ–163	na	Tamtam *et al.* (2008)

TMP	Germany	na	na	<LOQ–62	<LOQ–12	Burke *et al.* (2016)
	Spain	na	na	na	<LOQ–9.4	López-Serna *et al.* (2013)
	Kenya	4250–72850	90–150	<LOQ–11383	20–60	Segura *et al.* (2015) and K'oreje *et al.* (2016)
	USA	na	na	20**	2**	McEachran *et al.* (2016)
	India			<LOQ–660	<LOQ–55	Fick *et al.* (2009)
	South Africa	130*	160*	<LOQ–5875	na	Matongo *et al.* (2015a, b) and Segura *et al.* (2015)
	Mozambique	na	na	<LOQ–6223	na	Segura *et al.* (2015)
	Ghana	na	na	<LOQ–1374	na	Segura *et al.* (2015)
	China	2.3–813	0.7–108	<LOQ–19.0	<LOQ–10.5	Peng *et al.* (2014), Tong *et al.* (2014) and Dong *et al.* (2016)
	Bolivia	336.5**	145**	46–312	<LOQ–200.2	Archundia *et al.* (2017)
DOX	China	na	na	<LOQ–66.5	<LOQ–64.2	Tong *et al.* (2014)
	Spain				<LOQ–188	López-Serna *et al.* (2013)
	Australia	<LOQ–650	<LOQ–150	<LOQ–400	na	Watkinson *et al.* (2009)
	Ghana			<LOQ–50	na	Segura *et al.* (2015)
	Sweden	2480*	880*	na	na	Lindberg *et al.* (2005)
TET	South Africa	640–5680	1700*	2800*		Agunbiade and Moodley (2014)
	Spain	na	na	na	<LOQ–56.3	López-Serna *et al.* (2013)
	Ghana	na	na	<LOQ–465	na	Segura *et al.* (2015)
	Kenya			<LOQ–434	na	Segura *et al.* (2015)
	Belgium	1658*	<LOQ	na	na	Vergeynst *et al.* (2015)
	China	0.1–65.6	0.09–3.8	<LOQ–100	<LOQ–25.2	Tong *et al.* (2014), Dong *et al.* (2016) and Yao *et al.* (2017)
	China/Hong Kong	96–1300	180–620	na	na	Gulkowska *et al.* (2008)
AMO	Australia	<LOQ–6940	<LOQ–50	<LOQ–200	na	Watkinson *et al.* (2009)
	UK	na	na	39–245	na	Kasprzyk-Hordern *et al.* (2007)
	India	<LOQ–172.6	<LOQ–62.5	na	na	Mutiyar and Mittal (2014)
	Italy		<LOQ–120			Castiglioni *et al.* (2005)
	Germany	<LOQ–1270	<LOQ–187	na	na	Rossmann *et al.* (2014)
3TC	Kenya	30300–60680	19900–31070	<LOQ–167000	na	K'oreje *et al.* (2016)

	South Africa		na	<LOQ–132	na	Wood *et al.* (2015)
	Germany	720*	<LOQ	<LOQ	na	Prasse *et al.* 2010)
	Belgium	507	<LOQ	na	na	Vergeynst *et al.* (2015)
ZDV	Kenya	12100–20130	90–110	<LOQ–17000	20–30	K'oreje *et al.* (2016)
	Germany	310–380	98.2–564	4.5–170	na	Prasse *et al.* (2010)
NVP	Kenya	850–3300	1030–2080	510–5620	20–1600	K'oreje *et al.* (2016)
	South Africa	<LOQ–2100	<LOQ–350	<LOQ–177	na	Wood *et al.* (2015) and Schoeman *et al.* (2015)
	Germany	4.8–21.8	7.2–32.1	<LOQ–13	na	Prasse et al. (2010)

*mean concentration, **maximum concentration; na: not analysed; <LOQ: less than limit of quantification or not detected

1.4 Ecological sanitation and control environmental contamination

Waterless and source separation ecological sanitation has attracted a great deal of attention in the last three decades due to the potential economic and environmental gains (Hu *et al.* 2016, Simha and Ganesapillai 2016). Urine makes up of less than 1 % of the wastewater and contains majority of the nutrients and pharmaceuticals excreted by human, on average 88 % nitrogen, 67 % phosphorus, 73 % potassium and 64 % of active pharmaceutical ingredients (Lienert *et al.* 2007, Karak and Bhattacharyya 2011). Thus, waterless sanitation would in essence allow efficient nutrient recycling avoiding the high cost of synthetic fertilizers in a closed-loop fertility cycle (Ganesapillai *et al.* 2016). Secondly, with the rapid growth in global population, urbanization and improved standards of living, there is increased pressure in the existing centralized end-of-pipe technologies leading to incomplete removal of disease causing pathogens and nutrients which are ultimately discharged into the environment (Maurer *et al.* 2006, Pronk and Koné 2009, Simha and Ganesapillai 2016). Conventional sanitation systems require massive infrastructure and are often associated with high energy and water requirements (Hu *et al.* 2016). Thirdly, the majority of anthropogenic organic compounds such as pharmaceuticals are metabolized into polar species and are primarily excreted via kidneys in urine. As a result, urine contain high concentrations of organic micropollutants and source separation is an efficient way of preventing them from entering the WWTPs and environment by targeted treatments of the separated urine (Lienert *et al.* 2007, Bischel *et al.* 2015).

1.5 Analytical techniques

1.5.1 Solid Phase Extraction

Solid phase extraction (SPE) has arisen as one of the most preferred extraction methods for aqueous samples (Jones-Lepp *et al.* 2009). SPE offers some major benefits including: (i) reduction and simplification of the sample matrix that can compromise the analyte signal and contaminate the analytical instrument with each injection. (ii) The use of SPE significantly reduces to matrix effects on MS applications. (iii) SPE offers possibility to fractionate the sample extract, and (iv) SPE allows for the enrichment of samples with trace concentration of analyte due to the selective retention capability of the sorbents (Arsenault 2012).

A wide range of SPE sorbents such as reverse-phase (silica based materials such as C_8 and C_{18} which retain by hydrophobic interactions), normal phase (silica and alumina based on hydrophilic interactions), hydrophilic-lipophilic balance

(HLB), ion exchange (weak and strong cation and anion exchange), mixed mode (e.g. ion exchange and reverse phase) and functionalized resins based on styrene-divinylbenzene (PS-DVB)polymers (Buszewski and Szultka 2012, Quintana *et al.* 2014). The present study evaluated the use of Oasis HLB, Oasis MCX and Oasis MAX for sample enrichment and clean-up since they are water-wettable and have wide range pH stability and hence appropriate for aqueous sample extraction (Arsenault 2012). The three sorbents have been widely applied in the extraction of a wide range of pharmaceuticals in aqueous matrices (Benito-Peña *et al.* 2006, Vieno *et al.* 2006, Kasprzyk-Hordern *et al.* 2008a, Wood *et al.* 2015). Oasis HLB is a copolymer of N-vinylpyrrolidine and divinylbenzene monomers, which allows the retention of hydrophilic and lipophilic compounds by reverse phase and polar interactions. Oasis MCX is a mix mode strong cation exchange sorbent with of sulfonic acid and reversed phase retention groups widely applied in the extraction of weak basic compounds. Oasis MAX is a mix mode strong anion exchange bearing quaternary amine groups and reversed phase retention groups and is ideal in the extraction of weak acids (Arsenault 2012).

The greatest difficulty in a multiresidue SPE process is the optimization of experimental conditions since the compounds of interest normally exhibit a wide range of physico-chemical properties. For maximum pre-concentration and clean-up, all the SPE stages require to be optimized with respect to sorbent selection, sample pH and elution conditions (Jones-Lepp *et al.* 2009, Sosa-Ferrera *et al.* 2013, Quintana *et al.* 2014).

1.5.2 Liquid chromatography-mass spectrometry and calibration

Liquid chromatography with mass spectrometric detection has been effectively used in determination of many classes of APIs in environmental samples (Barceló and Petrovic 2007, Richardson and Ternes 2014). Chromatographic separation of LC coupled with high selectivity and sensitivity in MS/MS systems allows the trace detection and quantification of multiple analytes in the presence of sample matrix within a relatively short time and a few sample preparation procedures (Petrovic *et al.* 2005, Caliman and Gavrilescu 2009). One major benefit of an MS/MS detection system is that complete chromatographic resolution is not necessary and hence, multiresidue detection and quantification of closely eluting compounds is possible in a single rapid run (Petrovic *et al.* 2005). Reverse phase LC columns are commonly used to separate APIs with mobile phase consisting of an organic phase (mostly acetonitrile or methanol) and an aqueous phase with additives such as formic acid or ammonium acetate to enhance analyte ionization in the electrospray positive and negative modes, respectively (Kot-Wasik *et al.* 2007).

The LC-MS is interfaced soft ionization techniques predominantly with atmospheric pressure ionization either as electrospray ionization (ESI) or

atmospheric pressure chemical ionization (APCI) (Seifrtová *et al.* 2009). In the MS/MS systems the molecular ion (mostly the $(M+H)^+$ or $(M-H)^-$ ion commonly referred to as a precursor ion) is fragmented in a collision cell producing product ions which together with the precursor are used for the quantification and confirmation of compounds in a sample (El-Aneed *et al.* 2009). The isolation of specific precursor ion followed by fragmentation, isolation and detection of specific product ions commonly referred to as multiple reaction monitoring (MRM) is the basis of the high selectivity of the tandem MS. To ensure the correct identification of a target analyte, at least two MRM transitions are necessary in addition to the chromatographic retention time and ion ratios for different transitions (Gros *et al.* 2006). For maximum sensitivity, the MS parameters such as precursor and product ions, collision energy and cone voltages of each MRM transitions need to be optimized (Cimetiere *et al.* 2013)

One of the major challenges of ESI-MS/MS detection system in environmental samples analysis is its vulnerability to matrix related signal suppression or enhancement (Stahnke *et al.* 2012). Matrix effects (ME) have been found to negatively affect analytical figures of merit such as detection and quantification limits, accuracy, precision, reproducibility and linearity leading to unreliable quantitative data (Trufelli *et al.* 2011, Furey *et al.* 2013). Matrix effects are caused by such factors as the competition between endogenous sample components co-eluting with the analyte leading to compromised efficiency in ESI droplet formation and subsequently the amount of analyte gas phase ions reaching the detector (Smeraglia *et al.* 2002, Annesley 2003, Gosetti *et al.* 2010). Sample clean-up procedures and efficient chromatographic separations are some of the methods used to reduce matrix effects while isotopically labelled internal standards, matrix-matched standards and method of standard addition are used to compensate for ME (Trufelli *et al.* 2011, Quintana *et al.* 2014).

The use of internal standard calibration incorporating isotopically labelled analogues is the most preferred in quantitative chromatographic mass spectrometry (Wang *et al.* 2007). Internal standards can be used to correct for several analytical key variables when added at the beginning of the analysis including: analyte recovery during sample extraction, variability in extraction efficiency, injection volume variability, matrix effects and instrumental drifts (Stokvis *et al.* 2005, Guo *et al.* 2007, Xu *et al.* 2007, Lanuza 2011). Isotopically labeled standards have similar physico-chemical properties with the target analytes and will hence undergo similar degree of matrix effect and other analytical method related variations (Gros *et al.* 2012). In addition, the internal standards method is simple to execute, quick and efficient allowing a precise and accurate quantitative sample analysis. However, the use of isotopically labelled ISs are normally hampered by their high cost especially in multiresidue analysis where individual IS for each analyte are required. In addition, isotopically labelled IS for some

compounds are not commercially available (Stokvis *et al.* 2005, Gosetti *et al.* 2010, Lanuza 2011).

Standard addition calibration method offers the best strategy to minimize matrix related errors since it ensures near perfect match between the sample and the calibration standards. It however requires a calibration for each sample and does not account for instrumental drifts. In addition, the procedure is time consuming and requires large sample volumes; hence, not appropriate for multiple routine sample analysis (Ostroukhova and Zenkevich 2006).

Matrix-matched calibration is preferred to solvent based external calibration. In this method, the standards are prepared in a blank matrix that does not contain the analyte (Cuadros-Rodríguez *et al.* 2007). In many instances, the availability of uncontaminated blank matrix is difficult and the blank matrix used to prepare calibration standards will not have the exactly the same composition with the sample (Hernández *et al.* 2007). However, as a better compromise, a representative blank matrix can be selected for calibration purposes after thorough quality controls (Sargent 2013).

1.6 Removal of pharmaceuticals in WWTPs

1.6.1 Removal by conventional activated sludge process

Conventional municipal WWTPs as in Jyväskylä are specifically designed to remove a wide range of substances including particulate matter, nutrients, pathogens, carbonaceous biological matter and other macropollutants that can significantly raise the BOD/COD of the effluent (Zorita *et al.* 2009). The extent of removal of pharmaceuticals in a WWTP is governed by the physico-chemical property of a pharmaceutical and WWTP's associated factors such as design, sludge retention time (SRT), hydraulic retention time (HRT) and wastewater characteristics (Luo *et al.* 2014). However, with the typical low hydraulic retention time in most of the WWTPs, the overall removal is not sufficient and generally on average ranges between 10–90 % mainly because of the slow microbial degradation of the relatively polar APIs (Buttiglieri and Knepper 2008). Larsen *et al.* (2004) have identified four different approaches that can be used to improve the removal of micropollutants from wastewater that include; upgrading the existing WWTPs with new technologies, source separation methods, optimizing the existing wastewater treatment technology and source control of the micropollutants (Joss *et al.* 2008, Tambosi *et al.* 2010).

During wastewater treatment process, APIs may be removed from the aqueous phase by both biotic and abiotic processes. The main abiotic processes include sorption into the sludge, volatilization, isomerization/epimerization,

hydrolysis, and photolysis. Both sorption and volatilization involve processes which transfer the APIs from one environmental compartment to another while hydrolysis and photolysis leads to structural transformation or complete mineralization of the API (Boreen *et al.* 2003, Nikolaou *et al.* 2007, Radjenović *et al.* 2009). The biotic transformation is usually the main removal mechanism for most of the APIs whereby microorganisms are responsible for transformation or complete mineralization of the APIs (Kümmerer 2008, 2009c). Some APIs might inhibit the activity of the microorganism slowing down the biotic degradation process (Kümmerer 2008). As a result the conventional wastewater treatment process cannot be wholly relied upon as a means of effective removal of APIs from the wastewater streams.

1.6.2 Removal by wastewater stabilization ponds

Wastewater stabilization ponds (WSPs) as in Nairobi and Lusaka utilizes the natural attenuation processes for wastewater treatment (Mara 2006, Mahmood *et al.* 2013). They are preferred to conventional WWTPs in developing countries and small communities due to the low operation and maintenance cost with minimum electrical energy requirement and technical operation requirements (Amoatey and Bani 2011, Molinos-Senante *et al.* 2012, Zhang *et al.* 2012, Abdullahi *et al.* 2014). In addition, high wastewater treatment efficiency can be achieved in tropical and subtropical countries where the intensity of sun is high and minimum temperature variations.

WSPs have longer HRT compared to conventional treatment systems and have been found to remove the recalcitrant organic micropollutants better than activated sludge system due to the adsorption to the organic matter and photodegradation in the maturation ponds (Leclercq *et al.* 2009). Matamoros *et al.* (2016) conducted a comparative study of removal efficiencies of various emerging contaminants in different wastewater treatment technologies small communities in Spain. Overall, the WSPs had the highest removal efficiency of up to 82 % followed by rotating biological contactor, activated sludge and constructed wetland with removal efficiencies of 63 %, 62 % and 42 %, respectively. WSPs thus offer cheap and effective treatment process that significantly lowers the micropollutants concentration in aqueous wastewater especially in tropical and subtropical conditions. However, limitations such as need for large land area, regular removal of the sludge from the ponds, inefficiency in cold climates and odor slows down the uptake of the process especially in highly populated areas.

1.7 Post-treatment removal of pharmaceuticals in wastewater

1.7.1 Direct UV photolysis

Direct UV photolysis is one of the treatment processes that have been shown to remove many organic compounds including APIs from aqueous matrices (Yang *et al.* 2014). Low pressure (LP) UV (monochromatic light at 253.9 nm) is widely applied for the microbial disinfection of both drinking and wastewater and at the same time has potential to degrade some of the organic micropollutants (Sanches *et al.* 2010). The extent of photolysis is strongly dependent on the compound's molar extinction coefficients and quantum yields (Malley 2008, Wu and Linden 2008). The presence of conjugated π system as well as aromatic rings and heteroatoms are some of the indicators of good UV absorbers (chromophores) that readily undergo direct photolysis (Rivera-Utrilla *et al.* 2013). The UV light source (low pressure or medium pressure lamps), pH, temperature and presence of other matrices in the target water also play an important role in the removal of the APIs by direct UV photolysis (Boreen *et al.* 2003, Yang *et al.* 2014, Challis *et al.* 2014).

Among the compounds of interest to this study, the removal of sulfamethoxazole, trimethoprim and ciprofloxacin by direct UV photolysis have been previously studied in various aqueous matrices. For example, the removal for sulfamethoxazole, ciprofloxacin and trimethoprim was 98 %, 48 % and 7 % after 10 minutes of irradiation by 25W LP Hg lamp (De la Cruz *et al.* 2012). In most of the studies, poor removal of trimethoprim due to its relatively lower molar absorption coefficient and quantum yield has been reported (Guo *et al.* 2013, Carlson *et al.* 2015, Wu *et al.* 2016).

1.7.2 UV/Hydrogen peroxide advanced oxidation process

Advanced oxidation processes (AOPs) are defined as "processes that involve production of ample hydroxyl radicals to affect water purification" (Glaze *et al.* 1987). AOPs are used in water treatment to degrade recalcitrant compounds that are barely degraded by the convectional water treatment processes (O'Shea and Dionysiou 2012). The oxidation process relies on generation and utilization of free radicals and the most important is the hydroxyl radical (•OH) which has a high oxidation potential (2.8V) only lower than that of fluorine (3.03V) (Parsons 2004).

In the UV/H_2O_2 process, the H_2O_2 undergoes cleavage of the O–O when irradiated with UV light at a wavelength of less than 280 nm to generate two hydroxyl radicals per absorbed photon (Andreozzi 1999). One major limitation of photolysis of H_2O_2 is the low molar absorption coefficient of only 18.6 M^{-1} cm^{-1} at 254 nm implying that only a small fraction of incident light is utilized for radical formation (Oturan and Aaron 2014). Consequently, a high concentration of H_2O_2

is required in order to form sufficient hydroxyl radicals (Boal *et al.* 2015). However, at very high H_2O_2 concentration, H_2O_2 acts as hydroxyl radical scavenger limiting the efficiency of the radical formation (Boczkaj and Fernandes 2017).

The UV/H_2O_2 micropollutant removal process involves both hydroxyl radical reactions and direct photolysis and complete mineralization is possible with an extended UV irradiation and a high concentration of H_2O_2 (0turan and Aaron 2014). However, this is usually not economically feasible due to the high energy and oxidant demand (Ribeiro *et al.* 2015). Hence, UV/H_2O_2 process in most cases results to formation of oxidation products which are usually smaller in size, with high polarity and easily biodegradable (Klavarioti *et al.* 2009). Several studies have reported effective removal of multiple micropollutants by the UV/H_2O_2 process. For example, in the study by De la Cruz et al.(2012), the removal of trimethoprim by UV/H_2O_2 relative to direct UV photolysis after 10 minutes of irradiation with 25W LP Hg lamp was increased from 7 % to 66 % on the addition of 50 mg l^{-1} H_2O_2. The UV/H_2O_2 process has been applied in full scale water treatment for micropollutant removal and pathogen deactivation and several water treatment plants are in operation globally (Audenaert 2012). One such example is the PWN treatment plant in Andijk, Netherlands that was upgraded in 2004 with a treatment capacity of 4000 m^3 h^{-1}. The plant achieves 77 % removal of target organic micropollutants and efficient deactivation of pathogens (Kruithof *et al.* 2007).

1.7.3 UV/Chlorine advanced oxidation process

Recently, the use of UV/Cl_2 processes have been investigated and suggested to be effective in disinfection and degradation of recalcitrant organic micropollutants including APIs (Jin *et al.* 2011, Wang *et al.* 2016). Consequently, UV/Cl_2 process have been suggested as a possible alternative to the UV/H_2O_2 in the removal of organic micropollutants due to the higher HOCl UV absorbance and the lower radical scavenging relative to H_2O_2 (Watts and Linden 2007, Jin *et al.* 2011, Rosenfeldt *et al.* 2013, Fang *et al.* 2014, Kishimoto and Nishimura 2015). The UV quantum yields for hydroxyl radical formation of chlorine is higher than for H_2O_2 (Fang *et al.* 2014). However, the pH dependent aqueous chlorine species (HOCl and ClO^-) have different photochemical properties that influence the overall radical yield (Feng *et al.* 2007). HOCl is the more preferred species due to its higher quantum yield and scavenges the $HO^\bullet$ to a lesser extent (Rosenfeldt *et al.* 2013). Several comparative studies on the effectiveness of the removal of pharmaceuticals by UV/H_2O_2 and UV/Cl_2 processes have been made (Sichel *et al.* 2011, Yang *et al.* 2016). In their study, Sichel et al.(2011) compared the removal of some organic micropollutants by UV/Cl_2 and UV/H_2O_2 processes using a 40 W lamp, 5 mg l^{-1} H_2O_2 and 6 mg l^{-1} Cl_2 and the electrical consumption of 0.16 kWh m^{-3}. They found that the removal of sulfamethoxazole by UV/H_2O_2 was approximately 65 % and more than 90 % by UV/Cl_2 process. In their process evaluation, the energy

reduction for the UV/Cl_2 relative to UV/H_2O_2 was expected to be between 30–75 % and the overall cost reduction of 30–50 %. Despite the oxidation potential of the UV/Cl_2 reported from the bench scale and pilot experiments, the process is still developing and there is currently no reported full scale water treatment facility that incorporates the process.

2 OBJECTIVES OF THE STUDY

The main objective of this study was to determine the occurrence and mitigation of selected antibiotics and antiretroviral drugs in the urban hydrological cycles of Nairobi-Kenya, Jyväskylä-Finland and Lusaka-Zambia. The specific objectives were:

i. Develop a versatile and reliable multiresidue SPE-LC-ESI-MS/MS method for analysis of antiretroviral and antibiotic pharmaceutical compounds in aqueous samples (I, II).
ii. Determine the occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya (I).
iii. Determine the occurrence of selected drugs in source separated urine, surface water, wastewater and groundwater in Lusaka, Zambia (III).
iv. Evaluate post-treatment removal of antibiotics and antiretroviral drugs using direct UV photolysis as well as advanced oxidation processes (UV/Cl_2 and UV/H_2O_2) (IV).

3 MATERIALS AND METHODS

Here, a brief summary of the materials and methods is presented. More detailed information see I, II, III, IV.

3.1 Chemicals

The following pharmaceuticals including seven antibiotics and three antiretroviral drugs were selected in this study based on their consumption in Nairobi-Kenya, Lusaka-Zambia and Jyäskylä-Finland. The antiretroviral drugs were nevirapine (NVP), zidovudine (ZDV) and lamivudine (3TC) and antibiotics trimethoprim (TMP), sulfamethoxazole (SMX), ciprofloxacin (CIP), norfloxacin (NOR), tetracycline (TET), doxycycline (DOX) and amoxicillin (AMO).

3.2 Sampling

Data reported in this study is based on the samples collected from three urban hydrological cycles of Nairobi-Kenya, Lusaka-Zambia and Jyväskylä-Finland (I, II, II, IV). The sampling information is summarized in Table 3. After collection, all the samples were transported to the laboratory and stored at +4^{o}C waiting further processing within 48 hours (I, II) and one week (III, IV).

TABLE 3 Summary of the samples taken and the collection locations

	Location	Sample type	Sampling time	Number of samples	Extraction Volume (mL)
Nairobi-Kenya	Mathare River	SW-grab	October 2014	9	500
	Ngong River	SW-grab	October 2014	9	500
	Nairobi River	SW-grab	October 2014	18	500
	Athi River	SW-grab	October 2014	2	500
	Dandora WSPs	WWE-grab	October 2014	1	500
	JKUAT WSPs	WWE-grab	October 2014	1	500
Lusaka-Zambia	Chunga/Madimba residential areas	GW-grab	June 2016	26	200
	Chunga River	SW-grab	June 2016	2	200
	Matero WSPs	WWI/WWE-grab	June 2016	3	200
	Chunga/Madimba residential areas	SSU-grab	June 2016	10	20
Jyväskylä-Finland	Jyväskylä WWTP	WWI/WWE-composite 24h	September 2015, March 2016	2	200
	Jyväskylä WWTP	WWE-composite 24h	September 2016	Several 20 l canisters	10–100 mL
		Wastewater used in UV studies	October 2016 March 2017 June 2017		
	Lake Päijänne	SW-grab	March 2016	10	500 mL
	Lake Jyväsjärvi	SW-grab	October 2014	1	200–500 mL

GW: Groundwater; SSU: Source separated urine; SW: Surface water; WWE: Wastewater effluent; WWI: wastewater influent; WSPs: waste stabilization ponds

The selected APIs were extracted from the water using offline SPE with Oasis HLB cartridges 6 cc, 200 mg and 3cc, 60 mg (Waters, Milford, USA) (I, II, III, IV). The extraction volumes were as shown in Table 3 while the sample preparation procedures for the surface water, groundwater and wastewater are summarized in Fig. 2. Source separated urine was extracted with a similar procedure apart from the sample loading step which was done by passing 20 mL source separated urine slowly into Oasis HLB cartridges (3 mL 60 mg) using 20 mL luer syringes.

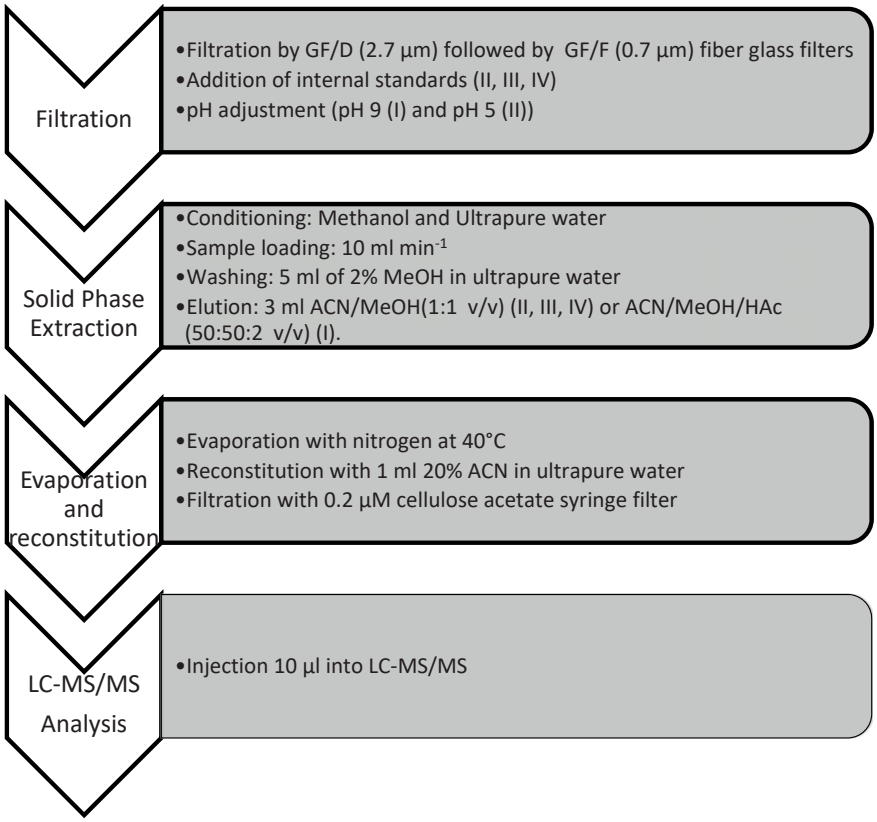

FIGURE 2 Sample preparation procedures for groundwater, surface water and wastewater.

3.3 Post-treatment removal of pharmaceuticals (IV)

The post-treatment removal of three antibiotics and three antiretrovirals was investigated in ultrapure water and wastewater effluents in batch process by recirculating 3 l water sample spiked with the target compounds at a flow rate of 0.5 l min^{-1} (see IV for detailed experimental set-up and procedure). UV irradiation experiments were performed using a 40 W low pressure mercury lamp (Aquada 2 UV system, Wedeco, Germany) emitting λ_{max}= 254 nm. Direct UV photolysis, UV/Cl_2 and UV/H_2O_2 processes in ultrapure water were conducted in the phosphate buffer at pH 7.5. Irradiation experiments in wastewater were conducted without buffering since the initial and final pH did not vary considerably (was within ±0.25 pH units). The initial concentration of the oxidants and APIs ranged from 4.1–85.2 mg l^{-1} and 1–20 µM, respectively. Samples were drawn at different

irradiation times and the residual oxidants quenched with sodium thiosulphate before further sample analysis procedures. The performance of UV photolysis, UV/Cl_2 and UV/H_2O_2 processes were evaluated based on the electrical energy per order of compound removal (E_{EO}), which is the energy required to remove or degrade 90 % of the target compound (Parsons 2004).

3.4 Analytical methods

3.4.1 SPE-LC-MS/MS optimization (I, II)

The APIs instrumental separation was performed on a Waters Alliance 2795 with a reversed phase C18 column (Waters XBridgeTM 3.5 µm, 2.1x100 mm with 3.5 µm, 2.1x10 mm guard column). The detection was achieved using Quattro Micro triple-quadrupole mass spectrometer in the positive electrospray ionization mode (ESI+) operated in the multiple reaction monitoring mode (MRM). Nitrogen was used as the desolvation and cone gas while argon was used as collision gas. The parameters optimized in the SPE-LC-MS/MS method are summarized in the scheme in Fig. 3. The ESI-MS/MS parameters were optimized by directly infusing individual APIs into the mass spectrometer using the syringe pump. The two strongest MS/MS transitions for each compound were respectively used for quantification and identification of the analytes.

The APIs were extracted from the aqueous matrix by offline SPE method. Oasis hydrophilic-lipophilic balance (HLB) cartridges, strong cation exchange (MCX) and strong anion exchange (MAX) cartridges were evaluated as SPE sorbents.

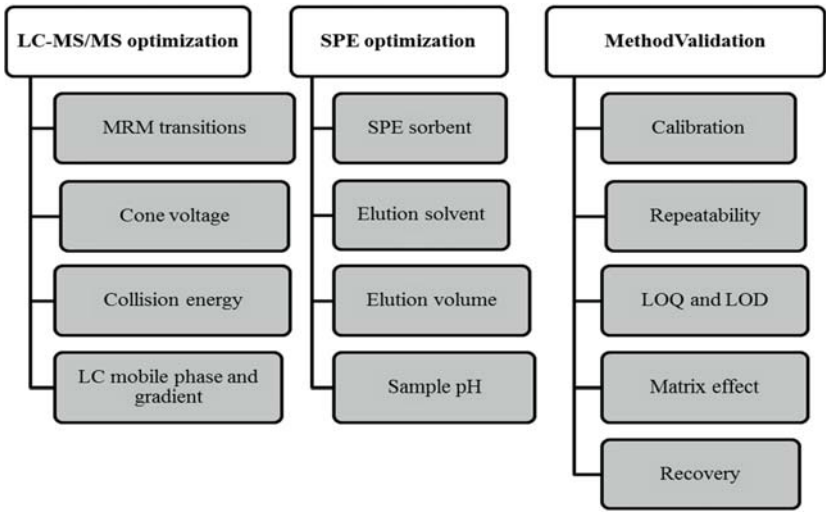

FIGURE 3 Scheme of various parameters optimized during the SPE-LC-MS/MS method development.

3.4.2 Matrix-matched and internal standard quantification (II)

One of the aims of this study was to develop an affordable, fast and accurate quantification method for the analysis of the target APIs in aqueous matrix with SPE-LC-ESI-MS/MS. Matrix-matched calibration was investigated as an alternative to the internal standard calibration method. To do so, seven compounds were simultaneously quantified in surface water and wastewater using calibration curves constructed using matrix-matched standards and corresponding isotopically labelled standards. The isotopically labelled internal standards included [$^{2}H_{8}$]-ciprofloxacin, [$^{2}H_{4}$]-sulfamethoxazole, [$^{13}C^{2}H_{3}$]-zidovudine, [$^{2}H_{4}$]-nevirapine, [$^{13}C^{2}H_{2}{}^{15}N_{2}$]-lamivudine and [$^{2}H_{9}$]-trimethoprim. [$^{2}H_{8}$]-Ciprofloxacin was used in the quantification of both ciprofloxacin and norfloxacin. The matrix-matched calibration curves were constructed by spiking varying concentrations of target analytes in surface water and subjecting the sample to the whole extraction process (I, II). The difference in the quantification results of the two calibrations was expressed as relative error with internal standard quantification as the reference (II).

4 RESULTS AND DISCUSSION

4.1 Analytical methods

4.1.1 LC-MS/MS optimization (I, II)

Various aspects of chromatographic sample separation and detection for aqueous sample analysis of the pharmaceuticals were optimized and the results are summarized in Tables 4 and 5. Gradient elution with acetonitrile and ultrapure water containing 0.1 % (v/v) formic acid as the mobile phases gave the best chromatographic elution of the analytes. The ESI-MS/MS parameters were optimized by direct infusion of the target compounds. The combination of chromatographic retention time, two MS/MS transitions and ion ratio between the quantification ion and qualification ion were used for analyte confirmation for the standards and the samples. A variation of within ±0.1 minutes and ±20 % for retention time and ion ratio were considered acceptable (European Commission 2002).

TABLE 4 LC-MS/MS instrumental conditions for the analysis of the pharmaceuticals

Parameter	Input
LC Column	C18 Waters XBridgeTM 3.5 µm, 2.1x100 mm with 3.5 µm, 2.1x10 mm guard column
Oven temperature	30°C
Mobile phase	A: Ultrapure water; B: Acetonitrile both containing 0.1 % (v/v) formic acid
Elution	0–2 min: constant ratio 80 % A and 20 % B; 2–5 min: gradient to 0 % A, 100 % B; 5–10 min: gradient to 80 % A and 20 % B; 10–12 min: constant ratio 80 % A and 20 % B. Column equilibration time 2 minutes before each injection.
Flow rate	0.25 ml min^{-1}
Injection Volume	10 µl
Desolvation gas	Nitrogen at flow rate 500 l h^{-1}
Cone gas	Nitrogen at flow rate 50 l h^{-1}
Collision gas	Argon pressure 2.8×10^{-4} mbar
Ionisation mode	ESI+

TABLE 5 Optimized MRM parameters for individual compounds: precursor, quantification and qualification ions and the corresponding cone voltage and collision energy; chromatographic retention time; ion ratio; instrumental LOQ and method LOQ in various matrices (wastewater influent (WWI), Wastewater effluent (WWE), Surface water (SW), Groundwater (GW).

Compound	Precursor ion (CV)[1]	Quantification ion (CE)[2]	Qualifier ion (CE)	RT[3]	Ion Ratio[4]	Instrumental LOQ (µg l^{-1})	Method LOQ (ng l^{-1})			
							SW/GW	WWI	WWE	SSU
CIP	332.1 (34)	288.0 (19)	314.1(19)	2.2	2.5	4	7	47	16	440
NOR	320.3 (30)	276.0 (18)	302.0 (25)	2.1	2.4	7	10	70	55	340
SMX	254.0 (28)	156.0 (18)	108.0 (17)	5.1	2.5	3	10	37	8	770
TMP	291.1 (34)	123.0 (19)	230.0 (19)	2.1	1.1	6	5	25	11	130
DOX	445.4 (30)	428.0 (25)	410.1(25)	5.8	5.3	8	44	19	17	880
TET	445.0 (25)	154.0 (25)	410.0 (20)	2.6	1.0	7	9	15	8	270
AMO	365.9 (15)	113.9 (19)	348.9 (9)	2.3	1.6	25	63	70	59	110
3TC	229.9 (17)	112.0 (18)	95.0 (29)	1.5	7.3	4	6	32	18	135
ZDV	268.2 (16)	127.0 (17)	110.1 (25)	2.3	9.2	23	44	56	40	1180
NVP	267.2 (40)	226.2 (29)	198.0 (29)	4.1	3.4	4	5	13	12	100
Internal standards										
[$^{2}H_{8}$]-CIP	340.4 (34)	296.1 (19)	322.1(19)	2.2	2.1					
[$^{2}H_{4}$]-SMX	258.2 (28)	159.9 (18)	111.9 (17)	5.1	3.2					
[$^{2}H_{9}$]-TMP	300.4 (34)	264.1 (26)	234.1(26)	2.1	1.1					
[$^{13}C^{2}H_{2}{}^{15}N_{2}$]-3TC	235.2 (17)	115.0 (18)	97.0 (29)	1.5	12.8					
[^{13}C $^{2}H_{3}$]-ZDV	272.1 (16)	130.9 (17)	113.9 (25)	2.3	13.6					
[$^{2}H_{4}$]-NVP	271.2 (40)	230.0 (29)	202.0 (29)	4.1	5.6					

[1]CV: Sample cone voltage

[2]CE: Collision energy in electron volts

[3]RT: Retention time in minutes

[4]Ratio between quantification and confirmation ion response

4.1.2 Solid phase extraction (I, II)

Due to a wide range of physico-chemical properties of the target pharmaceuticals, sorbent selection and extraction conditions that offer acceptable multiresidue SPE recoveries is essential. In this study, SPE was optimized with regard to sorbent, elution solvent, elution volume and sample pH using ultrapure water and surface water spiked with the target analytes. In sorbent selection, three polymeric sorbents including hydrophilic-lipophilic balance (Oasis HLB) strong cation exchange (Oasis MCX) and strong anion exchange (Oasis MAX) were tested using spiked ultrapure water (II). All the three sorbents have been widely used in extraction of pharmaceuticals in aqueous environmental samples (Pavlovic *et al.* 2007). The generic extraction protocols for the three sorbents were used to establish the sorbent with the highest multiresidue recovery for the target analytes (Arsenault 2012). The recoveries obtained are illustrated in Fig. 4 (II). The recovery for Oasis HLB, MCX and MAX ranged from 39–98 %, 14–98 % and 17–95 %, respectively. The ion exchange sorbents had at least one compound with significantly low recoveries leading to their exclusion in subsequent analysis. Generally, the average total recovery for all the compounds combined increased in the order Oasis MCX< MAX<HLB. Sample pH was evaluated using HLB and spiked surface water at 5 different pH levels **(II).** The optimum recoveries for most compounds were achieved at pH 5. Increasing or lowering the pH led to poor recoveries for some of the compounds. For example, 3TC is a basic compound with pKa of 4.3 implying that it is largely protonated at low pH resulting in poor retention by HLB. Other compounds that showed pronounced pH dependence included SMX, DOX, TET and AMO, which were better retained at lower pH. Overall, the optimum conditions for water extraction were achieved with Oasis HLB at pH 5 and elution with 4 mL ACN:MeOH 1:1 (v/v).

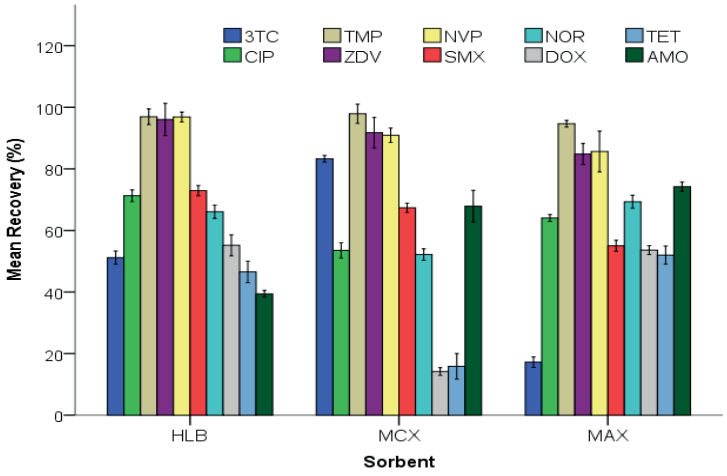

FIGURE 4 Percentage recoveries for various adsorbents, the error bars are ±1 SD.

4.1.3 Analytical method performance (I, II)

For detailed information on the determination of various analytical figures of merit see I, II**.** The parameters evaluated included, linearity, LOD, LOQ, repeatability, matrix effects and recovery. LOD and LOQ were for the various water matrices and source separated urine and were defined as the lowest concentration producing a signal-to-noise (S/N) ratio of 3 and 10 respectively (UNODC 2009). The results for the LOQ in 500 ml surface water, 200 ml wastewater and 20 ml source separated urine are presented in Table 5. The LOQ ranged from 5–63 ng l^{-1}, 13–70 ng l^{-1}, 8–59 ng l^{-1} and 135–1180 ng l^{-1} in surface water, wastewater influent, wastewater effluent and source separated urine, respectively. The LOQ achieved were considered sufficient for analysis of target analytes in aqueous environmental samples. The linearity of calibration curves was determined for both matrix-matched standards (11 points) and internal standards (7 points) at concentrations of between 10 ng l^{-1} – 5000 ng l^{-1} and 10 µg l^{-1} – 1000 µg l^{-1}, respectively. For all the compounds, the coefficients of determination were >0.99 over the whole calibration range indicating a linear fit. This was confirmed construction of residual plots from the regression analysis that showed random distribution about zero with relative residual values of < ±10 % (González and Herrador 2007).

Matrix effects were evaluated by comparing the signal of pure standards dissolved in the mobile phase to that of matrix-matched standards. ME of more than 15 % was considered significant (Yilmaz *et al.* 2009), as a result there was no significant ME for SMX, TMP, 3TC and NVP (II). ZDV had a signal enhancement of 33 % while AMO, CIP, DOX and TET had signal suppression of 17 %, 20 %, 26 % and 30 %, respectively. The ME was considered not to have significantly affected the accuracy of the quantification results since the use of matrix-matched and isotopically labeled internal standards has been found to largely compensate for matrix effects (Gosetti *et al.* 2010, Trufelli *et al.* 2011, Quintana *et al.* 2014).

Method precision was evaluated by computing interday relative standard deviation (RSD) of the responses generated by extraction of 500 ml surface water spiked at 0.1 µg l^{-1}, 0.5 µg l^{-1} and 2 µg l^{-1} with the target analytes. The RSDs for all the compounds were < 15 % indicating that the analytical method was repeatable and within acceptable limits (Tiwari and Tiwari 2010).

The recovery in trace environmental analysis is essential in establishing the efficiency of the analyte extraction process. In this study, the recoveries in surface water, wastewater effluents, wastewater influents and source separated urine were evaluated based on the methods described by IUPAC (Thompson *et al.* 1999) **(**II**).** The results are summarized in Table 6. The absolute recoveries for most compounds were above 50 % apart from 3TC, AMO, TET and DOX in wastewaters. However, the use of matrix-matched standards that were subjected to extraction process and internal standards compensated for the analyte losses. The recoveries for a majority of compounds in our study were

comparable with other previous studies surface water, wastewater influents and effluents as shown in Table 6.

TABLE 6 Mean absolute (%) recoveries of selected antibiotics and antiretroviral drugs in surface water (SW), wastewater effluents and wastewater influents by Oasis HLB together with some corresponding literature data

Analyte	SW		WWTP Effluent		WWTP Influent		Ref
	This study*	Literature	This study	Literature	This study	Literature	
3TC	51.7 (1.5)	20	45.3 (3.2)	70	41.2 (4.5)	30	1,2
ZDV	109.6 (4.5)	57	114.8 (6.2)	90	116.1 (2.9)	95	1,2
NVP	91.9 (2.3)	74	95.5 (3.7)	90	97.5 (4.4)	100	1,2
CIP	80.2 (2.2)	44–93.6	69.3 (2.7)	72	71.1 (3.2)	32	3–5
TMP	99.2 (2.2)	96.7	90.7 (5.2)	98	93.6 (1.5)	120	2,4–6
SMX	80.5 (2.6)	54.9–76.2	70.0 (4.6)	65–85	68.5 (5.3)	80	2,4–7
NOR	75.2 (4.0)	27	66.8 (2.2)	53–106	65.6 (4.6)	41	3,5,6
DOX	54.4 (2.7)	65	48.1 (3.5)	82	43.1 (7.8)	74.5	8–10
TET	52.4 (1.8)	72	42.5 (1.9)	91	40.8 (4.0)	64.2	8–10
AMO	52.9 (2.8)	No data	44.8 (4.4)	<10	40.9 (6.1)	<10	1

* Mean (standard deviation)

References: 1.Vergeynst *et al.* (2015); 2. Wood *et al.* (2015); 3. Rao *et al.* (2008) 4. Conley *et al.* (2008); 5. Vieno *et al.* (2006); 6. Renew and Huang (2004); 7. Zhang and Zhou (2007); 8. Liang *et al.* (2016); 9. Segura *et al.* (2015); 10. Tong *et al.* (2014)

4.1.4 Matrix-matched and internal standard quantification (II)

In this study, the feasibility of using matrix-matched calibration as an alternative to internal standard method without compromising on the accuracy of the analytical results was explored. The matrix-matched calibration curves were all constructed using surface water with no detectable quantities of target analytes since blank municipal wastewaters samples are not practically available. The proposed matrix-matched calibration procedure aimed at correcting for both matrix effects and loses associated with sample preparation (II). To do so, quantification of target analytes was done by the two calibration methods with the internal standard method as the reference. Deviation of matrix-matched quantification results from internal standard method was treated as relative error. The comparative results for quantification in surface water, wastewater effluents and wastewater influents are presented in Fig. 5 of II. In general, the results indicated a good agreement in surface water (±7 %) for all the compounds and underestimation in wastewater effluents and influents (of up to –20 %). The underestimation is a direct implication of the difference in the recovery between the three water matrices. The underestimation was within the acceptable limits –30 % – +20 % (Yang *et al.* 2005) and the proposed method can be used for quantification of the target compounds in the three water

matrices. However, in order to guarantee the integrity of the results while using matrix-matched calibration and in absence of isotopically labelled internal standards, a set of quality control samples can simultaneously be quantified with the more laborious method of standard addition.

4.2 Pharmaceuticals in the WWTPs (I, II, III)

Non-conventional (wastewater stabilization ponds) and conventional (activated sludge) WWTPs located in Nairobi-Kenya, Lusaka-Zambia and Jyäskylä-Finland, respectively were considered in this study (I, II, III). Dandora WSPs in Nairobi-Kenya receive approximately 80,000 m^3 day^{-1} of both domestic and industrial wastewaters (Pearson *et al.* 1996, Musyoki *et al.* 2013). Matero WSPs in Lusaka, Zambia receives domestic wastewater and has a maximum theoretical capacity of 7,100 m^3 day^{-1} (Wamukwamba and Share 2001). While the Jyväskylä activated sludge WWTP treats on average 37,000 m^3 day^{-1} of wastewater (Jyväskylän Seudun Puhdistamo Oy 2016). Results of the concentrations of the APIs in the three WWTPs are presented in Table 7. All the target compounds were detected in the analyzed wastewater samples with the highest concentrations in Lusaka followed by Nairobi while Jyväskylä had significantly lower concentrations. The mean effluent concentrations ranged from 80 ng l^{-1} for norfloxacin to 118970 ng l^{-1} for lamivudine in Lusaka, 61 ng l^{-1} for trimethoprim to 3940 ng l^{-1} for lamivudine in Nairobi and 8 ng l^{-1} for nevirapine to 537 ng l^{-1} for trimethoprim in Jyväskylä.

TABLE 7 Mean (±SD) concentrations in ng l-1 of selected antibiotics and antiretroviral drugs in WWTPs in Lusaka-Zambia, Nairobi-Kenya and Jyväskylä-Finland.

	Lusaka, Zambia June 2016		Nairobi, Kenya October 2014		Jyväskylä, Finland March, 2016	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
SMX	33300 (1890)	30040 (3420)	na	1940 (86)	220 (8)	111 (12)
CIP	740 (80)	230 (30)	na	66 (13)	429 (36)	77 (9)
NOR	100 (20)	80 (20)	na	na	242 (34)	98 (10)
DOX	4490 (810)	5280 (1190)	na	na	54 (6)	16 (1)
TET	220 (20)	4590 (540)	na	na	44 (5)	28 (2)
AMO	3270 (690)	5580 (1880)	na	na	116 (24)	69 (4)
TMP	32670 (1570)	1770 (160)	na	67 (6)	570 (30)	537 (16)
ZDV	66590 (4650)	37140 (2560)	na	513 (60)	62 (8)	37 (6)
3TC	118970 (9450)	55760 (5480)	na	3940 (352)	55 (3)	22 (2)
NVP	680 (60)	1720 (250)	na	1320 (39)	19 (4)	8 (2)

na: not analysed

Significantly high concentration of antibiotics and antiretroviral drugs was detected in Matero WSPs effluent in Lusaka, Zambia. This was attributed to high disease prevalence especially HIV/AIDS and poor performance of the WSPs that results from factors such as inadequate hydraulic retention time due to sludge accumulation amongst others (Brown *et al.* 2012). The adult HIV/AIDS prevalence Zambia as of 2015 was 12.9 % translating to about 1.2 million people of whom 63 % were under antiretroviral therapy ingesting a minimum daily dose comprising of at least three antiviral drugs and co-trimoxazole (sulfamethoxazole/trimethoprim in the ratio 5:1) (United Nations Programme on HIV/AIDS 2016, World Health Organization 2016a). As shown in Table 7, the concentration most anti-HIV medication is several times higher than the rest of the antibiotics. The concentrations of all anti-HIV medication in the Matero wastewater effluents apart from nevirapine were significantly higher than reported elsewhere. For example, the maximum concentration of lamivudine, zidovudine, nevirapine, sulfamethoxazole and trimethoprim were 31070 ng l^{-1}, 110 ng l^{-1}, 2080 ng l^{-1}, 4090 ng l^{-1} and 150 ng l^{-1}, respectively in Nairobi and Kisumu-Kenya (K'oreje *et al.* 2016).

Although the concentrations of antibiotics and antiretroviral drugs were lower in Nairobi-Kenya (Dandora WSPs) compared with Lusaka (Matero WSPs), similar occurrence pattern was observed, with the antretroviral drug lamivudine having the highest concentration of 3940 ng l^{-1}. By 2015, Kenya had about 1.6 million (5.4 % of the adult population) people living with HIV/AIDs and 64 % under antiretroviral therapy (AVERT 2016, United Nations Programme on HIV/AIDS 2017). This justifies the high concentrations measured for lamivudine, nevirapine and sulfamethoxazole in Nairobi. The effluent concentrations measured in Dandora WSPs were in the same order of magnitude for most of the compounds as previously reported in the other developing countries urban hydrological cycles apart from ciprofloxacin in South Africa with a concentration of 14100 ng l^{-1} (Agunbiade and Moodley

2016). For instance, the effluent concentration was 1600 ng l^{-1} for sulfamethoxazole and 160 ng l^{-1} for trimethoprim in South Africa (Agunbiade and Moodley 2014, Matongo *et al.* 2015b). Comparing the results with developed countries, the concentrations measured in the present study were at least one order of magnitude higher (Table 2). Although the influent concentration for Dandora WSPs was not measured, the hydraulic retention times of 60–90 days and near optimal performance of the stabilization ponds in the tropical environment leads to multiple physico-chemical removal pathways such as biodegradation, photodegradation, and adsorption to sludge. As shown in Table 2, the results of K'oreje *et al.* (2016) for Dandora WSPs confirms these assumptions since significantly lower concentrations were measured in effluents relative to influent for compounds as trimethoprim, sulfamethoxazole, lamivudine and zidovudine.

The concentrations of most of the antibiotics and antiretroviral drugs in Jyväskylä WWTP effluents were within the same range or lower than those previously reported in the WWTPs in Europe (Table 2 and papers I–III). For example, the concentration ranged from <LOQ–130 ng l^{-1} and <LOQ–110 ng l^{-1} for ciprofloxacin and norfloxacin, respectively in Finland (Vieno *et al.* 2007b); <LOQ–304 ng l^{-1} for sulfamethoxazole, <LOQ–1340 ng l^{-1} for trimethoprim and <LOQ–915 ng l^{-1} for doxycycline in Sweden (Lindberg *et al.* 2005); 98.2–564 ng l^{-1} for zidovudine, 7.2–32.1 ng l^{-1} for nevirapine and <LOQ–187 ng l^{-1} for amoxicillin in Germany (Prasse *et al.* 2010, Rossmann *et al.* 2014). The significantly lower concentration in Jyväskylä relative to Nairobi and Lusaka can be attributed to lower disease prevalence, low population density, higher dilution due to higher per capita water use as well as availability of efficient conventional wastewater treatment process. The removal of the antibiotics and antiretroviral drugs by Jyväskylä WWTP was more than 40% for most of the compounds apart from trimethoprim which most probably resulted from desorption from the particulate matter as well as possible deconjugation of metabolites during the treatment process as previously reported (Gulkowska *et al.* 2008).

4.3 Pharmaceuticals in surface waters (I, II, III)

Surface water samples from Nairobi-Kenya, Lusaka-Zambia and Jyväskylä-Finland were collected and analysed for the target analytes. There were a total of 38 surface water sampling locations from Nairobi River Basin while Jyväskylä and Lusaka had two sampling points each in Lake Päijänne and Chunga River, respectively (I–III). Table 8 broadly compares the occurrence of the pharmaceuticals in the three sampling areas.

Occurrence in Nairobi River Basin (I): Six compounds were analysed in Nairobi River Basin along the three main Rivers (Mathare, Nairobi and Ngong Rivers) that traverse the city and the Athi River to which the three rivers discharge their waters (I). The rivers run through several informal settlements

and sampling was done in the upstream, midstream and downstream of the informal settlements and the WWTP effluent discharge points. The concentrations for individual pharmaceuticals ranged from 510 ng l^{-1} (median 105 ng l^{-1}) measured for ciprofloxacin to 13800 ng l^{-1} (median 1830 ng l^{-1} for sulfamethoxazole. Sulfamethoxazole and ciprofloxacin had the highest and lowest detection frequency of 97.4 % and 60.5 %, respectively. The maximum concentrations for all the compounds were measured in river sections bordering the informal settlements along the sampled hydrological transect. The informal settlements in Nairobi host approximately 65 % of the city's population and occupy a land area of <10 % (Corburn and Karanja 2016). Consequently, the settlements are overcrowded and lack basic infrastructure such as access to clean water, sanitation and drainage (National Council for Population and Development 2013, African Population and Health Research Center 2014, Wang *et al.* 2014). The harsh socioeconomic conditions enhance the proliferation of diseases such as HIV/AIDS, tuberculosis and diarrhea (Kabiru *et al.* 2011, Corburn and Hildebrand 2015). A large proportion of raw waste generated from the informal settlements is directly discharged into surface water. The HIV/AIDS prevalence in the informal settlements is about 12 %, much higher than the national adult prevalence of 5.4 % (Corburn and Hildebrand 2015, AVERT 2016) and with a national antiretroviral coverage of 65 %, the amounts of active pharmaceuticals associated with HIV treatment directly discharged into the local rivers via untreated domestic waste is quite abundant. The high concentrations of the antibiotics and antiretroviral drugs detected in this study are within the same range or lower compared to a more recent study by K'oreje *et al.* (2016) in the same region (Table 2). The concentration ranged from 20–39000 ng l^{-1} for sulfamethoxazole, 30–7000 ng l^{-1} for trimethoprim, <LOQ–167000 ng l^{-1} for lamivudine, <LOQ–17000 ng l^{-1} for zidovudine and 510–5620 ng l^{-1} for nevirapine. Similar concentrations have been detected elsewhere within the sub-Saharan Africa. For example, the maximum concentration was 53828 ng l^{-1}, and 10568 ng l^{-1} for sulfamethoxazole in Mozambique and South Africa, respectively (Segura *et al.* 2015); 14300 ng l^{-1} for ciprofloxacin in South Africa (Agunbiade and Moodley 2016); 6323 ng l^{-1} and 1374 ng l^{-1} for trimethoprim in Mozambique and Ghana (Segura *et al.* 2015).

The concentrations detected in this region are several orders of magnitude higher than in developed countries as illustrated in (Table 2). Segura *et al.* (2015) examined the relationship between income inequality between countries and occurrence of antibiotics in surface waters. They identified the absence or inadequate wastewater collection and treatment in low income countries as the main reason for the significantly higher antibiotic concentration in surface waters. In addition, other factors such as higher infectious disease prevalence and availability of cheap over-the-counter antibiotics in the low income countries amplify the situation.

Occurrence in Chunga River (III): A total of seven antibiotics and three antiretroviral drugs were analyzed Chunga River-Lusaka. Lusaka and Nairobi have similar sociodemographic and infrastructure challenges. However, Lusaka has higher HIV prevalence than in Nairobi based on the national HIV

prevalence (as discussed in section 4.2). The detected concentrations in the two sampled contaminated sections of surface are shown in Table 8. Apart from norfloxacin which was not detected in any of the two sites, the rest of the compounds were detected at significantly high concentrations ranging from 400 ng l^{-1} for ciprofloxacin to 49700 ng l^{-1} for lamivudine. In addition to the similar detection pattern for the six compounds analyzed both in Nairobi and Lusaka, high concentrations were measured for doxycycline, tetracycline and amoxicillin in Chunga River. The maximum concentration was 3260 ng l^{-1}, 4220 ng l^{-1} and 3410 ng l^{-1} for doxycycline, tetracycline and amoxicillin, respectively. The concentrations were much higher than measured elsewhere. For instance, the maximum surface water concentration was 400 ng l^{-1} for doxycycline in Australia (Watkinson *et al.* 2009); 2800 ng l^{-1} and 465 ng l^{-1} for tetracycline in South Africa and Ghana, respectively (Agunbiade and Moodley 2014, Segura *et al.* 2015); 245 ng l^{-1} for amoxicillin in the UK (Kasprzyk-Hordern *et al.* 2007). The high concentrations were attributed to direct discharge of untreated domestic waste from the adjacent informal settlement of Madimba and discharges from the WWTPs.

TABLE 8 Mean (±SD where available) concentrations (ng l^{-1}) of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Chunga River and Lake Päijanne surface waters.

	Nairobi River Basin October 2014			Chunga River-Lusaka June 2016		Lake Päijänne March, 2016	
	Maximum	Median	Detection Frequency (%)	Site A	Site B	Site A	Site B
SMX	13800 (710)	1830	97.4	11800 (1200)	7810 (740)	26 (4)	14 (3)
CIP	510 (50)	105	60.5	400 (90)	540 (70)	52 (7)	34 (8)
NOR	na	na	na	nd	nd	54 (19)	22 (5)
DOX	na	na	na	2730 (610)	3260 (590)	nd[b]	nd
TET	na	na	na	2200 (700)	4220 (740)	nd	nd
AMO	na	na	na	2500 (660)	3410 (440)	nd	nd
TMP	2560 (400)	375	76.3	2410 (20)	510 (50)	15 (5)	10 (4)
ZDV	7680 (270)	680	86.8	1280 (400)	9670 (1290)	nd	nd
3TC	5430 (610)	1160	84.2	49700 (4000)	42630 (3660)	12 (2)	nd
NVP	4860 (194)	830	94.7	210 (30)	220 (30)	nd	nd

na: not analysed; nd: not detected

Occurrence in Lake Päijänne (II): The concentration of ten pharmaceuticals including seven antibiotics and three antiretroviral drugs were measured from two sampling sites at Hämeenlahti (A) and Konjakki (B) which are approximately 200 m and 4 km, respectively from Jyväskylä WWTP discharge point. The measured concentrations are shown in Table 8. Five of the ten

compounds were detected in the surface waters with a maximum concentration of 54 ng l^{-1} measured for norfloxacin. Of the two sampling points, Hämeenlahti (A) had a relatively higher concentration of the detected compounds owing to its close proximity to the discharge point. The concentrations measured in Lake Päijänne were comparable to previous studies in high income countries but much lower than in low and middle income countries as observed in Nairobi and Lusaka. For example, the maximum concentration was 62 ng l^{-1} for trimethoprim and 114 ng l^{-1} for sulfamethoxazole in Germany (Burke *et al.* 2016); 36 ng l^{-1} for ciprofloxacin in Finland (Vieno *et al.* 2007a); 163 ng l^{-1} for norfloxacin for France (Tamtam *et al.* 2008). In general, the concentrations of target pharmaceuticals detected in Lake Päijänne are quite low and ecological and health risks are not anticipated. However, precautionary measures at the point of release should continuously be undertaken since the long-term exposures to low doses of multiple pharmaceuticals remain poorly understood (Touraud *et al.* 2011, Li *et al.* 2014, Chèvre 2014).

4.4 Pharmaceuticals in groundwater (III)

The occurrence of 7 antibiotics and 3 antiretroviral drugs was determined from wells in Chunga area in Lusaka (III). Groundwater is a crucial resource in the study area since it is a major source for domestic water with boiling before use as one of the main treatment options (Nachiyunde *et al.* 2013). In addition, the area has a high water table, is densely populated and the local population mostly rely on pit latrines for sanitation making the groundwater vulnerable to contamination (Wamukwamba and Share 2001, Nkhuwa 2006, African Development Bank 2015). As discussed in sections 4.2, 4.3 and 4.5, the wastewater, surface water and source separated urine are highly contaminated by the majority of analysed antibiotics and antiretroviral drugs and major concerns on significant contamination groundwater arise. The detection frequency and concentrations for each compound are presented in Table 9.

TABLE 9 Summary of the detection frequency (%) and concentrations for the selected antibiotics and antiretroviral drugs in the Chunga-Lusaka groundwater samples in ng l^{-1}.

	Detection Frequency (n=26)	Range	Median
TMP	34.6	nd–140	60
CIP	19.2	nd–150	90
SMX	42.3	nd–660	100
AMO	11.5	nd–880	760
TET	0	nd	nd
NOR	0	nd	nd
DOX	0	nd	nd
3TC	0	nd	nd
ZDV	0	nd	nd
NVP	38.5	nd–410	150

nd: not detected

Five out of the ten analysed compounds were detected in the sampled groundwater with detection frequency ranging from 11.5 % for amoxicillin to 42.3 % for sulfamethoxazole in 17 shallow water wells and 2 boreholes. The lower detection frequency of the pharmaceuticals in the groundwater could be attributed to the sorption of the compounds by soil through multiple interaction mechanisms based on the soil properties and the pharmaceutical's molecular structure and physico-chemical properties (Thiele-Bruhn *et al.* 2004, Lees *et al.* 2016). Several experimental distribution coefficients (K_d) for antibiotics have been reported previously. Tetracyclines (e.g. tetracycline and doxycycline), fluoroquinolones (*e.g.* ciprofloxacin and norfloxacin) and trimethoprim have high distribution coefficients (K_d) in soils and are largely considered immobile while sulphonamides (e.g. sulfamethoxazole) and amoxicillin have relatively low a K_d and hence higher mobility (Thiele-Bruhn 2003, Germer and Sinar 2010, Kim *et al.* 2012, Kodešová *et al.* 2015, Deng *et al.* 2016).

Based on the aforementioned sorption properties, the relatively higher detection frequency for sulfamethoxazole amongst the compounds analysed in this study can hence be attributed to its higher mobility. In addition, it is hydrolytically stable and hard to degrade (Germer and Sinar 2010, Deng *et al.* 2016). However, the concentrations are much lower than measured in the surface water, wastewater and source separated urine. Similarly, the relatively immobile compounds such as ciprofloxacin and trimethoprim were detected at much lower frequency and concentrations while doxycycline, tetracycline and norfloxacin were not detected. Amoxicillin is one of the most commonly prescribed antibiotics and based on its sorption properties, migration to groundwater is highly expected. However, it has high potential to undergo hydrolysis in the chemically susceptible β- lactam ring (Cha *et al.* 2006) and thus detected only in three wells but at a higher concentration relative to the other

compounds which could possibly be as a result of contamination from adjacent pit latrines.

The concentrations of antibiotics detected in groundwater were comparable with other previous studies within one order of magnitude (Table 2). For example, the concentration ranged from <LOQ–443 ng l^{-1} for ciprofloxacin in Spain (López-Serna *et al.* 2013); <LOQ–113 ng l^{-1} for sulfamethoxazole in the USA (Schaider *et al.* 2014); 20–60 ng l^{-1} and for trimethoprim Kenya (K'oreje *et al.* 2016).

Among the three analysed antiretroviral drugs, only nevirapine was detected in the groundwater with a detection frequency and concentration range of 38.5 % and <LOQ–410 ng l^{-1}, respectively. The detection of nevirapine can be directly attributed to its non-biodegrability leading to persistence in the environment (Vaňková 2010, Jain *et al.* 2013). Lack of detection especially for lamivudine and zidovudine which were abundant in the wastewater, surface water and source separated urine was rather surprising since they are relatively hydrophilic. However, lamivudine is a basic compound that will tend to be sorbed in the soils of the study area that have an acidic pH of between 4.02–5.56 (Chabala *et al.* 2014). Similarly, zidovudine is a zwitterionic compound that will tend to be sorbed on both cationic and anionic surfaces. In addition, several other sorption and desorption mechanisms are possible which in turn require a more detailed soil-pharmaceutical interaction analysis including experimental studies for an all-inclusive justification. In a similar study by K'oreje *et al.* (2016) in Kenya, the concentrations of nevirapine and zidovudine in shallow water wells ranged from 20–1600 ng l^{-1} and 20–30 ng l^{-1} against a maximum concentration of 5620 ng l^{-1} and 17000 ng l^{-1}, respectively in surface water within the same vicinity. Based on the present study and study by K'oreje *et al.* (2016), it is clear that despite the high concentration in the surface water and wastewater, the groundwater contamination by the target analytes is minimal.

However, since the groundwater in the study area is directly consumed with little or no pre-treatment, the concentrations of antibiotics and antiretroviral drugs were much higher than reported in treated drinking water, usually well below 50 ng l^{-1} (World Health Organization 2011). In addition, the low concentration or absence of the parent antibiotic or antiretroviral compounds in this study has been cautiously interpreted since the analytical methods employed only measured the parent compounds. Even minor transformation of the parent compound which sometimes leave the pharmaceutical compounds active and whose risks cannot be ignored have not been accounted for. Consequently, to safeguard the human health and the environment comprehensive fate studies in highly contaminated environments as is the case in the study area are necessary.

4.5 Pharmaceuticals in source separated urine (III)

In order to establish the possible primary source of antibiotics and antiretroviral drugs in Chunga area aquatic environment, the concentration of pharmaceuticals in the source separated urine was determined from the available urine diverting dry toilets. Fig. 5 shows the maximum and median concentrations of detected antibiotics and antiretroviral in source separated urine. Zidovudine was not detected in any of the urine samples. The maximum concentrations of the other pharmaceuticals ranged from 2.8 µg l^{-1} for tetracycline to 12800 µg l^{-1} for trimethoprim. The detection frequency, range, median and mean concentrations are presented in Table 4 of III.

The concentrations of sulfamethoxazole, trimethoprim, lamivudine, ciprofloxacin, amoxicillin and norfloxacin were at least 2–4 orders of magnitude higher than in the surface water and wastewater samples from the same area. The detection pattern was quite similar to surface water and wastewater where trimethoprim, sulfamethoxazole and lamivudine were measured at elevated concentrations. In a similar study by Bischel *et al.* (2015) in eThekwini-South Africa the concentration for sulfamethoxazole and trimethoprim were 6800 µg l^{-1} and 1300 µg l^{-1}, respectively. Sulfamethoxazole was within the same order of magnitude as in the present study while trimethoprim was one order of magnitude lower. The high concentration of the target compounds in the source separated urine signifies that the role of source separation as a critical barrier to environmental contamination cannot be overlooked.

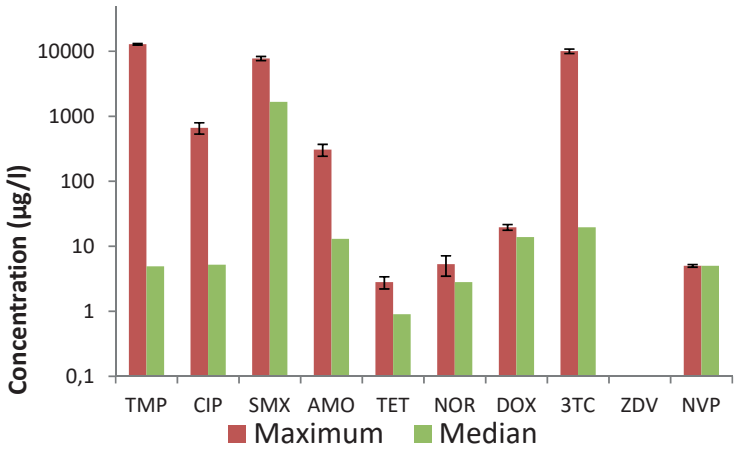

FIGURE 5 Maximum and median concentrations in µg l^{-1} of pharmaceuticals detected in source separated urine. The Y-axis is displayed in base 10 logarithimic scale.

From the results in this study, it is quite clear that urine source separation can be an important barrier in prevention of environmental contamination. Sequestering nutrients such as phosphorus and nitrogen through urine source separation can considerably reduce the cost of wastewater treatment and eutrophication in surface waters receiving discharges containing high nutrient concentration (Wilsenach and Van Loosdrecht 2003, Libralato *et al.* 2012).

To date, the main focus on ecological sanitation with urine source separation has been centred on pathogen deactivation and nutrient recycling. To deactivate disease causing pathogens, the World Health Organisation recommends storage of the urine for 6 months at 20°C (World Health Organization 2006b). However, recent studies have shown that pharmaceuticals are not sufficiently degraded during such storage. For example, Jaatinen *et al.* (2016) studied the removal of 4 antibiotics and 3 antiretroviral drugs in spiked source separated urine and the removal of 5 of the compounds was less than 50 % after six months of storage. A similar study by Bischel *et al.* (2015) that set to assess the presence of disease causing pathogens and pharmaceutical residues in eThekwini-South Africa source separated urine found that 11 out of the 12 monitored pharmaceutical were very stable to degradation.

The insufficient removal of pharmaceuticals during urine storage poses a major challenge to its direct application as a fertilizer in agriculture. Possible implications include the uptake of pharmaceuticals by the crops and translocation/accumulation in edible parts as well as qualitative and quantitative effects on soil microbial community (Kümmerer 2003, Li *et al.* 2013). There are several treatment options available for source separated urine aimed at either nutrient recycling or micropollutant removal that have previously been proposed (Escher *et al.* 2006, Maurer *et al.* 2006, Pronk and Koné 2009, Ledezma *et al.* 2015). However, majority of the processes have not been tested beyond the laboratory or pilot scale (Udert *et al.* 2015). Escher *et al.* (2006) investigated the removal efficiency of pharmaceuticals and hormones in source separated urine by ozonation, struvite precipitation, nanofiltration and electrodialysis. Both ozonation and struvite precipitation were able to remove more than 99 % of the pharmaceuticals. In the struvite precipitation, the residual pharmaceuticals were concentrated in the filtrate and nutrients recovered in the struvite. However, nutrients are usually not recovered if ozonation is the primary treatment process and thus, ozonation is ideal as a secondary treatment option for the pharmaceutical residues after nutrient recovery. Urine source separation can thus offer an opportunity to utilize advanced treatment processes for pharmaceutical residue removal and nutrient recycling with high efficiency that would otherwise not be economically feasible in the municipal wastewater treatment plants.

In developing countries, informal settlements and peri-urban areas are characterized by un-improved onsite sanitation systems that discharge human excreta directly into the environment. The high population density, water scarcity, lack of space and cost of infrastructure make building of centralized waterborne sewage networks an impractical option for improving sanitation.

Decentralized sanitation systems incorporating source separation with local treatment (onsite or close to the source) appears to be one of the most viable options (Libralato *et al.* 2012). The faecal matter can be efficiently digested anaerobically for energy production in the absence of toxic ammonia or dried while the separated urine can undergo advanced treatment for nutrient recovery and micropollutant degradation (Schouten and Mathenge 2010, Udert *et al.* 2016). The high population density in the informal settlements with shared toilets minimizes the distance between collection units favouring communal treatment centres.

4.6 Post-treatment removal of pharmaceuticals (IV)

4.6.1 Removal of pharmaceuticals by direct UV photolysis (IV)

The removal of compounds by direct UV photolysis is normally dependent on the energy of the incident radiation and the compound's molar extinction coefficients and quantum yields (Malley 2008, Wu and Linden 2008). Sulfamethoxazole and ciprofloxacin which have high molar absorption coefficients were efficiently degraded by direct UV photolysis by more than 90 % in 30 minutes. Similarly, even though the molar absorption for zidovudine is relatively low (7560 M^{-1} cm^{-1}), it was efficiently removed by direct UV photolysis probably due to a higher quantum yield. On the contrary, the removal of trimethoprim, nevirapine and lamivudine were significantly lower at 12.2 %, 13.4 % and 48.1 %, respectively, which was attributed to lower molar extinction coefficients and possibly lower quantum yields. Trimethoprim has been previously shown to have a quantum yield of 0.00118 mol $Einstein^{-1}$ much lower than sulfamethoxazole and ciprofloxacin with 0.033 mol $Einstein^{-1}$ and 0.0442 mol $Einstein^{-1}$, respectively (Guo *et al.* 2013, Carlson *et al.* 2015, Wu *et al.* 2016). Generally, the removal of sulfamethoxazole, ciprofloxacin and trimethoprim by direct UV photolysis was consistent with what has been reported in literature. For example, Kim *et al.* (2009) reported a removal efficiency of more than 90 % for sulfamethoxazole and approximately 10 % trimethoprim by direct UV photolysis of wastewater effluent using a low pressure mercury lamp (65 W) for 5 minutes. In a similar study, Yang *et al.* (2016) reported approximately 50 % reduction in CIP concentration in wastewater effluent when irradiated with a low pressure mercury lamp (10 W) for 3 minutes.

4.6.2 Effect of H_2O_2 and Cl_2 in UV post-treatment of wastewater (IV)

Addition of H_2O_2 and Cl_2 led to enhanced degradation of the target compounds especially for trimethoprim, nevirapine and lamivudine which had poor removal by direct UV photolysis. This was due to the oxidation by the highly reactive and non-selective hydroxyl radicals, chlorine radicals and direct

oxidation by chlorine. The effect of addition of the oxidants is shown in Fig. 6. With UV/H_2O_2 process, the removal of trimethoprim, nevirapine and lamivudine after 30 minutes of increased to 62.9 %, 52.9 % and 72.2 % respectively. The effect of addition of H_2O_2 in the removal of sulfamethoxazole, ciprofloxacin and trimethoprim by UV has been reported in literature. For instance, De la Cruz *et al.* (2012) found that sulfamethoxazole, ciprofloxacin and trimethoprim were entirely degraded in wastewater effluents by low pressure 25 W mercury lamp and 50 mg l^{-1} H_2O_2 after 30 minutes irradiation. As in the UV/H_2O_2 process, the UV/Cl_2 process showed increased removal of trimethoprim, nevirapine and lamivudine to 35 %, 20.8 % and 77.4 %, respectively. However, the removal by UV/Cl_2 process was much lower than UV/H_2O_2 but higher than direct UV photolysis. This was contrary to our expectations since the UV/Cl_2 has been proposed by several authors as possible alternative to UV/H_2O_2 (Sichel *et al.* 2011, Wang *et al.* 2015, Yang *et al.* 2016). However, the lower removal efficiency in the present study could be due to the higher dissolved organic carbon in the wastewater effluents of between 11.1–15.5 mg l^{-1} since the same experiment in ultrapure water gave higher removal efficiencies for UV/Cl_2 as relative to UV/ H_2O_2 (IV). These results are in line with those obtained by Jin *et al.* (2011) who evaluated the effectiveness of UV/Cl_2 and UV/H_2O_2 in the removal of cyclohexanoic acid in wastewater streams and found UV/Cl_2 to be less efficient than UV/H_2O_2 and thus not suitable for the degradation of cyclohexanoic acid in the studied wastewater stream.

Further evaluation on the removal of DOC by UV, UV/Cl_2 and UV/H_2O_2 in wastewater effluents and ultrapure water spiked target analytes showed that DOC removal increased in the order UV < UV/Cl_2 < UV/H_2O_2 indicating that complete degradation of DOC (mineralization) is higher in the UV/H_2O_2 process (IV).

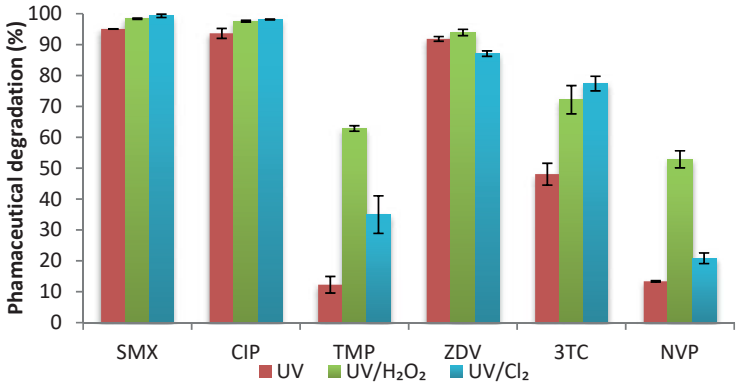

FIGURE 6 The removal of target antibiotics and antiretroviral drugs in wastewater effluent by direct UV photolysis, UV/H_2O_2 and UV/Cl_2 at electrical energy dose of 6.67 kWh m^{-3} and an initial concentration 20.4 mg l^{-1} H_2O_2 and 42.6 mg l^{-1} Cl_2. The error bars present the standard deviation (n = 3).

4.6.3 Energy consumption by UV post-treatment processes (IV)

The energy consumption for the removal for the target analytes was evaluated based on the electrical energy required for the removal of 90 % of the target APIs (Fig. 7). The electrical energy per order of compound removal (E_{EO}) for sulfamethoxazole, ciprofloxacin and zidovudine for the three processes did not show remarkable deference. However, there were major differences in E_{EO} for the compounds that were poorly removed by direct UV photolysis. For instance, relative to direct UV photolysis the E_{EO} for the UV/H_2O_2 process was 5.4, 4.1 and 2.4 times lower for trimethoprim, nevirapine and lamivudine respectively. Similarly, the E_{EO} for the UV/Cl_2 process relative to direct UV photolysis was 2.7, 1.7 and 3.4 times lower for trimethoprim, nevirapine and lamivudine, respectively. Overall, for the removal of the six compounds, the E_{EO} for the UV/H_2O_2 is 1.7 and 3.5 times lower relative UV/Cl_2 and direct UV photolysis, respectively. This implies that since the much of the UV post-treatment process costs are related to the electrical energy consumption, the UV/H_2O_2 process would be much superior to direct UV photolysis and UV/Cl_2 in post-treatment of wastewater.

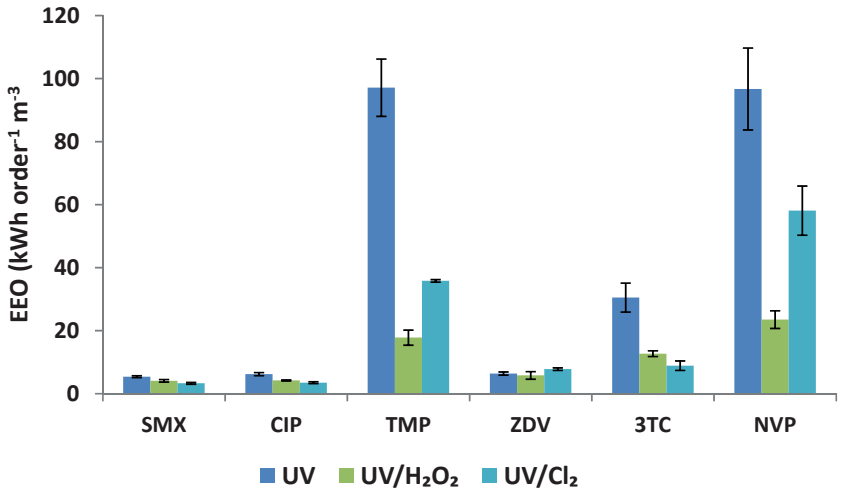

FIGURE 7 The electrical energy per order of compound removal (E_{EO}, kWh $order^{-1}$ m^{-3}) for direct UV photolysis, UV/H_2O_2 (20.4 mg l^{-1}) and UV/Cl_2 (42.6 mg l^{-1}) in treated wastewater effluent.

5 CONCLUSIONS

5.1 Occurrence and control of pharmaceuticals

The findings of this study enhance the knowledge on the occurrence and mitigation of selected antibiotics and antiretroviral drugs in urban hydrological cycles. Although there are many publications available on occurrence and fate of antibiotics in urban hydrological cycles particularly in high income countries, there is quite limited information available for low and middle income countries. Furthermore, there are only a few publications currently available globally on the determination of antiretroviral drugs in urban hydrological cycles. This study was performed in three urban hydrological cycles of Nairobi-Kenya, Lusaka-Zambia and Jyväskylä-Finland. The studied antiretroviral drugs included nevirapine, zidovudine and lamivudine and while the antibiotics were trimethoprim, sulfamethoxazole, ciprofloxacin, norfloxacin, tetracycline, doxycycline and amoxicillin.

The types of samples collected from each study area were: groundwater, surface water, wastewater influent, wastewater effluents and source separated urine from Lusaka; surface water and wastewater effluents from Nairobi; and surface water, wastewater effluent and wastewater influent from Jyväskylä.

From the study, the following conclusions can be drawn:

i. The analytical method developed allowed for the simultaneous quantification of the target analytes at environmentally relevant concentrations. The concentrations of antibiotics and antiretroviral drugs in Jyväskylä wastewater and surface water were within the same range or lower than those previously reported within Europe.

ii. Surface water and wastewater from Nairobi and Lusaka had significantly high concentrations of the target analytes primarily emanating from direct discharge of untreated wastes mostly from the informal settlements and wastewater treatment plants. In addition, the impact of high HIV prevalence was evident based on the high concentrations of antiretroviral drugs and associated antibiotics detected

in the samples. The concentrations detected in Lusaka groundwater were relatively low implying that a significant amount of the pharmaceuticals residues are sorbed and/or degraded in the soil. However, since the groundwater in the study area is consumed with little or no treatment, precautionary measures need to be taken to minimize human exposure.

iii. Exceptionally high concentrations of antibiotics and antiretroviral drugs were measured in source separated urine; this implies that source separation can be a significant barrier to environmental contamination by the pharmaceuticals. In addition, precautionary measures on the use of untreated source separated urine as a fertilizer need to be continuously undertaken to mitigate possible transfer of pharmaceuticals to food crops. However, since the majority of pharmaceuticals are strongly adsorbed in the soil, the direct use of source separated urine in forestry for energy production may be plausible.

iv. Effective removal of residual pharmaceuticals in wastewater is possible with UV-AOPs. However, despite the relatively good removal efficiencies by UV-based AOPs, the end-of-pipe technologies are not economically feasible in a majority of wastewater treatment facilities due to the high cost of energy, chemicals and infrastructure.

5.2 Suggestions for future work

The wastewater effluents and surface water in Nairobi and Lusaka are used for irrigation of food crops in the surrounding parcels of land. With the high concentrations detected in this study, there is need to evaluate the possible uptake of the antibiotics and antiretroviral drugs by the irrigated food crops. In addition, the compounds were found not to easily migrate to groundwater and thus the accumulation of some of the compounds in the soil column needs to be evaluated. Furthermore, the concentration of the compounds and their degradation products in the sediments and sludge of wastewater treatment plants and surface water need to be investigated.

An in-depth environmental and human risk assessment needs to be carried out. There is no toxicity data available for the antiretroviral drugs and experimental acute and chronic toxicity studies need to be undertaken for more accurate environmental risk assessment. Moreover, attention should be directed to the mixture effects of the pharmaceuticals to both terrestrial and aquatic organisms.

With the high concentration of antibiotics in the surface water and wastewater in Nairobi and Lusaka, there is a need to investigate the presence and possible propagation of antibiotic resistant genes in the sludge and sediments.

Acknowledgements

I am sincerely grateful to my supervisors Professor Tuula Tuhkanen and Professor Anthony Gachanja for introducing me to this topic and giving excellent guidance in every step of work. Tuula, you have been there for me and your constant advice and many insights on this work and my career have broadened my world of thinking and given me greater confidence. I will forever cherish the moments I worked with you. Anthony, it is because of your mentorship that I ended up in Jyväskylä, thank you for believing in me.

I am also grateful to my other co-authors Päivi Kosunen, Johanna Maldonado and James Nyirenda, for their great input in the experimental work and writing of the manuscripts.

I would like to thank Nab Labs Oy and Jyväskylän Seudun Puhdistamo Oy for their kind help with the sampling in Jyväskylä. I am also greatly indebted to Mr. Pentti Keskitalo of Universal Corporation Ltd-Kenya for the kind donation of pharmaceutical standards and assistance in the conveyance of samples from Nairobi to Jyväskylä. I am also grateful to the staff of Departments of Chemistry of Jomo Kenyatta University of Agriculture and Technology-Kenya and the University of Zambia for the support accorded during sample preparation. Sample collection in Lusaka and conveyance to Jyväskylä could not have been accomplished without the vital support of Sari Huuhtanen of the Global Dry Toilet Association of Finland as well as Mr. Obed Kawanga, Lydia Chibambo, Noah Chongo of the Network for Environmental Concerns and Solutions-Zambia and José Maldonado; many thanks to you all! I also would like to give my gratitude to Harrison Kwach of UN-HABITAT, Nairobi and Ms. Grace Githiri for their logistical support during the sampling process in Nairobi. Thanks to Preston Akenga for sacrificing lots of time during sampling and sample processing in Nairobi.

I would like to thank my follow-up group Dr. Heidi Ahkola (Finnish Environment Institute) and Dr. Anssi Vähätalo (University of Jyväskylä) for the great encouragement and support. I am also grateful to my external reviewers, Prof. Marina Trapido and Dr Anna-Lea Rantalainen for their insightful comments on this thesis. I greatly acknowledge the guidance given by the scientific editor Anssi Lensu. I would also like to thank Dr. Jerker Fick for accepting to be the opponent of this thesis.

I am sincerely thankful laboratory staff in Environmental science and Technology section and especially Mervi Koistinen and Leena Siitonen for their support in the laboratory and words of encouragement throughout this research. Am also grateful to my fellow PhD students and post-doctoral researchers including Yufei Gu, Juho Rajala Anna Suuronen, Hanna Arola, Hanna Aarnos, Andreas Eriksson, Anu Mikkonen, Alexey Ignatev, Cyril Rigaud for the support and fun filled company during my studies.

This study was funded by The Doctoral Programme in Biological and Environmental Science of the University of Jyväskylä. In addition, I would like to thank Maa- ja vesitekniikan tuki ry for the additional funds for consumables;

Finnish Doctoral Programme in Environmental Science and Technology (EnSTe) and Finnish University Partnership for International Development (UniPID) for the conference travel grants.

I am greatly indebted to my parents Keziah Mugure and Stephen Ngumba and my brothers and sisters for always encouraging me to explore my dreams and prayers you made on my behalf. I salute you for believing in me, giving me freedom to choose what I desired and for the many noble and sometimes painful sacrifices you made to shape my life. My heart felt regard goes to my late father in law, mentor and great friend Moses Kangau and mother in law Nancy Wairimu for their love, prayers and moral support. I am grateful to my friends James, Grace, Eva, Elvis, Miro, Richie, Lucy and Jacquiline here in Finland and Moses, Edna and Pius in Kenya for their constant unreserved practical support in many aspects of life.

Special thanks my wife Milkah Wambui and our sons Victor and Axel for always loving and cheering me up during this process. You were always around even during the times that I thought that it was difficult to continue, you managed to help me keep things in motion.

Words would never say how grateful I am to all of you!

YHTEENVETO (RÉSUMÉ IN FINNISH)

Antibioottien ja antiretroviraalisten lääkeaineiden esiintyminen ja kontrolli urbaanissa hydrologisessa kierrossa

Viimeisen parinkymmenen vuoden aikana on havaittu, että lääkkeiden aktiiviaineet (APIs) muodostavat ongelman esiintyessään urbaanissa hydrologisessa kierrossa. Tuhansia tonneja lääkeaineita (ja niiden metaboliitttija) päätyy ulosteiden, mutta ennen kaikkea virtsan, mukana jäteveden¬puhdistamolle ja sieltä osa päätyy ympäristöön. Osa voi päätyä suoraan käsittelemättömänä ympäristöön. Lääkeaineiden, jotka päätyvät ympäristöön pieninä pitoisuuksina mutta jatkuvasti, vaikutuksia kohdeorganismeihin ei ole tutkittu vielä tarkasti ja niitä ei täysin ymmärretä. Siitä huolimatta yleisesti tunnustetaan, että aktiiviaineet voivat aiheuttaa antibioottiresistenssin syntymistä, estrogeenivaikutuksia ja akuutteja toksisia vaikutuksia herkimmille eliöille.

Tässä väitöskirjassa on tutkittu eräiden antibioottien ja antiretroviraalisten lääkeaineiden esiintymistä vesistöissä (urbaanissa hydrologisessa kierrossa) kolmessa eri kohteessa: Nairobissa Keniassa, Lusakassa Sambiassa ja Jyväskylässä Suomessa. Tämän lisäksi tutkittiin mahdollisuutta lääkeainejäämien poistamiseen jätevedestä jälkikäsittelyn avulla. Antibiootit ja antiretroviraaliset yhdisteet valittiin tutkimuksen kohteeksi, koska niiden käyttö on yleistä esimerkiksi Nairobissa ja Lusakassa, ympäristöissä, jossa on erittäin korkea HIV/AIDS esiintyvyys sekä kattava lääkeohjelma.

Lääkeaineista ja niiden esiintymisestä ympäristössä kehitysmaissa tiedetään vielä suhteellisen vähän, koska lääkeaineiden ympäristöanalytiikka on kallista ja vaatii erityisiä laitteita ja osaamista. Kehittyneissä maissa monien lääkeaineiden käyttäytyminen on tutkittu hyvinkin tarkasti.

Tässä tutkimuksessa keskityttiin antiretroviraalisiin lääkeaineisiin (nevirapiini, zidivudiini ja lamivudiini) sekä seitsemään eri antibioottiin (trimetopriimi, sulfametoksatsoli, ciprofloksasiini, norfloksasiini, tetrasykliini, doksisykliini ja amoksisilliini). Näitä lääkeaineita tutkittiin erilliskerätystä virtsasta, jätevedestä, pintavedestä sekä kaivovesistä.

Ensiksi kehitettiin kiinteäfaasiuuttoon, nestekromatografiaan ja tandem- massaspektroskopiaan perustuva menetelmä, jolla voitiin tutkia yllä mainitut yhdisteet yhtaikaisesti. Kvantitoinnissa käytettiin sekä matrix-matched ulkoista standardia ja sisäisiä standardeja.

Kvantitointirajat vaihtelivat välillä 5–63 ng l-1 (pintavesi), 13–70 ng l-1 (käsittelemätön jätevesi), 8–59 ng l-1 (käsitelty jätevesi) ja 100–1180 ng l-1 (erilliskeräilty virtsa). Kaikki analyysimenetelmien kriteerit, kuten kalibraatio, matriisiefekti ja saanto, olivat riittävän hyviä tutkituille aktiiviaineille kussakin eri matriisityypissä. Tutkittujen lääkeaineiden pitoisuus vaihteli käsittelemättömissä jätevesissä välillä 10–570 ng l-1 (Jyväskylä) ja 80–118 970 ng l-1 (Lusaka). Käsitellyn jäteveden pitoisuudet olivat 8–537 ng l-1 (Jyväskylä), 80–55 570 ng l-1 (Lusaka) ja 66–3940 ng l-1 (Nairobi) Maksimikonsentraatio pintavesissä Jyväskylässä oli vain 54 ng l-1, kun vastaava pitoisuus Nairobissa ja Lusakassa oli useita kertaluokkia suurempi maksimikonsentraation ollessa Nairobissa 13 800 ng l-1 ja Lusakassa 49 700 ng l-1. Syy pintavesien ja jo-

kien korkeaan lääkeainepitoisuuteen Nairobissa ja Lusakassa johtuu suorista käsittelemättömistä jätevesiviroista ympäristöön, tiheästä asutuksesta, väestön korkeasta sairastavuudesta ja jätevesien tehottomista käsittelymenetelmistä.

Lusakassa tutkittiin erilliskäsiteltyjen virtsanäytteiden lääkeaine-pitoisuuksia sekä kaivovesien lääkeainejäämiä. Kaivovesistä tavattiin lääkeaineita yksittäistapauksissa ja pitoisuudet vaihtelivat välillä <LOQ – 880 ng l-1 huolimatta siitä, että kaivot saattoivat sijaita hyvinkin lähellä kuoppakäymälöitä, jotka ovat hyvin yleisiä tutkitulla alueella. Tämä voi johtua siitä, että lääkeaineet absorboituvat maa-ainekseen ja voivat muuntua maaperässä. Erilliskerätyssä virtsassa lääkeaineiden pistoisuustaso oli puolestaan hyvin korkea: 7740 µg l-1 (sulfametaksatsoli), 12800 µg l-1 (trimetopriini) ja 10010 µg l-1 (lamivudiini).

Olosuhteissa, joissa HIV/AIDS-sairastavuus ja lääkkeiden käyttö ovat runsaita ja joissa ei ole on järjestetty sanitaatiota, keskitettyä jäteveden keräilyä ja käsittelyä, virtsan ja ulosteiden erilliskeräily voi tarjota realistisen vaihtoehdon lääkeaineiden pääsyn estämiseen ympäristöön ja sitä kautta tapahtuvan altistuksen vähentämiseen.

Lääkeaineet eivät poistu perinteisissä jätevedenpuhdistusprosesseissa, joita tässä työssä pyrittiin tehostamaan lääkeaineiden poistumista edistävillä innovatiivisilla menetelmillä, kuten suoran UV-säteilyn avulla tai yhdistämällä se kloorin ja vetyperoksidin käyttöön. Tutkitut lääkeaineet olivat kolme antibioottia (trimetopriimi, sulfametaksatsoli ja ciprofloksasiini) ja kolme antiretroviraalista yhdistettä (nevirapiini, zinovudiini ja lamivudini). Sulfametaksatsoli, ciprofloxaciini ja ziduvudiini poistuivat jo pelkän UV-käsittelyn avulla. Kloorin ja vetyperoksidin lisäys tehosti UV-käsittelyä, koska silloin syntyvät radikaaliyhdisteet nopeuttavat hajoamista. Kolmesta tutkitusta menetelmästä UV/H_2O_2 -käsittelyn energiatarve oli pienin tutkittavan yhdisteen poistamiseksi 90 prosenttisesti.

Huolimatta suhteellisen tehokkaasta lääkeaineiden poistosta AOP-tekniikoilla, jäteveden jälkikäsittely ei ole taloudellisesti kestävä vaihtoehto johtuen korkeista energia- ja kemikaalikustannuksista sekä tarvittavan käsittelyinfran rakentamisen takia. Lisäksi jälkikäsittely on mahdollista vain alueilla, joilla on keskitetty jätevesien keräily- ja käsittelyjärjestelmä. Tämän takia source separation-tekniikat kuten kuivakäymälät, virtsan ja ulosteiden erilliskeräys, kunnollinen keräily ja turvallinen käsittely, vanhentuneiden ja käyttämättä jääneiden lääkkeiden keräys sekä antimikrobiologisten lääkkeiden myynnin kontrolloiminen käsikauppatavarana sekä yleisen tietoisuuden lisääminen voivat olla käyttökelpoisimpia tapoja ympäristön lääkekuormituksen vähentämiseksi.

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ORIGINAL PAPERS

I

OCCURRENCE OF SELECTED ANTIBIOTICS AND ANTIRETROVIRAL DRUGS IN NAIROBI RIVER BASIN, KENYA

By

Elijah Ngumba, Anthony Gachanja & Tuula Tuhkanen

2016

Science of the Total Environment. 539, 206–213

Science of the Total Environment 539 (2016) 206–213

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya

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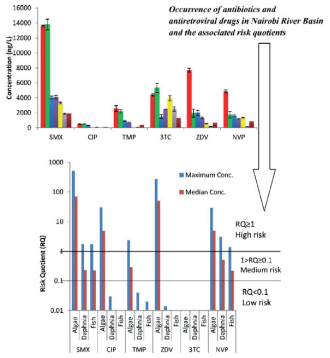
HIGHLIGHTS

- Measured concentrations were higher relative to cities in developed world
- Highest concentrations were measured within the informal settlements
- Some river waters were contaminated more than wastewater treatment plant effluents
- High risk quotients calculated signifying potential aquatic toxicity

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 28 August 2015
Accepted 28 August 2015
Available online xxxx

Keywords:
Antibiotics
Antiretroviral
Occurrence
Predicted no effect concentration
Measured environmental concentration
Risk quotient

ABSTRACT

In this paper, we investigated the occurrence of three antibiotics (sulfamethoxazole, trimethoprim and ciprofloxacin) and three antiretroviral (lamivudine, nevirapine and zidovudine) drugs in the Nairobi River Basin, Kenya. The analytical procedure involved extraction using solid phase extraction followed by liquid chromatography–electrospray ionization tandem mass spectrometry (SPE–LC–ESI–MS/MS). In this study, 40 sites were selected for sampling, including 38 sites along the rivers and 2 wastewater treatment effluent sites. All the studied compounds were detected with sulfamethoxazole having the highest detection frequency of 97.5% and ciprofloxacin had the lowest at 60%. The results showed that the concentration of the drugs increased in highly populated regions especially within the informal settlements. The maximum (median) concentrations in the river waters for sulfamethoxazole, trimethoprim, ciprofloxacin, lamivudine, nevirapine and zidovudine in ng/L were 13,800 (1800), 2650 (327), 509 (129), 5430 (1000), 4860 (769), and 7680 (660), respectively. The maximum concentrations in the river waters were generally higher than those of the wastewater treatment plant effluents signifying that the rivers are substantially contaminated by domestic wastewater. The environmental risk was evaluated by calculating the risk quotients (RQs) for algae, daphnia and fish based on the maximum and median concentrations of the analytes in the river basin and was expressed as the ratios of measured environmental concentrations (MEC) to predicted no effect concentrations (PNEC). The RQs ranged

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http://dx.doi.org/10.1016/j.scitotenv.2015.08.139

from 0 to 507.8 and apart from lamivudine that had a low RQ, all the other analytes had RQ > 1 at maximum and median measured concentrations for at least one taxonomic group. The high RQs are indicative of possible adverse ecological effects and calls for corrective and mitigation strategies.

1. Introduction

The occurrence of pharmaceutical residues in water systems has generated a lot of interest in the last two decades and currently a lot of analytical data on the environmental occurrence of pharmaceuticals in different environmental compartments has been published especially in developed countries. Several studies have documented that a significant part of consumed pharmaceuticals are excreted into the environment as fractions of various metabolites together with unchanged parent compounds largely through the urine and feces (Heberer, 2002; McArdell et al., 2003; Carballa et al., 2008; Kasprzyk-Hordern et al., 2008; Al Aukidy et al., 2012).

Some of the major concerns of the occurrence of pharmaceuticals in the environment are the development of antimicrobial resistance and possible toxicity to sensitive organisms (Kümmerer and Henninger, 2003; Backhaus et al., 2008; Martinez, 2009; Watts, 2011; Jain et al., 2013; Jiang et al., 2014; Richardson and Ternes, 2014). Discharges from wastewater treatment plants (WWTPs) have been identified as the primary point sources of pharmaceuticals into the aquatic environment with lesser contribution from secondary sources, such as hospital effluent and wastewater discharges from pharmaceutical companies (Leung et al., 2013). However, this is not usually the case in urban hydrological cycles of developing countries such as Nairobi Kenya where WWTPs serve only a fraction of the urban residents. This is specifically so because of the limited capacity of the WWTPs and a majority of the population resides in areas that are not connected to the sewerage system. The population of Nairobi is approximately 3.6 million people and about 2.5 million people live within the informal settlements that are characterized by overcrowding and poor sanitation, as well as exposure to diseases such as HIV/AIDS. A large proportion of wastewaters from these informal settlements are discharged directly into surface waters leading to large-scale contamination of the local rivers (APHRC, 2002; Index Mundi, 2013; National Council for Population and Development, 2013; Wang et al., 2014). According to UNAIDS and National AIDS and STI Control Program (NASCOP)-Kenya, HIV/AIDS adult prevalence in Kenya was approximately 6.2% in 2012. About 65% of the cases were under antiretroviral drugs which are administered together with antibiotics to prevent HIV induced infections. Further, HIV patients are prone to tuberculosis (TB) and as a result, a significant number is under TB antibiotic medication (NASCOP-Kenya, 2011). With the recommended dosage for antiretroviral therapy, each HIV patient (free of tuberculosis or any other opportunistic infection) ingests a daily dose of three first-line antiretrovirals and co-trimoxazole (sulfamethoxazole/trimethoprim combination antibiotic) (NASCOP-Kenya, 2011; WHO, 2013). Patients with other opportunistic infections are required to take extra drugs depending on the nature of infection. With such a high drug dosage, the amount of active pharmaceutical ingredients collectively released by this group of patients to the environment is quite significant.

Very little data is currently available on the occurrence and fate of pharmaceuticals in the Nairobi River Basin. To the best of our knowledge, only one research article by K'oreje et al. (2012) published indicative concentrations of some pharmaceuticals in Nairobi (K'oreje et al., 2012).

The aim of this study was to investigate the occurrence of the selected antibiotics and antiretroviral drugs within the Nairobi River Basin with emphasis on selected informal settlements in Nairobi-Kenya. In addition, an environmental risk analysis was undertaken in order to assess the potential environmental risk. Among the six drugs included in this study, nevirapine (NVP), zidovudine (ZDV) and lamivudine (3TC) are first-line antiretroviral regimen; Trimethoprim (TMP) and sulfamethoxazole (SMX) are used alongside antivirals to prevent HIV-induced infections as co-trimoxazole while ciprofloxacin (CIP) is commonly used to treat patients with persistent infections. Some of the physicochemical properties of the selected drugs are summarized in Table 1.

2. Materials and methods

2.1. Chemicals and standards

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany) and formic acid (98%) from Fluka (Darmstadt, Germany). Glass microfiber filters 47 mm GF/D (2.7 μm) and GF/F (0.7 μm) were obtained from Whatman (Maidstone, England). All the pharmaceutical standards (purity ≥95%) were a kind donation from Universal Corporation Ltd., Kenya. Oasis hydrophilic-lipophilic balanced (HLB; 6 cm^3, 200 mg) solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, USA). Ultrapure water was used throughout the study and was generated using Ultra Clear UV Plus and euRO 60 Reverse Osmosis unit (SG, Barsbuttel Germany). Unless otherwise indicated, all the chemicals used in the study were of analytical grade or above. Individual standards were prepared in a concentration of 1000 mg/L. Apart from ciprofloxacin which was dissolved in ultrapure water; all the other compounds were dissolved in methanol. The standards were subsequently diluted with 1:1 (*v*/*v*) methanol/ultrapure water to a pooled mixed standard of 10 mg/L as stock solution and stored at +4 °C in the dark.

2.2. Study area and sample collection

The study area and the sampling sites are shown in Fig. 1, and detailed site description provided in the supplementary information (Table S1). Nairobi River Basin is traversed by three main rivers that were subjects of this study i.e. Mathare, Nairobi and Ngong Rivers along with some of their tributaries. Ngong River runs across Kibera and Mukuru informal settlements. Nairobi River flows through a number of informal settlements such as Kawangware, Majengo, Kiambiu and Dandora. Mathare River traverses through Mathare slums before its confluence with Nairobi River. Kibera is the largest of these informal settlements and is home to approximately 1 million inhabitants (Erulkar and Matheka, 2007). 36 sampling sites were chosen along the three rivers; upstream, midstream and downstream sampling sites were selected with emphasis on river sections close to the informal settlements and the WWTP effluent discharge point. The water from the three rivers is discharged into Athi River outside Nairobi River Basin. Athi River was sampled from two sampling sites around Fourteen Falls (a popular fishing and recreation site) approximately 35 km downstream of Nairobi WWTP. In addition to the river sampling sites mentioned above, two WWTPs effluent sites were selected for sampling. The first effluent site was the Nairobi WWTP (also referred as Dandora stabilization ponds) that drains its effluent into Nairobi River within Nairobi River Basin. The second comparative site (located outside Nairobi River Basin) was Jomo Kenyatta University of Agriculture and Technology (JKUAT) WWTP which serves a population of approximately 20,000 residents. Both Nairobi WWTP and JKUAT WWTP use stabilization ponds (anaerobic, facultative and maturation ponds) with hydraulic retention time of 60–90 days. Nairobi WWTP

Table 1
Physicochemical properties of selected pharmaceuticals.

Compound	Abbreviation	CAS no.	Water solubility (mg/L)[a]	pKa[b, c]	Log Kow[a, c]
Sulfamethoxazole	SMX	723–46–6	610	5.6, 1.83	0.89
Ciprofloxacin	CIP	85,721–33–1	13,500	6.4, 8.2	0.28
Trimethoprim	TMP	738–70–5	400	7.2	0.91
Zidovudine	ZDV	30,516–87–1	20,100	9.7	−7.05
Lamivudine	3TC	134,678–17–4	70,000	4.3	−2.62
Nevirapine	NVP	129,618–40–2	0.7046	2.8	3.89

[a] Drugbank.
[b] (Babić et al., 2007).
[c] (USEPA and SRC, 2012).

receives approximately 80,000 m^3/day for both domestic and industrial wastewaters (Wang et al., 2014; Nairobi City Water and Sewerage Company, 2015). In total, 40 sampling sites were selected for this study, including 36 along the three rivers in Nairobi River Basin, 2 along the Athi River, and 2 from the WWTP effluents. Samples were collected in triplicates from the selected sites in October 2014 during the dry season in pre-cleaned 1 L reagent brown glass bottles. The samples were stored at +4 °C awaiting extraction within 48 h.

2.3. Physicochemical parameters

Four physicochemical water parameters (pH, conductivity, total organic carbon (TOC), and total nitrogen (TN)) were measured. pH and conductivity were measured onsite using a portable meter, while the TOC and TN content were measured using Shimadzu TOC/TN modules that conform to the Standard method for examination of water and wastewater 5310B and ASTM D5176, respectively (Shimadzu, 2012).

2.4. Sample extraction

Extraction of the target compounds from the water samples was carried out by solid phase extraction (SPE). Prior to the extraction, the water samples were filtered through 47 mm GF/D (2.7 μm) and GF/F (0.7 μm) glass microfiber filters (Whatman, Maidstone, England). After the filtration, sample pH was adjusted to 9 with aqueous NH_4OH to enhance the recovery of the analytes. Oasis HLB cartridges (6 cm^3, 200 mg, Waters, Milford, USA) were preconditioned with 6 mL methanol followed by 6 mL ultrapure water. Replicate 500 mL samples (n = 3) were then loaded using a vacuum manifold at a flow rate of approximately 10 mL per minute. After loading the cartridges were dried in vacuum for 5 min, then washed with 5 mL of 2% methanol solution in 5% aqueous NH_4OH, and dried for further 10 min. The analytes were eluted with 3 mL acetonitrile/methanol/acetic acid (50:50:2 *v*/*v*). The solvent was then evaporated in a stream of nitrogen at 40 °C, reconstituted to 1 mL with acetonitrile/water (20:80 *v*/*v*), and then filtered through a 0.2 μm cellulose acetate syringe filter before injection into an LC–MS/MS system.

2.5. Instrumental analysis

2.5.1. Liquid chromatography

Liquid chromatography was performed on a Waters Alliance 2795 (Milford, MA, USA) system consisting of a tertiary pump, a vacuum degasser, an autosampler and a column oven (set to 30 °C). Compounds were separated with a reversed phase C18 column (Waters XBridgeTM 3.5 μm, 2.1 × 100 mm with 3.5 μm, 2.1 × 10 mm guard column). The mobile phase consisted of ultrapure water (A) and acetonitrile (B), both containing 0.1% (*v*/*v*) formic acid. The flow rate was 0.25 mL/min, and the injection volume was 10 μL. The gradient of B was held at 20% in the first 2 min then linearly increased to 100% in 3 min. B was then lowered 20% in 5 min and held there for 2 min. The column was then equilibrated for 7 min before the next injection. The total run time for each injection was 19 min.

2.5.2. Mass spectrometry

Micromass Quattro electrospray triple-quadrupole mass spectrometer (Micromass, Manchester, UK), was used as the detector. Nitrogen was used as the desolvation gas (500 L/h) and as a cone gas (50 L/h). The desolvation temperature and the source temperature were 200 °C and 100 °C, respectively. Argon was used as a collision gas at a collision pressure of 2.8×10^{-4} mBar. Mass spectrometric analysis was performed in the positive electrospray ionization mode (ESI+), and the

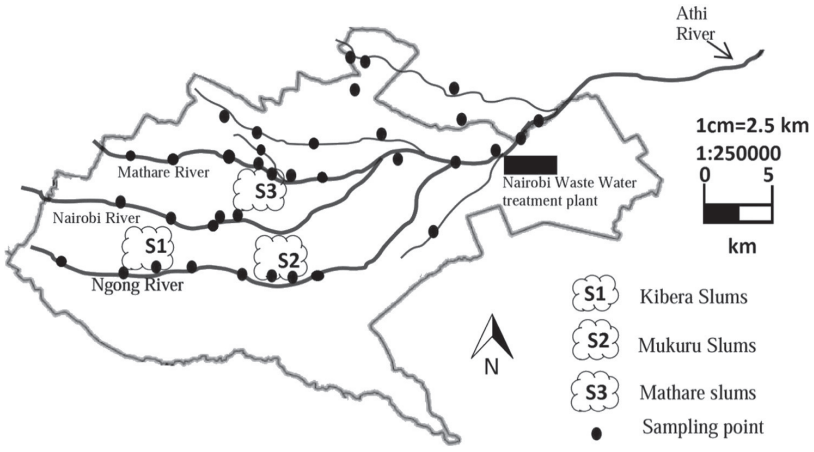

Fig. 1. Map of Nairobi River Basin and sampling sites. JKUAT WWTP and Athi River sampling sites not shown on this map.

spectrometer operated in multiple reaction monitoring (MRM) with a dwell time and interchannel delay of 200 ms. The Precursor and product ions, collision energies, and cone voltages were optimized for each analyte. This was done by continuously infusing 5 mg/L of pure analytes dissolved in 1:1 methanol/Ultrapure water (*v*/*v*) to the MS/MS system using a syringe pump at a flow rate of 10 μL/min. Two product ions were identified for each analyte; product ion 1 was used for quantification, while product ion 2 for confirmation. Table 2 summarizes the optimized LC–ESI–MS/MS conditions for the analysis of the target compounds.

2.6. Method validation

The method validation involved an experimental procedure that tested the calibration, limit of detection (LOD), and quantification (LOQ), instrumental repeatability and recovery. Matrix matched external standard method was used for quantification of the target analytes. To achieve this, calibration curves (11 points) were prepared by spiking 500 mL river water that had no detectable quantities of the target analytes with mixed standards at concentration levels between 0.05 μg/L–200 μg/L and subjecting them to the SPE process. The linearity of the calibration was evaluated based on the correlation coefficient (R^2) of the calibration curve. The LOD and LOQ for each analyte were defined as the lowest concentration producing a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LOD and LOQ were determined by analyzing spiked river water. The instrumental intra-day and inter-day repeatability were evaluated by injecting 1000 μg/L mixed standards (n = 6) and determining the relative standard deviation (RSD) within the responses. SPE recovery was evaluated by comparing the response of the spiked river water with matrix matched standard (Eq. 1). The matrix matched standard was prepared by spiking blank river water extract with the target analytes after the SPE process.

$$\textbf{Recovery}\ (\%) = \frac{C}{D}X100 \tag{1}$$

where *C* is the peak area determined after extraction of the spiked river water and *D* is the peak area for the corresponding matrix matched standard.

Matrix effect (ME) was evaluated using Eq. 2, as described in the literature (Caban et al., 2012)

$$\textbf{ME}(\%) = \frac{A}{B}X100 \tag{2}$$

where *A* is the peak area of the analyte recorded for the standard solution and *B* is the peak area of the analyte recorded for the river water spiked with the analyte after SPE.

2.7. Risk assessment

The environmental risk was assessed by computing the risk quotient (RQ) for algae, daphnia and fish. RQ was calculated as a ratio between the measured environmental concentration (MEC) and predicted no-effect concentration (PNEC) (Eq. 3) where RQ ≥ 1, 1 > RQ ≥ 0.1 and RQ < 0.1 indicates a high, medium and low risk, respectively, for the test organism (EMEA, 2006; Santos et al., 2007; López-Doval et al., 2012; X Van et al., 2014; Kosma et al., 2014; Pereira et al., 2015). The PNEC was estimated using acute toxicity data LC_{50} or EC_{50} divided by an assessment factor of 1000 (Eq. 4). Experimental toxicity data for the antibiotics is available in the literature and PNEC calculated through this data were used. However, limited data is available for the antiretroviral drugs and the LC_{50} or EC_{50} was estimated using Ecological Structure Activity Relationships (ECOSAR v1.10) software from U.S. Environmental Protection Agency.

$$\textbf{RQ} = \frac{\textbf{MEC}}{\textbf{PNEC}} \tag{3}$$

$$\textbf{PNEC} = \frac{\textbf{EC}_{50}\ \textbf{or}\ \textbf{LC}_{50}}{1000} \tag{4}$$

3. Results and discussion

3.1. Physicochemical parameters

The measured parameters are presented in the supplementary material (Table S4). In summary, the study demonstrated substantial changes in the water quality parameters as the rivers flowed through Nairobi River Basin. The TOC and TN values increased remarkably down the hydrological transect and ranged from 4.05–33.24 mg/L and 1.95–22.48 mg/L, respectively. The higher TN content can be attributed to the direct discharge of human waste into the rivers. In urban hydrological cycle, TOC and TN are good indicators of organic matter and human waste contamination, respectively. The maximum measured TOC and TN values were higher than those reported elsewhere (Charkhabi and Sakizadeh, 2006; Jekatierynczuk-Rudczyk, 2009).

3.2. Analytical method

The results for analytical method validation are summarized in Table 3. The calibration curves gave good linearity over the calibration range with the correlation coefficient $r^2 > 0.99$. The recoveries of the analytes from the spiked river water ranged from 57.51 ± 1.72% to 96.36 ± 5.62%. The recovery for most compounds was above 70% apart from 3TC which had a maximum recovery of 57.51%. The relatively low recovery of 3TC can be attributed to its high solubility in water that limited its retention in the SPE. The LOQ of the analytes varied from sample to sample, and ranged between 8 and 122 ng/L. ZDV had a relatively high LOQ (122 ng/L), which was attributed to its zwitterionic nature. In general, all the studied compounds were prone to matrix effect at varying degrees either as signal suppression or signal enhancement and ranged from 73 to 135% with ZDV experiencing highest signal enhancement, while 3TC had the highest signal suppression. Since the analytical calibration curves were constructed using matrix matched standards, the analyte concentrations in the samples were compensated for variations in SPE recovery and matrix effect. The intra-day and inter-days instrumental repeatability was evaluated based on the relative

Table 2
Optimum LC–ESI–MS/MS conditions for the analysis of antibiotics and antivirals in river water.

Analyte	RT (sd)[a]	Precursor ion $[M + H]^+$ (m/z)	Product ion 1 (m/z)	Cone voltage (V)	Collision energy (eV)	Product ion 2 (m/z)	Cone voltage (V)	Collision energy (eV)
CIP	2.05(0.08)	332.1	288.0	34	19	231.0	30	17
SMX	5.1(0.10)	254.0	156.0	28	18	92.0	28	18
TMP	2.02(0.06)	291.1	123.0	34	19	230.0	30	17
3TC	1.48(0.10)	229.9	112.0	17	18	95.0	30	24
ZDV	2.31(0.05)	268.2	127.0	16	17	142.1	24	27
NVP	4.09(0.06)	267.2	226.2	40	29	249.0	40	29

RT (sd) [a]—Retention time in minutes (standard deviation).

Table 3
Calibration, recoveries, matrix effect, repeatability LOD and LOQ for individual analytes.

Analyte	Linearity (r^2)	Percentage recovery (sd)[a]	% ME (sd)	Repeatability (RSD)		LOD (ng/L)	LOQ (ng/L)
				Intraday	Interday		
CIP	0.9987	87.94(5.82)	81.31(0.98)	2.83	3.52	4	12
SMX	0.9977	79.27(3.84)	104.21(0.73)	3.12	6.04	3	8
TMP	0.9985	91.88(3.15)	88.63(3.22)	3.69	4.58	7	24
3TC	0.9985	57.51(1.72)	73.94(0.98)	9.81	9.30	3	10
ZDV	0.9995	96.36(5.62)	135.87(1.95)	6.30	8.44	37	122
NVP	0.9980	87.40(3.17)	90.44(1.82)	2.60	4.52	4	12

(sd)[a]—Standard deviation.

standard deviations (RSDs) under similar experimental conditions. The intra-day and inter-days RSDs at 1000 μg/L ranged between 2.60–9.81 and 3.52–11.90, respectively and since the RSDs were <20%, the LC–MS/MS method was considered as repeatable and reliable. Chromatograms showing the MRM transitions for the standards, river water extracts and blank for the 6 analytes can be found in the Supplementary material (Figs. S1–S3).

3.3. Occurrence of selected drugs in the water samples

The results of the analysis of the samples showed that all the target analytes were detected in Nairobi River Basin at ng/L–μg/L levels. The maximum and median concentrations in the river waters and WWTPs effluents are illustrated in Fig. 3 while Table 4 gives global comparative concentrations of the target analytes. Comprehensive concentration and frequency of detection data for all sampling points are provided in the Supplementary material (Table S2). The detection frequency of the target compounds ranged from 60% to 97.5% (n = 40). SMX was the most predominant analyte with the detection frequency of 97.5% followed by NVP, ZDV, 3TC, TMP and CIP with the detection frequencies of 95%, 87.5%, 85%, 75%, and 60%, respectively. The detection pattern of the antibiotics and antiretroviral drugs was similar along the sampled hydrological transect. The maximum concentrations were detected in riverine sections, bordering informal settlements, while lower concentrations were detected in the upstream. Ngong River had the highest overall concentrations of all the target analytes and this can be attributed to the contamination emanating from highly populated Kibera and Mukuru informal settlements. The analytes were detected at relatively lower concentration at Athi River Fourteen Falls. This was attributed to the dilution of the waters emanating from Nairobi River Basin by rivers from less populated and contaminated areas.

Among the studied antibiotics, SMX had the highest maximum and median concentration; the overall concentration ranged from <LOQ–13,800 ng/L with a median of 1800 ng/L. The high concentration can be attributed to several factors. Firstly, SMX is one of the most commonly consumed antibiotic in Kenya (K'oreje et al., 2012). It has been recommended for prevention and treatment of opportunistic infections in HIV-infected adults in combination with TMP in a dose ratio of 5:1 (SMX: TMP). Secondly, SMX does not degrade quickly in the aquatic environment (Al-Ahmad et al., 1999; Radke et al., 2009; Straub, 2015).

TMP had the second highest concentration ranging from <LOQ–2650 ng/L with a median of 327 ng/L. It is a commonly consumed antibiotic in combination with SMX as co-trimoxazole due to their effectiveness against a broad range of infections and low cost (Global Antibiotic Resistance Partnership-Kenya Working Group, 2011).

The CIP concentration was significantly lower in the river waters relative to SMX and TMP. The concentration ranged from <LOQ–509 ng/L with a median of 129 ng/L. This lower concentration can be attributed to the lower consumption rate, since CIP is a more expensive antibiotic. It is used to treat infections that become resistant to the regularly used antibiotics (Global Antibiotic Resistance Partnership-Kenya Working Group, 2011).

The concentration of the antibiotics, detected in the studied rivers, was significantly higher compared to similar studies. For example, the maximum TMP concentration was 5.3 ng/L in South Korea (Kim et al., 2007) and 183 ng/L in South Wales, UK (Kasprzyk-Hordern et al., 2008). Similarly, maximum reported concentration for SMX was 11.4 ng/L in River Arno, Italy (Zuccato et al., 2010), 322 ng/L in Buyukcekmece Watershed, Turkey (Aydin and Talinli, 2013). CIP concentration in the present study was comparable with the Laizhou Bay, China at 346 ng/L (Zhang et al., 2012) but much higher than in Finland at 25 ng/L (Vieno et al., 2006) and in River Arno, Italy at

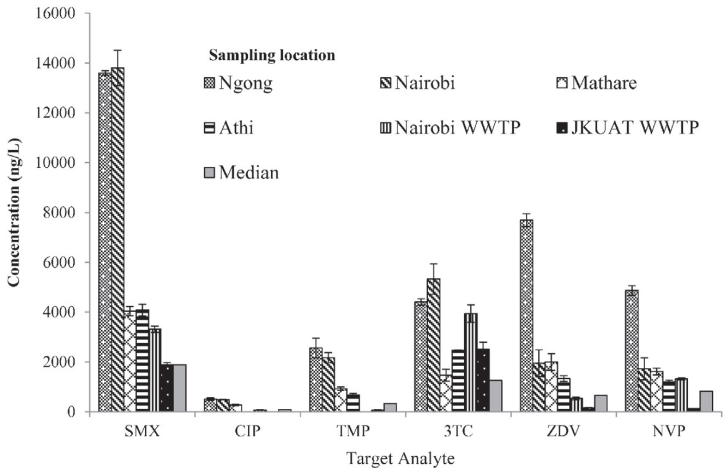

Fig. 3. Maximum and median concentrations of the antibiotics and antiretroviral drugs in the selected rivers and WWTPs. The error bars represent the standard deviation.

Table 4
Global comparison of antibiotics and antiretroviral concentrations (ng/L) in surface water and WWTP effluents.

Location	Sample type	SMX	CIP	TMP	3TC	ZDV	NVP	Reference
Nairobi, Kenya	River water	13,765	509	2650	5428	7684	4859	This study
Nairobi, Kenya	WWTP effluent	3336	67	66	3985	513	1357	This study
Buyukcekmece, Turkey	Lake water	322	13,567	n.a[a]	n.a	n.a	n.a	Aydin and Talinli (2013)
Laizhou Bay, China	River water	527	346	13,600	n.a	n.a	n.a	Zhang et al. (2012)
River Arno and Po, Italy	River water	11.4	37.5	n.a	n.a	n.a	n.a	Zuccato et al. (2010)
Hessian Ried & Ruhr, Germany	River water	n.a	n.a	n.a	Nd[b]	170	17	Prasse et al. (2010)
Nairobi, Kenya	River water	23,350	n.a	9480	3150	18,300	33,440	K'oreje et al. (2012)
Po Valley, Italy	WWTP effluent	317	499	130	n.a	n.a	n.a	Al Aukidy et al. (2012)
Madrid and Almeria, Spain	WWTP effluent	1142	5692	1416	n.a	n.a	n.a	Bueno et al. (2012)
Terrassa, Spain	WWTP effluent	1300	n.a	430	n.a	n.a	n.a	Radjenović et al. (2009)
El Ejido, Spain	WWTP effluent	1100	n.a	na	n.a	n.a	n.a	Muñoz et al. (2009)
Beijing, China	WWTP effluent	460	55	n.a	n.a	n.a	n.a	Gao et al. (2012)
Bangkok, Thailand	WWTP effluent	89	231	25	n.a	n.a	n.a	Tewari et al. (2013)
Valencia, Spain	WWTP effluent	60	1080	100	n.a	n.a	n.a	Gracia-Lor et al. (2012)
Hessian Ried & Ruhr, Germany	WWTP effluent	n.a	n.a	n.a	nd	564	32.1	Prasse et al. (2010)

n.a[a]—compound not analyzed; nd[b]–not detected.

37.5 ng/L (Zuccato et al., 2010). Previous studies by K'oreje et al. (2012) in the Nairobi River Basin reported higher indicative concentrations for SMX and TMP as 23,350 and 9480 ng/L, respectively. The high occurrence within the informal settlements can be attributed to the high disease prevalence especially HIV/AIDS. According to the Kenya HIV Estimates report (2014), the adult prevalence within Nairobi is 8% significantly higher than the national average of 6% (NACC, 2014) this difference can be attributed to the impact of the informal settlements. For example, in Kibera slums it is estimated that about 14% of the residents are infected with HIV, and the situation can be extrapolated to other informal settlements (UmandeTrust et al., 2007).

The concentration of antibiotics in effluent samples from the WWTP was significantly lower, than in the river samples. SMX, CIP and TMP mean concentrations for the samples from Nairobi WWTP were 1940 ± 86 ng/L, 66 ± 13 ng/L and 67 ± 6 ng/L, respectively. At JKUAT WWTP, SMX was the only detected antibiotic with a mean concentration of 3340 ± 90 ng/L. Overall, the effluents from the WWTPs had significantly higher SMX in comparison to those reported in literature. For example, the concentrations of SMX in Terrassa and Almeria, Spain were 1300 ng/L and 1142 ng/L in (Radjenović et al., 2009; Bueno et al., 2012), and elsewhere (Xu et al., 2007; Muñoz et al., 2009; Al Aukidy et al., 2012; Gao et al., 2012; Gracia-Lor et al., 2012; Tewari et al., 2013; Jiang et al., 2014). As for CIP and TMP, the concentration falls within the reported concentration range (Vieno et al., 2006; Gulkowska et al., 2007; Kim et al., 2007; Al Aukidy et al., 2012; Bueno et al., 2012; Gracia-Lor et al., 2012; Tewari et al., 2013). The relatively lower concentrations of the antibiotics can be attributed to the removal of the drugs during the wastewater treatment process which have been shown to remove up to 70% of the studied antibiotics (Kasprzyk-Hordern et al., 2009; Watkinson et al., 2009; Sui et al., 2010). Further the WWTP studied is based on stabilization ponds with high hydraulic retention time leading to a possibility of significant removal of the target analytes by photodegradation under the tropical solar irradiation (Andreozzi et al., 2003; Abellán et al., 2009; Keen and Linden, 2013). In addition to removal efficiency, the WWTP in Nairobi serves mostly the up market business and residential areas where population density and disease burden is not high.

The detection frequency and the maximum concentration of the three analyzed antiretroviral drugs did not show great variability. The concentration for 3TC, ZDV and NVP ranged from <LOQ–5430 ng/L, <LOQ–7680 ng/L and <LOQ–4860 ng/L with median concentrations of 1000 ng/L, 660 ng/L and 769 ng/L, respectively. This data is consistent with the consumption figures since the drugs constitute the first line daily dose antiretroviral regimen for people living with HIV and under antiretroviral therapy (NACC, 2014). This class of drugs has not been extensively studied in rivers and wastewater apart from studies reported for some rivers and WWTPs in Germany (Prasse et al., 2010). For instance, in Hessian Ried & Ruhr watershed, Germany 3TC was not detected but ZDV and NVP concentrations were 170 and 17 ng/L, respectively (Prasse et al., 2010). In the Nairobi River Basin, indicative concentrations of these drugs were previously studied and the maximum concentration measured were 3150 ng/L, 18,300 ng/L and 33,440 ng/L for 3TC, ZDV and NVP, respectively (K'oreje et al., 2012). The concentrations measured in the Nairobi River Basin by K'oreje et al. (2012) and in the present study vary considerably. This could be because in the study by K'oreje et al. (2012), the concentrations were indicative and, thus, cannot be used as the basis for concentrations comparison with certainty. It is, however, clear from the two studies that the Nairobi River Basin is significantly contaminated by antiretroviral drugs.

3.4. Risk assessment

The environmental risk assessment based on the calculated risk quotient for the maximum and median concentrations for the six analytes and three trophic levels are presented in Fig. 4 and Table S5 in the supplementary material.

The results show that, apart from 3TC whose RQ values were all negligible, all the other analytes had at least one taxonomic group with RQ > 1. Algae were found to be the most sensitive taxonomic group with a maximum RQ of 508.7, 271.5, 30.0, 29.1 and 2.32 for SMX, ZDV, CIP, NVP and TMP, respectively. The maximum detected concentration for SMX and NVP had RQ > 1 for daphnia and fish. RQ computed from median concentration were also significantly high, for SMX, NVP and CIP they were greater than 1 for algae. Further, the RQ takes into account only the parent molecule, however, it is only approximately 22.5% SMX, 50% TMP, 40% CIP, 70% 3TC, 25% ZDV and 2.7% NVP that are excreted unchanged (Singlas and Pioger, 1989; Johnson et al., 1999; Riska et al., 1999; Göbel et al., 2005; Wagenlehner et al., 2006; Voloshenko-Rossin et al., 2015), while the rest are excreted as metabolites that can show a pharmacological activity similar to the parent molecule (Besse et al., 2008). This indicates that the risk of contamination of the Nairobi River Basin by the analytes is high and the impact to the environment and human health by extension might be substantially higher than estimated. In addition, the risk was considered for individual compound, but it should be noted that pharmaceutically active compounds normally are simultaneously present in the environment, which increases the overall risk via the cocktail effect (Escher et al., 2011).

4. Conclusions

In this study we investigated the occurrence and risks of antibiotics and antiretroviral drugs in Nairobi River Basin. All the investigated

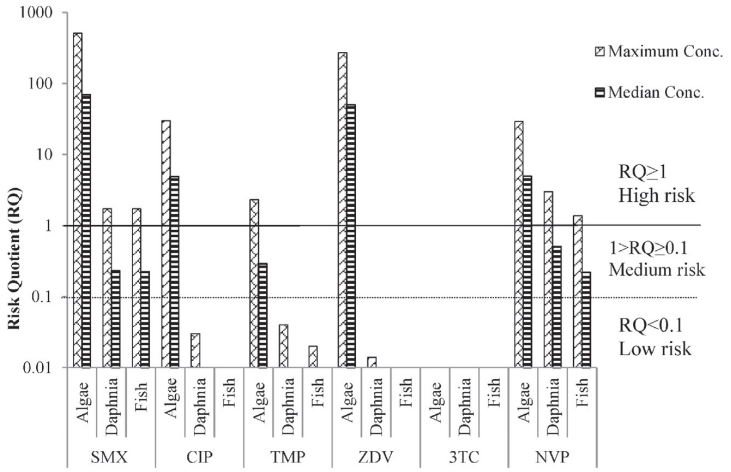

Fig. 4. Risk quotient for algae, daphnia and fish calculated using the measured maximum and median concentrations for the target analytes in the Nairobi River Basin.

antibiotics and antiretroviral drugs were detected in the river basin in ng/L–μg/L scale. Lack of access to wastewater management systems for majority of city residents, expansion of informal settlements and high prevalence of diseases (especially HIV/AIDS) contribute significantly towards the high antibiotic and antiretroviral loads in the Nairobi River Basin. The concentrations measured in this study are several orders of magnitude higher as compared to the developed world. The environmental risk assessment showed that most of the analytes posed medium to high risk for the selected aquatic organisms. From this study, it is anticipated that many other pharmaceuticals might occur at concentration levels exceeding the PNEC; thus, further comprehensive studies on seasonal variations, environmental fate of the parent molecules and metabolites, ecological and human health effects and risk control measures are recommended. In addition, incorporation of isotopically labeled internal standards in future studies can greatly improve the accuracy and reduce on the analysis time.

Acknowledgments

This work was financially supported by the University of Jyvaskyla Doctoral Program in Biological and Environmental Science. We thank Akenga Preston for the assistance given during sampling and sample preparation work in JKUAT, Kenya.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.08.139.

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SUPPLEMENTARY MATERIAL

Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya

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Table S1: Precise description of all sampling sites in Nairobi River Basin

Latitude	Longitude	Elevation	Abbreviation	Location (Description)
-1.2534	36.8217	5496	MR1a	Mathare River 1a (Limuru road)
-1.2630	36.8374	5394	MR1b	Mathare River 1b (Muranga road)
-1.2641	36.8490	5365	MR1c	Mathare River 1c (Entrance to slums)
-1.2638	36.8544	5314	MR1d	Mathare River 1d (Bondeni)
-1.2625	36.8593	5274	MR1e	Mathare River 1e (Confluence with NYS tributary),
-1.2470	36.8160	5531	MR2a	Mathare River 2a (NYS upstream),
-1.2588	36.8515	5359	MR2b	Mathare River 2b (NYS Tributary Thika Road)
-1.2610	36.8547	5304	MR2c	Mathare River 2c (NYS Tributary mid-stream)
-1.2642	36.8490	5372	MR3	Mathare Well 3
-1.2023	36.8968	5089	NR1a	Nairobi River 1a (Gathara-ini Kamiti road)
-1.2116	36.9041	5125	NR1b	Nairobi River 1b (Gathara-ini Thika road)
-1.2319	36.9271	5044	NR1c	Nairobi River 1c (Gathara-ini Mwiki tributary)
-1.2248	36.9901	4928	NR1d	Nairobi River 1d (Gathara-ini Eastern bypass)
-1.1843	36.8948	5045	NR2a	Nairobi River 2a (KIU up stream)
-1.1948	36.9034	5032	NR2b	Nairobi River 2b (KIU Northern bypass)
-1.1990	36.9207	5057	NR2c	Nairobi River 2c (Kiu Githurai)
-1.2074	36.9936	4917	NR2d	Nairobi River 2d (Kiu downstream)
-1.2749	36.9463	5158	NR3	Nairobi River 3 (Mihango tributary)
-1.2759	36.7345	5753	NR4a	Nairobi River 4a (Naivasha road)
-1.2674	36.7741	5696	NR4b	Nairobi River 4b (James Gichuru),
-1.2747	36.8119	5502	NR4c	Nairobi River 4c (Museum Hill)
-1.2750	36.8113	5498	NR4d	Nairobi River 4d (Museum Hill tributary)
-1.2811	36.8316	5430	NR4e	Nairobi River 4e (Racecourse)
-1.2433	36.9410	4995	NR4f	Nairobi River 4f (Njiru)
-1.2446	36.9888	4937	NR4g	Nairobi River 4g (Ruai Eastern bypass)
-1.2368	37.0118	4890	NR4h	Nairobi River 4h (Before WWTP)
-1.2218	37.0308	4862	NR4i	Nairobi River 4i (after WWTP)
-1.2276	37.0247	4887	NR5	Nairobi River 5 (WWTP Effluent)
-1.3092	36.7717	5753	KR1a	Ngong River 1a (Jamuhuri ground exit)
-1.3166	36.7751	5690	KR1b	Ngong River 1b (motoine confluence)
-1.3174	36.7786	5655	KR1c	Ngong River 1c (soweto)
-1.3170	36.7832	5637	KR1d	Ngong River 1d (Katwekera)
-1.3097	36.8288	5455	KR1e	Ngong River 1e (Mombasa Road)
-1.3076	36.8379	5419	KR1f	Ngong River 1f (Mukuru Fuata Nyayo)
-1.3106	36.8431	5391	KR1g	Ngong River 1g (Mukuru Kaiyaba)
-1.3160	36.8541	5347	KR1h	Ngong River 1h (likoni bridge)
-1.2498	36.9455	4995	KR1i	Ngong River 1i (Njiru downstream)
-1.0796	37.2465	4631	AR1	Athi River 1 (Ndonyo Sabuk Bridge)
-1.0802	37.2509	4596	AR2	Athi River (14 falls)
-1.0968	37.0250	4991	JKU	JKUAT WWTP

Table S2: Concentrations and detection frequency of the selected antibiotics and antiretroviral drugs for all sampling sites in Nairobi River Basin

	TMP	CIP	SMX	3TC	ZDV	NVP
MR1a	98(31)[a]	<LOQ[b]	564(23)	260(24)	136(63)	52(28)
MR1b	927(69)	144(16)	1870(477)	750(158)	335(3)	438(6)
MR1c	456(106)	218(30)	1550(51)	1470(83)	2000(334)	1550(318)
MR1d	509(40)	275(36)	4040(180)	1390(119)	1690(256)	1620(138)
MR1e	387(19)	<LOQ	1700(289)	1400(238)	1710(268)	882(18)
MR2a	<LOQ	<LOQ	46(15)	<LOQ	<LOQ	<LOQ
MR2b	512(54)	178(15)	652(108)	425(20)	961(104)	155(35)
MR2c	361(30)	<LOQ	140(52)	376(64)	1230(50)	75(11)
MR3	80(11)	88(34)	1737(95)	40(5)	274(12)	1026(41)
NR1a	<LOQ	<LOQ	32(11)	<LOQ	138(73)	60(1)
NR1b	146(29)	<LOQ	1790(157)	1310(183)	439(10)	641(78)
NR1c	<LOQ	<LOQ	162(39)	136(55)	<LOQ	256(24)
NR1d	<LOQ	<LOQ	2490(281)	291(6	189(18)	180(42)
NR2a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
NR2b	<LOQ	<LOQ	701(267)	<LOQ	100(40)	34(27)
NR2c	99(13)	65(12)	1030(325)	1020(79)	680(143)	414(53)
NR2d	<LOQ	<LOQ	2360(28)	498(73)	<LOQ	73(12)
NR3	<LOQ	<LOQ	31(22)	<LOQ	<LOQ	107(16)
NR4a	249(41)	79(21)	1680(14)	1440(194)	263(16)	776(298)
NR4b	297(29	40(11)	1920(168)	1320(547)	384(1)	943(205)
NR4c	869(7)	155(34)	2030(57)	296(54)	565(5)	574(20)
NR4d	781(24)	124(9)	1540(216)	<LOQ	930(132)	694(106)
NR4e	464(120)	175(33)	1940(201)	642(26)	674(66)	601(33)
NR4f	<LOQ	488(1)	4120(763)	2370(58)	1080(343)	1460(202)
NR4g	2170(203)	256(20)	5610(655)	1900(362)	1950(535)	1330(99)
NR4h	189(64)	330(30)	13800(708)	5330(613)	1830(128)	1720(438)
NR4i	522(87)	226(62)	8130(415)	4460(14)	921(7)	1490(86)
KR1a	69(4)	<LOQ	1240(17)	839(41)	644(92)	595(7)
KR1b	873(207)	125(2)	5620(278)	3300(620)	5160(425)	2030(127)
KR1c	675(51)	146(10)	8530(46)	3420(226)	4190(198)	1930(66)
KR1d	795(51)	204(39)	13560(90)	4400(127)	7690(265)	3970(832)
KR1e	453(96)	509(48)	4550(341)	2370(712)	881(194)	4870(194)
KR1f	2060(463)	311(104)	2440(312)	1460(24)	979(233)	1390(89)
KR1g	626(107)	286(4)	1780(179)	983(267)	424(139)	962(280)
KR1h	2560(397)	470(13)	1800(145)	1210(75)	1170(121)	982(284)
KR1i	86(7)	255(31)	8430(561)	1300(41)	480(52)	2430(31)
NR5	61(11)	66(9)	1900(69)	3940(352)	532(60)	1320(39)
JKU	<LOQ	<LOQ	3320(123)	2510(287)	164(11)	128(8)
AR1	625(63)	<LOQ	4240(120)	2470(126)	1420(112)	1150(42)
AR2	712 (34)	<LOQ	3920(92)	2480 (139)	1250 (205)	1250 (166)
Detection Frequency (%)	75	60	97.5	85	87.5	95

[a]Concentration in ng/L (sd) (n=3) and [b] <LOQ- Not detected/Below limit of quantification

1 Table S3: Structures of the antibiotics and antiretroviral drugs determined in Nairobi River Basin

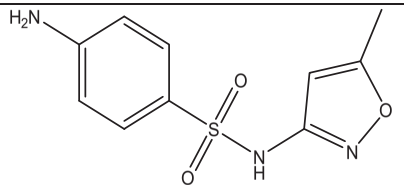
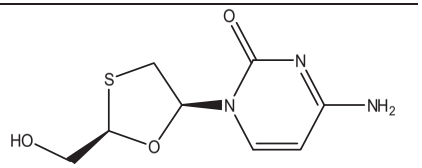
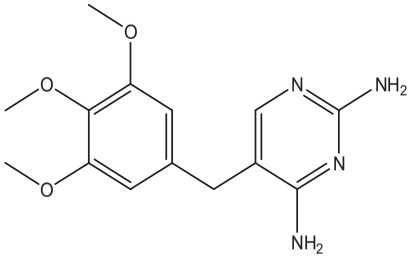
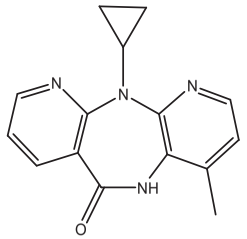
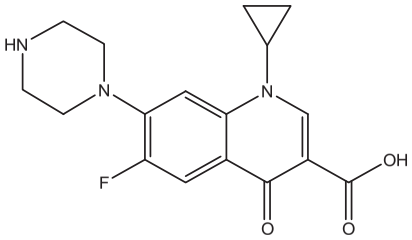
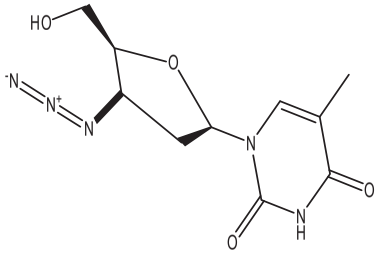
Antibiotics	Structure	Antiretrovirals	Structure
Sulfamethoxazole (SMX) Mw. 253.28		**Lamivudine** (3TC) Mw. 229.26	
Trimethoprim (TMP) Mw. 290.32		**Nevirapine** (NVP) Mw.266.30	
Ciprofloxacin (CIP) Mw. 331.35		**Zidovudine** (ZDV) Mw. 267.25	

Table S4: Physical chemical parameters for selected samples

Location	pH	Conductivity (µS/ cm)	TOC (mg/L)	TN (mg/L)
Nairobi River 2c	7.48	770	19.41	22.48
Nairobi River 4c	7.52	450	5.95	6.8
Mathare River 1b	7.8	890	33.24	13.6
Mathare River 2b	7.64	420	4.05	1.95
Nairobi River 4g	7.7	1020	17.11	17.61
Ngong River 1i	7.9	1100	32.4	22.43
Nairobi River 5 (WWTP Effluent)	8.13	1080	23.78	32.11

Table S5: Risk quotient (RQ) for algae, daphnia and fish calculated using the measured maximum and median concentrations for the target analytes in Nairobi River Basin

Compound	Taxonomic group	PNEC (µg/L)	Reference	Calculated RQ (Median)
SMX	Algae	0.027	(Ferrari et al., 2004)	508.7(69.94)
	Daphnia	12.97	(Mutiyar and Mittal, 2014)	1.72(0.23)
	Fish	8	(Mutiyar and Mittal, 2014)	1.725(0.225)
CIP	Algae	0.017	(Li et al., 2013)	30(4.89)
	Daphnia	15.51	(Li et al., 2013)	0.03(0.01)
	Fish	562.5	(Li et al., 2013)	0
TMP	Algae	11	(Ando et al., 2007)	2.32(0.29)
	Daphnia	54.8	(Park and Choi, 2008)	0.04(0)
	Fish	100	(Mutiyar and Mittal, 2014)	0.02(0)
ZDV	Algae	0.02	ECOSAR[a]	271.5(50)
	Daphnia	539.7	ECOSAR	0
	Fish	318.28	ECOSAR	0
3TC	Algae	7927.3	ECOSAR	0
	Daphnia	332.1	ECOSAR	0
	Fish	47662.1	ECOSAR	0
NVP	Algae	0.167	ECOSAR	29.14(4.96)
	Daphnia	1.621	ECOSAR	3(0.51)
	Fish	3.523	ECOSAR	1.38(0.22)

ECOSAR[a]- Ecological Structure Activity Relationships (ECOSAR v1.10) software from U.S. Environmental Protection Agency

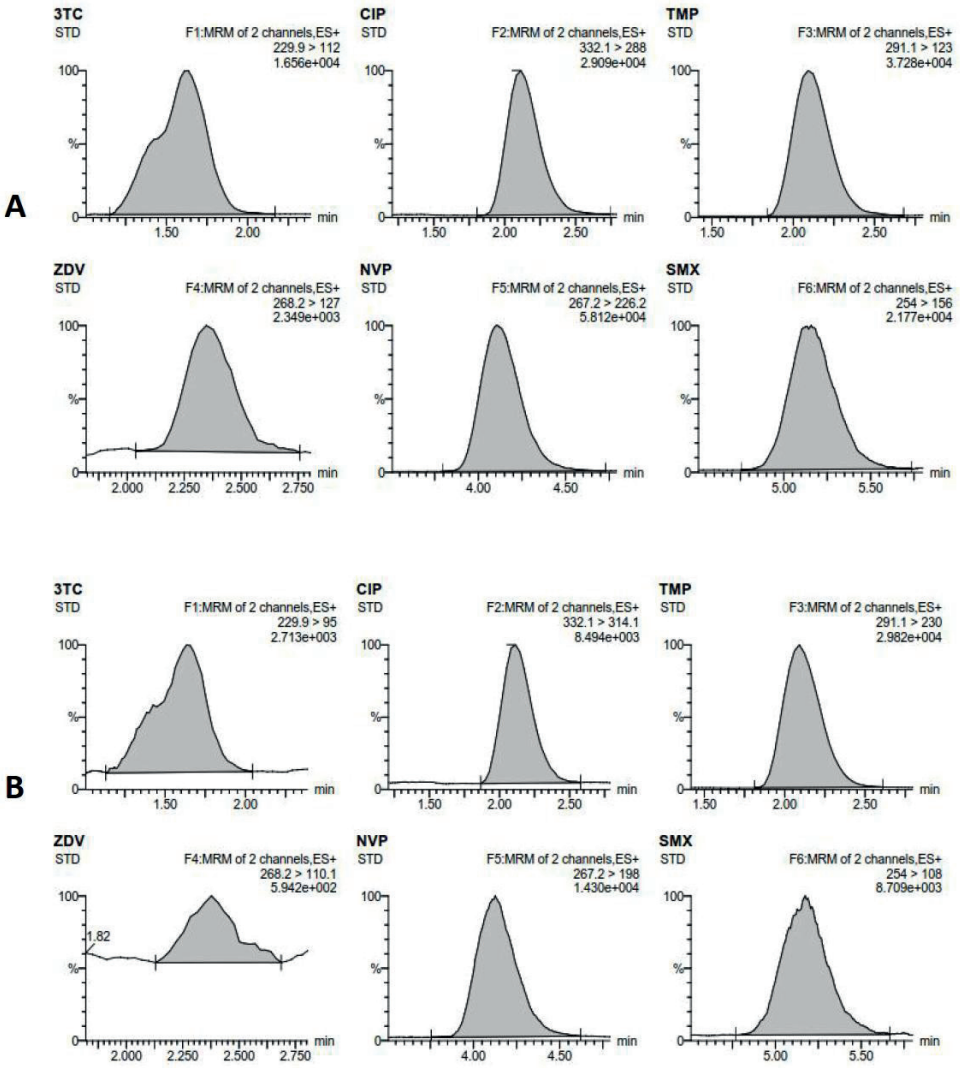


Figure S1: LC–MS/MS chromatogram showing the retention time and the two MRM transitions for a mixed standard. Transition A was used in quantification and B for confirmation

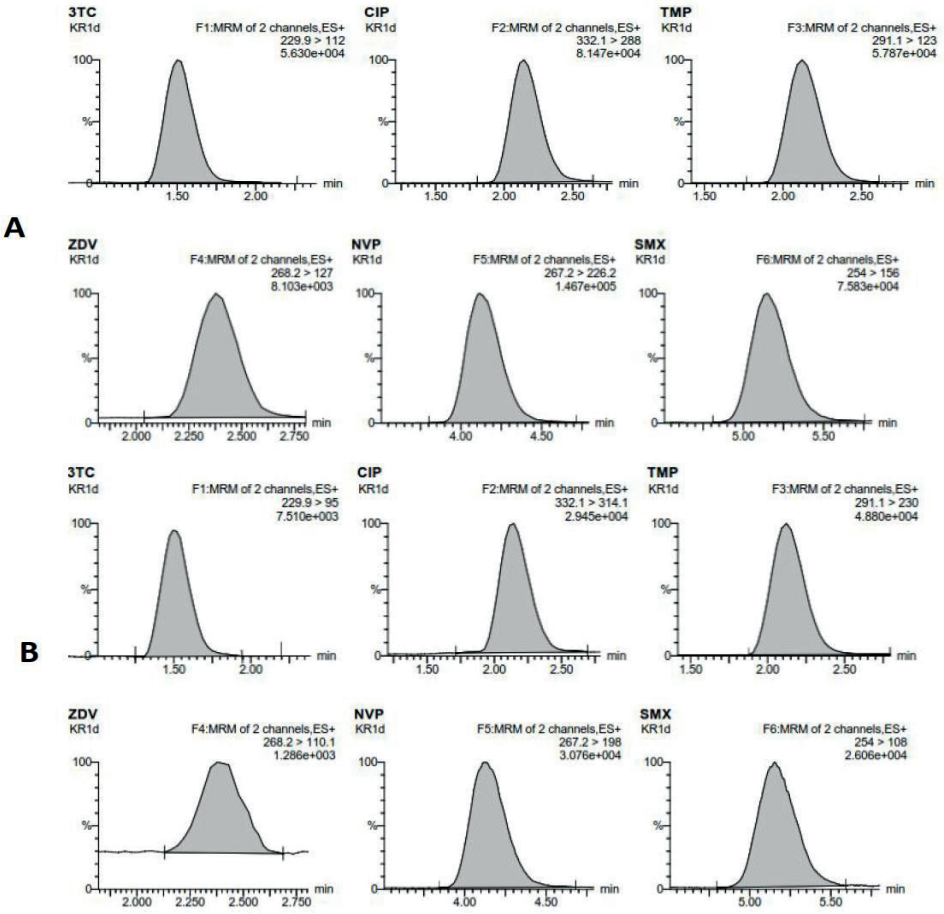


Figure S2: LC–MS/MS chromatogram showing the retention time and the two MRM transitions for one of the river water extract

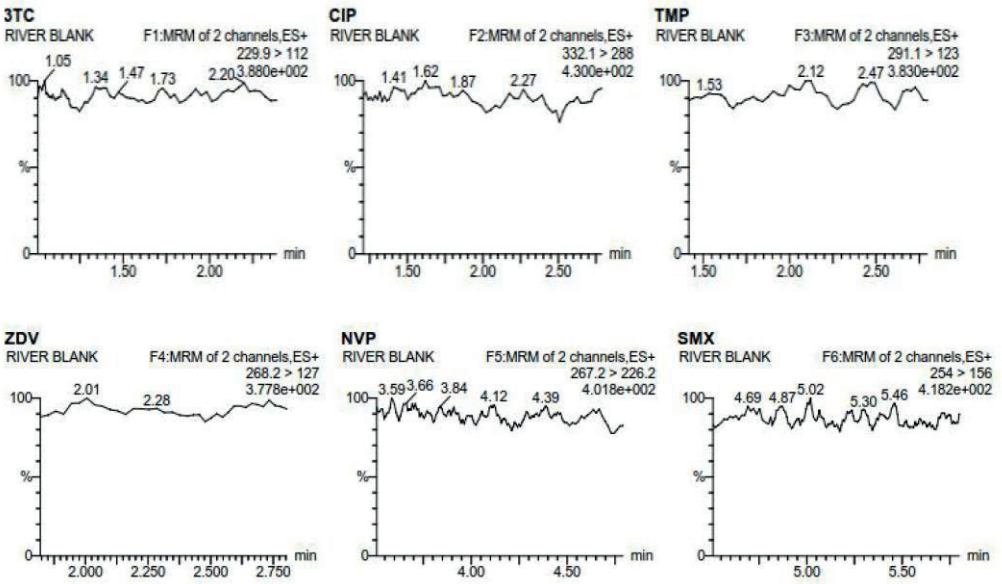


Figure S3: LC–MS/MS chromatogram showing extract from the river water used to prepare matrix matched standards

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II

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

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2016

Analytical Methods 8, 6720–6729.

Available at:
http://urn.fi/URN:NBN:fi:jyu-201611184665

III

OCCURRENCE OF ANTIBIOTICS AND ANTIRETROVIRAL DRUGS IN SOURCE SEPARATED URINE, GROUNDWATER SURFACE WATER AND WASTEWATER IN THE PERI–URBAN AREA OF CHUNGA IN LUSAKA, ZAMBIA

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Manuscript

IV

REMOVAL OF SELECTED ANTIBIOTICS AND ANTIRETROVIRAL DRUGS DURING POST–TREATMENT OF MUNICIPAL WASTEWATER WITH UV, UV/CHLORINE AND UV/HYDROGEN PEROXIDE

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