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Residue levels and discharge loads of antibiotics in wastewater treatment plants (WWTPs), hospital lagoons, and rivers within Lake Victoria Basin, Kenya

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Abstract The detection of antibiotics in water systems has instigated great environmental concern due to the toxicological effects associated with these compounds. Their discharge into the environment results from the ubiquity of use in medical, veterinary, and agricultural practices. Some of the effects of antibiotics include development of antibiotic-resistant bacteria, making it difficult to treat diseases, variation in natural microbial communities, and enzyme activities. In this study, the first comprehensive survey of some frequently used antibiotics namely ampicillin (AMP), amoxicillin (AMX), sulfamethoxazole (SMX), chloramphenicol (CAP), and ciprofloxacin (CPF) within Lake Victoria Basin of Kenya is presented. Sludge and wastewater samples were collected from wastewater treatment plants (WWTPs) and hospital lagoons within the study area. Samples were extracted and cleaned by solid-phase extraction, and analysis was carried out using high-performance liquid chromatography (HPLC). All wastewater samples and sludge collected contained quantifiable levels of the selected antibiotics. The highest concentrations were recorded for AMP with WWTPs and hospitals having 0.36 ± 0.04 and 0.79 ± 0.07 μg/L, respectively. In sludge samples, SMX recorded the highest concentrations of 276 ± 12 ng/g. The high levels in sludge indicate the preferential partition of antibiotics onto solid phase, posing great danger to consumers of crops grown in biosolid-amended soils. The daily discharge loads of antibiotics from nine WWTPs ranged between 80.75 and 3044.9 mg day^{-1} with a total discharge of 6395.85 mg day^{-1}, signifying a high potential of water resource pollution within the region. This report will aid in the assessment of the risks posed by antibiotics released into the environment.

Keywords Antibiotic residues · Wastewater treatment plants · Sludge · Pharmaceuticals · Discharge loads · Risk assessment

Introduction

Antibiotics are getting attention as a new class of emerging pollutants in aquatic environment. Their toxic effects on human, animal, and ecosystem health are not well documented (Yang et al. 2011; Cizmas et al. 2015). These compounds are discharged into the environment as a result of the ever-increasing and often indiscriminate use of antibiotics in medical, veterinary, and agricultural practices (He et al. 2014). After administration, antibiotics can be transformed into more polar and soluble forms as metabolites or conjugates of glucuronic and sulfuric acids

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(Heberer et al. 2002). Antibiotics and their metabolites are readily excreted through urine and feces. They eventually enter into wastewater treatment plants, providing a potential route of antibiotics loading into aquatic environment (Straub 2015). Dispersion of antibiotics into different environmental compartments may result from various sources such as municipal sewage, hospital wastewater, and effluent of wastewater treatment plants (Bottoni and Caroli 2015; Gurke et al. 2015). Recent studies have reported the accumulation of some antibiotics in sewage sludge, posing great danger to consumers of crops in sludge-amended soils (Jelic et al. 2011). In addition, other studies have shown the presence of these compounds in public drinking water wells where septic systems are prevalent (Ternes et al. 2001; Dinsdale et al. 2009). This raises a lot of concerns due to the recalcitrance of these chemicals to conventional wastewater treatment technologies and their toxicities (Clara et al. 2005; Li 2014). In the environment, pharmaceuticals are reported to have adverse effects on aquatic ecosystem, wildlife, and human health (Lemus et al. 2008; Gao et al. 2012; Helba et al. 2016). For example, carbamazepine has been reported to induce oxidative effects on cells of non-target species, such as mussels, hence affecting their health status (Tsiaka et al. 2013). Other pharmaceuticals are reported to have adverse effects on the renal system, resulting in kidney failure (Godoy et al. 2015). In addition, a study by Matozzo (2014) cited antibiotics causing variation of immune responses in aquatic invertebrates. In addition, some antibiotics are reported to induce pathogen resistance, posing great challenges in treatment of diseases (Gracia-Lor et al. 2012).

Among the frequently detected antibiotics in water resources worldwide are sulfonamides, tetracyclines, β-lactams, and macroclides (Liang et al. 2013; Li 2014; Matozzo 2014). In Kenya, some of the widely used antibiotics include but not limited to ampicillin, amoxicillin, sulfamethoxazole, chloramphenicol, and ciprofloxacin, which have been detected in water systems within Nairobi River (K'oreje et al. 2012). Another study by K'oreje et al. (2016) reported high levels of antibiotics and antiretroviral residues in water systems in Nairobi and Kisumu City, Kenya. Currently, there is limited literature on the levels of antibiotic residues in tropical systems especially the Lake Victoria Basin (LVB) of Kenya. The rapidly growing population and rapid urbanization within Lake Victoria catchment are causing strain to the limited waste disposal facilities available. Trace micropollutants such as perfluorinated surfactants, polyaromatic hydrocarbons, and pesticides have been detected within the LVB, indicating general pollution of the region (Chirikona et al. 2015; Lisouza et al. 2011; Werimo et al. 2009; Orata et al. 2009). Therefore, there is an urgent need to document the concentrations of antibiotics in order to assess the potential risks to aquatic ecosystem and human health. This study therefore reports, for the first time, the levels and discharge loads of five most commonly used antibiotics (ampicillin, amoxicillin, sulfamethoxazole, chloramphenicol, and ciprofloxacin) within LVB.

Materials and methods

Chemicals, standards and reagents

High-purity standards for chloramphenicol, ampicillin, sulfamethoxazole, ciprofloxacin, and amoxicillin were purchased from Sigma-Aldrich, Germany. Analytical-grade and high-performance liquid chromatography (HPLC)-grade water, methanol, and acetonitrile for extraction and analysis were purchased from Sigma-Aldrich through Kobian Kenya Limited. Solid-phase extraction cartridges and nylon microfilters were obtained from Estec Kenya Limited. All stock solutions were prepared using HPLC-grade methanol.

Study area and sampling

The study was conducted in major towns namely Kisii, Homabay, Kisumu, Kakamega, Mumias, Webuye, Bungoma, and Eldoret within LVB in Kenya (Fig. 1). Sludge and wastewater were collected from hospital lagoons and wastewater treatment plants (WWTPs) between October 2014 and September 2015. The selected WWTPs are used to treat wastewaters originating from domestic, hospital, and industrial areas, while their characteristics are presented in Table 1. During sampling, it was noted that effluents from the WWTPs were being discharged into rivers, which are tributaries of Lake Victoria. Wastewater samples were collected in 2-L amber glass bottles and taken to the laboratory in an ice box. The samples were then refrigerated at −4 °C prior to extraction within 48 h. Sludge samples were also collected, air-dried, ground, and passed through 200-mm sieve prior to extraction.

Extraction of samples for residue analysis

Wastewater samples were extracted by passing 300 ± 3.0 mL aliquots through a Phenomenex C_{18}

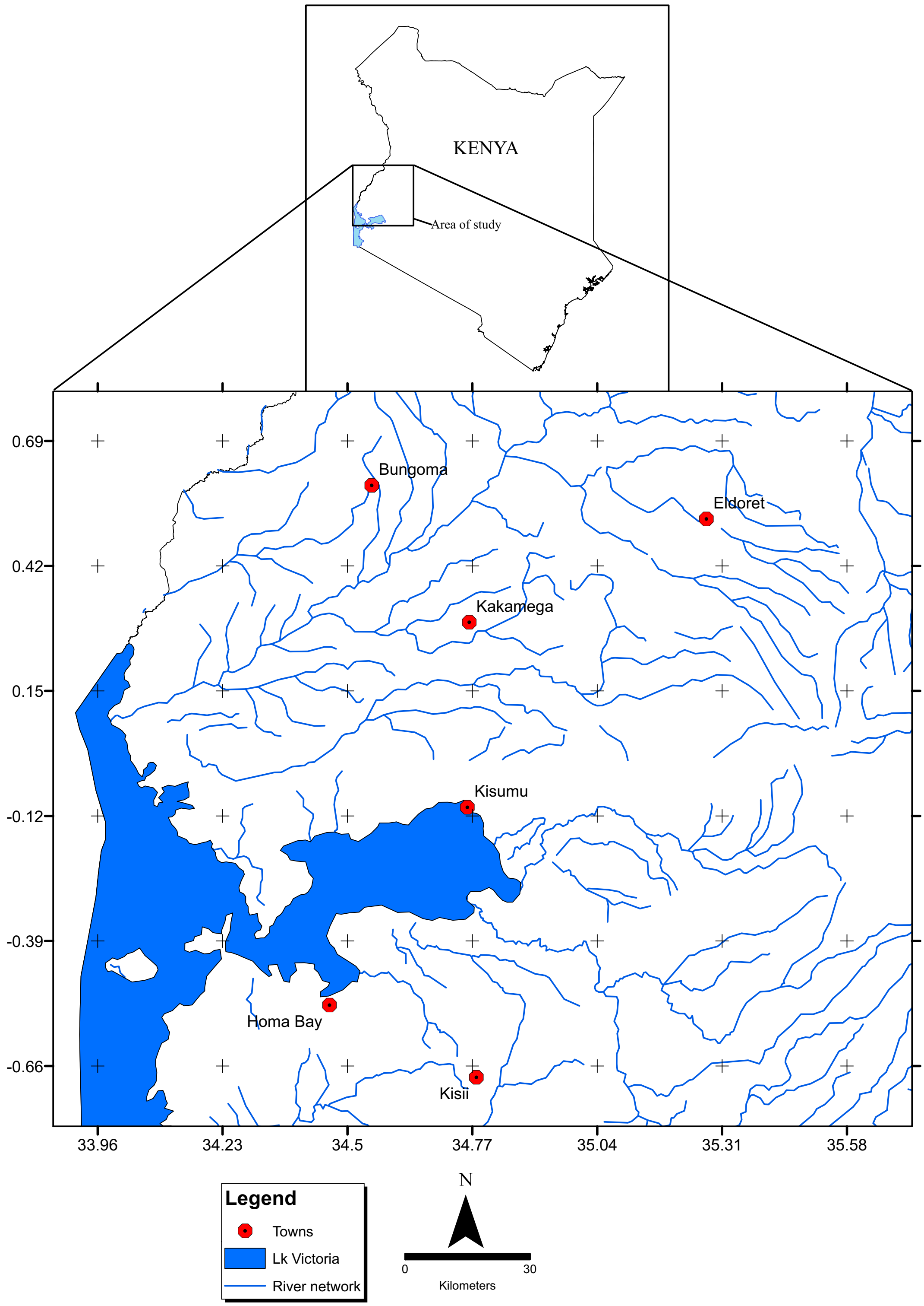

Fig. 1 A map of Lake Victoria Basin (Kenya) showing various towns where sampling was conducted

solid-phase extraction (SPE) cartridge that had been preconditioned with 10 mL of methanol and distilled water, successively. Elution was carried out using 10 mL of HPLC-grade methanol, which was then evaporated to

Table 1 Characteristics of WWTPs where samples were collected

Location of WWTP		Population served	Type of wastewater treated	Capacity (C_{WWTP}) (M^3 day^{-1})
Eldoret		165,450	Domestic	1600
Eldoret Hospital			Hospital	–
Bungoma		81,151	Domestic	1500
Bungoma Hospital			Hospital	–
Busia		61,715	Domestic	600
Kakamega	Kakamega Hospital	99,987	Hospital	–
	Shirere		Domestic	800
	Nabongo		Domestic	–
	MMUST		Institutional	425
Kisumu	Nyalenda	409,908	Domestic	6050
	Kisat		Domestic and industrial	2450
Homabay		54,040	Domestic	720
Kisii		83,460	Domestic	1400

Source: Technical report of the Lake Victoria North and South Water Services Board 2012

– Capacities not known

dryness under vacuum. The residue was redissolved to 2 mL with HPLC-grade methanol (Van der Ven et al. 2004). All samples were filtered using 0.45-μm nylon microfilters prior to injection to LC instrument. Sludge samples were extracted by weighing 0.5 ± 0.05 g of dry sample into a conical flask and adding 15 mL of HPLC-grade methanol. The samples were shaken for 8 h in an orbital shaker at 100 rpm, after which, the supernatant was filtered through glass wool that had been preconditioned with 10 mL of HPLC-grade methanol. The filtrate was evaporated to dryness and sample re-dissolved in 100 mL distilled water. Cleanup of samples was performed by SPE following the same procedure for wastewater samples (Nguyen et al. 2015).

Analysis by HPLC

A Shimadzu LC-20AD fitted with a SIL-20A (HT) autosampler and a SPD-20A prominence ultraviolet-visible (UV) detector was used for HPLC analysis. A reverse-phase Phenomenex (C_{18}) column (4.6-mm i.d. × 250 mm, 5-μm particle) was used for separation of the analytes (Van der Ven et al. 2004). Gradient elution was carried out using a mobile phase consisting of (50: 50 *v*/*v*) HPLC-grade acetonitrile/methanol (A) and water with 0.1 % formic acid (B) at a flow rate of 0.30 mL/min. The gradient program was set as follows: (0–3 min), A = 90 %; 8 min, A = 65 %; 17 min, A = 50 %; 20 min, A = 0 %; and 30 min, A = 90 %. Quantification was done using a UV detector at 250-nm wavelength (Park and Choung 2007). Analyte identification was based on comparison of chromatograms of unknowns with those of standards. Standards and blanks were measured periodically throughout the analysis for quality assurance. Quantitative analysis of antibiotics was achieved through the integration of selected HPLC chromatograms. All analyses were carried out in triplicate.

Discussions

The levels of selected antibiotics in sludge and sediment samples from hospitals, WWTPs, rivers, and streams within LVB, Kenya

All sludge samples contained quantifiable levels of the selected antibiotics. The mean concentrations in sludge and sediment samples from ten WWTPs and two hospitals, rivers, and streams within LVB are presented in Tables 2 and 3.

The average antibiotic concentrations in the sludge and sediment samples varied for individual compounds and amongst samples from different sources (Tables 2 and 3). The mean residue levels for all the five antibiotics in sludge were found to range from <50 to 276 ± 12 ng/g, while sediment samples recorded lower levels ranging from <50 to 94 ± 3 ng/g. This may be attributed to

Table 2 The distribution of selected antibiotics in sludge (ng/g) from WWTPs and hospitals in major urban towns within Lake Victoria Basin, Kenya

Study site	CPF	AMP	AMX	SMX	CAP
Bungoma WWTP	145 ± 9	143 ± 15	<LOQ	103 ± 7	106 ± 6
Bungoma Hospital	95 ± 6	231 ± 21	79 ± 10	205 ± 9	113 ± 8
Busia WWTP	94 ± 4	53 ± 6	<LOQ	154 ± 9	96 ± 8
Shirere WWTP	82 ± 6	<LOQ	<LOQ	105 ± 6	79 ± 7
Mumias Hospital	165 ± 8	151 ± 10	55 ± 5	276 ± 12	<LOQ
Homabay WWTP	82 ± 7	74 ± 3	<LOQ	121 ± 5	54 ± 3
Nabongo WWTP	98 ± 7	57 ± 8	<LOQ	94 ± 4	66 ± 11
MMUST WWTP	56 ± 2	<LOQ	<LOQ	89 ± 7	<LOQ
MMUST stream	76 ± 4	88 ± 9	50 ± 6	83 ± 5	84 ± 10
Kisat WWTP	92 ± 3	74 ± 5	<LOQ	101 ± 5	65 ± 9
Eldoret WWTP	67 ± 6	112 ± 6	<LOQ	84 ± 3	102 ± 7
Kisii WWTP	106 ± 5	108 ± 11	<LOQ	103 ± 2	<LOQ
Homabay WWTP	82 ± 7	74 ± 3	<LOQ	121 ± 5	54 ± 3

LOQ = 50 ng/g, $n = 3$

<*LOQ* less than limit of quantification

dilution in the river environment (Table 2). The maximum amount of antibiotics recorded was 276 ± 12 ng/g sulfamethoxazole (SMX) in sludge discharged from Mumias Hospital general ward (Table 2). The high levels of SMX in the sludge samples may be due to prescription patterns and the intrinsic properties of the compound. The low octanol-water partition of SMX (log *K*ow = 0.89) makes it preferentially adsorb onto sludge (Liang et al. 2013). Notably, amoxicillin levels were generally lower compared to the other antibiotics. This may be due to the unstable β-lactam ring in amoxicillin, which can be degraded by chemical hydrolysis and β-lactamase-producing bacteria present in WWTPs (Chagas et al. 2011; Kihampa 2014).

Table 3 The distribution of selected antibiotics in sediment samples (ng/g) from rivers and streams within Lake Victoria Basin, Kenya

Study site	CPF	AMP	AMX	SMX	CAP
MMUST stream (upstream)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MMUST stream (downstream)	51 ± 6	<LOQ	<LOQ	65 ± 5	<LOQ
Auji River	62 ± 7	74 ± 5	<LOQ	61 ± 6	54 ± 8
Kisat River (upstream)	<LOQ	52 ± 9	<LOQ	63 ± 3	59 ± 7
Kisat River (downstream)	62 ± 5	66 ± 6	<LOQ	85 ± 8	69 ± 4
Sosiani River (upstream)	<LOQ	54 ± 4	<LOQ	51 ± 5	54 ± 7
Sosiani River (downstream)	72 ± 7	94 ± 3	<LOQ	81 ± 5	94 ± 2

LOQ = 50 ng/g, $n = 3$

<*LOQ* less than limit of quantification

Antibiotic residues in river water and effluents from hospital lagoons and WWTPs within LVB, Kenya

Antibiotics were widely detected in effluents from wastewater treatment plants within the study region as shown in Table 4. To the best of our knowledge, this is the first survey on antibiotic residues within the LVB of Kenya.

Antibiotic levels in hospital effluents were high ranging from <0.05 to 0.79 ± 0.07 μg/L. Eldoret Hospital recorded the highest concentrations (ampicillin (AMP) = 0.79 ± 0.07 μg/L). This shows that hospitals are hot spots for antibiotic discharge into WWTPs. The mean antibiotic concentrations in effluents from the ten WWTPs studied ranged between <0.05 and 0.36 ± 0.04 μg/L. The highest levels in WWTPs were recorded in Nyalenda Kisumu with AMP levels of 0.36 ± 0.04 μg/L. Kisumu is the largest among the towns within the region with the highest population as shown in Table 1. The mean concentrations of antibiotics in rivers and streams within the region ranged from <0.05 to 0.29 ± 0.02 μg/L. Although the antibiotic residues were

Table 4 Concentration of antibiotics in effluents (μg/L) from hospitals and WWTPs within Lake Victoria Basin, Kenya

WWTP/hospital	CPF	AMP	AMX	SMX	CAP
Kakamega Hospital	0.42 ± 0.05	0.61 ± 0.08	0.12 ± 0.02	0.59 ± 0.06	0.07 ± 0.01
Shirere domestic	0.08 ± 0.02	0.13 ± 0.05	0.08 ± 0.03	<LOQ	<LOQ
Nabongo domestic	<LOQ	0.06 ± 0.01	<LOQ	0.07 ± 0.01	<LOQ
MMUST domestic	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	<LOQ	<LOQ
Mumias Hospital	0.09 ± 0.3	0.16 ± 0.05	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Bungoma Hospital	0.54 ± 0.02	0.17 ± 0.01	0.07 ± 0.02	<LOQ	0.08 ± 0.02
Bungoma domestic	0.08 ± 0.02	0.35 ± 0.06	0.07 ± 0.02	0.08 ± 0.03	0.06 ± 0.01
Homabay domestic	<LOQ	0.07 ± 0.03	<LOQ	<LOQ	0.06 ± 0.01
Busia domestic	0.07 ± 0.02	0.08 ± 0.03	<LOQ	<LOQ	<LOQ
Kisat domestic and Industrial	0.05 ± 0.01	0.20 ± 0.02	0.07 ± 0.02	<LOQ	<LOQ
Nyalenda domestic	0.07 ± 0.03	0.36 ± 0.04	0.07 ± 0.02	<LOQ	<LOQ
Kisii domestic	0.10 ± 0.06	0.12 ± 0.05	<LOQ	<LOQ	<LOQ
Eldoret Hospital	0.19 ± 0.04	0.79 ± 0.07	0.08 ± 0.03	0.06 ± 0.01	0.08 ± 0.02
Eldoret domestic	0.16 ± 0.05	0.34 ± 0.08	0.06 ± 0.01	<LOQ	<LOQ

LOQ = 0.05 μg/L, $n = 3$

<LOQ less than limit of quantification

lower in rivers and streams compared to WWTPs, they may pose long-term effects to aquatic ecosystem. The presence of higher levels of antibiotic residues downstream of the WWTP indicates the transfer of these compounds from WWTP effluents to rivers (Table 5). During sampling, it was observed that effluents from these WWTPs are discharged directly into rivers and streams, which are tributaries of the Lake Vitoria. This poses great health concerns due to the possible ecotoxicological effects of these compounds on aquatic ecosystem and human health (Liu et al. 2009).

Discharge loads of antibiotics from WWTPs

The daily discharge load from WWTPs was estimated based on the assumption that the concentrations of antibiotics in wastewater and sludge recorded were constant throughout the day (Nakata et al. 2005; Chirikona et al. 2015). This was done using Eq. 1:

$$\mathrm{Dd} = C_\mathrm{w} * C_\mathrm{WWTP} * 1000 * 10^{-3} \left(\mathrm{mg\,day}^{-1}\right) \quad (1)$$

where

DdDaily discharge of antibiotics (mg day^{-1}),

C_wConcentration of individual antibiotic in wastewater (μg L^{-1}) (obtained from Table 3),

C_WWTPCapacity of WWTP (M^3 day^{-1}) (obtained from Table 1),

1000Conversion factor from cubic meters to liters,

10^{-3}Conversion from nanogram to milligram.

Antibiotic discharge loads from the various WWTPs are shown in Table 6. The discharge loads were calculated only for WWTPs whose daily capacities are known. An estimated total amount of antibiotics discharged from the nine WWTPs was calculated using Eq. 2.

$$\mathrm{Dd}_\mathrm{Total} = \sum \mathrm{Dd}_\mathrm{WWTPs} \quad (2)$$

where

$\mathrm{Dd}_\mathrm{Total}$	Total amount of the five antibiotics discharged from the nine WWTPs studied,
$\sum \mathrm{Dd}_\mathrm{WWTPs}$ =	Sum of the daily discharge loads of antibiotics in the WWTPs (mg day^{-1})

The daily discharge loads from the nine WWTPs ranged between 80.75 and 3025 mg day^{-1} (see Table 6). Nyalenda WWTP in Kisumu recorded the highest amounts of antibiotic discharge of 3025 mg day^{-1}. This could be attributed to the high population in Kisumu city, which is the largest among

Table 5 Concentration of antibiotics in water samples (μg/L) from streams and rivers within Lake Victoria Basin, Kenya

Study site	CPF	AMP	AMX	SMX	CAP
MMUST stream (upstream)	<LOQ	0.05 ± 0.04	<LOQ	<LOQ	<LOQ
MMUST stream (downstream)	0.06 ± 0.02	0.07 ± 0.05	0.05 ± 0.03	<LOQ	<LOQ
Kisat river (upstream)	<LOQ	0.13 ± 0.07	<LOQ	<LOQ	0.05 ± 0.02
Kisat river (downstream)	0.06 ± 0.05	0.17 ± 0.06	0.06 ± 0.01	<LOQ	0.06 ± 0.08
Auji River	0.05 ± 0.02	0.29 ± 0.02	0.05 ± 0.01	<LOQ	0.06 ± 0.02
Sosiani River (upstream	0.10 ± 0.04	0.09 ± 0.07	<LOQ	<LOQ	0.05 ± 0.02
Sosiani River (downstream)	0.12 ± 0.07	0.24 ± 0.06	<LOQ	<LOQ	0.05 ± 0.02

LOQ = 0.05 μg/L, $n = 3$

<*LOQ* less than limit of quantification

the other towns studied (Table 1). The total discharge load from the nine WWTPs was 6395.85 mg day^{-1}.

Comparison of results with data from other parts of the world

Results from this study were compared with data from other countries worldwide. The concentrations of antibiotics obtained in effluents from WWTPs in this study ranged between <0.05 and 0.36 ± 0.04 μg/L. Hospital effluents recorded levels ranging from <0.05 to 0.79 ± 0.07 μg/L. These results are comparable to results from other studies elsewhere. A study by Li and Zhang (2011) reported antibiotics ranging from 0.01 to 0.233 μg/L in WWTPs in China. Another study by Giger et al. (2003) reported antibiotic levels ranging from 0.057 to 0.330 μg/L. However, a study by Kihampa (2014) recorded higher levels of up to 37 μg/L in WWTP effluent from Dar es Salaam City in Tanzania. This may be attributed to the high population in the capital city of Tanzania (Kihampa 2014).

Antibiotic residues in the sludge samples from WWTPs in the present study ranged between <50 and 154 ± 9 ng/g. Sludge from hospital lagoons recorded antibiotic residues between <50 and 276 ± 12 ng/g. Similar levels ranging between 299 and 455 ng/g were detected in sludge samples from Spain (Radjenović et al. 2009). The daily discharge loads of antibiotics from the nine WWTPs studied ranged between 80.75 and 3025 mg day^{-1}, which are comparable to other data elsewhere. For example, results obtained from China had daily discharge loads between 3000 and 5200 mg day^{-1} (Li and Zhang 2011), while in Michigan (USA), 4800 mg day^{-1} was reported (Nakata et al. 2005). The difference in discharge loads is largely dependent on the capacity of WWTP, population served, and the method of treatment employed (Li and Zhang 2011).

Table 6 Discharge loads (Dd) of antibiotics (mg day^{-1}) from WWTPs within LVB, Kenya

Study site	CPF	AMP	AMX	SMX	CAP	∑Dd
Shirere domestic	64	104	64	–	–	232
MMUST domestic	25.5	29.75	25.5	–	–	80.75
Bungoma domestic	120	525	105	120	90	960
Homabay domestic	–	50.4	–	–	43.2	93.6
Busia domestic	42	48	–	–	–	90
Kisat domestic and industrial	49	490	171.5	–	–	710.5
Nyalenda domestic	423.5	2178	423.5	–	–	3025
Kisii domestic	140	168	–	–	–	308
Eldoret domestic	256	544	96	–	–	896

– Not determined

Conclusions and recommendations

In this study, the concentrations of individual antibiotics varied for different sources. The highest individual antibiotic concentrations in wastewater and sludge were detected for ampicillin (0.79 ± 0.07 μg/L) and sulfamethoxazole (276 ± 12 ng/g), respectively. Sediment and water samples from streams and rivers were recorded up to 94 ± 3 ng/g and 0.29 ± 0.02 μg/L ampicillin, respectively. Total discharge loads of the selected antibiotics in the nine WWTPs were 6333.01 mg day^{-1} with Nyalenda in Kisumu contributing 47.3 %. The high levels of antibiotics recorded in sludge indicate that sorption may be one of the main controlling factors in removal of these compounds in WWTPs. The use of sewage sludge and treated wastewater in agriculture could introduce antibiotics into the food chain after plant uptake, hence the need to improve wastewater treatment methods in order to minimize release of antibiotics into surface waters, sludge, and soils. Eco-friendly prescription of antibiotics with low toxicity levels and less persistence in the environment is also necessary. In addition, emerging pollutants such as antibiotics should be included in environmental regulations and standards for water monitoring.

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