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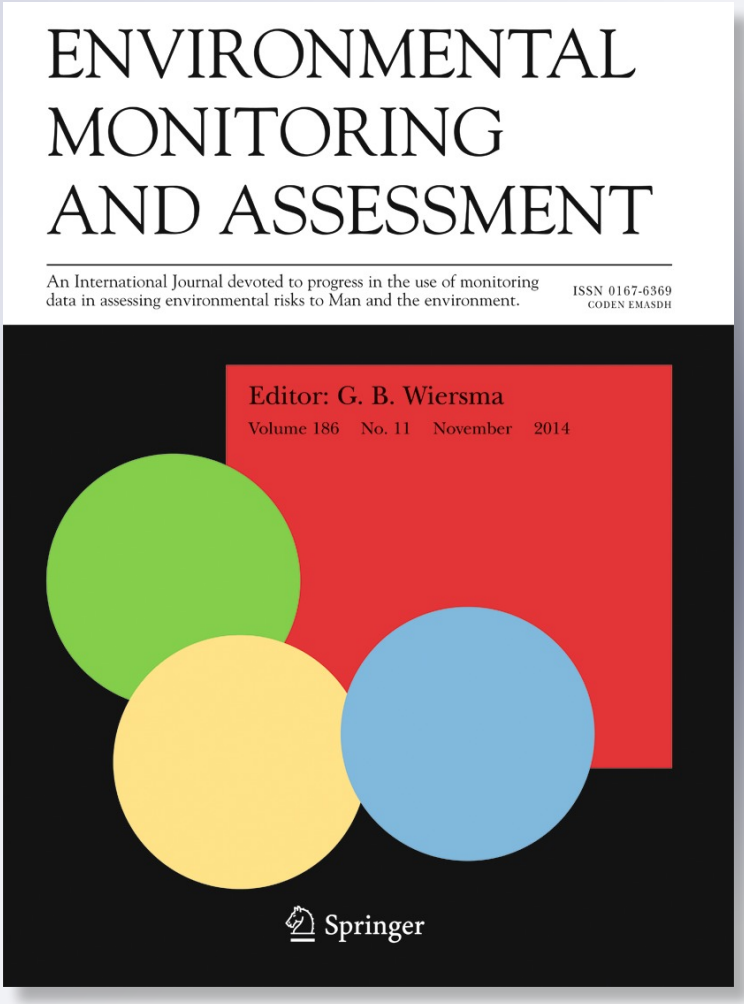

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Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa

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Abstract The occurrences of pharmaceuticals and personal care products as emerging organic contaminants (EOCs) have been reported in several countries of the world except from African countries. This study was therefore conducted to investigate the occurrence of nine antibiotics, five antipyretics, atenolol, bezafibrate, and caffeine in wastewater and surface water samples from the Umgeni River. The water samples were extracted with solid-phase extraction using hydrophilic-lipophilic balance (HLB) and C-18 cartridges for the acidic and neutral drugs, respectively. The quantification was carried out with high-performance liquid chromatography-diode array detector (HPLC-DAD) using the standard addition method. The method limits of detections were in the range of 0.14–0.97 μg/L while the recoveries were between 53.8 and 108.1 %. The wastewater had 100 % occurrence of the analytes studied, with caffeine having the highest concentration at 61 ±5 μg/L and nalidixic acid being the most observed antibiotic at 31±3 μg/L. The waste treatment process reduced the influent concentrations by 43.0–94.2 % before discharge except for atenolol removal that is lower. The concentrations of the analytes were lower in the surface water with most compounds having concentrations below 10 μg/L except acetaminophen and atenolol. The estuary mouth and Blue Lagoon had the highest concentrations of some of the compounds in surface water which depict downstream load. The factors governing the fate and mobility of these compounds in this environment are not fully understood yet and will require further studies.

Keyword Pharmaceuticals · Umgeni River · HPLC-DAD · Solid-phase extraction · Wastewater treatment

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Introduction

A wide variety of drugs are used in medical service deliveries and human healthcare globally. Likewise, diverse pharmaceuticals are used in livestock rearing and as veterinary drugs in animal treatments. Over 3,000 compounds have been approved as constituents of pharmaceuticals and medicinal products (Löffler and Ternes 2003). In recent years, the occurrences of these pharmaceuticals in the environment have started to raise serious concerns for both the scientific community and the general public. This is evidenced by an increase in the volume of published reports of these substances in the environment and their reported potentially toxic bioactivities (Xie and Ebinghaus 2008; Murray et al. 2010; Pal et al. 2010; Lapworth et al. 2012). These compounds have not been considered as risk prone nor monitored in the environment until recently (Aga 2008)

but it is being recognized that the presence of these pharmaceuticals in water supplies should be of interest to the public. There are presently no regulatory standards for them but they are considered to have potential for adverse human and environmental effects with increased risk potential on exposure (Celiz et al. 2009; Schriks et al. 2010). The study of pharmaceuticals and their metabolites in the environment has rapidly become a field of scientific research under environmental studies with a growing number of interests. Thus, these pharmaceuticals and other personal care products are referred to as emerging organic contaminants (EOCs). Some of their potential adverse effects are bacterial resistance to antibiotics, sterility, and feminization of aquatic animals, other drug resistance of pathogenic organisms among others (Carucci et al. 2006; Bolong et al. 2009; Schriks et al. 2010). There is therefore increasing global awareness of these contaminants and their toxic effects and therefore regulatory bodies are in the process of determining minimum standards for these substances in the water system.

Lindqvist et al. (2005) reported the occurrence of four acidic antiphlogistic pharmaceuticals (ibuprofen, naproxen, ketoprofen, and diclofenac) and an antihyperlipidemic drug (bezafibrate) in sewage treatment plants and in surface water receiving the treated wastes in Finland. Several other studies on EOCs have also been conducted and documented in different environmental compartments of various European countries and in the United States showing the occurrence of EOCs in varying concentrations (Ternes 1998; Heberer 2002a; Kolpin et al. 2002; Godfrey et al. 2007; Pal et al. 2010). A large number of studies have documented that the primary route of entry of EOCs into the environment are wastewater point sources from municipal waste treatment plants (Kolpin et al. 2002; Heberer et al. 2004), household discharges of unused drug wastes through septic systems, and excretion of unmetabolized products by humans (Swartz et al. 2006; Labadie et al. 2007; Dougherty et al. 2010). Other routes include the rinsing off of dermally applied products, the use of the wastewater systems for disposal of excess medication, and landfill leachates (Glassmeyer et al. 2008). Literature surveys have also revealed that EOCs are sourced from discharges of effluent from pharmaceutical industries, hospitals, and health service centers as well as veterinary drug applications from confined animal farms (Holm et al. 1995; Kümmerer 2001; Watanabe et al. 2010). EOCs enter the water system through these point sources and are combined with other diffused sources like run-off from various domestic sewers, manure applications of sewage to land surfaces for crop production purposes among others. Thus, EOCs have multiple sources of entry into the environment and their fate and mobility through the water system requires research.

Bolong et al. (2009) presented a review on EOCs in wastewater while Pal et al. (2010) and Murray et al. (2010) reviewed the presence of EOCs in freshwater environment. These reviews reported concentrations of over 50 different EOCs in water systems at the ng/L to μg/L level in the different environments. Lapworth et al. (2012) further reviewed the occurrence of a range of EOCs (as well as their degradates) in groundwater. Classes of EOCs reported in the reviews include human therapeutic pharmaceuticals, personal care compounds, veterinary medicines, lifestyle compounds or drugs of abuse, x-ray contrasting agents, steroids, surfactants, plasticizers, metabolic regulators, preservatives, and food additives, as well as a large range of other wastewater-related compounds. All these reviews were focused on EOCs that are under consideration for regulations or do not currently have drinking water standards in America and Europe. Important processes that control the fate of EOCs in the environment were identified as not yet well understood and are currently being studied. Also, the use of EOCs as tracers in groundwater studies is still being assessed, as well as their potential impact on water resources. The current gaps in literature are on the understanding of fate of EOCs in water systems, the evaluation of occurrence and concentrations of EOCs in African countries, and the potential treatment.

The limitation of the analysis of EOCs in the environment had been caused by the low concentration of these substances in the environment. The expected concentrations of these substances in the environment are in the sub-μg/L or ng/L level which necessitates the use of highly sensitive, specialized analytical instruments, and analyte preconcentration methods such as solid-phase extraction (SPE). Hyphenated chromatographic methods such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been adopted for the determination of these substances (Hirsch et al. 1998; Löffler and Ternes 2003; Labadie and Hill 2007). The LC-MS method is most preferred because it circumvents the time-wasting derivatization needed for the

Table 1 List of sampling sites in the downstream direction and types of samples analyzed

Site code	Site	Coordinate		Activities at the sampling sites
		South	East	
MDI	Midmar Dam Inlet	29° 29′ 16.05″	30° 09′ 23.10″	Dam for water supply (inlet)
MDO	Midmar Dam Outlet	29° 29′ 34.02″	30° 12′ 09.13″	Dam for water supply (outlet)
HF	Howick Falls	29° 29′ 18.18″	30° 14′ 19.70″	Water fall
AFI	Albert Falls Inlet	29° 26′ 31.94″	30° 14′ 47.10″	Dam for water supply
AFO	Albert Falls Outlet	29° 26′ 01.81″	30° 25′ 55.76″	Dam for water supply
ND	Nagle Dam	29° 35′ 08.42″	30° 37′ 23.94″	Dam for water supply
MUT	Msudunzi/Umgeni Tributary	29° 37′ 16.61″	30° 40′ 46.59″	Surface water/river
IDI	Inanda Dam Inlet	29° 39′ 05.20″	30° 48′ 06.24″	Dam for water supply
IDO	Inanda Dam Outlet	29° 42′ 55.74″	30° 52′ 07.69″	Dam for water supply
RH	Reservoir Hills axis	29° 47′ 08.05″	30° 56′ 25.51″	Domestic and farming area
UBP	Umgeni Business Park	29° 48′ 19.05″	30° 58′ 58.08″	Industrial and commercial activities
EWIn	Northern WWTP Influent	29° 47′ 47.08″	30° 59′ 50.01″	Influent of treated domestic wastewater from the city of Durban
EWOut	Northern WWTP after Treatment	29° 47′ 47.02″	30° 59′ 50.06″	Effluent after treated outlet before discharge
EWEff	Northern WWTP Effluent	29° 48′ 27.01″	30° 59′ 51.05″	Effluent of treated wastewater at the discharge into the river
BL	Blue Lagoon	29° 48′ 41.03″	31° 02′ 12.05″	Discharge point into the Indian Ocean

GC-MC method since most of the drugs of interest are polar substances. It also offers better sensitivity than the UV detector. However, there are some documented studies that used liquid chromatography-ultraviolet (LC-UV) methods (Blackwell et al. 2004; Benito-Pena et al. 2006; Esrafili et al. 2007).

There have been extensive studies on the occurrence and fate of emerging organic contaminants in the European Union countries and America but there is paucity of information on the occurrence and fate of EOCs in Africa. It is essential that the occurrence of EOCs be documented in Africa. Though, there have been studies on the classical persistent organic pollutants like PAHs, PCBs, and OCPs, there is no information on the occurrences and fate of EOCs in Africa which may be due to awareness and limitation of analytical method development/instrumentation. This study is therefore aimed at investigating the occurrence of 17 pharmaceuticals commonly used in South Africa—9 antibiotics, 5 antipyretics, 1 β-blocker, 1 lipid regulator, and a psychostimulant—in wastewater from a major domestic water treatment plant, Umgeni surface water and dams used for water supply in KwaZulu-Natal, South Africa. This paper also intends to present a method for determination of these pharmaceuticals using LC-UV where there is limitation of instrumentations like LC-MS/MS.

Experimental

Description of study site and sample collection

This study was conducted on the course of Umgeni River which is one of the major rivers in the KwaZulu-Natal province of South Africa between February and May, 2013. KwaZulu-Natal has the second largest population in South Africa with 10.267 million people according to the 2011 South African Census record (available online on: http://www.statssa.gov.za/Census2011/Products.asp). The Umgeni River transverses a catchment area of 4,418 km^2 and has a length of 257 km (Van der Zel 1975). The river has four dams located on its course (Midmar Dam, Albert Falls, Nagle Dam, and Inanda Dam) which were constructed for water supplies. The river empties into the Indian Ocean at the Blue Lagoon. The coordinates and identified activities of the selected sampling points (15) for this study in the direction of the downstream flow of the Umgeni River are presented in Table 1.

The sampling sites are classified into two based on associated activities. The first class is the region within Durban, represented in Fig. 1a, that is associated with anthropogenic activities (domestic, commercial, and industrial). The various districts, which the river

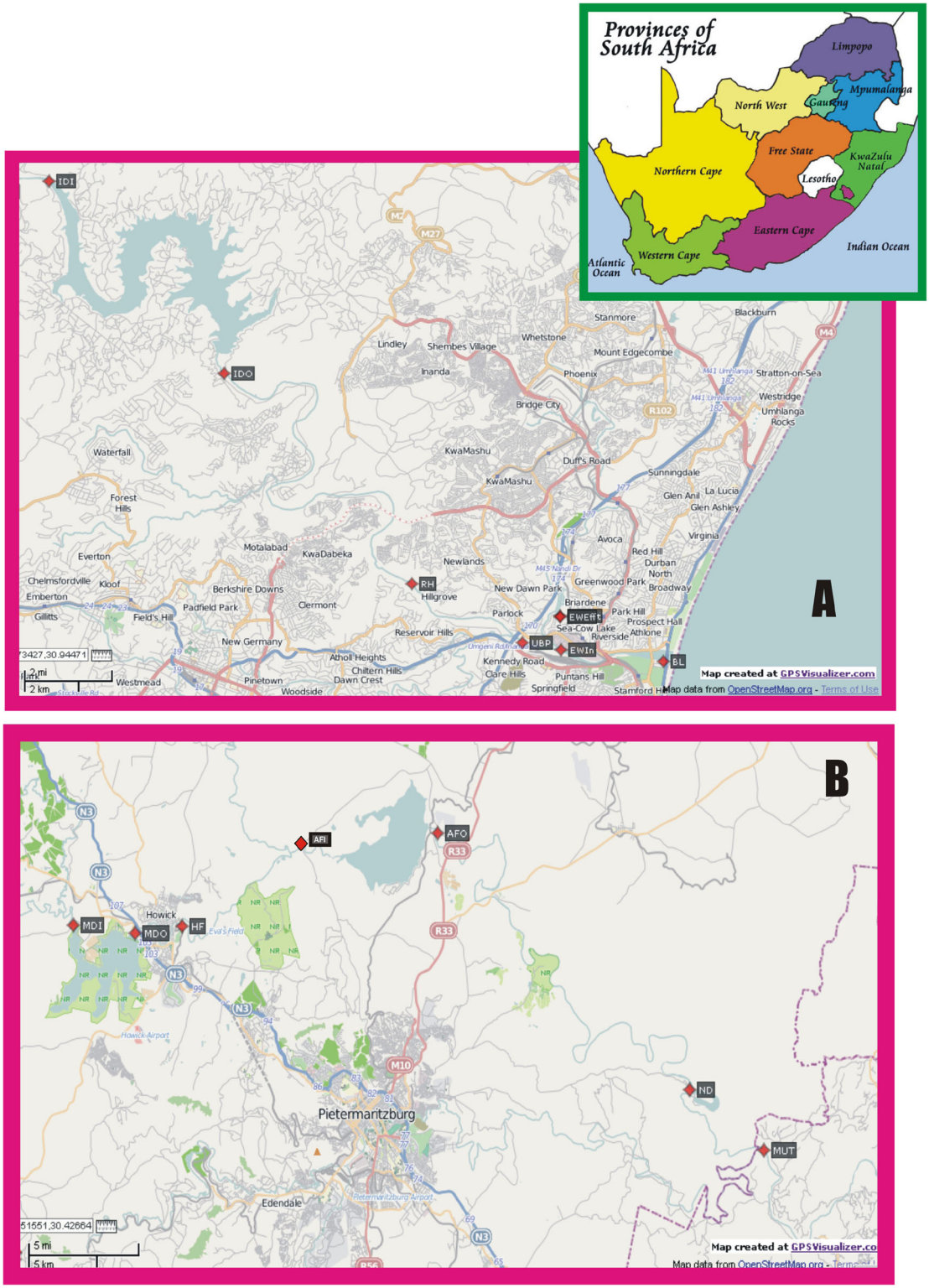

Fig. 1 Map of sampling site with the sample collection locations identified in *red diamond* shapes (maps were generated from GPS coordinates using an online tool—GPS visualizer)

transverses, have different domestic/residential facilities with sewers, industrial and commercial activities that are potential sources of the analytes of interest in this study. The sampling region also includes the wastewater treatment plant sampling points from which wastewater influent, effluents of treated domestic waste after treatment, and the discharged effluent into the river were collected. The second class of sampling sites is the dams on the course of the river (Fig. 1b) which were constructed for domestic and industrial water supply. Thus, evaluation of the targeted analytes in these dams can serve as a measure of exposure risk potentials to the consumers of this water. The maps of the two location-classes and the specific sample collection points are thus presented in Fig. 1. Water samples were collected from these sampling sites within the sampling period.

Sampling point BL is the Blue Lagoon which is the estuary discharge point of the river into the Indian Ocean. This point has a tendency of measuring the total load of waste/pollutants discharged into the river water course downstream as it empties into the ocean. The sample collection points EWIn, EWOut, and EWEff are the influents, outflow after treatment, and effluent discharge point of the domestic wastewater treated from the Northern Wastewater Treatment Plant (NWWTP), respectively. The NWWTP receives wastewater from the domestic sources through the influent point identified as EWIn, treats the wastewater after which it discharges the wastewater into the Umgeni River at site EWEff. Thus, EWIn, EWOut, and EWEff of this study were chosen to assess the impact of wastewater discharge on the surface water and the effectiveness of the treatment process on the removal of these compounds. Sampling site Umgeni Business Park (UBP) represents the area with high industrial and commercial activities. Sampling site RH is a domestic/residential area with subsistence agricultural activities nearby the river course.

The sampling sites IDI and IDO are the inlet and outlet into Inanda dam, respectively. The Inanda dam is an earth-filled dam, constructed in 1989 and located in the valley of a thousand hills approximately 42 km north of Durban. It is 23-km long, 1.5 km at the widest point, and 50-m deep at its deepest point. The surface area of the dam is 1,440 ha. It has a capacity of 256 million m^3 of water (Department of Water Affairs and Forestry 2008). The location labeled as MUT is the meeting point of the Umgeni River with one of its major tributaries, the Msunduzi River. Site ND is another water supply dam on the river course (Nagle Dam) while AFI and AFO are the sampling sites at the inlet and outlet of Albert Falls Dam. Albert Falls Dam has a gross capacity of 289 million m^3 and a surface area of 2,352.1 ha; the dam wall is 33-m high. Sample site HF represents the Howick Falls used for both domestic purposes and tourism. Howick Falls is approximately 95-m high and lies on the Umgeni River course. The outlet of Midmar Dam into the Umgeni River is the sampling site MDO and the inlet into the dam is the sampling site MDI. The dam which was established in 1965 has a surface area of 1,564 ha and a capacity of 177 million m^3 of water (UIDP 2001).

The water samples were collected in amber colored glass bottles that had been pretreated as specified by APHA (2005). The samples were kept in an ice chest at 4 °C and fixed with H_2SO_4 (50 %) during transportation to the laboratory. Temperature, pH, electrical conductivity, and total dissolved solids (TDS) were measured at

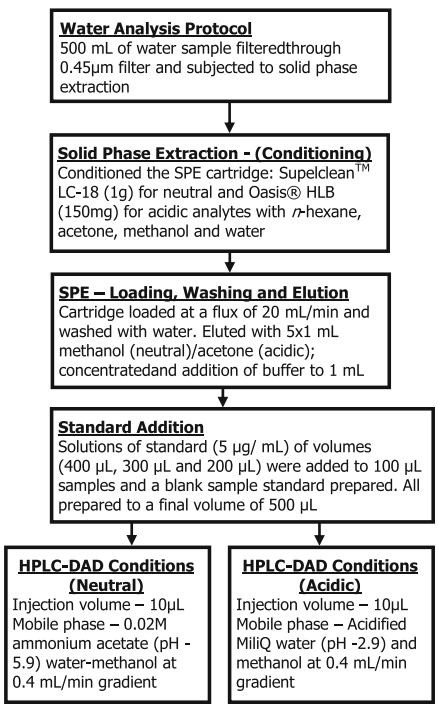

Fig. 2 Flow diagram of protocol for analysis

Table 2 HPLC method parameters

HPLC system (UFLC-XR)	Shimadzu LC-20ADXR; Degasser–DGU-20A_3; Autosampler–SIL-20AXR; DAD Detector–SPD-M20A		
Column type and temperature	Agilent C_{18}, 4.6 x 150 mm, 5 μm; 35 °C		
Injection volume	10 μL		
Elution solvent system	Buffer A: 90 % of 0.02 mol/L $AcONH_4$ buffer (pH 5.9) + 10 % CH_3OH		
	Buffer B: 30 % of Buffer A + 70 % CH_3OH–flow rate–0.4 mL/min		
Gradient (neutral)	Time	Buffer A (%)	Buffer B (%)
	0	100	0
	1	90	10
	3	80	20
	12	25	75
	18	0	100
	22	95	5
	30	100	0
Elution solvent system	Buffer A: 100 % CH_3OH		
	Buffer B: Acidified Milli-Q Water (pH 2.9)–flow rate–0.4 mL/min		
Gradient (acidic)	Time	Buffer A (%)	Buffer B (%)
	0	40	60
	5	100	0
	13	100	0
	18	40	60
	28	40	60

the collection site. Samples were refrigerated at 4 °C till analyses were completed.

Materials

The pharmaceutical standards were all purchased from Sigma-Aldrich through Capital Lab, South Africa. The standards were atenolol (product of India), tetracycline (product of USA), sulfamethoxazole, diclofenac sodium salt, and nalidixic acid (all products of Italy). Ciprofloxacin, aspirin, ampicillin, ketoprofen, bezafibrate, ibuprofen, acetaminophen, caffeine, streptomycin sulphate salt, erythromycin, chloramphenicol, and tylosin tartrate are all products of China. The details of structures, systemic names, uses, and chemical data of the pharmaceutical compounds investigated are obtainable on SciFinder. Methanol and acetone were HPLC Chromasolv® grade purchased from Sigma-Aldrich. Milli-Q water was generated from an Elix Millipore Water system. Other reagents used were AnalaR grade. The SPE cartridges used for the neutral analytes was Supelclean™ LC-18 (1 g) and for the acidic analytes Oasis® HLB (150 mg).

Analysis protocol

The protocol adopted for this study was adapted from the modification of the methods presented by Ternes et al. (2001) and Castiglioni et al. (2005). The flow diagram that summarizes the protocol is presented in Fig. 2.

Solid-phase extraction of water samples

The SPE cartridges were conditioned prior to sample loading with consecutive solutions of 6 mL of *n*-hexane, 2 mL of acetone, 10 mL of methanol, and 10 mL of distilled/deionized water. The water samples (500 mL each) were filtered with a 0.45-μm filter after which they were loaded onto the cartridges using a SPE manifold at 5 mL/min. After extraction, the cartridges were vacuum dried for 5 min. The extracts were eluted from the SPE cartridges using 5 × 1 mL methanol for neutral

pharmaceuticals and 5×1 mL acetone for the acidic pharmaceuticals. The eluates were concentrated with vacuum-drying (Castiglioni et al. 2005), reconstituted and phosphate buffer (pH 6) was added to neutral analytes, and ammonium acetate buffer (pH 4) was added to acidic analytes. The samples were made up to a final volume of 1 mL corresponding to a concentration factor of 500.

HPLC analysis and quantification

The identification and the quantification of the analytes were conducted with a Shimadzu LC-20ADXR having a degassing chamber—DGU-20A_3; auto sampler–SIL-20AXR; diode array detector (DAD)—SPD-M20A. The details of the HPLC system, the solvent system, and the method parameters for the two classes of analytes investigated are presented in Table 2. The quantification of the analytes was carried out using the standard addition method. The standard addition method has been documented in literature to correct for matrix effects which are a drawback of the LC-UV method (Fraga et al. 2000) and can enhance the signal above the detection limit of the instrument thus reducing the effects of instrument noise at low concentration. The limit of detection was calculated as three times the signal-to-noise ratio using the standard deviation of the five calibration intercepts divided by the slope while the limit of quantification is ten times this ratio.

The standard addition method was carried out by the addition of variable volume (V_s) of known concentrations (C_s) of standards to a known volume (V_i) of analyte (i) and made up to a total volume (V_T) to determine the unknown concentration of the analyte (C_i) using the expressions derived from peak area (P) and the total concentration (C_T) below:

$$P = kC_T \tag{1}$$

$$C_T = {}^{n_T}/_{V_T} = \frac{C_s V_s + C_i V_i}{V_T} \tag{2}$$

Thus:

$$P = k\frac{C_s V_s}{V_T} + k\frac{C_i V_i}{V_T} \tag{3}$$

From the plot of P against V_s, a ratio of the obtained intercept (c) to slope (m) will give an expression (Eq. 4) that could be used to evaluate the analyte concentration (C_i) since the concentration of the standard (C_s) and the volume of the analyte (V_i) used are known.

$$C_i = \frac{cC_s}{mV_i} \tag{4}$$

The recovery study of the analytes was undertaken under the same conditions expressed for the samples as a quality assurance step. Tap water (500 mL) was spiked with 50 μL of 100 mg/L of the analytes to make a final concentration of 10 μg/L. The recovery study was carried out in triplicate and the mean recovery with the percentage relative standard deviation calculated for each analyte. The sample analyses were carried out in triplicate to measure the reproducibility/precision of the method. Blank analyses were also carried out along with sample analysis to measure the possible contributions from external sources during analysis. The mean concentrations and the standard deviation of the analytes were calculated.

The data obtained were presented using univariate exploratory data analysis method (box and whisker plot). SPSS 17 software was used for the plots. Microsoft Excel 2010 was used to plot the stacked chromatograms at different wavelengths using the comma delimited data files from the HPLC instrument.

Results and discussion

Limit of detection and chromatographic separation

The typical chromatogram obtained for the methods reported for acidic compounds and neutral compounds are presented in Fig. 3a, b, respectively. Figure 3a shows eight acidic compounds at 220 and 240 nm maximum wavelengths while Fig. 3b presents the separation of nine neutral drug compounds at 220, 240, and 289 nm wavelengths. The peaks were well resolved with resolution values greater than 1 for all the analytes except erythromycin (Table 3). HPLC-UV method of analysis is less sensitive compared to the LC-MS methods as depicted by the method limit of detection and limit of quantification of the analytes studied (Table 3). The recovery results also showed that the method of analysis has good quality. The percentage recoveries obtained by the analytical methods were above 70 % in most of the analytes except for streptomycin and tetracycline which were 64 and 54 %, respectively. Tetracycline recovery has been reported

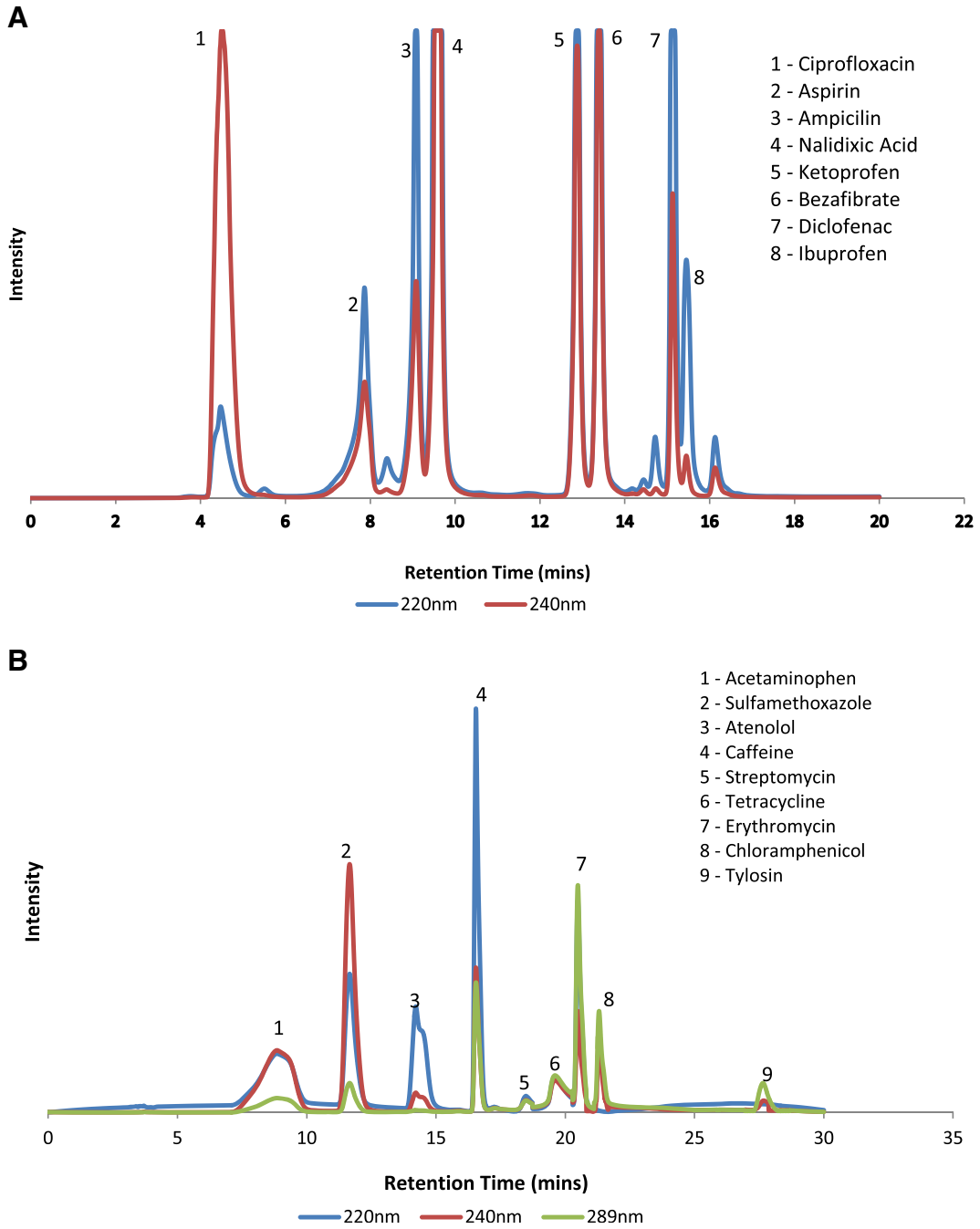

Fig. 3 The chromatogram of pharmaceuticals investigated **a** acidic compounds and **b** neutral compounds

in literature to be relatively lower due to complexation tendency with metals which may necessitate the use of EDTA (Miao et al. 2004). The results of the concentrations of the analytes investigated in this study are presented in the following sections based on their usage classifications.

Antibiotics and livestock prophylactics

The box and whisker plots of the concentration ranges of the nine antibiotics studied are presented in Fig. 4. The percentage detection frequency of the compounds investigated in all the 25 samples collected were as

Table 3 Chromatogram parameter, limits of detection and quantification; recovery results

Compounds (acidic)	R_T (min)	Peak resolution	LOD (μg/L)	LOQ (μg/L)	Linear range (μg/L)	Recovery ± RSD (%)	λ_{max} (nm)
Ciprofloxacin	4.453	1.58	0.70	2.34	1–1,000	80.4±8.1	240
Aspirin	7.758	4.71	0.27	0.89	1–1,000	106.8±4.0	220
Ampicillin	9.048	1.47	0.20	0.67	1–1,000	79.9±2.8	220
Nalidixic acid	9.674	1.80	0.97	3.25	0.5–1,500	92.5±5.0	220
Ketoprofen	12.885	1.98	0.31	1.04	0.5–1,000	91.1±5.5	220
Bezafibrate	13.303	2.08	0.50	1.66	0.5–1,500	76.4±5.0	220
Diclofenac	15.104	1.04	0.83	2.75	0.5–1,000	108.1±4.7	220
Ibuprofen	15.488	1.03	0.21	0.72	1–1,200	75.8±9.4	220
Acetaminophen	8.992	1.98	0.27	0.90	0.5–3,000	71.8±4.3	240
Sulfamethoxazole	11.746	1.76	0.31	1.04	0.5–2,000	87.4±5.1	240
Atenolol	14.674	3.13	0.37	1.25	0.5–3,000	107.3±2.8	220
Caffeine	16.985	2.12	0.38	1.25	0.5–3,000	98.5±7.3	220
Streptomycin	18.588	2.04	0.59	1.96	1–2,000	63.5±3.8	220
Tetracycline	19.787	1.03	0.50	1.65	2–2,000	53.8±3.1	220
Erythromycin	20.452	0.93	0.25	0.84	1–2,000	75.4±6.0	289
Chloramphenicol	21.466	1.28	0.97	3.23	0.5–3,000	72.8±7.3	289
Tylosin	28.535	1.71	0.14	0.46	1–2,000	83.6±7.0	289

follows: nalidixic acid (100), ampicillin (100), tylosin (96), sulfamethoxazole (92), ciprofloxacin (88), erythromycin (88), tetracycline (76), streptomycin (64), and chloramphenicol (36). The plots indicate that

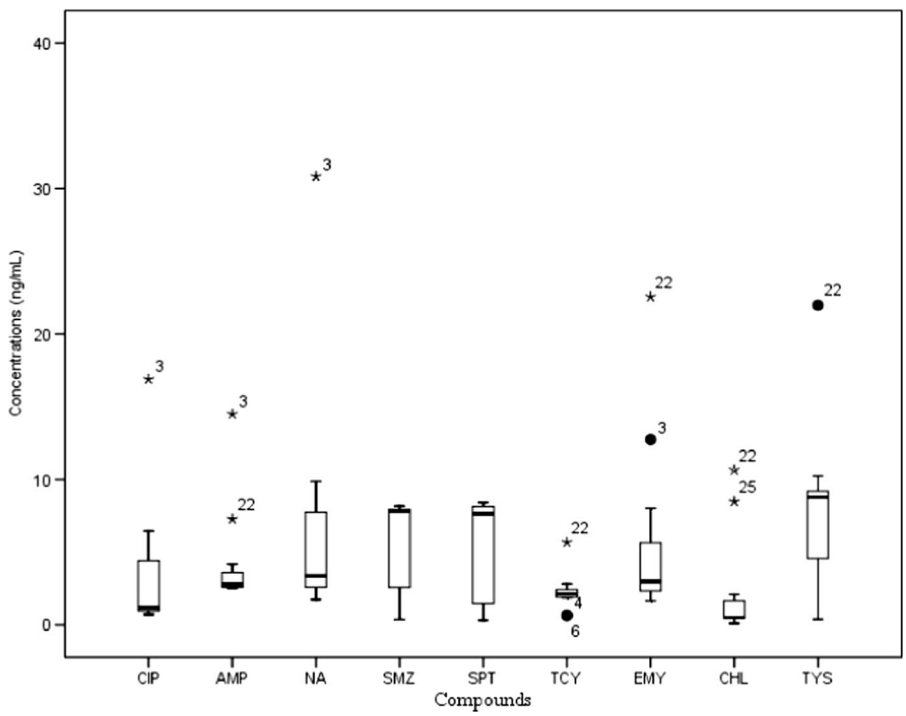

Fig. 4 Box and whisker plots of nine antibiotic drugs *CIP* ciprofloxacin, *AMP* ampicillin, *NA* nalidixic acid, *SMZ* sulfamethoxazole, *SPT* streptomycin, *TCY* tetracycline, *EMY* erythromycin, *CHL* chloramphenicol, and *TYS* tylosin

the highest concentrations of the analytes were observed in the wastewater influent samples (samples 3 and 22) for virtually all the antibiotics, as indicated by the outlier plots. Besides the extreme concentrations from the wastewater, the data pattern of ampicillin, tetracycline, and chloramphenicol showed narrow variations while the other six antibiotics had large concentration variations. It can be inferred based on the detection frequency information and the Q_2 (median) concentration values of the drugs that the relative abundance of the analytes ranked in increasing order of CHL<TCY<EMY<CIP<AMP<SMZ<SPT<NA<TY-S. Overall, the concentrations of the antibiotics in all samples were lower than 10 μg/L beside the wastewater influent samples where concentrations were higher. The observed concentrations of the antibiotic may not be absolutely due to anthropogenic activities because several antibiotics like the β-lactams, tetracycline, streptomycin, and others are produced by bacteria like Actinomycetes group and Streptomycetes (Soulides 1965; Kümmerer 2009) which may contribute to antibiotics load in the environment.

Tylosin had the highest Q_2 (median) concentration of 8.12 μg/L and was observed at a range of 0.21–21.99 μg/L. Concentration spread skewed towards the upper limit (negative skewedness) which indicates that larger frequencies of the samples have their detected concentrations below the Q_2 value. Tylosin is used as growth promoter and prophylactic in livestock farming. This result indicates that there is a high possibility of tylosin applied in livestock production being transported into the water system through wastewater discharge and other diffused sources of input. Tylosin was obtained at a lower average concentration of 1.15 μg/L in wastewater in Poudre River, USA (Yang and Carlson 2004) and between 0.06 and 0.18 μg/L in Arkansas streams, USA (Haggard et al. 2006). The concentration range obtained for streptomycin as presented in the box plot was 0.81–8.42 μg/L with a Q_2 value of 7.35 μg/L. Streptomycin concentrations observed in the water samples were of more frequent values below the Q_2 than above it (negative skewedness). Streptomycin and tetracycline are also reported to be used in plant agriculture to combat some bacterial infections in apple, pears, etc. (Kümmerer 2009; Stockwell and Duffy 2012). This can also contribute to the elevated concentration of these in the environment. Sulfamethoxazole concentration results were negatively skewed with a Q_2 value of 3.68 μg/L. A Q_2 concentration value of 12 ng/L was reported for sulfamethoxazole in US drinking water between 2006 and 2007 by Benotti et al. (2009) while Li et al. (2007) reported a concentration range of 0.33–0.61 μg/L for sulfamethoxazole in river and sewage water.

The concentrations of the other six antibiotics were positively skewed toward values higher than their Q_2 values. Nalidixic acid was in the concentration range of 1.73–30.84 μg/L and Q_2 value of 3.03 μg/L. A study by Watkinson et al. (2009) reported detection of nalidixic acid in hospital effluent, wastewater treatment plant influent and effluent, and in surface water in Australia with a maximum concentration of 0.75 μg/L. Ampicillin had a Q_2 concentration value of 3.16 μg/L and ranged from 2.52 to 14.48 μg/L. Benito-Pena et al. (2006) reported comparable ampicillin concentration results of 2.2–25.6 μg/L for industrial and wastewater treatment samples. The concentration of ampicillin in the wastewater sample was 14.48 μg/L but with a lower concentration range in the surface water. Ampicillin and the other antibiotics in this study are generally applied for both human and veterinary therapeutics which may indicate possible elevated usage. Erythromycin had a maximum concentration of 22.57 μg/L and a minimum of 0.58 μg/L, in the samples in which it occurred. Its spread is relatively wider and was frequently detected at concentrations above its Q_2 of 3.18 μg/L. Erythromycin and its degradation product were reported at concentrations up to 6.0 μg/L in wastewater effluents in Germany by Hirsch et al. (1999). Moreover, tetracycline values in the water system were between 0.64 and 5.68 μg/L. The sample concentration range of tetracycline was narrow in the surface water system. Li et al. (2010) reported tetracycline concentrations of 1 mg/L in wastewater and above 0.25 mg/L is surface water in China, which is much above the quantity detected in this study. In another study, Li et al. (2008) documented the presence of oxytetracycline at a concentration up to 19.5 mg/L in wastewater and 641 μg/L in receiving surface water. Another antibiotic investigated, ciprofloxacin, had a median (Q_2) concentration of 1.36 μg/L and a minimum observed concentration of 0.71 μg/L. The positive skewness of its data plot indicates that most of the ciprofloxacin concentrations in the samples were above the Q_2 and skewed towards the Q_3 value of 5.66 μg/L while its maximum value was 16.9 μg/L. Higher concentrations of ciprofloxacin of 28–31 mg/L have been recorded in pharmaceutical industries wastewater treatment plant samples in Sweden (Larsson et al. 2007) and

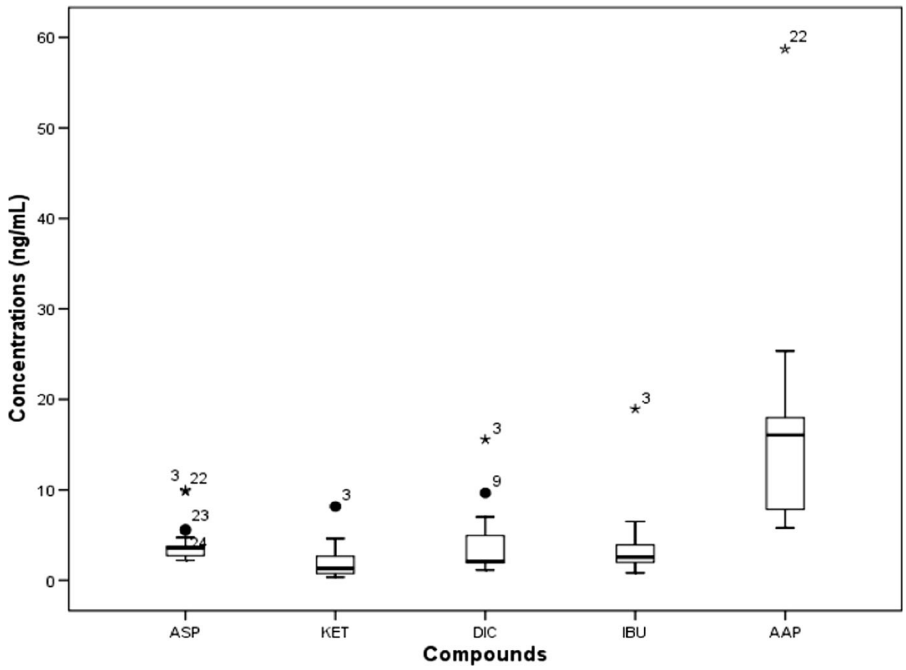

Fig. 5 Box and whisker plots of five analgesic/antipyretic drugs *ASP* aspirin, *KET* ketoprofen, *DIC* diclofenac, *IBU* ibuprofen, *AAP* acetaminophen

concentration between 2.5 and 6.5 mg/L in surface water from two lakes (Fick et al. 2009).

Chloramphenicol was the least detected analyte within this class of pharmaceuticals (with an occurrence percentage of 36 %). It has a narrow concentration variation of 0.5–10.7 μg/L. The Q_2 concentration of chloramphenicol detected was 1.20 μg/L and the concentration spread was positively skewed towards the upper percentile. Benito-Pena et al. (2006) also reported comparable chloramphenicol concentrations of 4.0–10 μg/L in industrial and sewage treatment plant wastewaters while Hirsch et al. (1999) reported a maximum concentration of 0.56 μg/L in a wastewater samples but lower concentrations in surface water samples.

Antipyretics

The concentration ranges of aspirin, ketoprofen, diclofenac, ibuprofen, and acetaminophen in all the samples from the studied sites are presented using a box plot (Fig. 5). All the antipyretic drugs studied were more ubiquitous than the antibiotics, with 100 % occurrence in the samples investigated. There were small concentration variations for all the analytes except for acetaminophen. The highest concentrations of the antipyretic drugs were also obtained in the influent samples collected from the wastewater treatment plant (samples 3 and 22). The ubiquitous nature may be because the drugs are over-the-counter drugs with no need of doctor's prescription. Generally, the concentrations of these analytes in the surface water were below 10 μg/L and there were narrow variations. Their concentration range in surface water is similar to that obtained for the antibiotics. The relative abundance of the drugs in the water system, expressed in ascending order using their Q_2 values, are ibuprofen (0.81 μg/L), ketoprofen (1.31 μg/L), diclofenac (2.16 μg/L), aspirin (3.54 μg/L), and acetaminophen (16.06 μg/L). Acetaminophen was the most concentrated in the samples with a concentration range of 5.8–58.7 μg/L. The other antipyretics were in the concentration range of 0.8–18.9 μg/L for ibuprofen, 0.4–8.2 μg/L for ketoprofen, 1.1–15.6 μg/L for diclofenac, and 2.2–10.0 μg/L for aspirin. The concentration spread of ketoprofen, ibuprofen, and diclofenac were positively skewed while that of aspirin and acetaminophen data were negatively skewed. Lindqvist et al. (2005) investigated the occurrence of four acidic antiphlogistic pharmaceuticals (ibuprofen, naproxen, ketoprofen, and diclofenac) and an antihyperlipidemic drug (bezafibrate) in sewage treatment plants and in receiving water in Finland and reported a comparable concentration range of 0.35–

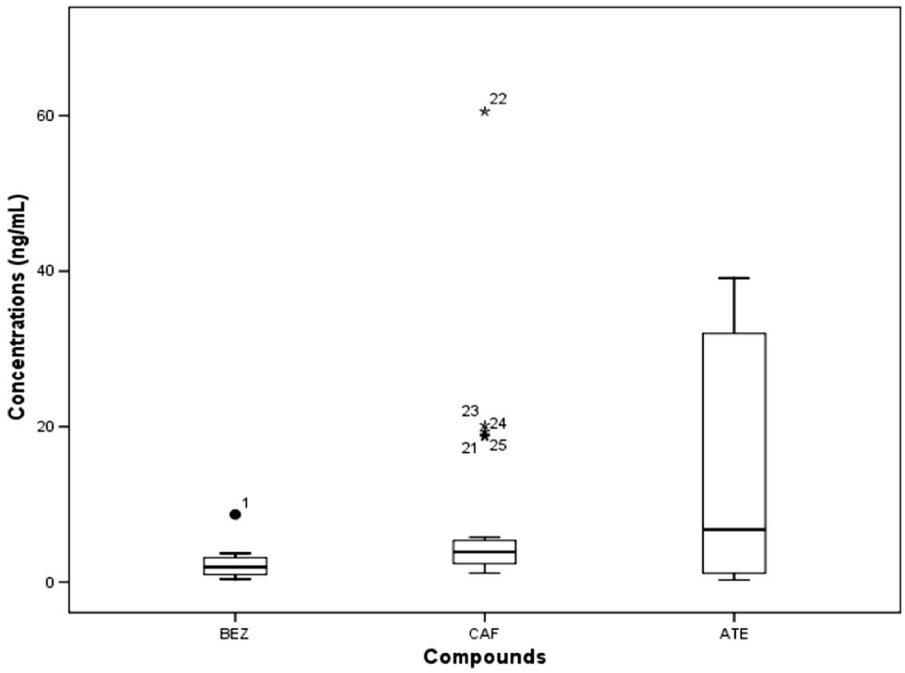

Fig. 6 Box and whisker plots of *BEZ* bezafibrate (anti-hyperlipidemia), *CAF* caffeine (psychostimulant), and *ATE* atenolol (β-blocker)

13.1 μg/L for the antiphlogistic drugs and 0.42 μg/L for the lipid regulator (bezafibrate). Rabiet et al. (2006) reported a maximum concentration of 11.3 μg/L for acetaminophen in wastewater influents and 10.6–68.1 ng/L in surface water in France while ibuprofen had a concentration of 0.51–1.35 μg/L in drinking and reclaimed wastewater and 3.23–25.8 μg/L in wastewater was reported in California, USA by Loraine and Pettigrove (2006). Investigation of antipyretics in raw and treated wastewater in Spain by Rodil et al. (2009) reported 2.2–6.1 μg/L aspirin, 2.3–10.4 μg/L ibuprofen, 0.48–0.76 μg/L diclofenac, and 0.14–0.35 μg/L ketoprofen. The concentrations of these analytes reported in literature were study site specific but are relatively comparable with the ones obtained in this study.

Others

Figure 6 is the box plot of the concentrations of atenolol, bezafibrate, and caffeine in the water samples. The percentage occurrence of these analytes in the samples investigated were atenolol (88), bezafibrate (92), and caffeine (96). The concentration variation of bezafibrate was narrow with symmetrical skewedness. It has a Q_2 concentration of 2.48 μg/L and occurred at a range of 0.81–8.71 μg/L. Atenolol had a wider concentration spread of 0.96–39.10 μg/L and a Q_2 value of 11.09 μg/L. Caffeine exhibited high (outlier) concentrations in five samples related to wastewater treatment and surface water (samples 21–25). The caffeine median concentration was 3.89 μg/L and the concentration range was 1.17–60.53 μg/L. Besides the samples with high concentrations of caffeine, the spread of the concentrations is symmetrical. Caffeine was detected in Spain at approximately 19 and 5 μg/L in wastewater treatment plant influent and effluent samples, respectively (Reyes-Contreras et al. 2012). A number of literature reports have also suggested that caffeine can be used as a tracer of sewage/wastewater contamination in surface waters (Heberer 2002b; Kolpin et al. 2002). The spatial pattern of the pharmaceuticals investigated in this study is presented in the next section.

Spatial distribution

Beside the general concentration pattern of the drugs investigated, it is essential to consider the spatial distribution pattern and the possible associated source inputs of the analytes based on the various anthropogenic activities within the sample collection points. The spatial distribution pattern is also useful in obtaining information on the fate and mobility of the compounds and the biogeochemistry of the environment responsible for their fate. The plots of the spatial pattern of the analytes

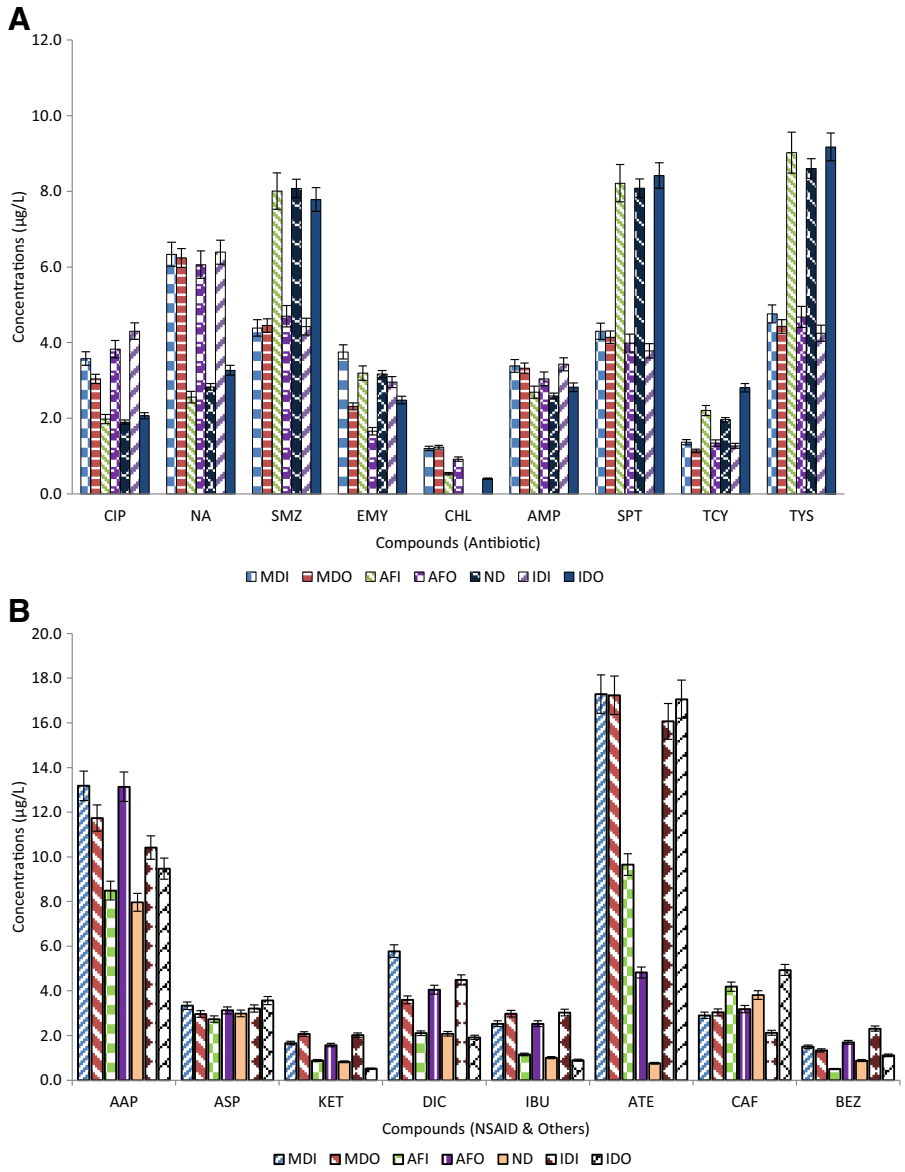

Fig. 7 **a** Plot of spatial concentrations distribution of nine antibiotics in seven dam-related samples (*MDI* Midmar Dam Inlet, *MDO* Midmar Dam Outlet, *AFI* Albert Falls Inlet, *AFO* Albert Falls Outlet, *ND* Nagle Dam, *IDI* Inanda Dam Inlet, and *IDO* Inanda Dam Outlet). **b** Plot of spatial concentrations distribution of five antipyretics and other drugs in seven dam-related samples (site label same as in **a**)

in this study are discussed based on three classifications of samples from dams, surface water, and wastewater.

Dam

The plots of the site concentration patterns of the 17 pharmaceuticals studied in the 7 sampling points from dams are presented in Fig. 7a, b. There is generally no remarkable concentration variation between the inlet and the outlet of Midmar Dam for the drugs except for erythromycin, acetaminophen, and diclofenac where there are decreases in concentrations at the outlet compared to the inlet. Midmar Dam and Inanda Dam had a relatively higher concentration of atenolol compared to other drugs. Ciprofloxacin and nalidixic acid exhibit similarities in their distribution patterns in Albert Falls. The concentrations of ciprofloxacin and nalidixic acid increased significantly from the entry site to the exit site.

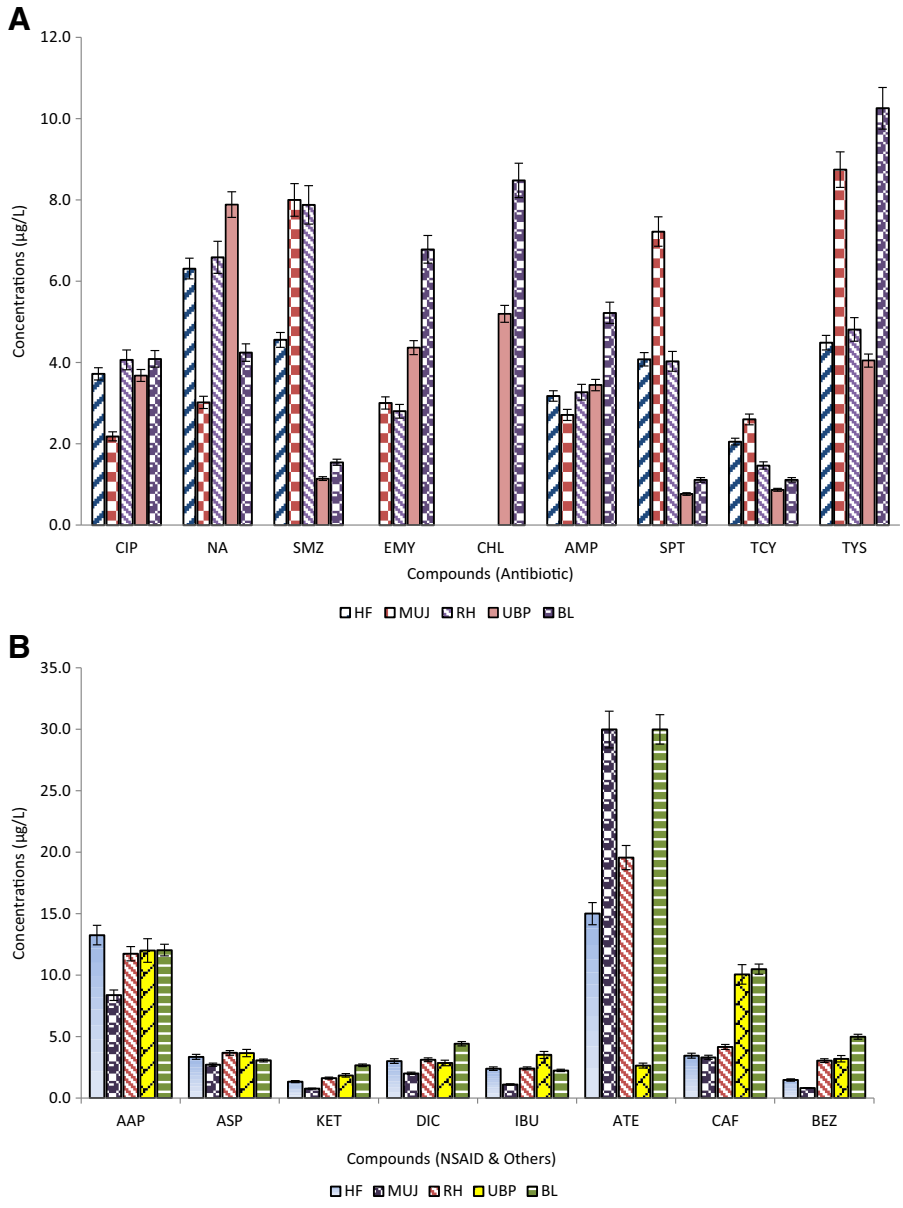

Fig. 8 **a** Plot of spatial concentrations distribution of nine antibiotic drugs in surface water samples (*HF* Howick Falls, *MUJ* Msudunzi/Umgeni Joint, *RH* Reservoir Hills axis, *UBP* Umgeni Business Park, *BL* Blue Lagoon). **b** Plot of spatial concentrations distribution of five antipyretics and other drugs in surface water samples (site label same as in **a**)

An increase was also observed for acetaminophen, diclofenac, and bezafibrate. The concentration increase of chloramphenicol and ampicillin in Albert Falls downstream is not remarkable. Conversely, Albert Falls had a drop in the concentrations of sulfamethoxazole, erythromycin, streptomycin, tetracycline, and tylosin from the inlet to the outlet which suggest that there are possible site-specific biogeochemical conditions that support the sequestration or degradation of these analytes thus reducing their concentration which may need further investigation for a better understanding. The concentration of atenolol also decreased significantly in Albert Falls downstream. The distribution pattern of sulfamethoxazole, streptomycin, and tylosin were also similar in Albert Falls inlet, Nagle Dam, and Inanda Dam outlet where higher concentrations were observed compared to other sites. Inanda Dam results depict a downstream increase in the concentrations of

sulfamethoxazole, chloramphenicol, streptomycin, tetracycline, and tylosin but a decrease in the concentrations of ciprofloxacin and nalidixic acid. This trend is the opposite of the distribution trend observed in Albert Falls which suggests that the chemistry of these environments and fates of the pharmaceuticals in them are not the same. Nagle Dam is relatively more pristine than the other dams with the least concentrations of most of the analytes studied except for sulfamethoxazole, streptomycin, and tylosin. Aspirin concentrations observed in the dams were closely related from one dam to the other. Moreover, similar distribution patterns were obtained for ketoprofen, ibuprofen, and bezafibrate in the dam. There were downstream concentration decreases in ketoprofen, ibuprofen, bezafibrate, and diclofenac in Inanda Dam as compared to their downstream increase observed in Albert Falls. This further confirms that the chemistry and fate of pharmaceuticals in Inanda Dam and Albert Falls inversely correlated while Midmar Dam exhibits no significant changes downstream. Caffeine concentrations in the dams did not vary significantly except in Inanda where there was an increase downstream. Chloramphenicol was below the detection limit in Nagle Dam and Inanda Dam inlet. The relative stagnancy of the water system in the dams may also contribute to the observed concentrations and variations.

Surface water

The sampling sites classified as surface water had a higher water flow rate compared to the surface water that was dammed which implies the possibility of higher mobility, variations, and perturbation. The surface water sites, however, have higher anthropogenic activities than the dams. These factors will contribute to the distribution trends of the studied pharmaceuticals at the sites. The site-based plots of the concentrations of the EOCs in the surface water samples are presented in Fig. 8a b. The general concentration trend indicates that the antibiotics in the surface water are slightly higher than in the dams except in few cases while the other classes of drugs investigated are of similar concentrations.

The Blue lagoon (BL) sampling site depicts the downstream accumulative load of the analytes being the estuary mouth. All the analytes investigated were detected at this site and it had the highest concentration of chloramphenicol, ampicillin, erythromycin, ciprofloxacin, tylosin, ketoprofen, diclofenac, atenolol, caffeine, and bezafibrate. None of the analytes investigated was least at the BL site but it has relatively lower concentrations of sulfamethoxazole, streptomycin, and tetracycline. Chloramphenicol was actually detected in only BL and Umgeni Business Park (UBP). UBP had the maximum concentrations of ibuprofen and nalidixic acid. The concentrations of the other drugs in UBP were lower than the other surface water sites except caffeine that is of comparable value with that at BL. It may be implied that the industrial/commercial activities at UBP may not be making significant EOCs contribution to the immediate environment since there is a channeling of the effluent for treatment in waste treatment plants. However, the contribution of caffeine in BL and UBP above other surface water sites may be an indicator of contributions from sewage/wastewater contaminations into surface waters since caffeine had been reported to be a useful tracer of this (Heberer 2002b; Kolpin et al. 2002).

Furthermore, samples collected at the Reservoir Hills (RH) axis had a concentration pattern similar to that at the UBP especially for acetaminophen, aspirin, ketoprofen, diclofenac, bezafibrate, ciprofloxacin, and ampicillin. Reservoir Hills, however, has relatively high concentrations of sulfamethoxazole, nalidixic acid, and atenolol. The results of the concentrations of the EOCs studied in the joining point between Umgeni River and Msudunzi River (MUT) indicates that there are possible combined contributions of streptomycin, tetracycline, tylosin, and atenolol from the tributary and from Nagle dam. The MUT site is downstream of Nagle dam which both have comparable concentrations of these analytes. Acetaminophen is the only substance higher in Howick Falls (HF) than the others. Erythromycin and chloramphenicol were below detection limits at Howick Falls. The concentrations of other substances in HF rank midway compared with the other sites but their occurrences and concentrations are evident.

Wastewater

Wastewaters from household/domestic activities, sewage wastes, and industrial activities have been extensively documented in literature to be the major sources of EOCs in the environment (Miao et al. 2004; Castiglioni et al. 2005; Bolong et al. 2009). This study also observed far higher concentrations of the 17 analytes studied in the influent samples into the wastewater treatment plant. Figure 9a, b present the results of

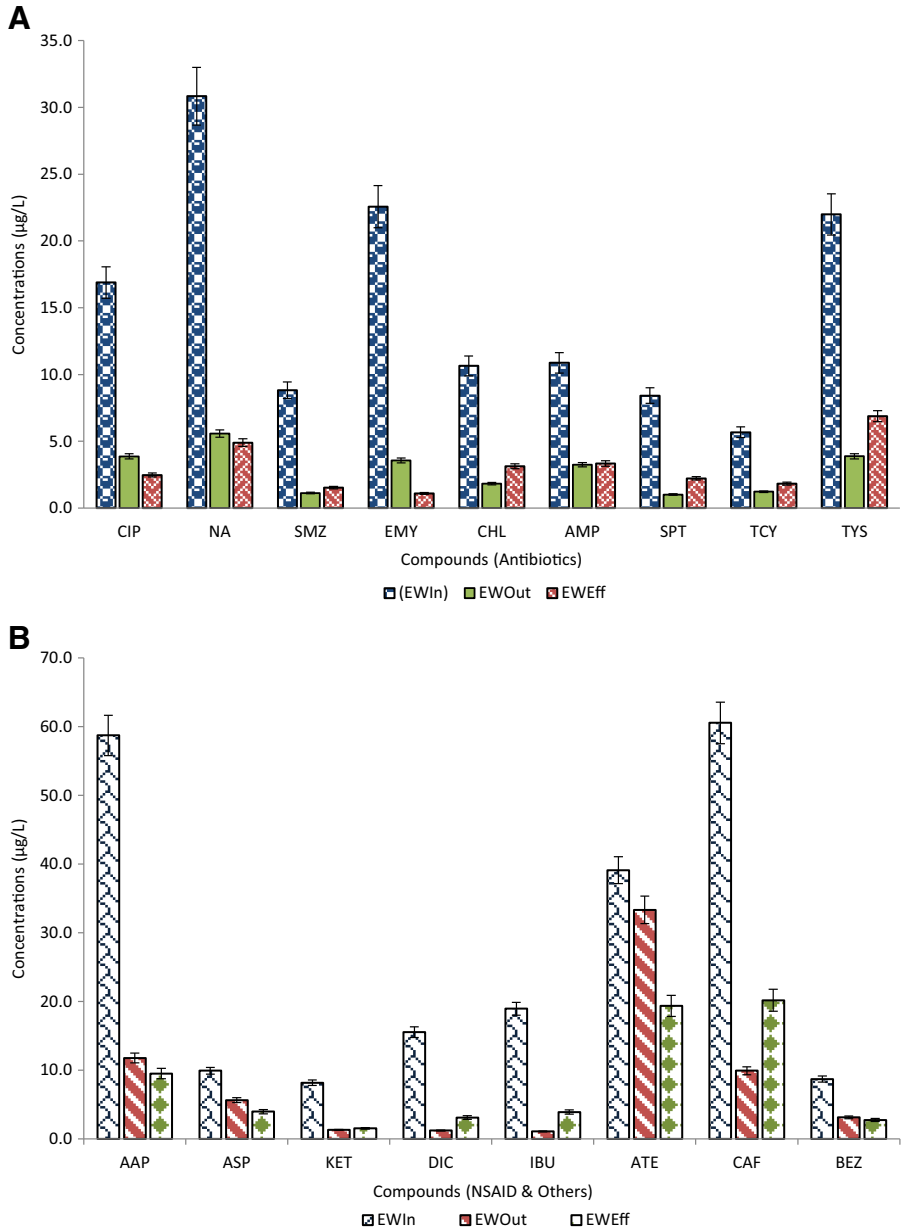

Fig. 9 **a** Plot of spatial concentrations distribution of nine antibiotic drugs in wastewater samples (*EWIn* Northern WWTP Influent, *EWOut* Northern WWTP after treatment, *EWEff* Northern WWTP Effluent). **b** Plot of spatial concentrations distribution of five antipyretics and other drugs in wastewater samples (site label same as in **a**)

the analytes in the wastewaters. The wastewater influent had the highest concentrations of the EOC analytes studied. Nalidixic acid was the antibiotic with the highest concentration in the wastewater (31±3 μg/L) followed by erythromycin and tylosin. The concentrations of chloramphenicol, ampicillin, sulfamethoxazole, and streptomycin were similar and below 10 μg/L in the influent wastewater while tetracycline had the lowest concentration. Caffeine and acetaminophen were the most abundant EOC in wastewater influent with concentrations of 61±5 and 59±4 μg/L. The caffeine concentration buttresses further its tracer capacity of wastewater impact on the surface water while the acetaminophen abundance is not unexpected since it is an over-the-counter drug and its administration requires no prescription. Atenolol concentration was notably high (39±

4 μg/L) in the influent sample. The studied analytes were all detected in the wastewater samples. The concentrations of aspirin, ketoprofen, and bezafibrate were closely related and lower than 10 μg/L. There are indications that the waste treatment process reduces significant portions of the analytes from the influent sample. The output samples after chlorination were significantly lower than the influent except that atenolol had a relatively high concentration in the treated sample.

The removal efficiency of the EOCs in the treatment process by the plant was measured by the ratio of the drop in concentration between the influent and the output after chlorination prior to discharge to the initial concentration in the influent. The efficiency of the treatment process on the antibiotic was in the range of 70.1–88.0 % removal with streptomycin having the highest removal percentage and ampicillin having the least. The concentrations of the antibiotics in the output samples were comparable to the concentrations obtained in the surface water environment. The treatment efficiency of atenolol was low (14.8 %) in the treatment process. Aspirin had 43.0 % removal from the influent. The other analytes had a percentage removal in the range of 63.9–94.2 % with ibuprofen having the highest percentage removal.

The sampling site labeled as EWEff is the discharge point of the wastewater treatment plant into the Umgeni River downstream after discharge and the observed concentrations of a significant number of the analyte indicate higher concentration than the concentrations in the effluent before discharge. There were increases in concentrations of seven analytes (caffeine, ibuprofen, diclofenac, chloramphenicol, streptomycin, tetracycline, and tylosin) between the treated wastewater before discharge and downstream samples after discharge which indicates possible contributions upstream of the discharge point into this discharge point. The Umhlangane River joins Umgeni River at this point and may be a possible source of additional input of these compounds. Study on the occurrence of pharmaceuticals in the Umhlangane River may thus be explored further. Ketoprofen, bezafibrate, ampicillin, sulfamethoxazole, and nalidixic acid had no significant changes in their concentrations before and after discharge. The other five analytes (ciprofloxacin, acetaminophen, aspirin, atenolol, and erythromycin) had a decrease in concentration downstream after discharge. The chemistry or other factors that are responsible for the fate and mobility of these analytes at the discharge point and downstream in the river are not yet fully understood and may require further investigation.

Conclusion

This study investigated the occurrence of 17 pharmaceuticals—9 antibiotics, 5 antipyretics, 1 β-blocker, 1 lipid regulator, and a psychostimulant in wastewater from a domestic wastewater treatment plant, Umgeni surface water, and dams along the Umgeni River used for water supply in KwaZulu-Natal, South Africa. The HPLC-DAD method for determination of these pharmaceuticals using standard addition and SPE preconcentration gives a relatively good recovery but the detection limits were higher than LC-MS methods reported in literature. All the compounds were detected in the wastewater at high concentrations but similar to those obtained from other countries in Europe and Asia. The wastewater treatment process reduced the influent concentrations of all studied pharmaceuticals by 43.0–94.2 % before discharge except for atenolol which had lower removal (14.8 %) after treatment. The frequency of occurrences and concentrations in surface water were lower than in the influent. Blue Lagoon which is the mouth of the river and the discharge point into the ocean had the highest concentrations of some of the studied compounds in surface water which depict the possibility of downstream load. The factors governing the fate and mobility of these compounds in the environment of this river are not fully understood yet and will require further studies.

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