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OCCURRENCE AND DISTRIBUTION PATTERN OF ACIDIC PHARMACEUTICALS IN SURFACE WATER, WASTEWATER, AND SEDIMENT OF THE MSUNDUZI RIVER, KWAZULU-NATAL, SOUTH AFRICA

FOLUSO O. AGUNBIADE*†‡ and BRENDA MOODLEY†

†School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, Durban, South Africa

‡Department of Chemical Sciences, College of Natural Sciences, Redeemer's University, Osun State, Nigeria

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Abstract: The paucity of information on the occurrence of pharmaceuticals in the environment in African countries led the authors to investigate 8 acidic pharmaceuticals (4 antipyretics, 3 antibiotics, and 1 lipid regulator) in wastewater, surface water, and sediments from the Msunduzi River in the province of KwaZulu-Natal, South Africa, using solid-phase extraction (SPE) and liquid chromatography–mass spectrometry (LC/MS). The method recoveries, limits of detection (LOD), and limits of quantification were determined. The method recoveries were 58.4% to 103%, and the LODs ranged between 1.16 ng/L and 29.1 ng/L for water and between 0.58 ng/g and 14.5 ng/g for sediment. The drugs were all present in wastewater and in most of the surface water and sediment samples. Aspirin was the most abundant pharmaceutical observed, 118 ± 0.82 μg/L in wastewater influent, and the most observed antibiotic was nalidixic acid (25.2–29.9 μg/L in wastewater); bezafibrate was the least observed. The distribution pattern of the antipyretic in water indicates more impact in suburban sites. The solid–liquid partitioning of the pharmaceuticals between sediment and water, measured as the distribution coefficient ($\log K_D$) gave an average accumulation magnitude of 10× to 32× in sediments than in water. The downstream distribution patterns for both water and sediment indicate discharge contributions from wastewater, agricultural activities, domestic waste disposal, and possible sewer system leakages. Although concentrations of the pharmaceuticals were comparable with those obtained from some other countries, the contamination of the present study site with pharmaceuticals has been over time and continues at present, making effective management and control necessary. *Environ Toxicol Chem* 2015;9999:1–11. © 2015 SETAC

Keywords: Emerging contaminants Solid-phase extraction Liquid chromatography–mass spectrometry (LC/MS) Water Sediment

INTRODUCTION

Regulation of pharmaceuticals and their treatment is not as stringent in African countries relative to developed economies, and the current wastewater treatment systems were not designed with the intent of managing pharmaceuticals as contaminants. These are strong justifications for investigating the occurrence, concentration, and distribution patterns of these contaminants in African countries. A recent global trend among environmental researchers and the scientific community emphasizes investigating the occurrences and concentrations of pharmaceuticals and other personal care product compounds, which are a class of emerging contaminants in the environment. This is predicated on the potential ecotoxicity of these compounds and their metabolites [1–3]. There are growing concerns over the toxicological effects of the exposure of these compounds to humans and the ecosystem [4,5]. The identified risks include development of microbial resistance to antibiotics and feminization or masculinization of aquatic organisms on exposure to hormones [6–8], among others. The environmental compartments that have been studied include wastewater treatment plant (WWTP) influents, effluents, and receiving surface water [9–11]; sediments [12,13]; biosolids and waste from confined animal farms [14]; and groundwater and drinking water [15,16]. The volume of studies in literature has shown that pharmaceuticals and personal care products (PPCPs) are ubiquitous contaminants globally, with diffused sources of input compared with classical pollutants. The discharges from WWTPs, which collect these contaminants from various sources with varying water matrix conditions, are well-identified sources of input into the environment. Thus, the evaluations of pharmaceuticals in WWTP samples for source identification and concentration contribution into the different environmental compartments has been carried out using WWTPs [10,17–19].

The study of pharmaceuticals in the environment is also becoming important because they differ from other classical contaminants that have previously been extensively studied, and there is increasing advocacy for focusing on monitoring pharmaceuticals [20]. They have widespread applications and acceptable uses with limited regulations, and some are grossly abused. Their primary input sources are widely varied, and source management difficult. Pharmaceutical compounds in the environment are not as persistent as the classical organic pollutants, but they have been reported in concentration ranges of sub-milligrams per liter to nanograms per liter, depending on the substance, the compartment of the environment in which they were investigated, and the level of application of the compounds in the studied areas. There is evidence of their potential adverse effect on lives within the ecosystem, even at these observed concentrations [21]. Presently monitored and regulated contaminants are most often from industrial or agricultural applications and are compounds that have been banned or have limited usage. Pharmaceuticals, however, are in use and are discharged by individuals, hospitals, and agro-based

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* Address correspondence to agunbiadef@run.edu.ng

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and pharmaceutical industries, making their entrance into the environment more continuous. Moreover, concurrent discharges occur across various areas of anthropogenic activities, from decentralized systems such as septic systems to washing off of dermal applications during bathing, and their natural reduction in the environment is less understood. Studies conducted in America and Canada support the view that PPCPs are being released from sewage septic tanks, decentralized system effluent, or onsite treatment plants into the water systems through the aquifers [22–26]. Dougherty et al. [16] reported that approximately 25% of the US population uses some form of decentralized system to treat and dispose of their wastewater, whereas the remaining 75% are connected to municipal sewers. A resulting effect is difficulty in estimating pharmaceuticals' steady-state concentration or understanding their fate and mobility in the environment toward appropriate management strategy. These indicate the need to evaluate these compounds in the centralized sewers and beyond for proper understanding of their distribution trend.

The studies about environmental pharmaceuticals in literature have been predominantly from Europe, North America, Australia, and some parts of Asia, where there are relatively stricter regulations on usage and prescription of drugs. Some studies of pharmaceuticals in the environments of South Africa have also been documented recently [27,28]. However, a paucity of information exists on their occurrence, concentrations, fate, and mobility in Africa. Their low concentration, high vapor pressure, good solubility in polar solvents, and low stability in heat have limited detection methods and favored the use of hyphenated liquid chromatographic methods. The need for analyte preconcentration methods has also been identified in literature. The present study is therefore focused on the application of solid-phase extraction (SPE) preconcentration method followed by liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS) in evaluating occurrences, concentrations, and distribution patterns of 8 acidic pharmaceuticals in wastewater, surface water, and sediments from the Msunduzi River in KwaZulu-Natal, South Africa. The pharmaceuticals investigated (Supplemental Data, Table S1) included 4 antipyretics (most frequently used without prescription and classified over the counter), 3 antibiotics, and 1 lipid regulator because of their ecotoxicity potential.

MATERIALS AND METHODS

Materials

External standard pharmaceutical compounds used for quantification were all purchased from Sigma-Aldrich through Capital Lab. The standards were diclofenac sodium salt and nalidixic acid (products of Sigma-Aldrich Italy). Ciprofloxacin, aspirin, ampicillin, ketoprofen, bezafibrate, and ibuprofen were products of Sigma-Aldrich China. The details of the chemical data of the pharmaceutical compounds are presented in Supplemental Data, Table S1. Methanol, acetonitrile, ethyl acetate, and acetone were high-pressure liquid chromatography 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 was 6 mL Oasis® HLB (150 mg sorbent mass).

Site description and sampling procedure

The sample collection sites were within the Msunduzi River (Figure 1). The river passes through the Msunduzi district (634 km^2) within Pietermaritzburg, the capital city of KwaZulu-Natal in South Africa. KwaZulu-Natal is the second most populated province in South Africa. The population of the

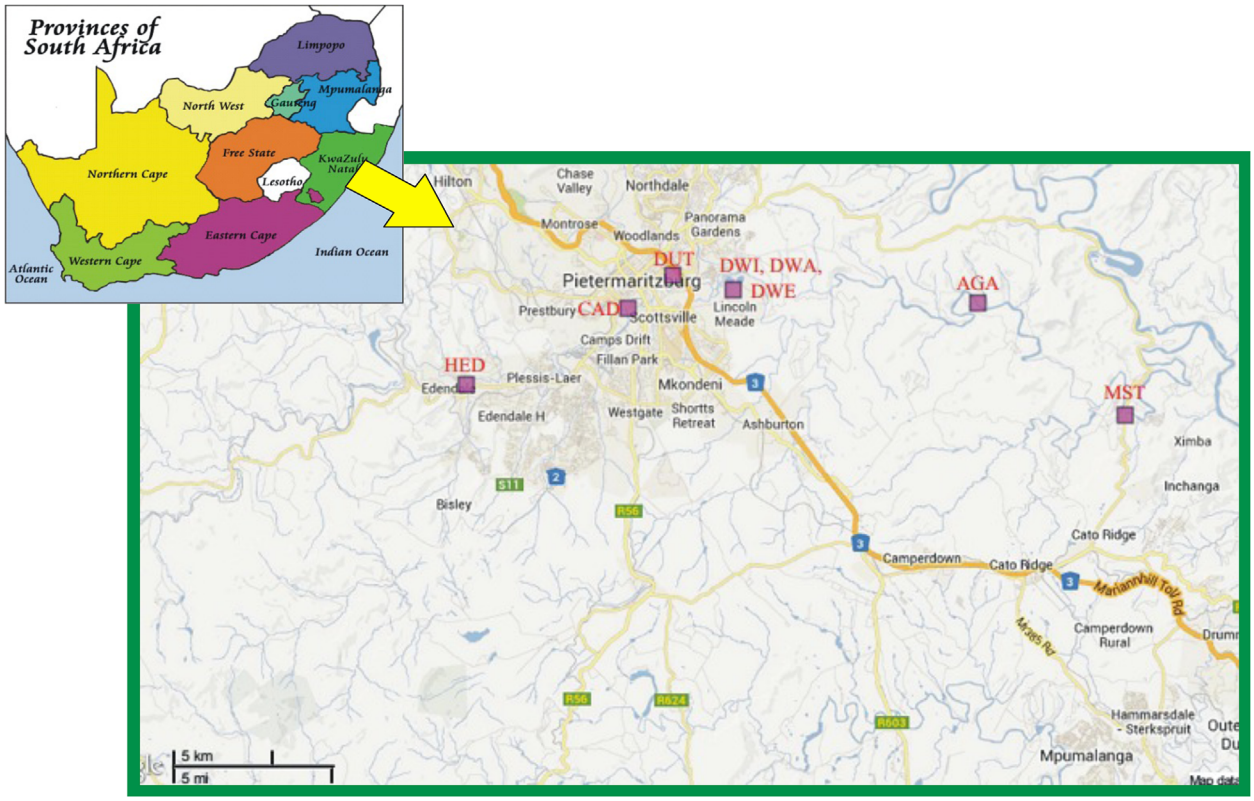

Figure 1. Map of the sampling sites with the representation of the sampling points on the Msunduzi River in the province of KwaZulu-Natal, South Africa.

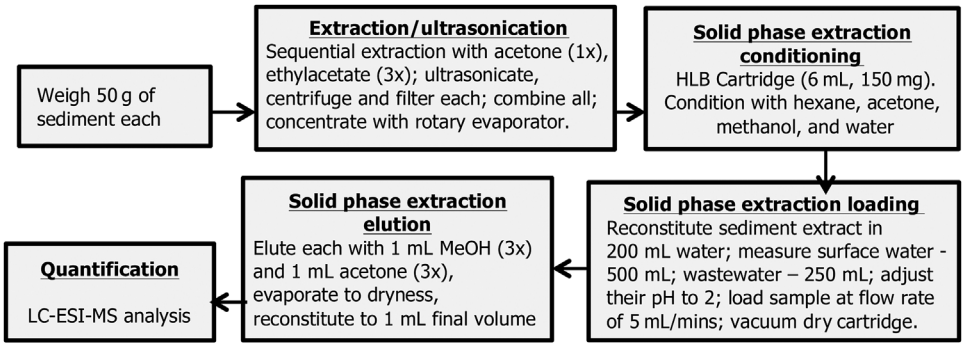

Figure 2. Flow diagram of the protocol used for the analysis. LC-ESI-MS = liquid chromatography–electrospray ionization mass spectrometry.

province as of the 2011 South African Census was 10.267 million people, and the Msunduzi municipality population is 618 536, with a growth rate of 1.1% [29]. The Msunduzi River drains approximately two-thirds of the metropolitan region. It passes through Henley dam, commercial farming settlements, and formal and informal settlements. It empties into the Umgeni River downstream of the Nagle Dam. The river catchment has several domestic and commercial activities, sewers, a WWTP, and industrial activities that are potential primary sources of the analytes of interest in the present study. In some of the sampling sites, there is evidence of freelance grazing of animals, confined animal grazing, poor sanitation, inadequate wastewater treatment services, and industrial pollution, which can result in high levels of contamination of the river. The coordinates and identified activities at the sampling points for the present study are presented in Supplemental Data, Table S2.

The sampling sites (Figure 1) include Darvill WWTP, where influent water sample, sample after treatment, and effluent of discharge-treated domestic waste into the river were collected. The treatment plant receives wastewater from domestic (30%) and hospital facilities, commercial sources, and industrial sources (70%). It is the major treatment plant handling all wastewater from the district. The treatment plant aerates by stirring the wastewater influent, after which it undergoes sedimentation, chlorination, and discharge into the receiving water. There are indications that the plant is handling more than its capacity. It has an installed treatment capacity of 65 ML/d but currently has a dry weather inflow load above 70 ML/d, with a wastewater quality compliance of 48% [30]. This indicates that the plant is handling more than its installed capacity, but attempts are being made to upgrade the Darvill WWTP to increase its handling capacity. The sampling sites CAD and DUT are in the core of the capital city, with higher anthropogenic activities, whereas site AGA hosts several commercial farming activities. Site HED is in the city suburb, whereas site MUT is a medium populated town. These identified sample collection sites along the river were studied in May 2013.

Water samples were collected in amber glass bottles at each sampling site before sediment collection, transported in an ice chest, and fixed with 1.5 mL concentrated H_2SO_4/L of sample collected. The samples were collected at the edge of the river flow and approximately 20 cm below the water surface. Samples were collected in triplicate and were kept in a refrigerator at 4 °C until analyses were completed. The conductivity, temperature, pH, and total dissolved solids of the water system were measured at the site. Sediment samples were collected with an Ekman grab sampler at an approximate depth of 0.25 m and stored in glass bottles. The sediments had loamy texture. The protocol recommended by APHA [31] for the treatment and cleaning of the sampling bottles before sampling was carried out.

Analysis protocol

The summary of the analytical protocol adopted for the present study is presented in a flow diagram (Figure 2). The methods were modifications of the methods presented by Löffler and Ternes [12], Lindqvist et al. [32], and Castiglioni et al. [33].

Sediment extraction

Sediment masses (50 g) were extracted successively in an ultrasonic bath with 45 mL of acetone/acetic acid [20:1 (v/v)] followed by 3 successive extractions with 45 mL ethyl acetate. The slurries of the solvent–sediment mixtures were thoroughly hand shaken and ultrasonicated for 25 min at 30 °C. The sonicated slurries were centrifuged and the supernatant solvent phases filtered. The different successive extracts were pooled together and evaporated using a Buchi rotary evaporator at 45 °C. The extracts were subjected to SPE cleanup.

Solid-phase extraction

The SPE cartridges' hydrophilic-lipophilic balance were conditioned before sample extraction with 6 mL *n*-hexane, 2 mL acetone, 10 mL methanol, and 10 mL double-distilled water. The sediment extracts were reconstituted in 200 mL double-distilled water, adjusted to pH 2 with 3.5 mol/L sulphuric acid before loading. The flow rate of the loaded sample was kept at 5 mL/min. The SPE of the water samples was carried out with 500 mL surface water and 250 mL wastewater, which were adjusted to pH 2 and filtered with 0.45-μm filters, after which they were loaded onto the cartridges using an SPE manifold at a flow rate of 5 mL/min. After extraction, the cartridges were vacuum dried for 5 min. The extracts were eluted from the SPE cartridges using 3 × 1 mL methanol and 3 × 1 mL acetone. The eluates were evaporated to dryness with vacuum-drying [33] and reconstituted with acetone/acetic acid (pH 2). The reconstituted samples were made up to a final volume of 1 mL.

Liquid chromatography–mass spectrometry analysis and quantification

The identification and the quantification of the analytes were conducted with LC-ESI-MS. The liquid chromatography (LC) system used was an Agilent LC 1200 series having a degassing chamber (G1322A), auto sampler (G1329A), diode array detector (DAD; G1315D), and column oven (G1316A). The details of the LC solvent system and the method parameters for

the analytes investigated are presented in Supplemental Data, Table S3. The mass spectrometer (MS) used was an Agilent 1100 series LC/MSD Trap—G2446C VL. The analytes were determined using selected reaction monitoring mode with the electrospray ionization (ESI) set in the negative mode at 350 °C. The nitrogen-collision–induced dissociation was achieved in a nebulizer set at 55 psi and with dry air (10 L/min). The settings of the ESI source and the MS parameters were automatically optimized, and the analytical parameters of the MS trap drive used include ion source (spray) voltage of 4.6 kV at a split rate of 1:10. The skim 1 and 2 voltages were –36.3 V and –6.0 V, respectively; the capillary exit voltage was –110.1 V, with its offset voltage at –73.8 V; the radio frequency (RF) amplitude was 150.0 V; and the MS peak-to-peak scanning range was 50 *m/z* to 2200 *m/z*.

Method validation

The limit of detection (LOD) was calculated by using external standards at 3× the signal-to-noise ratio based on the standard deviation of the 8 calibration curve intercepts divided by the slope, and limit of quantification was calculated at 10× this ratio. A recovery study of the analytes was undertaken under the same conditions expressed for the samples as a quality assurance step. The water and sediment samples were spiked and the recovery determined from the concentration differences between the spiked samples (standard addition) and the unspiked samples with 6 replicate analyses. Surface water (500 mL) and wastewater samples (250 mL) were spiked with the analytes at a spike concentration of 1 μg/L. Sediment samples (50 g) were spiked,with 1-μg analytes corresponding to 20 ng/g spike concentration. The spiked sediment was kept for approximately 14 h to allow the spiked compounds to integrate with the sample matrix. It was kept in a refrigerator during this period to limit the effects of biotransformation by microbial and enzymatic activities. The recovery study and sample analyses were carried out in triplicate to measure the reproducibility/precision of the method. The mean recovery values are presented in Table 1, with the percentage relative standard deviations. 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 deviations of the analytes are presented for the samples (Table 1).

RESULTS

Method validation

The total ion chromatograms and the structures of the external standards of the 8 analytes used in the method development and applied to the investigated samples in the present study are presented in Figure 3A through H, in order of increasing retention time. Analyte identification and quantification was carried out in the negative mode $[M–H]^-$. The total ion chromatograms of the analytes showed distinct peaks at the observed reported retention times with minimal deviation, except for aspirin, with a significant isomer peak $[M+H]^+$. The results of the retention times, the method detection limits, limits of quantification, and calibration curve parameters are presented in Supplemental Data, Table S4. The method was most sensitive to ciprofloxacin, with LODs of 1.16 ng/L in water and 3.86 ng/g in sediment. It had the least sensitivity to aspirin at LOD values of 29.1 ng/L in water and 15.5 ng/g in sediments. The low sensitivity of the method to aspirin is further observed with the lowest instrumental mass spectral signal to noise (S/N) ratio of 11.8 compared with the other analytes. Löffler and Ternes [12] applied a benchmark of S/N ratio > 10, which was also adopted as the minimum benchmark for quantification in the present study because interference and matrix effects are lower at higher ratios. Bezafibrate had the highest S/N ratio and signal strength, which also can be seen from its intensity in the chromatogram (Figure 3E). The calibration ranges of the analytes where linearity were above the stated S/N ratio are also presented in Supplemental Data, Table S4, alongside the fitness of the external calibration curve. The general calibration linear range observed for the compounds was 10 μg/L to 2000 μg/L, except for ampicillin, ibuprofen, and ketoprofen.

Table 1. Results of recovery data[a]

Compound	Wastewater	Surface water	Sediments
Ampicillin	70.0 ± 8.1	86.3 ± 4.2	95.2 ± 4.4
Ibuprofen	63.0 ± 2.1	91.3 ± 5.9	72.2 ± 3.9
Aspirin	71.3 ± 9.3	92.8 ± 10.2	68.5 ± 4.0
Nalidixic acid	70.0 ± 11.1	103.9 ± 10.9	82.4 ± 3.2
Ciprofloxacin	83.5 ± 12.3	99.2 ± 12.2	89.1 ± 1.5
Bezafibrate	91.2 ± 12.1	80.2 ± 12.4	58.4 ± 0.8
Ketoprofen	61.8 ± 8.4	102.7 ± 5.5	72.0 ± 4.7
Diclofenac	95.4 ± 13.4	100.8 ± 12.2	68.0 ± 0.7

[a]Data represent recovery ± relative standard deviation (percentages); $n = 3$.

The results of the recovery studies of the compounds in surface water, wastewater, and sediments are presented in Table 1. The results showed that the method of analysis has good quality, with good recoveries. The recovery studies were carried out on the different classes of samples to evaluate matrix/interference effects and ion suppression tendencies of the analytes in each sample matrix. The spiked concentration of the analytes (1 μg/L) in the surface water experiment is environmentally relevant because it falls within the range of concentrations reported in literature and obtained in the present study, but the spiked concentration is relatively lower than the concentrations of analytes obtained for the wastewater in the present study. This may be responsible for the relatively lower recoveries obtained from the wastewater-spiked samples compared with surface water for most of the analytes, except bezafibrate. Likewise, the recoveries of the analytes in the sediment samples were relatively lower than the surface water samples and lower than the wastewater samples in some cases, except in ampicillin, where it was higher than the water samples. The spiked concentration used in the sediment study (20 ng/g) was also environmentally relevant but higher than that of the water samples. Wastewater and sediment samples do have higher organic load and matrix interferences than the surface water, which are indicators of possible ion suppression in the ESI mode. Ion suppression and matrix effects in ESI of different sample matrices with high organic matter and total organic carbon contents have been reported in literature [34–36].

The results of the concentrations of the analytes investigated in the different environmental matrices in the present study are presented in the *Wastewater* section.

Wastewater

The results of analyte concentrations obtained in the wastewater are presented in Figure 4. Figure 4A presents the concentrations of the antipyretics, and Figure 4B represents the concentrations of antibiotics and the investigated lipid regulator. The analytes investigated in the study were detected in all of the wastewater samples. Aspirin was the most abundant, at 118 ± 0.82 μg/L in the influent sample from the WWTP.

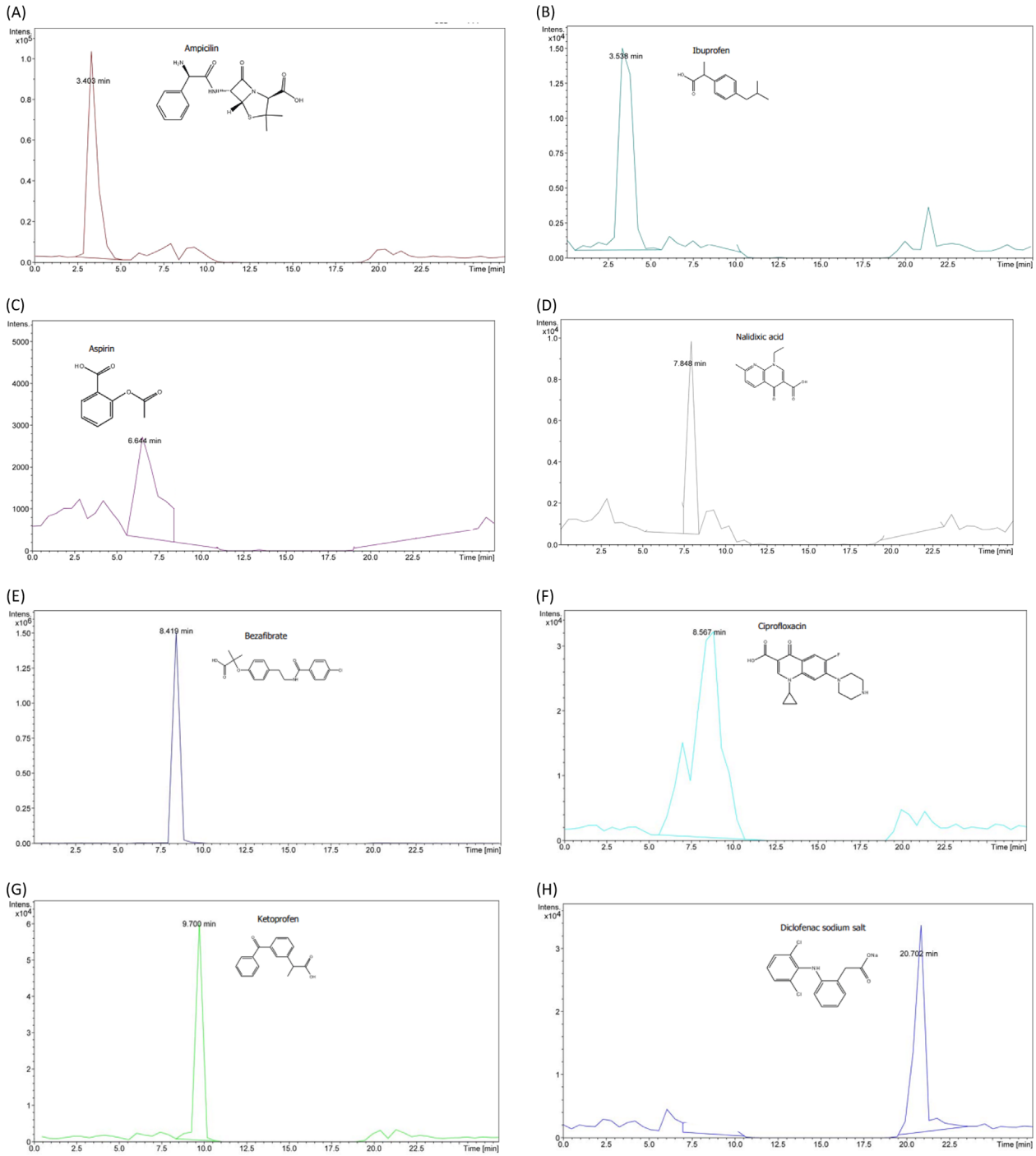

Figure 3. Total ion chromatograms of 8 pharmaceutical compounds: ampicillin (**A**); ibuprofen (**B**); aspirin (**C**); nalidixic acid (**D**); bezafibrate (**E**); ciprofloxacin (**F**); ketoprofen (**G**); and diclofenac sodium salt (**H**).

The concentration dropped to 47.1 ± 0.16 μg/L (60% reduction) in the WWTP sample after treatment. The sample collected downstream after the effluent discharge into the river contained 44.2 ± 0.18 μg/L aspirin, which is a minimal drop from the concentration discharged. Aspirin and its common metabolite (salicylic acid) were detected in wastewater influent samples in Canada at a median concentration of 330 μg/L and maximum concentration of 874 μg/L [37]. The occurrence of aspirin in the present study is comparable with results obtained in Canada. The observed concentration of aspirin in the wastewater influent can be related to the fact that it is an over-the-counter, nonprescription pharmaceutical drug and massively used as a pain reliever. Aspirin is used in South Africa in combination therapy with other antipyretics, with aspirin being the dominant ingredient. As of 2013, approximately 254 tonnes per annum of aspirin was sold in South Africa. Huschek et al. [38] put the estimate of the active substance of aspirin prescribed in Germany at 73.2 tonnes per annum, whereas the amount retailed was 775.4 tonnes. There is paucity of information on the retail rate of these drugs in South Africa (population, 51.2 million) but the possibility exists of related quantities between Canada (population, 34.9 million) and Germany (population, 81.9 million) based on related populations. The observed concentrations of another antipyretic drug, diclofenac, were lower than

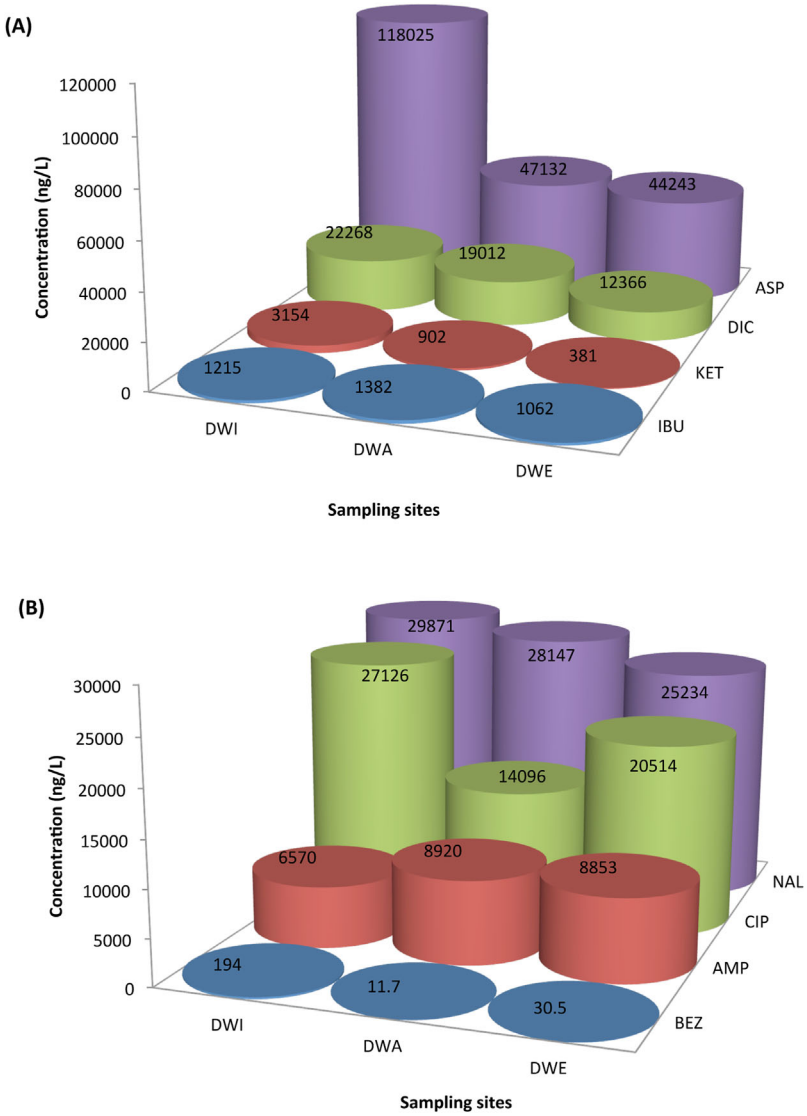

Figure 4. Mean concentration of analytes investigated in wastewater samples: (**A**) 4 antipyretics (ibuprofen [IBU], ketoprofen [KET], diclofenac sodium salt [DIC] and aspirin [ASP]); and (**B**) 3 antibiotics (ampicillin [AMP], ciprofloxacin [CIP], and nalidixic acid [NAL]) and 1 antihyperlipidemic (bezafibrate [BEZ]).

for aspirin in the wastewater samples. The influent sample had 22.3 ± 0.63 μg/L, which decreased to 19.0 ± 0.78 μg/L after treatment and 12.4 ± 1.56 μg/L downstream of the river/effluent mix. Ternes et al. [39] reported a comparable concentration of 15.3 μg/L to 19.4 μg/L for diclofenac from a German municipal WWTP. The predicted no-effect concentration reported for diclofenac in literature is 116 μg/L [32,40]. The concentration observed in the present study is much lower than the predicted no-effect concentration. The concentrations obtained in some other countries were relatively lower. Rabiet et al. [41] reported values of diclofenac in 2 WWTP effluents in France of 486 ng/L and 211. The concentrations of aspirin and diclofenac in the studied samples were higher than the concentrations observed for ketoprofen and ibuprofen. Ketoprofen was obtained in mean concentrations of 3.15 μg/L, 0.90 μg/L, and 0.38 μg/L in the WWTP influent, after treatment and effluent samples, respectively, whereas ibuprofen was present in a concentration range of 1.06 μg/L to 1.38 μg/L in these samples. Ketoprofen was obtained at a concentration of 1.2 μg/L in wastewater influent in Finland and with 78% contaminant removal after treatment [32]. Ibuprofen was detected in a concentration range of 5 μg/L to 8 μg/L in final wastewater effluent of 8 WWTPs in Canada [2]. Generally, the concentrations of these substances were comparable with occurrences reported in some other European and American countries. The treatment process (WWTP) did reduce the concentrations in some instances, as the values were lower after treatment than before treatment; but in some cases, no significant reduction occurred in the concentrations of these substances in the water system, because the treatment process is simply aeration, sedimentation, and chlorination.

The concentrations obtained for the 3 antibiotics and 1 lipid regulator, presented in Figure 4B, showed nalidixic acid as the most abundant, with a concentration range of 25.2 μg/L to 29.9 μg/L and with very minimal reduction from the wastewater influent downstream, as well as after treatment and the effluent discharged. This indicates little or no effect of the treatment process on this antibiotic in the WWTP. Nalidixic acid was reported in maximum concentrations of 0.04 μg/L in hospital effluent, 0.20 μg/L in WWTP influent, and 0.45 μg/L in the effluent water in Australia [42]. These concentrations were much lower that the concentrations obtained in the present study, which indicates a possibility of a higher degree of usage, of indiscriminate disposal or abuse of this drug in the study area,

or of additional sources of input that need further elucidation. Ciprofloxacin had a concentration of 27.1 ± 1.21 μg/L in the WWTP influent, with a 48% drop to 14.1 ± 0.62 μg/L after treatment. The concentration of ciprofloxacin increased after discharge of the treated wastewater into the river, which indicates the tendency of upstream contributions of ciprofloxacin into the river. Ciprofloxacin was observed at a concentration range of 27 ng/L to 514 ng/L in 8 WWTPs in Italy [33]. Hartmann et al. [43], however, reported a concentration range of 8 μg/L to 87 μg/L in hospital wastewater from Switzerland. The wastewater treated at Darvill WWTP is a combination of domestic and industrial wastewater (which includes hospitals) in the capital city, similar to the Switzerland study. The trend observed for ampicillin in the wastewater samples deviates from the norm. The after-treatment concentration (8.92 ± 0.70 μg/L) was higher than the influent sample load (6.57 ± 0.62 μg/L). The concentration of ampicillin in the downstream river/effluent mix decreased only marginally (8.85 ± 0.48 μg/L). This trend deviates from the pattern thus far observed and will require further study. Ampicillin has not been extensively studied in the environment compared with the other β-lactam drugs. Generally, there are indications of higher load and possible abuse of these drugs in the studied areas within the province compared with some other nations. Previous studies conducted in some provinces of South Africa reported 50% overprescription of antibiotics in public health facilities [44,45]. These coupled with the application of antibiotics in livestock farming and lack of a system for collecting unused drugs may be responsible for the observed concentrations.

The concentrations of the only lipid regulator investigated in the present study were much lower than those of all the other analytes studied. The concentration in the influent sample was 194 ± 5.6 ng/L, which was reduced by 94% to 11.7 ± 0.78 ng/L after treatment. After the discharge of the effluent into the river, the downstream concentration of the bezafibrate increased to 30.5 ± 0.59 ng/L, which is possibly attributable to contributions from the upstream surface water flow or may be related to sediment contribution from the river system perturbation at the discharge point. Bezafibrate was observed at higher average concentrations of 1878 ng/L in raw wastewater and 866 ng/L in treated wastewater in Spain [46] compared with the results obtained in the present study.

Surface water

The results of the antipyretics in the surface water are presented in Figure 5A. Their concentrations in the surface

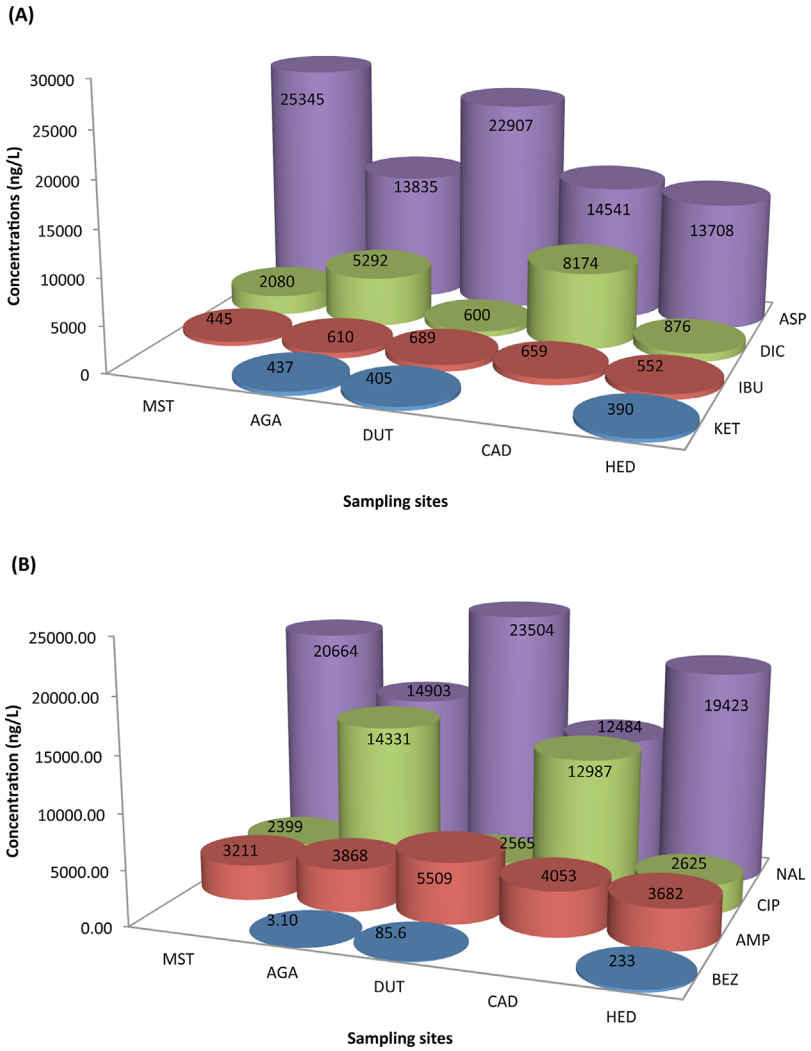

Figure 5. Mean concentration of analytes investigated in surface water samples: (**A**) 4 antipyretics (ibuprofen [IBU], ketoprofen [KET], diclofenac sodium salt [DIC] and aspirin [ASP]); and (**B**) 3 antibiotics (ampicillin [AMP], ciprofloxacin [CIP], and nalidixic acid [NAL]) and 1 antihyperlipidemic (bezafibrate [BEZ]).

water samples were expectedly lower than in the wastewater. All of the drugs were present in all of the surface water samples investigated, except ketoprofen. The direction of the flow of the river and the sampling point is Henley Dam (site HED), downstream to the town of Msunduzi (site MST). Aspirin had the highest concentration in the surface water, similar to the wastewater sample results. Aspirin is the most consumed pharmaceutical worldwide [41]. The concentrations of aspirin increased downstream of the river flow, from 13.7 ± 2.75 μg/L at site HED to 22.9 ± 3.14 μg/L in the more populated city center (site DUT). There was a marginal drop at the less populated sampling site and the commercial agricultural site (13.8 ± 0.72 μg/L), after which there was a notable rise to 25.3 ± 1.26 μg/L at site MST. The sampling point MST is an informal, suburban area with minimal facilities to manage contaminant discharges, and abuse of aspirin usage is likely in this area because of it being an over-the-counter drug. Also, the application of antipyretic drugs for pain relief may be more common among people involved in higher-energy, manual-labor and among lower-income earners, which is the case in most suburban, informal settlements [47]. Aspirin and its metabolite (salicylic acid) have been reported in surface water from different countries. Brun et al. [2] reported a concentration gradient of 0.2 μg/L to 4 μg/L aspirin downstream of a wastewater receiving stream in Canada. Watanabe et al. [48] simulated the concentration load of 68 μg/L and 119 μg/L aspirin in a lagoon receiving effluents from 2 dairy farms based on parameters such as volume of usage, excretion rate, and retention time/lagoon volume but without consideration for the attenuation and sequestration processes. The concentration observed in the present study falls between the simulated values and the naturally observed values reported in literature. Diclofenac was observed in the concentration range of 0.60 μg/L to 8.17 μg/L in these sites. The maximum concentration of diclofenac in the surface water was observed at the city center Camp drift sampling site (site CAD; Figure 5A), whereas the minimum was observed at another city center sampling site (DUT). A relatively significant concentration of diclofenac (5.29 ± 0.13 μg/L) was also observed at the sampling site that is in close proximity to commercial agriculture activities. Its presence at that site could be attributable to its application in livestock farming in the area. Ibuprofen was observed in a narrow concentration range of 445 ng/L to 689 ng/L across the surface water samples. The concentration change pattern of ibuprofen downstream of the river was similar to that observed for aspirin. Ellis [49] documented diclofenac concentrations of 20 ng/L and 100 ng/L upstream and downstream, respectively, for receiving surface water impacted with treated wastewater in the United Kingdom and ibuprofen concentrations of 900 ng/L upstream and 2000 ng/L downstream of the same surface water. In the present study, ketoprofen was not detected in 2 surface water samples and was the least detected antipyretic in the sampled sites. Ketoprofen was observed in a concentration range of 390 ng/L to 437 ng/L in sites where it occurred. A European Union–wide survey of 100 rivers conducted by Loos et al. [50] reported a maximum concentration of 239 ng/L for ketoprofen and an average of 10 ng/L. Ibuprofen was 31.3 μg/L maximum and 395 ng/L on average, and diclofenac was 247 ng/L maximum and 17 ng/L on average in EU surface water. Overall, the results obtained for the present study compared favorably with those of other countries except diclofenac, which is higher.

The concentrations of the antibiotics and antihyperlipidemic are presented in Figure 5B. The concentration of nalidixic acid was the most abundant and similar to the wastewater results. Its concentration in the surface water was in the range of 12.5 μg/L to 23.5 μg/L. The maximum nalidixic acid concentration was obtained at site DUT, and high concentrations also were found in the informal, suburban settlement sites (sites MST and HED). Ciprofloxacin had its maximum observed concentration in the surface water from the AGA site (14.3 ± 0.67 μg/L), which was followed by 13.0 ± 0.42 μg/L in the water from the city center CAD sampling site. The maximum concentration of ciprofloxacin reported in 2000 by a reconnaissance study of streams in the United States was 0.03 μg/L [51]. Batt et al. [52] also reported the downward concentration trend of ciprofloxacin in 2 New York rivers receiving treated wastewater outfall to be in the range of 0.03 μg/L to 5.6 μg/L, with the maximum concentration at the outfall point and a decrease in concentration downstream of the outfall. Ciprofloxacin in the present study was higher than in the US [52] study and a study of the Yangtze Estuary, China [53], but much lower than the concentrations reported in 2 surface water lakes in India (2.5 mg/L to 6.5 mg/L) [15]. Ampicillin in the surface water was observed to have a gradual increase from 3.68 ± 0.12 μg/L at site HED to 5.51 ± 0.52 μg/L at site DUT, after which there was a drop in concentration to 3.21 μg/L at site MST. The lipid regulator bezafibrate was not observed in the CAD and MST samples but had a relatively high concentration of 233 ± 2.1 ng/L at site HED compared with other sites. Loos et al. [50] carried out a Europe-wide river study and reported a maximum concentration of 1235 ng/L and an average concentration of 32 ng/L for bezafibrate, which is comparable with the result of the present study.

Sediment

The concentrations of the pharmaceuticals in the sediments from the study area are presented in Table 2. The occurrence and concentration trends of the antipyretics in the sediment were in the order of aspirin > diclofenac > ketoprofen > ibuprofen in

Table 2. Concentration of the analytes in the sediment samples (ng/g)

Site	Ibuprofen	Ketoprofen	Diclofenac	Aspirin	Ampicillin	Ciprofloxacin	Nalidixic acid	Bezafibrate
MST	6.85 ± 0.62	11.0 ± 0.92	222 ± 3.37	376 ± 21.8	87.2 ± 2.02	183 ± 4.14	307 ± 27.3	<LOQ
AGA	9.14 ± 0.16	9.12 ± 1.68	106 ± 20.3	300 ± 7.93	69.4 ± 20.1	63.6 ± 6.41	298 ± 7.54	80.3 ± 9.49
DWA	9.56 ± 0.41	38.4 ± 3.36	153 ± 7.62	366 ± 45.6	99.9 ± 4.30	<LOD	318 ± 6.90	<LOD
DWE	4.76 ± 0.10	29.6 ± 4.39	<LOD	212 ± 1.60	50.8 ± 2.66	72.0 ± 22.1	117 ± 23.1	1.43 ± 0.14
DWI	11.2 ± 0.66	57.4 ± 2.59	309 ± 15.3	427 ± 4.47	60.9 ± 4.38	60.4 ± 19.9	455 ± 12.2	8.00 ± 0.84
CAD	7.29 ± 0.55	12.0 ± 1.42	57.2 ± 1.05	370 ± 42.2	86.5 ± 1.87	<LOD	190 ± 10.7	1.73 ± 0.21
DUT	7.29 ± 1.30	8.94 ± 0.36	<LOD	304 ± 84.1	369 ± 19.0	13.6 ± 1.22	128 ± 3.16	<LOD
HED	9.14 ± 0.10	6.68 ± 0.21	82.8 ± 5.37	390 ± 13.1	77.1 ± 2.94	139 ± 15.6	243 ± 18.3	1.82 ± 0.22

LOQ = limit of quantification; LOD = limit of detection.

most of the sites. The maximum concentration of most pharmaceuticals was obtained from the biosolid separated from the WWTP inlet (site DWI). The highest concentration of the antipyretic drugs in the sediment was aspirin, which correlated well with the results obtained from water samples. Aspirin was observed in a concentration range of 212 ng/g to 427 ng/g. Diclofenac was not detected in sediment samples from 2 sites but occurred in the concentration range of 57.2 ng/g to 309 ng/g in other sites. A notable increase occurred in the concentration of diclofenac at sampling site MST. This may be as a result of site MST being a downstream location, which implies that the movement of the analyte and its subsequent deposit into the sediment phase at the site is more pronounced. Ketoprofen and ibuprofen were detected in all of the sediments in the ranges 6.68 ng/g to 57.4 ng/g and 4.78 ng/g to 11.2 ng/g, respectively. Reduction in concentration of the substances in the sediment after wastewater treatment was not significant. Diclofenac had the highest concentration drop between the influent biosolid and after treatment (50.5%). These observed concentrations of the analytes in the sediment are governed by a number of processes, such as transportation (mobility and dilution), sequestration (sedimentation, bioaccumulation, precipitation, adsorption on particulate, or volatilization), and degradation (hydrolysis, biotransformation, photo-transformations), among others [54,55]. These processes attenuate or transport the analytes between the mobile water system and the more stable sediment system, but their effects on the chemistry of emerging contaminants in the environment are yet to be adequately understood and may be site specific, depending on the chemistry of that environment. Thus, the variation pattern of aspirin observed in sediments collected from surface water sites indicates higher concentration in sites near suburban, informal settlements (sites HED and MST). The concentrations of these substances in the sediment from the river did not vary drastically downstream, probably because sediments are less mobile. The chemistry of these substances in the environment requires further elucidation to fully understand their fate. The half-lives of these pharmaceuticals are known to be shorter than the classical pollutants, and they are more susceptible to degradation (biological and photolytic). The presence of antipyretics in sludge, biosolids, and sediments has also been documented in literature by researchers. Ternes et al. [39], documented concentrations of diclofenac of 7020 ng/g in wastewater primary sludge and 310 ng/g in secondary sludge. The same study reported 120 ng/g for ibuprofen in secondary sludge.

Moreover, the order of the concentration of the antibiotics in sediments were generally nalidixic acid > ciprofloxacin > ampicillin in most of the sampling sites, except at the 2 sites where ciprofloxacin was below the detection limit (sites DWA and CAD). The WWTP influent biosolid had the highest concentration of nalidixic acid, but a different trend was observed for ciprofloxacin and ampicillin. It is noteworthy that the separated biosolids from the WWTP influent point (site DWI) is not a measure of accumulation over time but of source contributions, because the sample was not collected from stationary sediment but separated from a mobile inlet wastewater system. Nalidixic acid concentration dropped in the WWTP effluent discharge site to 117 ± 23 ng/g, which may indicate lesser accumulation downstream after discharge of the effluent although the concentration in the sediment after treatment is high (318 ± 6.9 ng/g). Comparison with the results of nalidixic acid in the wastewater indicates that the reduction is not from the treatment process but from the possibility of less partitioning or sequestration of nalidixic acid into the sediment at this discharge point, based on the biogeochemistry of the site. This may be a measure of the fate and mobility of Nalidixic acid because of its chemistry alongside other properties of the site. Nalidixic acid was also observed at a relatively high concentration in the agriculture activity site (298 ± 7.5 ng/g), which suggests the possibility of long-term contributions from agricultural applications. This trend was also observed for the other antibiotics (ampicillin and ciprofloxacin). The concentrations of ciprofloxacin and nalidixic acid were lower in the populated city center (sites DUT and CAD), but ampicillin was observed in high concentration at sampling site DUT (369 ± 19 ng/g). Although the source elucidation of ampicillin in the sediment at DUT may require further investigations, indiscriminate disposal of wastes into the river was observed at this site during sample collection and, together with its close proximity to residences, may suggest possible leakage from sewer systems. The results of ampicillin in the surface water corroborate this observation. The city suburban site HED also had high concentrations of ciprofloxacin and nalidixic acid. The lipid regulator bezafibrate was not detected in the discharge point sediment or at site DUT. It also had the lowest concentration among the pharmaceuticals investigated except in the agricultural-based site, where a quantum increase of the drug (80.3 ± 9.49 ng/g) was observed in the sediment, which was dissimilar to the observation in the water system.

Finally, the solid-water distribution coefficient (K_D) of the drugs, which is the measure of the extent of accumulation or transportation of the drugs between the liquid phase and the solid (sediment) phase, was calculated based on the ratio of their observed concentration in sediment (ng/g) to the concentration in the water (μg/L). This information is useful in understanding the fate and mobility of the substances for proper management. The result is presented as log K_D in Table 3. The average log K_D observed from the plot is between 1 and 1.5 for most of the drugs at most of the sites. This indicates that the concentrations of the pharmaceuticals in the sediment are of the magnitude of 10× to

Table 3. The partition coefficient (log K_D) of the drugs between sediment and water

Site	Ketoprofen	Ibuprofen	Diclofenac	Aspirin	Bezafibrate	Ampicillin	Ciprofloxacin	Nalidixic acid
MST	ND	1.39	2.03	ND	ND	1.43	1.88	1.17
AGA	1.32	1.17	1.30	1.34	4.41	1.25	0.65	1.30
CAD	ND	1.26	0.84	1.41	ND	1.33	ND	1.18
DUT	1.26	1.11	ND	1.12	ND	1.83	0.72	0.74
HED	1.37	1.08	1.98	1.45	0.89	1.32	1.72	1.10
DWI	0.96	1.26	1.14	0.56	1.61	0.97	0.35	1.18
DWA	0.84	1.63	ND	0.89	ND	1.05	ND	1.05
DWE	0.65	1.89	1.09	0.68	1.67	0.76	0.55	0.67

ND = not determined because the analyte is below limit of detection in 1 of the phases.

32× their concentrations in water. The value of K_D of a pharmaceutical in the environment is subject to properties of the soil/sediment, which includes pH, metal oxide presence, ionic strength, and cation exchange capacity, among others. This thus implies that K_D may vary with site and season. The values of K_D obtained for the present study are comparable with those reported in the literature [39,56]. The higher concentration in sediments than in the water system is expected because sediments are less mobile, serve as sinks, and can be a useful tool in measuring long-term contamination or years of contaminant through sediment coring.

In some cases the values of log K_D were greater than 1.5. Sites MST and HED for ciprofloxacin and diclofenac and site DWE for ibuprofen and bezafibrate were typical examples. This may be an indicator of long-term accumulative effect or impact, of possible abuse or indiscriminate disposal of unused drugs, or of possible biogeochemistry that favors the sequestration of these drugs into the sediment phase. An extreme condition was observed for bezafibrate at the AGA sampling site, where a log K_D value of 4.41 was observed for bezafibrate. Bezafibrate was found in abundance in the sediment but was less present in the water system. The trend is not well understood, but a contribution or transportation of bezafibrate may be made into this site, and the biogeochemistry of the site favors its distribution into the sediment phase. Overall, the results indicate that the contamination of the present study site with pharmaceuticals has been over time and continued until the present.

CONCLUSIONS

The present study applied an SPE pre-concentration method and LC-ESI-MS to investigate the occurrences of 8 acidic pharmaceuticals in water and sediments from the Msunduzi River of KwaZulu-Natal, South Africa. The method limits of detection and recoveries were determined for quality assurance and application for contaminant evaluation. The drugs were all present in the wastewater and in most of the surface water and sediment samples. The wastewater treatment process did not significantly reduce the contaminants and serves as a possible surface water and sediment impact source. Aspirin was the pharmaceutical observed in highest concentration in all of the samples analyzed, and the most abundant antibiotic observed was nalidixic acid. The occurrence and concentrations of the analytes investigated in the present study is comparable with results in some countries documented in the literature. The spatial distribution trend in the surface water indicates more antipyretic contamination in the suburban, informal resident sites, whereas most of the antibiotics were observed in the populated city center. The downstream distribution patterns indicate discharge contributions from wastewater, agricultural activities, domestic waste disposal, and possible leakages from the sewer system. The concentration partitions of the pharmaceuticals in the sediment from the water system measured as log K_D were of the magnitude of 10× to 32× the concentrations in water. The contamination of the present study site with pharmaceuticals has been over a period of more than 10 yr and continues today, and effective management and control is required. Further studies into the development of better water treatment technologies by the application of advanced oxidation processes such as photochemical and electrochemical oxidation to mineralize these pharmaceuticals will be required for effective management of the wastewater before discharge into the environment.

Supplemental Data—Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3144.

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Data availability—Data used for the present study are available upon request to the corresponding author (agunbiadef@run.edu.ng).

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