Research Article

Prevalence of Pharmaceuticals in Surface Water Samples in Ghana

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1.Introduction

The frequent detection of pharmaceutical residues in different sections of the environment is becoming an increasing cause of resistance and toxicity. The new Global Antimicrobial Resistance and Use Surveillance System report on antibiotics of the World Health Organization confirmed the widespread occurrence of antimicrobial resistance (AMR) across different countries [1]. Though the development of AMR is a natural evolutionary process for microorganisms, multiple studies have established cross-sectional relationships between antibiotic use and resistance [2–5]. The selective pressure caused by the extensive utilization of antimicrobials has been associated with accelerated AMR development. In the past two decades, the increased use of antibiotics outside the developed countries has led to higher global consumption of antibiotics [6]. This suggests that the direct and indirect effect of AMR will be grievous on low- and middle-income countries, such as Ghana, with constrained infrastructure, and human and financial resources to sufficiently offset drug-resistant epidemics.

The detection of antibiotic and analgesic residues in various aquatic compartments including municipal sewage, hospital sewage, groundwater, surface water, and drinking water at various concentrations [7–9] has been widely reported. While several of such reviews have highlighted aquatic monitoring in the European Union [10], the USA [11], and China [12], not much is known in the literature regarding the problem in sub-Saharan Africa [13]. Again, the lack of a universal view of what happens when these pharmaceuticals are released into the environment has lately become topical [14]. Although safety and toxicological studies have commonly provided some exposure to the side effects of these pharmaceuticals on human and animal health, the potential environmental consequences are less understood.
In Ghana, no policy or guidelines exist for the use of antibiotics in animals, and existing regulations meant to restrict antibiotic prescriptions for human use such as the Standard Treatment Guidelines (STG), Essential Medicines List (EML), and the National Health Insurance Scheme (NHIS) medicines list are not being adhered to [15]. Antibiotics are widely prescribed (with >40% of outpatient prescriptions containing antibiotics) and dispensed by unauthorized persons [16]. The story, arguably, may not be different from other classes of pharmaceuticals such as analgesics, antimalarial, antipyretics, antiseptics, and mood stabilizers.

According to Lerbech et al. [17], pharmaceutical products are widely available to the public from a lot of channels, such as hospitals and pharmacies, medical stalls, drug shops, and peddlers, despite severe restrictions. This widespread access has resulted in inappropriate use with residual consequences on the environment.

Recent research has also shown that edible crops such as tomatoes, cucumbers, carrots, and lettuce may absorb pharmacological chemicals from their growing environment through their roots [18, 19]. Through the food chain, bioaccumulation, and biomagnification, human and animal health may be endangered.

The activity of pharmaceuticals in water, soils, and plants is yet unknown and is very speculative. Biodegradation data analysis is also problematic due to the fact that kinetics is largely unknown [13].

Available literature has it that the levels of pharmaceuticals in surface and groundwater sources influenced by wastewater discharges are normally well below 0.1 µg/l (or 100 ng/l) while in treated drinking water are typically less than 0.05 µg/l (or 50 ng/l), an indication of no risk to human health [20]. That notwithstanding, the existing knowledge gaps linked to risks associated with long-term exposure to low concentrations of pharmaceuticals and the combined effects of blends of pharmaceuticals should be of concern [21]. Furthermore, because antimicrobial drug resistance is such an essential element of public health, we calculated the risk quotients (RQs) for antibiotic resistance in diverse water bodies.

This study, therefore, focused on the prevalence and risk assessment of 8 pharmaceuticals (chemical structures and physicochemical parameters are shown in Table 1) in surface water used for vegetable irrigation, which is under the influence of hospitals, sewage treatment plants, and market effluents in Kumasi and Sunyani metropolises of Ghana.

2. Materials and Methods

2.1. Chemicals and Reagents. Sigma-Aldrich (Dorset, UK) supplied tramadol hydrochloride (CAS #: 36282-47-0, >99 percent pure) and diazepam (CAS #:439-14-5, 98 percent pure), whereas Fluka (Steinheim, Germany) supplied amoxicillin trihydrate (CAS #: 267-87-780, 98 percent pure). Acetaminophen, ibuprofen, cefuroxime, and penicillin V were all donated by Phyto-Riker Pharmaceuticals Ltd., Ghana.

Merck supplied HPLC grade methanol (CAS #: 67-56-1, >99.9% pure) and acetonitrile (CAS #: 75-05-8, >99.9% pure). Distilled water was bought from KNUST Central Laboratory. Methanol was used to make the stock solutions, which were maintained frozen at −18°C.

2.2. Study Area. This study of pharmaceuticals was carried out in two cities in Ghana, West Africa (Sunyani and Kumasi), each of which has a rapid city growth and development. These cities were selected because studies have revealed that most wastewater is released to drains that discharge untreated into one of the streams flowing through or originating from Kumasi.

Sunyani is in the middle belt of Ghana. It is drained by seven tributaries of the Tano River (Figure 1(a)). These water bodies are located in the districts of Sunyani (stretches across four neighborhoods of the city), Akokora Kwadwo (Sunyani Estate), Agyei (number 2), Nsakonsuano (Nkwabeng North), Aboshyensua (Ministries Area), Danyame, and Tusua (Sunyani Estate).

Drainage at Kumasi is split into two halves, with 28% of the area draining to the west that ends up in Offin River. The reminder 78% drain southward entering into Oda River that is located on a drainage divide, and 28% of the developed area eventually drain to the west joining the Offin River. About 72% of the developed area drain to the Oda River in the south of the city. Most streams originate within the administrative boundaries of Kumasi. The only major inflow from outside sources comes from the Sisa and Wiwa rivers, which run towards the north of Kumasi (Figure 1(b)).

In the study city Sunyani, the water samples were collected from Akokora Kwadwo River at a point labeled SP, which receives effluent from the hospitals in the city. The second sampling point, SP2, was from the River Tusua, which receives wastewater from the main market in the city (Figure 1(a)).

In Kumasi, surface water was sampled from River Wiwi at a point indicated as KP1, which receives effluent from Ahensan waste stabilization ponds (WSPs), and River Oda, which receives effluent from Chirapatre WSPs that was sampled at point KP2. The surface water that receives effluent from pharmaceutical production companies was sampled at points KP3, KP4, and KP5. Subin River was sampled at a point KP6, after receiving wastewater from a hospital denoted Hospital A, while Wiwi River was sampled at point KP7, after receiving wastewater from hospital denoted Hospital B (Figure 1(b)).

2.3. Sampling Procedure. The three sample regions yielded a total of 9 sampling points (Figure 1). Two sampling periods were conducted, one in February and the other in May of the same year. Two replicates of composite water samples were obtained at each sampling station. A total of 1 L was collected at 30 mins interval from the same spot and placed in 1.5 L brown HDPE bottles (by pooling 200 mL aliquots for 5 times) In total, 54 water samples...
were collected and delivered to the CAN Laboratory of Department of Food Science and Technology of KNUST. Filtration was performed on the samples using grade 1 filter paper (Whatman®, Merck SA, an affiliate of Merck KGaA, Darmstadt, Germany). These filtered samples were placed into brown HDPE bottles in quantities of 500 mL each.

2.4. Solid-Phase Extraction of Analytes. The solid-phase extraction procedure described by Azanu et al. [13] was modified and used in this study. Water samples were cleaned up and concentrated on Oasis HLB (hydrophilic-lipophilic balance, 200 mg sorbent, 30 m, 6 cm³) cartridge supplied by Water Oasis (SPE) (Massachusetts, USA), and 2 mL MeOH, thereafter 2 mL distilled water, was used to condition the
SPE cartridge. At a flow rate of 1.5 mL/min, 500 mL of water samples was put into SPE columns. Dried SPE columns were washed with 3 mL of 5% MeOH. After permitting the sorbent under a vacuum to dry for a few minutes, the antibiotics were eluted with 3 mL MeOH at a flow rate of about 1 mL min$^{-1}$. Eluates were dried at 30°C with a moderate

Figure 1: A map showing the sampling points at (a) Sunyani metropolis and (b) Kumasi metropolis in Ghana.
nitrogen flow before being reconstituted in 1 mL 1% MeOH and injected into brown flatcap HPLC vials for analysis.

2.5. HPLC Analysis. Shraim et al. [22] presented a technique for analyzing pharmaceutical residues in surface water, which was significantly improved and used in this investigation.

2.5.1. Analgesics: Ibuprofen, Tramadol, Diazepam, and Acetaminophen. With a Wave Quest CE4300 UV/Vis Detector, a Cecil-Adept Binary Pump HPLC was used to develop the method for analyzing the analgesics (Cambridge, UK). The chromatographic separation of acetaminophen was achieved using a Zorbax Column (C18, 4.6 mm x 250 mm, 5 μm, Agilent Technologies Inc., Palo Alto, CA, USA) while for tramadol, ibuprofen, and diazepam was achieved using a SunFire Column (C18, 4.6 mm x 150 mm, 5 μm, Waters, Milford, MA, USA) preceded by a guard column (SunFire, C18, 4.6 mm x 10 mm, 5 μm, Waters, Milford, MA, USA) at 30°C. The mobile phase used was 40:60 (v/v), 0.1 M sodium acetate buffer (pH = 4): methanol and was pumped at 0.8 mL/min. A volume of 10 μL was injected into the HPLC for analysis.

2.5.2. Antibiotics: Amoxicillin, Cefuroxime, Trimethoprim, and Penicillin V. A gradient elution using a PerkinElmer Flexar UHPLC with a PDA Plus detector was used for the simultaneous analysis of the antibiotics (Cambridge, UK). The chromatographic separation of amoxicillin was achieved using a Zorbax Column (C18, 4.6 mm × 250 mm, 5 μm, Agilent Technologies Inc., Palo Alto, CA, USA) while for cefuroxime, trimethoprim, and penicillin V 4-point calibration curve was developed for each analyte across a concentration range of 1.0 to 80.0 ng/mL for measurement.

2.6. Validation of Analytical Procedure. A 5-point calibration curve was developed for each analyte across a concentration range of 1.0 to 30.00 ng/L for measurement. Precision and accuracy were determined by spiking a blank sample at low, mid, and high concentrations. The spiked samples were extracted and analyzed as described earlier. The average recoveries varied from 89 to 103%. The coefficients of variation of analyte concentration after 10 injections were below 15%. The correlation coefficients (r^2) for the calibration curves were all greater than 0.995. Analytical methods were evaluated for precision and accuracy. Limits of detection (LODs) were computed based on the ICH guidelines as 3.3 σ/S. LODs for the analytes were 1.0 ng/L (ibuprofen), 1.3 ng/L (tramadol), 1.1 ng/L (diazepam), and 1.1 ng/L (acetaminophen). Values reported in this study are the averages of at least triplicate measurements. Analysis of variance (ANOVA, α = 0.05) was undertaken to test whether the differences between three or more treatment groups are significant, while an independent sample t-test (α = 0.05) was employed to evaluate two treatment groups.

2.7. Risk Assessment. The risk quotient (RQ) of each antibiotic was computed as the ratio of maximum measured environment concentration (MEC) of an antibiotic to its projected no-effect concentration (PNEC), as prescribed by EMEA [23]. RQ was determined for fish, algae, and daphnid to cater to all levels in the food chain. PNEC data used in this study were obtained from the Ecological Structure-Activity Relationships (ECOSAR) model. PNEC values obtained from the model were multiplied by the assessment factor (AF), which takes care of the inherent uncertainty of measured concentrations. AF values of 100 were used for this study. For estimating the risk of antibiotic resistance, the MEC values and the AF of 1 were utilized. The MIC value for ciprofloxacin was 100 ng/L, whereas the MEC value for tetracycline was 15,000 ng/L, according to Gullberg et al. [26]. As a result, the MIC to produce antibiotic resistance employed in this investigation was 100 ng/L for all drugs detected in the worst-case scenario.

2.8. Statistical Treatment of Data. Data from the study were analyzed using Origin 2018 (OriginLab Corporation, Northampton, Massachusetts). Values reported in this study are the averages of at least triplicate measurements. Analysis of variance (ANOVA, α = 0.05) was undertaken to test whether the differences between three or more treatment groups are significant, while an independent sample t-test (α = 0.05) was employed to evaluate two treatment groups.

3. Results and Discussion

3.1. Pharmaceutical Compounds in Surface Water. In this study, 8 frequently dispensed pharmacologically active compounds including 4 antibiotics (amoxicillin, cefuroxime, trimethoprim, and penicillin V) and 4 analgesics (tramadol, ibuprofen, acetaminophen, and diazepam) were studied for their presence and levels in surface irrigation water.

Generally, 6 out of the 8 pharmaceutical analytes were detected in the surface water samples. The CEF (44.4%) recorded the minimum percentage detection, while AMX and IBU recorded 100% as maximum percentage detection. Antibiotics were the most prevalent chemicals and the therapeutic group with the overall highest concentration of surface water sample. The individual concentrations of the detected compounds were usually in the tens to hundreds of ng/mL range. Concentrations ranged from below detection (n.d) – 840.0 ng/L for TRIM, 145.0–659.0 ng/L for AMX, and n.d–68.0 ng/L for CEF were recorded in this study (Figure 2(a)).

The most concentrated analgesics and anti-inflammatory drugs were acetaminophen, ibuprofen, and diclofenac, with a concentration in the tens to hundreds of ng/L.
Individual concentrations ranged from 10.0–319.0 ng/L for IBU, n.d – 8.0 ng/L for DIA, and n.d–27.8 ng/L for PARA (Figure 2(a)). One-way ANOVA revealed a significant difference ($p = 0.001$) among all the pharmaceuticals detected. Considering the huge elimination rates for ibuprofen, acetaminophen, and ketoprofen revealed in wastewater treatment plants [27, 28], the high concentrations [27, 28], the high concentrations of these compounds in Ghana’s surface water could be linked to their widespread use as analgesics and anti-inflammatory drugs in human medicine and the easy access as over-the-counter drugs.

Patterns of pharmaceutical residues in environmental samples may provide clues to their sources and transport pathways, although the association is complicated [27]. Major sources of pharmaceutical residues identified were hospitals followed by pharmaceutical discharges. Sewage treatment plants and drains emanating from the marketplace are a minor source of pharmaceutical residues identified (Figure 2(b)). Large numbers of major hospitals and pharmaceutical companies’ wastewater drains are directly discharged into rivers and streams in Ghana. Routinely, wastewater be it partially treated or untreated is released into drains, smaller streams, and other tributaries of larger water bodies, which traditionally serve as irrigation water for many vegetable farmers. The practice of using such water bodies for irrigation has received wide reportage throughout sub-Saharan Africa, including Ghana [29–31].

The frequency of detection and average concentrations of antibiotics in the surface water samples were higher than those observed in other Ghanaian rivers [13], and this could be due to the dilution effect in the Ghanaian rivers with high flow.

Since indirect use of polluted water for irrigation is common in Ghana, these high concentrations also suggest an increased risk of uptake by vegetables. Exposure to these pharmaceuticals via the food chain, bioaccumulation, and biomagnification could endanger human and animal health. As a result, even at low concentrations, the negative health and environmental risks linked with exposure to a greater number of pharmaceuticals, their metabolites, and transformed products cannot be overlooked.

Generally, variations of concentration levels were observed due to different sampling campaigns (Figure 3), but they were not to a significant degree of variability. This behavior was observed at both study sites. The concentrations of pharmaceutical residues studied were slightly higher in the dry season samples than in the wet season water. The main factors governing variability in the occurrence of pharmaceuticals in surface water are dilution, flow fluctuations, temperature or UV radiation, degradation processes, and human consumption.

Pearson’s correlation revealed positive correlation (Figure 4) among the pharmaceutical analytes that detected with strong and significant correlation occurring between AMX and ACT ($r = 0.88, p = 0.001$), AMX and TRIM ($r = 0.70, p = 0.005$), and IBU and TRIM ($r = 0.72, p = 0.001$) (Figure 4).

### 3.2. Risk Assessment

#### 3.2.1. Toxicity

The maximum environmental concentration (MEC) of the studied pharmaceuticals in surface water samples for Daphnids (every species that belongs to the trophic level), fish, and algae PNEC and risk quotients (RQs) is presented in Table 2.

The risk quotients for algae were $9.09 \times 10^{-6}$ to $4.94 \times 10^{-1}$ for all, $5.33 \times 10^{-6}$ to $2.47 \times 10^{-1}$ for daphnids, and $1.78 \times 10^{-6}$ to $1.53 \times 10^{-1}$ for fish (Figure 5(a)).

The RQ values estimated for trimethoprim using the risk-ranking scale [33] were $4.94 \times 10^{-1}$ for algae, $2.47 \times 10^{-1}$ for daphnids, and $1.53 \times 10^{-1}$ for fishes, which indicate a low risk to all species belonging to their trophic levels. In the aquatic ecosystem, the remaining pharmaceuticals pose little risk to daphnids, fish, and algae. IBP was found to have a RQ of 1.2 in fish by Vazquez-Roig et al. [34], which compared to the RQ of 6.3 reported in this study. Flippin et [35] observed that a month-and-half exposure to low levels of IBP changed the reproduction trend of Japanese Medaka fish. Although there were fewer reproductions, the number of fertilized eggs was higher. IBP and other nonsteroidal anti-inflammatory medicines have been shown to reduce ovulation in mammals, including humans. [25].
Figure 3: Bar chart showing seasonal variation at various sampling sites for (a) TRIM concentration, (b) AMX concentration, (c) CEF concentration, (d) IBR concentration, (e) DIAZ concentration, and (f) ACT concentration.
This risk assessment includes flaws, which include paucity of long-term research on toxicology and the impossibility of conducting chronic investigations throughout the organism’s existence (especially in fishes). Antibiotics in the environment, on the other hand, are a problem that not only affects the ecosystem but also antibiotic resistance. Pharmaceuticals analyzed in this study are readily detected in the environment in view of their easy access over the counter that does not require any medical prescription.
which allows for self-medication in developing countries like Ghana [36]. These are found in the tens to hundreds of ng/L range. Through food quality, these concentrations may have an impact on the ecosystem and human health. Surface water is used for water contact activities such as drinking and food preparation in developing countries like Ghana [37]. Consequently, intake of these surface waters could expose people to antibiotic levels below the sub-MIC, which according to Gullberg et al. [26] have the potential to cause antibiotic resistance development. In Ghana, ibuprofen concentration as high as 30,000 ng/L was detected in sewage treatment plant effluent entering rivers [38], which is a thousand times higher than maximum concentrations of ibuprofen in sewage treatment plant effluent entering rivers in this study (14 ng/L). This could partly be due to relatively efficient sewage treatment facilities in Ghana [19], which decreases the pollution levels of STPs to surface water. Generally, the different consumption rates, sampling times, and seasonal effects could explain the significant variations of concentration levels observed across different STPs.

Tagoe and Attah [39] asserted that antibiotics of the β-lactam class, notably amoxicillin, are the most commonly prescribed in Ghana. This is followed by ampicillin and cefuroxime. This study agreed with the findings of Tagoe and Attah, with the maximum concentration of amoxicillin being 179.2 ng/L while that of cefuroxime was 47.0 ng/L. This trend appears different from the study by Azanu et al. [13] which reported cefuroxime and amoxicillin concentrations as high as 65 and 1.3 ng/L, respectively, for surface water used for irrigation. However, trimethoprim concentrations in the surface water reported in this study were comparable with a similar study performed by Azanu et al. [13].

Kümmerer and Henninger [40] calculated the PNEC concentration of amoxicillin to be 3.7 ng/L. The highest concentration obtained in this investigation was higher than the PNEC, indicating that bacteria in the sewage treatment facility may have an undesirable effect. There is a need for more research to determine their impact.

3.2.2. Antibiotics Resistance. According to Gullberg et al. [26], the estimated risk quotient for antibiotic resistance development for trimethoprim, 0.02–1.46 for amoxicillin, and 0.01–1.68 for cefuroxime was 0.24–4.20 (Figure 5(b)).

The RQ values were above 1 for all the antibiotics investigated in surface water impacted by hospitals and pharmaceutical companies’ wastewater. However, for all antibiotics investigated in surface water impacted by STPs and market wastewater, the RQ values were below 1. Gullberg et al. [26] found that MIC for the development of antibiotic resistance for ciprofloxacin and tetracycline is 100 ng/L and 15 g/L, respectively. This implies that there is a medium to high risk of antibiotic resistance development in the study area. The sub-MIC selective window is much larger than the classic selective window because antibiotic doses hundreds of times lower than the susceptible strains’ MIC can select resistant bacteria [41]. To determine the level of exposure and the danger involved, more research would be required. There is the need to assess the risk of food contamination, since point sources of emerging pollutants like antibiotics and analgesics monitored in this study are directly discharged into streams and rivers without treatment. Surface water used for agricultural purposes such as vegetable cultivation is rampant in Ghana, while several studies have proved that pharmaceuticals could be absorbed by plants [13]. The accumulation of pharmaceuticals in foodstuffs in the study areas would expose consumers to drug resistance development.

4. Conclusions

The findings of this study confirmed the presence of amoxicillin, trimethoprim, diazepam, ibuprofen, and acetaminophen, while tramadol, cefuroxime, and penicillin V were undetected in the surface water for vegetable irrigation in Sunyani.

Analgesics such as acetaminophen, ibuprofen, and diclofenac and antibiotics including amoxicillin, trimethoprim, and cefuroxime were found in this study. These pharmaceutical concentrations range from tens to hundreds of ng/L. Analgesics showed concentrations of up to 319.0 ng/L while the antibiotics up to 840.0 ng/L. The widespread usage of analogues and anti-inflammatory in Ghana, as most of them are easily obtained as an over-the-counter drug, could explain the high concentrations of these substances in the surface water. The point source of pharmaceutical residues entering into the surface water studied was in decreasing order as follows: hospital > pharmaceutical companies > sewage treatment plants > wastewater from market.

Based on the availability of long-term data, an environmental risk assessment was conducted. Because of the presence of ibuprofen, the results revealed that the lowest trophic level and fish are at risk. The estimated risk quotient values for antibiotic resistance were above 1 for all the antibiotics studied in surface water greatly influenced by hospitals and pharmaceutical companies’ wastewater except surface water impacted by sewage treatment plants (STPs) and market wastewater. The existence of pharmaceuticals in the environment is not solely a worry for the environment but also a human health threat in Ghana given the attending impact on drinking water and vegetable production.

Data Availability

All the data supporting the findings of this publication have been incorporated into the manuscript.

Conflicts of Interest

The authors attest that there are no conflicts of interest in respect of this publication.

Authors’ Contributions

David Adu-Poku contributed conceptualized the study, supervised the study, developed the methodology, provided the resources, performed investigation, and wrote the
manuscript. David Azanu conceptualized the study, developed the methodology, provided the resources, performed investigation, and wrote the manuscript. Selina Saah conceptualized the study, supervised the study, developed the methodology, provided the resources, contributed to project administration, and wrote the manuscript. William Ofori Appaw conceptualized the study, developed the methodology, provided the resources, and performed investigation. All the authors have agreed and given approval for the publication of this manuscript.

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