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Analysis and risk assessment of pharmaceutical residues in fish from three water bodies in Ghana

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ABSTRACT

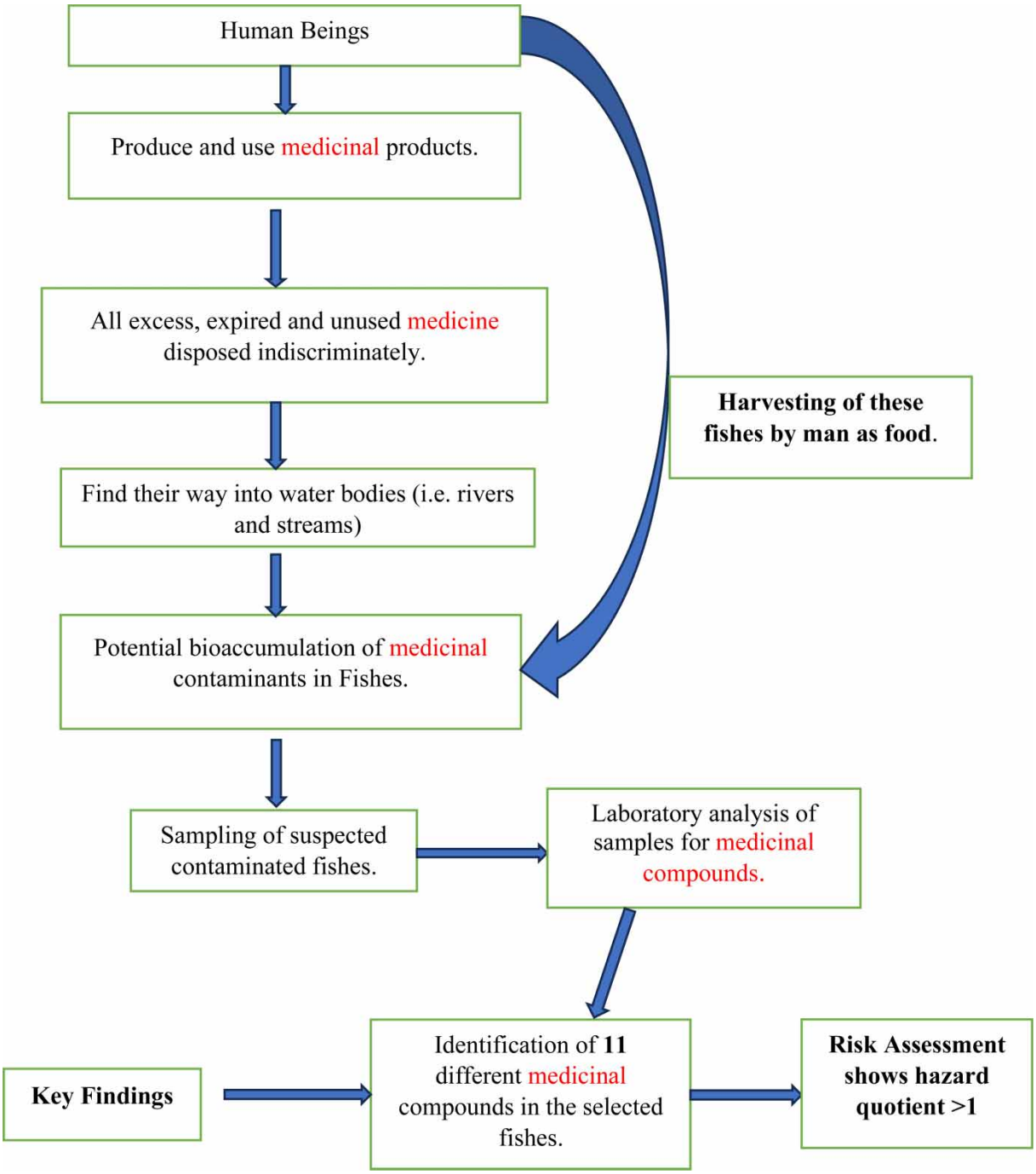
Illegal mining has overshadowed pharmaceutical pollution even though exposure to pharmaceutical waste is high. Consumption of fish potentially polluted with pharmaceuticals from the rivers continues with little concern or potential threat it poses. In the present study, the residues of one antibiotic (Chloramphenicol), five hormones (progesterone, 17-beta Estradiol, Estrone, 17a-Ethynylestradiol, and one), three environmental contaminants (4-para-nonylphenol, 4-tert-octylphenol, and Bisphenol A), one barbiturate (Primidone) and one analgesic (Diclofenac sodium salt), were investigated from fish samples from the rivers Pra, Narkwa, and the Volta. The results show a high concentration of drugs in River Pra in comparison to those in Rivers Narkwa and Volta. The hazard quotients (HQs) for the environmental contaminants were all above 1, except Bisphenol A. Furthermore, the HQs from this study suggest that consumers of fish from any of the three rivers stand a hazard risk of Chloramphenicol (19), 17a-Ethynylestradiol (4), Estrone (1.366), Diclofenac sodium salt (3.29), Progesterone (4.598), 4-tert-octylphenol (87.2), and 4-para-nonylphenol (7.252), but negligible risk against E2 (0.687), Primidone (0.014), Testosterone (0.16), and Bisphenol A (0.642). Of the fish species studied, the highest concentration of all pharmaceuticals put together is found in *Clarias gariepinus*, *Labeo senegalensis*, and *Chrysichthys nigrodigitatus* in that order.

Key words: fish, pharmaceutical residues, pollution, water bodies

HIGHLIGHTS

- There is a high potential for pollution of pharmaceuticals.
- River Pra is most polluted with pharmaceuticals.
- *Clarias gariepinus* is the most polluted fish species.
- The hazard quotients for the environmental contaminants were all above 1, except Bisphenol A.
- The hazard quotients from this study suggest that consumers of fish from any of the three rivers stand a potential hazard risk.

GRAPHICAL ABSTRACT



INTRODUCTION

The use of chemicals for industrial, domestic, commercial, and medicinal purposes will continue to increase as humans continue to find comfortable ways to experience and enjoy the full benefits of the environment. Unfortunately, these chemicals eventually find their way into the environment in their original state or in a different state depending on their usage. Whereas some chemicals such as heavy metals have documentation of their threat to the environment and living species, others such as pharmaceuticals have little or no documentation of the threat they pose to the environment.

According to Corcoran *et al.* (2010), pharmaceuticals are a large and diverse group of medicinal compounds used to diagnose, cure, mitigate, treat, or prevent diseases in humans and animals. Therapeutic properties (i.e., antibiotics, analgesics, antidepressants, etc.) are often used to classify pharmaceutical compounds. The presence of pharmaceuticals in the environment over the past three decades has gained much attention (Küster & Adler 2014) because they are sometimes excreted, changed, or unchanged in the environment. In addition, almost all of them are biologically active, which theoretically enables them to attack nontarget species exposed to them (Ojoghoro *et al.* 2021).

Unfortunately, the role of the metabolites and transformed products from the parent active pharmaceutical ingredients (API) are not well known (Kümmerer 2010), making their presence in the environment a very dicey situation. Furthermore, the different categories and their growing concentration levels identified through empirical studies are alarming. The use of antibacterials in aquaculture in the United States of America (USA) is about 92,500 and 196,400 kg per year, leading to an estimated range of 8.5 to 11.2 million kg annually of antibacterial use in agriculture (Mellon *et al.* 2001; Nawaz *et al.* 2002). In Germany, an estimated amount of 156 pharmaceuticals were detected in environmental media such as surface water, groundwater, and drinking water in the concentration range of 0.1–10.0 $\mu g/L^{-1}$ (aus der Beek *et al.* 2016). A study conducted in the 1990s discovered that for 1 kg of an active pharmaceutical compound manufactured, an amount of 50–100 kg of waste is generated (Kümmerer 2010). Globally, more than 600 pharmaceuticals are found in the environment (Küster & Adler 2014). It has been empirically established that 75% of the antibiotics used in Germany are excreted unchanged (Kümmerer & Henninger 2003 as cited in Wang *et al.* 2014). Globally, there are 71 countries where pharmaceuticals are found in the environment (aus der Beek *et al.* 2016). The veterinary use of the anti-inflammatory drug Diclofenac is responsible for the death of millions of vultures on the continent of Asia (Oaks *et al.* 2004 as cited in Hassan *et al.* 2018). It is known to cause kidney problems, heart attack, ulceration Johnson syndrome, and toxic epidermal necrolysis (Bendi & Suvvari 2020), while some known side effects of Chloramphenicol include breast cancer in postmenopausal women and contributing to weight loss (Hanekamp & Bast 2015). Drugs such as 4-tert-octylphenol and 4-paranonylphenol have been known to have effects on the reproductive health of mammals and acute toxicity to saltwater organisms, respectively (Du *et al.* 2008). Meanwhile, 17-beta Estradiol and 17a-Ethynylestradiol are toxic drugs known to cause breast cancer in postmenopausal women and decrease the production and release of prostacyclin in the epithelial tissue cells of humans (Stumpe & Marschner 2009). Bisphenol A in countries such as Canada has been declared as a dangerous chemical substance because of its fatal effects on the organs of human (Buka *et al.* 2009).

These pharmaceutical compounds enter the environment through nonpoint sources such as chemical and pharmaceutical manufacturing plants, effluents from sewage treatment plants (STPs), household waste, hospitals, veterinary medicine, and landfill effluent. In the past, there was this assumption that pharmaceuticals that enter the environment from chemical and pharmaceutical manufacturing companies are no cause for alarm; however, recent studies reveal otherwise. A study in Asian countries reveals several $mg\,L^{-1}$ of API in effluents from pharmaceutical manufacturing plants, whereas in Norway, it was discovered that local pharmaceutical manufacturing companies release higher quantities of certain antibiotics into the environment than the hospitals and the general public (Larsson *et al.* 2007; Li *et al.* 2008a, 2008b). Ruhoy & Daughton (2007) estimated that orphaned medications account for as many as 19.7 tons (19,700 kg) of APIs in U.S.A. sewage systems annually. According to Kümmerer (2010), there are higher concentrations of pharmaceuticals in hospital wastewater compared to municipal. However, hospital waste is not given very serious attention in developed countries because of the quantum of pharmaceutical usage compared to that from homes and municipalities. This situation may differ in developing countries as almost all hospitals in most developing countries operate without any treatment facilities as is the case in Ghana. Studies have shown that most people get rid of leftover and expired pills and liquid pharmaceuticals by pouring them into sinks, drains, and toilets (Kümmerer 2010), a widespread practice in Ghana. Over the years, traces of pharmaceuticals, typically at levels in the ng/L to low levels of the μg/L range, have been recorded in the water cycle, including surface waters, wastewater, groundwater, and, to a lesser extent, drinking water (Drover & Bottaro 2008). The state of pharmaceuticals in the environment in Ghanaian environment is yet to be established. However, considering the current trend in the use of pharmaceuticals: (a) through self-medication in homes; (b) in municipalities; (c) in hospitals, and their potential toxicity to the ecosystem, it is worth investigating.

There is little empirical evidence on the use of certain chemicals such as hormones in the aquaculture business in Ghana. Considering the increase in the demand for fish in recent years due to high population growth and the constantly expanding food-selling joints and local restaurants, the tendency for some fish farmers to introduce hormones to increase fish production, especially when one sex of the species can grow bigger and faster than the other sex (Hoga *et al.* 2018), is very high.

Usually, the primary steroid hormones employed are Estrone, estradiol, progesterone, Testosterone, and cortisol (Shore & Shemesh 2003). However, in this study, the steroids that will be investigated are progesterone, Estrone, Testosterone, and 17a-Ethynylestradiol. Substances such as nonylphenol ethoxylates are extensively used surfactants and incompletely biodegraded in the environment (Serino *et al.* 2012) will be investigated.

Finally, assessing and determining the presence and levels of the above pharmaceuticals in freshwater fishes that serve as the main source of fish for most domestic and local food-selling points is very crucial. The findings from this study will reveal the risk of potential chronic infections due to bioaccumulation of these pharmaceuticals because of the daily or frequent intake of such fishes.

MATERIALS AND METHODS

Study area and sampling

The Volta Basin occupies a land area of about 407,093 km^2 and stretches from Mali at latitude 14°30′N to Ghana at latitude 5°0′N. The Pra River is at the south of the Volta and rises in the Kwahu Plateau near Mpraeso and flows 240 km southward to enter the Gulf of Guinea. It is upstream of the Nakwa River which is along the coast in the central region of Ghana. The Nakwa River is located in the Densu River Basin between latitude 5°30′N–6°17′N and longitude 0°10′W. Fish samples were collected from February to March 2020 in the Pra, Narkwa, and Volta Rivers. In all, 20 fish samples were collected from the three rivers. The samples were cleaned up, washed (blended), placed in aluminum foil, and kept in a freezer for subsequent analysis at a temperature of –4 °C. The fish species and their potential as pollution sources are shown in Table 1.

Chemicals, reagents, and apparatus

All analytical compounds including pharmaceuticals and their standards were obtained from MECK Chemicals Limited. Absolute Methanol, Acetonitrile (ACN), Sodium Acetate (NaAct), magnesium sulfate ($MgSO_4$), 70% Ethanol, and Acetone were of the analytical grade and obtained from MECK Chemicals Limited. Prostate-Specific Antigen (PSA) was obtained for analysis. Standards of Chloramphenicol, Diclofenac sodium salt, Primidone, 17-beta Estradiol, 17a-Ethynylestradiol, Estrone, Testosterone, Progesterone, 4-tert-octylphenol, 4-para-nonylphenol, and Bisphenol A were used. A vortex mixer, a centrifuge, and a nitrogen generator (model 05B, System Instruments Co, Tokyo, Japan) were used to prepare the samples.

Buffer and standard preparation

An ultrapure distilled water from the Department of Water and Sanitation (University of Cape Coast, Ghana) Water and Environmental Quality Laboratory was used in the preparation of the above solutions. For the determination of the four acidic pharmaceuticals, a stock solution of 100 mM was prepared in the buffer solution of ammonium formate; 1.2 g of ammonium formate was dissolved in a 1000 mL volumetric flask, the pH was adjusted with formic acid to 3.4, and the mixture was filtered using a disc filter (0.45 μm). A mixture of ratios 80 mL: 13 mL: 7 mL of buffer: methanol: ACN, respectively, was then prepared for the mobile phase.

Table 1 | Fish species under study

Fish species	Identified chemical and potential pollutants	Author(s)
Clarias gariepinus	Zinc, manganese, lead, copper, chromium	Opasola *et al.* (2019)
Labeo senegalensis	Methyl mercury and mercury	Kwaansa-Ansah *et al.* (2013)
Brycinus nurse	Zinc and lead	
Chrysichthys nigrodigitatus	Lead	Kwaansa-Ansah *et al.* (2013)
Heterotranchis longifilis	Lead	Oribhabor & Edemiko (2016)
Parachanna obscura	Methyl mercury and mercury	Ezeonyejiaku & Obiakor (2016)
Synodontis eupterus	Methyl mercury and mercury	Kwaansa-Ansah *et al.* (2013)
Schilbe intermedius	Methyl mercury and mercury	Kwaansa-Ansah *et al.* (2013)
Sarotherodon melanotheron	Methyl mercury and mercury	Kwaansa-Ansah *et al.* (2013)
M. brachium rosenbergii	Mercury, cadmium, cobalt, copper, and zinc	Idrus *et al.* (2021)

Pharmaceutical standards (0.1 g) each were measured and dissolved into a 100 mL volumetric flask containing methanol. Using serial dilutions 10, 5, 0.1, 0.05 ppm, etc., molar concentrations were prepared from the 1,000 ppm concentrated solution for each standard and were then injected into the instrument to generate a calibration curve.

Sample preparation, extraction, and cleanup

The fish samples were extracted using the QuEChERs method with slight modification (Adjei *et al.* 2022). The fish samples were blended and freeze-dried (−4 °C) to remove all moisture from the fish. Two grams of each fish sample were measured and placed in a centrifuge tube. Ten mL of methanol and 2 g of MgSO4/NaAct were added to the sample and tightly covered and shaken vigorously by hand for 5 min. The shaking was repeated three times for a total of 15 min. It was then transferred to a centrifuge tube and centrifuged for 5 min at a speed of 4000 rpm. About 70–80% of the supernatant was transferred into a special Quencher tube. An equal proportion of $MgSO_4$/PSA was added to the supernatant and then centrifuged for another 5 min. The extract was then concentrated under liquid nitrogen to a total volume of 1.5 mL. It was then filtered with a disc filter (0.45 μm) and transferred into a 1.5 mL vial for HPLC analysis.

Analysis of pharmaceuticals via HPLC

The chromatographic separation was performed using HPLC using Shimadzu (Japan) Model GBM-20A containing a quaternary pump model Surveyor LC Plus, a manual injector valve of 20 μL Rheodyne. UV-vis photodiode array detector model Surveyor PDA with quartz cell with an optical path of 5.0 cm, ChromQuest software version 4.2 (Macherey-Nagel, Germany) for acquisition, and 20 signal recordings were used. The HPLC was fitted with column RP-18 ODS off base 250 × 4.6 mm (id) equipped with a guard column RP18 ODS 10 × 4.0 mm (id) both with particles of 5-micron pore size of 100 Å and carbon content of 15.5%. Before the first and after the last injection of the day, the column was cleaned with ultrapure water for 30 min at a flow rate of 0.5 mL min^{-1}. The initial conditioning of the stationary phase was performed by passing the mobile phase through the column for 20 min at a flow rate of 1.0 mL min^{-1}. After standard/sample injection (20 μL), the separation process was carried out. The temperature was fixed at 25 °C and a wavelength of 222 nm. After each analysis, the column was reconditioned for 10 min using the mobile phase at a flow rate of 1.0 mL min^{-1}. The concentration of the various pharmaceuticals studied was calculated using the calibration curve presented in Figure 1 which uses an equation of a straight-line $y = mx + c$. The antibiotics studied in this research are in the same range as the prepared standard curve hence it is used in the calculation of the concentrations.

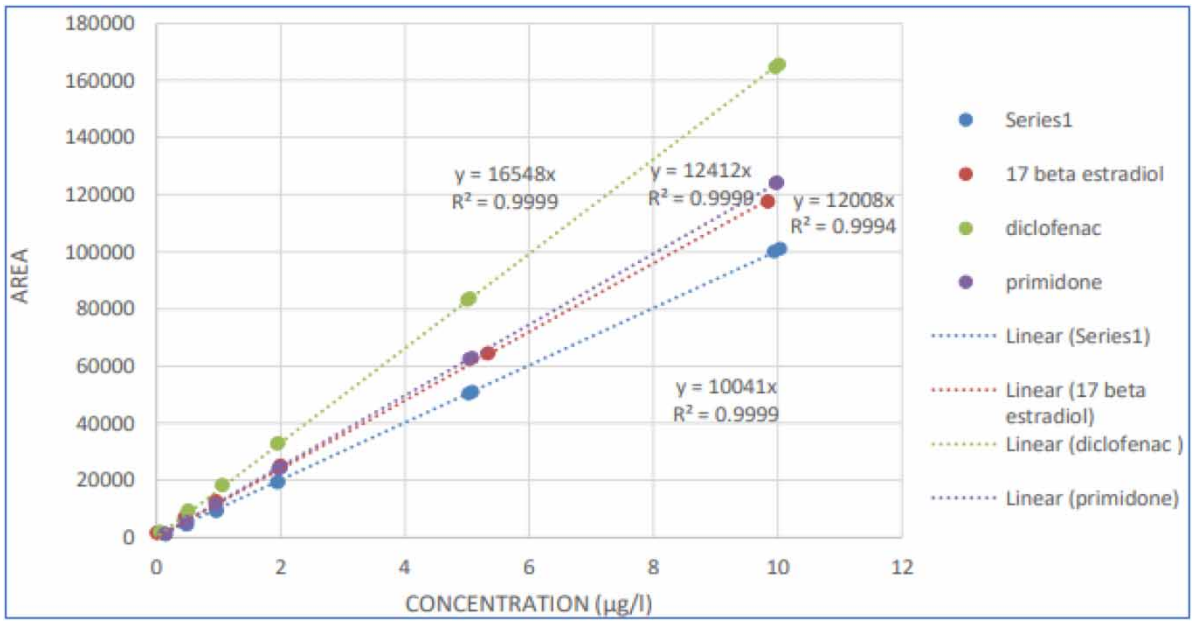


Figure 1 | Calibration curve for pharmaceutical standards.

Risk assessment

The hazard quotient (HQ) of the analyzed pharmaceuticals was determined according to the US EPA Human Health Risk Assessment with slight modifications (Fitzpatrick *et al.* 2017). Hazard is the potential for something to cause harm. The hazard could be acute or chronic. Acute hazards are hazards that pose obvious issues and would impact instantly, whereas chronic hazards are hazards that are not immediately apparent and can have more of a hidden issue, sometimes only arising after long periods. In this analysis, the HQ will be employed. HQ is used by regulatory authorities such as environmental protection agencies (EPA) of countries to describe the risk category of a chemical substance. A HQ is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. It is calculated by the relation:

$$\mathrm{HQ} = \frac{\text{Average daily dose}}{\text{Reference dose}} \tag{1}$$

HQs less than or equal to 1 indicate that adverse effects are not likely to occur, and thus can be considered to have negligible hazard. HQs greater than 1 are not statistical probabilities of harm occurring. Instead, they are a simple statement of whether (and by how much) an exposure concentration exceeds the reference concentration (RFC).

Quality control

Standard calibration curves were created for the analytes at concentrations 0.1, 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/L. For validating the calibration curve, an initial calibration verification (2.5 mg/L) was analyzed. To verify the robustness of the instrumental method, a continuous calibration verification standard of concentration of 10 mg/L was done before each batch analysis of the day. The calibration graph of the four standards (Chloramphenicol, 17-beta Estradiol, Diclofenac sodium salt, and Primidone) and their correlation coefficient (R^2) values of approximately 1 are presented in Figure 1. This graph shows that the regression equation explains 99% of the variation of the experiment data. Table 2 presents the percentage recovery or reproducibility of the pharmaceutical's Chloramphenicol, 17-Beta-estradiol, Diclofenac sodium salt, and Primidone. The recoveries were in the percentages of 106.5, 84.4, 94.52, and 106.165%, respectively, indicating that during calibration, 6.5% of Chloramphenicol was recovered than expected, furthermore, % recovery for 17-Beta-estradiol suggests that the expectation of a total recovery was not obtained and thus has about −15.6% deviation on average (Figure 1). Moreover, the reproducibility of Diclofenac was almost 100% with a % deviation of −5.48%. Lastly, Primidone reproducibility was higher than expected with a % deviation of +6.165%.

Results analysis

In total, 20 fish samples were collected and preserved in an ice char. The mean, maximum, and minimum pharmaceutical concentrations in the rivers are presented in Table 3. The only antibiotic, Chloramphenicol recorded maximum concentrations in the range of 0.343–4.154 μg/L in the three rivers with River Volta recording the least concentration of Chloramphenicol (Table 3). In recent years, ecotoxicology experiments carried out with fish and amphibians at the laboratory scale revealed that steroid hormones, both natural and synthetic, can adversely affect reproduction when present in water at extremely low concentrations: even sub-ng/L (Ojoghoro *et al.* 2021). Concerning the hormones, whereas progesterone recorded concentrations of 8.394, 8.062, and 6.243 μg/L among the Rivers Volta, Pra, and Narkwa in that order, the remaining hormones, 17-Beta-estradiol recorded a value of 5 μg/L in the Pra river with the other rivers recording values below 1 μg/L for the same hormone. The hormones Estrone, Testosterone, and 17a-Ethynylestradiol all recorded values above 1 μg/L in the Pra River, whereas Rivers Narkwa and Volta recorded values below 1 μg/L for the same hormones. Two of the three environmental contaminants, namely 4-tert-octylphenol and 4-para-nonylphenol, recorded values above 1 μg/L in all rivers understudies with the highest concentration of 12.192 μg/L of 4-para-nonylphenol being recorded in the Pra River. The Pra River still recorded the highest concentration of Bisphenol A, followed by the Volta and the Narkwa Rivers, respectively. The pharmaceutical Primidone was present in both the Pra and Narkwa Rivers in a range of 3.183–2.564 μg/L. Diclofenac sodium salt recorded concentrations above 3 μg/L in all rivers with the Pra recording the highest of the concentrations (Table 3). The chromatogram of the standards is shown in Figure 2.

Among the fish understudies, the fish that accumulated the highest concentration of all pharmaceuticals put together is *Clarias gariepinus*, followed closely by *Labeo senegalensis* and *Chrysichthys nigrodigitatus* in order (Table 4). The other

Table 2 | Quality assurance and percentage recovery

	Experimental concentration (μg/L)	Deviation (μg/L)	Deviation (%)	% Recovery
Expected: Chloramphenicol (μg/L)				
0.1	0.1475	0.0475	47.705	47.705
0.5	0.491	−0.009	−1.76	98.25
1	0.966	−0.034	−3.435	96.6
2	1.954	−0.046	−2.28	97.7
5	5.057	0.057	1.14	101.15
10	9.984	−0.016	−0.16	99.8
Expected: 17-beta Estradiol (μg/L)				
0.1	0.015	−0.085	−85.12	14.85
0.5	0.456	−0.044	−8.84	91.15
1	0.9525	−0.0475	−4.75	95.25
2	1.999	−0.001	−0.045	99.95
5	5.3395	0.3395	6.785	106.8
10	9.838	−0.162	−1.62	98.4
Expected: Diclofenac sodium salt (μg/L)				
0.1	0.061	− 0.039	−39.205	60.8
0.5	0.5115	0.0115	2.305	102.3
1	1.0605	0.0605	6.01	106.05
2	1.951	− 0.049	−2.455	97.55
5	5.026	0.026	0.52	100.5
10	9.9905	−0.0095	−0.095	99.9
Expected: Primidone (μg/L)				
0.1	0.146	0.046	45.835	145.85
0.5	0.4765	−0.0235	−4.775	95.25
1	0.9585	−0.0415	−4.16	95.85
2	1.982	−0.036	−1.82	99.1
5	5.0585	0.0585	1.17	101.15
10	9.9795	−0.025	−0.205	99.8

species whose cumulative pharmaceutical concentrations are within 20 μg/L are *Macrobrachium rosenbergii*, *Synodontis eupterus*, and *Heterotranchis longifilis*. The measured concentration of the fish species is presented in Table 5.

The species *C. gariepinus* recorded concentrations in the range of 4.154–8.062 μg/L for Chloramphenicol, 17-Beta-estradiol, Diclofenac sodium salt, and Progesterone. Concerning the remaining six pharmaceuticals, the species recorded concentrations in the range of 0.005–1.962 μg/L. The highest accumulated pharmaceutical in *C. gariepinus* is the hormone Progesterone with the least accumulated being the environmental contaminant (4-tert-octylphenol). All nine remaining fish species except *Sarotherodon melanotheron* accumulated high concentrations of Progesterone than all other pharmaceuticals. Aside from Progesterone, the order of pharmaceutical accumulation by the nine fish species is 4-Para-nonylphenol, Diclofenac sodium salt, 17-Beta-estradiol, Chlorophenicol, Estrone, Primidone, 17a-Ethynylestradiol, Bisphenol A, Testosterone, and 4-Tert-octylphenol. The highest concentration of Chlorophenicol, 17-Beta-estradiol, Diclofenac sodium salt, and Progesterone was found in *C. gariepinus* (Table 4). *C. nigrodigitatus* recorded the highest concentration of 4-Para-nonylphenol and 17a-Ethynylestradiol. For the pharmaceuticals Estrone and Testosterone, their highest concentration was found in *L. senegalensis*. The species *Parachanna obscura* has the highest accumulation of 4-Tert-octylphenol. The highest accumulation of barbiturate (Primidone) was found in *S. eupterus*. *M. rosenbergii* recorded the highest concentration of 2.578 μg/L for Bisphenol A, whereas the remaining species recorded values below 1 μg/L.

Table 3 | Measured pharmaceutical minimum and maximum concentrations (μg/L) in the three rivers

	River Pra μg/L			River Narkwa μg/L			River Volta μg/L		
Drugs	**Min**	**Max**	**Mean STD**	**Min**	**Max**	**Mean STD**	**Min**	**Max**	**Mean STD**
Chloramphenicol	0.57	4.154	1.958 ± 1.140	0.416	2.09	0.954 ± 0.398	0.19	0.343	0.245 ± 0.091
17-Beta-estradiol	0.887	5.262	3.214 ± 1.282	0.187	0.456	0.342 ± 0.058	0.214	0.046	0.392 ± 0.153
Diclofenac sodium salt	2.087	6.095	2.009 ± 1.585	3.12	3.427	0.314 ± 0.055	3.224	3.454	3.322 ± 0.151
Primidone	0.411	3.183	1.250 ± 1.108	0.336	2.564	0.921 ± 0.445	0.527	0.905	0.726 ± 0.242
Progesterone	0.463	8.062	4.675 ± 1.571	0.367	8.394	4.638 ± 2.064	2.967	6.243	4.373 ± 2.223
Estrone	0.91	1.962	1.351 ± 0.601	0.225	0.754	0.479 ± 0.109	0.431	0.571	0.508 ± 0.091
Testosterone	0.196	1.234	0.361 ± 0.253	0.262	0.359	0.297 ± 0.020	0.264	0.355	0.323 ± 0.056
17a-Ethynylestradiol	0.185	1.983	0.794 ± 0.436	0.08	0.276	0.225 ± 0.085	0.228	1.035	0.661 ± 0.488
Bisphenol A	0.222	2.578	0.618 ± 0.033	0.064	0.134	0.088 ± 0.010	0.063	1.611	0.575 ± 1.243
4-tert-octyphenol	−0.01	1.426	0.429 ± 0.085	0.315	1.972	0.937 ± 0.236	1.01	1.94	0.407 ± 0.070
4-para-nonylphenol	0.764	12.192	4.294 ± 0.220	0.993	2.111	1.387 ± 0.019	1.252	1.983	1.741 ± 0.445

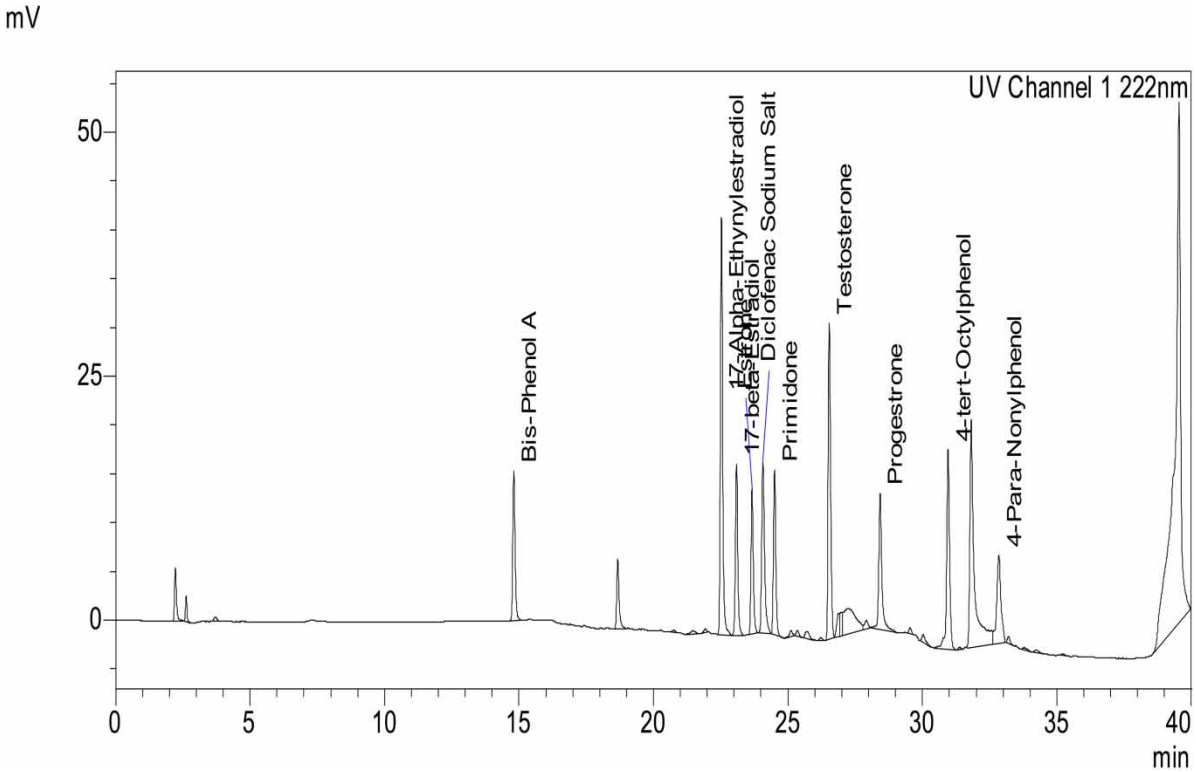


Figure 2 | A chromatogram showing all pharmaceutical analytes at a wavelength of 222 nm.

Risk assessment

The HQ for all pharmaceuticals was determined. The HQs for Chloramphenicol, 17a-Ethynylestradiol, Estrone, Diclofenac sodium salt, progesterone, 4-tert-octylphenol, and 4-para-nonylphenol were all above 1 indicating that the risk they pose cannot be ignored. However, 17-Beta-estradiol, Primidone, Testosterone, and Bisphenol A had HQs below the value of 1,

Table 4 | Total pharmaceutical concentration in fish species

Fish species	Pharmaceuticals concentration (μg/L)	Percentage concentration (%)
A	28.82	13.52
B	27.00	12.67
C	19.54	9.17
D	26.11	12.25
E	20.08	9.42
F	15.96	7.49
G	21.21	9.95
H	17.19	8.06
I	15.73	7.38
J	21.39	10.04
Total	**213.03**	**100**

A = *Clarias gariepinus*, B = *Labeo senegalensis*, C = *Brycinus nurse*, D = *Chrysichthys nigrodigitatus*, E = *Heterotranchis longifilis*, F = *Parachanna obscura*, G = *Synodontis eupterus*, H = *Schilbe intermedius*, I = *Sarotherodon melanotheron*, J = *Macrobrachium rosenbergii*.

Table 5 | Measured concentration (μg/L) of pharmaceuticals in fish species

Fish species	Chloro	17-Alpha	17-Beta	Estrone	Diclo	Primidone	Testosterone	Progesterone	4-tert	4-Para	Bisphenol A
A	4.154	0.821	5.262	1.962	6.095	0.892	0.585	8.062	0.005	0.764	0.222
B	0.921	1.071	4.395	3.325	4.765	1.872	1.234	6.156	0.04	2.712	0.497
C	1.475	0.662	1.722	1.469	2.626	3.078	0.234	4.487	0.919	2.286	0.572
D	0.57	1.983	2.027	0.947	2.661	0.823	0.23	3.555	0.694	12.192	0.426
E	1.614	0.85	1.36	0.963	2.923	0.439	0.216	4.378	0.314	6.728	0.298
F	2.835	0.274	0.906	0.829	2.237	0.517	0.221	3.87	1.055	2.666	0.548
G	2.262	0.397	1.263	1.199	2.932	3.183	0.208	6.613	−0.01	2.779	0.381
H	2.479	0.323	0.985	1.087	3.107	0.411	0.233	4.672	1.462	2.06	0.371
I	0.751	1.368	1.31	0.91	2.087	0.485	0.196	0.463	0.171	7.723	0.286
J	2.519	0.185	0.887	0.817	2.708	0.804	0.246	4.499	0.115	6.036	2.578

Chloro = chlorophenicol, 17-Alpha = 17a-Ethynylestradiol, 17-Beta = 17-Beta-estradiol, Diclo = Diclofenac sodium salt; A = *Clarias gariepinus*, B = *Labeo senegalensis*, C = *Brycinus nurse*, D = *Chrysichthys nigrodigitatus*, E = *Heterotranchis longifilis*, F = *Parachanna obscura*, G = *Synodontis eupterus*, H = *Schilbe intermedius*, I = *Sarotherodon melanotheron*, J = *Macrobrachium rosenbergii*.

indicating that the hazard they pose is negligible. Moreover, Chloramphenicol, 4-tert-octylphenol had HQs of 19 and 87.2, respectively, whereas Primidone and Testosterone had the least HQ of 0.014 and 0.16, respectively.

DISCUSSION

Indiscriminate disposal of waste in the selected water bodies, as well as some observations made during data collection at the sites, could account for the high concentration of pharmaceuticals observed in this study. Open defecation, sludge from water treatment plants, and crude dumping of untreated refuse near and into the water bodies, amongst others, which were observed at River Narkwa and River Pra are suspected to account for the levels of pharmaceuticals in the fish samples. The findings from this study will be of much relevance to all stakeholders and policymakers because of the high dependency of local communities on these fish species and the fact that the fish species are highly sought after by food joints and restaurants because of their high demand. The pharmaceutical 4-para-nonylphenol, which recorded the highest concentration in this study, is an environmental contaminant. Nonylphenol is one of the chemicals used in the manufacturing of antioxidants,

lubrication oil additives, emulsifiers, dish detergents, and solubilizers. Nonylphenol has a potential role as an endocrine disrupter since it can act with estrogen-like activity, and considering the measured HQ (Table 6), it poses a hazard to humans (Nyberg 2016).

4-tert-octylphenol, also an environmental contaminant as 4-para-nonylphenol, recorded the highest HQ in this study at 87.2 with a very low predictable no observed effect concentration (PNOEC) of 0.01ppb. The concentration of 4-tert-octylphenol among the 12 fish species in the three water bodies concerning their potential to cause harm to any consumer is a possibility as all species recorded higher values than the PNOEC (Adebola & Taiwo 2013). Amongst the environmental contaminants, Bisphenol A seems to be the only contaminant with a HQ of less than 1, indicating that the hazard posed by Bisphenol A in this study is negligible. The PNOEC of this contaminant according to a study conducted by Bergh & Budsberg (2005) is 0.55 ppb, which is higher than the average Bisphenol A concentration in this study.

There is no safe level of residue for Chloramphenicol or its metabolites in food that represents an acceptable risk to consumers according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA); however, Bergh & Budsberg (2005) suggest that the PNOEC for Chloramphenicol is 0.06 ppb. The measured concentration far exceeds the predictable 'no observable effect concentration', this situation is alarming and calls for immediate measures to establish how the levels entering the river could be prevented or stopped from any further increase. Concerning the potential of Chloramphenicol to cause harm, the HQ of 19 is an indication of its potential to cause harm since it far exceeds the value of 1 (Table 6). In addition, Chloramphenicol possesses a carcinogenic risk if the levels found in food are above 0.31 ppb (Mathys *et al.* 2019). The measured concentration of Chloramphenicol is almost four times the value found to cause carcinogenic risk, making the consumption of such fish species in the river very unsafe.

In humans, exposure to hormones can cause endocrine disorders, such as early puberty in children, advances in bone age, negative repercussions on growth, and modifications of sexual characteristics (Hoga *et al.* 2018). These disorders especially occur in children, because they are in a growing phase when puberty has not yet developed. Amongst the five hormones considered in this study, three of them, namely 17a-Ethynylestradiol, Estrone, and progesterone, pose a hazardous risk to consumers in the above challenges. Two of the three contaminants have a HQ that is about four times the PNOEC, and one (17-Beta-estradiol) is known to possess antibiotic properties (Medina-Estrada *et al.* 2018). 17-beta Estradiol, also known as E2 (due to its two hydroxyl groups), is a steroidal hormone derived from cholesterol and is the most predominant and potent sexual hormone at the reproductive stage of females. E2s are associated with reproductive and sexual functions; however, it is also involved in the development of different pathologies, such as cancer, autoimmune diseases, and infectious processes, where the hormone can alter the innate immune response (IIR) (Medina-Estrada *et al.* 2018). The levels of Testosterone and E2 in this study pose negligible hazards to humans because their HQs were below 1.

Primidone possesses efficacy for partial and secondarily generalized seizures and shows efficacy for juvenile myoclonic epilepsy, however, convincing evidence is lacking for primary generalized epilepsy (Johannessen 2004). Primidone poisoning from excessive intake can result in central nervous system (CNS) depression with dysarthria, nystagmus, and ataxia. Drowsiness, not often progressing to coma, may also occur (Jefferson & Morrow 1996). The HQ for Primidone in this study is

Table 6 | Hazard quotient of selected pharmaceuticals from three water bodies in Ghana

Pharmaceutical	Exposure/daily dose (ppb)	Ref PNOEC (ppb)	Source	Hazard quotient
Chloramphenicol	1.141	0.06	Bergh & Budsberg (2005)	19
17a-Ethynylestradiol	0.493	0.105	Bergh & Budsberg (2005)	4
17-beta Estradiol	0.816	1.188	Pereira *et al.* (2020)	0.687
Estrone	0.766	0.56	Bergh & Budsberg (2005)	1.366
Diclofenac	3.297	1	Schwaiger *et al.* (2004)	3.29
Primidone	0.989	69	Bergh & Budsberg (2005)	0.014
Testosterone	0.323	2	World Health Organization (2018)	0.16
Progesterone	4.598	1	Bergh & Budsberg (2005)	4.598
4-tert-octylphenol	0.872	0.01	Adebola & Taiwo (2013)	87.2
4-para-nonylphenol	2.393	0.33	Eu risk assessment of 2001	7.252
Bisphenol A	0.353	0.55	Bergh & Budsberg (2005)	0.642

0.014 ppb, which is negligible and possesses no reason for alarm. Besides, the PNOEC for Primidone is 69 (Bergh & Budsberg 2005), and the exposure concentrations in this study are way below.

A study by Marmon *et al.* (2021) shows that NSAIDs in the aquatic environment have the potential to cause adverse effects in the wild fish population under chronic exposure. As per the HQ of Diclofenac sodium salt (3.29), found in this study, it is inferred that the levels of Diclofenac in the water bodies pose a hazard to consumers of fish from such an environment.

The species *C. nigrochigatus*, recording the highest pharmaceutical residue in the study, can be attributed to its feeding habits and its ability to feed at deeper depths in water (Gunder 2004). In addition, the high concentration of nonylphenol can be attributed to the level of pollution in these water bodies. The organism was obtained from the River Pra, and during the sample collection, a high level of suspended pollution was observed, so with the high levels obtained in this study, there is no room for surprise. *Paranchana obscura* recorded the lowest pharmaceutical concentration of 4-tert-octylphenol. The levels of Diclofenac were relatively high amongst all fish species, whereas levels of Testosterone and 4-tert-octylphenol were relatively low in all fish species.

CONCLUSION

In the present study, the residues of Chloramphenicol, 17-Beta-estradiol, Primidone, and Diclofenac sodium salt obtained in samples from River Pra and River Narkwa were analyzed. From the results, it can be concluded that the levels of drugs in River Pra were all higher compared to those in River Volta and Narkwa. The HQs for the environmental contaminants were all above 1, except Bisphenol A. Furthermore, HQs from this study suggest that consumers of fish from any of the three rivers stand a hazard risk of Chloramphenicol, 17a-Ethynylestradiol, Estrone, Diclofenac sodium salt, Progesterone, 4-tert-octylphenol and 4-para-nonylphenol, but negligible risk against E2, Primidone, Testosterone, and Bisphenol A. Of the fish species studied the highest concentration of all pharmaceuticals put together is found in *C. gariepinus*, then *L. senegalensis*, and *C. nigrodigitatus* in that order. Since the levels of pharmaceutical residues in River Pra were higher than those of River Volta and Narkwa, it is advised that supervisory authorities be set to control the activities and apprehend illegal dumping of any form of waste into the river. Furthermore, routine assessment or monitoring of pharmaceutical residues in water bodies at both regional and national levels should be conducted in each other to reduce the concentration of pharmaceuticals in water bodies.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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