Analysis and Risk Assessment of Pharmaceutical Residues in Fish from Three (3) Water Bodies in Ghana.

Albert . Ebo Duncan (aduncan@ucc.edu.gh)
University of Cape Coast https://orcid.org/0000-0002-0073-7294
Christian Adoko
University of Cape Coast
Martha Osei-Marfo
University of Cape Coast https://orcid.org/0000-0001-6044-9334
Samuel Barnie
University of Cape Coast School of Physical Sciences https://orcid.org/0000-0001-7958-6718
Aboagye George Sakyi
University of Cape Coast
Joseph Adjei
University of Cape Coast

Research Article

Keywords: Pollution, Water bodies, Pharmaceutical Residues, Fish

Posted Date: May 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1585710/v1

Abstract

Pharmaceutical pollution of rivers in Ghana is overlooked due to the overshadowing problem of illegal mining. However, a greater percentage of water bodies (especially rivers) in Ghana are potentially exposed to pharmaceutical waste and pollution. The uncontrolled disposal of pharmaceuticals from industry, hospitals, households, municipalities, and other consumers into rivers is very alarming. Humans continue to fish from these polluted water bodies with little concern or knowledge of the potential threat the pharmaceutical residues accumulated in these fishes pose to their health. In the present study, the residues of one **(1)** antibiotic (Chloramphenicol), five **(5)** hormones (progesterone, 17-b-estradiol, estrone, 17-a-ethynylestradiol, and testosterone), three **(3)** environmental contaminants (4-para-nonylphenol, 4-tert-octylphenol, and Bisphenol A), one **(1)** Barbiturate (Primidone) and one **(1)** analgesic (Diclofenac sodium salt), were investigated from fish samples from the rivers Pra, Narkwa, and the Volta. The fish samples were analyzed using high-performance liquid chromatography (HPLC). The results show a high concentration of drugs in River Pra in comparison to those in River Narkwa and Volta. The hazard quotients for the environmental contaminants were all above one **(1)**, except Bisphenol-A. Furthermore, hazard quotients from this study suggest that consumers of fish from any of the three rivers stand a hazard risk of Chloramphenicol (19), 17-a-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, then Labeo senegalensis, and Chrysichthys nigrodigitatus in that order.

1 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 different state depending on their usage. Whereas some of the 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 for diagnosis, cure, mitigation, treatment, or prevention of 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 **(3)** decades has gained much attention (Küster & Adler, 2014; Boxall, 2004) because they are sometimes excreted changed, or unchanged into the environment. In addition, almost all of them are biologically active which theoretically enables them to attack non-target species exposed to them (Ojoghoro, Scrimshaw, & Sumpter, 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. A study in the United States of America (USA) reveals that the use of antibacterial in aquaculture 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 in the USA (Nawaz et al, 2001; Mellon et al, 2001). 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 0.1–10.0 μgL^{-1} (aus der Beek, Weber, Bergmann, Gruttner, & Carius, 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, there are more than 600 pharmaceuticals that are found in the environment (Küster & Adler 2014). A study by Kümmerer & Henninger, (2003) shows that 75% of the antibiotics used in Germany are excreted unchanged. Globally, there are 71 countries where pharmaceuticals are found in the environment (aus der Beek, Weber, Bergmann, Gruttner, & Carius, 2016). A study in Asia reveals that veterinary use of the anti-inflammatory drug diclofenac is responsible for the death of millions of vultures in the continent (Oaks et al.,2004).

The pharmaceutical compounds enter the environment through non-point 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 the chemical and pharmaceutical manufacturing companies are no cause for alarm, however, recent studies reveal otherwise. A study in Asian countries reveals several mgL^{-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 (Kümmerer & Henninger, 2003; Larsson, de Pedro, & Paxeus, 2007; Li, Yang, Hu, Ren, Zhang, & Li, 2008; Li, Yang, Hu, Zhang, Chang, & Jin, 2008). A study by Ruhoy & Daughton (2007), estimates orphaned medications account for as many as 19.7 tons of APIs into U.S.A. sewage systems

annually. A study by Kümmerer in 2010 reveals that there are higher concentrations of pharmaceuticals in hospital wastewater as compared to that of the municipal. However, hospital waste is not given very serious attention in developed countries because of the quantum of pharmaceutical usage as compared to that from homes and the municipal. 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 very common practice in Ghana. Over the years, traces of pharmaceuticals, typically at levels in the ng/L to low levels of µg/L range, have been recorded in the water cycle, including surface waters, wastewater, and 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) municipalities; c) hospitals and their potential toxicity to an 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 17-alphaethynylestradiol. Substances such as nonylphenol ethoxylates, which are extensively used surfactants (Belmont and Metcalfe, 2003; Serino, Luche, Gres, Baylac, Bergé, Cenac, & Burcelin, 2012) and are incompletely biodegraded in the environment (Mann & Boddy, 2000; Cesari, Martin, Calin, Pentimalli, Bichi, McAdams, & Croce, 2003; Chang, Wang, Kanamori, Shih, Kawai, Lee, & Esashi, 2005) 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 as a result of the daily or frequent intake of such fishes.

2 Materials And Methods

2.1 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 Sulphate ($MgSO_4$), 70% Ethanol, Acetone, were of the analytical grade and obtained from MECK Chemicals Limited. Prostate-Specific Antigen (PSA) was obtained for the analysis. Standards of chloramphenicol, Diclofenac sodium salt, Primidone, 17-β-estradiol, 17-a-ethynylestradiol, Estrone, Testosterone, Progesterone, 4-tert-octylphenol, 4-para-nonylphenol, and bisphenol A were used.

A vortex mixer, a centrifuge, a nitrogen generator (model 05B, system instruments Co, Tokyo, Japan) were used to prepare the samples. 15 mL quencher containers were used to hold the samples during the preparation to break the tissues into bits' puree, sharp knife aided the cutting, an aluminum foil was used to preserve the blended samples and was kept in a freeze dryer at − 40^{o}C.

2.2 Buffer and Standard Preparation

An ultrapure distilled water from the Department of Water and Sanitation 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 for the buffer solution of ammonium formate. A 1.2g 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 ratio 80mL: 13mL: 7mL for buffer: methanol: acetonitrile respectively were then prepared for the mobile phase.

Pharmaceutical standards (0.1g) each was measured and dissolved into a 100mL volumetric flask containing methanol. Using serial dilution, 10ppm, 5 ppm, 0.1 ppm, 0.05 ppm, etc., molar concentrations were prepared from the 1000 ppm concentrated solution for each standard and were then injected into the instrument for the generation of a calibration curve.

2.3 Sampling

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, mushed (blended), placed in an aluminum foil, and kept in a freezer for subsequent analysis

at a temperature of – 4^0C.

2.3.1 Sample Preparation, Extraction, and Cleanup

The fish samples were blended and freeze-dried (-4^0C) to remove all moisture from the fish. Two grams (**2g**) of each fish sample were measured and placed in a centrifuge tube. 10 mL of methanol and 2 g of MgSO4/NaAct were added to the sample, and tightly covered and shaken vigorously by hand for 5 minutes. The shaking was repeated three times for a total of 15 minutes. It was then transferred to a centrifuge tube and centrifuged for five (**5**) minutes at a speed of 4000 rpm. About 70–80% of the supernatant was transferred into a special Quenchers tube. An equal proportion of $MgSO_4$/PSA was added to the supernatant and then centrifuged for another 5 minutes. 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.

2.4 Analysis of Pharmaceuticals via HPLC

The chromatographic separation was performed using HPLC from Shimadzu (Japan) Model GBM-20A containing quaternary pump model Surveyor LC Plus, 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 recording 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 clean with ultrapure water for 30 minutes at a flow rate of 0.5 $mLmin^{-1}$. The initial conditioning of the stationary phase was performed by passing the mobile phase through the column for 20 minutes at a flow rate of 1.0 $mLmin^{-1}$. After standard/sample injection (20µL), the separation process was carried out. The temperature was fixed at 25^0C. The pharmaceuticals are recorded at their known wavelengths. After each analysis, the column was reconditioned for 10 minutes using the mobile phase at a flow rate of 1.0 $mLmin^{-1}$.

2.5 Risk Assessment

Hazard is the potential for something to cause harm. A 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 hazard quotient (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 hazard quotient 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:

$$HQ = \frac{AVERAGEDAILYDOSE}{REFERENCEDOSE}$$

A hazard quotient less than or equal to **1** indicates 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).

3 Analysis And Results

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 1. The only antibiotic, Chloramphenicol recorded maximum concentrations in the range of 0.343–4.154ug/L in the three rivers with River Volta recording the least concentration of chloramphenicol (Table: 1). In recent years, ecotoxicology experiments carried out with fish and amphibians at the laboratory scale reveal that steroid hormones both natural and synthetic can adversely affect reproduction when present in water at extremely low concentrations: even sub-ng/L (Ojoghoro, Scrimshaw, & Sumpter, 2021). Concerning the hormones, whereas progesterone recorded concentrations of 8.394, 8.062, and 6.243ug/L among the Rivers Volta, Pra, and Narkwa in that order, the remaining hormones, 17 – beta-estradiol recorded a value of 5ug/L in the Pra River with the other Rivers recording values below 1ug/L for the same hormone. The hormones Estrone, Testosterone, 17-a-Ethynylestradiol all recorded values above 1ug/L in the Pra River whereas Rivers Narkwa and Volta recorded values below 1ug/L for the same hormones. Two of the three environmental contaminants namely 4-tert-octylphenol, and 4-para-nonylphenol recorded values above 1ug/L in all the rivers understudy with the highest concentration of 12.192ug/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 ug/L. Diclofenac sodium salt recorded concentrations above 3ug/L in all the rivers with the Pra recording the highest of the concentrations (Table 1).

Table 1
Measured Pharmaceutical Minimum and Maximum Concentrations(ug/L) in the Three Rivers

Drugs	River Pra ug/L		Mean STD	River Narkwa ug/L		Mean STD	River Volta ug/L		Mean STD
	Min	Max		Min	Max		Min	Max	
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
17-a- 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

Among the fishes understudy, the fish that accumulated the highest concentration of all pharmaceuticals put together is Clarias gariepinus, followed closely by Labeo senegalensis and Chrysichthys nigrodigitatus in that order (Table 2). The other species whose cumulative pharmaceutical concentrations are within 20ug/L are Macorobrachium rosenbergii, Synodontis eupterus, and Heterotranchis longifilis.

Table 2
Total Pharmaceutical Concentration in Fish Species

Fish Species	Pharmaceuticals Concentration (ug/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 = Macorobrachium rosenbergii

The species Clarias gariepinus recorded concentrations in the range of 4.154–8.062ug/L for Chlorophenicol, 17-Beta-estradiol, Diclophenac sodium salt, and Progestrone. Concerning the remaining six (6) pharmaceuticals, the species recorded concentrations in the range of 0.005–1.962ug/L.The highest accumulated pharmaceutical in Clarias gariepinus is the hormone (Progestrone) with the least accumulated being the environmental contaminant (4-tert-octylphenol). All the nine (9) remaining fish species except Sarotherodon melanotheron, accumulated high concentration of Progestrone than all the other pharmaceuticals. Aside Progesterone, the order of

pharmaceutical accumulation by the nine (9) fish species is 4-Para nonylphenol, Diclophenac sodium salt, 17-Beta-estradiol, Chlorophenicol, Estrone, Primidone, 17-Alpha-ethynylestradiol, Bisphenol A, Testosterone, and 4-Tert-octylphenol. The highest concentration of Chlorophenicol, 17-Beta-estradiol, Diclophenac sodium salt, and Progestrone was found in Clarias gariepinus (Table 3). Chrysichthys nigrodigitatus recorded the highest concentration of 4-Para nonylphenol and17-Alpha-ethynylestradiol. For the pharmaceuticals Estrone and Testostrone their highest concentration was found in Labeo senegalensis. The species Parachanna obscura has the highest accumulation of 4-Tert-octylphenol. The highest accumulation of barbiturate (Primidone) was found in Synodontis eupterus. Macorobrachium rosenbergii recorded the highest concentration of 2.578ug/L for Bisphenol A whereas the remaining species recorded values below 1ug/L.

Table 3
Measured Concentration (ug/L) of Pharmaceuticals in Fish species

Fish Species	Chloro	17-Alpha	17-Beta	Estrone,	Diclo	Primidone	Testosterone	Progestrone	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 = 17-alpha-ethynylestradiol, 17-Beta = 17-Beta-estradiol, Diclo = Diclophenac 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

3.1 Risk Assessment

The hazard quotient for all the pharmaceuticals was determined. The hazard quotient for chloramphenicol, 17-a-ethynylestradiol, Estrone, Diclofenac sodium salt, progesterone, 4-tert-octylphenol, and 4-para-nonylphenol were all above one indicating that the risk they pose cannot be ignored. However, 17-beta-estradiol, Primidone, Testosterone, and bisphenol A had hazard quotients below the value one, indicating that the hazard they pose is Negligible. Also chloramphenicol, 4-tert-octylphenol had hazard quotients of 19 and 87.2 respectively. Whereas Primidone and Testosterone had the least hazard quotient of 0.014 and 0.16 respectively.

Table 4
Hazard quotient of selected pharmaceuticals from the three water bodies in Ghana

Pharmaceutical	exposure (ppb)	Ref PNOEC (ppb)	Source	Hazard quotient
Chloramphenicol	1.141	0.06	Bergh 2005	19
17-a-ethynylestadiol	0.493	0.105	Bergh 2005	4
17-beta-estradiol	0.816	1.188	Pereira, et al. 2020	0.687
Estrone	0.766	0.56	Bergh 2005	1.366
Diclofenac	3.297	1	Dossier, 2011; Schwaiger et al, 2004	3.29
Primidone	0.989	69	Bergh 2005	0.014
Testosterone	0.323	2	JECFA 2018	0.16
Progesterone	4.598	1	Bergh 2005	4.598
4-tert-octylphenol	0.872	0.01	Adebola & Fagbemigun, 2013	87.2
4-para-nonylphenol	2.393	0.33	Eu risk assesment of 2001	7.252
Bisphenol A	0.353	0.55	Bergh 2005	0.642

4 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, crude dumping of untreated refuse near and into the water bodies, amongst others which were observed at river Narkwa, and river Pra is suspected to account for the levels of the pharmaceuticals in the fish samples. 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 the potential role as an endocrine disrupter since it can act with estrogen-like activity, and considering the measured hazard quotient (Table 4), it poses a hazard to humans (Nyberg, 2016).

4-tert-octylphenol, also an environmental contaminant as 4-para-nonylphenol, recorded the highest hazard quotient 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 (3) water bodies concerning their potential to cause harm to any consumer is a possibility as all the species recorded higher values than the PNOEC (Adebola & Fagbemigun, 2013). Amongst the environmental contaminants, bisphenol A seems to be the only contaminant with a hazard quotient of less than one **(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, 2005 is 0.55ppb, 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, a study by Bergh (2005), suggest that the PNOEC for chloramphenicol is 0.06ppb. 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 rivers could be prevented or stopped from any further increase. Concerning the potential of chloramphenicol to cause harm, the hazard quotient of 19 is an indication of its potential to cause harm since it far exceeds the value of one **(1)** (Table 4). In addition, chloramphenicol possess a carcinogenic risk if levels found in food are above 0.31ppb (Mathys et al, 2019). The measured concentration of chloramphenicol is almost four **(4)** 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 (5) hormones considered in this study, three **(3)** of them namely 17-a-ethynylestradiol, Estrone, and progesterone pose a hazardous risk to consumers in the above challenges. Two of the three contaminants have a hazard quotient that is about four **(4)** times the PNOEC, and one (17-beta Estradiol) is known to possess antibiotic properties. (Medina-Estrada et al, 2018). 17β-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. E2's 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 hazard quotients were below one.

Primidone possesses efficacy for partial and secondarily generalized seizures and shows efficacy for juvenile myoclonic epilepsy, however, convincing evidence is lacking for the primary generalized epilepsies (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 hazard quotient for Primidone in this study is 0.014ppb, which is very negligible and possesses no reason for an alarm. Besides the PNOEC for Primidone is 69 (Bergh 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. Per the hazard quotient of Diclofenac sodium salt (3.29), found in this study, it is inferred that levels of diclofenac in the water bodies pose a hazard to consumers of fish from such environment.

The Species *Chrysichthys nigrochigatus* recording the highest pharmaceutical residue in the study can be attributed to its feeding habits and its ability to feed at deeper depth in water. (Gunda, 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 river Pra, and during the sample collection, the level of pollution was observed, and 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 the fish species, whereas levels of testosterone and 4-tert-octylphenol were relatively low in all the fish species.

5 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 levels of drugs in River Pra were all higher as compared to those in River Narkwa. Also, chronic daily intake values of chloramphenicol and 17-beta estradiol in this study suggest that they pose a negligible carcinogenic risk to consumers of fish from both rivers. Chloramphenicol residue levels in this study pose no acute or chronic risk/ hazard to consumers of fish from both rivers. Primidone levels in this study pose chronic hazard risk but no acute hazard risk, whereas diclofenac poses both acute and chronic toxicity in both rivers. The risk quotient for 17-beta estradiol suggests that it poses a health risk to consumers of fishes from River Pra but not Narkwa. Since the levels of pharmaceutical residues in river Pra were relatively higher than that of River Narkwa, it is advised that supervisory authorities be set to control the activities and apprehend people dumping 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 other to reduce the concentration of the pharmaceuticals into water bodies.

Declarations

Data Availability

The primary data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

Acknowledgments

The author would like to thank the technicians and staff of the Department of Forensic Science of the University of Cape Coast (UCC) for providing laboratory space, facilities, and assistance to support the study

Funding statement

The authors received no funding from any organization or institution, authors funded the research themselves.

Consent to Publish

All authors have given their consent for the publication.

Ethical Approval

This is not applicable in Ghana for this type of research conducted.

Consent to Participate

This is not applicable

Authors	Contributions
Albert Ebo Duncan	Introduction and discussion
Christian Adokoh	Methodology and discussion
Martha Osei-Marfo	Discussion and methodology
Samuel Barnie	Discussion and Conclusion
George Aboagy Sekyi	Laboratory analysis discussion
Joseph Adjei	Laboratory analysis discussion

References

1. Adebola A, Taiwo K (2013) F. Determination of nonylphenol, octylphenol and bisphenol-A in water and sediments of two major rivers in Lagos, Nigeria. *Journal of Environmental Protection*, *2013*
2. aus der Beek T, Weber FA, Bergmann A, Gruttner G, Carius A (2016) Pharmaceuticals in the environment: global occurrence and potential cooperative action under the strategic approach to international chemicals management (SAICM). *Ger. Fed. Environ. Agency*, *94*
3. Belmont MA, Metcalfe CD (2003) Feasibility of using ornamental plants (Zantedeschia aethiopica) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants—a laboratory-scale study. Ecol Eng 21(4–5):233–247
4. Bergh MS, Budsberg SC (2005) The coxib NSAIDs: potential clinical and pharmacologic importance in veterinary medicine. J Vet Intern Med 19(5):633–643
5. Boxall AB (2004) The environmental side effects of medication: How are human and veterinary medicines in soils and water bodies affecting human and environmental health? EMBO Rep 5(12):1110–1116
6. Cesari R, Martin ES, Calin GA, Pentimalli F, Bichi R, McAdams H, Croce CM (2003) Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25–q27. *Proceedings of the National Academy of Sciences*, *100*(10), 5956–5961
7. Chang C, Wang YF, Kanamori Y, Shih JJ, Kawai Y, Lee CK, Esashi M (2005) Etching submicrometer trenches by using the Bosch process and its application to the fabrication of antireflection structures. J Micromech Microeng 15(3):580
8. Corcoran J, Winter MJ, Tyler CR (2010) Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. Crit Rev Toxicol 40(4):287–304
9. Drover VJ, Bottaro CS (2008) Determination of pharmaceuticals in drinking water by CD-modified MEKC: Separation optimization using experimental design. J Sep Sci 31(21):3740–3748
10. Gunder H (2004) "Clarias gariepinus" (On-line), Animal Diversity Web. Accessed July 25, 2020 at https://animaldiversity.org/accounts/Clarias_gariepinus/
11. Hoga CA, Almeida FL, Reyes FG (2018) A review on the use of hormones in fish farming: Analytical methods to determine their residues. CyTA-Journal of Food 16(1):679–691
12. Jefferson JA, Morrow JI (1996) 8. Anticonvulsants. Human Toxicology. Elsevier Science BV, pp 319–334
13. Johannessen SI (2004) Therapeutic drug monitoring of antiepileptic drugs. Handbook of Analytical Separations, vol 5. Elsevier Science BV, pp 221–253
14. Karmakar S, Das SA, Chhaba B, Rout SK NONYLPHENOL:ITS TOXIC EFFECTS ON AQUATIC ORGANISMS
15. Kümmerer K (2010) Pharmaceuticals in the environment. Annu Rev Environ Resour 35:57–75

16. Kümmerer K, Henninger A (2003) Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin Microbiol Infect 9(12):1203–1214
17. Küster A, Adler N (2014) Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Philosophical Trans Royal Soc B: Biol Sci 369(1656):20130587
18. Larsson DJ, de Pedro C, Paxeus N (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater 148(3):751–755
19. Li D, Yang M, Hu J, Ren L, Zhang Y, Li K (2008) Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river. Environ Toxicol Chemistry: Int J 27(1):80–86
20. Li D, Yang M, Hu J, Zhang Y, Chang H, Jin F (2008) Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river. Water Res 42(1–2):307–317
21. Mann RM, Boddy MR (2000) Biodegradation of a nonylphenol ethoxylate by the autochthonous microflora in lake water with observations on the influence of light. Chemosphere 41(9):1361–1369
22. Mann RM, Boddy MR (2000) Biodegradation of a nonylphenol ethoxylate by the autochthonous microflora in lake water with observations on the influence of light. Chemosphere 41(9):1361–1369
23. Marmon P, Owen SF, Margiotta-Casaluci L (2021) Pharmacology-informed prediction of the risk posed to fish by mixtures of non-steroidal anti-inflammatory drugs (NSAIDs) in the environment. Environ Int 146:106222
24. Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Tsai LH (2019) Single-cell transcriptomic analysis of Alzheimer's disease. Nature 570(7761):332–337
25. Medina-Estrada I, Alva-Murillo N, López-Meza JE, Ochoa-Zarzosa A (2018) Immunomodulatory effects of 17β-estradiol on epithelial cells during bacterial infections. *Journal of Immunology Research*, *2018*
26. Mellon M, Benbrook C, Benbrook KL (2001) Hogging it.Estimates of antimicrobial abuse in livestock,7–9
27. Nawaz MS, Erickson BD, Khan AA, Khan SA, Pothuluri JV, Rafii F, Cerniglia CE (2002) Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. In *Research Perspectives Journal, online*
28. Nyberg L (2016) *Assessing the impact of emerging contaminants on anaerobic microbial communities* (Doctoral dissertation, Purdue University)
29. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Khan AA (2004) Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427(6975):630–633
30. Ojoghoro JO, Scrimshaw MD, Sumpter JP (2021) Steroid hormones in the aquatic environment.Science of The Total Environment,148306
31. Ruhoy IS, Daughton CG (2007) Types and quantities of leftover drugs entering the environment via disposal to sewage revealed by coroner records. Sci Total Environ 388(1–3):137–148
32. Serino M, Luche E, Gres S, Baylac A, Bergé M, Cenac C, Burcelin R (2012) Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 61(4):543–553
33. Tyumina EA, Bazhutin GA, Gómez ADPC, Ivshina IB (2020) Nonsteroidal Anti-inflammatory Drugs as Emerging Contaminants. Microbiology 89(2):148–163

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Highlights.docx