Targeted detection of pharmaceutical residues in fish and macro invertebrates around the waste water treatment at the University Teaching Hospital of Yaoundé, Cameroon

ABSTRACT

Introduction:Hospital effluents are one of the main sources of dissemination of pharmaceutical residues in hydro system. Worldwide, analysis of these effluents has revealed the presence of various substances. Very few studies assessing the contamination of solid matrices by pharmaceutical residues have been carried out in Africa,specifically inCameroon.

General Objective: The aim of this study was to detect pharmaceutical residues in solid environmental matrices around the discharge of the effluent from theYaoundéUniversity Teaching Hospital.

Methods: Macro invertebrates and fish were collected in three sampling sites prior and after the waste water treatment plant of the YaoundéUniversity Teaching Hospital. Once in the laboratory, samples were freeze dried and ground. Powders were sent for analysis at the Sefako Makgatho Health Sciences University in South Africa.The analysis was carried out using an ultra-high-performance liquid chromatography coupled with a triple quadrupole tandem mass spectrometer.

Results: A total of ninety-nine pharmaceutical residues related to seventeen pharmacological classes were detected in all samples ofmacro invertebrates (72) and Nile tilapia (55 for fish inside and 58 for fish flesh) collected.Antibiotics were the most represented (23.2%), followed by anti-inflammatory (18.2%), and anti-parasitics (9.1%). More than half (53.6%) of the drug residues detected in the fish samples were of thethe anti-inflammatory (21,71%), antihypertensive (17,67 %) and antibiotic (13,13 %) classes.The number of drug residues detected in the macroinvertebrates before the treatment plant (41) was smaller than those detected after the treatment plant (67).

Conclusion: The YaoundéUniversity Teaching Hospitaleffluent constitutes an important contamination source of the hydro system with pharmaceutical residues. This surely has an impact on solid matrices and human health that need additional research to be well understood and addressed.

Key words: Ecopharmacovigilance, pharmaceutical residues, Hospital effluent, solid matrices, Cameroon.

INTRODUCTION

The incorrect disposal of hospital waste water is one of the common pathways of pharmaceuticals into the environment. Even though, these waste water undergoes some form of treatment prior to release into the environment [1],it has been reported worldwide that wastewater treatment plant (WWTP) processes, even the one of hospitals, do not eliminate drugs residues and as such quite a large number of pharmaceuticals have been detected in WWTP effluents [1-6].Based on literature review over a 22 years period (2000–2022), pharmaceuticals residues were only monitored in 28 out of 54 African countries[7]. Very few studies assessing the contamination of living organisms by pharmaceutical residues have been carried out in Africa, specifically in Cameroon [8-10]. The weakness of pharmaceutical disposal and wastewater treatment protocols and policies could be one of the reasons which allows improper disposal with no repercussion.

This implies that the potential human health risks and ecotoxicity effects of pharmaceuticals residues to aquatic life in Africa is unknown. The current study has focused on target detection of pharmaceutical residues in solid environmental matrices around the discharge of the effluent from the Yaoundé University Teaching Hospital**.**

MATERIALS AND METHODS

Study period: The study was carried out from March 2022 to December 2023.

Study sites

Four Sampling sites of fish and macroinvertebrates were identified fromthewater was treatment point (WWTP)from the watercourse after the discharge of effluent and water pondwhere there was the discharge of effluent from University Teaching Hospital of Yaoundé (UTHY). Figure 1 represents the cartography of the study area.

Figure 1: Different study sites (Tchadji, Cameroun, Yaoundé, 2021)

The four study sites selected were:

- Olezoa (figure 1 A)
- Wastewater treatment plant of the Yaoundé University Teaching Hospital (Figure 1B)
- A watercourse located after the treatment plant (Figure 1C)
- Melen pond (Figure 1D)

Samples collection and preparation

Fish

Random fish sampling was carried out. We caught the one available in the Melen pond during the study period. They were dissected to remove brain, heart, liver, kidney, gonads, gills, liver and muscles. Brain, kidney, heart and gonads were mixed to obtain a sample mass that could be easily extracted. Liver, muscles, and gills were preserved separately. The different parts were then frozen (-80°C) in an

EVERMED freezer then freeze-dried for 96 hours using a CHRIST BETA 1-8 Bioblock Scientific freeze dryer.

- **Macroinvertebrates**

Macroinvertebrates were collected at each study station. The different samples were placed per family in 60 mL pill bottles previously filled with alcohol 95°C. Once in the laboratory, the samples were identified and numbered, then frozen (−80 °C) and cryonized using a CHRIST BETA 1-8 Bio-block Scientific freezedryer for 96 hours.

- **Sample conservation and transport**

The powder obtained from fish and macroinvertebrates samples, was crushed and stored in sealed plastic bags using a laminator before being deposited at DHL agency in Yaoundé for shipment to South Africa to the University of Sefako Makgatho Health Sciences University, where the detection and identification drugs residues were carried out.

- **Selection of drugs to be detected and quantified**

The selection of pharmaceutical compounds was initially made on the basis of drugs consumed in the hospital (UTHY) with the hypothesis that the latter are those likely to be found in wastewater. To do this, we took inspiration from the list of medication frequently prescribed at the UTHY in 2014[11, 12]. The pharmaceutical products selected were the therapeutic classes most found within the list of drugs most prescribed in the hospital. This information was cross referenced with literature data on occurrence and persistence in hospital effluents and the environment. Then, some available pharmaceuticals products were included in the database. Thus, the selectioncriteriausedwere as follows :

- The representativeness of the therapeutic class and chemical family of the compound ;
- The affiliation of the compound to molecules with a large prescription volume;
- The availability of an analytical standard;
- The reported presence of the compound in hospital effluent.

- **Selection of aquatic organisms**

The organisms collected during the study were freshwater fish available in the Melen pond and benthic macroinvertebrates. These two groups of organisms live in mud and would be the most prone.

- **Chemicals and reagents used for detection**

High performance liquid chromatographic-grade methanol was purchased from Sigma Aldrich (Johannesburg, South Africa) while ultra-high purity water was prepared in the laboratory using Milli-QRO4 system 117 (Millipore, Bedford, MA, United States). The detected compounds were all analytical grades and supplied in powder form by Merck Chemicals (Pty) Ltd. (Johannesburg, South Africa).

- **Analysisof macroinvertebrates and fish samples**

Upon their arrival in South Africa, samples were analysed through the methods developed by Ncube et al [1].Analyses were done usinga Dionex Ultimate 3000 UHPLC system from Thermo Scientific (Sunnyvale, California, United States) with a quadrupole time-of-flight mass spectrometric detector from Bruker Daltonics (Bremen, Germany) (UHPLC-QTOF-MS).Prior to HPLC analysis, a solid-phase extraction was carried out on the samples. A total of seven samples (labelled from letter A to letter G) were analysed and correspond to the following pats: A and B: fish muscles; C and D: fish inside; E: Macroinvertebrates from Olezoa; F: Macroinvertebrates after the treatment plant of University Teaching Hospital of Yaoundé; G: Macroinvertebrates in Melen pond.

RESULTS

Sampling

The macroinvertebrates collected belonged for the most part to the families Chironomidae andBaetidae. The table I groups together the different families collected.

Table I: Inventory of macroinvertebrates collected for the determination of drug residues

Branch	Class	Order	Family	Gender and/or species	OL	SE	AE	ME
ARTHROPODS	Insects	Diptera	Chironomidae	S/F Tanypodinae	65	/	/	/
				s/F Chironominae TR Chironomini	/	54	92	27
		Ephemeroptera	Baetidae	*Cleon*sp	/	8	/	/
				*Baetis*sp	/	60	/	1
		Heteroptera	Belostomatidae	*Abedus*sp	/	5	/	16
			Notonectidae	*Notonecta*sp	/	14	/	/
			Corixidae	*Corixa*sp	/	1	/	/
		Odonata	Cordulegasteridae	*Cordulegaster*	/	2	/	/
			Libellulidae	*Libellula*sp	/	/	27	/
				Orthetrumcaffrum	/	/	/	2
			Coenagrionidae	*Enallagmacyathigerum*	/	/	/	5
MOLLUSCS	Gasteropodes	Basomatophora	Lymnaeidae	*Radix*	/	/	/	4
				Stagnicola	/	/	32	14
			Physidae	*Physaacuta*	/	25	1	/
					/	/	/	/
ANNELIDES	Clitellata	Huridinea	Glossiphonidae	*Hemiclepsismarginata*	/	3	/	/
				Haementeriacostata	/	1	/	/

SE: Water treatment plant AE: Watercourse after water treatment plant ME: Melen Pond OL: Olezoa

The fish caught were those available at the Melen fish pond. It was tilapia (*Oreochromis niloticus*). Their number was twenty (20) and the weight in the fresh state varied between 189 g and 628 g. Sizes ranged from 23 cm to 32 cml. Tables II and III are summaries of the weight of fish and different part taken fresh as well as their length.

Table II: Weight of different parts of fish collected at cool and dry state

Parts collected	**Brain**	**Kidney**	**Heart**	**Gonads**	**Liver**	**Gills**	**Muscles**	**B+H+K+G**
Cool state (g)	3	11	3	20	59	173	533	37
Dry state (g)	/	/	/	/	23,6	69,2	213,2	14,8

B: Brain; H: Heart; K: Kidney; G: Gonads

Table III: Weight and length of collected tilapia

Weight (g)	469	386	316	403	275	395	489	337	332	277	419	289	431	638	406	254	189	214	232	193
Length (cm)	28,5	28	26,5	27,5	25	27,5	30,5	27	25,5	24,5	27,5	24,5	28	32	30	24	21,5	23,5	24	23

Detection and identification of drug residues

The presence of numerous pharmaceutical residues was highlighted in all samples. Seventeen drugs classes were detected and identified with a total of 99 actives molecules. Thirty-one drugs residues were identified in all samples. They were as follows: 17-alpha ethinylestradiol, 6-alpha methylprednisolone, aceprometazine, acetaminophen, acetyl salicylic acid, atenolol, bifenazate, caffeine, cimaterole, dichloroacetic acid, diethylstilbestrol, fenoterol, dimetridazole, flumequine, gemfibrozil, hydrocortisone, ibuprofen, lidocaine, mefenamic acid, metoprolol, naproxen, prednisolone, primidone, procaine, progesterone, propranolol, spectinomycin, sulfamidin, sulfamethazine and terbutalin.

- **Distribution per study site**

The greatest number of therapeutic families identified was in the Melen pond. The distribution was almost similar for all study sites thus the family of antibiotics was the most represented (23,2%) followed by that of anti-inflammatory drugs (18,2%), then antiparasitics (9, 1%), antihypertensive, antiasthmatics, hormones and antifungal. Each therapeutic family detected was having 6 to 7 pharmaceuticals compounds as shown in Figure 2.

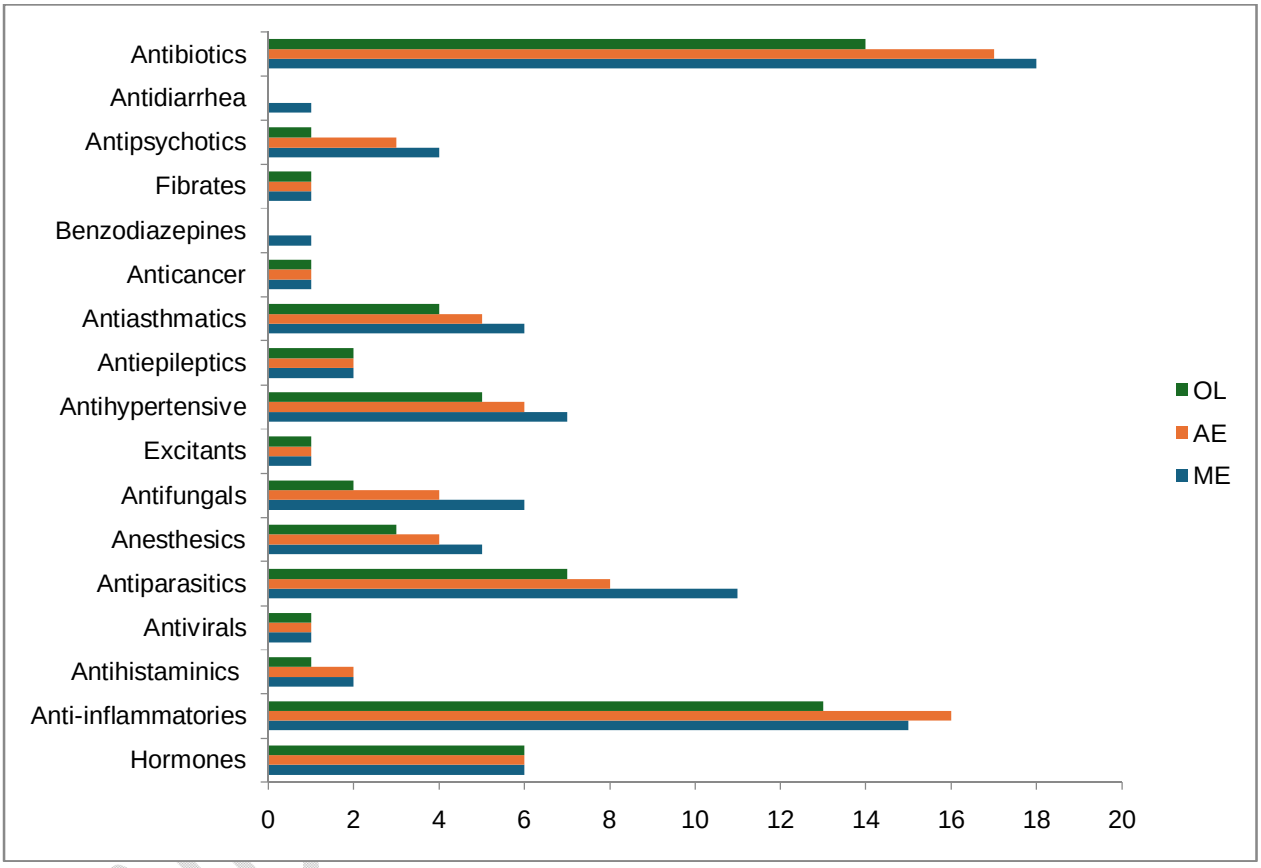

Figure 2: Pharmacological families identified at each study site

OL: Olezoa AE: Water course after water treatment plan ME: Melen pond

- **Distribution in each matrix**

The distribution of identified drug residues was almost uniform across all matrices: 39 % for macroinvertebrates, 30% for fish flesh and 31% for fish viscera (Figure 2). Benthic macroinvertebrates constituted the major reservoir of these compounds.

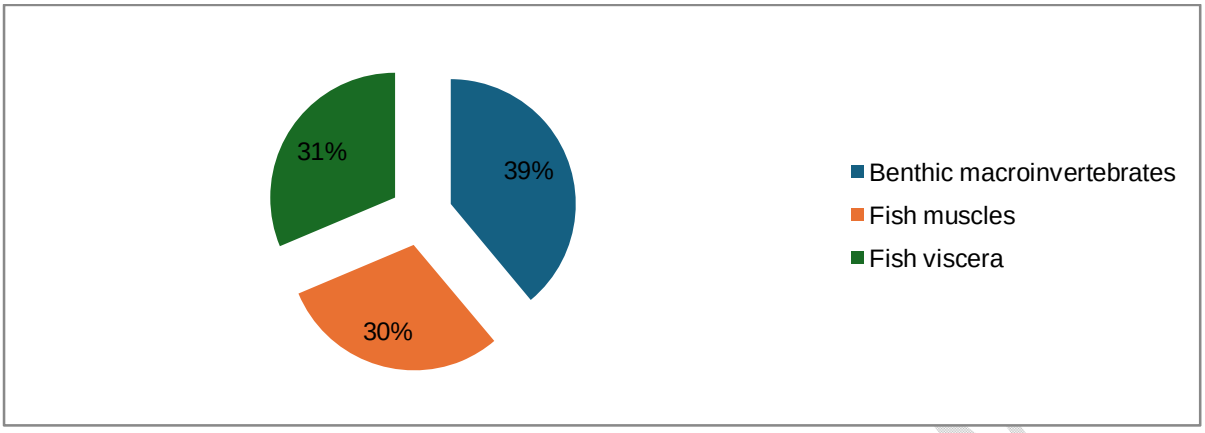

Figure 3: Distribution of drug residues in each matrix

- **Macroinvertebrates**

The number of therapeutic classes of drug residues detected in macroinvertebrates before water treatment plant (41) was lower than the one detected after the water treatment plant (67) (Figure 4).

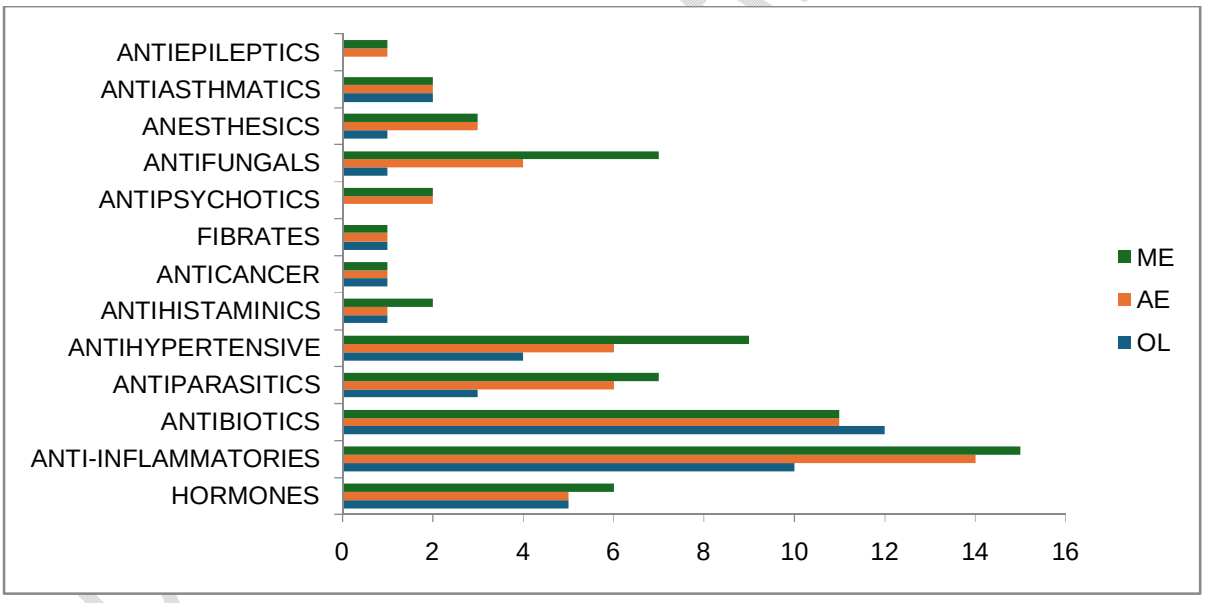

Figure 4: Therapeutic classes of drugs residues detected in macroinvertebrates.

OL: Olezoa AE: Water course after water treatment plant ME: Melen pond

- **Fish**

The drugs residues detected and identified in fish samples belonged for the most part (53, 6%), to the classes of anti-inflammatories (21. 71%), antihypertensive (17.73%)and antibiotics (13.13%) (Figure5).

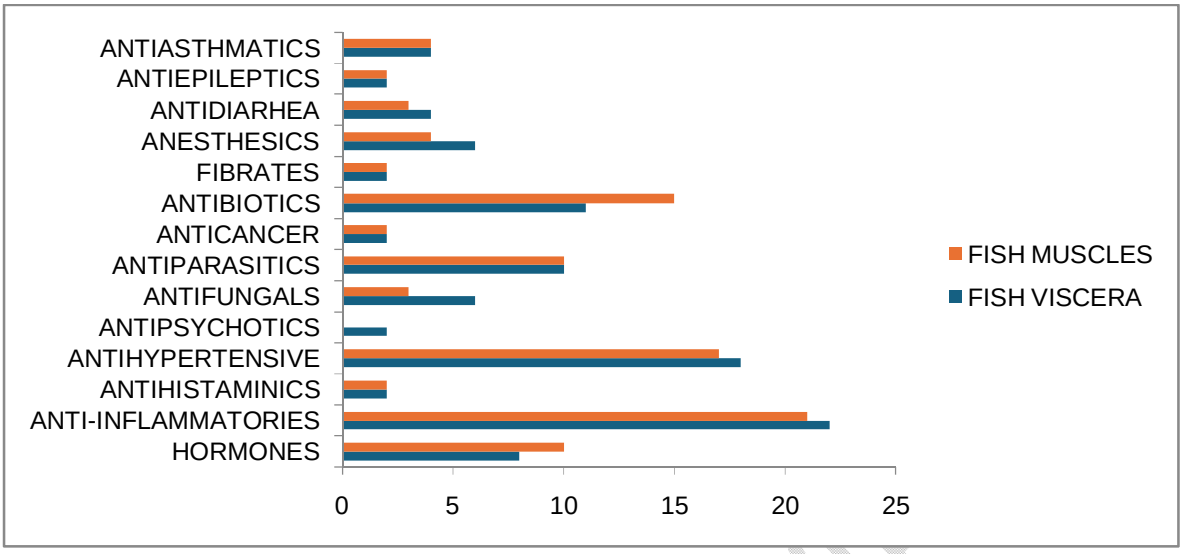

Figure 5: Summary of drugs residues identified and detected in fish samples

- **Class of drugs residues detected**

Figure 6Asummarises the class of antibiotics where 5 families were identified and detected. The quinolones family had the greatest number of detected compounds (7) followed by sulfamids family(6).This was followed by the cyclinsand aminosides families with 3 compounds each.

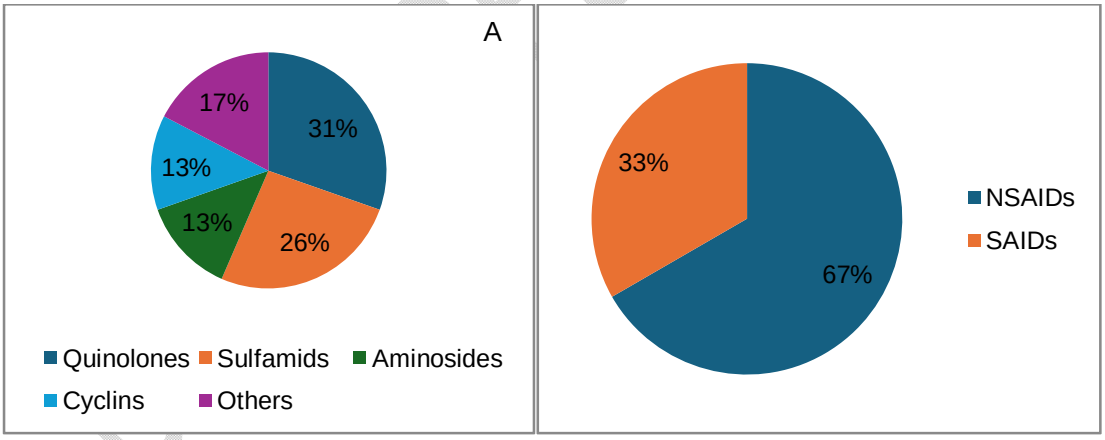

Figure 6: Percentage of each family in antibiotic(A)and anti-inflammatories (B) class

SAIDs: Steroidal Anti-Inflammatoires Drugs NSAIDs: Non-Steroidal Anti-inflammatories Drugs

The anti-inflammatories class, on one handwas made up of pharmaceutical compounds that were -morphine, including NSAIDs (Non- Steroidal anti-inflammatories) andSAI (Steroidal Anti-inflammatories) (Figure 6 B). Antiparasitics were the third most represented class with the families of anthelminthic and antiprotozoal (Figure 7A). Figure 7B represents

the different families of drugs in the antihypertensive class identified during the study. Beta-blockers were the most represented (4 compounds).

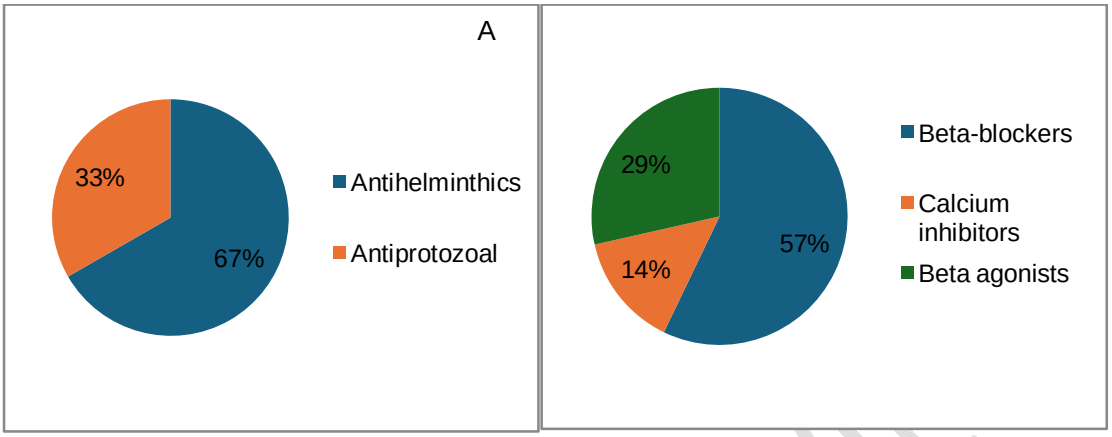

Figure7: Percentage of each familyof identified antiparasitics (A) and antihypertensive

Discussion

This study highlighted a significant distribution of drugs residues in all the studied sites.This distribution was considerable given the fact that these residues are related to more than a dozen of pharmacological classes. This study confirmed a centred and uncontrolled discharge of pharmaceuticals waste in hospital effluents. The major part of drugs residues detected (80, 61%) was identified for the first time in Cameroon more particularly in this study site. Most of the work carried out in Cameroon has been related to surface and underground water waste.[3, 6, 13, 14]. None of them had been interested in organic materials and specifics of hospital drug related was. Studies on hospital waste has mostly involved solid waste infectious materials, especially of potential pathogenic transmission.

Macroinvertebrates

The benthic macroinvertebrates constituted the major recipient of these compounds.The number of drug residues detectedin macroinvertebrate samples before the hospital treatment (41) was lower than those detected after the treatment plant (67), which demonstrated the non-functionalityof water treatment plant and drug environmental pollution through hospital waste water.

Fish

More than half(53.6%) of detected drugs residues in fish samples belonged to anti-inflammatories (21,71%), antihypertensive (17, 67 %) and antibiotics (13, 13 %). The

freshwater fish tilapia. In this study were considered as great bio accumulatorsbecause of their high fat content [15]. In addition, they live in contact with the bottom of the water, which increased their tendency to bioaccumulate. The bioaccumulative capacity of medicinal substances such as antibiotics has been demonstrated in fish liver and brain tissue [14]

All the tilapia caught randomly werefemales. The recommended sex ratio for production and reproduction of tilapia is 1 male for 2-3 females [15]. However, during our work, we obtained a sex ratio of zero in favour of females. This could be due to the ubiquityofthe different samples'endocrine disruptors ((17αethinyl oestradiol, 17 β oestradiol and bisphenol A) known to feminize.

Families of drugs residues detected and identified

Regarding the families of drug residues detected, a table made in 2014 at UTHY [11]revealed the five most consumed families. Antibiotics were the most consumed class of drugs detected at UTHY in 2014 [11]and were among the molecules with a high probability of being found. Their detection and identification in the present work supported this hypothesis. Among the families of antibiotics detected, quinolones was the most represented with lomafloxacin, ofloxacin, norfloxacin, nalidixic acid, marbofloxacin and sparfloxacin. They have been reported in some earlier studies as poorly biodegradable [9]. Their detection in our study corroborated this property. Lincomycin, from the lincosamid class, was also detected that has been shown to have poorly biodegradable properties like macrolids.

Analgesics represented the fifth most used class at UTHY in 2014[11]. The probability of finding them in the UTHY hospital effluent was therefore high. Paracetamol, one member of this therapeutic class, was found in all analysed samples. It also constituted a molecular witness to environmental pollution by drugs. Paracetamol which is an over the counter drug in Cameroon and highly auto medicated by the population was the most consumed molecule in the analgesic class. This trend has been shown by many studies around the world [8-9, 17-24], tending to confirm the place of analgesics as one of the groups of most consumed medications. One reason that could justify this ubiquity would be its great ability for binding to solid materials, thus limiting its hydrolysis and therefore its degradation [13]. Ibuprofen, which was identified during our analysis, NSAID, was known to be generally used in outpatient treatment. Packer *et al*. in 2003,demonstrated in a pharmacokinetics study that 15% ibuprofencan be excreted unchanged[42].Furthermore, it has been proven that analgesics/anti-inflammatories have endocrine disrupting effects. The National Institute of Health and

Medical Research(INSERM) in2013 demonstratedthat paracetamol, aspirin and indomethacininhibited the production of testosterone in adults [16]. Some studies first carried out on rats showed adrop-in testosterone production and feminization of new borne male rats. Subsequently, the researchers exposed testicular explants from adult men to different doses of aspirin, paracetamol and indomethacinduring at least 24 hours at concentrations similar to those found in plasma when taking these molecules. They noted a drop in the production of testosterone, prostaglandins and insulin like factor 3 (ILF-3) [16]. Later, in 2022, some studies were done on opioids (tramadol, codeine, morphine, fentanyl, loperamide, dextromethorphan, pholcodin, buprenorphine) on the endocrine disrupting effects which depended on the doses received and the duration of use. This study led to a reduction of sex hormones production in both women and men, abnormalities in the menstrual cycle, hot flashes, reduced libido, infertility and erectile dysfunction, gynecomastia, milk secretions outside of breastfeeding in women, metabolic disorders in the regulation of sugar levels even in the absence of diabetes, osteoporosis and thyroid dysfunction. These effects differed depending on the substance, the route of administration, and individual factors. The effects were more marked when opioids had long duration of action and weretaken for a long period in high doses[17]. It should be noted that the opioid class of drugs is among the most consumed and prescribed in Cameroon to managed chronic pain[11] . Their presences in the environment are therefore not trivial and increase the risk and duration of exposure of these substances.

Gemfibrozil from fibrates therapeutic family was detected and identified in all sampleseven though of less use in Cameroon.[11], its physicochemical properties could explain its presence. This molecule has been shown to be possibly adsorb on solid phases [18].

Antihypertensive represented the third therapeutic class of drugs most consumed within the UTHY in 2014 [11]. Beta blockers such as Atenolol, Propranolol and Metoprolol were the compoundsmost detected and identified in this class of drugs. One of the reasons of their identification would be their use in outpatient treatment, with the consequence of a constant entry into the waters by poor disposal of the unused drugs, although they are not the most consumed molecules in this large group [11]. The prevalence of hypertension in Cameroon is 33 % [123]. Several studies also reported the detection of beta-blockers as drug residues in the environment [3, 6, 8, 14, 19]. They also reported the great ecotoxicological danger of these compounds, although at concentrations higher than those found in the natural environment.Due to the physicochemical property, beta-blockers are stable in aquatic

environment (Propranolol, Atenolol, Metoprolol) which can their accumulation, an increase the tendency to adsorb on sold matter particularly Propranolol[20]. This made them available to living organisms in this environment (fish, macroinvertebrates).

Among the antihistaminic, astemisole and aceprometazine could be identified. Astemisoleis a long lasting antihistaminic that was introduced to the market in 90s. It is also attributed an antimalarial property with a similar mechanism of action to chloroquine. Its presence could be explained either by the purchase of the drug from street or by the persistence of astemisole in the environment or by substitution of chloroquine by astemisole in preparations supposed to contain it.

Drinking water and consumption of aquatic organisms are two routes by which humans would be exposed to drugs polluting the aquatic environment. Therefore, the possible risks of exposure to human health are subject of particular interest in countries where drinking water is a rare commodity. Several quantitative studies on drug exposure at tracelevels in different parts of the world have demonstrated very low risks for human health based on toxicological data [20-22]. However, these studies do not exclude possible effects on human health because they were based on information that only considered long-term effects. Furthermore, knowledge on the effects of drug mixtures is limited[22, 23]. It should also be noted that the majority of studies did not focused on living organisms to assess health risks although in our current situation the water treatment plant are not well adopted to deal with drug waste which is not only an environmental risk, but a health challenge problem of drug resistance [24, 25].

Another risk of drug residue in the environment would be the development of antimicrobial resistance. The presence of antibiotics in treated waste water could lead to high mortality and morbidity as well as an increase in incurable infectious diseases [25]. Antimicrobial resistance has become a major challenge in therapy because it compromises the effectiveness of antibiotics resulting in therapeuticfailure, high health costs and an increase in morbidity and mortality [26]. Take for example the case of the multi-resistant bacteria *Klebsiella pneumonie*, it cannot be treated with any antibiotic currentlyon the market [25]**.** Furthermore, the abuse and misuse of antibiotics could cause risk to human health by promoting antibiotic-resistant bacteria and antibiotic resistance genes in the aquatic environment [27, 28]. The bacterial community capable of withstanding this antimicrobial pressure, will survive and multiply, leading to more antibiotic- resistant groups in the environment [26]. These genes can be transferred horizontally from animals pathogens to

human being through different classes of antibiotics used in human and veterinary medicine, especially those with the same mechanism of action [29], which constitutes a major health problem.

CONCLUSION

The aim of this study was to detect pharmaceutical residues in solid environmental matrices around the discharge of the effluent from the Yaoundé University Teaching Hospital. The distribution of identified and detected drug residues was nearly uniform in all matrices. Benthic macroinvertebrates were the majority recipient of drug compounds. The largest number of therapeutic family identified was in Melen pond. Five therapeutic classes (antibiotics, anti-inflammatories, antihypertensive, antiparasitics and hormones) were mostly represented. All the analysed samples contained drugs residues. This confirms exposure of aquatic organisms to drug residue and the potential risk to human health.

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