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PHYSICOCHEMICAL AND ECOTOXICOLOGICAL ASSESSMENT OF WASTEWATER FROM THE YAMOUSSOUKRO REGIONAL HOSPITAL CENTER TREATMENT PLANT

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ABSTRACT

The lack of health care facilities in developing countries leads to the hospitalisation of many sick patients, who release drug residues into wastewater through urine. As a result, treatment plants can no longer effectively treat this wastewater. This study aims to assess the ecotoxicological risks of Pharmaceutical Compounds (PhCs) in wastewater from the treatment plant at the Yamoussoukro Regional Hospital Centre (YRHC). To this end, 24 PhCs, including antibiotics, analgesics/anti-inflammatories, antiepileptics, steroid hormones, antidepressants, beta-blockers, alkaloids, and antimetabolites, were analysed by HPLC-MS/MS. Ecotoxicological risks were evaluated using the Risk Quotient (RQ). The results revealed relatively high concentrations of compounds such as sulfamethoxazole (1,612 ng/L), diclofenac (2,690 ng/L), caffeine (13,535 ng/L), carbamazepine (3 ng/L), ibuprofen (1,739 ng/L), desvenlafaxine (2,570 ng/L), levofloxacin (1,907 ng/L), and hydroxyl-diclofenac (5,621 ng/L). In terms of RQ, caffeine, carbamazepine, desvenlafaxine, ibuprofen, and sulfamethoxazole posed a minimal risk (RQ < 0.1). In contrast, diclofenac, levofloxacin, and hydroxyl-diclofenac presented a moderate risk, with RQs of 0.26, 0.051, and 0.64 respectively. Moreover, the cumulative RQ (≈ 1.01) indicates that YRHC effluents pose a significantly high overall risk. Finally, these results underscore that effluents containing drug residues or PhCs must be properly treated before discharge into the lake, their receiving environment.

Introduction

The contamination of aquatic ecosystems by Pharmaceutical Compounds (PhCs) is increasingly recognised as a global concern (Nantaba *et al.*, 2020). Hospitals carry out a wide range of activities (care, diagnosis, hygiene, maintenance, etc.), which involve the use of persistent substances such as surfactants, disinfectants, cleaning products, and pharmaceuticals. Unfortunately, these PhCs contained in hospital effluents often escape treatment processes and end up in waterways (Li *et al.*, 2020). Indeed, hospitals are considered major contributors to environmental pollution (Hoang *et al.*, 2024). Studies assessing the risks of PhCs in the environment indicate that they can cause chronic effects related to growth inhibition, altered feeding behaviour, reproductive impacts, and increased mortality in certain aquatic organisms (Orias & Perrodin, 2013; Tell *et al.*, 2019; Bouzas-Monroy *et al.*, 2022). In addition, PhCs can accumulate in edible fruits and vegetables (González García *et al.*, 2018) and even in drinking water (Sharma et *al.*, 2019).

The situation is particularly critical in developing countries, where only 10% to 20% of wastewater is treated. The remainder is discharged directly into aquatic environments, causing significant environmental concerns (Rasool *et al.*, 2018). In Côte d'Ivoire, market gardening is increasingly being developed on the shores of lakes due to climate change, which is seriously disrupting agricultural productivity. Fishing is also practised near these lakes, despite the discharge of wastewater, including hospital effluent from Yamoussoukro. This situation poses many harmful consequences for aquatic life, the environment, and even human health. For example, Nantaba *et al.* (2020), and Madikizela and Ncube (2021) reported the ecotoxicological risks of selected PhCs in Lake Victoria's water and of non-steroidal anti-inflammatory drugs in the South African aquatic environments, respectively.

To understand the impact of hospital effluents on the aquatic environment and human health, an appropriate study is needed to characterise and assess the risks of PhCs. To the best of our knowledge, no scientific study in Côte d'Ivoire has yet assessed the ecotoxicological risks associated with PhCs. The objective of this study is to evaluate the quality of hospital wastewater at the Yamoussoukro Regional Hospital Centre (YRHC) treatment plant and assess the environmental risk of its pharmaceutical compounds.

Materials and Methods

Site presentation

The study was conducted in Yamoussoukro, the political capital of Côte d'Ivoire, located between 6°40' -7°00' N, and 5°10' -5°20' W (Figure 1). A notable feature of Yamoussoukro is its network of 10 artificial lakes, which are considered tourist sites due to the presence of caimans. Various gardening activities, including the cultivation of tomatoes, lettuce, and cucumbers, are carried out around these lakes. The YRHC is the primary healthcare facility in this autonomous district and operates a bacterial bed treatment plant for its wastewater. Effluents from this plant are discharged directly into one of Yamoussoukro's lakes. Samples were collected from these effluents to assess their physicochemical characteristics and their impact on the receiving environment.

Chromatographic Analysis of Emerging Contaminants

All detailed extraction and analytical procedures were previously developed and described according to Ouarda *et al.* (2019). PhCs were quantified by high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (Thermo Scientific TSQ Quantiva). Samples were prepared in a 10 mL amber glass vial, 2 mL aliquots were injected into the HPLC system for analysis. PhCs were preconcentrated on an in-line solid-phase extraction (SPE) cartridge (Hypersil Gold, Thermo Scientific), which is integrated into the HPLC system and maintained at room temperature. Cartridges were conditioned with eluent prior to sample injection.

Analysis was performed using electrospray ionisation (ESI) in both positive and negative modes, with a C18 reversed-phase column maintained at a temperature of 30˚C. The mobile phase consisted of ultrapure water, HPLC grade methanol, and 1% formic acid. PhCs recoveries averaged 70%. For PhCs quantification, an internal standard was added to synthetic solutions containing PhCs at concentrations ranging from 10 ng/mL to 25 µg/mL, and a nine-point calibration curve was used to quantify PhCs in YRHC samples. The limit of detection was determined by the signal-to-noise ratio (S/N = 3), and the limit of quantification was determined by the signal-to-noise ratio (S/N = 10). To avoid sudden pressure increases and damage to the HPLC autosampler, samples were filtered through 3 µm glass paper prior to injection into the HPLC system.

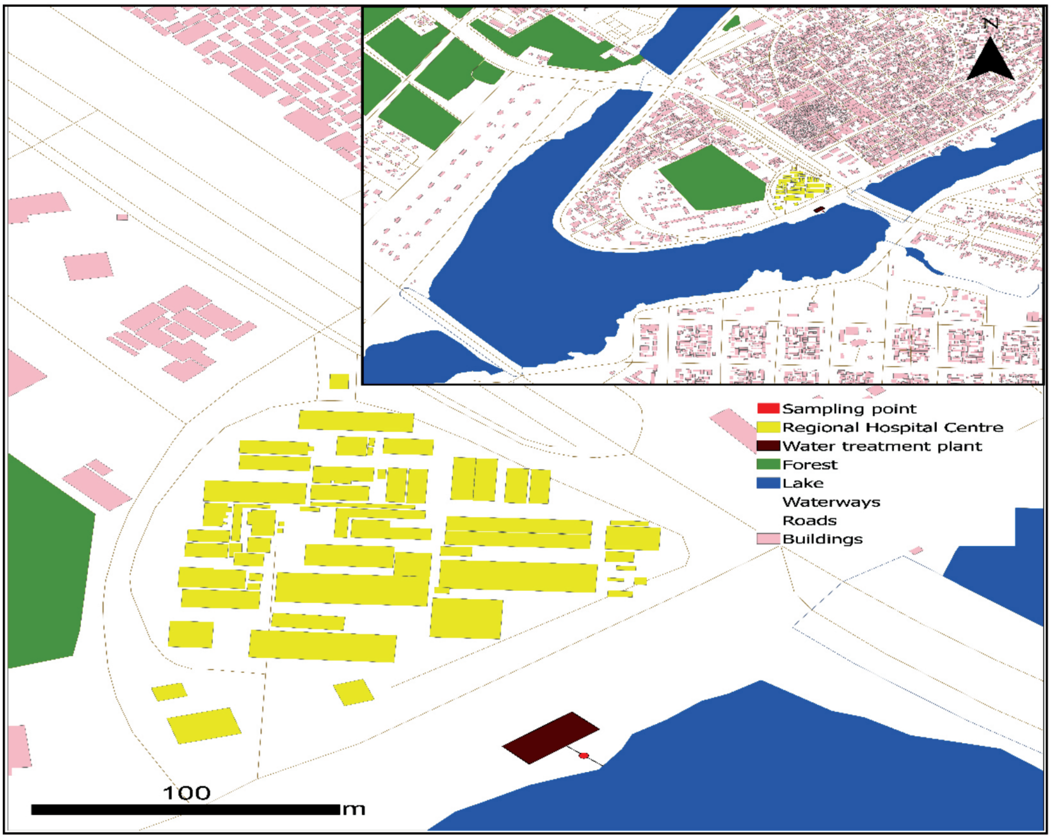

Figure 1: Synoptic view of the study and sampling area

Ecotoxicological Analysis

The environmental risk associated with each PhC in the hospital effluent was assessed using the Risk Quotient (RQ), which quantifies potential hazards to aquatic organisms exposed to these compounds. The RQ is calculated as the ratio of the residual concentration of the targeted phytochemical, or Predicted Environmental Concentration (PEC), to the Predicted No-Effect Concentration (PNEC) in the environment. According to European community guidelines, the RQ is calculated using Equation (1) and Equation (2) (Gosset *et al.*, 2021).

$$RQ_y = PEC_y/PNEC_y \quad (1)$$

$$RQ_{mix} = \sum_{y=1}^{n}/RQ_y \quad (2)$$

Where is the risk quotient of contaminant *y* in the environment; is predicted environmental concentration of contaminant *y* (ng/L); is the concentration of contaminant *y* that has no effect on the environment (ng/L); and, is the risk quotient for all compounds. The potential for environmental risk depends on the position of the RQ value within a defined range: RQ ≥ 1 indicates high risk; 0.1 < RQ < 1 indicates medium risk; and, RQ < 0.1 indicates low risk (Gani *et al.*, 2021).

To effectively protect ecological resources, we adopted the PNEC selection criterion based on the approach of Tell *et al.* (2019). This approach recommends using the lowest PNEC value in ecotoxicological risk assessment. As sampling was carried out at the outlet of the YRHC wastewater treatment plant, the dilution factor was taken into account to better reflect environmental realities (Keller *et al.*, 2014). The relationship between the dilution factor and expected measured concentrations of PhCs can be obtained using Equation (3):

$$PEC_y = MEC_y/DF \qquad (3)$$

Where *MEC*y is the concentration of contaminant *y* measured at the treatment plant outlet (ng/L) and *DF* is the Dilution Factor. For the case of Cote d'Ivoire, the annual median dilution factor was estimated at 516.59, according to Keller *et al.* (2014).

Results and Discussion

Physicochemical Characteristics of the Effluent from the Wastewater Treatment Plant (WWTP)

The characteristics of the effluents from the YRHC treatment plant are presented in Table 1. These results indicate that the nitrate and pH levels comply with WHO discharge standards (Squilbin & Yourassowsky, 2005; Todedji *et al.*, 2020). However, the other parameters, including COD, BOD, conductivity, ammonium, and orthophosphate, did not comply with WHO discharge standards.

Conductivity is a measure of the concentration of ions present in water and is commonly used as an indicator for water quality control in hospital effluents (Gaur *et al.*, 2021). It provides information on the water's ability to conduct electrical current and reflects the salinity level of wastewater. In this study, the conductivity value was found to be 3,453 µS/cm, which is notably high and may indicate the presence of a large quantity of dissolved salts. These salts are likely to be discharged into the lake downstream of the wastewater treatment plant (WWTP), potentially posing health risks for the local population. The area surrounding the lakes is used for market gardening, with crops such as tomatoes, cucumbers, and salads being cultivated. These lakes also serve as a water source for crop irrigation.

Additionally, village wells are constructed very close to these lakes. The presence of high salt concentrations in water intended for human consumption can have detrimental health effects. Excessive intake of salts through drinking water has been associated with increased blood pressure, a known risk factor for cardiovascular diseases (El-Ogri *et al.*, 2016). Therefore, the high conductivity levels observed in these effluents warrant further investigation and targeted mitigation measures to protect public health.

The measured concentrations of ammonium and orthophosphate do not comply with the discharge standards established by the World Health Organisation (WHO). The presence of ammonium in the effluent is likely due to pollution from human urine (Aissi, 2013), while orthophosphate ions may originate from phosphorus-based detergents. Ammonium and phosphorus are essential nutrients for plant growth; however, their elevated concentrations in the YRHC effluent may contribute to the eutrophication of the lake located downstream of the treatment plant.

Table 1: Characteristics of effluent from the YRHC WWTP

Parameters	Average Values	WHO Standards
Temperature (°C)	27.3 ± 0.4	30
pH	7.5 ± 0.3	6.5 - 8.5
Conductivity (µS/cm)	3,453 ± 1,637	< 2,000
Nitrate (mg/L)	0.17 ± 0.05	< 2
Orthophosphate (mg/L)	1.7 ± 0.3	1
Ammonium (mg/L)	11.9 ± 4.5	0.2
BOD (mg/L O_2)	511 ± 62	< 150
COD (mg/L O_2)	1,352 ± 148	< 300

The characterisation of the hospital effluent also revealed that the Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values exceeded WHO standards for wastewater discharge. The COD/BOD ratio (2.65) is greater than 2, which indicates the presence of organic compounds that are difficult to biodegrade (Shah *et al.*, 2014) such as pharmaceutical molecules. Similar average values for COD (785 mg/L O2) and BOD (221 mg/L O2) were obtained by Hocaoglu *et al.* (2021) from wastewater around 1,156 hospitals in Turkey. Additionally, Elmountassir *et al.* (2019) reported COD and BOD concentrations of 1,593.6 mg O2/L and 131.28 mg O2/L, respectively, in effluents from the Hassan II hospital in Fez, Morocco.

PhCs in Effluent from RHCY

Analysis of wastewater samples collected at the outlet of the treatment plant revealed the presence of 24 PhCs in the YRHC effluent. These PhCs include eight steroid hormones (17 α-ethynylestradiol, 17 β-estradiol, estrone, estriol, progesterone, testosterone, medroxyprogesterone, and norethindrone), four antibiotics (ciprofloxacin, clarithromycin, sulfamethoxazole, and levofloxacin), four antidepressants (desvenlafaxine, venlafaxine, fluoxetine, and its metabolite norfloxetine), four anti-inflammatory drugs (diclofenac, ibuprofen, and their hydroxylated derivatives), one antiepileptic (carbamazepine), one beta-blocker (acebutolol), one alkaloid (caffeine), and one antimetabolite (methotrexate).

Some PhCs were detected at concentrations below the detection limit (Table 2), while others exhibited significantly high levels (Table 3). Accordingly, statistical analysis, including mean, standard deviation, and Coefficient of Variation (CV), was performed on PhCs present at concentrations well above the detection limit. The analysis revealed relatively high standard deviations, indicating substantial variability in concentrations depending on the wastewater sampling periods (Table 3), with peak levels observed between 10 a.m. and 4 p.m. This temporal variability in the concentrations of PhCs such as sulfamethoxazole, levofloxacin, ibuprofen, caffeine, carbamazepine, and diclofenac is further confirmed by CV values greater than 10, with the exception of diclofenac and desvenlafaxine.

According to Briton *et al*. (2018), CV values exceeding 10 signify a strong dispersion of the data around the mean. Among these compounds, caffeine showed the highest concentration (13,535 ng/L), likely due to its widespread administration to patients.

Caffeine

Caffeine, an alkaloid that primarily stimulates the central nervous system, is a significant contaminant in freshwater environments, particularly in developing countries (Li *et al.*, 2020). The characterisation of the YRHC effluent revealed caffeine concentrations as high as 13,535 ng/L, accounting for approximately

Table 2: PhCs detected below their detection limits

PhCs	Contents (ng/L)	PhCs	Contents (ng/L)
Estrone	< 0.5	17 β-Estradiol	< 10
Acebutolol	< 0.5	Testosterone	< 10
Estriol	< 10	Progesterone	< 1
Hydroxy-Ibuprofen	<1	Fluoxetine	< 1
Ciprofloxacin	< 10	Venlafaxine	< 1
Clarithromycin	< 0.1	Medroxyprogesterone	< 10
17 α-Ethynylestradiol	< 20	Norethindrone	< 20
Norfluoxetine	< 5	Methotrexate	< 0.5

Table 3: PhCs above the detection limit determined at different times of the day

PhCs	Content (ng/L) per Sampling Period			Statistical Parameters		
	6 a.m. to 9 a.m.	10 a.m. to 4 p.m.	6 p.m. to 9 p.m.	Average (ng/L)	Standard Deviation	CV (%)
Diclofenac	2,670	2,847.245	2,552.755	2,690	148	5.5
Hydroxy-diclofenac	4,848.11	6,680.67	5,334.214	5,621	949	16.9
Carbamazepine	2	4,1	2.9	3	1	35.1
Caffeine	11,200	19,012.05	10,392.9	13,535	4,760	35.2
Desvenlafaxine	2,563	2,634	2,513	2,570	61	2.4
Ibuprofen	1,391.2	2,253.98	1,571.81	1,739	455	26.2
Levofloxacin	1,302	2,729	1,690	1,907	737	38,7
Sulfamethoxazole	1,250	2,681	905	1,612	941	58.4

45.9% (w/w) of the total pharmaceutical residues detected in the wastewater.

This high prevalence of caffeine is not surprising, given its widespread occurrence in foods, beverages, and pharmaceuticals (such as analgesics). The concentration found in this study exceeds that reported by Kosma *et al.* (2014), who measured 5,810 ng/L in wastewater from certain sewage treatment plants in Greece. Conversely, it remains lower than the levels reported by Subedi *et al.* (2017), who observed concentrations ranging from 30,000 to 61,000 ng/L.

These differences can be attributed to the high consumption of caffeine-containing products on the one hand and a deficit in hospital wastewater treatment on the other. In this case, the high concentration of caffeine in YRHC effluent highlights the inadequacy of the existing wastewater treatment system (Kosma *et al.*, 2014; Kleywegt *et al.*, 2019). Therefore, implementing effective caffeine removal processes is strongly recommended to mitigate environmental impacts.

Anti-inflammatories

Anti-inflammatory compounds such as diclofenac, hydroxy-diclofenac, and ibuprofen were detected in YRHC effluents at concentrations of 2,690 ng/L, 5621 ng/L, and 1,739 ng/L, respectively. Hydroxy-ibuprofen was detected at trace levels (< 1 ng/L). These findings corroborate reports of diclofenac and ibuprofen in African hospital wastewater (Madikizela *et al.*, 2017). For instance, ibuprofen concentrations of 6 ng/L were measured in Uganda's largest lake (Nantaba *et al.*, 2020), while South African wastewater reached 221,000 ng/L (Madikizela *et al.*, 2017). Unfortunately, these compounds, like diclofenac, persist in the environment due to their stability and hydrophilic nature (Tiedeken *et al.*, 2017). This was also noted by Sathishkumar *et al.* (2020), who reported concentrations of 13,480 ng/L in wastewater in Nigeria and 2,770 ng/L in wastewater in Poland.

Antibiotics

Four antibiotics were detected in YRHC effluents, with varying concentrations as indicated in Table 2, representing approximately 11.84% (w/w) of the pharmaceutical contaminants (PhCs) concentration. Among these antibiotics, levofloxacin (1,907 ng/L) and sulfamethoxazole (1,612 ng/L) are present in high concentrations because they are commonly used to treat bacterial infections (such as sinusitis, acute pneumonia, urinary tract infections, chronic prostatitis, and gastroenteritis) in patients (Swain *et al.*, 2020). This confirms that these wastewaters originate from hospitals where these antibiotics are administered to treat ill patients, who subsequently release drug residues through urine. The bioresistance and antibacterial properties of these antibiotics increase the likelihood of their presence in surface waters (Salem *et al.*, 2024). Similarly, related studies have revealed the presence of sulfamethoxazole, with maximum values of 8 ng/L in Kenyan surface waters and 59,000 ng/L in Nigerian rivers (Madikizela *et al.*, 2017).

Other Molecules Detected

In addition to the main drug residues mentioned above, desvenlafaxine was found at a high concentration (2,570 ng/L) in this hospital effluent, likely due to its abundance. As an antidepressant, desvenlafaxine is indicated for the symptomatic treatment of depressive disorder (Endicott *et al.*, 2014). It is probably the most commonly administered drug to patients due to its short-term efficacy compared to other antidepressants listed in Table 2. This hypothesis is further supported by Tiwari *et al.* (2021), who reported a concentration of 7,650 ng/L of desvenlafaxine in wastewater. Similarly, Castillo-Zacarías *et al.* (2021) observed desvenlafaxine at a concentration of 109,000 ng/L in the effluent of a wastewater treatment plant. Furthermore, the high solubility of desvenlafaxine in water may contribute to its persistence in surface waters, which could potentially be used for the production of drinking water (Franek *et al.*, 2015). Therefore, it is important to monitor these drug residues in hospital effluents to consider their elimination through appropriate treatment processes (Hossen *et al.*, 2024).

Ecotoxicological Risk Assessment

Currently, there are no comprehensive regulatory frameworks governing most pharmaceutical residues in the environment. However, comparing observed environmental concentrations with ecotoxicological benchmarks and calculating the Risk Quotient (RQ) provides valuable guidance for prioritising the development of regulatory measures (Madikizela & Ncube, 2021). The environmental risk associated with each PhC and that of all PhCs were assessed.

The lake situated downstream of the WWTP receives the entire effluent from the YRHC. For each PhC, the RQ is calculated as the quotient of its residual concentration by its concentration with no effect on the environment, multiplied by the dilution factor specific to Côte d'Ivoire, which is 516.19 (Keller *et al.*, 2014). The predicted no effect concentration (PNEC) values vary depending on the sensitivity of target organisms (daphnids, fish, microorganisms). Several compounds of ecotoxicological concern were identified, including steroid hormones (17 β-estradiol, PNEC = 0.008 ng/L), anti-inflammatories, and antibiotics (ciprofloxacin, PNEC = 36 ng/L).

Furthermore, levofloxacin has a PNEC of 72 ng/L, diclofenac has a PNEC of 200 ng/L (Orias & Perrodin, 2013), and hydroxyl-diclofenac has a PNEC of 170 ng/L (Kallio *et al.*, 2010). Alkaloids such as caffeine have a PNEC of 12,000 ng/L (Bouzas-Monroy *et al.*, 2022). Finally, the other compounds have PNECs as follows: Carbamazepine, PNEC 2500 ng/L (Bergmann *et al.*, 2011); ibuprofen, PNEC = 200 ng/L (Orias & Perrodin, 2013); sulfamethoxazole, PNEC = 590 ng/L (Tell *et al.*, 2019); and desvenlafaxine, PNEC = 32,000 ng/L (Bouzas-Monroy *et al.*, 2022). The aforementioned information was employed as the foundation for the risk assessment. Thus, Table 4 depicts the risk level per compound, as well as the overall risk for all these compounds. Indeed, levofloxacin, diclofenac, and hydroxyl-diclofenac present a moderately high risk. In contrast, ibuprofen, caffeine, carbamazepine, desvenlafaxine, and sulfamethoxazole all present minimal risks. However, since the RQ of all detected compounds is equal to one, this indicates a high level of risk for aquatic organisms.

Furthermore, the cumulative risk was also highlighted by Riva *et al.* (2019), following the mixing of several pharmaceutical compounds from wastewater samples in a receiving environment. Similar results were observed by Spilsbury *et al.* (2024), who found it necessary to treat effluents by ozonation to substantially reduce the concentration of pharmaceutical compounds (azithromycin, diclofenac, venlafaxine, clarithromycin, and mycophenolic acid).

Finally, these studies reveal that the discharge of several pharmaceutical compounds from drug residues constitutes a danger for receiving environments such as surface water. In view of the above, it is important to strengthen the capacity of the WWTP of YRHC by integrating advanced oxidation post-treatment for the simultaneous degradation of several pharmaceutical molecules.

Conclusions

This study focused on the physicochemical and ecotoxicological characterisation of wastewater from YRHC to evaluate its potential impact on downstream environments. The findings showed that not only did the conventional parameters of this effluent fail to comply with discharge standards, but it also contained drug residues. A total of 24 pharmaceutical compounds (diclofenac, caffeine, ibuprofen, carbamazepine, desvenlafaxine, levofloxacin, sulfamethoxazole, and hydroxyl-diclofenac, among others) were identified as drug residues by HPLC.

The results demonstrated that diclofenac, hydroxyl-diclofenac, and levofloxacin posed significant risks to fish and algae in the aquatic environment. Conversely, pharmaceuticals such as caffeine, carbamazepine, desvenlafaxine, ibuprofen, hydroxyl-diclofenac, and sulfamethoxazole presented low Risk Quotients (RQs). However, considering these eight major pharmaceuticals collectively, the overall RQ is high, reflecting the danger to which aquatic species are exposed. Finally, it is urgent to reinforce the treatment capacity of this wastewater treatment plant (WWTP) in order to effectively treat hospital effluent and protect aquatic life.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Table 4: Risk level of the various PhCs

Compounds	MEC (ng/L)	PEC (ng/L)	PNEC (ng/L)	RQs	Risk Level	Comment
Diclofenac	2,690	5.207	20	0.26		Medium
Ibuprofen	1,739	3.366	200	0.017		Low
Caffeine	13,535	25.814	12,000	0.002		
Carbamazepine	3	0.006	2,500	< 0.001		
Desvenlafaxine	2,570	4.975	32,000	< 0.001		
Levofloxacin	1,907	3.692	72	0.051		Medium
Sulfamethoxazole	1,612	3.120	590	0.005		Low
Hydroxyl-diclofenac	5,621	10.881	17	0.640		Medium
All compounds				≈ 1.00		High

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