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Assessment of Non-Steroidal Anti-Inflammatories in River Waters of Northeastern of Brazil: Occurrence and Environmental Risk

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The environmental impact of pharmaceuticals is a significant concern, as understanding their presence, spread, and harm in coastal regions remains limited. The occurrence of non-steroidal anti-inflammatory drugs (NSAIDs), namely, mefenamic acid (MFN), diclofenac (DCF) and naproxen (NPX) were studied in river waters. Four sample collections were carried out in five points in the dry and rainy seasons. Solidified floating organic drop microextraction (SFODME) coupled with high performance liquid chromatography using diode array detection (HPLC-DAD) were applied to quantify the presence of MFN, DCF and NPX in river water samples. DCF had the highest concentration (640 ng L^{-1}), followed by NPX (410 ng L^{-1}). MFN was not detected in any point within the used working range (limit of detection (LOD) of 0.07 µg L^{-1}). Additionally, an assessment was conducted to evaluate the environmental risk associated with the pharmaceuticals detected in freshwater, specifically in various organisms including algae, mollusks, amphibians, fish, and cnidarians, spanning different trophic levels. The ecotoxicological assessment showed risks ranging from low to high, indicating deleterious effects on several exposed species. No high environmental risk was detected in the sampling areas. Although no immediate negative effects were detected, the potential impact on non-target species should not be disregarded.

Keywords: NSAIDs, SFODME, HPLC, water quality, environmental monitoring

Introduction

Recently, there has been a heightened focus on evaluating the occurrence of pharmaceuticals in natural waters and their impact on ecosystems. With advancements in analytical technology, researchers are now capable of measuring extremely low concentrations (such as ng L^{-1}) with high accuracy. This has allowed for the detection of these xenobiotics in a variety of aquatic systems, including surface waters, groundwater, wastewater (treated and untreated), and tap water.[1-4]

Pharmaceuticals play a key role in maintaining the health of populations. However, their use also has negative impacts on natural biota, humans and animals. Each day, massive amounts of these compounds are introduced into the sewage system and reach wastewater treatment plants (WWTPs). These WWPTs were primarily proposed to remove floating solids and organic matter, and their ability to remove micropollutants may be limited. The efficiency of pharmaceutical removal in WWTPs exhibits a wide range of variability, with some compounds passing through nearly intact while others are removed with an efficiency of nearly 50% .[5] The incomplete removal of these compounds leads to their continuous discharge into the environment, potentially causing persistent exposure

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of water organisms to pharmaceuticals, as well as their bioactive metabolites and transformation products.[6] In some cases, these products have shown similar toxicological effects in the aquatic environment,[7] specifically, nonsteroidal anti-inflammatory drugs (NSAIDs) which are a significant group of pharmaceuticals frequently used in both human and veterinary medicine.[8]

Recently, the European Union implemented regulatory guidelines to assess the occurrence of pharmaceuticals in aquatic environments. This guidance was established through the Directive 2015/495/EU,[9] which modified Directives 2000/60/EC and 2008/105/EC. In accordance with this directive, a watch list of pharmaceuticals has been created and is regularly updated with information gathered from European Union countries. This directive includes a watch list with various pharmaceutical class, including the NSAID diclofenac (DCF).

Usually, the concentration of pharmaceuticals in environmental matrices is on the order of ng L^{-1}. Sometimes, the incorporation of preconcentration and clean-up procedures is still necessary.[10,11] New methods of extraction have been proposed to quantify such microcontaminants, including microextraction and various approaches, such as, dispersive liquid-liquid microextraction using vortex, ultrasound, ionic-liquids and solidified floating organic drop (SFOD). SFOD offers several advantages, including fast, efficient, sensitive, selective, environmentally friendly and compatible with high-performance liquid chromatography (HPLC).[12-15] Briefly, a small volume of an organic solvent (referred to as the extracting solvent) and a dispersing solvent are rapidly introduced into the aqueous sample. This injection induces a turbulent cloud within the sample, leading to the formation of dispersed organic droplets distributed throughout the aqueous phase. Following this, equilibrium is attained, and the mixture undergoes vortexing and/or centrifugation, concentrating the extracting solvent at the surface of the aqueous sample. Subsequently, the system is subjected to an ice bath for a brief period, causing the remaining frozen droplet of organic solvent to collect at the upper portion of the aqueous sample. This frozen droplet is then gathered into a vial for subsequent HPLC analysis.[15]

Although the present information and data concerning the occurrence, fate and behavior of NSAIDs in surface waters is extensively documented,[6] such knowledge in coast and river water is still very limited and recent.[16-18] In this way, this work aims to evaluate the occurrence of three NSAIDs, namely, diclofenac (DCF), naproxen (NPX) and mefenamic acid (MFN) in Munim River, in a total of 18 samples, collected in dry and rainy season. Compounds were selected based on their high consumption and to previous studies reporting their presence in Brazilian waters.[19-22] Subsequently, a preliminary environmental risk assessment was conducted for the detected NSAIDs, considering the available ecotoxicological data pertaining to aquatic species. By addressing the limited knowledge in river waters, selecting relevant compounds, conducting a comprehensive sample collection, and performing an environmental risk assessment, this work offers a novel contribution to the field of NSAIDs occurrence and behavior in aquatic environments.

Experimental

Reagents and standards

The analytical standards of anti-inflammatory drugs, including DCF (≥ 98%), NPX (≥ 98%), and MFN (≥ 98%), as well as the extracting solvent 1-dodecanol (≥ 98%) were provided from Sigma-Aldrich (St. Louis, USA). Methanol (MET) and acetonitrile (ACN) were both analytical grade and acquired from Merck (Darmstadt, Germany). Ultrapure water was obtained using a Milli-Q Ultra purification system, from Millipore (Bedford, USA). Orthophosphoric acid (≥ 85%) and sodium chloride (≥ 99%) were from Isofar (Duque de Caxias, Brazil). Individual stock solutions (100 mg L^{-1}) of each analytical standard in methanol were prepared. The working solutions were prepared by diluting the stock solution in ultrapure water to achieve the desired concentrations.

Solidified floating organic drop microextraction (SFODME)

SFODME method was applied according with the optimized procedure described by Silva *et al.*[15] Briefly, SFODME was performed injecting a mixture composed of 150 μL of dispersive solvent (ACN) and 30 μL of extracting solvent (1-dodecanol), which was immediately transferred to a conical tube containing 5 mL of aqueous sample acidified to pH 2 with 85% H_3PO_4 (v/v) and NaCl 2.5% (m/v). The tube was then vortexed (20 s) (Vortex MX-S, Scilogex, Bedfordshire, UK), centrifuged (5000 rpm for 4 min) (centrifuge Q222T, Quimis, Diadema, Brazil) and placed in an ice bath (12 min) to solidify the organic phase. The solid extract was removed, stored in a 2 mL vial, and allowed to melt at room temperature. Afterward, it was analyzed by high-performance liquid chromatography (HPLC) with diode array detector (DAD). Prior to HPLC analysis, all the samples were filtered using nylon filter membrane (0.45 μm) from Millipore (Darmstadt, Germany).

Chromatographic analysis

In this work, a HPLC Shimadzu (Kyoto, Japan) model LC-20AT Prominence equipped with detector DAD Shimadzu (Kyoto, Japan) model SPD-20A at 230 nm was used. The analytes were separated on a Phenomenex Luna C18, 250 × 4.6 mm, 5 µm particles (Torrance, USA) (reversed-phase column), using a mobile phase composed of ACN and H_2O (acidified to pH 2.24 with 85% H_3PO_4 (v/v)), 60:40 (v/v) ratio, flow of 1.2 mL. An analytical curve was drawn based on the fortification of ultrapure water at concentrations ranging from 0.15 to 3 µg L^{-1}, followed by the application of the above described SFODME procedure. Calibration curves were prepared by dilution of each stock solution. Every standard solution was analyzed in quintuplicate.

Analytical performance SFOME-HPLC-DAD method

Based on each calibration curve, limit of detection (LOD) and limit of quantification (LOQ), precision (based on the coefficient of variance (CV)), accuracy (percentage of recovery) and enrichment factor (EF). The recovery (in percentage) was determined by dividing the average experimental concentration from HPLC analysis by the expected concentration. The EF was calculated from the ratio between the concentration obtained by the method and the initial concentration of the added analyte.

LOD and LOQ were calculated according to the following equations:

$$LOD = 3 \times (s/S) \quad (1)$$

$$LOQ = 10 \times (s/S) \quad (2)$$

where s is the estimated standard deviation of the regression equation and S is the slope of the calibration curve.[23]

Study area

River water samples were collected in the Munim River basin, which is a part of the Western Northeast Atlantic Hydrographic Region (NAWHR). The main channel of the river has a length of 441.57 km.[24] The Munim River basin includes 27 municipalities with an estimated population of 320,000 people.[24] In 2021, according to Brazilian Institute of Geography and Statistics (IBGE),[25] only about 2.5% of sewage in this area is collected and treated (730.2 kg biochemical oxygen demand day^{-1}). The climate in the region is characterized by two distinct seasons: a rainy season (from December to May) and a dry season (from June to November), as reported by INMET (National Institute of Meteorology) in 2021.[26] In addition to inadequate sanitation, the Munim River basin has several environmental issues, such as the sale of locally extracted sand and gravel through dredging, as well as the destruction of riparian vegetation to facilitate commercial soybean and eucalyptus farming in the Chapadinha micro-region. The result is erosion and silting of river channels, which increases the risk of aquatic contamination from domestic sewage, as *per* studies by Ribeiro *et al.*,[27] Ribeiro and Nunes[28] and Teles and Rocha.[29]

To assess the occurrence of the NSAIDs (DCF, NPX and MFN) in the Munim River, samples of river water were collected along five georeferenced sampling points in the basin (Figure 1), as follows: points P1 in Mangabeiras village (3°48'33.9"S, 43°24'33.6"W) and P2 in Riacho Fundo village (3°42'19.9"S, 43°31'45.7"W), both in the municipality of Chapadinha which has the largest population in the basin. Sampling point P3 was located in the municipality of Nina Rodrigues (3°27'35.7"S, 43°54'09.0"W), where two major tributaries, the Preto River and the Iguará River, flow into the Munim River. Another two sampling points were located in the tourist hub of Munim: point P4 in Axixá (2°50'14.3"S, 44°03'03.4"W) near São José Bay, and point P5 in Icatu (2°46'34.0"S, 44°04'03.0"W) at the mouth of the river, which was included starting in the third sample collection.

Sample collection

Four sample collections were carried out in the dry (November 2019, November 2020 and June 2021) and rainy (December 2020) seasons. All the samples were collected in triplicate in the morning (to ensure the same collection conditions). Water samples were collected using 1 L of amber glass bottles at a depth of 20 cm. Immediately after the collection, the samples were kept at 4 °C, filtered to remove particulate matter. The hydrogen potential (pH) was measured and adjusted before subjecting them to the SFODME procedure.

Physicochemical characterization

The characterization of the water matrix was made in terms pH, temperature, transparency, turbidity, dissolved oxygen (DO), conductivity, salinity and total dissolved solids (TDS).

A pH meter (Hanna Instruments, Woonsocket, USA) model HI9126 and a bench top digital pH meter (Hanna Instruments, Woonsocket, USA) model HI 2221 were used to measure the hydrogen potential (pH) and water temperature. Water transparency was assessed with a

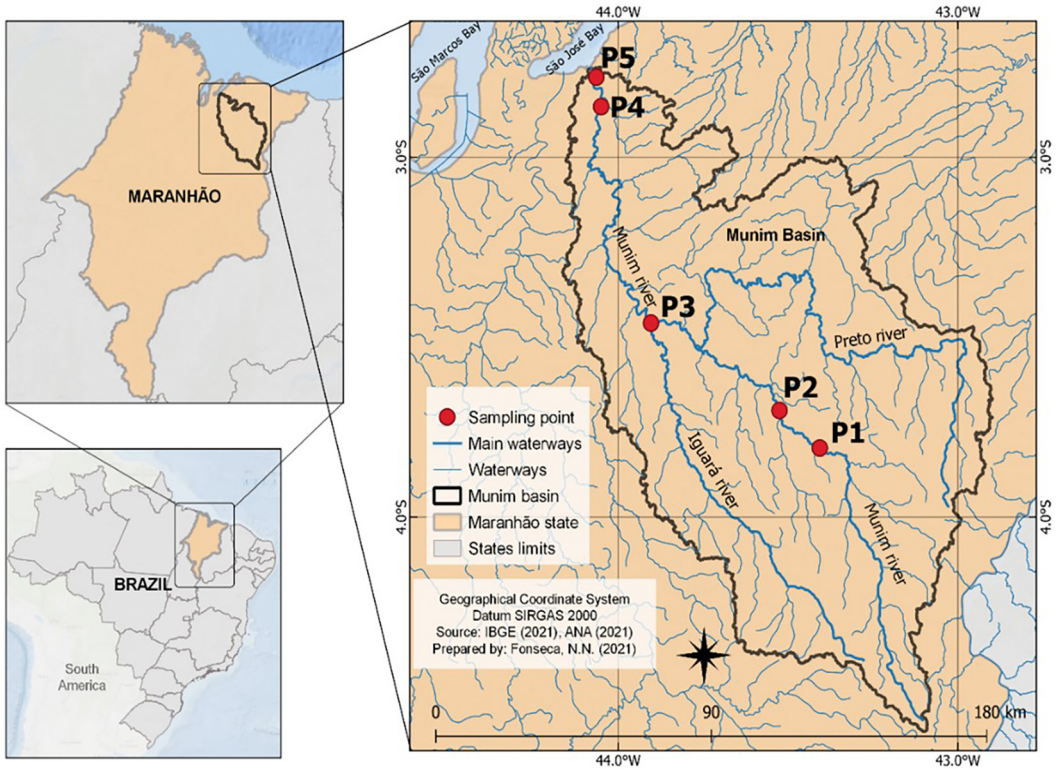

Figure 1. River water sampling points in the study area: sampling points of the river water in the study area. P1: Mangabeiras Village/Chapadinha, P2: Riacho Fundo Village/Chapadinha, P3: Nina Rodrigues, P4: Axixá, and P5: Icatú.

Secchi disk. A turbidity meter (Policontrol, Diadema, Brazil) model AP2000 WT IP 67 was used to measure turbidity. For the DO levels, it was used an oximeter (Hanna Instruments, Woonsocket, USA) model HI 9146, and a multi-parameter conductivity meter (ION, Shanghai, China) model COM-500 was used for conductivity, salinity, and TDS.

To perform microbiological analysis of the water, the multiple tube technique was utilized to determine the most probable number (MPN 100 mL^{-1}) of thermotolerant coliforms. The analysis was conducted according with the Standard Methods for the Examination of Water and Wastewater-APHA/American Public Health Association 2017 recommendations.[30,31] This analysis was used to verify the presence of thermotolerant coliforms. These contaminants can interact with pharmaceuticals, influencing their biodegradation, bioavailability, and ultimate fate.[32] The use of the established methodology ensures the accuracy and reliability of the water quality assessment results.

The collected data was analyzed based on the parameters of the current Brazil's National Environmental Council (CONAMA) resolutions[33,34] and was compared to the measurements available in recent QUALIÁGUA reports released by SEMA (State Secretariat for the Environment and Natural Resources)[35] as well as literature data.[36] The water quality index was calculated using the protocol suggested by the Canadian Council of Ministers of the Environment (CCME).[37]

A detailed description of the characterization results can be found in Supplementary Information (SI) section.

Ecotoxicological risk assessment (ERA)

The ecotoxicological risk assessment (ERA) was determined as described by Shanmugam *et al.*[38] To assess the potential risk, the risk quotient (RQ) was calculated by dividing the measured environmental concentrations (MEC) by the predicted no effect concentrations (PNEC) for the exposed organisms. The PNEC values were obtained from data on toxicological assays for aquatic organisms available in the ECOTOX Knowledgebase of the United States Environmental Protection Agency.[39] The PNEC values were calculated based on the lowest NOEC (no observed effect concentration) found in the database for aquatic organisms with an assessment factor (AF) of 10 for NOEC, according to equation 3.

$$\mathrm{PNEC} = \left(\frac{\mathrm{NOEC}}{\mathrm{AF}\ (10)} \right) \quad (3)$$

The values considered for MEC were the highest concentrations detected at each sampling point. Risk

Table 1. Linear range, determination coefficient (r^2), limit of detection (LOD), limit of quantification (LOQ), coefficient of variance (CV), extraction recovery (ER) and enrichment factor (EF) from the analytical curve obtained by SFODME-HPLC-DAD for naproxen (NPX), diclofenac (DCF) and mefenamic acid (MFN)

Analyte	Linear range / (μg L^{-1})	r^2	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	CV[a] / %	ER[a] / %	EF[a]
NPX	0.15-3.0	0.9996	0.05	0.15	2.6	102 ± 3	170 ± 4
DCF	0.15-3.0	0.9987	0.09	0.31	3.4	88 ± 3	147 ± 5
MFN	0.15-3.0	0.9993	0.07	0.24	3.8	107 ± 4	179 ± 7

[a]Mean ± standard deviation (n = 5) obtained for the concentration of 3.0 μg L^{-1} of NPX, DCF, and MFN under the optimized SFODME-HPLC-DAD conditions.

classification for aquatic organisms considers RQ values of < 0.1 (low), > 0.1 (medium) and > 1 (high) risks.[40]

Results and Discussion

Analytical performance of the SFODME-HPLC-DAD method

Five analytical standards were prepared in concentration ranging from 0.15 to 3 μg L^{-1}. The standards were subjected to SFODME procedure. The analytical performance was assessed in terms of determination coefficient (r^2), LOD, LOQ, coefficient of variance (CV), extraction recovery and enrichment factor, as shown in Table 1.

The analytical method demonstrated good linearity with a satisfactory curve, as evidenced by r^2 values ranging from 0.9987 to 0.9997. Notably, the method achieved better values for LOD, ranging from 0.05 to 0.09 μg L^{-1} and LOQ ranging from 0.15 to 0.31 μg L^{-1} compared to those previously reported in the literature by Silva *et al.*[15] The calculated parameters, including CV (ranging from 2.6 to 3.8%), recovery (88-107%), and EF (147 to 179), showed in Table 1, were all satisfactory in relation to the concentration of analyte in the samples, as previously described by Brito *et al.*,[41] and Silva *et al.*[15]

Evaluation of the presence of NSAIDs in real samples

Results obtained on the occurrence of NSAIDs in real samples (Munim River, Brazil), are showed in Table 2, which presents the five points collections among different periods. All samples were subjected to the optimized SFODME method and analyzed by HPLC-DAD. Figure S8 in SI section shows the chromatograms of the freshwater sample with and without of 3 μg L^{-1} of NSAIDs. In this sense, it can be confirmed that other contaminants or NSAIDs under study were not present before the sample was spiked.

The analyses revealed the presence of DCF and NPX at four out of the five water sampling points, in maximum concentrations of 640 ± 0.04 and 410 ± 0.04 ng L^{-1}, respectively, as shown in Table 2. The presence of DCF and NPX could be linked to their widespread use in recent years. In fact, both NSAIDs have become increasingly popular and were even listed among the top 100 best-selling medications in Brazilian drugstores and pharmacies in 2020, ranking 10th and 77th, respectively.[20] On the other hand, MFN was not detected in any of the collected water samples. This may be due to its Log_{KOW} of 5.12, which is the highest among the studied NSAIDs (DCF 4.51 and NPX 3.18), hindering its solubility in water and therefore resulting in undetectable concentrations in water samples. The physicochemical properties of the NSAIDs under study are present in Table S3 (SI section).

Table 2. Detected NSAIDs concentrations in water samples from the Munim River, MA

Sampling point	Period	Concentration ± standard deviation / (ng L^{-1})		
		Mefenamic acid	Diclofenac	Naproxen
P1	Nov 19[a]	< LOD	< LOD	< LOD
	Nov 20[a]	< LOD	< LOD	410 ± 0.04
	Dec 20[b]	< LOD	< LOD	< LOD
	Jun 21[a]	< LOD	< LOQ	< LOQ
P2	Nov 19[a]	< LOD	< LOD	< LOD
	Nov 20[a]	< LOD	< LOD	< LOQ
	Dec 20[b]	< LOD	< LOD	< LOD
	Jun 21[a]	< LOD	< LOD	< LOD
P3	Nov 19[a]	< LOD	< LOD	< LOD
	Nov 20[a]	< LOD	< LOQ	410 ± 0.02
	Dec 20[b]	< LOD	< LOD	< LOD
	Jun 21[a]	< LOD	< LOD	< LOD
P4	Nov 19[a]	< LOD	640 ± 0.04	< LOD
	Nov 20[a]	< LOD	360 ± 0.08	< LOQ
	Dec 20[b]	< LOD	< LOD	< LOD
	Jun 21[a]	< LOD	< LOD	< LOD
P5	Dec 20[b]	< LOD	< LOD	< LOD
	Jun 21[a]	< LOD	< LOD	< LOD

[a]Dry season; [b]rainy season. LOD: limit of detection. LOQ: limit of quantification.

DCF and NPX were detected only in samples collected during the dry season, which is indicative of a point source of contamination, such as domestic wastewater. Given the lower rainfall in this season, the river's volume is smaller, increasing its exposure to pharmaceutical contaminants from point sources that receive untreated urban sewage.[42] This was particularly evident at point P1, where the river's volume declined considerably. Both DCF and NPX were detected, with NPX being detected more frequently (Table 2). In addition, the relative proximity of point P1 to the urban area (9.5 km), where there is no sewage treatment plant (STP) and only a low percentage of households have proper sanitation (16.1%), may also have contributed to the presence of NSAIDs.[25]

On the other hand, P2 was less strongly impacted by the presence of NSAIDs than P1, probably due to its location (19.1 km from the urban area), low riverine population density and the use of septic tanks.

Sampling point P3 has an estimated population of around 15,000 people, with only 6.8% of them having access to proper sanitation.[25] At this point, several significant tributaries of the Munim River, including the Preto River and the Iguará River, converge into it. The presence of pharmaceutical residues in the collected sample at this specific location could be attributed to the urban population residing near the banks of the Munim River. Located in the municipality of Axixá, sampling point P4 is situated downstream and is exposed to tidal influence owing to its proximity to São José Bay. The population of Axixá is estimated to be approximately 12,000, with only 33.1% of households having access to appropriate sewerage.[25] Despite the tidal influence, the higher density of the urban population along the riverbank in this area is likely resulted in a higher concentration of DCF detected at this sampling point. Sampling point P5, situated close to the river mouth and São José Bay, did not register any detectable levels of NSAIDs. This could be attributed to the substantial dilution factor in the estuarine area, irrespective of the sampling period.

Although the concentrations of NSAIDs detected along the Munim River are comparable to those found in other studies conducted in Brazil (as shown in Table 3), the results cannot be directly compared with those of other hydrographic basins due to the unique anthropic activities and land use practices in each region of the country.

To illustrate the differences among hydrographic basins, a study applied by Perin *et al.*[22] discovered a concentration of 578.21 ng L^{-1} of MFN in Lake Guaíba, RS. This lake is

Table 3. Comparison of mefenamic acid (MFN), diclofenac (DCF) and naproxen (NPX) concentrations in the Munim River basin and concentrations in other Brazilian water bodies

Compound	State	Water body	Detected concentration / (ng L^{-1})	Reference
MFN	Rio Grande Do Sul	Dilúvio Stream	N.D.	43
	Rio Grande Do Sul	Lake Guaíba	578.21	22
	Maranhão	Munim River	N.D.	this study
DCF	Pernambuco	Beberibe River	190-193000	44
	São Paulo	Anhumas e Pinheiros Stream	96-115	45
	Paraná	Upper Iguaçu Basin	> 9-285	46
	Mato Grosso Do Sul	Onça Stream	120-8250	21
	São Paulo	Monjolinho River	< 0.04-385.6	47
	Minas Gerais	Paraopeba River Basin	136.6-2620	48
	São Paulo	Piraí River	9.11-29.2	49
	São Paulo	Jundiaí River	37.3-328.5	49
	São Paulo	Piraí River	4.88-29.7	50
	São Paulo	Jundiaí River	26.5-277	50
	Maranhão	Anil River and Bacanga River	< 100-463	51
	Maranhão	Munim River	< 140-640	this study
NPX	Mato Grosso Do Sul	Onça Stream	70-21.285	21
	São Paulo	Monjolinho River	0.10-655.2	47
	São Paulo	Jundiaí River	5.14-98.6	49
	São Paulo	Piraí River	5.67	50
	São Paulo	Jundiaí River	6.83-145	50
	Maranhão	Munim River	< 70-410	this study

N.D.: not detected.

an urban freshwater course receiving substantial amounts of domestic, hospital, and industrial effluents from the entire metropolitan region of Porto Alegre, in contrast to the Munim River. On the other hand, Américo-Pinheiro *et al.*[21] reported similar concentrations to those observed in this study at specific points in the Córrego da Onça drainage basin in Mato Grosso do Sul. They concluded that an increase in water temperature, combined with sunlight exposure, resulted in the degradation of DCF and NPX, causing a decrease in their concentrations in the water.

In another study, Chaves *et al.*[51] discovered a range of emerging contaminants, including DCF, and identified a concentration range similar to that observed in this study in the Anil and Bacanga rivers (Table 3). The behavior of pharmaceuticals residues in aquatic environments is influenced by various biotic and abiotic factors, and additional studies are needed to evaluate the biogeochemical characteristics of such contaminants in these environments.[32] The main pathway of DCF degradation in water is photodegradation. For NPX, photochemical degradation can occur directly and indirectly when the dissolved organic matter absorbs sunlight, producing reactive oxygen species, hydroxyl radicals or superoxide ions. These reactive species then react with the original compound, generating byproducts that may be more persistent and more toxic.[19,52] Thus, concentrations of the original NSAIDs (DCF and NPX) found in the environment can be lower than those of their degradation products, which can be more harmful to the environment.

While Brazil's National Environmental Council (CONAMA) does not establish limits for DCF and NPX concentrations in river waters, concentrations ranging from < 0.04 ng L^{-1} to 193 µg L^{-1} of these drugs have been detected in samples of surface water in several regions of the country (Table 3).[34] This clearly indicates of the necessity for legislation and monitoring to safeguard aquatic life.

Ecotoxicological risk assessment (ERA)

Numerous studies conducted worldwide have explored the toxicity of emerging contaminants to assess the potential risks posed to aquatic organisms. These studies are now available in databases like the ECOTOX Knowledgebase, enabling researchers and regulators to assess ecotoxicological risks and bridge the knowledge gap.[39]

Table 4 presents the ecotoxicity data for DCF and NPX, along with RQ values associated with the concentrations observed in this study. It is important to note thar the same toxic substance may pose varying ecotoxicological risks to different species that share the same environmental compartment, which is freshwater. The RQ for DCF was classified as low for algae and fish species at points P1 and P3 and RQ medium for mollusks and amphibian species. However, at point P4, the RQ was deemed low only for fish species, but medium for alga and high for other aquatic species. NPX's RQ was considered low for one fish species and cnidarian at all sampling points. For amphibian species, the RQ was low at P2 and P4, and medium at P1 and P3. Nevertheless, the behavior of drugs in aquatic environments is impacted by various biotic and abiotic factors, highlighting the need for further research to comprehensively evaluate their biogeochemical behavior.

Table 4. Organism type, evaluated effect, predicted no effect concentration (PNEC), measured environmental concentration (MEC) and risk quotient of the presence of NSAIDs detected in the basin of the Munim River, MA, Brazil

Compound	Organism	Evaluated effect	PNEC / (ng L^{-1})	MEC / (ng L^{-1})				Risk quotient (low < 0.1< medium <1 high)			
				P1	P2	P3	P4	P1	P2	P3	P4
DCF	*Microcystis aeruginosa* (alga)	population growth rate	2000	170	–	170	640	0.085	–	0.085	0.320
	Dreissena polymorpha (mollusk)	physiological and morphological changes and mortality	385					0.442	–	0.442	1.662
	Xenopus sp. (amphibian)	growth rates	296.2					0.574	–	0.574	2.161
	Danio rerio (fish)	embryo mortality	10000					0.017	–	0.017	0.064
NPX	*Hydra vulgaris* (cnidarian)	abnormal morphology	100000	410	70	410	70	0.004	0.001	0.004	0.001
	Limnodynastes peroniir (amphibian)	developmental, growth and morphological alterations	1000					0.410	0.070	0.410	0.070
	Danio rerio (fish)	sexual differentiation and abnormal physiologic	10000					0.041	0.007	0.041	0.007

DCF: diclofenac; NPX: naproxen; NOEC (concentration at which no effect is observed) was used to calculate the PNEC were taken from the United States Environmental Protection Agency (USEPA) using the ECOTOX database.[53]

According to Nesbitt,[54] the concentration of 100 ng L^{-1} of NPX, which is within of the range founded in this study (Table 2), was found to reduce egg fertilization rates in one fish species. The European Union suggested this concentration level as an environmental quality standard for DCF, considering the potential risks already documented.[55] Currently, DCF and NPX are not on any watch list as a potentially hazardous compound, however further studies are needed to establish a safe concentration for aquatic life.[55-59]

It is important to highlight that the evaluations presented in Table 4 are of low frequency and only considers the compounds individually, any conclusion should be prudent. However, when different contaminants and their degradation products mix in the water column, synergistic interactions can occur, leading to complex mixtures with significant ecotoxicity. This has negative impacts not only on aquatic biota but also on riparian vegetation.[52,60-65]

Conclusions

In this study, the occurrence of residues of diclofenac and naproxen was identified along the course of the Munim River, MA, Brazil, at maximum concentrations of 640 and 410 ng L^{-1}, respectively. These residues were predominantly detected in the dry season, indicating the presence of point sources such as the input of domestic effluents resulting from the absence of sanitation, which is common throughout the basin. According to the ecotoxicological risk assessment, the concentrations of the pharmaceuticals DCF and NPX in the waters of the Munim River can be harmful to aquatic life at certain trophic levels. This study provides the first data on DCF and NPX concentrations in the waters of the Munim River basin, contributing to reduce the existing gap in knowledge about the impacts of the presence of these contaminants on water quality, and to underpin new investigations and environmental policies.

Supplementary Information

Supplementary information (figures and tables) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Naldirene N. Fonseca was responsible for methodology, validation, formal analysis, investigation, data curation, writing original draft; Érika M. L. Sousa for conceptualization, formal analysis, writing review and editing; Jeiza F. Pinheiro for conceptualization, writing review and editing; Lanna K. Silva for conceptualization, formal analysis, writing review and editing; Hélio O. Nascimento for conceptualization, formal analysis, writing review and editing; Ronaldo F. Nascimento for supervision, writing review and editing; Gilmar S. Silva for supervision, writing review and editing; José H. G. Rangel for methodology, formal analysis, resources, supervision, project administration and funding acquisition.

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