

Spatial and seasonal variation in pollution from pharmaceuticals and personal care products and the ecological risks in the Upper Paraná River Basin – Brazil

Variação espacial e sazonal na poluição por produtos farmacêuticos e de higiene pessoal e riscos ecológicos na bacia do Alto Rio Paraná – Brasil

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ABSTRACT

Both pharmaceuticals and personal care products (PPCPs) contaminate river water in Brazil. To determine the extent of such contamination in the rivers and tributaries of important basins in the state of Mato Grosso do Sul, Brazilian Midwest, this study aimed to examine the distribution of PPCPs in these waterways and then perform an ecological risk assessment (ERA). To accomplish this, water samples were collected from four river basins and their sub-basins, all of which are located in the southern part of the Brazilian state of Mato Grosso do Sul. PPCP concentrations were quantified, along with assessments of the *in situ* physicochemical characteristics of the water. ERA was evaluated as a risk quotient (RQ) based on the maximum measured concentration of PPCPs in the samples. The relationship among the concentrations of PPCPs in aqueous samples was examined to assess the distribution of these substances in the environment. The highest concentrations of PPCPs involved caffeine, particularly during the dry season, indicating an intense route of contamination at sampled sites. Among other compounds, pharmaceutical compounds such as diclofenac, ibuprofen, and naproxen were prevalent, especially during the dry season, albeit in slightly lower concentrations. RQ results showed that most compounds investigated represent moderate to high toxicological risk to the aquatic ecosystem. These results suggest that PPCPs are widely distributed in the studied area and provide a benchmark for comparison, or starting point, for future monitoring and environmental recovery programs, as well as encouraging revisions to current legislation.

Keywords: pharmaceutical compounds; risk analysis; contaminants in rivers.

RESUMO

Tanto os produtos farmacêuticos quanto os de higiene pessoal (PFHP) contaminam as águas dos rios no Brasil. Para determinar a extensão dessa contaminação em rios e seus afluentes em importantes bacias hidrográficas no estado de Mato Grosso do Sul, centro-oeste brasileiro, o presente estudo avaliou a distribuição de PFHP nesses cursos d'água e analisou os riscos ecológicos (ARE) dessas águas. Para tanto, foram coletadas amostras de água de quatro bacias hidrográficas e suas sub-bacias, todas localizadas na parte sul do estado de Mato Grosso do Sul, Brasil. As concentrações de PFHP foram quantificadas junto com avaliações das características físico-químicas *in situ* da água. A ARE foi avaliada por meio do quociente de risco (QR) com base na concentração máxima medida de medicamentos nas amostras. A relação entre as concentrações de PFHP em amostras aquosas foi analisada para verificar a distribuição desses medicamentos no meio ambiente. As concentrações mais altas de PFHP envolveram cafeína, especialmente durante a estação seca, indicando uma rota intensa de contaminação nos locais amostrados. Entre outros compostos, compostos farmacêuticos como diclofenaco, ibuprofeno e naproxeno foram predominantes, especialmente durante a estação seca, embora em concentrações ligeiramente menores. Os resultados de QR mostraram que a maioria dos compostos investigados representa um risco toxicológico moderado a alto para o ecossistema aquático. Esses resultados sugerem que PFHP são amplamente distribuídos na área estudada e fornecem uma referência para comparação, ou seja, um risco de risco elevado.

Palavras-chave: compostos farmacêuticos; análises de risco; contaminantes em rios.

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Introduction

Emerging contaminants (ECs) have drawn the attention of multiple environmental authorities around the world owing to the inherent risks. EC pollution of water bodies has increased in recent years as industrial and technological developments have improved the quality of life of people around the world (Patel et al., 2020; González-González et al., 2022).

Among ECs, pharmaceuticals and personal care products (PPCPs) are the most commonly detected substances in various environmental matrices, including freshwater (surface and groundwater), seawater, sediments, and aquatic biota (Ebele et al., 2020; Verbinnen et al., 2024). Water contamination by PPCPs is expected to increase in the coming years based on the increased global consumption of pharmaceuticals for human medicine (IQVIA, 2019; OECD, 2019).

Many studies have shown that wastewater treatment plants (WWTPs) are not able to completely remove PPCPs (Castiglioni et al., 2006; Petrie et al., 2015), making them the main route of entry of these substances into the aquatic environment and leading to the contamination of various watercourses (Castiglioni et al., 2018; Söregård et al., 2019; Feo et al., 2020; Zaid et al., 2020). When WWTPs are available, wastewater products are treated up to the secondary stage, which does not completely capture and remove pharmaceutical micropollutants (Starling et al., 2019). In addition, in low- and middle-income countries, where access to wastewater infrastructure is often limited or non-existent, contaminants enter the environment more diffusely (Kookana et al., 2014).

As pharmaceuticals remain in WWTP effluents for longer periods, the entry of pharmaceuticals into tributaries of river systems may be continuous and increasing, thereby increasing their “pseudo-persistence” in these environments (Brooks et al., 2006). Consequently, the rivers that receive this input through their tributaries become the main sink for these micropollutants in the majority of industrialized regions of the world (Quadra et al., 2021; Ranjan et al., 2022). In addition, increased urbanization and unplanned industrialization around rivers and streams have contributed to their degradation through the dumping of untreated wastewater into these water sources (Chaves et al., 2021).

Common classes of pharmaceutical pollutants

Among the PPCPs with widespread occurrence in aquatic environments, caffeine stands out for its predominance in surface waters in different parts of the world (Zhou et al., 2016; Fekadu et al., 2019; Korekar et al., 2020). Globally, caffeine is a widely consumed substance. Its use and presence are common in foods, beverages, and even in the formulation of many prescription and over-the-counter (OTC) medicines used worldwide (Kleywegt et al., 2019; Li et al., 2020).

Due to the reported high residual concentrations of caffeine in the aquatic environment, major concerns have been raised about the potential negative effects of caffeine residues on ecological safety and

human health (Li et al., 2020). In addition, it has been demonstrated that long-term exposure to caffeine at trace levels can lead to the death of some aquatic organisms (Pires et al., 2016). Therefore, the assessment of general stress as a sensitive early warning indicator of the life status of organisms has been recommended to be included as a toxicity screening tool in the assessment of the effects of toxic compounds on organisms (Aguirre-Martínez et al., 2016).

Other pharmaceutical residues found in the environmental matrix are non-steroidal anti-inflammatory medicines (NSAIDs), which represent one of the most important therapeutic classes found in aquatic ecosystems worldwide (Aus der Beek et al., 2016). Diclofenac, naproxen, ibuprofen, and piroxicam are the most common NSAIDs detected in the aquatic environment (Fekadu et al., 2019). NSAIDs are commonly and extensively used to treat pain and inflammation in human and veterinary medicine, including agriculture and aquaculture, due to their analgesic, antipyretic, and anti-inflammatory properties (Boxall et al., 2012). After use, NSAIDs are excreted in their native form or as metabolites and thus enter aquatic ecosystems (Parolini, 2020).

Despite the fact that acute toxicity from NSAIDs only occurs at high, unrealistic concentrations, sublethal effects have been observed at low, environmentally relevant concentrations of all these pharmaceutical compounds. Thus, further studies represent a priority in order to improve the knowledge on NSAID toxicity and mechanism(s) of action in freshwater organisms and to shed light on their real ecological hazard toward freshwater communities (Aus der Beek et al., 2016; Parolini, 2020).

In Brazil, the indiscriminate use of medicines, their purchase without a prescription, and their irregular disposal after the expiration date all contribute to an increase in the pollution of aquatic environments by pharmaceuticals (Freitas and Radis-Baptista, 2021). Although national legislation on the disposal of pharmaceutical compounds is recent (Brasil, 2020), no restrictions have been imposed on concentrations or maximum permissible limits of these substances in environmental matrices exposed to contamination (Beretta et al., 2014). This probably contributes to the increasing and widespread contamination by different PPCPs in the country's waterways (Montagner et al., 2019), which impacts local ecology and poses risks to aquatic biota (Fent et al., 2006; Hejna et al., 2022).

Normally, before a pharmaceutical product can obtain marketing authorization, an ecological risk assessment (ERA) must demonstrate that it poses no risk to the environment (Pereira et al., 2020). The ERA compares the predicted environmental concentrations (PECs) with the predicted no-effect concentrations (PNECs) of different trophic levels of aquatic organisms. Once the medicine product is already on the market, measured environmental concentrations (MECs) can be used instead of PECs to reflect the actual concentration in the aquatic environment (Pereira et al., 2017).

In addition to the ERA for human medicines, the risk quotient (RQ) is calculated by dividing the PEC or MEC by the PNEC for each

medicine, considering different trophic levels (Pereira et al., 2020). These tools are crucial for studies involving PPCPs, as realistic toxicity data on the actual risk to the environment and human health is required (Heath et al., 2016; Kümmerer et al., 2016). This information clarifies the possible mechanisms of seasonal distribution and the likely impact of these substances on the aquatic environment (EMEA, 2006), as well as informing the development of strategies to reduce their effects (Pereira et al., 2017).

This study complements previous studies on the occurrence of PPCPs in surface waters in the state of Mato Grosso do Sul and their potential ecotoxicological risks in these areas (Sposito et al., 2019; Minillo et al., 2023). Considering the continuing and persistent nature of PPCPs dumping, this study aimed to monitor seasonal variations in PPCPs concentrations along the Ivinhema, Amambai, Maracaí, and Iguatemi Rivers in order to establish the ecological risk of these xenobiotic substances on aquatic organisms. It is anticipated that our results will form a database on the fate, distribution, and environmental risks of PPCP residues in these rivers for use in future environmental monitoring and restoration programs for these basins.

Material and methods

study area

The Upper Paraná River Basin is the most urbanized region in South America (Dagosta et al., 2024), with high population and industrial density. This higher population density leads to the proliferation of a larger number of hydroelectric dams that affect water characteristics, changing sediment loads and nutrient availability. Despite this, some basin portions are less occupied with smaller levels of water pollution. In the Mato Grosso do Sul region (Figure 1), the Ivinhema, Amambai, Maracaí, and Iguatemi Rivers, along with smaller watersheds, are almost free of dams and have lower population densities, but they are still impacted by the effects of conversion to agriculture and pasture.

The combined Ivinhema, Amambai, Maracaí, and Iguatemi river system is part of a large drainage area in the southern area of the state, serving more than 33 municipalities, with an estimated population of 508,000 inhabitants and covering an area of approximately 65,138 km² (Supplementary Material 1). This river system, with its large drainage basin, is uniquely characterized by: (1) proximity to densely populated areas with medium- and small-sized cities (Supplementary Material 1); (2) different land uses across municipalities, e.g., crop cultivation and sugarcane farming in the Ivinhema River Basin, sugarcane farming and pasture in the Amambai River Basin, and pasture in the Maracaí and Iguatemi River Basins (Supplementary Material 2) (IBGE, 2022); and (3) the presence of WWTPs with an average treatment rate of 12.5%±12.3% (IBGE, 2022), discharging treated sewage into these rivers and their tributaries (Supplementary Material 2).

In addition, smaller micro-regions are integrated within large river basins, including, for example, micro-watersheds, or sub-basins, and

even micro-watersheds, all part of the drainage system. Such micro-regions in Mato Grosso do Sul (MS) state receive an average rainfall of 1.663 mm year⁻¹ with an irregular pattern of rainfall concentrated in specific months (Arai et al., 2012; Farias and Berezuk, 2018). This could result in severe flow fluctuations and significant differences in contaminant dilution over the three river systems evaluated in this study.

Samples

A total of 208 river stretches were sampled during the dry (May–August) and wet seasons (November–February) from 2023 to 2024. Sampling sites were selected with the aim of including different longitudinal sections (headwaters to river mouth), major watersheds, and distance to urban areas. In each sample site, we obtained a set of environmental variables, including water pH, oxygen concentration (mg.L⁻¹), water electric conductivity (µS.cm⁻¹), water temperature (°C), and turbidity (NTU), using a Horiba U53 multiprobe sensor (Supplementary Material 3). Geographical coordinates and altitude (m.a.s.l.) were obtained using a GARMIN GPS.

We generated a circular buffer with a radius of 5 km and evaluated the main soil use from data found in the MapBiomas Initiative for 2022. Then, we estimated the percentage of forest, planted forest, wetland, pasture, urban area, and sugarcane cultivation within these buffer zones. We also obtained data about the percentage of houses with a septic system (IBGE, 2021).

Water samples

Surface water samples were taken at each sampling site using amber glass bottles, which were sealed and transported to the laboratory in thermal boxes refrigerated with ice at 4°C until extraction or analysis of the target compounds.

Extraction of target compounds

For the extraction of PPCPs, 5 mL of water samples were initially vacuum-filtered in a closed glass system using a 0.45 µm cellulose acetate membrane (Advantec MFS, Inc., Pleasanton, CA, USA). The filtered samples were then transferred to 15 mL Falcon-type tapered tubes for centrifugation, followed by dispersive liquid–liquid microextraction (DLLME). DLLME involves two solvents: a dispersing solvent and an extracting solvent, and the sample in aqueous phase (Rezaee et al., 2006; Ojeda and Rojas, 2011). In this study, 500 µL of methanol (MeOH) (PA-grade Synth) served as the dispersing agent, and 500 µL of carbon tetrachloride (CCl₄) (Merck/SA anhydrous, ≥99.5% degree of purity) was used as the extracting agent, according to Martins et al. (2012). The tubes were shaken for 2 min and then centrifuged at 3,500 rpm for 3 min in a bench centrifuge. The organic phases contained in the bottom of the tubes were then transferred to a glass tube (10 mL) and dried (45°C) in the dark. Eluate resuspension was performed with 300 µL of methanol (MeOH) (PA Synth grade), and 25 µL of the sample was injected into the high-performance liquid chromatography (HPLC) system for analysis.

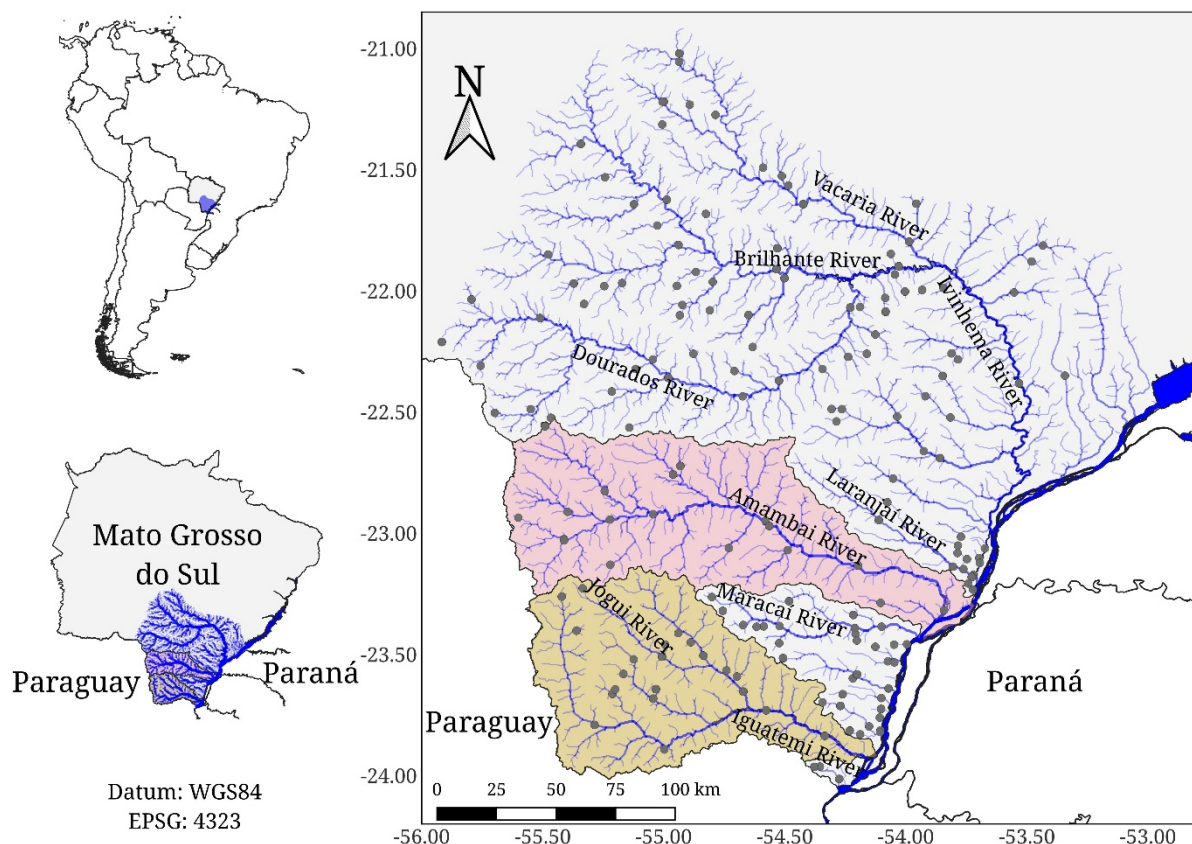


Figure 1 – Location of sample area in Upper Paraná River Basin, Mato Grosso do Sul, Brazil, from 2023 to 2024.

Chromatographic analysis of pharmaceutical compounds

The determination of target compounds (caffeine – Caf, diclofenac – Dic, ibuprofen – Ibu, naproxen – Nap, and piroxicam – Pir) present in the samples was performed using a Shimadzu HPLC system equipped with two modules: LC-20AT and LC-20AD, integrated via the CBM-20A Prominence Communications Bus Module. The system featured a Rheodyne injector (Rohnert Park, CA, USA) equipped with a 20 µL loop and a Shimadzu SPD-M20A Prominence Diode Array Detector, using wavelengths from 220 to 280 nm and employing LC solution software. The HPLC-grade organic solvents used were trifluoroacetic acid (PA Vetec), acetonitrile (HPLC JT Baker grade), methanol (PA-grade Synth), and aqueous water solvent (Milli-Q®). The chromatographic columns used were the Shimadzu Shim-pack C18 (250×4.6 mm ID, 5.0 µm particles) and the Zorbax ODS C18 (150×4.6 mm ID, 5.0 µm particles). All samples were injected in triplicate in a 25 µL volume.

Pure commercial standards (Sigma-Aldrich) of five pharmaceutical compounds with claimed purity of 97–99% were used for the analysis. The optimization of the method, as well as the identification and construction of the analytical curve, followed the procedures of the National Institute of Metrology, Quality and Technology (INMETRO, 2010). Preparation of the standard solutions was built on analytical curves with a concentration range of the stock solution (20 µg/L)

plus 1 mL of methanol (100%), all of which were kept at 15°C in the dark until use. Detailed information on the five target compounds and internal standards is provided in Supplementary Material 4. The coefficients of determination of the calibration curves of each pharmaceutical compound were consistently above 0.9800 for most analytes. Method quality parameters such as linearity, limits of detection (LOD) and quantification (LOQ), and the recovery of the target compounds at the concentrations used are shown in Supplementary Material 5. The identified chromatographic peaks of these target compounds were determined based on their respective retention times and spectrophotometric profiles, based on the literature.

Risk assessment

The ecological risks of PPCPs in aquatic environments were assessed using the RQ method (European Commission, 2003), calculated as Equation 1:

$$RQ = MEC/PNEC \quad (1)$$

Where:

MEC: the measured environmental concentration;

PNEC: the predicted no-effect concentration for non-target organisms.

To evaluate risk ranges, criteria were adopted for RQ interpretation of the risk ranking: $RQ < 0.01$ (minimal risk); $0.01 \leq RQ < 0.1$ (low risk); $0.1 \leq RQ < 1$ (medium risk); and $RQ \geq 1$ (high risk) (Hernando et al., 2006). The MEC values of PPCPs detected at the collection sites were used. The PNEC values for freshwater organisms were calculated from chronic (NOEC) and acute (EC_{50}) effect values obtained using three trophic levels (microcrustaceans, fish, and algae) with respective evaluation factors of 100 and 1,000. Acute and chronic toxicity data were obtained from traditional endpoints, including mortality, growth, and reproduction. The lowest PNEC values were selected to estimate the worst ecological threats (Supplementary Material 6) for RQ calculation.

Statistical analysis

To evaluate the influence of environmental variables on pharmaceutical compounds in sampled sites, we used a conditional inference tree using the function “ctree” (Hothorn et al., 2006) in the “partykit” package (Hothorn and Zeileis, 2015). In these procedures, the concentration of pharmaceutical compounds was used as a response variable. Basin, season, altitude, percentage of forest, planted forest, pasture, wetland, urban area, sugarcane farming, and percentage of houses with sewage treatment were used as predictor variables.

RQs were converted into categorical variables (1=minimal risk, 2=low risk, 3=medium risk, and 4=higher risk) to evaluate the levels of pollution by pharmaceutical compounds between watersheds and seasons. We also used categorical RQs to determine the number of sample sites exhibiting RQ values indicative of pollution by pharmacological compounds, thereby generating a relative value of all RQs (sum of four RQs/16). A matrix of categorical RQs was used to generate a principal coordinate analysis (PCoA) using Gower's distance to represent ordination graphs representing the main variation source in water pollutants by pharmaceutical compounds. The PCoA was realized using the “cmdscale” function. The “wascores” function in the “vegan” package (Oksanen et al., 2022) was used to associate pharmaceutical compounds with ordination axes of PCoA.

The same distance matrix generated for calculating RQ (Gower's distance) was used for permutational multivariate analysis of variance (PERMANOVA) using basin and season included as explanatory variables. This procedure was performed using the “adonis2” function in the “vegan” package. All analyses and graphs were generated in the R platform (R Core Team, 2022).

Results

Out of five pharmaceutical compounds, four were detected in sampled rivers; piroxicam concentrations were below the detection limit. Caffeine concentration varied from 0 to 43.35 $\mu\text{g/L}$ (mean=6.88 $\mu\text{g/L}$, standard deviation [SD]=10.66). Ibuprofen concentration varied from 0 to 323.92 $\mu\text{g/L}$ (mean=55.91 $\mu\text{g/L}$, SD=63.07). Diclofenac concentration varied from 0 to 165.24 $\mu\text{g/L}$ (mean=15.69 $\mu\text{g/L}$, SD=29.63), and

naproxen concentration varied from 0 to 126.34 $\mu\text{g/L}$ (mean=15.17 $\mu\text{g/L}$, SD=26.47). Detailed information on the concentrations found in the target compounds is provided in Supplementary Material 7. Three of four compounds varied mainly in response to season (caffeine, ibuprofen, and diclofenac), with higher concentrations in the dry season. However, naproxen varied mainly in response to watershed, followed by season and finally by percentage of wetland habitats (Figure 2).

We observed that all sampled sites had a higher risk for caffeine in the whole studied area during the dry season. On the other hand, during the rainy season, nearly 40% of the samples presented a higher RQ for caffeine, highlighting the Amambai and Maracá River Basins. The other sites presented a minimum RQ in the rainy season (Figure 3). For ibuprofen concentration, we observed a higher RQ during the dry season, followed by a minimal risk, while in the rainy season, we observed a predominance of minimal risk in the Amambai, Iguaçu, and Ivinhema River Basins, but an opposite result was observed in the Maracá River with a slightly higher risk (Figure 4). Naproxen concentration predominated in the dry season in the whole study area, with nearly 10% of the samples considered at medium risk in the Ivinhema River Basin. However, in the rainy season, we also observed a higher frequency of samples with minimum risk in all basins (Figure 5). Finally, the concentration of diclofenac was similar to that described for ibuprofen, with a higher RQ during the dry season, followed by a minimal risk, while in the rainy season, we observed a predominance of minimal risk in the Amambai, Iguaçu, and Ivinhema basins, but an opposite result was observed in the Maracá River, with a slightly higher risk (Figure 6). Detailed information on the target compound RQs found at the collection points is provided in Supplementary Material 8.

PCoA showed that 81.1% of data variation can be explained by two axes, with the first axis explaining 65.1% and the second axis explaining 16% of data variation. PERMANOVA results showed that temporal variation was more important (pseudo $F=100.33$; $p < 0.001$) than spatial variation (pseudo $F=7.2$; $p < 0.001$) (Figure 7). In a complementary way, all analyzed compounds were more associated with the dry season.

Considering the spatialization of the relative RQ, we observed that all basins presented higher pharmaceutical pollution levels, except the upper portion of the Amambai and Maracá Rivers and small streams of the Ivinhema River Basin with less urbanization (Figure 8).

Discussion

Spatiotemporal distribution of pharmaceutical compounds among river basins

Studies show that concentrations of pharmaceutical compounds present in aquatic environments can vary by region and/or country in terms of quantity, variety, different prescription practices, and degree of economic and health infrastructure (Santana et al., 2021). Our results show that PPCP concentrations varied among sampled basins. It is

likely that heterogeneity in the range of values of the pharmaceutical compounds detected stems from variability in the sampling sites, consumption of these substances according to season, and variations in the characteristics and efficiency of the sewage treated and discharged into watercourses throughout the day (Sousa et al., 2014; Wang and Wang, 2016). In addition, variability could have been affected by outbreaks of infectious diseases in humans or livestock (Hong et al., 2020). Moreover, variations in the concentrations of pharmaceutical compounds in rivers and streams are generally attributed to biodegradation, sorption, dilution, photodegradation, as well as the input of sewage into stretches close to urban areas (Zhou et al., 2016).

This study showed a significant presence of caffeine and NSAIDs at different sampling points in the basins, especially in stretches around urban areas. This effect is to be expected, given that high concentrations of pharmaceutical compounds are common in rivers and streams close to human activities, compared to more remote locations, such as rural areas. In general, the proximity of small watersheds to urban centers tends to favor a greater input of micropollutants into the aquatic environment from domestic and industrial effluents that are discharged in these areas (Zaied et al., 2020).

Among the PPCPs with widespread occurrence among the monitored basins, caffeine showed a striking predominance at all collection points, especially during the dry season, when high concentrations were found. The persistent levels of these pharmaceutical compounds were close to those previously reported in surface waters in different parts of the world (Zhou et al., 2016; Fekadu et al., 2019; Korekar et al., 2020). The high input of caffeine in the aquatic environment has likely resulted from its diverse, widespread use and the increase in its concentration in bodies of water over the last two decades (Korekar et al., 2020).

Our results showed the omnipresence of caffeine in the three basins evaluated, which is indicative of anthropogenic contamination. The concentration of this stimulant can be influenced by water characteristics (Daneshvar et al., 2012). Normally, its half-life in aqueous media is close to 1.5 days (Lam et al., 2004), but it can be extended between 100 and 240 days under variable environmental conditions (Hillebrand et al., 2012) that favor its longer permanence in the aquatic environment. Its high human consumption and specific anthropogenic nature (Ferreira, 2005), as well as its relative stability under environmental conditions (Korekar et al., 2020), make caffeine a recognized marker of anthropogenic activity in surface waters (Carvalho et al., 2022).

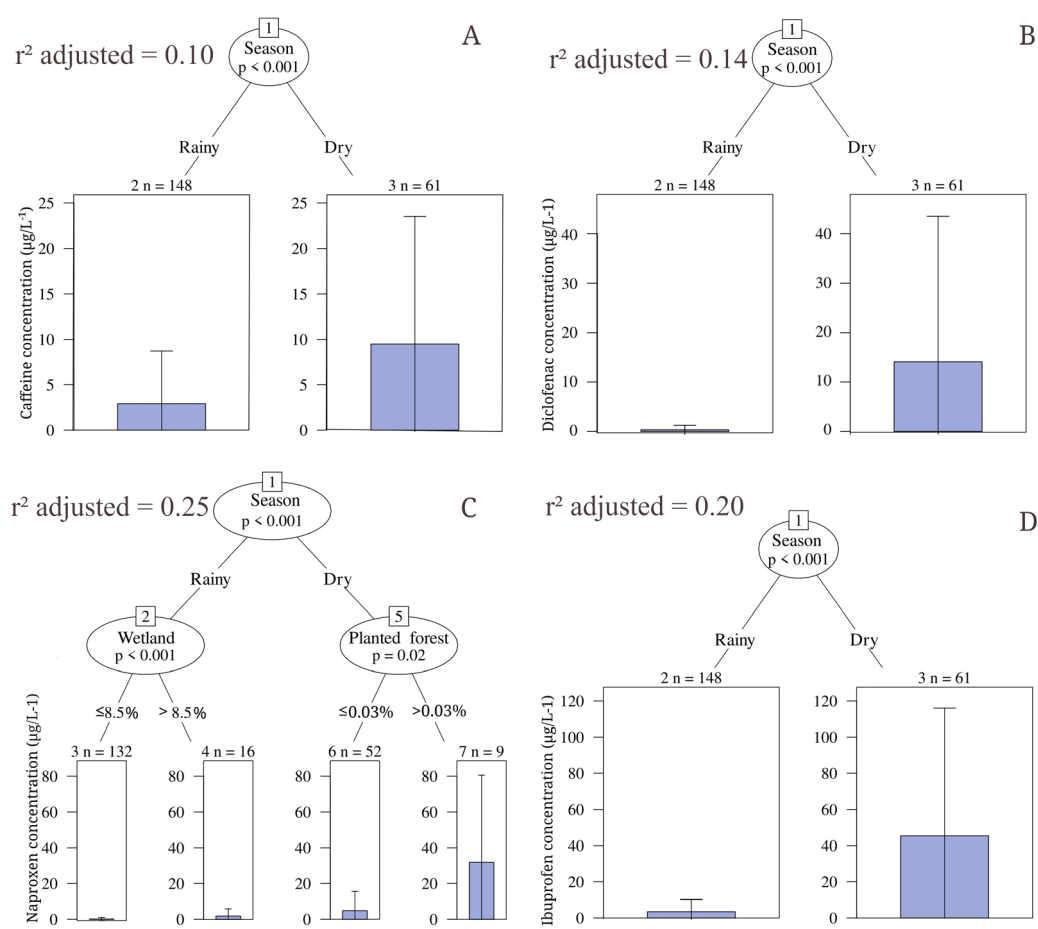


Figure 2 – Conditional inference trees of pharmaceutical contaminants (A=caffeine, B=naproxen, C=ibuprofen, and D=diclofenac) in sampled rivers of the Upper Paraná River Basin.

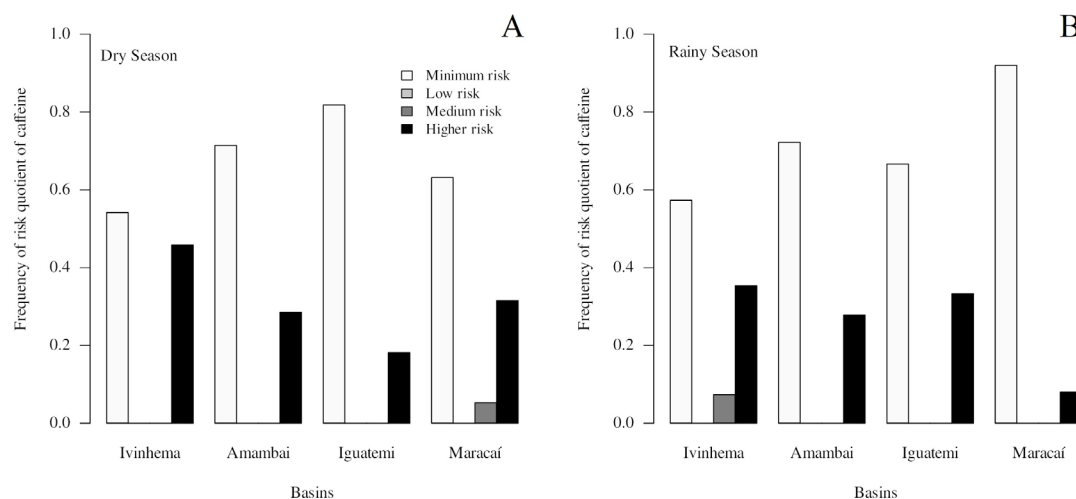


Figure 3 – Frequency of risk quotient levels for caffeine concentration during dry and rainy seasons in the Upper Paraná River Basin.

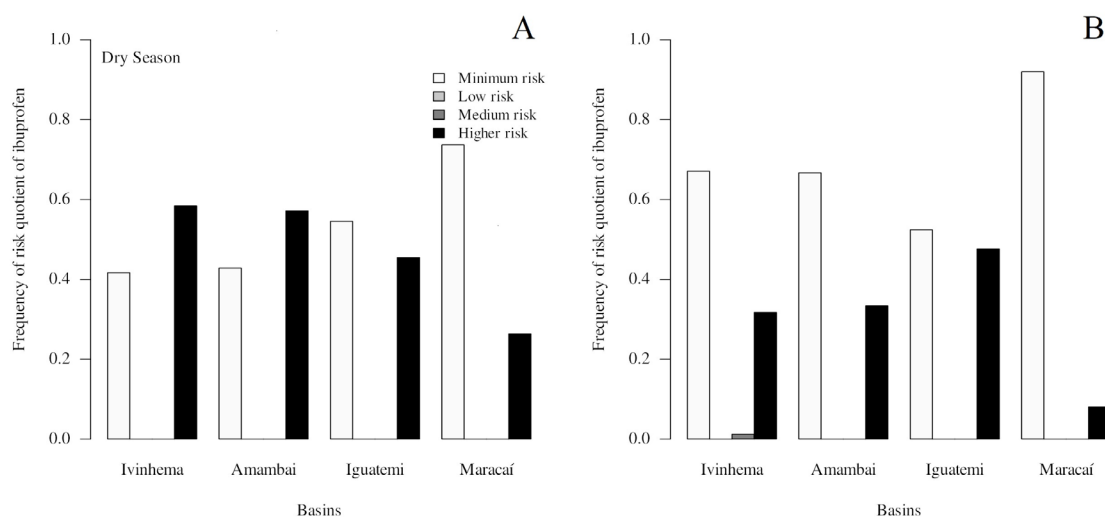


Figure 4 – Frequency of risk quotient levels for ibuprofen concentration during the dry and rainy seasons in the Upper Paraná River Basin.

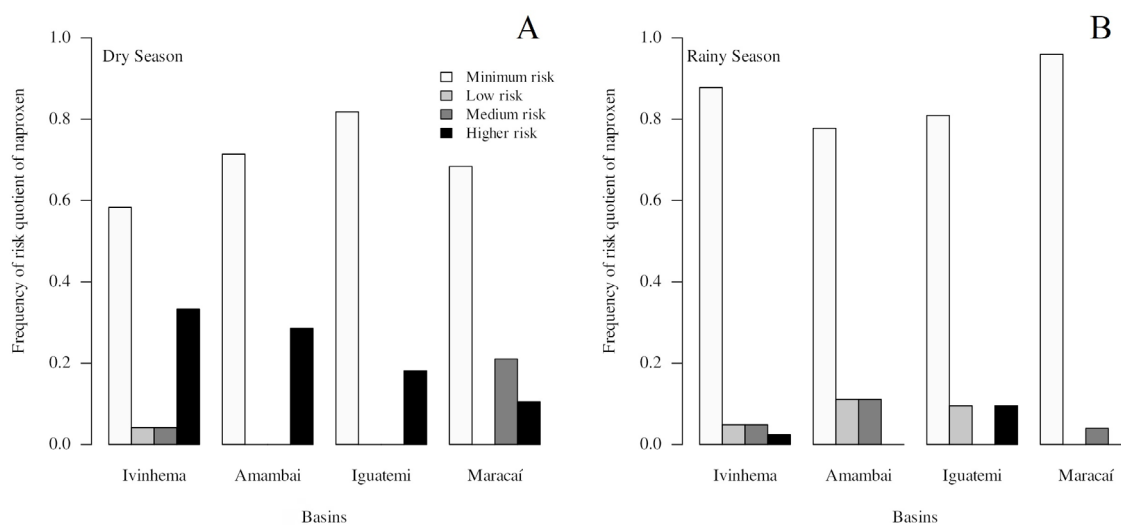


Figure 5 – Frequency of risk quotient levels for naproxen concentration during the dry and rainy seasons in the Upper Paraná River Basin.

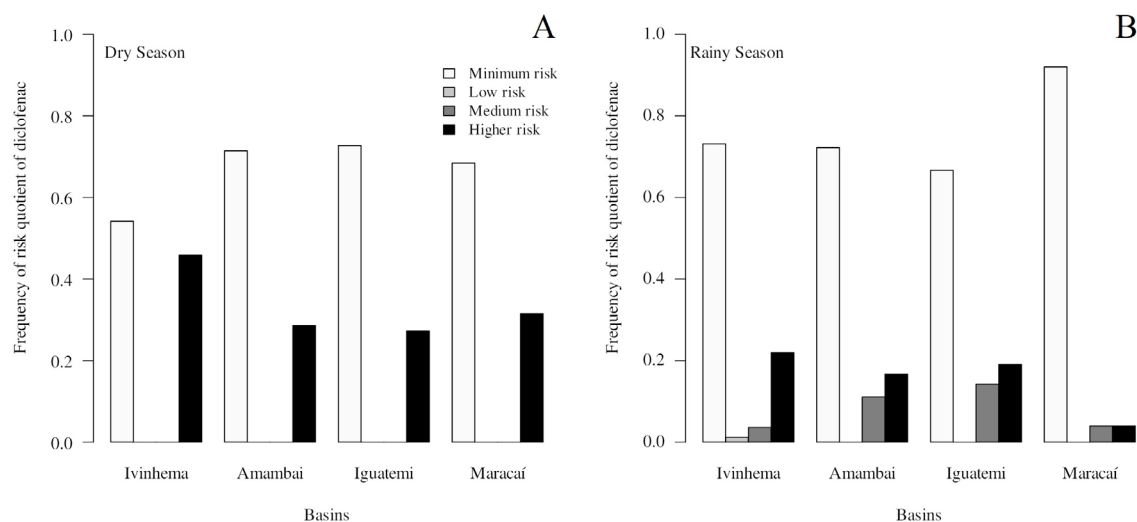


Figure 6 – Frequency of risk quotient levels for diclofenac concentration during the dry and rainy seasons in the Upper Paraná River Basin.

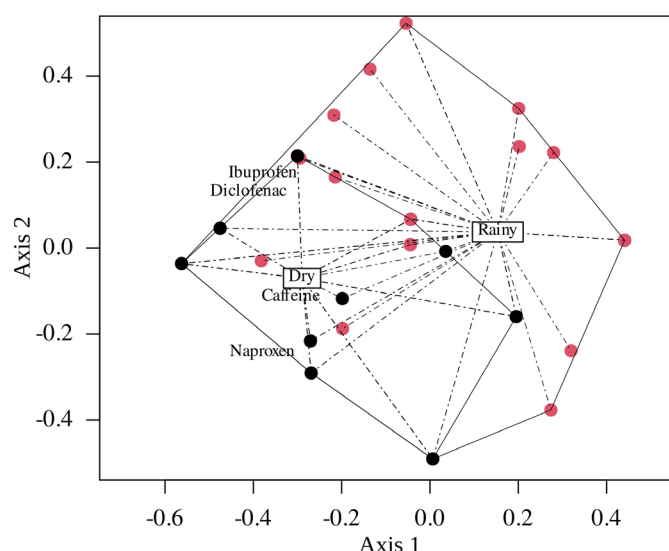


Figure 7 – Scatterplot of principal coordinate analysis using risk quotients of pharmaceutical compounds grouped by season in the Upper Paraná River Basin in Mato Grosso do Sul State, Brazil.

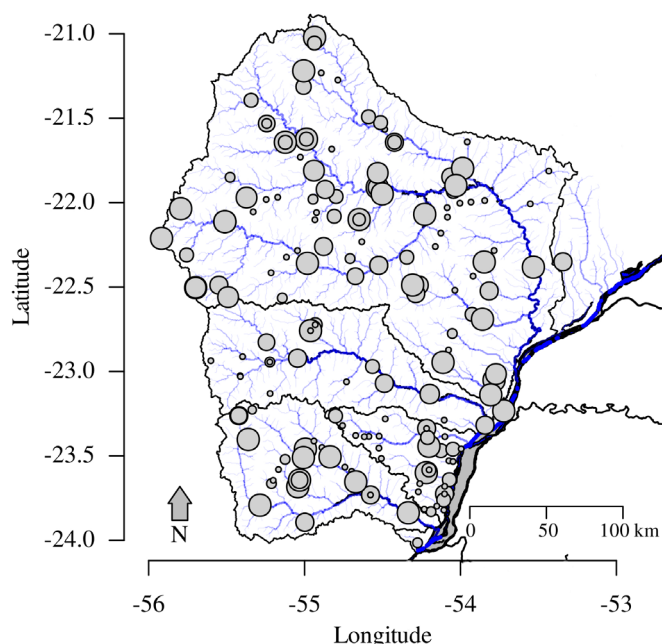


Figure 8 – Spatial distribution of relative risk quotient for pharmaceutical compounds from 2023 to 2024 in the Upper Paraná River Basin, Brazil. The size of circles, spatially located in sample sites, is proportionate to the risk quotient.

The high concentration of caffeine in most samples studied could be due to its use in a variety of drinks and numerous food products and the high consumption of OTC medicines containing this stimulant in their composition. Results of the distribution and occurrence of caffeine at the monitored sites suggest that its contamination arises from anthropogenic pressures in these areas.

The occurrence of pharmaceutical residues in aquatic environments is controlled by a number of environmental factors. For example, seasonality is an important factor in determining the distribution and concentration of pharmaceuticals in the environment (Pan et al., 2020; Singh and Suthar, 2021). The higher levels of caffeine during the dry season may be due to lower flow of rivers and tributaries, favoring its accumulation in surface waters.

The low flow of the monitored watercourses, which is common during this period, may have also contributed to the increase in pollutants contained in the treated effluents from the WWTPs situated close to municipalities. Gumbi et al. (2017) reported a significant contribution of pharmaceutical compounds to watercourses due to recycled water from WWTPs entering the stormwater system. It is likely that a similar situation occurred during the dry season in this study. This would explain the greater presence of caffeine in this matrix compared to the wet season.

Other pharmaceutical residues contained in the environmental matrix evaluated, such as diclofenac, naproxen, and ibuprofen, showed a trend similar to that observed for caffeine, with variations along rivers and their tributaries. The NSAIDs detected had a slight increase in their concentrations in the dry season compared to that of the rainy season, probably owing to illnesses associated with the onset of winter, such as more frequent outbreaks of flu and fever common in the dry period (Hong et al., 2020). In addition, we should consider how seasonal dilution effects, i.e., changes in water flow and volume, particularly during different seasons, impact the concentration of pollutants in water bodies.

The quantity of pharmaceutical compounds is generally lower in the rainy season compared to the dry season (Ohoro et al., 2021). Furthermore, in the dry season, the volume of rivers and streams decreases, consequently reducing the dilution capacity of water bodies (Pereira et al., 2017; Starling et al., 2019). Previous studies have revealed a similar pattern of antibiotic concentrations in water and sediment, which tend to be higher during the dry season. This suggests that low water flow reduces the dilution of inputs (Ohoro et al., 2021).

The presence of NSAIDs in the watercourses sampled reveals a worrying reality since anthropogenic inputs are increasingly common in environmental matrices. This class of pharmaceutical compounds has become increasingly common in aquatic ecosystems around the world and accounts for more than 15% of all pharmaceutical products found in the environment (Parolini, 2020). In Brazil, as in many other countries, NSAIDs do not require prescriptions to be purchased in pharmacies (Bisognin et al., 2018). In this context, the indiscriminate use of these medicines, their purchase without a prescription, and their irregular disposal after the expiry date can contribute to increased pollution of aquatic environments by pharmaceutical products (Freitas and Radis-Baptista, 2021). Furthermore, issues relating to poor basic sanitation infrastructure contribute to the contamination of these xenobiotic compounds in watercourses. In 2017, approximately 39.7% of Brazilian municipalities had no wastewater treatment systems (IBGE, 2024), making the rivers of industrialized or highly populated areas prone to contamination by PPCPs due to anthropogenic action (Oliveira et al., 2024). These factors are relevant and may have contributed to the increase in these inputs for contamination of NSAIDs in the monitored basins.

The prevalence of PPCPs found in the watercourses sampled may be associated with their high consumption and use in the study area. The seasonal and spatial distribution variations in their concentrations at the monitored sites probably follow land use patterns in sub-basins and anthropogenic activities. Studies of the Jiulong River Basin in southeastern China have shown drug contamination in rural areas due to the agricultural use of sludge from WWTPs contaminated with pharmaceutical compounds (Hong et al., 2020). These results underscore the significance of secure strategies for the utilization of solid

waste from WWTPs in agricultural regions, which will avert recurrences of this nature in the domain of the present investigation.

When scanning the samples analyzed in the present study, no piroxicam was found among the PPCPs tested. The absence of the pharmaceutical compounds in the matrix may be related to a combination of factors that limited their occurrence, e.g., their low consumption in the population compared to other NSAIDs that are normally prescribed. Studies report lower consumption of piroxicam based on adverse side effects, e.g., gastrointestinal disorders and cardiovascular, hepatic, and renal risks (Hunt et al., 2009; Scarpignato, 2013). Other studies point to economic benefits associated with limiting the use of piroxicam, such as the reduction of costs associated with the risk of health complications (Maciá Martínez, 2015), as well as the risk and harm to human health compared to other NSAIDs (EMA, 2007).

RQ assessment

The risk of contamination from pharmaceutical compounds in environmental matrices has been extensively reported in the literature (Pereira et al., 2020; Bouzas-Monroy et al., 2022). In particular, the use of ERA methods, such as RQ, makes it possible to interpret likely effects expected for a given environmental contaminant after the application of appropriate screening methods (Li et al., 2020). RQ is a point estimate of the relationship between the predicted or measured exposure concentration, either PEC or MEC, and the PNEC of a given substance. If the RQ value is above 1, then the exposure concentration exceeds the concentration value below which no adverse effects are expected, indicating potential risks to organisms (Hernando et al., 2006).

The results of the present study were based on a “worst case scenario,” in which pharmaceutical compounds present in the aqueous matrix were determined according to seasonal and spatial variations, and then expressed as RQ values for each pharmaceutical compound residue relative to the basins evaluated. Among the PPCPs, caffeine, especially distributed between the Amambai and Maracá River Basins, showed the highest ecological risk values. The proximity of the watercourses to the more populated urban areas may have contributed to the high RQ values, especially in the dry season. However, the entry of these PPCPs from diffuse sources of contamination into sampled stretches must be considered, as must their individual transfer and transport characteristics, as described in studies of Chinese rivers and their tributaries in peri-urban areas (Ke et al., 2024).

The effects of caffeine on aquatic biota are widely described in the literature (Li et al., 2020). The detection ranges of these pharmaceutical compounds in the basins are relevant to the risks for aquatic biota. For example, studies have shown significant effects on locomotor activity in zebrafish larvae after exposure to relevant environmental concentrations (1, 10, and 100 µg/L) of caffeine (Zhou

et al., 2019). Other studies have reported that residues of this medicine cause alterations in psychomotor functions/behavioral profile, cell cycle regulation, and the generation of reactive oxygen species (ROS) in a wide range of aquatic organisms (Cervený et al., 2022). In addition, ecotoxicological studies on caffeine have demonstrated neurotoxic, biotransformation, oxidative stress, and genotoxic effects in non-target aquatic organisms (Muñoz-Peñuela et al., 2022; Diogo et al., 2023).

Caffeine, a central nervous system stimulant and an addictive substance (World Health Organization, 2020), is considered relevant for its potential neurotoxic effects, which have attracted special attention in recent studies (Aguirre-Martínez et al., 2016; Li et al., 2020). One characteristic associated with the substance effects is its ability to attenuate actions on enzyme receptors, such as acetylcholinesterase (AChE), a co-responsible enzyme involved in neurotransmission, causing its inhibition (Fiani et al., 2021). Laboratory studies show that AChE activity decreases in zebrafish embryos exposed to different concentrations of caffeine. Muñoz-Peñuela et al. (2022) tested *Astyanax altiparanae* (=lacustris) at concentrations close to those found in the three monitored basins (27.5 µg/L) and observed inhibition of AChE activity in muscle tissue. Aguirre-Martínez et al. (2016) evaluated the neurotoxicity of caffeine in molluscs (*Ruditapes philippinarum*), finding that it caused a significant decrease in AChE activity. Consequently, the risks of caffeine to aquatic biota warrant attention, as unexpected effects on neurobehavioral parameters manifest themselves at concentrations that are frequently detected in natural aquatic ecosystems (Diogo et al., 2023). This is worrying, considering that environmentally relevant concentrations of these pharmaceutical compounds are capable of inhibiting AChE activity in different aquatic organisms, causing damage to locomotor activities and compromising normal behavior, including feeding, escape response, and reproduction (Muñoz-Peñuela et al., 2022).

With regard to NSAIDs, the results showed ecological risk largely ranging from medium to high across the monitored basins. Although the main purpose of this class of pharmaceutical compounds is mainly symptomatic treatment, their increasingly common presence in the aquatic environment can cause adverse effects when tested on various non-target organisms as a model (Placova et al., 2023). For example, the specific side effects of naproxen include teratogenic activity in hydrozoans (*Hydra attenuata*) (Quinn et al., 2008) and a reduction in the growth rate of microcrustaceans (*Daphnia magna*) (Kwak et al., 2018) after exposure at environmentally relevant levels. Similar to naproxen, ibuprofen has been reported to have toxic effects and to reduce growth rates in phytoplankton species such as *Chlorella vulgaris* (Geiger et al., 2016) and *Navicula* sp. (Ding et al., 2017), respectively. In addition, various effects on fish have been reported, such as genotoxicity in tilapia (*Oreochromis niloticus*) (Ragunetti et al., 2011); tissue deformities in the gills, liver, and kidneys of cat-

fish (*Clarias gariepinus*) (Ogunwale et al., 2021); and nephrotoxicity and immunosuppressive activity in catfish (*Rhamdia quelen*) (Mathias et al., 2018) exposed to these substances. The negative effects of diclofenac exposure include anomalies in zebrafish (*Danio rerio*) embryos (Ribeiro et al., 2015), damage to the gills, liver, and kidneys of adult carp (*Cyprinus carpio*) (Derakhsh et al., 2017), and mortality in adult three-spined catfish (*Gasterosteus aculeatus*) in mesocosm experiments (Joachim et al., 2021). The uptake of diclofenac in aquatic ecosystems can pose risks to ichthyofauna in these contaminated environments (Joachim et al., 2021).

The results obtained in this study show that the study sites, especially those close to urbanized areas, are vulnerable and susceptible to continuous anthropogenic disturbance (Brooks et al., 2006). The presence of pharmaceutical compounds, whether from stimulants (e.g., caffeine) or medicines (e.g., NSAIDs), can trigger a series of deleterious biological effects on aquatic biota at biochemical, cellular, and individual levels, in environmentally relevant concentrations (Das et al., 2019; Diogo et al., 2023). The importance of this is revealed by the high RQ values of contaminants found in the sampled sites in the present study. Therefore, we can infer the use of this metric as a useful tool for assessing local and regional threats arising from contamination by pharmaceutical compounds.

Pharmaceuticals in the environment are a global challenge that calls for multi-stakeholder and multi-sector approaches to prevent, reduce, and manage their release into the environment (Auser Beek et al., 2016). The authors of these studies recommend including more comprehensive assessments, such as the Strategic Approach to International Chemicals Management, a study by the United Nations Environment Program. This reinforces the importance of promoting the discussion of environmental regulations to include pharmaceutical residues in monitoring programs and standards related to the disposal of treated wastewater (Starling et al., 2019; Chaves et al., 2021; Oliveira et al., 2024).

Conclusion

The occurrence of different PPCPs in the rivers and tributaries of the studied watersheds shows the importance of controlling effluents discharged into these watercourses from anthropogenic activities and evaluating contaminants that are not removed by WWTPs. The presence of pharmaceutical compound residues in watercourses is a significant threat to aquatic biota, which was proven by the ecological risk analysis of this matrix. More detailed future studies integrating other environmental compartments, e.g., sediment, are proposed to assess the extent of environmental damage caused by PPCPs in the aquatic ecosystem as a whole. Meanwhile, the present study may serve as a database to predict and evaluate the efficiency of possible future pollution measures in the restoration program of these basins, including risk analysis protocols for sites impacted by pharmaceutical residues.

Authors' Contributions

Minillo, A.: conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing—original draft; writing—review and editing. **Isique, W. D.:** formal analysis; methodology; validation; investigation. **Córdova Junior, R. F. S.:** data curation; formal analysis; investigation; methodology. **Pinho, H. L. L.:** data curation; formal analysis; investigation; methodology. **Cardoso, C. A. L.:** conceptualization; methodology; validation; investigation; visualization; writing—original draft. **Súarez, Y. R.:** conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; writing—review and editing.

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