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Occurrence and ecological risk assessment of pharmaceutically active compounds in neotropical small basins, Brazil

Ocorrência e avaliação de risco ecológicos de compostos farmaceuticamente ativos em pequenas bacias neotropicais, Brasil

Alessandro Minillo[1]*, William Deodato Isique[2], Claudia Andrea Lima Cardoso[3] and Yzel Rondon Súarez[1]

[1]Laboratório de Ecologia, Centro de Estudos em Recursos Naturais – CERNA, Universidade Estadual de Mato Grosso do Sul – UEMS, Rodovia Dourados-Itahum, Km 12, 79804-970, Dourados, MS, Brasil

[2]Laboratório de Saneamento, Departamento de Engenharia Civil – DEC, Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Alameda Bahia, 550, 15385-000, Ilha Solteira, SP, Brasil

[3]Laboratório de Análise Instrumental, Centro de Estudos em Recursos Naturais – CERNA, Universidade Estadual de Mato Grosso do Sul – UEMS, Rodovia Dourados-Itahum, Km 12, 79804-970,Dourados, MS, Brasil

*e-mail: alminillo@yahoo.com.br

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Abstract: Aim: The aim of our study was to evaluate the contamination levels of selected pharmaceutically active compounds (PAC) and their potential ecological threat to forested streams. **Methods:** Samples of stream water were collected in the second largest city in Mato Grosso do Sul State in center-west of Brazil. Physicochemical parameters and concentrations of PAC were quantified in samples collected in six field campaigns. Ecological risk assessment (ERA) based on risk quotient (RQ) was performed based on the maximum measured concentration of PAC in water. **Results:** Six pharmaceutical compounds were successfully quantified in the forested streams, namely caffeine, naproxen, diclofenac, estriol, estradiol and ethinylestradiol. From the point of view of ecological risk, ethinylestradiol (22,57), estradiol (1,46), diclofenac (16.99) and caffeine (5.30) can be considered as priorities PAC, as they present moderate to high risks to aquatic organisms and may also cause damage to the food chain. **Conclusions:** This study provides valuable information to emphasize the importance of continuous monitoring of forested streams in the west central region of the country, as well as efforts to control the input of these micropollutants into watercourses.

Keywords: pharmaceuticals; estrogens; risk assessment; environmental contaminants.

Resumo: Objetivo: O objetivo do nosso estudo foi avaliar os níveis de contaminação de compostos farmaceuticamente ativos selecionados e sua potencial ameaça ecológica em riachos florestais. **Métodos:** Amostras de água de córregos foram coletadas na segunda maior cidade do estado de Mato Grosso do Sul no centro-oeste do Brasil. Parâmetros físico-químicos e concentrações de CFA foram quantificados em amostras coletadas de seis campanhas de campo. A avaliação de risco ecológico (ARE) por meio do quociente de risco (QR) foi realizada com base na concentração máxima medida de CFA na água. **Resultados:** Seis compostos farmacêuticos foram quantificados com sucesso nos riachos florestais, a saber, cafeína, naproxeno, diclofenaco, estriol, estradiol e etinilestradiol. Do ponto de vista do risco ecológico, o etinilestradiol (22.57), o estradiol (1.46), o diclofenaco (16,99) e a cafeína (5,30) podem ser considerados como CFA prioritários, pois apresentaram riscos moderados a altos para os organismos aquáticos e podem levar a danos ao longo da cadeia alimentar. **Conclusões:** Este estudo fornece informações valiosas para reforçar a importância do monitoramento contínuo dos córregos florestados na região centro-oeste do país, bem como os esforços para controlar a entrada desses micropoluentes nos cursos d'água.

Palavras-chave: fármacos; estrógenos; análise de risco; contaminantes ambientais.

1. Introduction

Pharmaceutical and personal care product pollution (PPCPs) is an unprecedented local and global public health problem, even if it is not perceived by the public as an environmental and health threat (Owens, 2015; Bunke et al., 2019). In recent years, one of the major environmental and public health concerns is the discharge of active pharmaceutical ingredients (PAC) into water bodies (Patel et al., 2020). Part of this preoccupation is because their hazardous concentrations do not necessarily follow a typical dose-response curve of toxicity, by which their cumulative quantities of contaminant could cause a deleterious effect (Khetan & Collins, 2007; Freitas & Radis-Baptista, 2021).

Inputs of PPCPs to the environment generally include known sources such as hospitals, animal husbandry, wastewater from households and pharmaceutical manufacturing, and wastewater treatment plants (Pal et al., 2010; Stefanakis & Becker, 2016). Other pathways, such as municipal, industrial, and agricultural waste disposal, excretion/disposal of pharmaceuticals, and accidental releases, also play a role in introducing these micropollutants into environmental matrices (Petrie et al., 2015).

The PAC arouse concern by represent one major category of anthropogenic contaminants present in the aquatic environment, with high global consumption and a representative incidence in surface and groundwater samples, degrading water quality, as documented in several publications (Zhou et al., 2019; Richardson & Kimura, 2020; Liu et al., 2020; Vieira et al., 2022; Wilkinson et al., 2022).

Several aspects of drug residue pollution analysis, such as occurrence, monitoring, treatment, emission control, and environmental effects of drugs, are proving necessary (Freitas & Radis-Baptista, 2021). This problem has led to numerous studies addressing the ecological risks of various pharmaceuticals and other chemical micropollutants in the aquatic environment in different countries (Hernando et al., 2006; Ashfaq et al., 2019; Peteffi et al., 2019; Liu et al., 2020).

Relevant studies indicate that most PAC lack concrete and conclusive toxicity data and pose a real risk to ecological and human health (Heath et al., 2016; Kümmerer et al., 2016). In the past, work on the environmental risk of pharmaceuticals was often conducted using the simple and deterministic risk quotient (RQ) method, expressed by the measured environmental concentration (MEC) for a given compound divided by the toxicologically relevant predicted no-effect concentration (PNEC) of the substance, as recommended by international agencies for the ecological risk assessment (ERA) of pharmaceuticals (EMA, 2006).

The ERA for pharmaceutical pollution represents important information that allows analyzing the effect of these compounds in the aquatic environment (Gavrilescu et al., 2015). The information obtained from this analysis makes it possible to clarify the possible mechanisms of seasonal distribution and the probable impact of these micropollutants on the environment (EMA, 2006). In addition, they pave the way for the adoption of measures that allow the development of environmental policies to address priority problems and the definition of strategies to reduce the impact of these substances on the aquatic environment (Pereira et al., 2017). This is the situation we will evaluate in the proposed study.

Brazil is among the largest consumers of pharmaceutical products in the world (Aragão et al., 2020), which contributes to the detection of various compounds in the environment (Marson et al., 2022). The state of Mato Grosso do Sul is one of the few regions in the country where part of the biome (e.g., Cerrado, Atlantic Forest and Pantanal forests) is still preserved, with a great diversity of animal and plant species and the environmental services they provide to ecosystems. However, the rapid growth of large urban and industrial centers, combined with the advance of agribusiness, threatens and causes changes in these landscapes (Bellón et al., 2020), such as the loss of ecological integrity of aquatic ecosystems (Sundar et al., 2020), with records of toxicogenetic damage in the water of important rivers of the region (Sposito et al., 2019), possibly related to the presence of emerging contaminants on site (Sposito et al., 2018)

Little is known about the pollution of streams in the state of Mato Grosso do Sul by PAC and the risks to aquatic life. The ability to assess current levels of drug residues in water bodies and the resulting ecological risks will allow us to anticipate and mitigate the associated future harms. In this study, we examined the presence of PAC in selected stream waters in an important urban center in Mato Grosso do Sul state. The specific objectives of this study are to (1) evaluate the occurrence and spatial and temporal distribution characteristics of PAC in surface water samples of selected streams and (2) evaluate the ecological risks associated with the presence of micropollutants in these watercourses.

2. Materials and Methods

2.1. Study area

Dourados is the second-largest city (population of 227,990) (IBGE, 2020) in Mato Grosso do Sul State, Brazil. This municipality has several forest streams that are crucial to regulate the hydrological regime, maintain biodiversity and develop ecological sustainability for important rivers in the central-west region of the country (IMASUL, 2021). However, these streams have been affected by anthropogenic activities, such as urbanization, agriculture, and consequent reduction of vegetation around the watercourses, compromising water quality due discharge of effluents, leading to the degradation of aquatic biota and changes as the loss of environmental integrity of the aquatic (Viana et al., 2014; Santana et al., 2015).

The study area is composed of three small basins of the Upper Paraná Basin, Brazil. These streams present different levels of urbanization. Água Boa Stream is a more urbanized small basin with headwaters located inside the area of municipality with three sewage treatment stations in its watershed. On the other hand, Engano Stream has its headwaters in the periurban portion of Dourados city, while its middle and lower forks are in agricultural areas. Finally, the Curral de Arame Stream is entirely located in a rural area with good forest cover surrounding its headwaters free from perceptible pollution sources (Figure 1).

2.2. Data sampling

Samples were taken bimonthly from August/2019 to July/2020. In each sampling site, basic water variables were measured (pH, water conductivity, water temperature, water turbidity and concentration of dissolved oxygen), using a U52 Horiba multiprobe. At each sampling site, surface water samples were collected in pre-cleaned amber glass bottles that were sealed and transported to the laboratory in thermal boxes to quantify the target compounds.

2.3. Analytical methods

2.3.1. Sample preparation and dispersive liquid-liquid microextraction

For extraction of the target compounds, 5.0 ml of the water samples were first vacuum filtered in a closed glass system using a 0.45-µm cellulose acetate membrane (Advanted MFS, Pleasanton, CA, USA) and then transferred to Falcon conical tubes to centrifuge 15.0 ml, followed by dispersive liquid-liquid microextraction (DLLME). The DLLME procedure involves two solvents, dispersing and extracting, and sample in aqueous phase (Ojeda & Rojas, 2011; Rezaee et al., 2006). 500 µL of methanol (PA grade Synth) was used as dispersant, followed by 500 µL of acetonitrile (HPLC JT Baker grade) as extractant, according to Martins et al. (2012). The tubes were shaken for 2.0 min and then centrifuged in a tabletop centrifuge (3,500 rpm for 3 min). 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. The eluate was resuspended with 300.0 µl of methanol (PA Synth grade), and 25.0 µl of the sample was injected

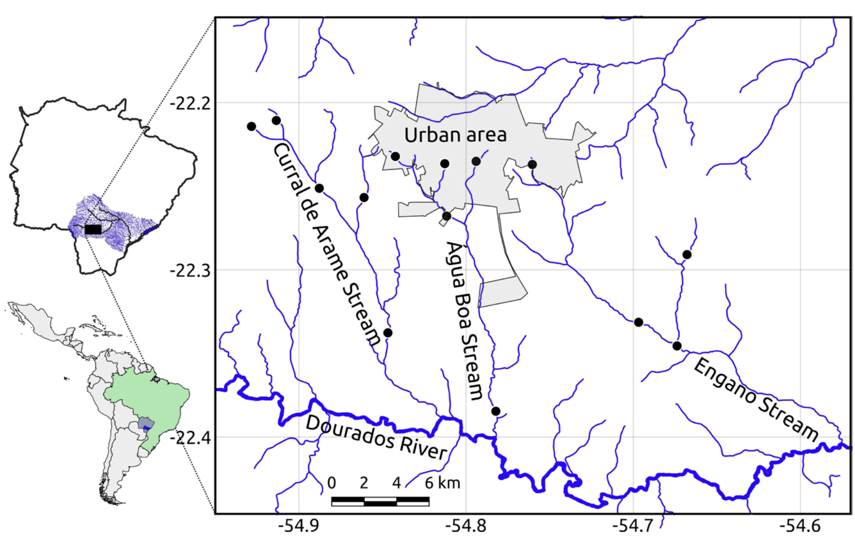

Figure 1. Map with the location of the study area with emphasis on the points of the streams monitored in the city of Dourados, Mato Grosso do Sul State.

into a high performance liquid chromatograph (HPLC).

2.3.2. Analysis of pharmaceutical compounds

Analyses to determine the target compounds present in water, caffeine, diclofenaco, ibuprofen, naproxen, and piroxicam, were performed using a Shimadzu HPLC equipped with two modules, LC -20AT and LC -20AD, of the CBM-20A Prominence Communications Bus Module, a 20 µL Rheodyne injector (Rohnert Park, CA, USA), and an SPD-M20A Prominence Diode Array Detector. Wavelengths from 220 to 280 nm were used and the LC solution software was employed.

The organic HPLC solvents used were trifluoroacetic acid (PA Vetec), acetonitrile (HPLC JT Baker grade), methanol (PA grade Synth), and aqueous solvent (MilliQ). The chromatography columns used were Shim-pack C18 HPLC column (250 × 4.6 mm ID, 5.0 µm particles) and Zorbax ODS C18 HPLC column (150 × 4.6 mm ID, 5.0 µm particles). All samples were injected in triplicate with a volume of 25.0 µL. Analyses for the determination of the hormones estriol, estradiol, and ethinylestradiol present in water were performed using an Agilent HPLC (Agilent, 1200 - F1) instrument with fluorescence detector, Agilent ODS 4-Pack C18 column (12.5 mm x 4.6 mm ID, 5.0 µm particles), and autosampler injector SL (G1367B), using wavelengths from 280 to 310 nm. The HPLC grade organic solvents used were acetonitrile acidified with H_2SO_4 (Verbinnen et al., 2010) and aqueous solvent (MilliQ). All samples were injected in triplicate in a volume of 25.0 µL.

Pure commercial standards (Sigma-Aldrich) of eight pharmaceutical compounds with purity ranging from 97 to 99% were used for the analysis. Optimization of the method and identification and preparation of the analytical curve were performed according to the procedures of the National Institute of Metrology, Quality and Technology (INMETRO, 2010). The preparation of the standard solutions was based on analytical curves with a concentration range of the stock solution (20 µg/L) plus 1 ml of methanol (100%), which were stored in the dark at 15 °C until use. Ultrapure water was supplied by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Detailed information on the 8 pharmaceutical compounds and internal standards is provided in the following repository: https://doi.org/10.48331/scielodata.XO1XDK. The coefficients of determination of the calibration curves of each drug were consistently above 0.9800 for most analytes. The validation of the chromatographic method studied was performed using protocols described in the literature (Brasil, 2003; Ribeiro et al., 2008). The analytical curves for pharmaceutical compounds and hormones were prepared according to specific methods (Nebot et al., 2007; Verbinnen et al., 2010). Standard solutions were injected in duplicate and peak area was measured. Method quality parameters such as linearity, limits of detection (LOD) and quantification (LOQ), and recovery of target compounds at the concentrations used were considered. The identified chromatographic peaks of these target compounds were determined based on their respective retention times and spectrophotometric profiles based on the literature.

2.4. Risk evaluation

Ecological risk associated with pharmaceutical compounds in stream surface waters was assessed based on the risk quotient (RQ), defined as the ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) of the target. Conventional methods were applied according to the Guidance for Risk Assessment of New and Existing Medicinal Products in the Environment (EMA, 2006) and the related technical guidance document prepared by the European Commission (European Commission, 2003) and calculated using Equation 1 as follows:

$$RQ = MEC / PNEC \tag{1}$$

In general, the PNEC estimate is derived from the toxicity data divided by the assessment factor. If only acute toxicity data (EC50 - LC50) are available, the assessment factor is 1000. If observed values for concentration without effect are available for one, two or three trophic levels, an assessment factor of 100, 50 or 10 can be set (European Commission, 2003).

To better evaluate risk ranges, RQ ratios were classified into four levels: no ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), medium ($0.1 \leq RQ < 1$) and high ($RQ \geq 1$) ecological risk to aquatic organisms. (Hernando et al., 2006). To define the worst-case scenario, the maximum detected levels of the selected pharmaceutical micropollutants in the streams were used. When more than toxicity data were available for contaminants, the smaller value was chosen to estimate the worst-case ecological threat PNEC values for the detected pharmaceutical compounds were taken from the published literature on toxicological data of pharmaceutical compounds for sensitive species, when available (Caldwell et al., 2012; Loos et al., 2018; Zhou et al., 2019; Ecotox Centre, 2021).

2.5. Statistical analysis

To evaluate statistical difference in limnological water characteristics among small basins and seasons (wet and dry), we realized a multivariate analysis of variance (MANOVA), using the 'MANOVA' function. Subsequently, we also used the 'manova.aov' function to evaluate the role of each individual variable to differentiate among groups. The Bartlett test (bartlett.test function) and Shapiro-Wilk test (shapiro.test function) were used to measure variance homogeneity and data normality.

Analysis of variance was also used to measure variation in mean concentrations of pharmaceutical compounds and log-transformed (ln+0.1) in this case. All statistical analysis was performed on R environment (R Core Team, 2021).

3. Results

3.1. Limnological characteristics of streams

Considering all analyzed variables, we observed significant differences in water physicochemical parameters, mainly by seasons (MANOVA: Seasons $F=43.44$; $p<0.001$ and Basins $F=9.83$; $p<0.001$), but also water conductivity, water turbidity and dissolved oxygen concentration which mainly varied by small basins, with water temperature varying only by the sampled seasons. Water pH did not vary among small basins or sampled seasons. Água Boa small basin presented higher values of water conductivity, but smaller values of water turbidity and oxygen concentration (Figure 2).

3.2. Occurrence and concentrations of PAC in water

For all data concerning the standard substances used, we recorded the concentrations, frequency

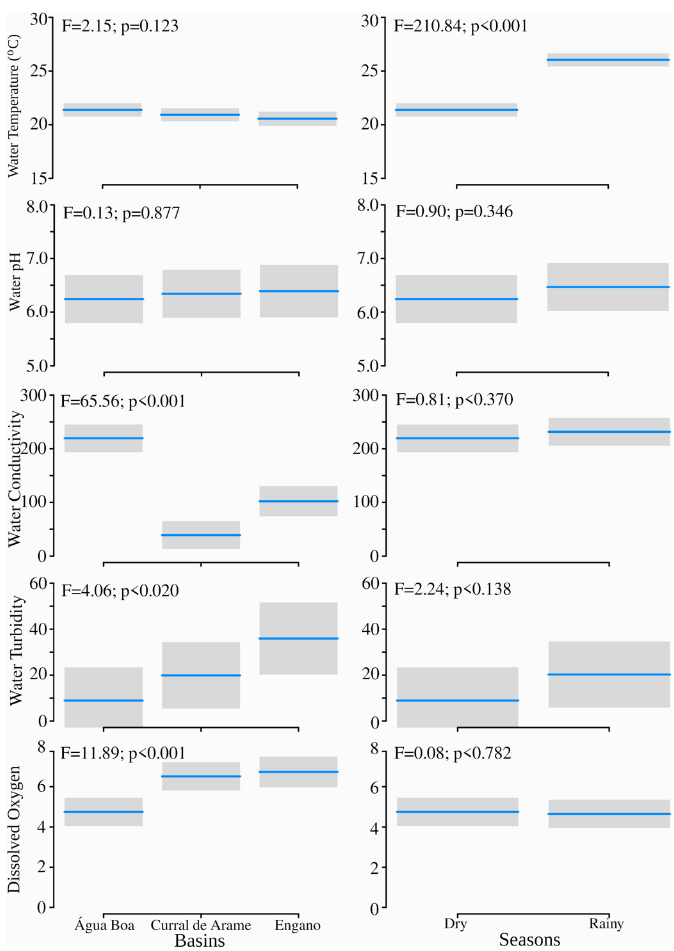

Figure 2. Spatial (small basins) and seasonal variations in water physicochemical characteristics.

of occurrence of six pharmaceutical compounds, represented by stimulants, painkillers and hormones (Figure 3). Caffeine was the most frequent PAC in all small basins (76.7 to 100%) with higher concentrations in evaluated samples (1.04 to 1.69 µg/L). Painkillers and non-steroidal anti-inflammatory drugs (NSAIDs) compounds were also frequent, mainly naproxen (97%) and diclofenac (90%), with maximum concentrations of 0.681 and 0.849 µg/L, respectively. Estrogenic hormones, such, as estriol (73.3%), estradiol (73.3%) and ethinylestradiol (63%), were also frequent with maximum concentrations of 0.064, 0.587 and 0.790 µg/L, respectively.

All evaluated contaminants had higher detection frequency in samples from Água Boa Stream. For Engano Stream, NSAIDs and stimulants presented higher frequency of detection, while hormones had lower frequency of detection in water samples. On the other hand, in Curral de Arame Stream, only the evaluated NSAIDs were detected, while hormones were below detection limits during the whole sample period.

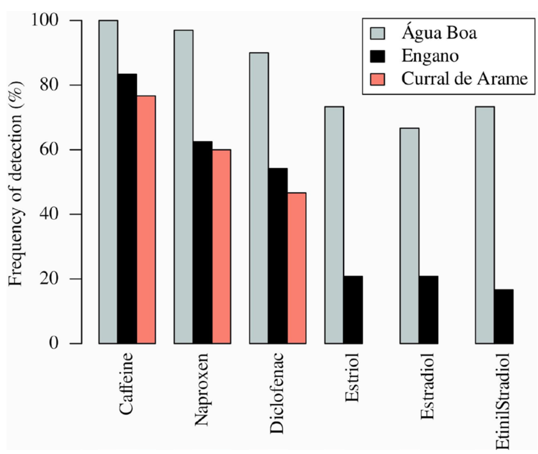

Figure 3. Frequency of detection of evaluated emergent contaminants at sampled small basins in Dourados River, Upper Paraná River.

Considering spatial variations in stimulants and painkillers, we observed a significant difference among small basins, with higher concentrations in Água Boa Stream; however, only caffeine and naproxen varied temporally, with higher concentrations in the dry season (Figure 4).

For hormone concentrations, we also observed variation among sampled small basins, but also temporal variations in these pharmaceutical compounds (Figure 5). As also observed for painkillers and stimulants, we observed higher concentrations of evaluated hormones at Água Boa small basin located in a more urbanized portion.

3.3. Environmental risk assessment

The maximum RQ presented larger values in Água Boa Stream during the whole sample period and for Engano Stream during the dry period and beginning of the rainy season (Figure 6). For Curral de Arame Stream, maximum RQ values were smaller than those seen in other sampled small basins (Figure 6).

For Água Boa Stream, we observed that ethinylestradiol was the contaminant with highest RQ for the whole sampled year. For Engano Stream, three contaminants alternated according to the one with highest RQ (ethinylestradiol, estradiol and diclofenac), and for Curral de Arame Stream, caffeine and diclofenac also alternated in RQ predominance (Figure 7).

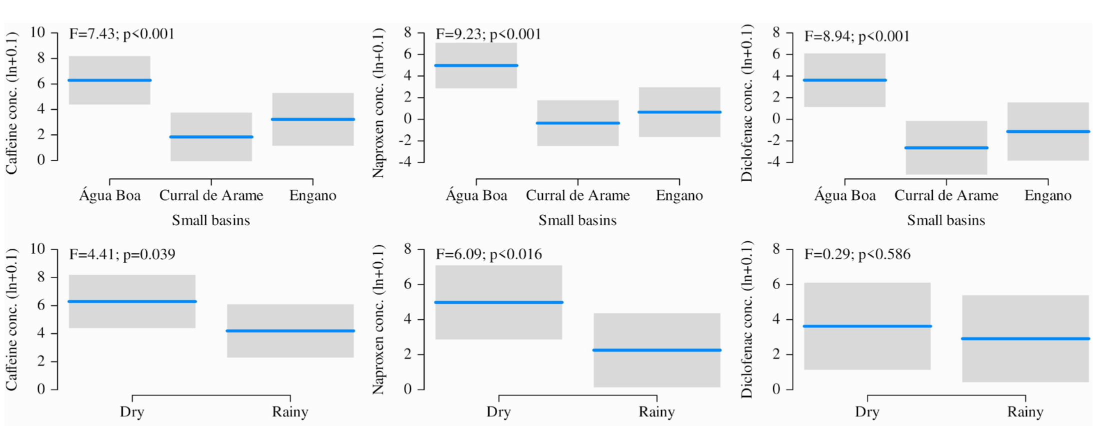

Figure 4. Temporal and spatial variations in concentrations of stimulants and NSAIDs in sampled small basins at Dourados River basin, Upper Paraná River.

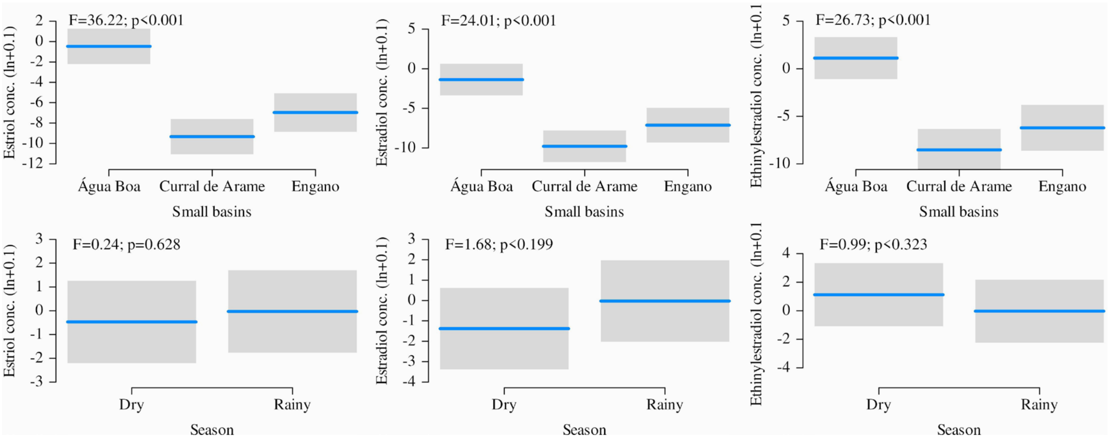

Figure 5. Temporal and spatial variations in concentration of hormones in sampled small basins at Dourados River basin, Upper Paraná River.

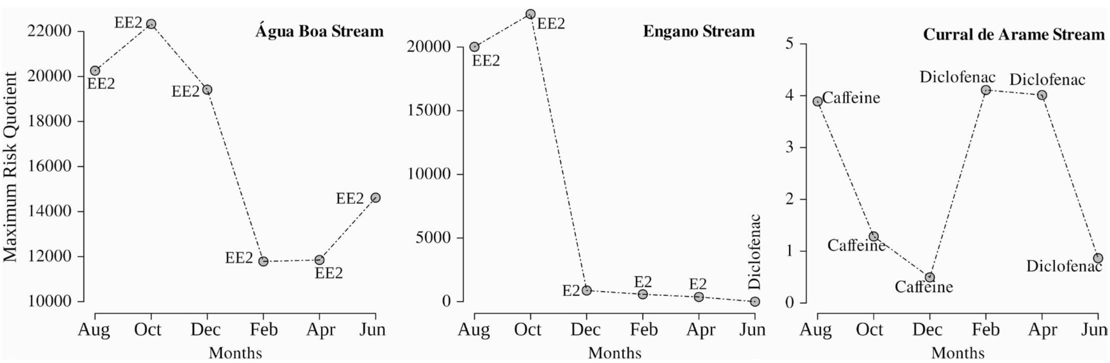

Figure 6. Temporal variation in maximum RQ for sampled streams in Dourados River, Upper Paraná basin. E2= estradiol; EE2= ethinylestradiol.

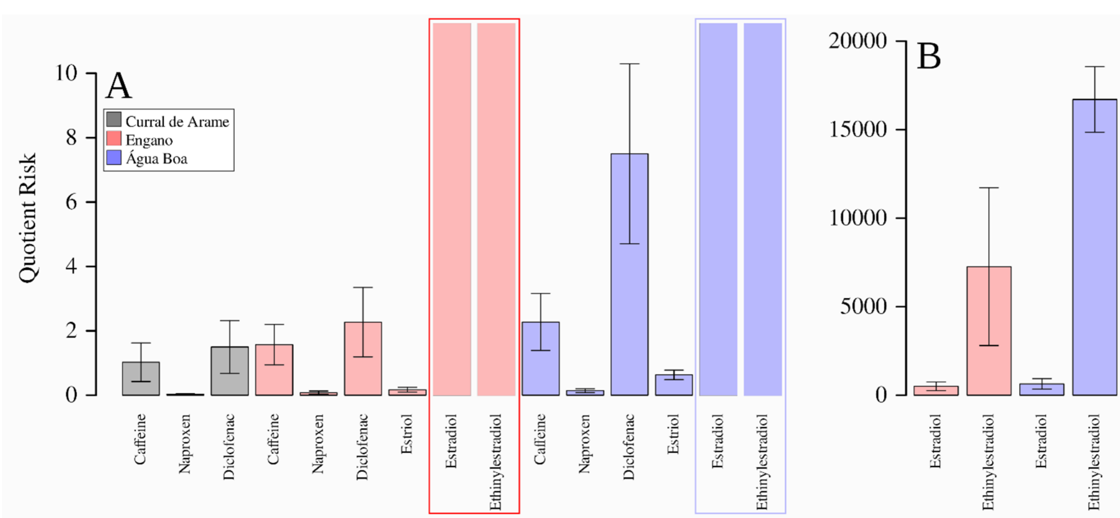

Figure 7. Variation in RQ (on a logarithmic scale) of emerging contaminants considering the most sensitive effects among the streams evaluated (A) and zoom in RQ for estradiol and ethinylestradiol (B) by scale difference. Bar error indicates standard error.

4. Discussion

4.1. Basic limnological characteristics

Considering all results of multivariate analysis of variance (MANOVA), temporal variations are considered more important than spatial variations. Despite this, only for water temperatures we detect significant differences (mean temperature: dry season=20.98 and wet season=25.63). This difference (4.65 °C) is small in comparison and expected in neotropical streams; however, smaller confidence intervals for these estimated temperatures generated higher statistical difference ($F=210.84$, $p<0.001$) and influenced MANOVA results. Then, comparing small basins by degree of urbanization, we detected water conductivity, water turbidity and dissolved oxygen concentrations to be a better predictor of water quality.

Differences in stream turbidity are generally associated with 1) differences in riparian vegetation, as deforested streams may have higher sediment loads, and 2) differences in soil geomorphology, which also increase sediment loads to streams. In our study, we found no differences in soil properties because they were spatially close and subject to the same soil properties. However, urban streams did not exhibit higher turbidity. In contrast, the Engano stream and, to some extent, the Curral de Arame stream showed different turbidity values, which can be explained by agricultural use.

Oxygen concentration is often used as an indicator of water quality, and Brazilian legislation (Brasil, 2005) proposes that this variable must be at least 5 mg/L to maintain biodiversity and also to allow human use, which facilitates water treatment. In our data, the Água Boa stream had an average concentration of 4.69 mg/L, a value well below that recommended by CONAMA Resolution 357 (Brasil, 2005). Such low levels can act as an environmental filter, eliminating more common fish species and leading to the increase of exotic and tolerant species, as observed by Ferreira et al. (2021), who compared this small basin with others in the same region.

Water conductivity is not considered in Brazilian water quality assessment laws (CONAMA 357/2005), as this variable may be elevated by many nutrients at higher concentrations. Complementary to this, pristine environments in the same region (e.g. Miranda basin) may have higher water conductivity, due to limestone soils (Oliveira & Ferreira 2003). However, when considering these sampled small basins, water conductivity clearly functions as an indicator of water quality in response to geomorphologic homogeneity leading to higher conductivity as observed in response to wastewater discharge in the sampled streams. Small tributaries with preserved riparian vegetation had a conductivity of 45 µS/cm (Curral de Arame stream), while streams with an intermediate degree of anthropogenic influence due to lower levels of urbanization and agricultural deforestation had a conductivity of 108 µS/cm (Engano stream). Finally, urbanized streams with a higher wastewater load in the tributaries had a conductivity of 225 µS/cm (Água Boa Stream).

The physicochemical evaluation of the water samples of the streams, highlighting the parameter of conductivity, showed as an indicator the main degradation of the aquatic environment, probably due to the presence of effluents released as a result of human activity. A work carried out in an important watercourse (Jundia River) in the state of São Paulo, southeastern Brazil, showed a good correlation between the conductivity of the water and the drug residues present on site (e.g., diclofenac, propranolol, ibuprofen, atenolol and carbamazepine). The authors demonstrated that the correlation coefficients with electrical conductivity exceeded those of caffeine, a compound that has been widely used in recent years as an anthropogenic indicator of wastewater discharge into water bodies (Sousa et al., 2014).

Recent studies have shown the potentially toxicogenic character of water from tributaries (Dourados and Brilhante) of the region analyzed in bioassays with *Allium cepa* and *Astyanax lacustris*, which could be associated with the effects of the aforementioned physicochemical parameters on these watercourses (Sposito et al., 2019). Rocha et al. (2018) observed DNA alterations in erythrocytes of *A. lacustris* exposed to water samples of the streams investigated in the present study, highlighting the sensitivity of the test organism due to the presence of the chemical compounds and interaction with physicochemical parameters in aquatic environments.

Much of the stewardship of water resources, particularly in developing countries, has neglected streams in rural and urban areas, so little is known about the impact of anthropogenic disturbance of the landscape on pollutant loads emanating from tributaries and discharged into the main rivers and reservoirs that supply cities, industries, and irrigated crops (Medeiros et al., 2017). We can assume that the type of land use associated with wastewater disposal within the sub-basin directly contributes to the type of pharmaceutical compounds found in it, which favors the continuous input of these micropollutants into the studied watercourse. The use of physicochemical parameters of the water may in some cases be an indication of the presence of micropollutants (e.g., pharmaceutical residues), which was noted in the present study and should be taken into account.

4.2. Distribution and seasonality of PAC in streams

The presence of PPCPs in aquatic ecosystems is a global problem and a challenge for various international environmental regulators. Similar to other countries, the presence of pharmaceutical micropollutants in sampled streams is of concern given the uncertainty of adverse effects on aquatic communities (Gavrilescu et al., 2015). Some aspects associated with elevated concentrations of pharmaceutical compounds in surface waters are due to a combination of factors, such as seasonal variation in environmental characteristics and wastewater discharges, heterogeneity of sampling sites (Sousa et al., 2014), and patterns of use of pharmaceuticals in local populations (Ebele et al., 2020). Soil and water properties may also influence the rate of biotransformation of each contaminant.

Our data show spatial differences in drug residues in the small basins studied, with higher levels in most urbanized small basins, and temporal significance for only two of the six contaminants studied (caffeine and naproxen). In general, the dry season has higher concentrations of pharmaceutical micropollutants affected by the absence of precipitation, which affects the lower dilution of these contaminants, which in turn increases their concentration in water (Montagner & Jardim 2011; Sousa et al., 2014; Ide et al., 2017; Mizukawa et al., 2019).

It is interesting that most studies encountering an increase of pharmaceutical contaminants in streams are realized near larger cities when, in the dry period, a larger proportion of water volume in streams is from domestic sewage, while streams near smaller cities do not experience this variation in pollutant concentration. Accordingly, effluents of domestic sewage in the dry season for our study area represent a smaller proportion of stream volume, decreasing the importance of this type of contaminant in the dry season. Small basins sampled in these studies represent nearly half the population of Dourados city since another part of the urban area is included in another watershed (Brilhante River), which was not evaluated in the present study.

Our results also showed a clear spatial difference in concentration of pharmaceutical compounds in association with highly urbanized small basins presenting higher concentrations of contaminants and higher levels of RQ for Água Boa Stream, diverging at low occupation density observed in Curral de Arame Stream where hormones were apparently below detection limits.

Stimulants and NSAIDs were the only emergent contaminants detected at Curral de Arame, the evaluated urbanized small basins. These results also clearly show the effect of urbanization on the concentration of emergent contaminants; however, the presence of stimulants and painkillers also suggests that small ranches with livestock and other animals can produce superficial contamination of small streams, as also observed in other studies (Petrie et al., 2015; Hanna et al., 2018).

Seasonal variations in concentrations of some pharmaceutical micropollutants are complex and difficult to understand. They may reveal variations in drug consumption during certain periods of the year and outbreaks of infectious diseases in regional populations (Petrie et al., 2015). In addition to the environmental impact of these micropollutants on aquatic biota, these data show the abundance of micropollutants among populations along the year and in different parts of the cities according to the river basins that receive wastewater from different parts of the urban areas studied. Recently, in another study, SARS-CoV-2 was detected in urban sewage in different cities, also suggesting the need for monitoring of sewage as a method of evaluating public health (Prado et al., 2020; Fongaro et al., 2021).

4.3. RQ for target PAC

Our data showed temporal differences in RQ for each sampled small watershed, with estradiol and ethinyl estradiol predominating in Água Boa and Engano creeks, but caffeine and diclofenac in Curral de Arame creek. Then, we found differences in the nature of the emerging pollutants and also in the magnitude of the RQ between the small basins. The results are similar to those found in other studies in Brazil (Mizukawa et al., 2018; Peteffi et al., 2019; Montagner et al., 2019) and other countries (Garrido et al., 2016; Pereira et al., 2017; Zhang et al., 2018; Wee et al., 2019; Molnar et al., 2021).

Estrogens have a short half-life (from hours to a few days), but continuous input into aquatic systems can lead to pseudo-persistence of this pollutant (Hernando et al., 2006) and therefore have strong effects on aquatic life such as crustaceans and fish (Sumpter & Jobling, 2013), altering the life cycles and population dynamics of affected species, and thus changing the diversity and species composition in aquatic communities (Baldigo et al., 2015).

Low concentrations of estradiol could change brain aromatase expression in *Jenynsia multidentata* (Guyón et al., 2012), while ethinylestradiol is considered fifty times more potent, leading to feminization of *Pimephales promelas* males (Kidd et al., 2007). Other works also reported similar results for other fish species (Arnold et al., 2014; Adeel et al., 2017), but the consequences on population dynamics and conservation of fish assemblages were not evaluated (Wedekind 2014; Jackson & Klerks 2020). Recent study showed deleterious effects of ethinylestradiol by interfering with nervous system development, both centrally and peripherally, with negative effects on regeneration and swimming behavior. Survival of fish and other aquatic species may be at risk in chronically ethinylestradiol-contaminated environments (Nasri et al., 2021). Thus, more urbanized streams that frequently present little richness and tolerant species can be defined not only by biological interactions among native and exotic species in altered environments, but also population dynamics caused by sexual hormones.

Diclofenac concentration also showed potential risk to aquatic biodiversity, and its presence in water reflects an increase in these medicines consumed by human and animal populations (Lonappan et al., 2016), ultimately affecting the aquatic food chain (Bonnefille et al., 2018). The elevated observed Log Kow (4.51) can bioaccumulate in organisms (Brozinski et al., 2013; Palma et al., 2014; Zhou et al., 2019), disrupting the function of kidney, gills, and endocrine system in fish (Derakhsh et al., 2020; Godoi et al., 2020).

RQ for caffeine was also higher (mainly above 1) in Curral de Arame small basin, and this contaminant is usually a proxy of other hormones and painkillers occurring in water samples. Usually, deleterious effects of pollutants are conditioned to their environmental concentrations, toxicity and mechanisms of action (Halling-Sørensen et al., 1998; Fent et al., 2006). Despite the higher biodegradation facility of caffeine, recent studies showed deleterious effects on aquatic organisms (Vieira et al., 2022; Wilkinson et al., 2022), such as oxidative balance in fishes (Santos-Silva et al., 2018), inducing an increase in plasma vitellogenin in male individuals of *Carassius auratus* exposed to a few days of lower concentrations (Li et al., 2012), up to extreme skeletal deformations and reduced growth in exposed larvae of an endemic neotropical catfish (*Rhamdia quelen*) (Santos et al., 2022). Therefore, caffeine may be a strong agent of estrogenicity in aquatic organisms (Montagner et al., 2014), suggesting caution in evaluating caffeine in monitored streams and further studying the effects on native fauna (Santos-Silva et al., 2018; Godoi et al., 2020) and human populations.

PPCPs are an environmental concern because their residues are increasingly common in the aquatic environment (Wilkinson et al., 2022) and their adverse effects on various organism have been widely described (Fent et al., 2006; Wee et al., 2019). The presence of PAC in aquatic systems, such as streams, is increasing due to its continuous release from anthropogenic activities (e.g., wastewater discharges), which fosters its "pseudo-persistence" in these locations and risks to biota (Brooks et al., 2006). Monitoring these environments requires efforts by managers and environmental control agencies to mitigate this problem (Patel et al., 2020). This study demonstrated the importance of taking action along these lines to better assess the impacts and risks of these micropollutants along small basins monitored.

Streams are complex ecosystems and are highly sensitive to anthropogenic impacts, as water and biological composition depend on local and regional characteristics such as land use, deforestation, and hydrologic features (Allan, 2004; Hamid et al., 2020). This means that streams and other aquatic systems near urban areas may be affected by pollutant inputs that alter the original characteristics, which may change the natural diversity/composition of the assemblage, but may also affect human health through bioaccumulation during fish consumption (Rajeshkumar & Li, 2018). In this study, pharmaceuticals were ubiquitous in the surface waters of the streams evaluated, with varying concentrations of these compounds found. This can have negative impacts on aquatic biota (e.g., growth, mortality, reproduction or developmental, behavioural effects, and molecular, cellular, tissue level changes) (Molnar et al., 2021), as shown by the extent of ERA in the small basins studied. The information obtained may contribute to a better understanding of the effects of concentrations of these xenobiotic compounds and provide important support for future and more comprehensive studies.

5. Conclusion

The present study demonstrated a wide occurrence and spatio-temporal distribution of pharmaceutical residues in the streams urban and rural. The results indicate that the continuous input of these micropollutants may lead to adverse effects on freshwater communities. This could be related to diffuse pollution caused by urban expansion and the reduction of green areas in the micro-basins, as well as to the clandestine connection of sewage outlets to the rainwater drainage systems that increases the input of various water contaminants. Subwatershed land use patterns and measures to control pollution from untreated wastewater in urban areas should be strengthened in the future.

This study highlights the potential risks of these PACs to aquatic life. There are knowledge gaps in water quality monitoring related to pharmaceutical residues in the country (e.g., limited risk characterization in sediments, reduced and unknown ecotoxicological data from pharmaceutical mixtures), which poses a challenge for implementing a multi-barrier approach to safe water monitoring in Brazil.

This is because understanding risk perception is an important factor in adopting risk prevention and interventions to protect environmental quality. Therefore, new studies are needed at temporal and spatial scales. They can contribute to natural resource management at the local, community, and regional levels and stimulate public policies for water safety and water quality improvement in urban and rural streams.

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Data availability

Data used in this article was uploaded to Acta Limnologica Brasiliensia's Dataverse at SciELO Data and can be downloaded at https://doi.org/10.48331/scielodata.XO1XDK.

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