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Emergent contaminants in spring rivers and their relation to the benthic macroinvertebrates

Contaminantes emergentes em rios de abastecimento e sua relação com macroinvertebrados bentônicos

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ABSTRACT

To determine the presence of emergent contaminants in aquatic environments and to evaluate responses of the dominant taxa of benthic macrofauna, seven stations were sampled along three rivers that serve as public water suppliers in three sampling campaigns, in the Upper Iguassu Basin, Brazil. Concentrations of ethinylestradiol, fenofibrate, ibuprofen and triclosan were detected in the water and sediment. To correlate patterns of distribution and abundance benthic fauna with the various contaminants found a redundancy analysis (RDA) was applied and showed positive relationships between faunal groups, that indicate stress (such as Tubificinae), and emerging pollutants (such as ibuprofen and ethinylestradiol). The analysis also showed that the most influential variables in the distribution of the fauna were exclusively anthropogenic, which shows that these compounds can be harmful and that the rivers destined for the supply are receiving pollutant loads.

Keywords: Bioindicators; Water supply; Emerging contaminants.

RESUMO

Com o objetivo de determinar a presença de contaminantes emergentes em corpos aquáticos e avaliar respostas dos grupos dominantes da macrofauna bêntica foram amostrados sete pontos distribuídos ao longo de três rios de mananciais em três campanhas amostrais, na bacia do Alto Iguaçu, Brasil. Foram detectadas concentrações de etinilestradiol, fenofibrato, ibuprofeno e triclosan na água e no sedimento. A fim de correlacionar os padrões de distribuição e abundância da fauna bêntica com os contaminantes encontrados, uma análise de redundância (RDA) foi aplicada e mostrou relações positivas entre grupos taxonômicos indicadores de estresse (como Tubificinae) e alguns dos contaminantes emergentes (como ibuprofeno e etinilestradiol). A análise mostrou, também, que as variáveis mais influentes na distribuição da fauna eram exclusivamente antropogênicas, o que mostra que estes compostos podem ser nocivos e que os rios destinados ao abastecimento recebem cargas poluentes.

Palavras-chaves: Bioindicadores; Abastecimento; Contaminantes emergentes.

INTRODUCTION

Emergent contaminants are new chemical compounds, synthetic or natural, detected as a result of new analytical technologies. Pharmaceuticals, personal care products (PCPs), and endocrine disrupters (EDCs) are some of these contaminants considered potentially endangering aquatic organisms (Mons et al., 2013; Sauvé & Desrosiers, 2014; Rodriguez-Narvaez et al., 2017; Gogoi et al., 2018; Pesqueira et al., 2020; Richardson & Kimura, 2020). These compounds are generally released into the aquatic environment by point sources, mainly of drugs excreted and/or directly disposed into the domestic sewage network, in leaking landfills and aquaculture areas, in addition to illegal and uncontrolled disposal of drugs (Behera et al., 2011; Archer et al., 2017; Sophia & Lima, 2018; Richardson & Kimura, 2020). The removal of these compounds from conventional wastewater treatment plants (WWTP) is often inefficient (Li et al., 2016; Fischer et al., 2017**;**Gogoi et al., 2018; Pesqueira et al., 2020) and a significant part of the non-degraded compounds tends to remain in the aqueous phase (Carballa et al., 2007).

Pharmaceuticals, as naproxen, ibuprofen, diclofenac, acetylsalicylic acid, salicylic acid, paracetamol, and ketoprofen, are frequently found in surface waters (Pal et al., 2010; Selke et al., 2010; García et al., 2013; Kosma et al., 2014; Caracciolo et al., 2015; Eslami et al., 2015; Le Coadou et al., 2017; Wilkinson et al., 2017; Galindo-Miranda et al., 2019; Pulicharla et al., 2020). Ecotoxicological studies have shown that ibuprofen was toxic to algae, invertebrates, and fish (Ferrari et al., 2003; Ginebreda et al., 2010; Brozinski et al., 2013; Mezzelani et al., 2018; Yildirim et al., 2021) and fenofibrate can act as an EDC affecting the endocrine system of fish, amphibians, and bivalves (Ruyter et al., 1997; Fent et al, 2006; Maskrey et al., 2021).

The compound triclosan is one of the most commonly PCPs found in surface waters. This compound tends to accumulate in sediment and organisms due to its low solubility in water, and may affect the survival and growth of invertebrates (Dussault et al., 2008; Brausch & Rand, 2011; Von Der Ohe et al., 2012; Kosma et al., 2014; Haman et al., 2015; Li et al., 2016; Ma et al., 2018; Peng et at., 2018). Parabens have been detected both in aqueous and in sediment habitats. These compounds showed low acute toxicity in *in vivo* tests using laboratory animals (Soni et al., 2005; Bazin et al., 2010; García et al., 2013; Kosma et al., 2014; Haman et al., 2015; Santos et al., 2017). However, there is yet no information on the chronic effects of parabens in aquatic environments (Brausch & Rand, 2011).

The female sex hormones (FSH) can influence the hormonal system and reproductive cycles of several organisms, being considered endocrine disrupters (EDC). Among the natural FSHs we can mention estradiol and estrone as the compounds that have been raising greater concern; along with the synthetic ethinylestradiol (Diniz et al., 2010; Wijekoon et al., 2013; Torres et al., 2015; Ide et al., 2017; Torres et al., 2021). Due their hydrophobic behavior, the sediment is an important compartment in reducing the concentrations of these substances in the aqueous phase (Araújo, 2006).

Sterols can be used as indicators of the anthropogenic contribution to organic matter, as well as for the differentiation between fecal matter sources. However, the presence of certain sterols alone is not sufficient to determine the origin or degree of local contamination, so different ratios of sterols have been proposed for this purpose (Grimalt et al., 1990; Takada et al., 1994; Mudge & Seguel, 1999; Zhang et al., 2008; Bujagić et al., 2016; Frena et al., 2016; He et al., 2018).

The anthropic pressure on aquatic environments directly affects its inhabitants. Freshwater benthic macroinvertebrates have been widely used as bioindicators. These organisms inhabit the bottom sediment and present limited mobility being directly in contact with lipophilic compounds adsorbed by the sediments. In addition, the macroinvertebrates present different degrees of tolerance to pollution gradients with a relatively long life cycle (Queiroz et al., 2008; Esteves, 2011; Egres et al., 2012; Leite et al., 2014; Bem et al., 2015; Brauko et al., 2015; Nicacio & Juen, 2015; Clemente et al., 2018; Xu et al., 2018).

Although benthic bioindicators are widely used to determine organic contamination, their relation with emergent compounds sampled *in situ* is practically unknown. Thus, the objectives of this work were: i) to use benthic macroinvertebrates as an indicator of anthropic disturbance, in rivers destined to the supply of adjacent populations; as well as ii) characterize the health of the rivers in relation to the inputs of drugs, PHPs and HSF, relating them in a multivariate way with the structure of the benthic macrofauna.

MATERIAL AND METHODS

Study area

The sub-basin of the Itaqui River and the Pequeno River are important suppliers for the metropolitan region of Curitiba (RMC) (Figure 1). The occupation of the Itaqui River's sub-basin occurred in the last decades in a disorderly way and the areas around the river, from its source to its mouth, including its flood areas, show signs of irregular occupation. In the Itaqui sub-basin there was a wastewater treatment plant, WWTP Martinópolis, which was shut down in 2014. Downstream of the WWTP is the Itaqui lagoon, resulting from the impoundment of the river by a small dam, which was built for the operation of a fishpond (Andreoli et al., 1999; Faria et al., 2010; Yamamoto, 2012).

The sub-basin of the Pequeno River develops towards the Iguassu River, in the east-west direction; its mouth is downstream of the Iguassu Captation, which was diverted, part of its flow, through the Extravasor Channel, upstream. Most of its route is located in the Environmental Protection Area (EPA) of the Pequeno River, while its mouth is located in an urbanized region (Chueh & Santos, 2005; Schechi et al., 2013).

The Extravasor Channel was constructed to minimize the anthropic impacts in the region of springs and increase the drainage channel of the Iguassu River (Monteiro, 2006; Superintendência de Desenvolvimento de Recursos Hídricos e Saneamento Ambiental, 2007). Another function of the Channel is to improve the conditions of water abstraction for the water treatment plant (WTP) Iguassu that supplies Curitiba.

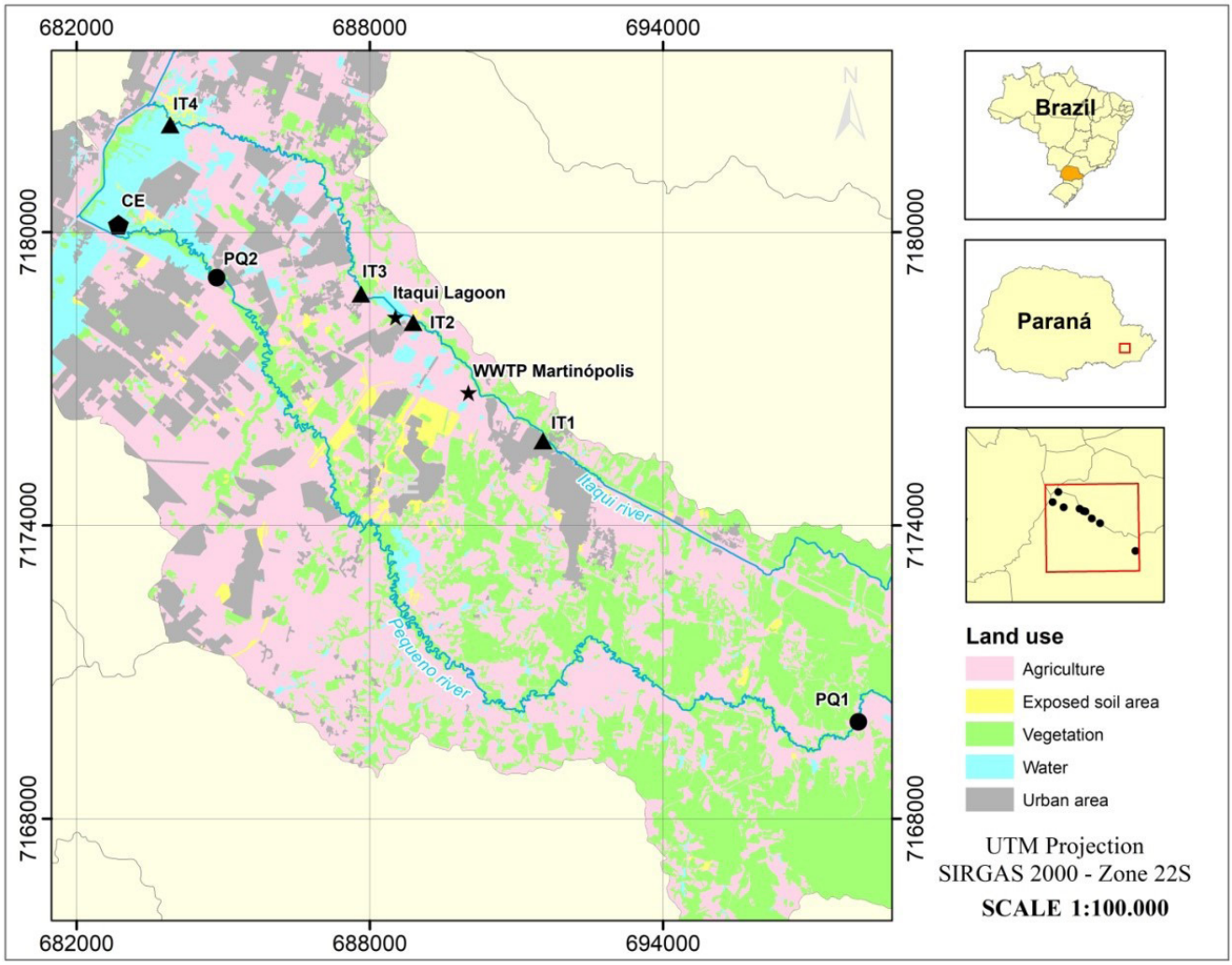

Figure1. Map of the sample stations in the Pequeno (PQ1, PQ2), Itaqui (IT1, IT2, IT3, IT4) Rivers and Extravasor Channel (CE), and the Itaqui lagoon and WWTP Martinópolis; and the land uses around the sample stations.

Samplings

Samplings occurred at four stations along the Itaqui River (IT1, IT2, IT3, and IT4), two stations in the Pequeno River (PQ1 and PQ2) and a station in the Extravasor Channel (CE) (Figure 1), in three campaigns, in January 2016, May 2016 and October 2016). In the first sampling station of Itaqui River signs of irregular occupation in this area are evident, the peak flow rate at this stations is 18 $m^3 s^{-1}$. The second sampling station counts with a peak flow rate of 39.5 $m^3 s^{-1}$, and it is located downstream of the WWTP Martinópolis. The position of the third sampling station is downstream of the Itaqui lagoon and has a peak flow rate of 30.7 $m^3 s^{-1}$. The fourth sampling station has a peak flow rate of 34.2 $m^3 s^{-1}$, and it is located at the mouth of the river. The sample station PQ1 is located inside of the EPA of the Pequeno River and the sample station PQ2 is located in an urbanized area. The peak flow rate in those stations is, approximately, 62.4 $m^3 s^{-1}$ and 43.3 $m^3 s^{-1}$, respectively.

The water was collected with a 5 L Van Dorn bottle, stored in amber bottles, preserved at 4 °C, and transported immediately to the laboratory for analysis of emerging contaminants and organic matter. For the analysis of nutrients, an additional 500 mL was collected in a PET bottle, previously decontaminated with 5% hydrochloric acid. All the materials used in the analysis of the emerging contaminants were decontaminated as recommended by Ghiselli (2006). For the fauna sampling a modified Petersen dredge (0.0180 m2) was used, and at each station, three replicates were collected at the riverbanks, spaced approximately one meter apart to avoid interference between the replicates. The samples were then conditioned in plastic bags and fixed in 70% ethyl alcohol solution for laboratory processing. In each station, one sediment sample was also collected at each station for analysis of emerging contaminants and nutrients with the same sampler. The number of samples for the physical-chemical analyses of the sediments was due to the low spatial variability of these compounds in this matrix. In the laboratory, 200 g of sediment was dried in an oven at 100ºC, for analysis of total phosphorus, nitrogen, and organic carbon, in addition to granulometry. Approximately 50 g of sediment was frozen for analyzes of emerging contaminants.

Physical and Chemical Parameters

The methodologies used to measure nutrient concentrations in water followed the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2005). The spectrophotometer used was a Cary 50 Bio, from Varian. The total phosphorus and nitrogen concentrations in the sediment followed the methodologies of Andersen (1976) and Smart et al. (1983). The granulometric analyses were performed in the Laboratory of Analysis of Minerals and Rocks (LAMIR) using the laser diffraction method. Before the measures, 50 g of sediment has sifted into the sieves with open 0.062 mm and 0.004 mm. The equipment used was the granulometer laser of the brand Microtrac; model S3500, means of deionized water.

Extraction of emerging contaminants and sterols and chromatographic analysis

For the extraction of the emerging contaminants in the water, the method proposed by Ide et al. (2017) was used; the cartridges used for solid phase extraction were Agilent Sampli 1,000 mg - C18 6 mL brand. After extraction and concentration of the samples, an aliquot of the extract was separated for analysis by High Performance Liquid Chromatography (HPLC) and another for analysis in the Gas Chromatograph coupled to mass spectrometry operating in *tandem* mode (CG-MSMS). The extraction of the emergent contaminants in the sediment was carried out adapting the methodology described by Martin et al. (2010). The sediment used to extract emerging contaminants was previously frozen and then dried in a lyophilizer. After extraction of the contaminants from the sediment, the same procedures proposed by Ide (2014) were carried out for solid phase extraction.

For the chromatographic determination of the emerging contaminants, both in water and sediments, three different methodologies were used. The first methodology was proposed by Mizukawa (2016) where the concentrations of paracetamol (PARA), diclofenac (DIC) and ibuprofen (IBU) were determined. For the analysis of these compounds an Agilent model 1260 HPLC was used, equipped with a 600 bar quaternary pump, with an octadecylsilane (Eclipse Plus C18) column with 5 μm pore diameter, 250 mm length and 4.6 mm of internal diameter, and a detector with photodiode array, model 1260. Isocratic elution with mobile phase composed of 75% acetonitrile and 25% ultrapure water acidified at pH 3.0 was used, the injection of the sample was 5 μL a flow rate of 1.0 mL min^{-1}. The monitored ions were: 210 nm (ibuprofen) and 274 nm (paracetamol and diclofenac). The analysis time for each sample was 7 minutes.

The second methodology proposed by Ide et al. (2017) analyzed acetylsalicylic acid (ASA), salicylic acid (AS), ketoprofen (CET), naproxen (NPX), estradiol (E2), ethinyl estradiol (EE2) and estrone (E1). The compounds were analyzed in the same equipment described above. In this case 5 μL sample was injected at a flow rate of 1.0 mL min^{-1}. The isocratic elution had the composition of 1:1 acetonitrile and ultrapure water, with pH adjusted to 3.0 in the mobile phase. Monitored wavelengths: 230 nm (AAS, AS and NPX), 254 nm (CET) and 280 nm (E1, EE2 and E2). The analysis time was 12 minutes per sample.

For the determination of methylparaben (METP), ethylparaben (ETP), propylparaben (PROP), butylparaben (BUTP), benzylparaben (BZP), triclosan (TRC) and fenofibrate (FNF) was adapted to the methodology proposed by Mizukawa (2016). Gas chromatography (Agilent Technologies model 7890A) was coupled to a triple quadrupole mass spectrometer (model 7000) with autosampler (PalSampler). Before the samples were injected, they were derivatized with the addition of 50 μL of N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA). After, they were dried in an oven at 60°C for 30 minutes. Then 1 μL of the sample was injected in splitless mode using a silica capillary HP-5msi 30 m x 0.25 mm x 0.25 μm silica capillary. The entrainment gas used was the helium gas, injected at a constant flow of 1 mL min^{-1}. The oven temperature programmed into 3 ramps, from 100 °C to 325 °C at a rate of 10 °C min^{-1}, remaining for 1 minute. The injector and transferline temperatures were 280 °C and the source ion temperature was 270 °C. For mass spectroscopy analysis, multiple reaction monitoring was used, with fragmentation occurring by electron impact at 70 eV. The analysis time is 33 minutes per sample.

Extraction of sterols, coprostanol (COP), cholesterol (COLE), β-sitoterol (SIT), epicoprostanol (ECOP), cholestanone (COLA) and stigmasterol (ESTI) followed the methodology adapted from Mater et al. (2004). The reconstituted aliquot was analyzed by gas chromatography (Agilent Technologies model 7890A) coupled to a triple quadrupole mass spectrometer (model 7000) with autosampler (PalSampler). Prior to the injection the samples were derivatized in the same manner as described above. After that, 1 μL of the sample was injected in splitless mode using a silica HP-5msi 30 m x 0.25 mm x 0.25 μm silica capillary column. The entrainment gas used was the helium gas, injected at a constant flow of 1.2 mL min^{-1}. The temperature of the kiln is programmed in two ramps, from 40 °C to 250 °C at a rate of 120 °C min^{-1}, remaining for 0.5 minute and from 250 °C to 310 °C. The injector and transferline temperatures were 280 °C and the source ion temperature was 300 °C. For mass spectroscopy analysis, multiple reaction monitoring was used, with fragmentation occurring by electron impact at 70 eV. The analysis time is 15.5 minutes per sample.

The standards used for the determination of the concentrations of the emerging contaminants and sterols, as well as standard HPLC solvents, are available from Sigma Aldrich.

Processing of benthic macroinvertebrates

In the laboratory, the macrofauna samples were washed in a set of 2.0, 1.0 and 0.5 mm mesh apertures. The material retained at the 0.5 mm aperture was then screened under a stereoscopic microscope, and fauna was identified to the lowest possible taxonomic level and then stored in the Francisco Borsari Netto Environmental Engineer Laboratory (LABEAM). To identify some organisms (such as Chironomidae larvae) it was necessary to use an optical microscope, slides were used to identify the structures of the organisms, however the organisms were not fixated. The fauna identification was based on the following keys and identification guides: Brinkhurst & Marchese, 1989;

Domínguez & Fernandez, 2001; Bouchard, 2004; Mugnai et al., 2010; Strixino, 2011; Bolton, 2012.

Statistical analysis

The redundancy analysis (RDA), a method of linear ordering, was performed to assess the relationships between the fauna and the abiotic variables (predictors) (concentrations of ibuprofen, triclosan, fenofibrate, propylparaben, butylparaben, total ammoniacal nitrogen, and orthophosphate in the aqueous matrix, ethinylestradiol in sediment and water, estradiol, methylparaben, coprostanol, and total phosphorus in the sedimentary matrix), as well as the variation in the distribution of the sampling stations along the rivers and campaigns. Each sampling station in each campaign was considered individually in the analysis (n = 21). Prior to analysis, the collinearity between each pair of abiotic variables was tested by Spearman correlations for the exclusion of covariates (r = 0.95). Then, the abiotic variables that presented no collinearity were transformed (square root or log) for normalization. Only the numerically dominant macroinvertebrates taxa (*Helobdella* ssp., *Tubificinae* sp., *Chironomus*, *Caladomiya* and *Polypedilum*), which represented 85% of the total abundance, were analyzed due their high abundance and their expected effect on ecological processes (Avolio et al., 2019). The biotic matrix was then transformed using the distance of Hellinger to reduce the heterogeneity of the data, since species sampled along an environmental gradient tend to have unimodal distributions and, consequently, many null values in the matrix. The RDA was conducted according to Borcard et al. (2011) with standardization of all abiotic variables. The statistical significance of the correlations was evaluated with Monte Carlo permutation tests under 9999 permutations. The RDA was performed using the Vegan package (Oksanen et al., 2008) in the software R (R Development Core Team, 2019).

RESULTS AND DISCUSSION

A total of 46 taxa of benthic macroinvertebrates were found throughout the sampling period, with densities ranging from 55.6 individuals m^{-2} to 16 166.7 individuals m^{-2} (Table 1 and 2). The leeches represented 57.4% of this total, and 55.7% of them belonged to the genus *Helobdella* (Hirudinea: Glossiphoniidae). Degraded environments usually exhibit low diversity of hirudineas allied to high abundances, since they are considered highly tolerant organisms, occurring in coastal zones of lakes and rivers, and even in urban rivers considerably degraded (Miserendino & Gullo, 2014; Alba-Tercedor et al., 2017; Cortelezzi et al., 2018). Although the occurrence of a high number of species may indicate a preserved aquatic environment, since the richness of leeches decreases in relation to organic pollution (Gullo & Darrigran, 1991; Cortelezzi et al., 2018), the number of species found, nine in total, corresponds to a single genus (*Helobdella*) considered resistant to environmental disturbance (Koperski, 2017; Cortelezzi et al., 2018), which suggests that the environment started to be affected.

The second dominant group was the oligochaetes, mainly Tubificinae (Oligochaeta: Naididae), that represented 17.8% of the total abundance. The presence of these individuals is directly associated with nutrition and food availability, with many species thriving in sediments rich in organic matter (Clemente et al., 2018).

Table 1. Total abundance of Hirudinea and Oligochaeta taxons found in the sampling points IT1, IT2, IT3, IT4, PQ1, PQ2 and CE in the three sampling campaigns.

	Family	Subfamily	Genus	Species	Density (individuals.m^{-2})
Hirudinea	Glossiphoniidae		*Helobdella*	sp1	16 166.7
				sp2	2 833.3
				sp3	1 833.3
				sp4	1 388.9
				sp5	5 500.0
				sp6	1 500.0
				Helobdella lineata	1 777.8
				spx	222.2
				spy	666.7
Oligochaeta	Naididae	Tubificinae		sp1	5 888.9
				sp2	3 166.7
				sp3	611.1
			Spirosperma		222.2
		Naidinae	*Dero*		388.9
			Dero (Dero)		333.3
			Dero (Aulophorus)		55.6
			Nais		3 111.1
			Pristina		555.6
			Paranais		166.7
				sp	166.7
				sp	500.0
	Narapidae		*Narapa*	*Narapabonettoi*	111.1

Table 2. Total abundance of Diptera, Hemiptera, Odonata and Tricoptera taxons found in the sampling points IT1, IT2, IT3, IT4, PQ1, PQ2 and CE in the three sampling campaigns.

	Family	Subfamily	Genus	Species	Density (individuals.m^{-2})
Diptera	Chironomidae	Chironominae	*Chironomus*		2 444.4
			Denopelopia		111.1
			Tanytarsus		500.0
			Goeldichironomus		111.1
			Caladomyia		1 944.4
			Polypedilum		2 000.0
			Fissimentum		222.2
			Riethia		55.6
		Tanypodinae	*Paramerina*		55.6
			Ablasbesmia		55.6
		Orthocladiinae			55.6
				sp1	166.7
	Ceratopogonidae				55.6
	Tabanidae				55.6
Hemiptera	Mesovellidae				55.6
	Belostomatidae		*Belostoma*		55.6
				sp	111.1
	Corixidae				55.6
Odonata	Libellulidae		*Idiataphe*		55.6
Tricoptera	Caenidae				55.6
	Odontoceridae				55.6

Species of the Tubificinae subfamily, considered to be pollution tolerant, tolerate anaerobic conditions and are therefore used as indicators of organic contamination (Wetzel, 2001; Rafia & Ashok, 2014; Vivien et al., 2019). Therefore, the combination of the dominance of a diversified group of leeches and oligochaetes (in a much lower density) may be a consequence of a moderately disturbed environment, and the presence of many agricultural areas close to the sampling points (Esteves, 2011; Protasov et al., 2019).

Despite the presence of animals typically associated with contamination, high quality environmental indicators were also found, such as Caenidae **(**Arthropoda: Ephemeroptera) and Odontoceridae (Arthropoda: Tricoptera) and Tanypodinae and Orthocladiinae (Arthropoda: Chironomidae) (Callisto et al., 2001; Sanseverino & Nessimian, 2008; Das & Maity, 2021). This indicates that the studied rivers may be receiving pollutant loads, but still maintain their quality associated to the presence of more sensitive taxa in some stations, such as PQ1 (Esteves, 2011; Ochieng et al., 2019; Protasov et al., 2019)

As for the emergent contaminants, there was a great variation of the concentrations between samplings, both in the aqueous and in the sediment matrix. Triclosan was quantified in all samplings and their concentrations ranged from 50.7 ng L^{-1} to 1 070.6 ng L^{-1} (Figure 2a). Fenofibrate was quantified only at the first sampling, while ibuprofen at 86% of the station of the third sampling. Concentrations of ibuprofen ranged from 441.5 ng L^{-1} (IT4) to 1 466.3 ng L^{-1} (IT2), and concentrations of fenofibrate ranged from 40.2 ng L^{-1} (IT3) to 1602.2 ng L^{-1} (CE). Studies in water suppliers in Europe, Canada, the United States and Mexico found ibuprofen and triclosan concentrations ranging from 2.5 ng L^{-1} to 734 ng L^{-1}, lower values than those found in this study, suggesting a greater contribution of these substances to water sources in the region (Mompelat et al., 2009; Carmona et al., 2014; Aristizabal-Ciro et al., 2017).

The hormone ethinylestradiol (EE2) was found at two stations in the third sampling campaign, ranging from 140 ng L^{-1} to 242.8 ng L^{-1}, while estrone (E1) and estradiol (E2) were found in a single sampling station, IT1 and PQ2, at the concentrations of 97.5 ng L^{-1} and 872.4 ng L^{-1}, respectively. Ide et al. (2017) found concentrations of ethinylestradiol at stations near the study area ranging from 390 ng L^{-1} to 1480 ng L^{-1}. The hormones E1, EE2 and E2 were not detected in the first and second campaigns in the water matrix. As for estradiol, the authors found a concentration of 1 260 ng L^{-1}. In the sediment estradiol concentrations ranged from 28.8 ng g^{-1} to 155.9 ng g^{-1}, while those of ethinylestradiol ranged from 18 ng g^{-1} to 808.2 ng g^{-1}. Concentrations of triclosan, fenofibrate and ibuprofen in the sediments were below detection and quantification limits.

These compounds may have chronic effects on the fauna, bioaccumulating or affecting the growth rates, and the reproduction cycle, altering the ecosystem balance and consequently the quality of the water used in the supply (Dussault et al., 2008; Brausch & Rand, 2011; Peng et al., 2017; Parolini, 2020). In addition, water treatment plants (WTP) do not have specific treatments for these compounds, with the possibility of arriving at the residences and being ingested. Some of these compounds have a carcinogenic potential in humans, such as dioxins from the degradation of triclosan (Moraes et al., 2015; Wang et al., 2018).

Parabens were detected in the surface water of all samples at a frequency of 92%, with the highest concentration found at the third sampling (1 852.2 ng L^{-1}) of methylparaben at the

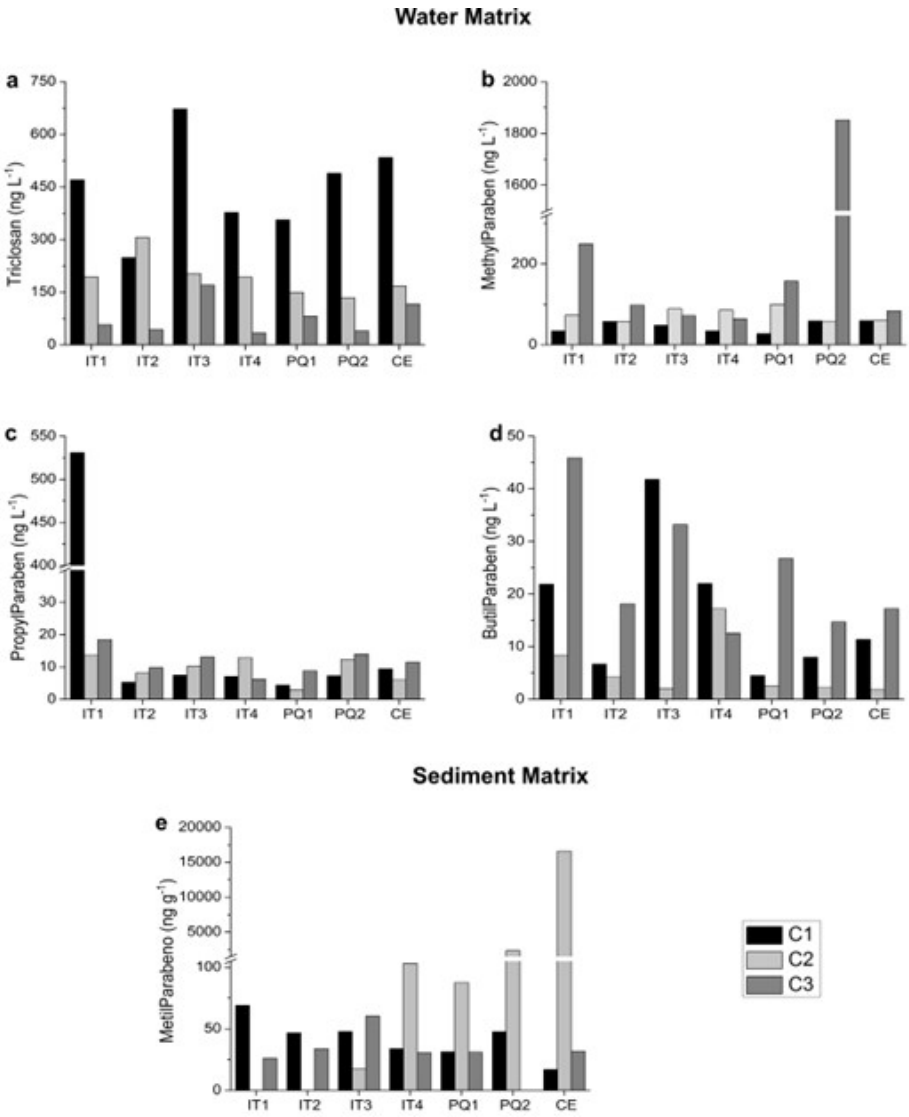

Figure 2. Triclosan (a), methyl- (b), propyl-(c) and butylparabens(d) concentrations on water surfaces; and methylparaben (e) concentrations in sediment on the three sampling campaigns.

PQ1 site (Figure 2b). In the sediment, concentrations of up to 103.2 ng g^{-1} of methylparaben (METP_S) were found (Figure 2e), but the concentrations of propylparaben and butylparaben were below detection. Studies in water supply were found to contain concentrations up to 140 ng L^{-1} of this compound (Carmona et al., 2014; Haman et al., 2015; Aristizabal-Ciro et al., 2017). Carmona et al. (2014) detected a concentration of 476 ng g^{-1} of methylparaben in the sediment of Turia River Basin (Valencia, Spain), and the concentrations found in this study are below the concentrations found by the authors. However, the concentrations in the aqueous matrix are above the concentrations found in non-target areas for supply in other countries. The sediments act as a sink for parabens, due the lipophilic nature of these compounds, and the low concentrations found suggest that the presence of these compounds in the environment is recent (Peng et al., 2017; López-Ortiz et al., 2018)

As for the sterols, the concentrations of sitosterol were generally much higher than the concentrations of coprostanol, except in the Extravasor Channel (CE), where the concentrations found were similar (Table 3). Gonzales-Oreja and Saiz-Salinas (1998) suggested in their study that values above 500 ng g^{-1} of coprostanol in the sediment indicate contamination by sewage, since this sterol is present in human faeces in concentrations 10 times higher than the others (Leeming et al., 2015; Bujagić et al., 2016; Nakagawa et al., 2019). Although the concentrations of coprostanol were found to be higher than 500 ng g^{-1}, the high concentration of sitosterol suggests that most of the organic matter present in the rivers sampled comes from higher plants. Stigmasterol containing mostly natural sources had mean concentrations ranging from 98.8 ng g^{-1} (IT4) to 8 235 ng g^{-1} (IT3), while cholesterol and cholestanone, sterols of human and animal origin, had mean concentrations of 4 053.3 ng g^{-1} (PQ1) and 3 468.1 ng g^{-1}. Epicoprostanol, the isomer

Table 3. Average and standard deviation between collections of the concentration of sterols (ng g^{-1}), total nitrogen concentrations (TN), total phosphorus (TP) and granulometric characteristics in sediment and the ratios for determination of fecal pollution and determination of sources of sterols. And the average and standard deviation between collections of nutrient concentrations in surface waters.

Sediment Matrix							
Stations	**IT1**	**IT2**	**IT3**	**IT4**	**PQ1**	**PQ2**	**CE**
Coprostanol	3 451.8 ± 2 056.1	2 347.9 ± 2 394.8	2 544.7 ± 171.6	392.8 ± 254	294.2 ± 131.2	1 227.3 ± 728.5	13 222 ± 20 206.9
Epicoprostanol	6 670.7 ± 6 637.9	3 568.1 ± 2 583.7	14 226.9 ± 10 395.9	263.3 ± 46.1	3 103.4 ± 2 965.7	1 836.9 ± 989.2	14 213.5 ± 23 952.4
Cholesterol	1 734.4 ± 1 194.9	1 865.2 ± 1 266.8	2 595. 2 ± 988.6	284.8 ± 192.3	4 053.3 ± 5 654.5	668.3 ± 313.3	2 031.6 ± 3 310.5
Cholestanone	1 661.1 ± 2 163.5	1 012 ± 1 029.5	3 468.1 ± 1 623.2	9.3 ± 6.1	901.4 ± 1 051.8	437.3 ± 290.5	5 645.1 ± 9 664.5
Stigmasterol	1 070 ± 1 279.9	2 922.8 ± 3 415.1	8 235 ± 7 455.1	98.6 ± 46.6	3 303.7 ± 3 991.4	350.9 ± 196.8	3 073.6 ± 4 667.2
Sitosterol	18 965.3 ± 25 370.9	50 448.7 ± 77 658.9	35 154.6 ± 24 331	292.9 ± 211.5	21 847.8 ± 18 771.9	2 380.7 ± 1 621.2	13 300.9 ± 21 172.1
Cop/Epicop*	0.79 ± 0.71	0.50 ± 0.29	0.27 ± 0.22	1.48 ± 0.87	0.12 ± 0.05	0.64 ± 0.06	2.85 ± 1.74
Epicop/Cop*	1.96 ± 1.16	2.78 ± 2.17	5.51 ± 3.7	0.82 ± 0.41	9.13 ± 4.79	1.56 ± 0.16	0.55 ± 0.51
Cop/Chol*	3.00 ± 2.34	1.04 ± 0.93	1.08 ± 0.45	2.08 ± 2.12	0.29 ± 0.34	1.93 ± 1.35	8.43 ± 6.69
Cop/ (Cop+Chol)*	0.73 ± 0.17	0.66 ± 0.07	0.44 ± 0.12	0.97 ± 0.02	0.34 ± 0.14	0.74 ± 0.01	0.88 ± 0.17
TN (mg g-1)	1.859 ± 2.16	3.119 ± 3.09	4.815 ± 4.19	0.765 ± 0.76	3.214 ± 3.04	1.644 ± 1.58	2.094 ± 1.67
TP (mg g-1)	0.047 ± 0.01	0.053 ± 0.02	0.056 ± 0.01	0.014 ± 0.01	0.038 ± 0.006	0.028 ± 0.02	0.022 ± 0.01
%#	36.4 ± 18	51.8 ± 28	14.9 ± 3	12.5 ± 8	53.7 ± 11	50.2 ± 5	74.9 ± 20
Diameter (μm)+	17.21 ± 7.1	10.46 ± 5.5	33.23 ± 3.9	43.11 ± 21.4	10.02 ± 2.6	9.03 ± 1.9	6.72 ± 2.6
Water Matrix							
Stations	**IT1**	**IT2**	**IT3**	**IT4**	**PQ1**	**PQ2**	**CE**
N- Ammoniacal (mg L^{-1})	0.136 ± 0.07	0.524 ± 0.34	0.451 ± 0.29	0.024 ± 0.04	0.001 ± 0.002	0.147 ± 0.04	0.047 ± 0.03
Nitrate (mg L^{-1})	0.478 ± 0.57	0.731 ± 0.83	0.607 ± 0.64	2.602 ± 4.24	0.326 ± 0.44	0.392 ± 0.56	0.392 ± 0.50
Orthophosphate (mg L^{-1})	0.004 ± 0.004	0.037 ± 0.02	0.042 ± 0.03	0.027 ± 0.02	0.0006 ± 0.001	0.011 ± 0.001	0.014 ± 0.01

*Ratio: Cop/Epicop:> 1.5 fecal pollution indicative (Zhang et al.,2008; Bujagić et al., 2016); Epicop/Cop: <0.2 indicative of fecal pollution and >0.8 without pollution (Mudge & Seguel, 1999; Frena et al., 2016); Coprostanol/Cholesterol: >1.0 indicative of fecal pollution (Takada et al., 1994; Frena et al., 2016); Coprostanol/ Coprostanol+Cholesterol: >0.7 pollution indicative (Grimalt et al., 1990; Bujagić et al., 2016). In which: Epicop- Epicoprostanol, Cop- Coprostanol, Chol- Cholesterol. # (%) percentage of fine sediments and + mean grain diameter

of coprostanol, had mean concentrations of up to 14 226.9 ng g^{-1}. The mean concentrations of sterols can be observed in Table 3.

The steroid ratios of coprostanol/epicoprostanol and epicoprostanol/coprostanol (Table 3), used to identify possible fecal contamination, suggest that there is no indicative of this type of contamination in most of the stations sampled, with the exception of CE. Coprostanol/cholesterol ratio, which differentiates the anthropogenic and other biogenic sources from natural sources, showed values above 1 meaning a contribution of anthropogenic sources to the sampled rivers, excepting at station PQ1, located within an area of environmental protection. The values of the coprostanol/(coprostanol+ cholesterol) ratio also showed the anthropic interference in the rivers sampled.

The concentrations of nutrients in the surface water varied greatly among the stations, the smallest of which were found in PQ1 (Table 3). These results agree with the concentrations of sitosterol and those of sterols found at this site, indicating a small anthropogenic influence, e.g. sewage discharge (Mudge & Duce, 2005; Antanasijević et al., 2018). The increase of organic matter from sewage discharge can lead to an increase of resistant taxons, such as Tubificinae, and an imbalance in the local ecosystem (Esteves, 2011; Bem et al., 2015, Clemente et al. 2018).

The total phosphorus and nitrogen concentrations in the sediment can be observed in Table 3, as well as the grain diameter and the percentage of fine material at each sampling station. Station IT4 presented the highest mean grain diameter and the lowest percentage of fine sediment stations. These characteristics may explain the lower concentration of sterols in the region, since coarser sediments with diameters greater than 50 μm (sand) are known to have no adsorption capacity for various chemical compounds.

The Redundancy Analysis (RDA) model, which correlated the physical-chemical variables with the fauna found along the rivers, significantly explained 59.2% of the total data variation, with the first axis explaining 22.2% and the second 13.4% ($p < 0.001$, Monte Carlo test)(R^2 0.60)(Figure 3).

The RDA shows that the most influential compounds on the environmental gradient were butylparaben (BUTP) and the forms of total ammonia nitrogen (NH4) in the aqueous matrix, both presented the longest vector length in the graphic. Forms of total ammonia nitrogen are related to disturbed environments, as well, the presence of butylparaben (Esteves, 2011; Bem et al., 2015; Haman et al., 2015). The Itaqui River stations were grouped oppositely to the other rivers, associated with high amounts of BUTP and NH4 in the water and estradiol in the sediment (E2_S). The Itaqui River was strongly correlated with the presence of drugs, while the first station of the Pequeno River is inversely correlated with them, indicating a smaller anthropogenic influence at this station. The second station of Pequeno River, located in an urbanized

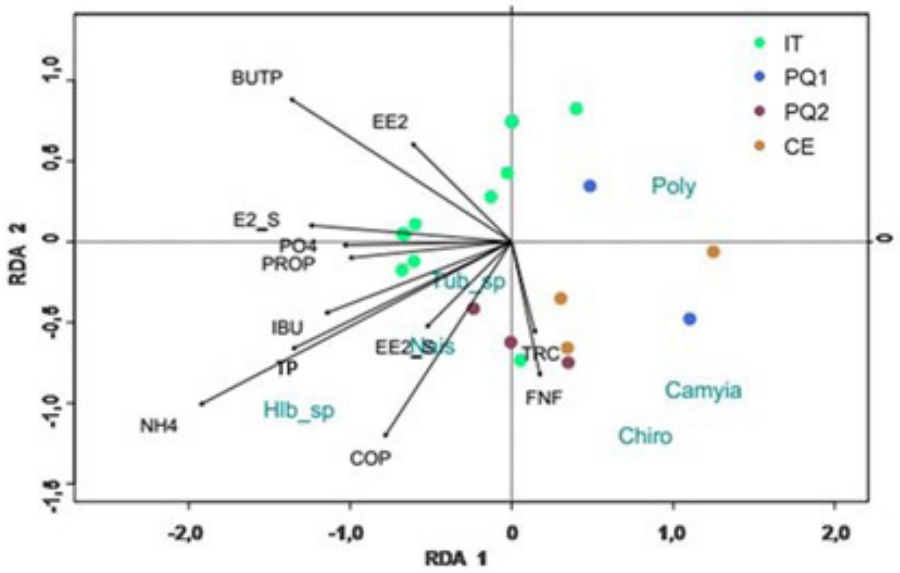

Figure 3. Redundancy analysis triplot (RDA) showing the relationship between chemical variables (black arrows), fauna keychains (blue) and samples (circles). In which: Hlb_sp - *Helobdella* sp; Tub_sp - Tubificinae sp.; Chiro - *Chironomus* sp.; Camyia - *Caladomyia* sp.; Poly - *Polypedilum* sp.

area, is strongly correlated with triclosan (TRC) and fenofibrate (FNF), in addition to high concentrations of coprostanol (COP) in the sediment, an indication of the presence of sewage in the region. The Extravasor Channel (CE) is significantly influenced by TRC and FNF drugs.

The distribution of the fauna along the rivers followed similar patterns, with opposite groups formed along axis 1. The more tolerant organisms like oligochaetes and leeches were correlated to chemical indicators of contamination, such as NH4 and TP. The taxa grouped in the genus Helobdella (Hlb_sp) were strongly correlated (angle > 30 °, vector distance approximately 2.0) with increasing concentrations of NH4, COP, TP and ibuprofen (IBU) and less related to the hormone ethinylestradiol from the sediment (EE2_S) correlated (angle > 30 °, vector distance approximately 0.5). However, the oligochaetes from the Tubificinae (Tub_sp) family were poorly correlated with these compounds. Individuals of the genus *Nais* were significantly correlated with the concentrations of COP in the sediment. Studies have shown that oligochaetes are resistant to some emerging contaminants, such as ibuprofen (Muñoz et al., 2015; Trombini et al., 2020), which the pattern founded in this study suggest that Tubificinae might be used as an bioindicador for emerging contaminants.

The insects of the genus *Chironomus* and *Caladomyia* were detected more frequently in the second station of the Itaqui River and in the Extravasor Channel, being present in all sampling campaigns. These genera were inversely correlated with the pollution gradient, although they were associated with the presence of TRC and FNF. The genus *Polypedilum* was not positively correlated with any indicator of anthropic activity. Within the Chironominae subfamily, the genus *Chironomus* shows the highest tolerance to contamination (Proulx et al., 2018). The genus *Polypedilum* shows degrees of tolerance similar to that of the genus *Chironomus*, while the genus *Caladomyia* is considered more sensitive (Callisto et al., 2001; Strixino, 2011). The fact that these genera have been correlated with compounds such as TRC and FNF suggests that these organisms may have some resistance to these drugs (Martínez-Paz, 2018; Planelló et al., 2020).

Triclosan is considered to be a toxic compound, and studies under controlled conditions with *Chironomus* individuals have demonstrated that small concentrations (100-440 μg L^{-1}) of this compound can cause delays in the development of larvae (Dussault et al., 2008) Parabens also had chronic effects on aquatic invertebrates, which may interfere with the mobility, growth and reproductive cycles of these organisms, as well as ibuprofen (Cleuvers, 2004; Brausch & Rand, 2011). The presence of these compounds and their correlation with the taxa *Helobdella* ssp., *Nais*, *Tubificinae* sp., *Caladomyia* and *Chironomus* in the Itaqui and Pequeno Rivers, although weak as in the case of triclosan, is worrisome, since a small increase in the concentrations can cause a population imbalance to irreversible levels, although more studies are necessary to show those effects. The loss or substitution of benthic taxa that play key roles in aquatic ecosystems as the basis of the food chain and nutrient cycling may lead to loss of ecosystem function and complete change in the quality of spring waters.

CONCLUSIONS

Spring rivers are important environments, and the quality of their water is essential for the water supply of urban centers. The rivers integrate all the result of the human activities in the surrounding areas and this means that they are very closely connected to the terrestrial environment, as in the case of the use and occupation of the land. The compounds ibuprofen, triclosan, butylparaben, and the hormones estradiol and ethinylestradiol were found in water and sediment. Thus, the connections between fauna and chemical variables suggest that the studied

environment begins to reflect changes occurring in the vicinity as the increase of the occupation in the environment. However, the physical and chemical changes of the environment have not yet been sufficient to eliminate pollution-sensitive species such as trichopterans insects and the genus *Caladomyia* during the period of the samplings, constant release of contaminants can lead to a change in the ecosystem, mostly because the chronic effect of the emerging contaminants. Although present in almost all aquatic ecosystems, the interactions between emergent contaminants and the benthic fauna indicative of environmental quality in real field situations are rarely studied. Benthic macrofauna can be a useful tool for monitoring environmental quality in relation to new contaminants. Cause-and-effect relationships have yet to be thoroughly investigated with the use of more comprehensive sampling designs and field manipulative experiments using different concentrations and exposure times, but our results already raise important signs of possible impacts of these contaminants on the benthic fauna.

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Authors contributions

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