Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems

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ABSTRACT

Urban areas in the Brazilian Amazon have grown at an unprecedented rate during the last years. About 90% of the wastewater produced by these urban areas are discharged untreated into Amazonian freshwater ecosystems, constituting a potential environmental pathway for pharmaceuticals and other chemicals consumed by modern societies (e.g. psychostimulants, personal-care products, hormones). The distribution of these chemicals into the Amazon River and their potential risks for freshwater biodiversity have not been evaluated so far. Here, we show the results of the largest chemical monitoring campaign conducted in the Amazon region. We assessed exposure patterns for 43 pharmaceuticals and other urban contaminants in 40 sampling sites distributed along the Amazon River, three major tributaries (Negro, Tapajós and Tocantins Rivers), and four large cities of the Brazilian Amazon (Manaus, Santarém, Macapá, Belém). We assessed risks for freshwater biodiversity using species sensitivity distributions and mixture toxicity approaches. We found that urban areas constitute important hot-spots for chemical contamination, with mixtures containing up to 40 different compounds and exposure concentrations reaching the world's maxima for some of them. We show that chemical pollution can result in long-term effects for up to 50–80% of aquatic species next to urban areas. Moreover, we identified several ubiquitous compounds which can be used as tracers of anthropogenic pressure in the Amazon basin. We conclude that the chemical burden created by urbanization significantly contributes to a biodiversity loss in the region and should be further controlled.

1. Introduction

The Amazon is the largest river basin globally and contains about 40% of the world's remaining tropical rainforest, hosting a vast diversity of terrestrial and aquatic organisms (Laurance et al., 2001; Tisseuil et al., 2013). It is estimated that about 30 million people live in the Amazon basin nowadays, most of whom live in Brazil (WWF, 2020). About two thirds of people in the Brazilian Amazon live in large cities such as Manaus, Belém, Macapá or Santarém. These cities have more than doubled their population within the last 50 years (Fig. 1), and there are prospects for a continued population increase in the future (Côrtes et al.,

2020). Only about 12% of the population inhabiting these cities were connected to sewage treatment facilities in 2018 (SNIS, 2020), and this figure is expected to be even lower for smaller urban settlements. This implies that most wastewater produced by the inhabitants in the region is discharged untreated into small tributaries or directly into the Amazon River.

Untreated wastewater contain large amounts of toxic ammonia and particulate organic matter, which can contribute to eutrophication and anoxia in freshwater ecosystems (de Carvalho Aguiar et al., 2011; Jaiswal and Pandey, 2019). Furthermore, they are considered a major environmental pathway for pharmaceuticals (Fekadu et al., 2019;

Valdez-Carrillo et al., 2020). Pharmaceutical compounds and their metabolites are biologically active substances and can, therefore, affect the physiological status of a wide range of living organisms (Boxall et al., 2012). Several studies show that environmental concentrations of pharmaceuticals can affect the behaviour, growth, and reproduction of aquatic organisms (Brodin et al., 2013; Arnold et al., 2014), ultimately affecting the structure and functioning of ecosystems. Moreover, urban wastewater also contains a wide range of personal-care products, psychostimulants, synthetic hormones, biocides and other compounds consumed by modern societies (Castiglioni et al., 2006; Pedrouzo et al., 2011), which form complex chemical mixtures.

Anthropogenic contamination has been suggested as one of the main drivers of freshwater ecosystem degradation in the Amazon (Castello et al., 2013). Some studies report the occurrence of metals derived from mining (Capparelli et al., 2020), selected pharmaceuticals and illicit drugs (Thomas et al., 2014), pesticides (Pires et al., 2020) and microplastics (Gerolin et al., 2020) in relatively small river transects or tributaries. However, the large-scale distribution of anthropogenic contaminants and their impacts on freshwater biodiversity have not yet been quantified.

In this study we provide the first large-scale exposure assessment of pharmaceuticals and other substances related to human presence (psychostimulants, personal-care products, hormones) in different sampling sites distributed along the Amazon River, three major tributaries (Negro, Tapajós and Tocantins Rivers), and smaller streams crossing the cities of Manaus, Santarém, Macapá and Belém, covering a distance of more than 1,500 km. We evaluated 43 active ingredients and metabolites belonging to the following chemical groups: antibiotics, anti-arrhythmic, anti-hypertensives, lipid regulators, anti-diabetics, gastrointestinal protectants, anti-inflammatories, analgesics, anti-depressants, anti-epileptics, anxiolytics, psychostimulants, adrenergics, antihistaminics, hormones (synthetic and natural) and fragrances. Most of the compounds investigated here were identified in a previous widescope screening, which included over 900 pharmaceuticals, illicit

drugs and metabolites (Fabregat-Safont et al., 2021). In the current study, we focus on the characterization of pharmaceutical exposure in areas with direct wastewater discharge and areas with high dilution capacity. Moreover, we quantified short and long-term risks for freshwater ecosystems using probabilistic risk assessment methods, and identified compounds that should be continuously monitored due to their significant environmental impacts. Our study demonstrates that urban areas in the Amazon constitute contamination hot-spots for pharmaceuticals and other substances consumed by modern societies (psychostimulants, hormones), which significantly contribute to a biodiversity loss in the region.

2. Materials and methods

2.1. Sampling campaign

A field sampling campaign was performed between November 16th and December 8th of 2019, after the dry season. Water samples were taken from 40 different locations in the Amazon River (upper and lower reach, I and II, respectively, n = 11), in three major tributaries (Negro River (n = 5), Tapajos River (n = 2) and Tocantins Rivers (n = 2)), and in smaller tributaries and streams crossing the urban areas of Manaus (n = 8), Santarém (n = 3), Macapá (n = 3) and Belém (n = 6) (Fig. 1). The samples in the Negro River included two locations in the Anavilhanas National Park (N1 and N2), and two samples in the dilution area of the city of Manaus (N4 and N5). The majority of samples in the Amazon River were collected relatively close to small urban areas. Two samples collected in Belém (B3 and B4) were taken in the discharge area of two small tributaries into the Tocantins River, and one of the samples collected from the Tocantins River (TO2) was taken downstream of the city of Belém to evaluate the potential influence of the urban discharge. The sampling locations in Macapá, as well as in the Tocantins River and Belém, were subjected to some tidal effects. In these locations, samples were taken when there was a low tide, so that we avoided dilution by up-

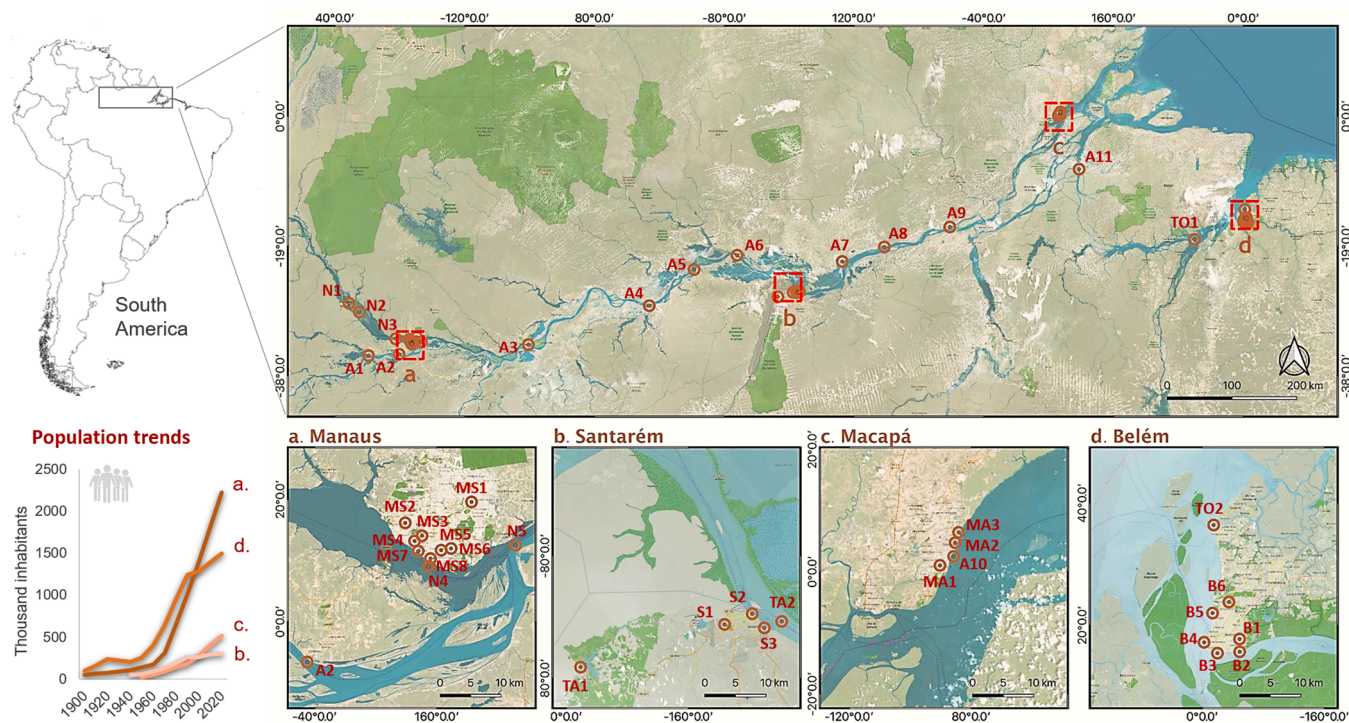**Fig. 1.** Map of sampling locations and population trends of the largest cities in the Brazilian Amazon. Sampling site initials refer to: N: Negro River (n = 5); M: Manaus (n = 8); A: Amazon River (n = 11); TA: Tapajós River (n = 2); S: Santarém (n = 3); MA: Macapá (n = 3); Tocantins River (n = 2); B: Belém (n = 6). The population data series for the monitored urban areas was retrieved from IBGE, 2020.

stream tidal currents. Further details on the sampling sites are provided in the Supplementary Information (Table S1). Sampling was performed from boats or urban bridges by using a pre-washed metal bucket and collecting water from a depth of approximately 20–30 cm. Water samples (2 L) were introduced into amber glass bottles and stored at −4 °C (under dark conditions) for a maximum of 48 h until further processing.

2.2. Sample processing and chemical analyses

Water samples were first filtered through a 0.7 μm glass fiber filter (Merck Millipore, Cork, IRL). Then they were subjected to a clean-up and pre-concentration procedure. Batches of 4–8 samples collected from nearby areas were treated using a portable solid-phase extraction (SPE) system. Two different SPE procedures and analytical approaches were implemented due to the different polarity of the studied substances. As for fragrances, 1 L of the sample was passed over SPE cartridges (Oasis HLB Waters, 6 cc, 500 mg) preconditioned with 6 mL of MeOH and 6 mL MilliQ Water. For the rest of the compounds, 100 mL of sample was adjusted to pH 8–9 by adding NH4OH at 32%. Then, 100 μL of an isotope-labelled internal standard (IS) solution containing 25 μg/L of IS (Table S2) was added to the water sample. SPE cartridges (Oasis HLB, 200 mg/6 cc, Waters, Mildford, MA, USA) were preconditioned with 6 mL of methanol, 6 mL of ultrapure water and 6 mL of ultrapure water at basic pH (8–9). The water samples were passed through the SPE cartridges using a vacuum manifold (Phenomenex, Torrance, CA, USA). After loading, the cartridges were rinsed with 10 mL of ultrapure water and dried for 10 min under full vacuum (5 bar) to eliminate residual water. The loaded SPE cartridges were properly labelled, sealed with parafilm, and stored at −20 °C (dark conditions). The SPE cartridges for fragrance analysis were shipped to and analysed at the Earth and Environmental Sciences Department of the University of Milano Bicocca (Italy), while the SPE cartridges used for the rest of the compounds were analysed at the IMDEA Water Institute (Spain). The fragrance SPE cartridges were eluted with 15 mL of n-hexane, 10 mL of n-hexane: methylene chloride (30:70), and 6 mL of ethyl-acetate, were evaporated to 0.05 mL and transferred into glass vials. Samples with concentrations higher than 50 ng/mL were diluted before re-injection for the quantification analysis. The SPE cartridges used for the rest of the compounds were eluted with three aliquots of 4 mL of methanol. The obtained extracts were evaporated to dryness at 45 °C, 0.2 Torr using a SpeedVac concentrator (Thermo Scientific, Massachusetts, USA), reconstituted with 0.5 mL of methanol:water (10:90, v/v), and vortex stirring for 1 min. Next, the SPE extracts were centrifuged for 5 min at 13,000 rpm (MiniSpin centrifuge, Eppendorf, USA) and transferred into amber glass vials. The SPE extracts (200 times pre-concentrated) as well as their corresponding dilutions - by one forth, using methanol:water (10:90, v/v) - were used for the quantitative analysis. Diluted extracts were only used to reduce matrix effects when the concentrated ones indicated the presence of the target compound.

The identification and quantification of fragrances were performed by GC–MS using the MSD 5977B system equipped with GC 8860 (Agilent Technologies, CA, USA) in selected ion monitoring (SIM) mode. Separations were achieved by a GC-column HP-MS5, 30 m, 0.25 mm, 0.25 μm (Agilent Technologies) with a 1.5 mL/min of carrier gas flow (He). Before injection, the Agilent 7693A Automatic Liquid Sampler added 0.2 μL of internal standard (atrazine-d5) to each of the nine calibration levels and samples. The linearity of the detector response was tested in the range from 0.1 μg/L to 50 μg/L. A summary of the chromatographic conditions and instrumental parameters is provided in Tables S3 and S4.

The identification and quantification of the rest of the compounds were carried out following the methods described by Rico et al. (2019), using high-performance liquid chromatography (HPLC, 1200 Agilent series, Palo Alto, CA, USA), coupled to an Agilent 6495 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Palo alto, CA, USA) in positive and negative mode. Ions were generated using an electrospray ion source

with Agilent Jet Stream Technology. Descriptions of the optimum chromatographic conditions, instrumental parameters, and Multiple Reaction Mode (MRM) transitions are provided in Tables S3 and S4. The LOQs were determined as the lowest concentration whose quantification transition presented a signal-to-noise ratio (S/N) = 10, and qualification transition was detected accomplishing abundance criteria. The LODs were determined as the minimum detectable amount of analyte with a signal-to-noise ratio (S/N) = 3, maintaining abundance criteria between transitions. The method linearity for each compound was established from the corresponding LOQ level to a maximum concentration of 20 μg/L, using external standards over a two-concentration range: 100 ng/L (for low concentration levels) and 20 μg/L (for high concentration levels). The standard regression line was obtained as the mean of three injections of each calibration point, which had a regression coefficient (R^2) of 0.99.

The recovery percentages for the studied compounds (except for fragrances) were assessed for every batch of samples included in the solid-phase extraction procedure using samples fortified at 100 ng/L. There were three batches of samples taken in areas with low urban impact and three in areas with high urban impact, so the results of the recovery analysis are presented separately for samples with low and high urban impact (Table S5). The recovery efficiency for fragrances was checked at two fortification levels (10 ng/mL and 1000 ng/mL) for each selected compound in three replicates for each level. Mean recoveries ranged between 70% and 130%, with a Relative Standard Deviation (RSD) that was below 20% (n = 3) in the majority of the cases (Table S5). The sensitivity of the method was estimated by establishing the methodological quantification limits (MQLs), considering the achieved recoveries and the pre-concentration factor (LOQ/200) applied in the solid-phase extraction protocol. The MQLs and the method detection limits (MDLs) are shown in Table S5. When the achieved recoveries fell outside the mentioned interval (70–130%), the chemical concentrations were corrected using the calculated recovery percentages for the spiked sample in each batch. Moreover, field blanks formed by 100 mL of ultrapure water (pH adjusted to 8–9) containing 100 μL of IS solution were made and analysed concurrently with the samples of every SPE batch to detect possible contamination. Only traces of some compounds were found in the field blanks, which were also used to correct the chemical concentrations.

2.3. Chemical exposure assessment

Based on the results of the chemical analyses, we calculated the sum of compounds monitored above the MDL in each sample, together with the total chemical concentration and the relative contribution of each compound class to the total chemical concentration. The statistical differences between the exposure concentrations in the urban areas and the rest of samples, and between the different sample groups (i.e., Amazon River upper and lower reach, main tributaries and cities) were evaluated with a Permutation Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) test based on Euclidean distances with 999 Monte Carlo permutations using the PRIMER software (Clarke and Gorley, 2015). A Redundancy Analysis (RDA) with 999 Monte Carlo permutations was additionally performed with the Canoco v5.0 software (Ter Braak and Šmilauer, 2012) to visualize the differences between the sample groups in the urban areas, and in the Amazon River and its tributaries. All statistical analyses were performed by using a log x + 1 data transformation, and replacing the concentrations detected below the MDL by zeros, and the concentrations between the MQL and the MDL by MQL/2.

2.4. Ecological risk assessment

Ecological risks were calculated using the Species Sensitivity Distribution (SSD) approach. SSDs are cumulative distribution functions of toxicity data for a given compound and are used to calculate the fraction

of species that will be potentially affected given an exposure concentration (Posthuma et al., 2002). In this study, Potentially Affected Fractions (PAFs) were calculated for individual compounds, and the multi-substance Potentially Affected Fraction (msPAF) was calculated for contaminant mixtures according to de Zwart and Posthuma (2005). SSDs parameters were derived from toxicity data available in public databases and chemical registration reports (for a detailed description see Posthuma et al., 2019), and from calculated toxicity values based on read-across information (i.e., quantitative structure-activity relationships) using the US EPA ECOSAR software (Mayo-Bean et al., 2012). The SSD parameters, μ (median of the log-transformed toxicity values) and σ (standard deviation of log-transformed toxicity values or slope), for short and long-term effects, were calculated with acute (Effect Concentration for the 50% of the individuals, EC50) and chronic toxicity data (No Observed Effect Concentrations, NOECs), respectively, and using a log-normal distribution. In the selection of toxicity data, the following organism groups were included: algae, bacteria, invertebrates and fish. In some cases, NOEC to EC50 (3 cases in the short-term effect SSDs) and acute-to-chronic extrapolation techniques (4 cases in the short-term effect SSDs, and 12 cases in the long-term effect SSDs) were used to increase the number of toxicity data points available to build the SSDs (Table S6). Finally, the robustness of the SSDs to predict ecological risks was evaluated based on the following criteria: (1) number of available toxicity data, (2) origin of the toxicity data (i.e., experimental, extrapolated or read-across), and (3) type of extrapolation (in case the data was extrapolated). The SSD parameters used in this study are provided in Table S6, together with their evaluation criteria. SSDs based on experimental data for ≥ 5 species were assumed to be sufficiently representative for the total number of species in the aquatic environment. Calculations based on SSDs with a lower number of toxicity data, or based on extrapolated or read-across data, were considered first estimates, requiring additional laboratory toxicity data and/or field experiments to confirm their risks.

The PAF associated with each chemical concentration in each sample was calculated using the Microsoft Excel © function shown in Eq. (1).

$$PAF_x = NORM \cdot DIST(MEC_x, \mu_x, \sigma_x, 1) \tag{1}$$

where PAF_x is the PAF for the compound x in the sample, MEC_x is the logarithm of the measured environmental concentration for the compound x, and μ_x and σ_x are the median and slope parameters of the SSD for the compound x, respectively.

Mixture toxicity for each sample was calculated considering the Toxic Mode of Action (TMoA) of the different compounds forming the mixture. Initially, compounds belonging to the same chemical group were assumed to have the same TMoA, as they are expected to affect the same receptor and physiological system. However, when the SSD slopes of the chemicals belonging to the same group deviated more than 10% from the others, they were assigned to a separate TMoA following de Zwart and Posthuma (2005) (see Table S6). Subsequently, we calculated the msPAF for the compounds belonging to each TMoA (msPAF_TMoA) assuming concentration addition. For this, we first calculated the Hazard Unit (HU) for each compound in each sampling site to adjust for differences in the potency of the evaluated compounds within the same TMoA. The HUs were calculated by dividing the MEC by the μ of the SSD for the same compound. The msPAF_TMoA for each TMoA were calculated using the following Microsoft Excel © function (Eq. (2)):

$$msPAF_{TMoA} = NORM \cdot DIST(HU_{TMoA}, 0, \sigma_{TMoA}, 1) \tag{2}$$

where HU_{TMoA} is the sum of the HUs for each compound in the TMoA, and σ_{TMoA} is the average σ for all compounds in the TMoA group.

The total toxicity of the sample (msPAF_Total) was calculated using Eq. (3) and assuming response addition among the different TMoAs.

$$msPAF_{Total} = 1 - \prod_{i=1}^{n} (1 - msPAF_{TMoA,i}) \tag{3}$$

Finally, the msPAF_Total for each sample was represented with the relative contribution of each chemical group to the total mixture toxicity.

The single compound PAF and the msPAF_Total represent the fraction of species of the ecosystem that will be affected (i.e., the EC50 or NOECs are exceeded after short and long-term exposure, respectively) by the exposure to an individual compound or a chemical mixture, respectively. The hazardous concentration for 5% of species (i.e., which results in a protection level of 95% of species) is usually applied as concentration threshold to benchmark unacceptable ecological effects in chemical risk assessment (Posthuma et al., 2002). In our study, sampling sites with PAF or msPAF_Total above this threshold were classified as severely impacted by the monitored chemicals, while sampling sites with values between 1% and 5% were considered to have a low-to-moderate impact.

3. Results

3.1. Chemical exposure assessment

The results of our study show that 40 out of the 43 studied compounds were quantified above the limit of detection at least once within the study area (Table 1; Table S7). Chemical exposure was significantly different in the samples taken in urban areas as compared to those taken in the Amazon River and its major tributaries (PERMANOVA, pseudo-$F_{1,39} = 81$, Monte Carlo $p = 0.001$). The number of substances identified was notably higher in the tributaries crossing urban areas (23–40 per sample) than in the Amazon River and its major tributaries (9–17), as well as the total exposure concentration (Fig. 2). As shown by the PERMANOVA (Table S8) and the RDA (Fig. 3), chemical exposure was found to be relatively homogenous in Manaus, Macapá and Belém, but significantly different from Santarém, which showed a lower number of compounds and a lower total concentration (Fig. 2). The exposure pattern in the Negro River (the less impacted by urbanization) was not statistically significant to that measured in other parts of the Amazon River (Table S8). However, a trend was observed showing an increase in the number of compounds and concentrations in the lower reaches of the Amazon River (Amazon I and II), and in other major tributaries (Tapajós, Tocantins; Figs. 2 and 3), as compared to the Negro River.

Several compounds were measured at very low concentrations in relatively remote areas of the Negro River, such as the Anavilhanas National Park (samples N1 and N2), and in some areas of the Amazon River subjected to high dilution (Table 1). Caffeine and nicotine were found in the Anavilhanas National Park at very low concentrations (<30 ng/L), and in all samples taken along the Amazon River and its major tributaries (with concentrations up to 600 ng/L). Acetaminophen (paracetamol) was found in the dilution area of Manaus at 20 ng/L (N5), and at concentrations up to 200 ng/L in the Amazon River next to the cities of Parintins and Jurutí (A4 and A5). The anti-diabetic metformin was found in concentrations up to 77 ng/L in the Tocantins River (TO2). Moreover, some antibiotics (clarithromycin, sulfamethoxazole, trimethoprim), anti-hypertensives (atenolol and its metabolite atenolol acid), anti-inflammatories (ibuprofen, ketoprofen), antidepressants (venlafaxine), anxiolytics (lorazepam), analgesics (codeine), and fragrances (galaxolide, musk ketone) were detected at trace concentration levels (ng/L) in several sampling sites, being more frequently detected in the dilution areas of Manaus (N4 and N5) and Belém (TO2; Table S7).

The concentrations in the small tributaries and streams crossing urban areas were, on average, about two orders of magnitude higher than those measured in the dilution areas of the Amazon River and its main tributaries. Overall, the total mass of contaminants was dominated by anti-diabetics, psychostimulants, and analgesics (Fig. 2). The anti-diabetic metformin was found at concentrations up to 24,000 and 31,000 ng/L in the streams crossing Belém and Manaus, respectively (Table 1). Caffeine and its metabolite paraxanthine were found in all urban streams, with maximum concentrations of 12,000 and 1,200 ng/

Table 1

List of measured compounds and minimum-maximum concentrations (ng/L) in the different sample groups. Metabolites are indicated in italics. 'Lower than' values indicate that the compound has been detected below the MQL. n.d.: not detected. n: number of samples in each group.

Compounds	Negro River (n = 5)	Manaus (n = 8)	Amazon River I (n = 6)	Tapajós River (n = 2)	Santarém (n = 3)	Amazon River II (n = 5)	Macapá (n = 3)	Tocantins River (n = 2)	Belém (n = 6)	All samples (n = 40)
Antibiotics										
Lincomycin	n.d.	2.0–8.8	n.d.	n.d.	n.d.	n.d.	2.1–7.0	n.d.	2.4–11	n.d. – 11
Clarithromycin	n.d. – <0.1	2.8–22	n.d. – <0.1	<0.1	n.d. – <1.0	n.d. – 0.2	1.0–27.9	n.d.	<1.0–28	n.d. – 28
Metronidazole	n.d.	70–159	n.d.	n.d. – <0.1	<0.2–2.7	n.d.	2.1–88	n.d.	<0.2–75	n.d. – 159
Sulfamethoxazole	n.d. – 0.6	294–893	1.2–1.4	n.d. – 1.3	2.3–3.6	1.1–1.9	193–794	<0.6–4.6	6.6–434	n.d. – 893
N4-acetylsulfamethoxazole	n.d.	184–679	n.d. – <0.1	n.d.	n.d. – 5.3	n.d. – <0.1	26–313	n.d. – 0.7	1.6–233	n.d. – 679
Trimethoprim	n.d. – 1.0	42–143	n.d. – 1.2	<0.1–0.6	<0.2–1.3	<0.1–0.7	4.6–50	<0.1–0.4	<0.2–45	n.d. – 143
Anti-arrhythmics										
Flecainide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives										
Atenolol	n.d. – 1.9	45–194	n.d. – 1.2	n.d.	1.7–9.3	n.d. – 0.9	32–230	n.d. – 2.9	3.8–282	n.d. – 282
Atenolol acid	n.d. – 0.7	142–683	n.d. – 0.6	n.d. – <0.1	0.4–1.2	n.d. – 0.6	7.8–303	<0.1–1.2	2.3–485	n.d. – 683
Enalapril	n.d.	32–103	n.d.	n.d.	n.d.	n.d.	4.4–23	n.d.	29–56	n.d. – 103
Furosemide	n.d.	14–133	n.d.	n.d.	n.d.	n.d.	5.7–62	n.d.	36–92	n.d. – 133
Propranolol	n.d. – 7.2	5.5–26	n.d.	n.d.	n.d.	n.d.	n.d. – 8.0	n.d.	9.3–15	n.d. – 26
Valsartan	n.d.	90–1306	n.d.	n.d.	<2.0–5.9	n.d. – 4.7	152–2914	n.d. – 3.0	14–3391	n.d. – 3391
Lipid regulators										
Atorvastatin	n.d.	1.1–2.5	n.d.	n.d.	n.d.	n.d.	n.d. – 6.7	n.d.	n.d. – 7.3	n.d. – 7.3
Gemfibrozil	n.d.	1.8–20	n.d.	n.d.	n.d. – <1.0	n.d.	1.4–18	n.d.	n.d. – 6.6	n.d. – 20
Anti-diabetics										
Metformin	<3.0	6214–30742	<3.0–18	<3.0–9.6	22–167	<3.0–42	411–9204	9.7–77	77–23762	<0.3–30742
Gastrointestinal protectants										
Omeprazole	n.d. – 1.1	n.d. – 2.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d. – 0.7	n.d. – 2.7
Anti-inflammatories										
Diclofenac	n.d.	46–167	n.d.	n.d.	<3.0	n.d.	32–155	n.d.	<3.0–142	n.d. – 167
Ibuprofen	n.d. – <0.5	360–1803	<0.5	<1.5–1.5	3.2–12	<0.5–3.2	119–870	<0.5–1.7	4.7–799	n.d. – 1803
Ketoprofen	n.d.	14215,0	n.d. – 28	n.d.	<10	n.d.	<10–42	n.d.	<10–69	n.d. – 69
Naproxen	n.d.	177–473	n.d.	n.d.	n.d.	n.d. – 6.3	14–384	n.d.	4.5–470	n.d. – 473
Analgesics										
Acetaminophen	<0.7–19.1	4057–17605	3.7–226	<0.7–15	26–127	<0.7–10	90–7486	<0.7–9.7	45–9122	<0.7–17605
Codeine	n.d. – <0.1	2.4–13	n.d. – 0.2	n.d. – 5.3	<0.4–1.0	n.d.	1.0–71	n.d. – 0.3	<0.4–46	n.d. – 71
Anti-depressants										
Venlafaxine	<0.2–3.5	19–76	< 0.2	n.d. < 2.0	<5.0–5.8	<2.0–73	9.9–128	<2.0	<5.0–73	<0.2–128
Anti-epileptics										
Carbamazepine	n.d. – <0.1	95–240	n.d. – <0.1	<0.1	3.5–48.6	<0.1–0.4	42–178	<0.1–11	2.3–207	n.d. – 240
Carbamazepine epoxide	n.d.	6.9–25	n.d.	n.d.	<0.2–12	n.d.	3.1–16	n.d.	<0.2–27	n.d. – 27
Anxiolytics										
Diazepam	n.d.	n.d. – 7.8	n.d.	n.d.	n.d.	n.d.	<0.7–3.8	n.d.	n.d. – <0.7	n.d. – 7.8
Lorazepam	n.d.	4.8–66	n.d. – 0.6	n.d.	n.d. – <2.0	n.d. – 0.4	6.6–31	n.d.	n.d. – 30	n.d. – 66
Psychostimulants										
Benzoylecgonine	n.d. – 35	448–1958	5.3–89	n.d. – 7.8	4.0–14	n.d. – 4.3	55–340	2.2–5.0	26–695	n.d. – 1958
Caffeine	15–45	7033–12237	14–61	158–163	185–3289	49–573	2004–7249	61–133	284–10265	14–12237
Paraxanthine	<0.3	217–808	<0.3	3.2–4.2	52–180	<3.0–7.6	54–711	< 3.0	9.3–1246	<0.3–1246
Nicotine	0.8–118	310–997	7.5–292	185–608	195–1165	54–127	111–490	108–299	360–1670	0.8–1670

Table 1 (*continued*)

Compounds	Negro River (n = 5)	Manaus (n = 8)	Amazon River I (n = 6)	Tapajós River (n = 2)	Santarém (n = 3)	Amazon River II (n = 5)	Macapá (n = 3)	Tocantins River (n = 2)	Belém (n = 6)	All samples (n = 40)
Cotinine	n.d.	7.4–221	n.d.	n.d.	<0.2–34	n.d.	26–207	n.d.	8.5–194	n.d. – 221
Adrenergics										
Salbutamol	n.d.	0.2–1.8	n.d.	n.d.	n.d.	n.d.	<0.2–4.6	n.d.	n.d. – 5.4	n.d. – 5.4
Anti-histaminics										
Loratadine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Androgens										
Testosterone	n.d. – 2.4	n.d. – 36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d. – 16	n.d. – 36
Estrogens										
17α-Ethinylestradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17β-Estradiol	n.d.	n.d. – 9.2	n.d.	n.d.	n.d.	n.d.	n.d. – 7.9	n.d.	<4.0–5.7	n.d. – 9.2
Estriol	n.d.	41–166	n.d.	n.d.	n.d.	n.d.	13–81	n.d.	n.d. – 102	n.d. – 166
Estrone	n.d.	20–70	n.d.	n.d.	<3.0	n.d.	7.3–56	n.d. – 0.9	<3.0–82	n.d. – 82
Fragrances										
Galaxolide	n.d. – 71	382–2978	n.d. – 86	n.d. – 0.3	n.d. – 116	n.d. – 54	65–862	n.d.	n.d. – 2142	n.d. – 2978
Musk ketone	n.d. – 1.9	16–78	0.1–1.5	n.d. – 0.3	n.d. – 1.7	n.d. – 0.4	0.8–6.5	n.d.	n.d. – 23	n.d. – 78
Tonalide	n.d.	43–1219	n.d.	n.d.	n.d.	n.d.	n.d. – 209	n.d.	n.d. –738	n.d. – 1219

L. Also, nicotine and its metabolite cotinine were found in these samples, with parent compound concentrations up to 1,700 ng/L. Benzoylecgonine, one of the main metabolites of cocaine, was found in concentrations up to 2,000 ng/L in Manaus. It was also detected in all urban areas at relatively high concentrations (100 ng/L), and at very low concentrations in some areas of the Amazon River and its tributaries. The analgesic acetaminophen, the anti-hypertensive valsartan and the anti-inflammatory ibuprofen were consistently found in all urban areas, with concentrations up to 18,000, 3,400 and 1,800 ng/L, respectively. The sum of antibiotic concentrations in the urban streams was 500–1,700 ng/L, with sulfamethoxazole and its metabolite (N4-acetylsulfamethoxazole) having the largest contribution. The natural estrogenic hormones estrone and estriol were frequently detected in the urban samples at concentrations of nearly 100 ng/L, while 17β-estradiol was found at low concentration levels (few ng/L). The androgenic hormone testosterone was found in few urban samples at very low concentration levels. Fragrances such as galaxolide and tonalide were found at relatively high concentrations in the streams of Manaus, Macapá and Belém, reaching concentrations up to 3,000 and 1,200 ng/L, respectively (Table 1).

3.2. Ecological risk assessment

The risk assessment performed using acute toxicity data indicated insignificant risks in the Amazon River and its major tributaries (msPAF$_{Total}$ < 1%). However, high short-term risks were calculated in sampling sites within or close to urban areas, with msPAF$_{Total}$ values exceeding 5% of species in nearly 90% of the samples taken in Manaus, Macapá and Belém. The maximum msPAF$_{Total}$ values calculated for these samples were 18%, 11% and 16%, respectively (Fig. 4; Table S9). The compounds with the highest contribution to short-term effects were estrone and paraxanthine, which had individual PAFs exceeding 5%. Other substances with a notable contribution to short-term effects were furosemide and 17β-estradiol, with calculated PAFs ranging between 1–4% and 1–2%, respectively (Fig. 4; Table S10).

The risk assessment performed using chronic toxicity data showed insignificant risks for the samples taken in the Negro River (msPAF$_{Total}$ < 1%). However, low-to-moderate risks (1%<msPAF$_{Total}$ < 5%) were calculated for 50% of the samples taken in the Amazon River, the two samples in the Tapajós River, and one of the two samples in the Tocantins River. In most cases, the calculated msPAF$_{Total}$ was slightly above 1%, except for sample A8, which was 3% (Fig. 4; Table S9).

Severe long-term risks were calculated for the samples taken in urban areas, with msPAF$_{Total}$ values ranging between 58 and 77% in Manaus, 3–20% in Santarém, 23–58% in Macapá, 6–69% in Belém (Fig. 4; Table S9). Long-term risks were dominated by psychostimulants, analgesics, estrogens, anti-inflammatories and anti-hypertensives (with chemical class PAFs above 5% in at least one sampling site; Fig. 4). Particularly, caffeine was the compound showing the highest long-term toxicity (with single compound PAFs up to 41%), followed by acetaminophen (22%), estrone (17%), furosemide, ibuprofen, paraxanthine (12%), benzoylecgonine (9%) and 17β-Estradiol (8%; Table S10). The risk calculations for these substances were based on SSDs built with experimental toxicity data and a sufficient number of species (≥5), with the exception of furosemide (4 species), and the metabolites benzoylecgonine and paraxanthine (which were based on read-across extrapolations for 3 species; Table S6). This indicates that the robustness of the assessment for the latter three substances is lower as compared to the other compounds, and a refined assessment could be performed as soon as additional experimental toxicity data becomes available.

4. Discussion

This study shows that the streams crossing the main urban areas of the Amazon region constitute major pathways for pharmaceuticals and other chemicals related to human presence (i.e., life-style compounds,
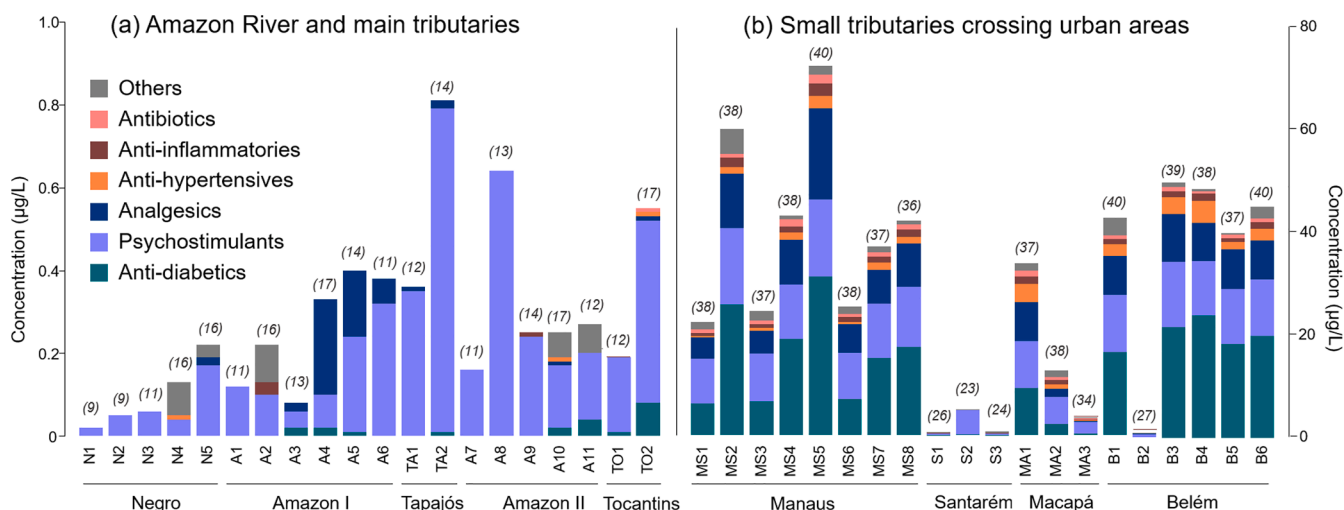
Fig. 2. Relative contribution of each chemical class to the total measured concentrations. (a) Concentrations in the Amazon River and its main tributaries. (b) Cocentrations in the streams and tributaries crossing urban areas. Note that the concentration scale in the panel (a) and (b) are different. Numbers on top of the bars indicate the number of compounds identified in each sample.
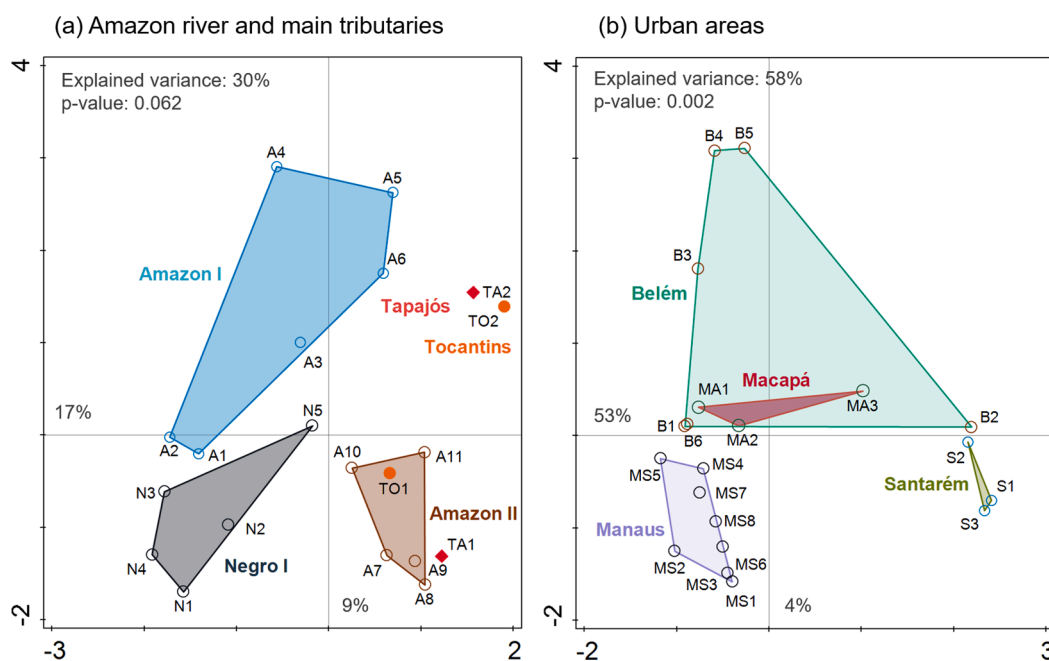

Fig. 3. Chemical exposure differences among sample groups. (a) Redundancy analysis (RDA) biplot showing differences in chemical exposure patterns between different sections and tributaries of the Amazon River. (b) RDA biplot showing differences in chemical exposure patterns between tributaries crossing the main urban areas of the Brazilian Amazon.

home-care products) into the Amazon River. Some chemical concentrations in the streams of Manaus, Macapá and Belém resemble exposure profiles shown by untreated sewage or very contaminated surface water bodies in other parts of the world (Fekadu et al., 2019; Valdez-Carrillo et al., 2020). On the other hand, we found a lower contamination level in the samples taken in Santarém, which may be related to the lower population density and the higher dilution rate of urban wastewaters, as there is no evidence of lower chemical consumption or higher implementation of sanitation measures in this city.

This is the first time that metformin is measured in surface waters of Latin America (Valdez-Carrillo et al., 2020) and, to the best of our knowledge, the concentrations found in the streams of Manaus and Belém constitute the maximum values reported in the literature so far

(Fekadu et al., 2019). This compound is usually found at high concentrations (up to 100,000 ng/L) in the influents of sewage treatment facilities, but it is usually well eliminated by conventional wastewater treatments, so concentrations in surface waters are typically much lower (Scheurer et al., 2012; Oosterhuis et al., 2013). The concentrations of caffeine and its metabolite paraxanthine monitored in urban areas fall above the 99th centile of the global exposure distributions reported by Rodríguez-Gil et al. (2018). Moreover, commonly used substances such as acetaminophen, valsartan and ibuprofen were consistently found in all urban streams, with concentrations that are in the range of those found in very polluted rivers of Latin America and Africa (Fekadu et al., 2019; Valdez-Carrillo et al., 2020). It is noteworthy the high concentrations of benzoylecgonine (metabolite of cocaine) found in Manaus,
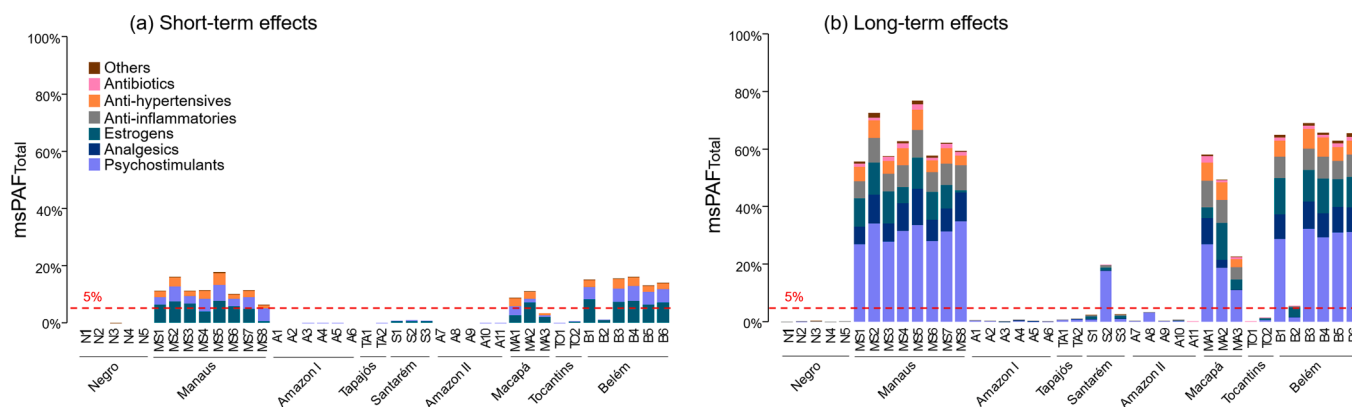
Fig. 4. Results of the ecological risk assessment. (a) and (b) show the multi-substance Potentially Affected Fraction of species (msPAF$_{Total}$) affected by short and long-term exposure, respectively, and the relative contribution of each chemical class to the mixture toxicity. msPAF$_{Total}$ values above 0.05 (i.e., affecting more than 5% of species) are considered to result in unacceptable ecological risks. Only chemical classes with a relevant contribution to the total mixture toxicity (i.e., msPAF above 0.01 in at least one sample) are shown, while the rest are grouped under ´Others´.

and its widespread occurrence in some areas of the Amazon River and its tributaries. Previous studies demonstrate that this metabolite was commonly found in the same streams, together with high concentrations of cocaine (Thomas et al., 2014), which may indicate an elevated consumption as well as the possibility to have cocaine processing laboratories in the study area (Fabregat-Safont et al., In press).

It is estimated that the Amazon basin receives about 12×10^{12} m^3 of water annually (Sioli, 1984), and the volume of urban wastewater contributing to it is lower than 0.01% (SNIS, 2020). In line with this, our study demonstrates that exposure concentrations in the urban discharge areas of the Amazon River and tributaries are about two orders of magnitude lower that those measured in urban streams, and decrease further down-stream. However, despite the high dilution potential, we identified some compounds that are ubiquitous in several parts of the Amazon River. These include primarily caffeine and nicotine, but also acetaminophen, and could be used as tracers of human activity (Buerge et al., 2003; Senta et al., 2015).

It should be noted that exposure patterns of the monitored compounds could vary over the year and that the grab sampling method used here could introduce some bias in our risk calculations. However, we consider that the exposure assessment performed in this study is relatively worst-case, as the study was conducted in a period with low river flow and low chemical dilution potential. Under such conditions, we found low-to-moderate risks in areas of the main river close to urban emission points, with 1–3% of species being affected by long-term exposure. Establishing contaminant thresholds for Amazonian freshwater ecosystems is challenging given the high degree of ecological speciation and biological trait variability (Haffer, 2008; Smith et al., 2014), which usually contributes to a wide species sensitivity range to chemicals (Van den Berg et al., 2019). It is estimated that there are about 3,500 fish species in the Amazon (Junk et al., 2007), so establishing a threshold of 1% would imply an impact on 35 species, and this figure may be even larger for aquatic invertebrates and plants. Previous studies have compared the sensitivity of Amazonian freshwater organisms and their temperate counterparts to pesticides, showing little or non-significant differences (Rico et al., 2010; Rico et al., 2011; de Souza et al., 2020). However, our knowledge on the sensitivity of Amazonian freshwater organisms to pharmaceuticals is too limited. Further investigations are needed to test the impact of the contaminant mixtures identified in this study on species assemblages representative of the Amazonian region, as well as to quantify direct and indirect effects on structural and functional ecosystem parameters.

Our study shows that streams crossing urban areas of the Brazilian Amazon constitute major hotspots of chemical contamination. Particularly, caffeine, acetaminophen, estrone, ibuprofen, and 17β-estradiol were identified as a major threat for freshwater organisms, while

furosemide, paraxanthine and benzoylecgonine were identified as potential hazardous substances given the limitation on the amount of toxicity data available. We estimated that the percentage of freshwater species affected by mixtures of these contaminants reaches 50–80% in most urban areas. At the measured exposure levels, caffeine can affect the regenerative capacity of worms (Pires et al., 2016) and reduce the biomass of freshwater biofilms (Lawrence et al., 2012); while anti-inflammatory drugs such as paracetamol or ibuprofen can cause oxidative stress, genetic alterations and impair the reproductive success of aquatic invertebrates and fish (Flippin et al., 2007; Parolini, 2020). Finally, estrone and 17β-estradiol have been reported to contribute to the feminization of fish and can result in a decline of fish population abundances (Senta et al., 2015). Therefore, severe effects on biodiversity are expected in streams directly impacted by urban wastewater contamination.

We must acknowledge that the calculations provided here could underestimate the risks for substances with low polarity (i.e., fragrances, anti-inflamatories and hormones; Table S5) and that the compounds evaluated here only constitute a small fraction regarding the number of substances emitted via untreated wastewater. In fact, in urban streams, we noted black sediments and water smell characteristic of anoxic conditions produced by an excess of organic matter, and identified large amounts of plastic and metal waste. Follow-up studies should be dedicated to study the contribution of untreated wastewater emissions to eutrophication and anoxia in Amazonian freshwater ecosystems, and to quantify the ecological risks caused by other potentially toxic substances such as metals, pesticides or microplastics, which will be addressed in follow-up studies within the SILENT AMAZON project (www.silentamazon.com).

Our study confirms that the Amazonian region is well behind achieving the sustainable development goals set for 2030 by the United Nations (UN, 2016), in particular Goal 6.3 (to improve water quality via the reduction in pollution, elimination of dumping and to minimize the release of hazardous chemicals and untreated wastewater into the aquatic environment). In this regard, we support the implementation of measures to minimize the environmental impacts of wastewater emissions. These may include, for example, (1) the expansion of the sanitary system and household's connectivity, (2) the implementation of new sewage treatment facilities and upgrading of the few existing ones, and (3) the implementation of nature-based solutions, such as constructed wetlands, to increment the natural attenuation of contaminants.

The potential decrease of invertebrates and fish biomass next to urban areas of the Brazilian Amazon can be intrinsically related to a loss of food resources for reptiles, birds and humans (by reducing fish captures), and may also have detrimental effects for basic ecological functions such as organic matter decomposition (Posthuma and de Zwart,

2006; Pilière et al., 2014). At a broader scale, the water quality impacts by urban pollution may add up to those caused by agriculture (Schiesari et al., 2013) or mining (Capparelli et al., 2020), and together with other pressures such as damming, droughts or deforestation (Castello et al., 2013; Latrubesse et al., 2017), can contribute to set-up barriers for migratory species and contribute to a biodiversity loss (Castello and Macedo, 2016). Therefore, we conclude that the protection of Amazonian freshwater biodiversity requires the implementation of monitoring programs to regularly assess and control the chemical status of freshwater ecosystems in areas with significant demographic pressure.

CRediT authorship contribution statement

Andreu Rico: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing - original draft. **Rhaul de Oliveira:** Conceptualization, Investigation, Writing - review & editing. **Gabriel Silva de Souza Nunes:** Investigation, Writing - review & editing. **Cristiana Rizzi:** Formal analysis, Methodology, Writing - review & editing. **Sara Villa:** Conceptualization, Methodology, Writing - review & editing. **Isabel López-Heras:** Formal analysis, Methodology, Writing - review & editing. **Marco Vighi:** Conceptualization, Writing - review & editing. **Andrea Viviana:** Waichman Conceptualization, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106702.

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.

Arnold, K.E., Brown, A.R., Ankley, G.T., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Phil. Trans. R. Soc. B 369, 20130569.

Boxall, A.B., et al., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ. Health Perspect. 120, 1221–1229.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339, 814–815.

Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.R., 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environ. Sci. Technol. 37, 691–700.

Capparelli, M.V., et al., 2020. An integrative approach to identify the impacts of multiple metal contamination sources on the Eastern Andean foothills of the Ecuadorian Amazonia. Sci. Total Environ. 709, 136088.

Castello, L., Macedo, M.N., 2016. Large-scale degradation of Amazonian freshwater ecosystems. Glob. Change Biol. 22, 990–1007.

Castello, L., et al., 2013. The vulnerability of Amazon freshwater ecosystems. Conserv. Lett. 6, 217–229.

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography– tandem mass spectrometry. Anal. Chem. 78, 8421–8429.

Clarke, K.R., Gorley, R.N., 2015. Getting started with PRIMER v7. Primer–E. Plymouth, Plymouth Marine Laboratory, p.20.

Côrtes, J.C., D'Antona, Á.D.O., Ojima, R., 2020. Extended urbanization and rural reconfiguration in the Amazon: a theoretical-methodological proposal based on demographic and spatial indicators. Rev. Bras. Est. Urb. Reg. 22.

de Carvalho Aguiar, V.M., Neto, J.A.B., Rangel, C., 2011. M., Eutrophication and hypoxia in four streams discharging in Guanabara Bay, RJ, Brazil, a case study. Mar. Pollut. Bull. 62, 1915–1919.

de Souza, T.C., da Silva, S.L.R., Marcon, J.L., Waichman, A.V., 2020. Acute toxicity of deltamethrin to Amazonian freshwater fish. Toxicol. Environ. Health Sci. 1–7.

de Zwart, D., Posthuma, L., 2005. Complex mixture toxicity for single and multiple species: Proposed methodologies. Environ. Toxicol. Chem. 24, 2665–2676.

Fabregat-Safont, D., Ibáñez, M., Bijlsma, L., Hernández, F., Waichman, A.V., de Oliveira, R., Rico, A., 2021. Wide-scope screening of pharmaceuticals, illicit drugs and their metabolites in the Amazon River. Water Research 200, 117251. https://doi.org/10.1016/j.watres.2021.117251.

Fekadu, S., Alemayehu, E., Dewil, R., Van der Bruggen, B., 2019. Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. Sci. Total Environ. 654, 324–337.

Flippin, J.L., Huggett, D., Foran, C.M., 2007. Changes in the timing of reproduction following chronic exposure to ibuprofen in Japanese medaka, *Oryzias latipes*. Aquat Toxicol. 81, 73–78.

Gerolin, C.R., Pupim, F.N., Sawakuchi, A.O., Grohmann, C.H., Labuto, G., Semensatto, D., 2020. Microplastics in sediments from Amazon rivers, Brazil. Sci. Total Environ. 749, 141604.

Haffer, J., 2008. Hypotheses to explain the origin of species in Amazonia. Brazil J. Biol. 68, 917–947.

IBGE, 2020. Brazilian Institute of Geography and Statistics. Brazilian Cens. Available at: https://www.ibge.gov.br/ Accessed on September 23rd of 2020.

Jaiswal, D., Pandey, J., 2019. Hypoxia and associated feedbacks at sediment-water interface as an early warning signal of resilience shift in an anthropogenically impacted river. Environ. Res. 178, 108712.

Junk, W.J., Soares, M.G.M., Bayley, P.B., 2007. Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. Aquat. Ecos. Health Manage. 10, 153–173.

Latrubesse, et al., 2017. Damming the rivers of the Amazon basin. Nature 546, 363–369.

Laurance, W.F., et al., 2001. The Future of the Brazilian Amazon. Science 291, 438–439.

Lawrence, J.R., et al., 2012. Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities. Environ. Toxicol. Chem. 31, 508–517.

Mayo-Bean, K., Moran, K., Meylan, B., Ranslow, P., 2012. Methodology document for the ecological structure-activity relationship model (ECOSAR) class program. US-EPA, Washington DC, p.46.

Oosterhuis, M., Sacher, F., ter Laak, T.L., 2013. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Sci. Total Environ. 442, 380–388.

Parolini, M., 2020. Toxicity of the non-steroidal anti-inflammatory drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: A review. Sci. Total Environ. 720, 140043.

Pedrouzo, M., Borrull, F., Pocurull, E., Marcé, R.M., 2011. Presence of pharmaceuticals and hormones in waters from sewage treatment plants. Wat Air Soil Pollut. 217, 267–281.

Pilière, A., et al., 2014. Comparing responses of freshwater fish and invertebrate community integrity along multiple environmental gradients. Ecol. Ind. 43, 215–226.

Pires, A., et al., 2016. Long-term exposure to caffeine and carbamazepine: Impacts on the regenerative capacity of the polychaete *Diopatra neapolitana*. Chemosphere 146, 565–573.

Pires, N.L., Passos, C.J.S., Morgado, M.G., Mello, D.C., Infante, C.M.C., Caldas, E.D., 2020. Determination of glyphosate, AMPA and glufosinate by high performance liquid chromatography with fluorescence detection in waters of the Santarém Plateau, Brazilian Amazon. J. Environ. Sci. Health B 55, 794–802.

Posthuma, L., De Zwart, D., 2006. Predicted effects of toxicant mixtures are confirmed by changes in fish species assemblages in Ohio, USA, rivers. Environ. Toxicol. Chem. 25, 1094–1105.

Posthuma, L., Traas, T.P., Suter, G.W., 2002. Species sensitivity distributions in risk assessment. CRC Press, Boca Raton (FL), 564 p.

Posthuma, L., van Gils, J., Zijp, M.C., van de Meent, D., de Zwart, D., 2019. Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12,386 chemicals. Environ. Toxicol. Chem. 38, 905–917.

Rico, A., Geber-Corrêa, R., Campos, P.S., Garcia, M.V., Waichman, A.V., van den Brink, P. J., 2010. Effect of parathion-methyl on Amazonian fish and freshwater invertebrates: a comparison of sensitivity with temperate data. Arch. Environ. Contam. Toxicol. 58, 765–771.

Rico, A., Waichman, A.V., Geber-Corrêa, R., van den Brink, P.J., 2011. Effects of malathion and carbendazim on Amazonian freshwater organisms: comparison of tropical and temperate species sensitivity distributions. Ecotoxicology 20, 625–634.

Rico, A., et al., 2019. Identification of contaminants of concern in the upper Tagus river basin (central Spain). Part 1: Screening, quantitative analysis and comparison of sampling methods. Sci. Total Environ. 666, 1058–1070.

Rodríguez-Gil, J.L., Cáceres, N., Dafouz, R., Valcárcel, Y., 2018. Caffeine and paraxanthine in aquatic systems: Global exposure distributions and probabilistic risk assessment. Sci. Total Environ. 612, 1058–1071.

Scheurer, M., Michel, A., Brauch, H.J., Ruck, W., Sacher, F., 2012. Occurrence and fate of the antidiabetic drug metformin and its metabolite guanylurea in the environment and during drinking water treatment. Water Res. 46, 4790–4802.

Schiesari, L., Waichman, A., Brock, T., Adams, C., Grillitsch, B., 2013. Pesticide use and biodiversity conservation in the Amazonian agricultural frontier. Phill. Trans. Royal Soc. B 368, 20120378.

Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E., Castiglioni, S., 2015. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res. 74, 23–33.

Sioli, H., 1984. The Amazon, Limnology and landscape ecology of a mighty river and its basin. Dr. W. Junk Publishers. Boston. 800 pp.

Smith, B.T., et al., 2014. The drivers of tropical speciation. Nature 515, 406–409.

SNIS, 2020. Brazilian National System of Information about the sanitation system. Indicators for water supply and sewage. Available at: http://www.snis.gov.br/+ Accessed on September 23rd of 2020.

Ter Braak, C.J.F., Šmilauer, P., 2012. Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.0). Microcomputer Power, Ithaca, NY, USA, p.496.

Tisseuil, C., Cornu, J.-F., Beauchard, O., Brosse, S., Darwall, W., Holland, R., Hugueny, B., Tedesco, P.A., Oberdorff, T., 2013. Global diversity patterns and cross-taxa convergence in freshwater systems. J. Anim. Ecol. 82, 365–376.

Thomas, K.V., et al., 2014. Screening for selected human pharmaceuticals and cocaine in the urban streams of Manaus, Amazonas, Brazil. J. Am. Wat. Res. Associat. 50, 302–308.

UN, United Nations, 2016. Transforming our world: The 2030 agenda for sustainable development. Available at: Transforming our world: the 2030 Agenda for Sustainable Development | Department of Economic and Social Affairs (un.org).

Valdez-Carrillo, M., Abrell, L., Ramírez-Hernández, J., Reyes-López, J.A., Carreón-Diazconti, C., 2020. Pharmaceuticals as emerging contaminants in the aquatic environment of Latin America: a review. Environ. Sci. Pollut. Res. 27, 44863–44891.

Van den Berg, S.J., et al., 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. Environ. Sci. Technol. 53, 6025–6034.

WWF, World Wide Fund for Nature, 2020. Inside the Amazon. Available at: https://wwf.panda.org/knowledge_hub/where_we_work/amazon/about_the_amazon.