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Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon

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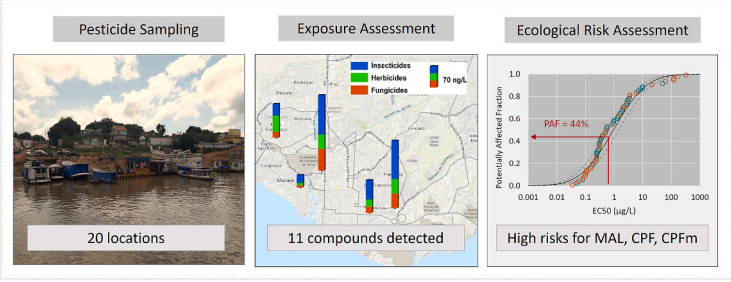
HIGHLIGHTS

- Eleven pesticides were detected in Amazonian freshwater ecosystems.
- A high number of compounds was found in areas impacted by urbanization.
- Largest prevalence for: chlorpyrifos, carbendazim, diuron, atrazine, terbuthylazine.
- Malathion, chlorpyrifos, and chlorpyrifos-methyl pose high ecological risks.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of pesticides in households and peri-urban areas of the Amazon has increased notably during the last years. Yet, the presence of these contaminants in Amazonian freshwater ecosystems remains unexplored. Here, we assessed the exposure to 18 pesticides and 5 transformation products in the Amazon River and in the urban streams of Manaus, Santarém, Macapá, and Belém (Brazil). Pesticide concentrations were analyzed by liquid and gas chromatography methods. Ecological risks were assessed following a two-tiered approach. First, hazard quotients and an overall hazard index were calculated using toxicity data for standard test species of primary producers, invertebrates, and fish. Second, the pesticides showing moderate-to-high ecological risks in the first tier were evaluated using Species Sensitivity Distributions (SSDs). Our study shows that pesticides are widespread in urban and peri-urban areas of the Brazilian Amazon. The frequency of detection was higher in urban streams than in the Amazon River, with some samples taken in Manaus, Santarém, and Belém containing up to 8 compounds. Most pesticides were measured at relatively low concentrations (ng L^{-1}), except for malathion, carbendazim and the bulk concentration of chlorpyrifos, which were monitored at concentrations above 100 ng L^{-1}. Based on the first-tier assessment, we found moderate-to-high risks for freshwater invertebrates for

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malathion, chlorpyrifos, and chlorpyrifos-methyl, and moderate risks for malathion to fish. The risk assessment performed with SSDs indicated high risks of malathion and chlorpyrifos-methyl in urban areas, with up to 15% and 5% of invertebrate species potentially affected, respectively. The bulk concentrations of chlorpyrifos resulted in high risks in some urban areas (14–22% of species affected) and in areas of the main river (32–44%) impacted by agriculture. We conclude that pesticide residues may contribute to a biodiversity impact in the Amazon and should be further monitored in urban and peri-urban areas, particularly after heavy rainfall events.

1. Introduction

The Amazon region is a biome of global importance, hosting a vast diversity of aquatic and terrestrial organisms and playing a key role in global carbon sequestration, water cycling, and climate regulation (Nobre et al., 2016). However, its position in preserving global biodiversity and preventing climate change has not been considered in the development plans implemented in the region, which are responsible for increasing population mobility, disorderly urbanization, and environmental degradation (Barbieri and Monte-mór, 2007). At the end of the 1960s and 1970s, the Brazilian military government encouraged mineral exploration, agribusiness, highways, hydroelectric dams, and large enterprises in the Amazon. Such policy promoted dramatic changes in population dynamics and altered the land occupation patterns in the region. As a result, the Brazilian population living in the Amazon increased from 7% to 13% between 1950 and 2010 and concentrated in urban areas (Castro et al., 2019). Today, about 80% of the Brazilian Amazon population lives in cities (IBGE, 2020).

The rise in urban population has increased the demand for food and boosted small-scale agricultural activities around the large metropolitan areas of the Amazon (Römbke et al., 2008; Parry et al., 2010). The cultivation of non-native fruits and vegetables to serve local urban markets has enhanced pesticide use to combat insect and fungi pests and to prevent competition with other plants (Waichman et al., 2002; Schiesari et al., 2013). Moreover, the lack of education and training of small farmers has contributed to the incorrect use of pesticides in the region. As previously reported, farmers use pesticide doses higher than recommended, apply pesticides at too frequent intervals, and discard packages in inappropriate places (Waichman et al., 2007). Therefore, it is expected that these practices, together with the high rainfall rates in the region, contribute to the spread of pesticides into freshwater ecosystems.

In addition to inadequate agricultural practices, the lack of urban sanitation is another factor contributing to the degradation of freshwater ecosystems in the region. Rapid urbanization hampers the implementation of sanitation at the same rate as cities grow. On average, only 14% of the total Amazonian households are served with sewage collection and treatment, with percentages ranging from 6% to 26% (Viana et al., 2016). Due to the lack of sanitation, the prevalence of endemic vector diseases is high in urban areas of the Amazon (Castro et al., 2019). Pesticides are widely used to control disease vectors such as mosquitoes and barbers, as well as urban and domestic pests such as ants, termites, and cockroaches (Fernandes et al., 2020). Although there are no specific data on the use of domestic pesticides in the Amazon, the study conducted by Diel et al. (2003) indicates that 89% of Brazilian households use pesticides for these purposes.

Some studies claim that the concentration of the populations in urban centers decreases the overall human footprint on the Amazon (Wright and Muller-Landau, 2006; Young, 2006; Parry et al., 2010). However, other studies have shown that the intensification of peri-urban agriculture promoted by the population concentration in urban areas and the lack of a proper sanitation system strongly affects the structure and functioning of streams and rivers in the Amazon (Cak et al., 2016). The few existing studies assessing the impacts of urbanization on the water quality of Amazonian freshwater ecosystems have focused on evaluating loads of organic matter, nutrients and basic water quality parameters (Couceiro et al., 2007; Pinto and Pascoaloto, 2009; dos Sousa et al., 2011), and to a lower extent, the presence of emerging contaminants such as pharmaceuticals, personal-care products and illicit drugs (Thomas et al., 2014; Fabregat-Safont et al., 2021; Rico et al., 2021). However, to date, no studies have assessed the environmental exposure to pesticides in urban and peri-urban areas of the Amazon.

The objective of this study was to assess the occurrence of pesticides in urban areas of the Brazilian Amazon and to assess their risks for freshwater ecosystems. For this, we analyzed exposure concentrations of 18 pesticides and 5 transformation products in samples taken in the Amazon River and in streams crossing the cities of Manaus, Santarém, Macapá, and Belém. Through this study, we identified pesticide compounds that may be contributing to a biodiversity loss in freshwater ecosystems surrounding urban areas and provide recommendations to guide further pesticide monitoring efforts in the region.

2. Materials and methods

2.1. Sampling campaign

A sampling campaign was performed between the 17th of November and the 7th of December of 2019. The period coincided with the end of the dry season, in a moment in which some showers were starting to occur (principally in Manaus). Water samples were collected from 20 different locations (Fig. 1). Samples were taken from the Amazon River, upstream of Manaus (n = 1), next to the urban area of Manacapuru; from the Anavilhanas National Park, Negro River (n = 1), which was expected not to show pesticide impacts due to the low population density in the area; and from streams crossing the urban areas of Manaus (n = 6), Santarém (n = 3), Macapá (n = 4) and Belém (n = 5). The sampling locations in Macapá and the Tocantins River and its tributaries in Belém were subjected to tidal effects. Therefore, samples were taken in these locations with a low tide to avoid dilution by upstream tidal currents. 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 filtered through a 0.7 μm glass fiber filter (Merck Millipore, Cork, IRL). Then, they were subjected to solid-phase extraction (SPE) in batches of 4–8 samples. Water samples (500 or 1000 mL) were loaded into SPE cartridges (Oasis HLB Waters, 500 mg) previously conditioned with 5 mL methanol and 5 mL of ultra-pure water in duplicate. 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 labelled, sealed with parafilm, and stored at −20 °C.

Two different analytical approaches were implemented: method A and B (Table 1). The analysis by method A was done at the Institute of Chemistry of the University of Campinas (Brazil). The analysis by method B was done at the Earth and Environmental Sciences Department of the University of Milano Bicocca (Italy). For method A, the analytes were recovered from the SPE cartridges with 4 mL of methanol followed by 4 mL of acetonitrile. The extract was reduced to dryness under a gentle stream of nitrogen gas, then brought to a final

concentration factor of 1000x using water:methanol 70:30 (v/v). The final extract was filtered in a syringe with a pore size of 0.22 μm and quantitatively transferred to a vial. Chromatographic separation was performed on an Agilent HPLC 1200 coupled with a Zorbax SB-C18 column (30 × 2.1 mm i.d., 3.5 μm). Target compounds were analyzed in an Agilent QqQ 6410B by electrospray ionization (ESI) combined with multiple-reaction monitoring (MRM). For the compounds analyzed in positive ionization mode, the mobile phase was composed of solvent A (0.01% formic acid in water) and solvent B (100% methanol). For the ones with negative ionization, the mobile phase of solvent A was 0.01% ammonium hydroxide in water, while solvent B was the same. The conditions for the chromatography and mass spectrometry method used in method A are summarized in Table S2. At the same time, the MRM transitions and the respective collision energies for the different compounds are provided in Table S3. Method validation was performed according to the National Institute of Metrology Standardization and Industrial Quality (Inmetro) and the Brazilian Health Regulatory Agency guidelines (Anvisa). For this, we evaluated linearity (always above 0.99), as well as the method recovery and precision (expressed as relative standard deviation) at three fortification levels (in triplicate).

For method B, 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. Then, they were evaporated to 0.05 mL and transferred into glass vials. Samples with concentrations higher than 50 ng mL^{-1} were diluted before re-injection for the quantification analysis. The identification and quantification 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^{-1} 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 of 0.1–100 μg L^{-1}. A summary of the chromatography and mass spectrometry conditions, as well as the MRM transitions and collision energies used by method B, are provided in Tables S4 and S5, respectively. For these compounds, we evaluated linearity (always above 0.99), the method recovery, and precision (expressed as relative standard deviation) at two fortification levels (in triplicate). Additionally, we analyzed the bulk concentration of the insecticide chlorpyrifos and chlorpyrifos-methyl in the water sample, which accounts for the dissolved fraction and the fraction sorbed to particulate organic matter. This was done as we expected to find notable differences between the dissolved and the bulk concentration given to its hydrophobic properties (log K_{ow} = 4.7 and 4, respectively). The extraction method used to analyze the insecticide concentrations in suspended solids is described in the Supplementary Information.

The analytical method recoveries and precision levels for the analyzed compounds are provided in Table S6, together with the calculated limits of detection (LODs) and quantification (LOQs). Measured water concentrations were corrected when the average recovery fell outside the recommended range (70–120%) following the European Commission guidelines (EC, 2000). This was the case of malathion, fipronil, atrazine and carbendazim (Table S1). For these substances, the uncertainty regarding the exposure and risk characterization may be higher as compared to the other substances. Two field blanks (based on Milli-Q water) were analyzed with the described methodologies along with the analyzed samples. No pesticide residues were found in the field blanks.

2.3. Ecological risk assessment

Risks for freshwater ecosystems were calculated following a tiered approach. The first-tier assessment was based on Hazard Quotients (HQs). HQs were calculated for primary producers, invertebrates, and fish using acute toxicity data. HQs for each pesticide and species combination were derived by dividing the measured environmental concentration by a Predicted No Effect Concentration (PNEC) or reference value, derived by dividing the toxicity value of the selected sentinel species by an assessment factor of 100. PNECs for primary producers were calculated with the EC50–72/96 h (growth inhibition) for the green algae *Raphidocelis subcapitata*. PNECs for freshwater invertebrates were calculated with EC50–48 h (immobilization) for *Daphnia magna*, while PNECs for fish were estimated with the LC50–96 h for the fish *Oncorhynchus mykiss*. Toxicity data for the pesticides detected in this study were retrieved from the Pesticide Properties Database (Lewis et al., 2016) and are shown in Table S7. Finally, the Hazard Index (HI) was calculated for each taxonomic group as the sum of the individual

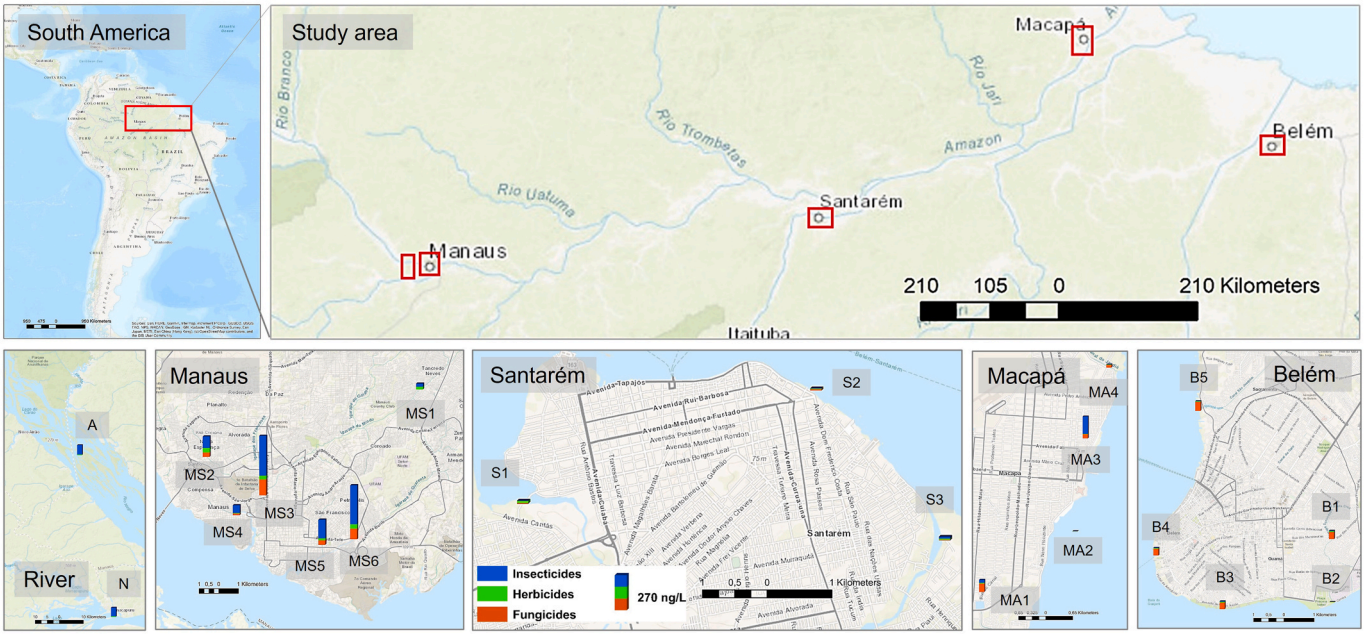

Fig. 1. Sampling locations and sum of exposure concentrations in the sampling locations. GPS coordinates and further details on the sampling locations are provided in the Supplementary Information (Table S1).

Table 1
List of evaluated compounds, frequency of detection (% of samples), measured concentrations (ng L^{-1}), and the total number of compounds found in the different samples. Sample codes refer to the Amazon River (A), Negro River (N), and the streams in Manaus, Santarém, Macapá, and Belém. Transformation products are indicated in italics. 'Lower than' values indicate that the compound was detected below the LOQ. Bulk concentrations of chlorpyrifos and chlorpyrifos-methyl are indicated in bold (between brackets). Empty cells mean the compound was not detected in the given sample.

		River		Manaus						Santarém			Macapá				Belém				
Pesticides	Freq (%)	A	N	MS1	MS2	MS3	MS4	MS5	MS6	S1	S2	S3	MA1	MA2	MA3	MA4	B1	B2	B3	B4	B5
Insecticides																					
Carbofuran[a]	0% [c]																				
Imidacloprid[a]	0% [c]																				
Malathion[a]	50%	42	46	42	154	535	104	242	523				46		300						
Chlorpyrifos[b]	80% [c]	**(700)**	**(365)**	**(0.5)**	<1.3**(4)**	<1.3**(4)**		2**(2)**		<1.3**(2)**	**(0.4)**	**(0.9)**	**(3)**	**(3)**	**(186)**		<1.3**(0.3)**	**(4)**	3**(100)**	**(0.7)**	[d]
Chlorpyrifos-methyl[b]	50% [c]	**(0.2)**			**(0.4)**	**(0.01)**	**(0.1)**			**(0.02)**		12**(12)**		**(0.01)**	**(0.3)**				<1.5**(0.3)**	**(0.1)**	[d]
Fipronil[a]	55% [c]							6			2	2	9	2	4	2	6		4	6	4
Herbicides																					
Diuron[a]	70% [c]			3	9	13	3	1	4	1	1	3	4		2				3	2	3
Metolachlor[b]	40% [c]			3	27	7		5	13			0.3					4		2		
2,4-D[a]	35%			3		4			3	5							3			4	2
Pendimethalin[b]	0%																				
Ametrine[a]	0% [c]																				
Atrazine[a]	70% [c]	3	3		15			4	25	12	4	3		3		3	3	4	7	7	4
2-hydroxyatrazine[a]	0%																				
Deethylatrazine[a]	0%																				
Deisopropylatrazine[a]	0%																				
Terbuthylazine[b]	70%			28	11	26	5	16	11			3	9	<0.2	5	3	7		4	1	
Terbuthylazine-desethyl[b]	0%																				
Simazine[a]	0% [c]																				
Hexazinone[a]	0% [c]																				
Tebuthiuron[a]	10% [c]								3									2			
Fungicides																					
Carbendazim[a]	80% [c]				58	214	22	61	142	6	11	6	147		72	47	125	3	117	119	161
Azoxystrobin[a]	0%																				
Tebuconazole[a]	0%																				
Total compounds		**4**	**3**	**6**	**8**	**8**	**5**	**8**	**8**	**5**	**4**	**8**	**6**	**5**	**7**	**4**	**7**	**4**	**8**	**8**	**5**

[a] Compounds analyzed by method A.
[b] Compounds analyzed by method B.
[c] Compound banned according to the European Commission Regulation 1107/2009.

HQs assuming additivity. Ecological risks were classified as low or insignificant when the HI was lower than 1, moderate when the HI was between 1 and 10, and high when the HI was higher than 10.

The compounds that showed moderate or high risks in the first-tier assessment were evaluated using acute Species Sensitivity Distributions (SSDs; Posthuma et al., 2002). The toxicity data used to build the SSDs were obtained from the US EPA ECOTOX database (ECOTOX, 2020). The exposure duration and assessment endpoints of the toxicity data selected for this study were based on the criteria described by Rico et al. (2019). The SSDs were calculated using the ETX 2.3 software (Van Vlaardingen et al., 2004), which is based on a log-normal distribution. We calculated the Potentially Affected Fraction (PAF) of species by each exposure concentration of the monitored pesticides following the methods established by Aldenberg and Jaworska (2000). Ecological risks were defined as low or insignificant when the PAF was lower than 5% of species and high when the calculated PAF was equal to or higher than 5% of species.

3. Results and discussion

3.1. Pesticide exposure concentrations

The results of our study show that all evaluated samples contained pesticide residues, with some samples taken in the urban streams of Manaus, Santarém, and Belém containing up to eight different compounds (Table 1). The sample taken in the Negro River contained the lowest number of compounds. The largest total dissolved pesticide concentrations (>100 ng L^{-1}) were found in the urban streams of Manaus (MS2, MS3, MS4, MS5, MS6), Macapá (MA1, MA3) and Belém (B1, B3, B4 B5; Fig. 1). Overall, the compounds with the highest contribution to the total pesticide concentration were insecticides, followed by fungicides and herbicides. Among insecticides, the compound with the highest total concentration was malathion, with a maximum concentration of 535 ng L^{-1} in MS3. The fungicide showing the highest water concentration was carbendazim, with concentrations up to 214 ng L^{-1} in MS3. Regarding herbicides, most exposure concentrations were relatively low (few ng L^{-1}), except for metolachlor, atrazine, and terbuthylazine, with concentrations up to 25–28 ng L^{-1} in some streams of Manaus. Chlorpyrifos and chlorpyrifos-methyl were primarily sorbed to the particulate organic matter that was retained in the filters. The highest bulk concentrations of chlorpyrifos were recorded in the samples taken in the Amazon River (700 ng L^{-1}), in the Negro River (365 ng L^{-1}), and in one stream of Macapá (186 ng L^{-1}) and one of Belém (100 ng L^{-1}), while chlorpyrifos-methyl was found at very low concentrations (Table 1).

The compounds with the highest frequency of detection were the insecticide chlorpyrifos and the fungicide carbendazim (found in 80% of the samples), followed by the herbicides diuron, atrazine, and terbuthylazine (70%), and the insecticides fipronil (55%), malathion and chlorpyrifos-methyl (50%; Table 1). All these compounds (except for malathion) were found in all urban areas. Some of these pesticides are among the most used in Brazil. For example, atrazine occupies 5th place in sales in Brazil, malathion 8th, and chlorpyrifos 10th (IBAMA, 2020). The most frequently detected pesticides are regularly used in agriculture. However, malathion and chlorpyriphos are also used within urban settlements. Malathion is used for insect control in urban gardens (Andrighetti et al., 2013). Although chlorpyrifos is only allowed for agricultural use in Brazil and has severe restrictions for domestic use, it continues to be widely used inside households against ants (da Cruz et al., 2000) and to combat cockroaches in the form of granulated baits (Brasil, 2004).

Our study shows that the number of compounds and the total dissolved exposure concentrations are generally larger in the streams of Manaus, followed by Belém and Macapá (similar concentrations) and Santarém. This may be explained by the larger concentration of peri-urban agricultural activities in Manaus as compared to other cities. In addition, there were heavy rainfall events during or shortly before the samplings performed in Manaus, which could have influenced runoff and transport from upstream areas, including small plantations and back-yard agriculture in the outskirts of the city. This supports the need to conduct follow-up studies assessing concentration peaks after heavy rainfall events in the region. It is important to highlight the presence of pesticides in the waters of the Negro River where there is little development of agriculture due to the low fertility of soils (Quesada et al., 2011). Thus, the pesticides found are possibly of domestic origin, used to control insects, such as ants, and vectors of diseases, mainly mosquitoes.

Most of the monitored pesticides (8 out of 11) are currently banned in Europe due to their potential environmental and/or human health hazards (Table 1). Brazil has a simplified system of registration of pesticides, which is based on equivalence with authorizations in Europe and North America. This means that compounds that were authorized elsewhere are allowed to be used in Brazil for similar uses. Since the Brazilian registration system is not regularly updated, the patent expiration or ban in the reference countries is not incorporated into the Brazilian system, resulting in the continued use of obsolete products, which generally have greater toxicity and environmental persistence. Furthermore, the regulatory monitoring of pesticides in Brazil focuses on comparing measured concentrations with thresholds established for drinking water, rendering the health of freshwater ecosystems unattended (Brovini et al., 2021). Further work should be undertaken to develop agricultural and domestic pesticide exposure scenarios for the Brazilian Amazon and to update the current ecological risk assessment scheme.

3.2. Ecological risk assessment

The first-tier risk assessment performed with the measured concentrations of the pesticides indicated potential toxicological risks for invertebrates. HIs larger than 1 were calculated in 12 samples, with 6 showing high ecological risks (HIs >10; Fig. 2). The toxicity of the sample was generally dominated by one or two compounds, with malathion having the largest contribution to the calculated HIs, followed by chlorpyrifos (in samples B3 and MS5) and chlorpyrifos-methyl (in sample S3). The rest of compounds had a very low contribution to the total toxicity for invertebrates (with HQs<0.1). The first-tier risk assessment indicated moderate toxicological risks (1 < HIs<10) for fish in 4 out of the 20 evaluated samples. In these samples, the toxicity was clearly dominated by malathion. The calculated first-tier risks for primary producers were low or insignificant in all samples. The first-tier risk assessment performed with the bulk concentration of chlorpyrifos indicated moderate-to-high risks for invertebrates (RQs>1 in 11 samples). The acute RQs for invertebrates reached values above 100 in the samples taken in the Amazon River, in the Negro River, and one stream of Macapá (MA3) and Belém (B3; Fig. 3). The same calculations performed with chlorpyrifos-methyl indicated low or insignificant risks, except for sample S3, which indicated moderate risks for invertebrates (RQ = 2).

Based on the first-tier risk assessment, invertebrate SSDs were built for malathion, chlorpyrifos, and chlorpyrifos-methyl, including only insect and crustacea (Fig. 4), as they are significantly more sensitive than the rest of invertebrate taxa (Maltby et al., 2005). Based on the dissolved concentrations, the highest toxicological risks were found for malathion, with PAFs equal or above 5% of species in five samples taken in Manaus (MS2, MS3, MS4, MS5, and MS6) and one taken in Macapá (MA3), reaching a maximum value of 15% of species potentially affected. Chlorpyrifos-methyl also showed a PAF of 5% of species in one sample (S3). However, it must be noted that the calculated SSD for this compound was based on toxicity data for 5 taxa (Table S8), which is lower than the minimum number of toxicity data recommended (Wheeler et al., 2002), so the uncertainty around this risk calculation is larger than for the other evaluated compounds. The risk assessment for chlorpyrifos based on dissolved concentrations showed insignificant

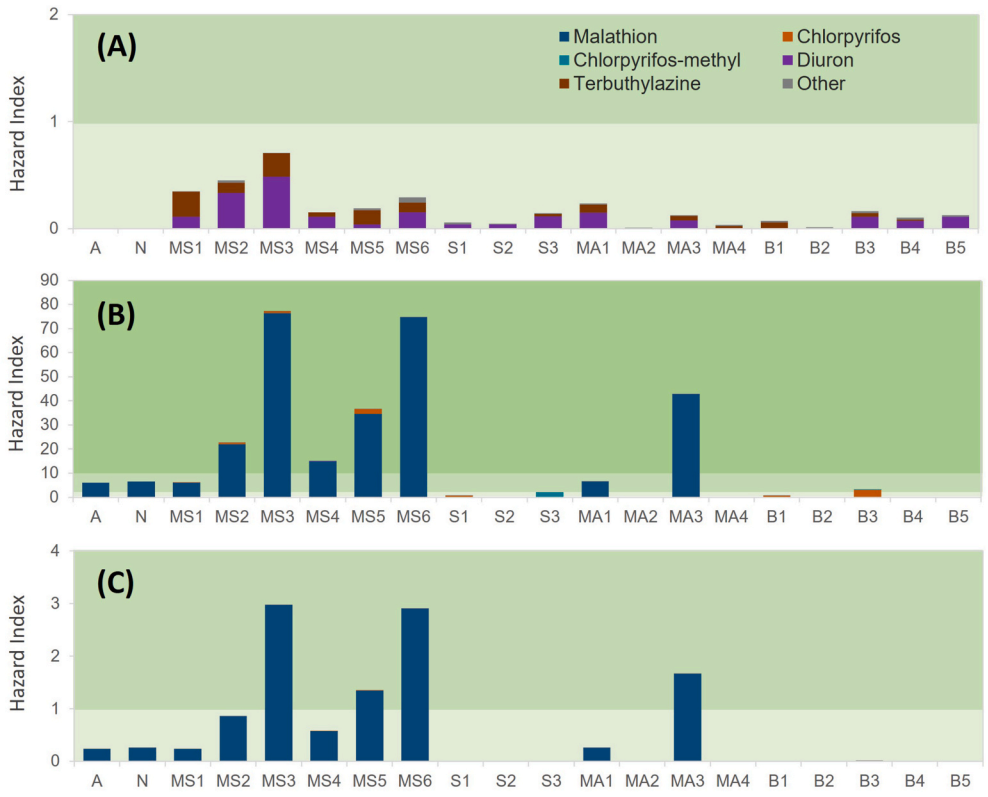

Fig. 2. Calculated Hazard Index for (A) primary producers, (B) invertebrates, and (C) fish, and contribution of the different pesticides to the total toxicity. Only compounds with Hazard Quotients above 0.1 are displayed, while the rest are grouped as "Others". The shaded area in light, medium, and intense green color indicates low (HI < 1), moderate (1 ≤ HI ≤ 10), and high (HI > 10) ecological risks, respectively. A: Amazon River; N: Negro River; MS: Manaus; S: Santarém; MA: Macapá; B: Belém. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

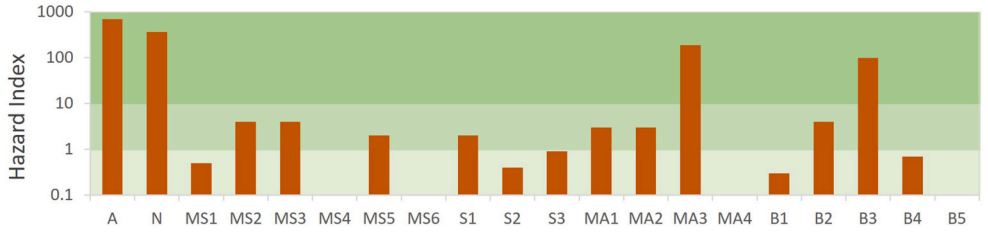

Fig. 3. Hazard Quotients for invertebrates calculated with the bulk concentration of chlorpyrifos in the different samples. The shaded area in light, medium, and intense green color indicate low (HQ < 1), moderate (1 = HQ ≤ 10), and high (HQ > 10) ecological risks, respectively. A: Amazon River; N: Negro River; MS: Manaus; S: Santarém; MA: Macapá; B: Belém. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

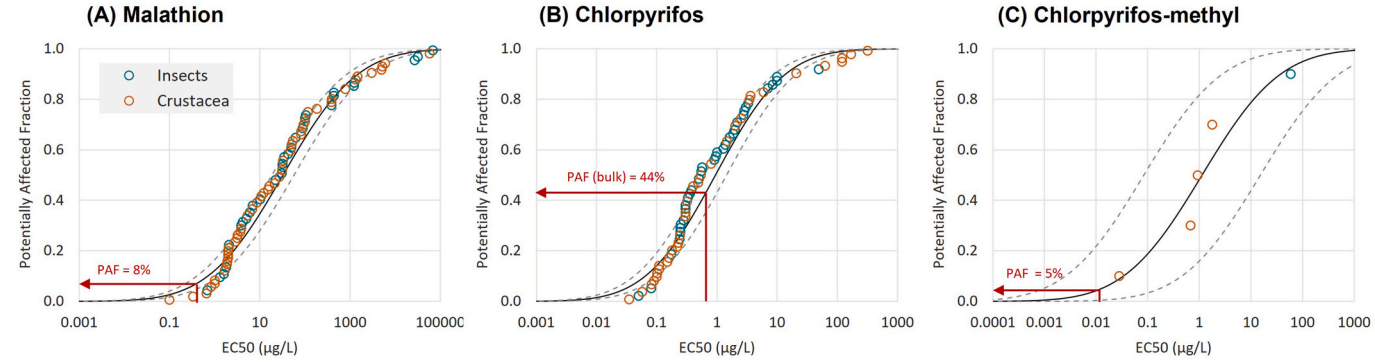

Fig. 4. Invertebrate Species Sensitivity Distributions (SSDs) for malathion, chlorpyrifos, and chlorpyrifos-methyl. The graphs also show the Potentially Affected Fraction (PAF) of species calculated with the highest measured pesticide concentration. The number of taxa available and the parameters of the calculated SSDs are provided in Table S8.

risks (with PAFs<1%). However, the risk assessment performed with the bulk concentration of chlorpyrifos resulted in PAFs above 5% in 4 samples. The highest PAF was calculated in the sample taken in the Amazon River (44% of species), followed by the Negro River (32%) and two samples taken in urban streams: Macapá (22%, MA3) and Belém (14%, B3).

According to the results of the first-tier risk assessment, an SSD for malathion and fish species was built (Table S8). However, the calculated PAF for the measured concentrations resulted in values below 0.1% (Table 2), indicating that the direct effects posed by this substance to fish communities are insignificant.

Overall, this study shows that pesticide pollution from urban and peri-urban areas can notably affect the biodiversity of Amazonian freshwater ecosystems. Although the number of substances contributing to the risk is low (principally one in each sample), the measured concentration levels are expected to affect (at least temporarily) some insect and crustacean populations. Based on the toxicity data used to construct the SSDs, we may expect a decline of small crustaceans (Cladocera) and insects (Ephemeroptera) in the areas impacted with the highest malathion and chlorpyrifos concentrations (Fig. 4), while some large crustaceans such as freshwater shrimps may also be impacted by chlorpyrifos-methyl. It must be noted that the toxicity data used to build the SSDs used in this study was primarily obtained from laboratory experiments performed with species representative of the temperate region. However, first investigations on the toxicity of pesticides to Amazonian freshwater organisms did not identify significant differences in sensitivity between Amazonian and temperate taxa (Rico et al., 2010, 2011; de Souza et al., 2020), suggesting that the evaluation of pesticide risks based on SSDs that integrate Amazonian (or other tropical taxa) and temperate aquatic organisms will not yield unprotective results at the community level. However, some populations may be more sensitive to the effects of given pesticides under (sub-)tropical conditions. For example, several authors have found that some Ephemeroptera, Diptera and Cyclopoid taxa are significantly more sensitive to the insecticide imidacloprid than their temperature counterparts (Sumon et al., 2018; Merga and Van den Brink, 2021; Van de Perre et al., 2022), and Daam and Rico (2018) found that (sub-)tropical shrimps are generally more sensitive than *Daphnia magna* to selected pesticide groups (e.g. sodium channel modulators, GABA-gated chloride channel antagonists). The use of native species for the first-tier risk assessment of pesticides in the tropics will reduce uncertainties in the extrapolation of temperate toxicity and will provide opportunities for the biomonitoring of pesticide in the field (Daam and Rico, 2018; Raymundo et al., 2019). Therefore, further research towards characterizing the sensitivity of Amazonian local taxa and the development of standard test protocols is recommended.

The high risks calculated for chlorpyrifos sorbed to particulate organic matter in the Amazon River (near Manacapuru) and in the Negro River suggest that this pesticide may affect some invertebrate taxa with specific feeding habits (e.g., filter feeders, detritivores). Follow-up investigations should be conducted to assess the occurrence of chlorpyrifos in sediments adjacent to agricultural areas and to assess their transport capacity downstream. Furthermore, the decline of invertebrate biomass in lakes and other refugee areas next to the Amazon River may contribute to a food source decline for fish and other predators (birds). Therefore, it is of paramount importance to reinforce pesticide monitoring and regulation in urban and peri-urban areas and to invest in education programs to foster safe pesticide use practices in the region.

4. Conclusions

Here we provide the first comprehensive evaluation of pesticide contamination in urban and peri-urban areas of the Brazilian Amazon. We identified the presence of 11 compounds in surface water samples (4 insecticides, 6 herbicides and 1 fungicide). The compounds showing the highest prevalence in the analyzed samples were: chlorpyrifos,

Table 2
Calculated invertebrate Potentially Affected Fraction (%) for the pesticides that showed potential risk (HQ > 1) in the first-tier assessment. PAFs equal to or higher than 5% indicate high ecological risks and are marked in bold. n.d.: compound not detected in the sample. -: filter was lost.

	River		Manaus						Santarém			Macapá				Belém				
Pesticides	A	N	MS1	MS2	MS3	MS4	MS5	MS6	S1	S2	S3	MA1	MA2	MA3	MA4	B1	B2	B3	B4	B5
Invertebrates																				
Malathion	4	4	4	**8**	**15**	**7**	**10**	**15**	n.d.	n.d.	n.d.	4	n.d.	**12**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorpyrifos (dissolved)	n.d.	n.d.	n.d.	<0.1	<0.1	n.d.	0.2	n.d.	<0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.	0.3	n.d.	n.d.
Chlorpyrifos (bulk)	**44**	**32**	<0.1	0.4	0.4	n.d.	0.2	n.d.	0.2	<0.1	<0.1	0.3	0.3	**22**	n.d.	<0.1	0.4	**14**	<0.1	-
Chlorpyrifos-methyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**5**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.
Fish																				
Malathion	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.	n.d.	<0.1	n.d.	<0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

carbendazim, diuron, atrazine, and terbuthylazine; while the compounds showing the highest water concentrations (>100 ng L^{-1}) were malathion, carbendazim and chlorpyrifos (sorbed to particulate organic matter). Besides the complexity of the pesticide mixtures identified in Amazonian streams (with some samples containing up to 8 different compounds), our study indicated only a reduced number of substances driving ecological risks. Particularly, malathion, chlorpyrifos, and chlorpyrifos-methyl were identified as posing a potential ecotoxicological risk for freshwater invertebrates. Our study adds crucial information to understand the fate and ecotoxicological risks of pesticides in urban and peri-urban areas of the Northern part of Brazil. Moreover, it suggests that continued monitoring of selected pesticides should be performed in urban streams and in nearby areas of high ecological value, particularly during the rainy season and after heavy rainfall events.

Author contributions

A. Rico: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Writing - original draft. R. Oliveira: Conceptualization; Investigation; Methodology; Writing - review & editing. G. Silva de Souza Nunes: Investigation; Methodology; Writing - review & editing. C. Rizzi: Investigation; Methodology; Writing - review & editing. S.Villa: Investigation; Methodology; Writing - review & editing. B. De Caroli Vizioli: Investigation; Methodology; Writing - review & editing. C.C. Montagner: Investigation; Methodology; Writing - review & editing. A.V. Waichman: Conceptualization; Formal analysis; Investigation; Methodology; Writing - review & editing; Writing - original draft.

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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.132821.

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