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Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon

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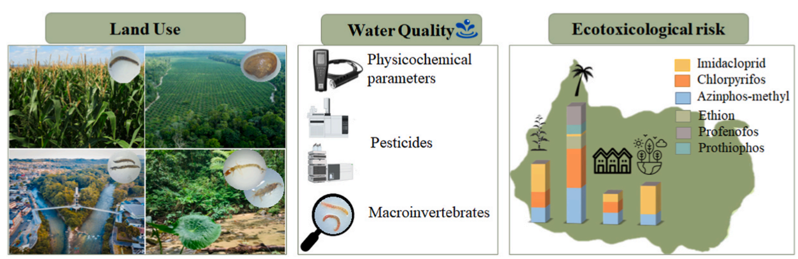
HIGHLIGHTS

- Water quality and pesticide risks were evaluated in the Napo River basin.
- Urban areas and palm oil plantations affect water quality and ecological status.
- 23 pesticides were detected in freshwater ecosystems.
- 20% and 68% of sites present acute and chronic ecological risks, respectively.
- Ecological risks were dominated by organophosphates and imidacloprid.

GRAPHICAL ABSTRACT



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ABSTRACT

The Ecuadorian Amazon has experienced a significant land use change due to the demographic increase and the expansion of the agricultural frontier. Such changes in land use have been associated to water pollution problems, including the emission of untreated urban wastewater and pesticides. Here we provide the first report on the influence of urbanization and intensive agriculture expansion on water quality parameters, pesticide contamination and the ecological status of Amazonian freshwater ecosystems of Ecuador. We monitored 19 water quality parameters, 27 pesticides, and the macroinvertebrate community in 40 sampling locations of the Napo River basin (northern Ecuador), including a nature conservation reserve and sites in areas influenced by African palm oil production, corn production and urbanization. The ecological risks of pesticides were assessed using a probabilistic approach based on species sensitivity distributions. The results of our study show that urban areas and areas dominated by African palm oil production have a significant influence on water quality parameters, affecting macroinvertebrate communities and biomonitoring indices. Pesticide residues were detected in all sampling sites, with carbendazim, azoxystrobin, diazinon, propiconazole and imidacloprid showing the largest prevalence (>80% of the samples). We found a significant effect of land use on water pesticide contamination, with residues of organophosphate insecticides correlating with African palm oil production and some fungicides with urban areas. The pesticide risk assessment indicated organophosphate insecticides (ethion, chlorpyrifos, azinphos-methyl, profenofos and prothiophos) and imidacloprid as the compounds posing the

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largest ecotoxicological hazard, with pesticide mixtures potentially affecting up to 26–29% of aquatic species. Ecological risks of organophosphate insecticides were more likely to occur in rivers surrounded by African palm oil plantations, while imidacloprid risks were identified in corn crop areas as well as in natural areas. Future investigations are needed to clarify the sources of imidacloprid contamination and to assess its effects for Amazonian freshwater ecosystems.

1. Introduction

The northern Amazon of Ecuador has been characterized as one of the areas with the highest biodiversity in the world (De la Torre et al., 2012; Viteri-Salazar and Toledo, 2020). It is made up of important wetlands and hosts the main hydrographic basins of Ecuador, which give rise to the Napo River, the main tributary of the upper Amazon River. In recent decades, the Ecuadorian Amazon has experienced a significant land use change due to the expansion of the agricultural frontier, which includes the substitution of forested land by cattle raising areas and monocultures of African palm oil, corn, naranjilla, cacao and banana (López, 2022; Vasco et al., 2021). The expansion of the agricultural frontier has been accelerated by the population growth in the region (Huera-Lucero et al., 2020) and the establishment of mining and other industrial activities (Capparelli et al., 2021; Galarza et al., 2021), which are supported by different public policies (Viteri-Salazar and Toledo, 2020). The expansion of the productive sector and the fast population increase have made it difficult for local governments to establish suitable management plans to control wastewater and solid waste emissions, and to regulate the use of pesticides in agriculture. In the Ecuadorian Amazon, 56% of untreated wastewater is discharged directly into rivers and only 25% of wastewater receives any short of treatment (Capparelli et al., 2021). Moreover, chemical pollution from agriculture is expected to reach freshwater ecosystems and protected natural areas, thus having negative impacts for biodiversity in the region (Moulatlet et al., 2021).

Although there is little information on the use of pesticides in the Ecuadorian Amazon, Vasco et al. (2021) recently found that the majority of the Kichwa and mestizo populations of the northern Ecuadorian Amazon use pesticides in their agricultural production. According to FAO. (2021), Ecuador is among the ten countries that increased the use of pesticides per area of cultivated land (14 kg ha^{-1}) in 2019 due to changes in farming practices and the industrialization of agriculture. One of the consequences of pesticide use in the Ecuadorian Amazon basin is the increased occupational exposure of agricultural workers (Hurtig et al., 2003). In Latin America, most people in charge of pesticide applications lack knowledge on proper pesticide use and handling (Carriquiriborde et al., 2014; Waichman et al., 2002), and have limited knowledge as to what the consequences of chemical pollution may be for human and ecosystem health (Mollocana Lara and Gonzales-Zubiate, 2020).

In general, water quality monitoring studies in Ecuador are scarce due to limited economic investments and the lack of analytical capabilities. The Environmental Quality and Effluent Discharge Regulation for Water Resources (TULSMA) of Ecuador (MAE, 2015) establishes the parameters for the control of water pollution, which includes water quality criteria for few physicochemical parameters (i.e., pH, dissolved oxygen, nitrite, nitrate), and the maximum total concentration of organophosphate and organochlorine pesticide residues that may be permitted in freshwater ecosystems. However, monitoring studies assessing exposure to insecticides of other chemical groups, herbicides or fungicides are very limited. Some research studies have identified the presence of carbamates, neonicotinoids and triazines in surface water samples collected in the Guayas river basin (Deknock et al., 2019) and in mangrove areas (Andrée et al., 2021) of Ecuador, which are highly impacted by coastal rice and banana plantations. These studies pointed at the herbicide diuron, the fungicide carbendazim, and the insecticide cadusafos as the substances posing the largest ecotoxicological hazard for aquatic organisms. Moreover, these studies suggested the need to perform large-scale monitoring campaigns to characterize pesticide contamination and risks for freshwater ecosystems in the Amazonian part of the country, where different agricultural practices are employed.

Therefore, the main objectives of this study were: (1) to assess the influence of different agricultural crops and urbanization on water quality parameters of the Ecuadorian Amazon, (2) to identify individual compounds and pesticide mixtures that may be posing an ecotoxicological risk for freshwater ecosystems, and (3) to assess the impact of anthropogenic pollution on Amazonian freshwater ecosystems using macroinvertebrate biomonitoring indices. For this, we monitored 19 water quality parameters and 27 pesticides belonging to eight groups (i.e., strobilurin, benzothiazinone, carbamate, conazole, neonocotinoid, triazine, pyridalyl, and organophosphorus) in 40 sampling locations of the Napo basin (northern Ecuador). The sampling locations were selected as being representative of different land use impacts, including natural areas, urban areas, and areas with intensive agriculture dominated by corn and palm oil production. Moreover, the benthic macroinvertebrate community was analyzed, and two biotic indices were calculated to determine the ecological status of the sampled rivers. Our study provides the first report of pesticide concentrations in aquatic ecosystems of the Ecuadorian Amazon. The data generated by this study fills an important knowledge gap and provides a baseline to guide further environmental monitoring efforts in the region. Furthermore, it supports the development of environmental policies for the regulation and control of chemical use in agricultural production areas of the Amazon.

2. Methodology

2.1. Study area and sampling

Forty sampling sites were selected along the tributaries of the Napo River (Northern Ecuador; Fig. 1). Sampling was conducted between February 15th and March of 2022, in the dry season, which can be considered the period in which there is less chemical dilution in surface water bodies. During the field sampling period, average precipitation was approximately 12 mm day^{-1} and temperature 23 °C. Out of the 40 sampling sites, 3 were located in natural areas (NAs), 7 in urban areas (UAs) and 30 in areas impacted by different agricultural crops: 12 by corn crops (CCs) and 18 by African palm crops (PCs) (Fig. 1). Detailed information of the different sampling sites is provided in Table S1. Water samples were collected using plastic bottles (1 L) for the analysis of anions, cations and dissolved organic carbon (DOC) and amber glass bottles (1 L) for pesticides. All samples were collected, as far as possible, in the middle section of each tributary. Then they were transported to the laboratory using refrigerators and stored at −20 °C until further analysis. Additionally, benthic macroinvertebrates were collected using a D-shaped hand net (mesh size 500 μm). With the hand net, a stretch of approximately 10 m was sampled for 3 min in riversides, ponds, under the rocks, and leaf packs to collect organisms from all representative habitats (for further details see: Chancay et al., 2021; Gabriels et al., 2010; Galarza et al., 2021). The macroinvertebrate samples were cleaned on spot with sorting trays and the biological material was stored with alcohol (70%) until further analysis in the laboratory.

2.2. Physicochemical parameter analysis

Water pH, temperature (Temp), electric conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO) were measured *in-situ* using a previously calibrated YSI professional plus multiparameter. DOC, turbidity, color, anions and cations were analyzed in the laboratory. DOC concentrations were measured using a total organic carbon analyzer (TOC-L Shimadzu, Japan). Turbidity was read with the HACH TL 2300 turbidimeter. Apparent color was determined by the method 8025 (HACH, 2014). The water anions (F^-, Cl^-, NO_3^-, NO_2^-, SO_4^-, PO_4^{3-}) and cations (Ca^{2+}, Mg^{2+}, Na^+, NH_4^+, K^+)were analyzed using an ion chromatograph equipped with a Shodex IC-52 4 E column, according to Pfaff (1996) and with a Shodex IC YS-50 column as described in Thomas et al. (2002). To assess whether physicochemical parameters varied from local normative, these values were compared to the environmental quality standards established by the Ecuadorian legislation (MAE, 2015).

2.3. Pesticide analysis

Water samples were filtered through a 0.7 μm glass fiber filter (Merck Millipore, Cork, IRL). Further processing varied according to the analytical method for the detection of the selected pesticides. Organophosphate pesticides were analyzed using an in-house method based on gas chromatography (GC, 2030 Shimadzu) with mass spectrometry detector (MS) (GC-MS) at the Universidad Regional Amazónica Ikiam (Ecuador). The rest of compounds (i.e., strobilurin, benzothiazinone, carbamate, conazole, neonocotinoid, triazine and pyridalyl pesticides) were analyzed by liquid chromatography (1200 Series, Agilent Technologies) coupled to a mass spectrometer triple quadrupole (QQQ) (6495, Agilent Technologies) by electrospray ionization (ESI) and equipped with Jet Stream technology (LC-MS/MS) at the Water Quality Lab of the IMDEA Water Institute (Spain).

The extraction of organophosphate pesticides was carried out using the procedure described by Cruzeiro et al. (2015). Oasis HLB cartridges (Waters, 6 cc, 200 mg) were conditioned with 5 mL of EtOAc and MeOH and then 2.5 mL of MilliQ water. Then, 250 mL of sample were passed through the SPE cartridges (Oasis HLB 6 cc, 200 mg, Waters). Afterwards, the cartridges were rinsed with 10 mL of MilliQ water and dried for 5 min under full vacuum (10 bar) to eliminate residual water. The loaded cartridges were labeled, wrapped in aluminum foil, and stored at −20 °C until analysis. The analytes were eluted with 6 mL EtOAc and concentrated to dryness under a gentle nitrogen stream for approximately 1 h. The extracts were reconstituted with 200 μL of hexane for analysis by GC-MS. The chromatographic conditions are given in Table S2 and the retention times (Tr) in Table S3.

The rest of samples were pre-concentrated using Oasis HLB cartridges (Waters, 6 cc, 200 mg) previously preconditioned with 6 mL of MeOH and 6 mL of MilliQ Water. Next, the cartridges were rinsed with 10 mL of MilliQ water and dried for 5 min under full vacuum (10 bar) to

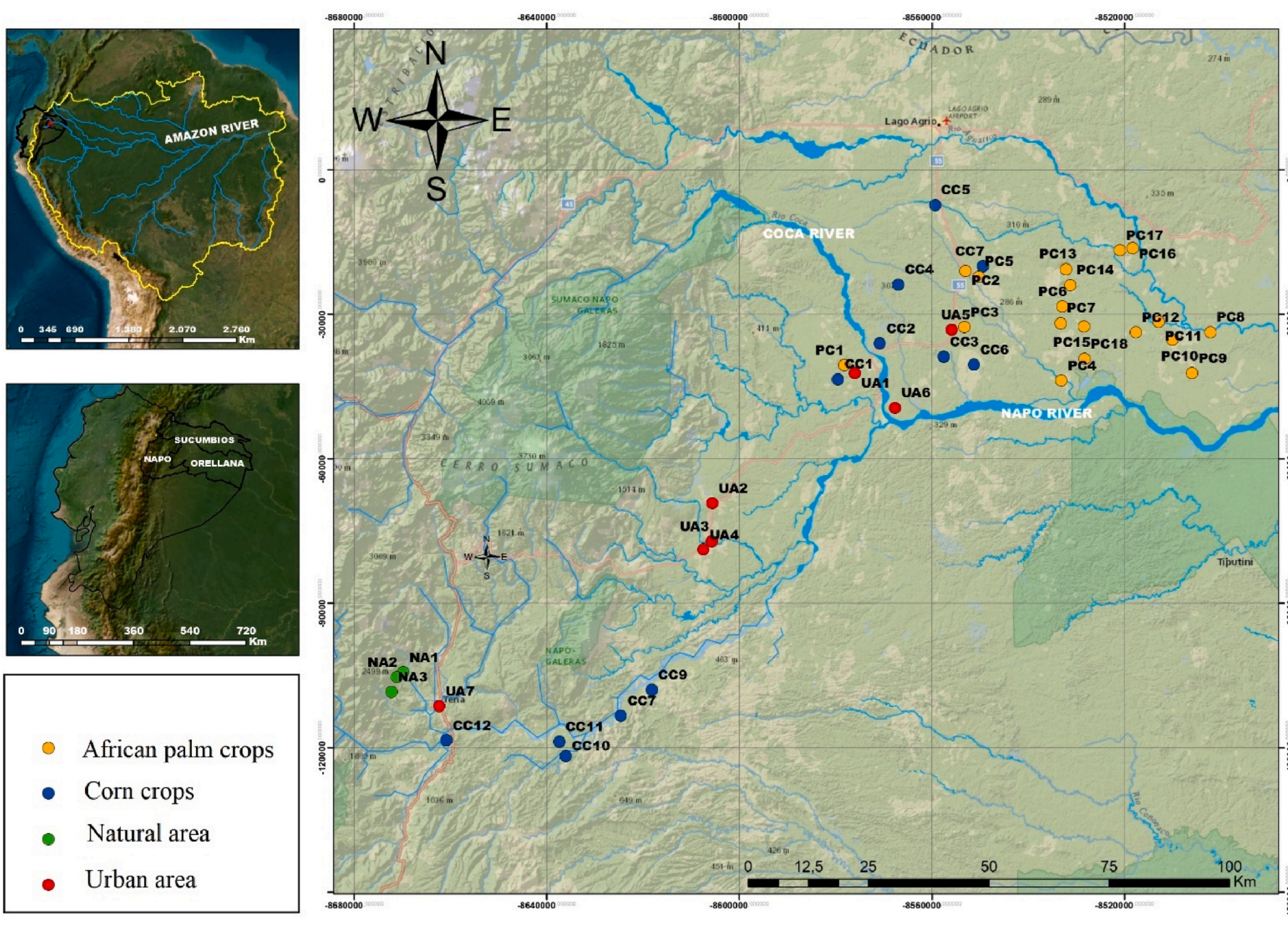

Fig. 1. (A) Location of the study area in the Amazon basin (red area), including the perimeter of Ecuador (black line) and the Amazon basin (yellow line). (B) Location of the Napo basin, which includes the provinces of Sucumbios, Napo and Orellana. (C) Sampling locations in Napo River basin. Different sample codes and colors refer to sampling sites with different land use impacts: African Palm Crops (PC), Corn Crops (CC), Urban Area (UA) and Natural Area (NA). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

eliminate residual water. The loaded cartridges were labeled, wrapped in aluminum foil, and stored at −20 °C. The SPE cartridges for analysis were transported to the IMDEA Water Institute (Spain). All samples were processed in batches of 8 samples with one positive control and one blank per batch. The blanks were used to detect possible contamination during the extraction process and contained 100 mL of MilliQ Water and 50 μl of internal standard (IS) solution (100 μg L^{-1}) (Table S4). All samples contained 50 μl of IS solution and the positive controls contained samples fortified at 100 μg L^{-1} with a pesticide mix and 50 μl of IS solution. Analytes were eluted with two aliquots of methanol (4 + 4 mL) and two aliquots of acetonitrile (4 + 4 mL). The extracts were concentrated at 45 °C in a SpeedVac for approximately 3 h and reconstituted with 1 mL of MeOH:water (10:90, v/v) and vortex stirred for a few seconds. The reconstituted samples were transferred to an amber glass vial for analysis using LC-MS/MS. Operation parameters for analytical equipment are given in Table S2, while information on the retention times (Tr), the multiple-reaction monitoring transition, and the collision energy (EC) for the different compounds is provided in Table S3.

The instrumental detection limit (LOD) and the instrumental quantification limit (LOQ) were determined by the method based on the signal/noise ratio (S/N). The LOD value was established as the minimum concentration that provided a 3xS/N signal, while the LOQ value is the minimum concentration that provided a 10xS/N signal, maintaining the abundance of the qualifying transition with respect to the quantifying one.

The methodological detection and quantification limits (MDL and MQL) were established taking into account the LOD and LOQ values, the pre-concentration factor applied in the sample treatment process and the validated recovery percentages for each compound. The recovery percentages of the 27 pesticides were assessed at two fortification levels with three replicates. The recovery percentages varied from 50% to 120% and the measured concentrations were corrected when the average recovery was lower than 70% following the guidelines of the European Commission (2000). The MQL, MDL and the recovery percentages of the analytical methods are provided in Table S5.

2.4. Ecotoxicological risk assessment

The ecotoxicological risks of pesticides for aquatic ecosystems were calculated based on Species Sensitivity Distributions and the multi-substance Potentially Affected Fraction (ms-PAF) approach (de Zwart and Posthuma, 2005). Acute and chronic SSD parameters (median, μ, and standard deviation, σ) were obtained from Posthuma et al. (2019) and were calculated with acute (EC50: Effect Concentration for the 50% of the individuals) and chronic (NOEC: No Observed Effect Concentrations) toxicity data for a wide range of aquatic species (i.e., algae, bacteria, invertebrates, and fish) using a log-normal distribution. 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 or read-across), and (3) any potential acute-to-chronic or EC50-NOEC extrapolation. The SSD parameters used in this study are provided in Table S6, together with their evaluation criteria.

For the msPAF calculation, compounds belonging to the same chemical group were assumed to have the same Toxic Mode of Action (TMoA). However, when the SSD slopes of the chemicals belonging to the same chemical group deviated more than 10% from the others, they were assigned to a separate TMoA, yielding a total of 12 TMoAs for the acute risk assessment and 12 TMoAs for the chronic risk assessment (Table S6). The msPAF for the compounds belonging to the same TMoA was calculated assuming concentration addition. For this, the Hazard Unit (HU) for each compound in each sampling site was calculated by dividing the measured concentration by the μ of the SSD for the same compound. The msPAF for compounds with the same assigned TMoA was calculated using the following Microsoft Excel © function:

$$msPAF_{TMoA} = NORM.DIST\ (HU_{TMoA}, 0, \sigma_{TMoA}, 1) \qquad \text{Equation 1}$$

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

The msPAF for all TMoAs represented in the mixture was calculated assuming response addition according to:

$$msPAF_{Total} = 1 - \prod_{i=1}^{n} \left(1 - msPAF_{TMoA,i}\right) \qquad \text{Equation 2}$$

The msPAF for all TMoAs represents the fraction of species of the ecosystem that will be affected by the exposure to the pesticide mixture measured in the sample. Samples with calculated msPAFs exceeding 5% of species were considered to pose high ecological risks (for further details see Posthuma et al., 2002).

2.5. Macroinvertebrate identification

Collected macroinvertebrates were classified and identified taxonomically to the family level using standard taxonomic keys (e.g., Domínguez and Fernadez, 2009). The status and composition of the macroinvertebrate communities were evaluated by the Andean–Amazon Biotic Index (AAMBI) (Carrera and Fierro, 2021) and the Biological Monitoring Working Party Colombia (BMWP-Col) index. The indices assign a value between 1 and 100 to each macroinvertebrate family, with a tolerance score of 1 for contamination-tolerant families, and 100 for the most sensitive families. The AAMBI was selected because it was considered an adequate index for the study area and has been used in several studies in the Amazon basin of Ecuador (Capparelli et al., 2021; Galarza et al., 2021). The BMWP-Col was considered an appropriate index for Ecuador since it was developed in a region of Colombia with similar environmental conditions (Damanik-Ambarita et al., 2016; Deknock et al., 2019; Cabrera et al., 2021).

2.6. Effects of land use on water quality

The relationship between the different sample groups (natural areas, urban areas, corn crops and African palm crops) and the physicochemical parameters and the pesticide concentrations was evaluated by performing two independent Redundancy Analysis (RDA), with the sample group representing the different land uses as independent test variable. The relationship between land use (urban areas, corn crops, African palm crops and natural areas) and macroinvertebrate communities at the family level was evaluated by performing a Canonical Correspondence Analysis (CCA). Finally, an analysis of variance (ANOVA) and a Tukey's post hoc test were used to determine differences between the sampling site groups (natural areas, urban areas, corn crops and African palm crops) and the macroinvertebrate indices (AAMBI and BMWP-Col), and a Spearman's correlation analysis was performed to assess the relationship between the individual physicochemical parameters and the calculated msPAF values with the two macroinvertebrate indices. All multivariate analyses were performed with log (x+1) transformed data using the CANOCO Software, version 5 (Braak et al., 2012). The ANOVA, the Tukey test and Spearman's correlation analysis were performed using the software R (R Core Team., 2022).

3. Results

3.1. Physicochemical parameters

Table 1 shows a summary of the physicochemical parameters measured in the different sample groups, together with the parameters that exceed the Water Quality Criteria for the Protection of Aquatic Life of Ecuador (TULSMA) (MAE, 2015). Nitrite exceeded the established threshold (0.2 mg L^{-1}) in 8%, 6% and 14% of the samples taken in corn

Table 1

Summary of physicochemical parameters measured in the water samples of the different land uses. Mean, maximum (Max) and minimum (Min) values by sampling area. <LOQ indicates that the ion was detected but the concentration fell below the LOQ. Values highlighted in bold are above (NO_3^-, NO_2^-) or below (pH, DO) the established thresholds for the Protection of Aquatic Life of Ecuador (TULSMA) (MAE, 2015). The complete dataset is provided in Table S7.

			Agricultural Areas						Urban Areas			Natural Areas		
			Corn			African palm oil								
Parameters	Units	TULSMA	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
pH		6.5–9	7	8	**6**	7	8	**4**	7	8	**5**	8	8	8
EC	μScm^{-1}		102	160	40	94	155	32	165	432	19	35	42	30
DO	%	>80	**72**	95	**2**	**67**	99	**37**	**55**	83	**9**	85	88	83
Temp.	°C		25	28	23	25	28	24	25	27	24	20	20	19
Color	Pt–Co		67	341	11	69	166	9	107	188	37	13	13	12
Turbidity	NTU		5	15	1	6	19	1	11	27	2	1	2	1
TDS	$mg\ L^{-1}$		65	102	28	61	101	20	91	190	13	25	31	21
DOC	$mg\ L^{-1}$		3	4	1	5	14	0.04	19	100	3	1	2	1
Fluoride	$mg\ L^{-1}$		0.09	0.12	0.07	0.07	0.10	0.06	0.10	0.13	0.05	0.09	0.11	0.08
Chloride	$mg\ L^{-1}$		1	2	0.52	1	4	0.45	5	16	0.74	0.75	0.81	0.72
Nitrite	$mg\ L^{-1}$	0.2	0.09	**0.24**	<LOQ (0.025)	0.07	**0.25**	<LOQ (0.025)	0.14	**0.41**	<LOQ (0.025)	<LOQ (0.025)	<LOQ (0.025)	<LOQ (0.025)
Nitrate	$mg\ L^{-1}$	13	2	7	0.33	2	10	0.29	6	**35**	0.32	0.94	1	0.85
Phosphate	$mg\ L^{-1}$		0.52	0.87	<LOQ (0.025)	0.39	0.51	<LOQ (0.025)	0.84	3	0.37	<LOQ (0.025)	<LOQ (0.025)	<LOQ (0.025)
Sulfate	$mg\ L^{-1}$		1	3	0.68	0.96	2	0.50	4	18	0.61	3	6	0.84
Sodium	$mg\ L^{-1}$		3	7	0.87	3	8	0.85	7	21	2	2	2	2
Ammonium	$mg\ L^{-1}$		0.004	0.04	<LOQ (0.025)	0.07	1	<LOQ (0.025)	2	9	<LOQ (0.025)	<LOQ (0.025)	<LOQ (0.025)	<LOQ (0.025)
Potassium	$mg\ L^{-1}$		2	3	0.56	1	2	0.06	3	8	0.93	0.46	0.59	0.38
Magnesium	$mg\ L^{-1}$		4	8	1	4	7	1	3	6	0.76	0.45	0.67	0.27
Calcium	$mg\ L^{-1}$		10	20	2	8	14	1	10	16	0.86	2	3	2

crops, African palm oil crops and urban areas, respectively. Nitrate exceeded the threshold (13 mg L^{-1}) in 14% of urban areas, and the measured pH was below the established range in some samples taken in the agricultural and urban areas. The dissolved oxygen concentration was lower than the established threshold in 63% of samples, all of them corresponding to the agricultural and urban land uses. Urban areas and agricultural areas were also associated with higher EC, TDS, temperature and concentrations of dissolved nutrients and major ions (Cl^-, Ca^{2+}, Mg^{2+}, K^+ and Na^+).

The RDA shows that land use significantly explains the variation in the physicochemical parameters analyzed in this study (Monte Carlo p-value: 0.01; Fig. 2A). The RDA biplot shows that urban areas were positively correlated with the concentration of DOC, NH_4^+, Cl^-, K^+ and water color, while temperature and Mg^{2+} showed a positive correlation with agricultural areas, principally African palm crops (Fig. 2A). Both, urban and agricultural areas showed a positive correlation with water turbidity and the concentration of PO_4^{3-}. Natural areas were characterized by presenting higher DO concentrations (above 83% of saturation), lower concentration of dissolved ions and DOC (1 mg L^{-1}), and a basic pH (around 8) (Table 1; Fig. 2A).

3.2. Pesticide concentrations

All samples collected contained pesticide residues. The highest

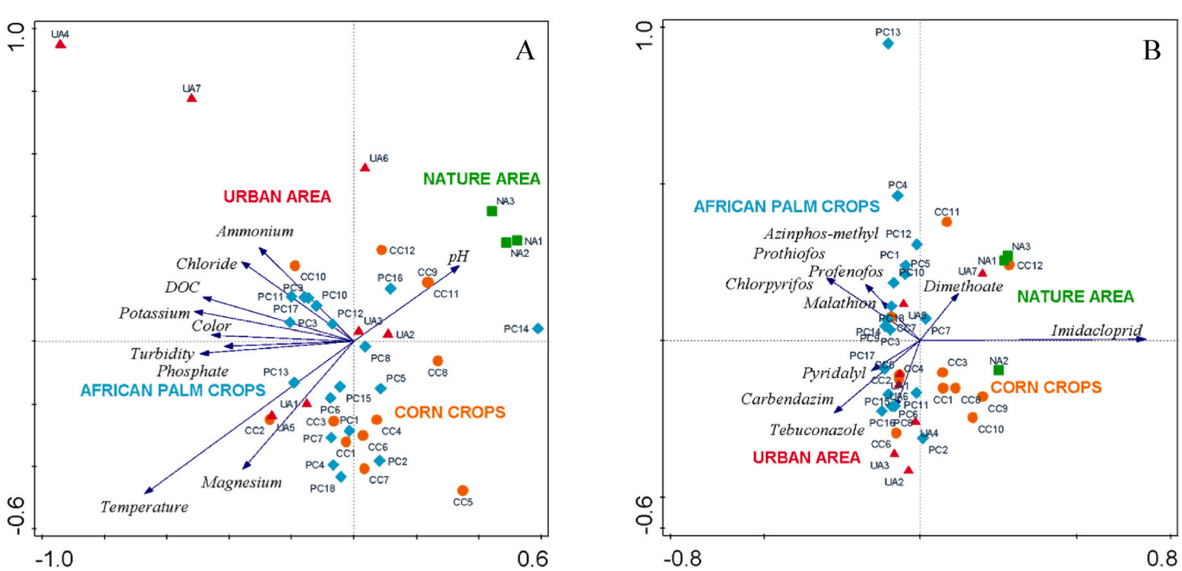

Fig. 2. Multivariate analysis (Redundancy Analysis) showing (A) the influence of land use on the water physicochemical parameters, and (B) the influence of land use on pesticide exposure concentrations. In A, land use explains 19% of the physicochemical parameter variation, out of which 78% is displayed in the x-axis and 6% in the y-axis. In B, land use explains 21% of the pesticide exposure concentrations, out of which 90% is displayed in the x-axis and 6% in the y-axis. Only the 10 most relevant physicochemical parameters and pesticides are displayed in A and B, respectively.

number of pesticides was found in the African palm crop samples (23), followed by the samples taken near urban areas (20) and in areas dominated by corn crops (19). The samples taken in the natural areas contained the lowest number of pesticides Berens et al., 2021. The pesticides carbendazim, azoxystrobin, diazinon, propiconazole and imidacloprid showed the highest occurrence, being present in more than 80% of the samples (Table 2). The compounds that showed the highest concentrations (above 100 ng L^{-1}) were: imidacloprid (3233 ng L^{-1}), ethion (2199 ng L^{-1}), azoxytrobin (249 ng L^{-1}), prothiofos (164 ng L^{-1}) and azinphos-methyl (112 ng L^{-1}).

The RDA shows that land use significantly explained the variation of pesticide exposure concentrations in the environmental samples (Monte Carlo p-value: 0.002; Fig. 2B). The RDA biplot shows a positive correlation between the concentration of organophosphorus insecticides (chlorpyrifos, azinphos-methyl, prothiofos, profenofos and malathion) and the African palm crops, while urban areas were positively correlated with the fungicides carbendazim and tebuconazole, and the insecticide pyridalyl. Natural areas were positively correlated with the insecticides imidacloprid and dimethoate (Fig. 2B).

3.3. Ecotoxicological risk assessment

The calculation of the acute msPAF showed that 20% of the sampling sites present a high ecological risk (msPAF >5%). All other sampling sites presented moderate to low risks (msPAF <5%). The maximum msPAF value was found in African palm crops (12%), caused by a peak of the insecticide ethion. Natural areas presented a maximum msPAF of 10%, dominated by the insecticide imidacloprid (Fig. 3A). The SSDs used to assess the acute risks of ethion and imidacloprid were based on more than 10 toxicity values obtained from laboratory toxicity studies, indicating a high robustness of the acute risk assessment for these compounds (Table S6).

The calculation of the chronic msPAF showed that 68% of the sampling sites present high ecological risks (Fig. 3B). This analysis indicates that the monitored pesticide mixtures could affect up to 29%, 28%, 27% and 26% of the species in the African palm crops, urban areas, corn crops and natural areas respectively. Toxic pressure in natural areas was dominated by imidacloprid and azinphos-methyl, while in urban areas it was dominated by imidacloprid and chlorpyrifos. In African palm crops, it was dominated by the insecticides chlorpyrifos, ethion, azinphos-methyl, prothiophos and profenofos, while in corn crops it was dominated by imidacloprid, chlorpyrifos and azinphos-methyl (Fig. 3B). The other compounds had a low contribution to the calculated chronic toxic pressure. It should be noted that SSDs used to calculate the chronic risks were based on a large number of experimental chronic toxicity data (>10 toxicity data points) only for azinphos-methyl, chlorpyrifos and profenofos (Table S6). However, the chronic SSD for ethion and imidacloprid relied on some acute-to-chronic extrapolations, and for prothiophos it was based on a minimum number of read-across toxicity data obtained from the Quantitative Structure Activity Relationships contained in the US EPA ECOSAR software (Mayo-Bean et al., 2012). Therefore, for ethion, imidacloprid and prothiophos the risk assessment could be improved by using a larger number of chronic toxicity data obtained under laboratory conditions, preferably from tropical regions.

3.4. Macroinvertebrate community composition

In total, 2200 individuals belonging to 52 families and 6 classes were collected. Overall, the macroinvertebrate dataset was dominated by insecta (37 taxa), gastropoda (6 taxa), hirudinea (4 taxa), bivalve (3 taxa), crustacea (1 taxon), and arachnida (1 taxon). The families showing the greatest overall abundance were Chironomidae insects (876

Table 2
Summary of the pesticide concentrations in the water samples. The table shows the frequency of detection as well as the mean, maximum (Max) and minimum (Min) concentration (ng L^{-1}) by sample group. "ND" indicates that the compound was not detected in the sample. "<" indicates that the pesticide was detected but the concentration fell below the LOQ. [a] Compounds analyzed by LC-MS-MS. [b] Compounds analyzed by GC-MS. The complete dataset is provided in Table S8.

Pesticides		Agricultural Areas						Urban Areas			Natural Areas		
		Corn crops			African palm crops								
	Freq (%)	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Fungicides													
Azoxystrobin [a]	88	4	14	ND	18	249	<3	12	68	<3	ND	ND	ND
Carbendazim [a]	93	0.6	3	<0.14	2	4	ND	8	31	ND	0.2	0.2	<0.14
Difenocazol [a]	60	2	6	ND	1	3	ND	2	4	ND	ND	ND	ND
Propiconazol [a]	85	6	62	ND	0.7	4	ND	0.9	7	ND	ND	ND	ND
Tebuconazole[a]	70	4	27	ND	0.4	2	ND	1	5	ND	ND	ND	ND
Pyrazophos[b]	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Herbicides													
Bentazone[a]	5	ND	ND	ND	0.4	4	ND	ND	ND	ND	ND	ND	ND
Propazine[a]	43	0.08	0.2	ND	0.2	1	0	0.3	0.9	ND	ND	ND	ND
Simazine[a]	23	0.5	3	ND	2	13	0	1	4	ND	ND	ND	ND
Terbuthilazyne[a]	18	0.01	0.07	ND	0.09	0.8	0	0.2	0.8	ND	ND	ND	ND
Terbutryn[a]	30	0.9	10	ND	0.3	2	0	0.2	1	ND	ND	ND	ND
Insecticides													
Azinphos-methyl[b]	33	13	54	ND	21	112	ND	16	56	ND	36	57	ND
Chlorpyrifos[b]	45	7	22	ND	10	31	ND	9	17	ND	ND	ND	ND
Demeton-S-methyl[b]	28	7	20	ND	8	41	ND	12	86	ND	ND	ND	ND
Diazinon[a]	88	1	2	<1	0.7	1	ND	0.8	1	ND	1	1	<1
Dimethoate[b]	28	8	47	ND	23	87	ND	7	47	ND	ND	ND	ND
Ethion[b]	23	6	71	ND	128	2199	ND	29	78	ND	ND	ND	ND
Imidacloprid[a]	85	415	3233	ND	2	7	ND	214	1484	ND	2472	2877	2217
Malathion[b]	8	ND	ND	ND	2	16	ND	2	15	ND	ND	ND	ND
Methidathion[b]	3	3	31	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pirimicarb[a]	15	2	6	ND	0.7	6	ND	0.9	6	ND	ND	ND	ND
Profenofos[b]	8	ND	ND	ND	9	69	ND	ND	ND	ND	ND	ND	ND
Prothiofo[b]	5	ND	ND	ND	14	164	ND	ND	ND	ND	ND	ND	ND
Pyridalyl[a]	15	0.13	2	ND	0.8	8	ND	2	14	ND	ND	ND	ND
Spinosina-A[a]	8	ND	ND	ND	0.4	6	ND	1	8	ND	ND	ND	ND
Tolclofos-methyl[b]	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pirimiphos methyl[b]	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

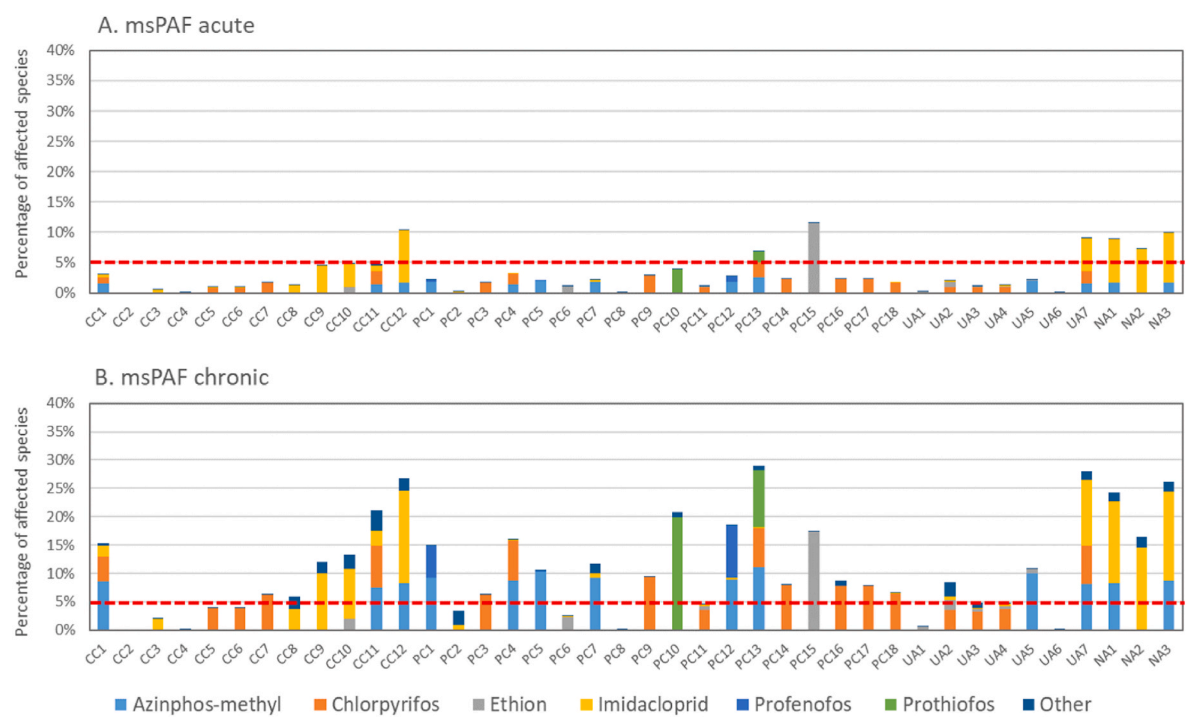

Fig. 3. Calculated multi-substance Potentially Affected Fraction (msPAF) for each sampling site based on (A) acute toxicity data and (B) chronic toxicity data. Only compounds with msPAF higher than 1% are displayed, while the rest are grouped as Others. Samples with msPAFs higher than 5% (dashed line) are expected to have high ecological risks.

individuals) and Cochliopidae snails (477 individuals). The complete dataset is provided in Table S9.

The CCA shows a significant influence of land use on the macroinvertebrate community composition (Monte Carlo p-value: 0.01). Natural areas showed optimum conditions for most of the insect and crustacean families (Fig. 4) and showed a high taxonomic richness

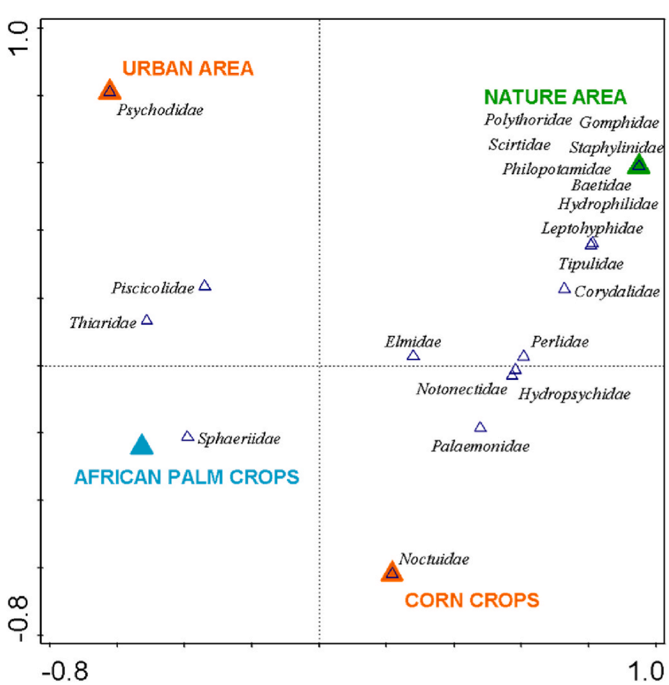

Fig. 4. Multivariate analysis (Canonical Correspondence Analysis) showing the influence of land use on the macroinvertebrate community. Land use explains 13% of the total macroinvertebrate community variation, out of which 55% is displayed in the x-axis and 23% in the y-axis.

(average value: 9 families/sample) and abundance (average value: 141 individuals/sample). In urban areas there was a low taxonomic richness (2 families/sample) and a medium-to-high abundance (97 individuals/sample) of macroinvertebrates. The most common family found in urban areas was Piscicolidae (leech), which is considered moderately tolerant. The African palm crops were associated with the Sphaeriidae family, which is considered tolerant, and showed a very low richness (1 family/sample) and abundance (12 individuals/sample). Corn crops were associated with the families Noctuidae, the Hemiptera Notonectidae which is considered moderately tolerant, and with the Palaemonidae family, which is considered sensitive, and showed a medium taxonomic richness (3 families/sample) and abundance (75 individuals/sample).

Both indexes (AAMBI and BMWP-Col) showed similar trends. AAMBI ranged from 0 to 84 and the BMWP-Col from 0 to 93. The average AMMBI scores in urban areas (9), African palm crops (11) and corn crops (16) indicate bad to very bad ecological status, while in the natural areas the ecological status was moderate-to-high. Similarly, the BMWP-Co scores indicated urban areas (15) as having very bad ecological status, African palm crops (23) and corn crops (19) having bad ecological status, and natural areas (87) having moderate-to-high ecological status. The indexes showed significant differences with respect to the different land uses (Fig. 5). The natural areas presented significantly better ecological status compared to urban and agricultural areas ($p < 0.05$), while the urban areas and agricultural areas did not show significant differences ($p > 0.05$).

The Spearman correlation analysis between the macroinvertebrate indices (AAMBI and BMWP-Col), the physicochemical parameters and the calculated msPAF are shown in Fig. 6. A strong negative correlation was observed between macroinvertebrate indices and the msPAF chronic, while the relationship with msPAF acute was very weak. Of the 18 physicochemical parameters, 11 (nitrates, nitrites, ammonium, phosphate, sodium, DOC, turbidity, phosphates, potassium, EC, and STD) presented a strong negative correlation with the macroinvertebrate indices, while pH and DO showed a mild positive correlation with the macroinvertebrate indices.

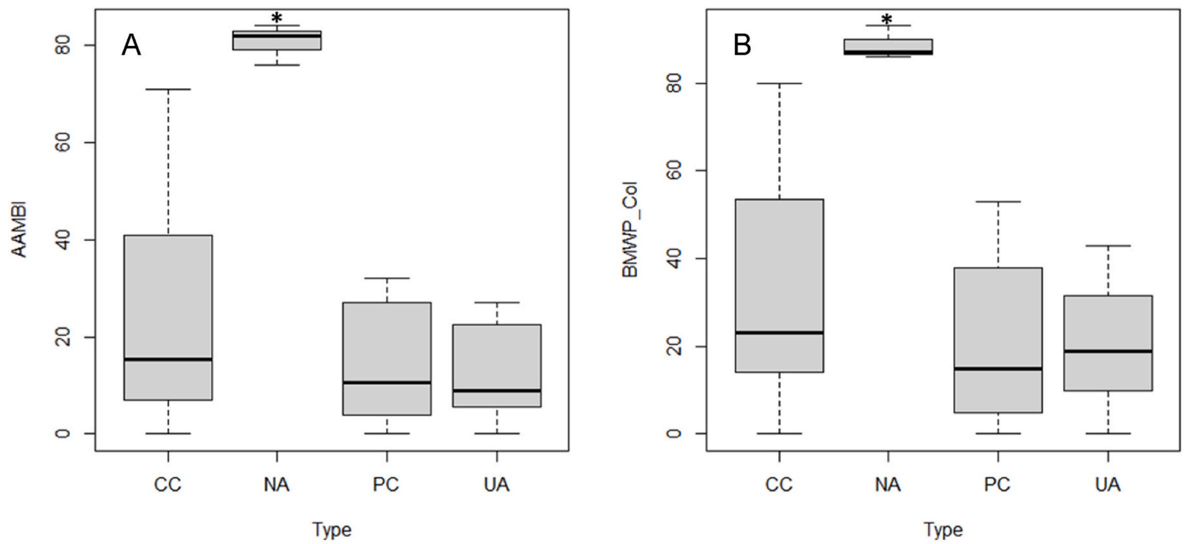

Fig. 5. Box plots showing the distribution of the AAMBI macroinvertebrate index (A) and the BMWP-Col macroinvertebrate index (B) in the different sample groups. NAs = Natural areas, CCs = Corn cultivation areas, PCs = African palm oil cultivation areas, UAs = Urban areas. Significant differences (Tukey test, p-value <0.05) are indicated by an asterisk. Data are given as box and whisker plots showing the median, the 25% and 75% interquartile limits, and the minimum and maximum values.

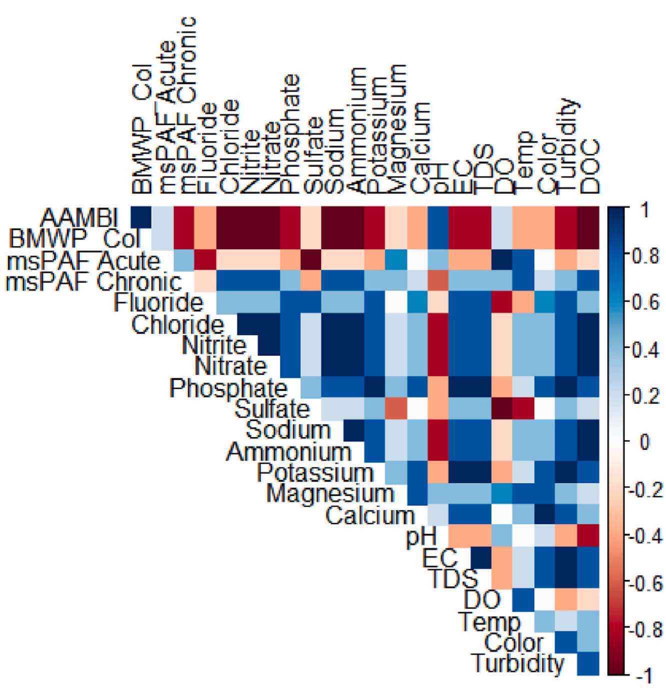

Fig. 6. Correlation matrix between the macroinvertebrate indices (AAMBI and BMWP-Col), the measured physicochemical parameters and the calculated acute and chronic msPAFs. Colors indicate the Spearman's correlation coefficient. Blue color indicates a positive correlation between the combination of parameters, while red color indicates a negative correlation. The color intensity is related to the strength of the correlation as displayed in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Effects of land use on chemical and ecological status

Sampling sites located in urban and agricultural areas (corn crops and African palm oil plantations) presented lower chemical and ecological status compared to sampling sites located in natural areas with less anthropogenic influence. This is in line with former studies that report poor macroinvertebrate richness and abundance in rivers that flow through African palm oil plantations (Mercer et al., 2014) and urban areas (Galarza et al., 2021). Luiza-Andrade et al. (2017) found that the species composition of the ephemeropteran, plecopteran and trichopteran groups was affected by palm oil plantations, making agricultural expansion a major concern. In general, the results of our study show that macroinvertebrates classified as sensitive to contamination were not present in rivers next to urban areas and African palm oil plantations; while the Chironomidae family was predominant in urban and agricultural areas. According to Galarza et al. (2021), the Chironomidae family was also found in high abundance in river dumps that connect with the upper Napo River. In addition, this family was the most abundant in the Coca and Aguarico rivers of the Ecuadorian Amazon (Cabrera et al., 2021) and in the Guayas River basin (Damanik-Ambarita et al., 2016), which can be explained by its capacity to tolerate high organic pollution levels, low oxygen concentrations and its multivoltine reproductive characteristics, which allow rapid recolonization from water bodies with less fluctuant environmental conditions (Mercer et al., 2014).

Compared with other land uses, urban areas were the main source of ion contamination, including inorganic nitrogen and DOC, which is in line with other environmental monitoring studies performed in the region (Capparelli et al., 2021; Galarza et al., 2021). The sample collected at the AU3 site presented the highest concentration of nitrates and the AU7 sample presented the highest concentration of DOC. This is due to the discharge of untreated wastewater from households directly into Amazonian rivers (Galarza et al., 2021), causing such high concentrations of nitrogen and DOC. The regulations of TULSMA require private industries to monitor and reduce their wastewater discharge; however, the lack of governmental control, together with limited penalties for non-compliance with these regulations, make companies to act freely in this respect.

In agricultural areas, a decrease in water pH and an increase in temperature, DOC, EC, turbidity, and nutrients were observed compared to natural areas. However, only African palm crops were significantly correlated with parameters of temperature, pH, turbidity, and phosphate. The African palm is the most widespread crop in the northern Amazon of Ecuador, specifically in the provinces of Sucumbíos and Francisco de Orellana, with a cultivated area of 48,700 ha; while corn has a cultivated area of 2972 ha (INEN, 2019). Luiza-Andrade et al. (2017) reported that the effects of palm oil plantations on the macroinvertebrates were related to changes in water physicochemical parameters, and the amount of organic matter debris and reduced vegetation cover in streams of the Brazilian Amazon. This is in line with the results of our study. In addition, we found a significant negative correlation between the chronic msPAF values, physicochemical parameters (nitrates, nitrites, ammonium, phosphate, sodium, DOC, turbidity, phosphates, potassium, EC, and STD) and the macroinvertebrate indices. The negative correlation indicates a potential negative impact of these parameters on the macroinvertebrate community. This suggests that the listed parameters may be important factors influencing the abundance and diversity of macroinvertebrates and that long-term pesticide pollution could also be associated to the deterioration of the ecological status of freshwater ecosystems. This is the first study in which the AAMBI and the BMWP-Col have been applied in the Ecuadorian Amazon and indicate that both can be used as indicators for the ecological status assessment of Amazonian freshwater ecosystems, although their specificity to identify pesticide impacts has been questioned (Deknock et al., 2019). Further studies are needed to establish thresholds for the ecological status classification of water bodies in the Ecuadorian Amazon as well as to test their response to specific contaminant groups, including pesticides, metals, and other emerging contaminants (e.g. pharmaceuticals, home-care products, biocides).

4.2. Pesticide exposure and risk assessment

A total of 27 pesticides were analyzed, out of which 23 were detected in the upper basin of the Napo River. Of these pesticides, only carbendazim is prohibited in Ecuador since 2019 due to concerns about its potential impacts on public health and environmental side effects (AGROCALIDAD., 2019). Despite its ban, we found a high prevalence of carbendazim in the collected samples (93%), with the highest concentration being 31 ng L^{-1} in urban areas. Rico et al. (2021) reported its presence in 80% of the water samples collected in urban streams of the Brazilian Amazon, with concentrations up to 214 ng L^{-1}. Fungicides such as carbendazim are used in urban areas as additives to reduce the deterioration of construction materials by microbial activity, and they can also be used as worm control agent to protect amenity turf (Merel et al., 2018).

Our study identified several organophosphate compounds (diazinon, ethion, chlorpyrifos and azinphos-methyl) that have been banned in countries with less permissible environmental regulations, such as the members European Union (EU, 2022). In our study, organophosphate compounds were mainly associated to African palm crops, where they are applied to kill insect pests, through direct spray over palms and fruit bunches (Muhamad et al., 2010). Organophosphates inhibit the activity of the enzyme acetylcholinesterase and are extremely toxic to aquatic invertebrates (Fulton and Key, 2001). Most organophosphate compounds have a relatively low environmental persistence, although some others, such as chlorpyrifos, tend to sorb to soils and particulate organic matter. Indeed, chlorpyrifos residues have been detected in soils of oil palm plantations up to seven days after its application (Muhamad et al., 2010). The heavy precipitation events that occur in the northern Ecuadorian Amazon can transport contaminants from the soil and vegetation to downstream freshwater ecosystems, thus being the most likely entry route for these compounds into the monitored freshwater ecosystems.

The total concentration of organophosphate pesticides in all sampling sites was below the maximum permitted threshold established by the Ecuadorian regulations (10 μg L^{-1}). However, based on the risk assessment performed here, 68% of the sampling sites presented high long-term risks, with those being dominated by mixtures formed by 2–4 organophosphorus insecticides (ethion, chlorpyrifos, azinphos-methyl, profenofos and prothiofos) together with imidacloprid. This study suggests that the pesticide limits set by the Ecuadorian government are not stringent enough to protect aquatic ecosystems. This is supported by several studies that have evaluated the toxicity of organophosphorus insecticides on Amazonian freshwater invertebrates under laboratory conditions (Rico et al., 2010, 2011) and in microcosm studies performed in other tropical regions (Daam et al., 2008), which have set safe environmental concentrations in the order of 0.1 μg L^{-1} for short-term exposure.

Imidacloprid was found in agriculture and in urban areas but showed the largest prevalence in natural areas. Imidacloprid remains as one of the most widely used pesticides worldwide in developing regions (Hladik et al., 2018). Besides its agriculture use, imidacloprid has been detected in various natural areas around the world. For example, Benton et al. (2017) reported concentrations of 379 ng L^{-1} in streams of the Great Smoky Mountains National Park (USA), with no observed negative effects on aquatic macroinvertebrate communities. Berens et al. (2021) reported concentrations of 1.9 ng L^{-1} in water bodies of mixed-use forest areas (Kawishiwi River, USA). In forest and natural conservation areas, imidacloprid is used to control sucking insect pests such as aphids, whiteflies, leaf and planthoppers, thrips, some micro-lepidoptera, and coleopteran pests (Jeschke et al., 2011). Due to its high solubility in water, imidacloprid can be transported to surface waters and shows high toxicity towards aquatic insects (Raby et al., 2018), which is explained by its agonistic effect to the acetylcholine receptor (Jeschke et al., 2011). Several studies have indicated significant changes in the structure of macroinvertebrate and zooplankton communities after prolonged exposure to concentrations that are two orders of magnitude lower than the ones measured in this study. For example, Merga and Van den Brink (2021) found significant effects of imidacloprid in the macroinvertebrate community of freshwater microcosms deployed in Ethiopia at concentrations of 100 ng L^{-1}, while Sumon et al. (2018) found long-term effects in sub-tropical species of Ephemeroptera at concentrations of 30 ng L^{-1}. On the other hand, Rico et al. (2018) found a significant abundance decline of Chironomids and Ephemeroptera (*Cloeon dipterum*) after a single pulse of 200 ng L^{-1} under Mediterranean conditions. Therefore, it is expected that the imidacloprid concentrations in agricultural and natural areas of the Napo River basin are contributing to a deterioration of the ecological status of freshwater ecosystems. Furthermore, several studies show that the uptake and toxicity potential of pesticides such as imidacloprid can be accented by an increase in water temperatures (Camp and Buchwalter, 2016; Kang et al., 2019; Huang et al., 2023), which may explain the large toxicity potential of this compound under tropical conditions.

Overall, our study shows that insecticide use in agriculture, urban areas and nature conservation areas of the Ecuadorian Amazon is likely to cause significant effects on non-target aquatic organisms. Recently, Ecuador has taken some steps to control the environmental effects of agricultural pesticides, including the establishment of a national pesticide monitoring program and the adoption of stricter regulations on pesticide labeling and storage based on the Andean technical manual (SGCAN, 2002). However, as in many other Latin American countries, the pesticide registration system of Ecuador is too basic, and relies on exposure and effect evaluations carried out in other parts of the world (mostly temperate regions) (Vryzas et al., 2020; Daam, 2022). Further investments are needed to develop risk prioritization studies under similar agricultural scenarios as well as to develop exposure and effect studies considering local conditions.

5. Conclusions

This study shows the outcomes of the first large-scale monitoring of water quality and pesticide residue contamination in aquatic ecosystems of the Ecuadorian Amazon. It was found that intensive agriculture, specifically African palm oil crops, and urban areas affect water quality, causing an increase in the concentration of nutrients and a reduction of oxygen concentrations, which influence macroinvertebrate communities. Moreover, pollution by organophosphate insecticides and imidacloprid are expected to pose high risks to aquatic ecosystems. Ecological risks of organophosphate insecticides have been associated to their use in African palm oil plantations, while imidacloprid has been detected at hazardous concentrations in corn crop areas as well as in nature conservation areas. Future investigations are needed to understand the sources of imidacloprid contamination and to assess its effects for Amazonian freshwater ecosystems. Finally, this study shows that the current pesticide standards set for the diagnostic risk assessment of organophosphates in Ecuador are unprotective and should be urgently revised and extended to account for other pesticide groups.

Credit author statement

M. Cabrera: Investigation; Methodology; Formal Analysis; Writing - original draft. M.V. Caparelli: Conceptualization; Methodology; Formal analysis; Investigation; Writing - review & editing. C. Ñacato-Ch.: Investigation; Conceptualization; Writing - review & editing. G.M. Moulatlet: Investigation; Conceptualization; Writing - review & editing. I. López-Heras: Investigation; Methodology; Writing - review & editing. M. Díaz González: Investigation; Methodology; Writing - review & editing. D. Alvear-S.: Investigation; Methodology; Writing - review & editing. A. Rico: Investigation; Conceptualization; Methodology; Supervision; Formal analysis; Funding acquisition; Writing - original draft; 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.

Data availability

Data will be made available on request.

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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.2023.139286.

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