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Starting from the bottom: Contrasted trophic transfer of antibiotics and pesticides through a riverine food web

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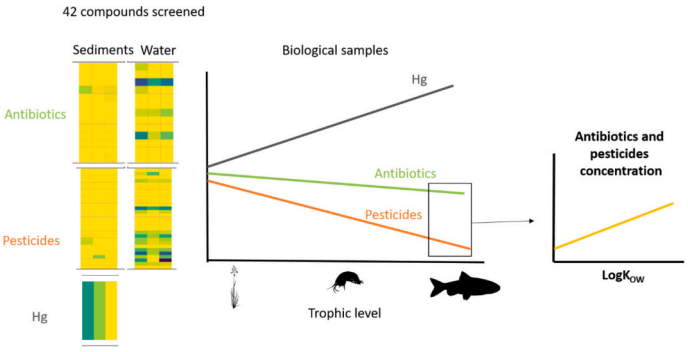
HIGHLIGHTS

- 19 pollutants among 42 (pesticides and antibiotics) were detected in biota, 20 in water, and 9 in sediments of the Orge River.
- Mercury biomagnified in the riverine food web.
- Detection of pesticides and antibiotics decreased along the trophic chain.
- Body burden of pesticides and antibiotics increased with Log K_{OW}.
- Complex pollutant dynamics make biota monitoring challenging for pesticides and antibiotics.

GRAPHICAL ABSTRACT



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ABSTRACT

Among the various forms of pollution arising from human activities, the release of trace elements, pesticides and pharmaceutical residues in freshwater poses a major threat to the health and stability of these ecosystems. Once ingested by aquatic organisms, some pollutants bioaccumulate over time and biomagnify along trophic webs, while others are metabolised and/or eliminated. Understanding the trophodynamics of trace elements, pesticides and antibiotics according to their chemical properties is thus crucial. In this study, we investigated the occurrence and fate of mercury (Hg), well-studied in trophodynamic studies, pesticides and antibiotics, much less investigated despite being biologically-active compounds, in the trophic webs of the Orge River, a tributary of the Seine River, France. We sampled primary producers, macroinvertebrates, and fish, as well as sediments and water, and searched a total of 42 chemicals. We detected 19 compounds in biological samples, 9 in sediments, and 20 in water. The fate of pollutants along the trophic chain varied, with the biomagnification of Hg as predicted, and a reduction in number of antibiotics and pesticides detected along the trophic chain. This pattern suggests the metabolism and excretion along the food chain. Pollutant concentrations in biological samples were best explained by chemical properties, with $LogK_{OW}$ emerging as the main predictor in our models, while other tested variables (molecular weight, solubility, $LogK_{OC}$, and K_M) did not show significant effects. The differential

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transfer of pollutants along the trophic chain and the influence of chemical and biotic properties underscore the complex interactions between environmental pollution and living organisms.

1. Introduction

Human-induced pressures such as habitat destruction, pollution, overexploitation, and climate change threaten freshwater ecosystems, precipitating a global conservation crisis (Dudgeon et al., 2006). Among the various forms of pollution arising from these activities, chemical contamination by pesticides and pharmaceutical residues poses a major threat to the health and stability of these environments, and are found worldwide (Arnold et al., 2014; Schäfer et al., 2011). Antibiotics are among the most commonly found pharmaceutical in surface water with concentrations reaching 10, 15, 50 and 450 $\mu g.L^{-1}$ in America, Europe, Africa and Asia, respectively (Danner et al., 2019). Pesticides are mainly found in the ng to $\mu g.L^{-1}$ range (Anderson et al., 2015; Cedergreen and Rasmussen, 2017). These chemical substances are often transported to water bodies through agricultural runoff (Singh et al., 2023) as well as domestic and industrial discharges (Cardoso et al., 2014).

The entry of these contaminants into aquatic organisms can occur through various pathways, including direct absorption from water, ingestion of contaminated sediment particles, and consumption of contaminated prey (Katagi, 2010). Once ingested, pollutants can either be directly excreted (Rainbow, 2002), or bioaccumulated over time (i.e. stored in the tissues of organisms over time) and biomagnified (i.e. increasing levels with increasing trophic level, Yang et al., 2020). Trophodynamic studies mainly focused on persistent organic pollutants or trace metal elements such as mercury (Hg), which are known to bioaccumulate and biomagnify throughout the trophic chain (Córdoba-Tovar et al., 2022; Lavoie et al., 2013). Conversely, biodilution, which corresponds to the decline of contaminant levels with increasing trophic levels, may also be observed, as a consequence of excretion and metabolic transformation of some pollutants by several organisms (Goutte et al., 2020; Zhou et al., 2020). Biomagnification or biodilution can also be influenced by chemical properties such as particles adsorption ability (Zhou et al., 2020), degradation half-lives (Matthee et al., 2023), organic carbon/water coefficients ($logK_{OC}$, Matthee et al., 2023), metabolic transformation rate and hydrophobicity ($logK_{OW}$, Walters et al., 2016). However, most studies regarding the trophic transfer of metals, pesticides, or pharmaceuticals in aquatic systems tend to focus on specific contaminant classes or isolated compartments of the trophic network. Few investigations have simultaneously assessed the dynamics of differing pollutants within a single ecosystem using a multi-contaminant, multi-compartment approach, taking into account their chemical properties, despite the increasing prevalence of these mixed pollutants in freshwater environments (Goutte et al., 2020). The interplay of their differing physicochemical properties, metabolic pathways, and trophic-level interactions remains insufficiently understood in complex trophic webs.

In this study, we investigated the presence and fate of Hg, pesticides and antibiotics in the complete trophic network of the Orge River, a tributary of the Seine river, by sampling sediments, water, primary producers, macroinvertebrates, and fish. We related concentration to trophic level, using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes as proxies. We also investigated which chemical properties influenced pollutants concentrations in this freshwater ecosystem. Our goal was to determine how these contaminants are distributed and transferred within the trophic network, and assess how their contrasting physico-chemical properties shape their environmental fate.

2. Methods

2.1. Sampling

Sampling was performed during spring, the 19, 26 and 28 of April 2022 (no precipitation over this 9 day-period, which could have influenced contaminant levels), along a 100 m-transect in the Orge River at Viry-Châtillon, a few kilometres upstream of the confluence with the Seine River (48°40′23″N; 2°21′30″E, see Appendix A). Temperature (°C), pH, conductivity ($\mu S.cm^{-1}$) and dissolved oxygen ($mg.L^{-1}$) were measured at each sampling event using a Portable Multi-Parameter Analyzer (RS Pro) (Mean ± SE: temperature = 14.53 ± 1.53, pH = 7.57 ± 0.18, conductivity = 641 ± 43.22, dissolved oxygen = 10.35 ± 0.05). Three surface water samples were collected in a 1 L-HDPE bottle, in order to compute background contamination levels at each visit; aliquots were filtered on GF/F filters to determine the suspended matter content. The dissolved phase is collected in a 250 mL flask or Erlenmeyer flask and adjusted to approximately pH 4 with orthophosphoric acid (99 %, then 5 % for fine-tuning). Correspondingly, three composite surface sediment samples (0–2 cm) were taken at 1 m from the riverbank using a stainless-steel spoon. This distance was chosen because the Orge River is not wadeable across its full width, but the shallow nearshore zone allowed consistent sampling. The number of samples was selected to ensure representative coverage of the study area while considering logistical constraints and the homogeneity of sediment composition observed during preliminary site assessments. We did not assessed the specific composition of sediments in our study site, but a study conducted close to our sampling point in February 2021 reveal a composition of sediments dominated by quartz (>60 %), with feldspars (~10 %) and minor carbonates (~5 %), containing an average of 5.3–8.2 % organic carbon, and a grain size between 43 and 656 μm (Le Gaudu et al., 2022).

In total, 62 biological samples were collected. Twenty-one fish, corresponding to 10 fish species (number within brackets correspond to number of individuals per group, *Barbus barbus* [2], *Carassius gibelio* [3], *Cottus gobio* [1], *Squalius cephalus* [1], *Gasterosteus aculeatus* [3], *Gobio gobio* [3], *Barbatula barbatula* [3], *Perca fluviatilis* [1], *Lepomis gibbosus* [1], *Tinca tinca* [3]) were collected by electrofishing (Martin pecheur®, DREAM Electronique, 240 W, under permit AP 2022-DDT-SE-87). Such sampling strategy allowed the collection of species with contrasted feeding and thus supposedly diverse trophic levels. Fish were identified, anesthetized in diluted eugenol (1/2 in ethanol, 0.5 mL of the dilution in 5 L of water, tested to ensure correct anesthetic responses in the species) and then euthanized by eugenol overdose (dilution 1/2 in ethanol, 3 mL of the solution in 5 L of water), followed by severing of the nervous system. Individuals were then measured and weighted (Ohaus, Valor2000W).

Twenty-three pools of macroinvertebrates (pools were only constituted of individuals captured the same day on the same location, corresponding to 9 genus, *Asellus* [4 pools of 76 to 100 individuals], *Calopteryx* [1 pool of 41 individuals], *Corbicula* [1 pool of 8 individuals], *Gammarus* [5 pools of 40 to 85 individuals], *Hirudiniformes* [1 pool of 12 individuals], *Lymnaea* [4 pools of 1 to 3], *Myxas* [1 pool of 1 individual], *Planorbis* [2 pools of 5 individuals], *Unio* [4 pools of 1 to 2 individuals]) were sampled using two devices, one for benthic fauna (a 0.05 m^2 Surber-type sampling device; mesh 300 μm) and one for organisms associated with macrophytes (1 × 1 mm mesh hand net). Individuals were identified and pooled for further analyses. Additionally, 18 samples of primary producers were hand sampled, belonging to *Carex* [2], *Chara* [4], *Cladophora* [6], leaf litter [3], crisp-leaved pondweed [1], and submerged roots [2].

2.2. Isotopic analyses and trophic level

For both isotopic and pollutant analyses (see below), entire organisms were used (excluding mollusc shell): individuals were collected, surface-cleaned, freeze-dried, ground, and homogenized prior to analysis. Isotopic analyses were carried out at LIENSs laboratory (La Rochelle, France). We directly analysed the bulk material without delipidation or decarbonation. Sample of 0.5 mg (for fish) and 2 mg (for macroinvertebrates and primary producers) were analysed with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112). Results are presented as standard delta (δ) notation as parts per mil (‰) deviation relative to Vienna Pee Dee Belemnite and atmospheric N_2 (air) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Internal laboratory standards USGS-61 (Caffeine) and USGS-62 (Caffeine) for both C and N were used to check accuracy. Foodweb structure, represented according to $\delta^{13}C$ and $\delta^{15}N$ values, are represented in Appendix B. The trophic level of each 62 samples was then evaluated using the following equation:

$$TL_{conso} = 2 + \frac{\delta^{15}C_{consumer} - \delta^{15}N_{base}}{3.4}$$

where TL_{conso} represents the trophic level of a consumer organism, $\delta^{15}N_{conso}$ (‰) and $\delta^{15}N_{base}$ (‰), respectively, represent the ratio of stable nitrogen isotopes for the consumer and the organism assigned to the base of the trophic network. In our case, the baseline was defined using *Gammarus* sp., which was chosen because it was consistently present and abundant across all sub-sites, ensuring comparability among samples. Although *Gammarus* sp. is generally considered herbivorous or detritivorous, we acknowledge that its trophic position may slightly exceed 2 due to occasional omnivorous feeding behavior. Nevertheless, it remains a commonly used baseline taxon in similar studies where primary consumers are not directly accessible (e.g. (Goutte et al., 2020). Selecting a lower baseline (e.g., primary producers with TL = 1) would result in elevated consumer trophic levels, which would be ecologically unrealistic for the species present in our system. As such, this choice provides a pragmatic and ecologically relevant reference point for estimating trophic levels. As described in (Goutte et al., 2020), the constant 2 corresponds to the estimated trophic position of the organism at the base of the food web; 3.4 ‰ is the mean trophic fractionation of $\delta^{15}N$ across two successive trophic levels (Post, 2002). Mean $\delta^{13}C$ and $\delta^{15}N$, as well as mean trophic position for each species (fish) or genus (primary producers and macroinvertebrates) are given in Appendix C.

2.3. Mercury analyses

Total Hg (hereafter Hg) was measured on samples originating from whole individuals that were freeze-dried and homogenized, as described by Chouvelon et al. (2009). Mercury analyses were carried out with an Advanced Mercury Analyzer (ALTEC AMA 254), on dried sediments and tissues aliquots ranging from 4 to 50 mg, weighed to the nearest 0.01 mg. For Hg determination, the metal was evaporated by progressive heating up to 800 °C, then held under oxygen atmosphere for 3 min, and finally amalgamated on a gold trap. Afterwards, the net was heated to liberate the collected Hg, which was measured by atomic absorption spectrophotometry at 254 nm. TORT-3 (NRCC) certified reference material was used to control quality. The recovery was 98.8 ± 1.9 % of the certified value. Detection limit of the AMA was 0.1 ng. The results of Hg concentrations are further expressed in $ng.g^{-1}$ dry weight.

2.4. Pesticides and antibiotic analyses

Biological samples as well as the 3 water samples and the 3 sediments samples were analysed, following Marchand et al. (2024). All were analysed through solid phase extraction and chromatographed by an Agilent 1260 liquid chromatograph (LC) interfaced to a 6465 triple quadrupole mass spectrometer system (MS/MS) (Agilent Technologies). For biological samples, analyses were performed on whole organisms, as recommended in (Borgå et al., 2012) using the same dried samples as used for isotopic analyses. Gut contents were not removed prior to analysis, in order to reflect the total contaminant burden of the organism as encountered in the environment. Since no dissection or selective tissue removal was performed, lipid content is integrated across the whole body, and no lipid normalization was applied. This approach ensures consistency among samples and avoids introducing artificial variation due to differential tissue composition. A total of 42 pollutants, i.e. 13 antibiotics (Acetoxolinic acid, Amoxicillin, Ciprofloxacin, Clarithromycin, Enoxacin, Enrofloxacin, Erythromycin, Flumequine, Norfloxacin, Ofloxacin, Sulfamethoxazole, Tetracycline, Trimethoprim) and 29 pesticides (3,4-Dichloroaniline, Atrazine, Bentazone, Carbendazim, Chlortoluron, Deethylatrazine (DEA), Deisopropylatrazine (DIA), Diflufenican, Hydroxyatrazine, Imidacloprid, Imidacloprid Olefin, Imidacloprid Urea, Irgarol, Isoproturon, Metazachlor, Metolachlor, Metolachlor OXA, Metolachlor ESA, Nicosulfuron, Oxadiazon, Pendimethalin, Penoxsulam, Propanil, Prosulfuron, Simazine, Tebuconazole, Tebufenozide, Terbutylazine, Terbutylazine Desethyl) were quantified (see Table 1). These compounds are expected to be found in the sampled area (Marchand et al., 2024) and were quantified in water ($ng.L^{-1}$), sediment and biological samples (both $ng.g^{-1}$ dry weight).

Two ultrasonic extractions are performed on 1 g of dried biological samples: one with an acidic buffer (10 mL of orthophosphoric acid/MeOH 20/80 mixture and 100 µL of 1 M Na2 EDTA) and another with a basic buffer (10 mL of NaOH 2.5 %/MeOH 20/80 mixture and 100 µL of 1 M Na2 EDTA).

After centrifugation at 2500 rpm for 5 min, the supernatant is collected in a 22 mL amber glass tube before being reconcentrated using an EZ2 and then under a nitrogen stream until a residual volume of 5 mL is obtained. After transferring to 250 mL glass bottles, 200 mL of MQW (Milli-Q Water) are added, and the pH of the solution is adjusted to 7 using 5 % sodium hydroxide NaOH.

Purification then takes place using a multi-cartridge system with, from top to bottom: a SAX (strong anion exchange) cartridge (0.5 g, 6 mL, Agilent), a PSA (primary secondary amine) cartridge (0.5 g, 3 mL, Agilent), and an Oasis HLB (Hydrophilic-Lipophilic Balance) cartridge (0.5 g, 6 mL). These cartridges are conditioned with 10 mL of MeOH followed by 10 mL of MQW. Elution is performed with 2 × 5 mL of MeOH into 22 mL amber tubes. The samples are then reconcentrated a second time using the EZ-2 and then under a nitrogen stream until the meniscus is reached. They are reconstituted in 200 µL of MQW/MeOH (50/50), then applied to filter tubes, rinsing twice with 70 µL of MeOH. After centrifugation at 5000 rpm for 5 min, the filtrates are transferred (using a Pasteur pipette) into 2 mL amber vials with flat-bottom restrictors.

Water samples are extracted using HLB Chromabond cartridges (150 mg; 6 mL, preconditioned with 2 × 5 mL of MeOH +5 mL of MQW). Elution is performed with 2 × 5 mL of MeOH. The samples are reconcentrated under EZ-2 (100 % MeOH; 5) and then under a nitrogen stream until the meniscus, deposited on filter tubes (nylon; 0.2 µm VWR), then the amber flask is rinsed twice with 70 µL of 0.1 % formic acid MeOH before centrifuging the filter tubes at 6000 rpm for 2 min. The filtrate is collected in a 2 mL flat-bottom tube with a restrictor (400 µL), rinsing with 50 µL of 0.1 % formic acid MeOH.

Sediments samples are extracted using Chromabond-HLB cartridges (6 mL × 150 mg × 30 µm, Macherey-Nagel). The columns are conditioned with 5 mL of MeOH followed by 5 mL of MQW. After passing the entire sample through, the pump is left on vacuum for 3 min to dry the columns. Elution is carried out with 2 × 5 mL of MeOH.

The limits of detection (LOD) and limits of quantification (LOQ) of the analytical method were calculated using the signal-to-noise (S/N) ratios, as previously described in (Marchand et al., 2024). LOD and LOQ were determined by extracting spiked (100 $ng.g^{-1}$ for sediment $n = 3$, 50 $ng.g^{-1}$ for water, n = 3, and biological matrices, n = 3) samples. The

Table 1
Mean concentration and frequency of 42 chemical compounds (antibiotics [13] and pesticides [29]) in sediments (ng.g^{-1}) and water (ng.L^{-1}) compartments.

Type	Compound	Water (n = 3)				Sediments (n = 3)			
		Median	Mean	Max	Frequency	Median	Mean	Max	Frequency
Antibiotics	Acoxolinic acid	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.50	1.50	33.33
	Amoxicillin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Ciprofloxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Clarithromycin	21.4	23.4	42.0	100.00	<LOQ	<LOQ	<LOQ	0.00
	Enoxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Enrofloxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Erythromycin	7.9	9.8	14.5	100.00	<LOQ	<LOQ	<LOQ	0.00
	Flumequine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Norfloxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.86	2.58	33.33
	Ofloxacin	<LOQ	2.3	6.9	33.33	3.73	5.93	11.52	100.00
	Sulfamethoxazole	49.6	50.3	68.3	100.00	<LOQ	<LOQ	<LOQ	0.00
	Tetracycline	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Trimethoprim	<LOQ	2.8	8.3	33.33	<LOQ	0.12	0.35	33.33
Pesticides	3.4-Dichloroaniline	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Atrazine	8.3	8.9	11.2	100.00	<LOQ	<LOQ	<LOQ	0.00
	Bentazone	39.2	208.7	586.8	66.67	<LOQ	<LOQ	<LOQ	0.00
	Carbendazim	12.5	12.0	23.4	66.67	<LOQ	5.40	16.19	33.33
	Chlortoluron	65.6	49.5	70.8	100.00	<LOQ	<LOQ	<LOQ	0.00
	Desethylatrazine	17.4	18.3	25.9	100.00	<LOQ	<LOQ	<LOQ	0.00
	Desethylterbuthylazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Desisopropylatrazine	7.6	8.8	14.4	100.00	<LOQ	3.13	9.40	33.33
	Diflufenican	12.5	10.7	14.8	100.00	<LOQ	2.48	7.45	33.33
	Hydroxyatrazine	31.3	25.9	33.1	100.00	1.86	1.34	1.96	100.00
	Imidacloprid	9.8	10.6	13.4	100.00	<LOQ	<LOQ	<LOQ	0.00
	Imidacloprid Olefin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Imidacloprid Urea	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Irgarol	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Isoproturon	<LOQ	0.7	2.2	33.33	<LOQ	<LOQ	<LOQ	0.00
	Metazachlor	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor	8.2	6.1	10.0	100.00	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor ESA	42.6	42.5	54.5	66.67	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor OXA	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Nicosulfuron	<LOQ	0.7	2.2	33.33	<LOQ	<LOQ	<LOQ	0.00
	Oxadiazon	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Pendimethalin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Penoxsulam	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Propanil	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Prosulfuron	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Simazine	7.4	8.3	11.2	100.00	<LOQ	<LOQ	<LOQ	0.00
	Tebuconazole	<LOQ	<LOQ	<LOQ	0.00	0.35	0.66	1.62	66.67
	Tebufenozide	6.4	10.2	24.4	66.67	<LOQ	<LOQ	<LOQ	0.00
	Terbuthylazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00

ratio between spiked amount and S/N ratios was multiplied by 3 to determine LOD and by 10 for LOQ. These validations allowed the evaluation of recovery ratios for this protocol (mean recovery rate ± SD; sediments = 92.1 ± 11.1; water = 92.1 ± 5.4; biological matrices = 86.5 ± 7.9, see Appendix D). Blanks and quantification controls were added for each analytical run.

2.5. Statistical analyses

All statistical analyses were performed using R 3.6.3 (R Core Team, 2020) and Rstudio v1.1.419. We computed Linear Models (LMs), or binomial, quasipoisson and gamma Generalized Linear Models (GLMs) from the R base environment, and quasibinomial Generalized Linear Mixed Models (GLMMs) from the *MASS* package (Ripley et al., 2013). For LMs, normality was checked (shapiro test) and data were log10+1 or square root (sqrt) transformed when residuals did not follow the normality assumption. For all test, models accuracy was also tested using the check_model function from the *performance* package (Lüdecke et al., 2020).

2.5.1. Pollutants along the trophic chain

We analysed the Hg concentration, the total number (number of compounds) of antibiotics, pesticides and of both antibiotics and pesticides, found in each sample. This approach was chosen due to the high diversity and high number of compounds detected, with many compounds not being detected above their LOQ, which made analysing individual contaminant concentrations, and sum of all concentration, across trophic levels impractical within the scope of this study. By focusing on the number of detected compounds, we aimed to provide a broader ecological perspective on the distribution of chemical diversity across the food web. When an antibiotic or pesticide concentration could not be computed because the value was <LOQ, the value was replaced by 0. This decision was made to minimize the potential for artificial inflation of mean and additives values, especially important when the contaminant is rarely detected. This approach avoids overestimating the presence and impact of compounds that were not quantifiable, ensuring a more conservative and representative analysis of contamination levels across samples. Additionally, by focusing on detected values and treating undetectable concentrations as zero, we aim to provide a clearer picture of the distribution and prevalence of detectable contaminants, which is particularly relevant for understanding environmental and public health implications.

To check for the accumulation properties along the trophic chain, we tested Hg concentration (sqrt transformed), number of pollutants, and number of pesticides according to trophic level, using LMs. The effect on total number of antibiotics was tested using a quasipoisson GLM.

Trophic Magnification Factors (TMF), a metric used to quantify the increase in concentration of a contaminant with the trophic level (TL), was calculated for Hg following (Goutte et al., 2020; Zhou et al., 2020): $LogC_{biota} = a + b * TL$; $TMF = 10^b$. TMFs could not be computed for

antibiotics and pesticides, as most compounds are rarely detected in biological samples (see Table 2, mean frequency of detection in primary producers = 9.52 % [with 28 compounds never found above their LOQ], in macroinvertebrates = 5.80 % [with 27 compounds never found above their LOQ], and in fish = 2.04 % [with 35 compounds never found above their LOQ]). Particularly, 23 compounds (5 antibiotics and 18 pesticides) were never found in biological samples: amoxicillin, atrazine, bentazone, clarithromycin, desethylatrazine, enoxacin, enrofloxacin, erythromycin, imidacloprid, imidacloprid olefin, imidacloprid urea, irgarol, metazachlor, metolachlor OXA, nicosulfuron, oxadiazon, penoxsulam, propanil, prosulfuron, simazine, tebufenozide, terbuthylazine, desethylterbuthylazine.

2.5.2. Chemical properties

According to Zhou et al. (2020), we retrieved molecular properties of the studied pollutant from PubChem: $LogK_{OW}$ (logarithm of the octanol-water partition coefficient), molecular weight ($g.mol^{-1}$), solubility at 25 °C ($mg.L^{-1}$), $LogK_{OC}$ (soil organic carbon-water partitioning coefficient, $cm^3.g^{-1}$), and K_M (metabolic biotransformation factor). Data are provided in Appendix D. For many emergent pollutants and particularly antibiotics, no data were available for some molecular properties, and they were thus left as NA (Appendix D). For pollutants not detected in any sample, we treated their concentrations as missing values (NA) and excluded them from the regression analyses rather than assigning zeros, to avoid biasing the estimation of concentration–property relationships. This concerned 14 compounds that were never found neither in biologic, water or sediments compartments (amoxicillin, enoxacin, enrofloxacin, imidacloprid-olefin, imidacloprid-urea, irgarol, metazachlor, metolachlor OXA, oxadiazon, penoxsulam, propanil, prosulfuron, terbutylazine, terbutylazine-desethyl). All molecular properties ($LogK_{OW}$, molecular weight, solubility, $LogK_{OC}$, K_M) were first included simultaneously in a single Gamma GLM, with concentration of each pollutant in biological samples as a dependent variable. Variance inflation factors indicated no problematic collinearity among predictors. We then applied a stepwise drop-down selection procedure to identify the most parsimonious model.

3. Results

3.1. Compounds found in samples

Out of the 42 compounds (13 antibiotics and 29 pesticides) analysed, we detected 9 compounds at least once above their LOQs in sediments (4 antibiotics and 5 pesticides), and 20 (5 antibiotics and 15 pesticides) in water samples (Fig. 1, Table 1). In biological samples, 19 compounds (8 antibiotics and 11 pesticides) were detected at least once above their LOQs: 14 in primary producers (6 antibiotics and 8 pesticides), 15 in macroinvertebrates (5 antibiotics and 10 pesticides), and 7 in fish (3 antibiotics and 4 pesticides, Fig. 1, Table 2). The compound found with the highest concentration in water was the diazine herbicide bentazone,

Table 2
Mean concentration and frequency of detection (above LOQ) of 42 chemical compounds (antibiotics [13] and pesticides [29]) in biologic ($ng.g^{-1}$) samples.

Type	Compound	Primary producers (n = 18)				Macroinvertebrates (n = 23)				Fish (n = 21)			
		Median	Mean	Max	Frequency	Median	Mean	Max	Frequency	Median	Mean	Max	Frequency
Antibiotics	Acoxolinic acid	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.20	3.11	8.70	<LOQ	0.05	1.13	4.76
	Amoxicillin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Ciprofloxacin	<LOQ	1.34	24.15	5.56	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Clarithromycin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Enoxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Enrofloxacin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Erythromycin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Flumequine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.07	1.69	4.35	<LOQ	0.45	9.42	4.76
	Norfloxacin	<LOQ	0.50	6.34	11.11	<LOQ	1.85	22.68	8.70	<LOQ	3.78	55.93	9.52
	Ofloxacin	<LOQ	3.92	42.84	22.22	<LOQ	0.08	1.91	4.35	<LOQ	<LOQ	<LOQ	0.00
	Sulfamethoxazole	<LOQ	5.09	91.63	5.56	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Tetracycline	<LOQ	1.24	19.39	11.11	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Trimethoprim	<LOQ	1.65	7.45	44.44	<LOQ	0.44	2.79	39.13	<LOQ	<LOQ	<LOQ	0.00
Pesticides	3,4-Dichloroaniline	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.16	1.91	8.70	<LOQ	<LOQ	<LOQ	0.00
	Atrazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Bentazone	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Carbendazim	<LOQ	2.09	37.66	5.56	<LOQ	1.33	30.62	4.35	<LOQ	<LOQ	<LOQ	0.00
	Chlortoluron	<LOQ	2.64	21.29	33.33	<LOQ	21.07	484.71	4.35	<LOQ	<LOQ	<LOQ	0.00
	Desethylatrazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Desethylterbuthylazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Desisopropylatrazine	<LOQ	4.09	38.47	16.67	<LOQ	1.79	41.17	4.35	<LOQ	<LOQ	<LOQ	0.00
	Diflufenican	4.55	8.15	64.14	83.33	3.59	21.83	180.69	86.96	1.03	3.48	42.13	52.38
	Hydroxyatrazine	1.24	1.08	2.87	72.22	<LOQ	0.02	0.55	4.35	<LOQ	<LOQ	<LOQ	0.00
	Imidacloprid	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Imidacloprid Olefin	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Imidacloprid Urea	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Irgarol	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Isoproturon	<LOQ	0.18	0.58	44.44	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.02	0.33	4.76
	Metazachlor	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.26	5.91	4.35	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor ESA	<LOQ	<LOQ	<LOQ	0.00	<LOQ	0.12	2.72	4.35	<LOQ	<LOQ	<LOQ	0.00
	Metolachlor OXA	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Nicosulfuron	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Oxadiazon	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Pendimethalin	<LOQ	0.53	3.93	22.22	<LOQ	0.19	1.68	13.04	<LOQ	0.09	1.82	4.76
	Penoxsulam	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Propanil	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Prosulfuron	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Simazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Tebuconazole	<LOQ	0.76	7.40	22.22	<LOQ	2.72	17.89	43.48	<LOQ	0.05	1.12	4.76
	Tebufenozide	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00
	Terbuthylazine	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00	<LOQ	<LOQ	<LOQ	0.00

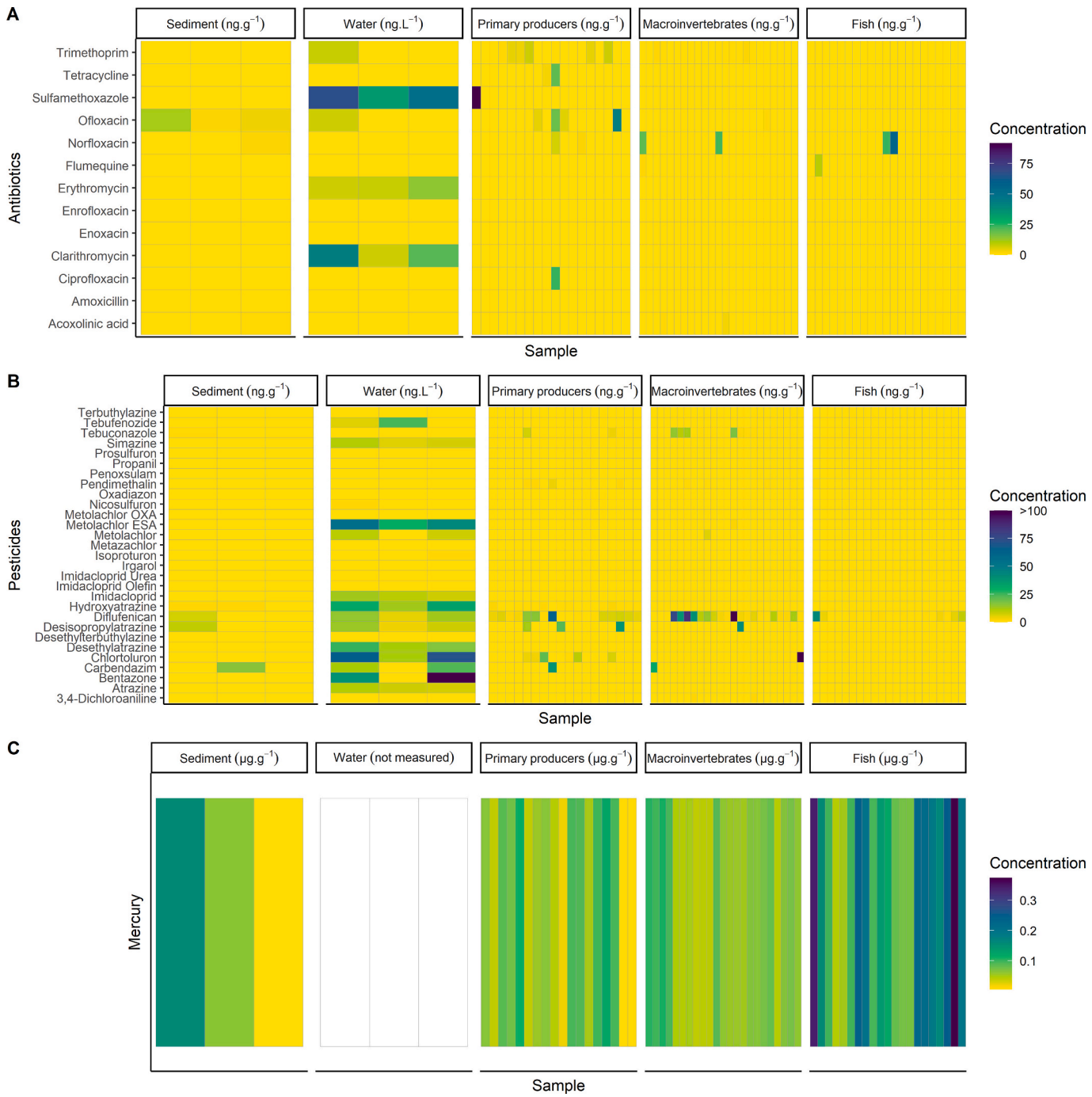

Fig. 1. Heatmap of 42 chemical compounds (antibiotics [13] and pesticides [29]) found in biologic ($N = 62$), sediments ($N = 3$) and water (N = 3) compartments. X axis represent each sample collected for each compartment, and Y axis the name of (A) antibiotic or (B) pesticide that had been screened. For (C) mercury no data were collected for samples in water. For clarity, values <LOQ were replaced by 0.

with a mean concentration of 208.67 ng.L^{-1} (Fig. 1, Table 1). Concentrations of all compounds in the sediments were much lower with mean concentration ranging from <LOQ to 5.93 ng.g^{-1} (Fig. 1, Table 1). The compound found with the highest concentration in primary producers was the sulfonamide antibiotic sulfamethoxazole, with a maximal concentration of 91.63 ng.g^{-1} (Fig. 1, Table 2). The compound found with the highest concentration in macroinvertebrates was the urea herbicide chlortoluron, with a maximal concentration of 484.71 ng.g^{-1} (Fig. 1, Table 2). The compound found with the highest concentration in fish was the fluoroquinolone antibiotic norfloxacin, with a maximal concentration of 55.93 ng.g^{-1} (Fig. 1, Table 2). The herbicide diflufenican was the compound with the highest prevalence, being found in 83.33 %, 86.96 % and 52.38 % of the primary producers, macroinvertebrates and fish samples, respectively (Fig. 1, Table 2).

3.2. Pollutants along the trophic chain

Mercury concentration increased along the trophic chain (Table 3, Fig. 2A), with a trophic magnification factor of 1.57. In contrast, number of antibiotics detected in each individual marginally decreased along the trophic chain (Table 3, Fig. 2B). Number of pesticides detected in each individual decreased along the trophic chain (Table 3, Fig. 2C). Number

Table 3
Statistical analyses for the effect of trophic level on mercury concentration and pollutants number, and of chemical properties on pollutants concentration in biological samples. $LogK_{OW}$ (logarithm of the octanol-water partition coefficient), molecular weight ($g.mol^{-1}$), solubility at 25 °C ($mg.L^{-1}$), $LogK_{OC}$ (soil organic carbon-water partitioning coefficient, $cm^3.g^{-1}$), and K_M (metabolic biotransformation factor). For chemical properties, as a drop down selection was processed, for the discarded parameters, the last values before exclusion from the model were retained. Only $LogK_{OW}$ was retained in the final model. Antibiotics and pesticides values for which concentrations were < LOQ were replaced by 0.

Dependant variable	Explanatory variable	Estimate	SE	t-value	*p*-value	
Mercury	Trophic level	0.070	0.015	4.690	<0.001	*
Number of antibiotics	Trophic level	−0.402	0.227	−1.776	0.081	.
Number of pesticides	Trophic level	−0.954	0.203	−4.694	<0.001	*
Number of pollutants	Trophic level	−1.201	0.262	−4.584	<0.001	*
Pollutants concentration in biological samples	$LogK_{OW}$	0.189	0.092	2.059	0.040	*
	Molecular weight	<−0.001	<0.001	1.033	0.302	
	Solubility	0.003	0.004	0.814	0.416	
	$LogK_{OC}$	<0.001	<0.001	−1.243	0.214	
	K_M	−0.003	0.127	−0.251	0.802	

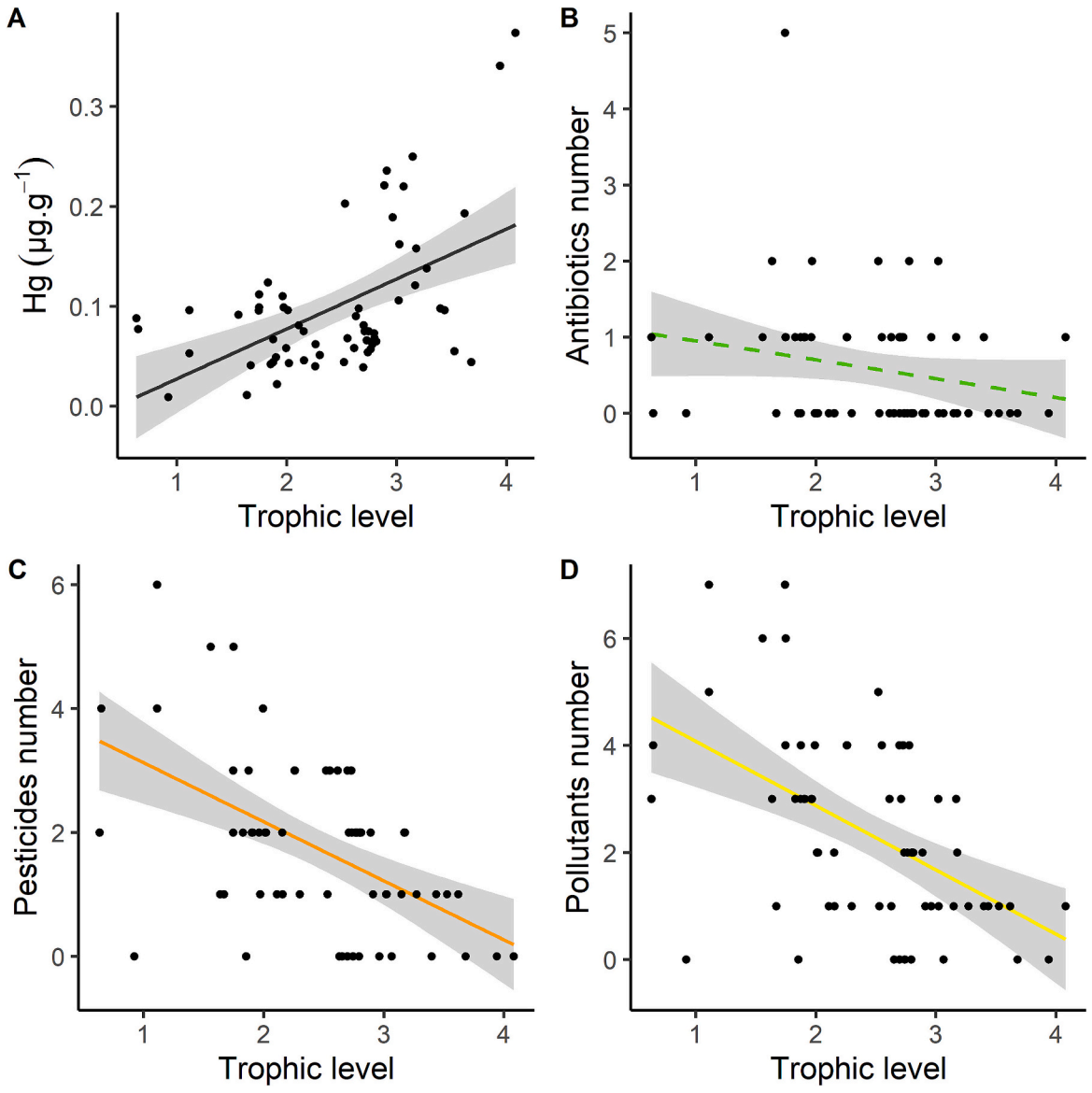

Fig. 2. (A) Mercury concentration, number of (B) antibiotics, (C) pesticides and (D) pollutants (antibiotics and pesticides), detected in 62 individuals along the trophic chain, among the 42 chemical compounds tested (antibiotics [13] and pesticides [29]). Dotted line indicates a marginally significant relationship.

of pollutants detected in each individual decreased along the trophic chain (Table 3, Fig. 2D).

3.3. Chemical properties

Concentration of pollutants in biological samples increased with increasing $LogK_{OW}$, and was not influenced by the molecule solubility, soil organic carbon-water partitioning, molecular weight, and metabolic biotransformation factor (Table 3, Fig. 3).

4. Discussion

Our findings revealed significant contamination across all sample types, with, across 42 pollutant compounds scanned, 19 compounds detected in biological samples, 9 in sediments, and 20 in water. The distribution of pollutants along the trophic chain varied, with Hg showing biomagnification, while detection of antibiotics and pesticides overall decreased along the trophic chain, but with differing patterns specific to each contaminant. The chemical property that mostly explained pollutant concentrations in biological samples was $LogK_{OW}$.

4.1. Pollutants found in the system

The widespread detection of pollutants across different sample types underscores the extensive contamination in the studied ecosystem, which is located in a highly urbanized area with multiple sources of pollution, including discharges from wastewater treatment plants and runoff from artificial surfaces, as found in many freshwater bodies (Arnold et al., 2014; Schäfer et al., 2011). Importantly, atrazine is found in water despite its ban in the European Union in 2003 (Bethsass and Colangelo, 2006), as well as imidacloprid, banned in 2018, though reauthorized by derogation for sugar beet in 2021 (Perrot et al., 2024), which suggests the environmental persistence of these compounds for several years and decades. Diflufenican was the compound with the highest prevalence in all biological samples, which might be due to its widespread use in agriculture and its persistence in the environment (Time to 50 % dissipation [DT_{50}] = 95 days, lab, 20 °C, Lewis et al., 2016).

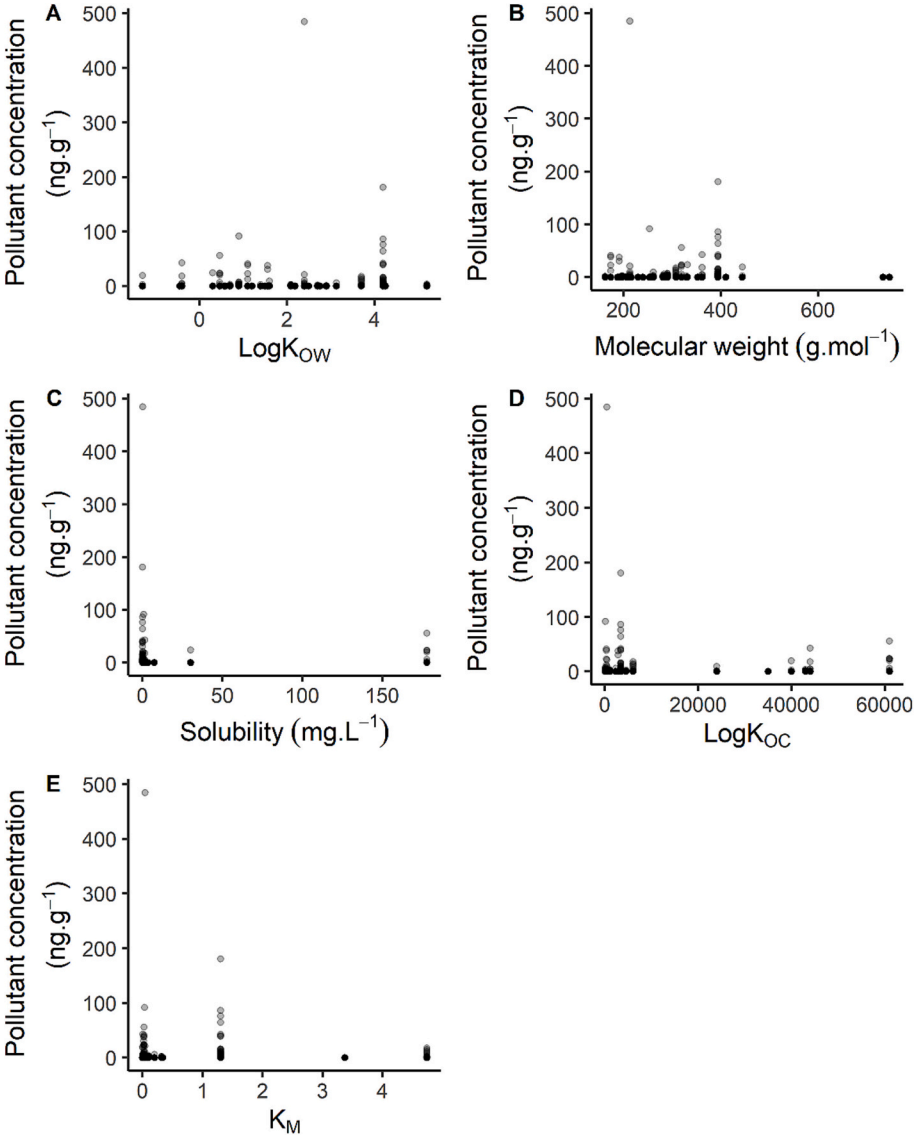

Fig. 3. Pollutant concentration (sum of the concentration of the 42 chemical compounds: antibiotics [13] and pesticides [29]) found in 62 biological samples (ng.g^{-1} dry weight), according to their chemical properties: $LogK_{OW}$ (logarithm of the octanol-water partition coefficient), molecular weight (g.mol^{-1}), solubility at 25 °C (mg.L^{-1}), $LogK_{OC}$ (soil organic carbon-water partitioning coefficient, cm^3.g^{-1}), and K_M (metabolic biotransformation factor). Individual data points are plotted with partial transparency so that overlapping values appear darker, with deeper colors indicating higher densities of points.

The presence of 19 out of 42 compounds in biological samples highlights the potential for transfer of most of the compounds we studied. Importantly, some pollutants are also neither found in the biota or in water or sediments samples, but some contaminants are found only in water but not in the biota (clarithromycin, erythromycin, atrazine, bentazone, desethylatrazine, imidacloprid, nicosulfuron, simazine and tebufenozide), which can be due to their molecular properties (Zhou et al., 2020) (see below), persistence in water and sediment matrices (Froger et al., 2023) and/or bioavailability. Conversely, some compounds are found in biological samples, but neither in water or sediments samples (i.e. ciprofloxacin, flumequine, tetracycline, 3,4-dichloroaniline and pendimethalin). While some discrepancies might be explained by differing limits of quantification (LOQ) between biological, sediments and water samples, this may also highlight spatial remobilization or displacement of pollutants, which can occur for both water and sediments (Ciszewski and Grygar, 2016; He et al., 2021). Individuals can also be polluted from other points when they move, or have been polluted at a different time, highlighting long-term processes of accumulation and depuration, this snapshot of contamination at a given time limiting our ability to capture the full temporal dynamics of pollutant distribution. This finding thus highlights that overall concentration of pollutants in water and sediments on a site at a given time is not systematically representative of all compounds that might be found in the biota.

4.2. Pollutants along the trophic chain

Our findings are in line with the biomagnification of Hg, as documented in numerous studies (Córdoba-Tovar et al., 2022; Lavoie et al., 2013). This pattern aligns with mercury's strong affinity for organic matter (Wang et al., 2022). The biomagnification of mercury can be attributed to its persistence and ability to bind with proteins of organisms (Liu et al., 2011; Okpala et al., 2018). Mercury's low depuration rate and its ability to form methylmercury (Peng et al., 2016), further exacerbate its accumulation in aquatic food webs. The TMF (1.57) estimated from our data set was lower than TMFs of Hg usually found in freshwater habitats (mean TMF = 4.3 ± 4.8, Lavoie et al., 2013). Since this value can vary with latitude and according to the habitat type (Borgå et al., 2012; Lavoie et al., 2013), we considered that our results are consistent with previous studies.

Trophodynamics of antibiotics and pesticides in freshwater ecosystems are much less investigated. The decrease in the number of these pollutants can be explained by their specific chemical properties. Indeed, the positive relationship with $LogK_{OW}$ suggests that more lipophilic compounds are likely to accumulate in biological tissues (Geyer et al., 2000), and differing metabolization/excretion patterns for hydrophilic compounds as compared to hydrophilic ones (Benedetti et al., 2009).

Overall, the reduction in the total number of detected pollutants across the trophic chain is primarily explained by the decline in pesticide occurrence, suggesting that these compounds are more readily metabolized or excreted by organisms at successive trophic levels (Goutte et al., 2020). This reduction in pesticide load at higher trophic levels could be due to the metabolism of these compounds by the organisms at each step of the trophic chain (Büyüksönmez et al., 1999; Tiryaki and Temur, 2010) or the preferential uptake of more hydrophilic pesticides by primary producers (Ge et al., 2014; Hsu and Kleier, 1996). This dilution effect may also be influenced by the metabolic capacity of organisms at higher trophic levels to biotransform and then excrete metabolized pesticides (Rőszer, 2014), intake and elimination rates being species dependent (Matthee et al., 2023). We found a weaker reduction of antibiotics number along the trophic chain. The difference with pesticides might result from the differential degradation or metabolism of specific antibiotics, leading to the persistence of more recalcitrant compounds (Bilal et al., 2019). Accumulation or degradation and excretion of antibiotics may depend on physiological state (Long et al., 2024; Matthee et al., 2023), but also on specificities of the host such as the activity of drug-metabolizing enzymes and the relative contribution of different excretory organs (Matthee et al., 2023), or $LogK_{OW}$ of antibiotics, which are relatively low. Our approach highlights the trophic-level-dependent variation in exposure to a wide range of substances, offering valuable insights into potential ecological implications that may not be fully captured by focusing solely on individual contaminant concentrations. Still, the concentrations of the detected compounds in our study were generally low, and often near or below LOQs, even in this highly urbanized river system where contamination was expected to be elevated. This suggests that many of the targeted current-use pesticides and antibiotics are not strongly bioaccumulating or persisting in higher trophic levels, which might for example depend on the environment properties including soil characteristics (Svendsen et al., 2020) and environmental conditions and hydrological dynamics (Tamtam et al., 2008). The concentrations of key detected compounds such as bentazone, chlortoluron, norfloxacin, and diflufenican were below reported effect thresholds for aquatic organisms (e.g. see Mäenpää et al., 2003; Sikorski and Bęś, 2024; Valiente Moro et al., 2012), indicating a low likelihood of acute toxic effects. However, the simultaneous detection of multiple compounds across matrices raises the possibility of mixture effects, which are increasingly recognized as a potential driver of sub-lethal impacts on aquatic organisms even when individual compounds occur below effect thresholds (Arnold et al., 2014; Relyea, 2009). Further research is needed to disentangle these complex interactions, particularly by examining how chemical properties, biological pathways, and environmental conditions converge to shape pollutant behavior in ecosystems.

5. Conclusion

Our study reveals widespread contamination across compartments, with 19 pollutants detected in biota, including substances banned for over a decade, underscoring the persistence of legacy pollutants. Pollutant transfer along the trophic chain varied by compound: mercury biomagnified, while detections of pesticides and antibiotics showed a decrease along the trophic chain. These trends were linked to chemical properties, particularly $LogK_{OW}$, and presumed organism-level metabolism. Notably, discrepancies between pollutants in abiotic matrices and biota highlight the limits of water or sediment monitoring alone. By integrating trophic position, compound properties, and multiple matrices, our work provides a comprehensive picture of pollutant dynamics in urban freshwater systems.

CRediT authorship contribution statement

Léa Lorrain-Soligon: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Etienne Marchand:** Writing – review & editing, Methodology, Investigation. **Fabrice Alliot:** Writing – review & editing, Methodology, Investigation, Data curation. **Paco Bustamante:** Writing – review & editing, Methodology, Investigation. **Fabienne Petit:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Aurélie Goutte:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180617.

Data availability

The data that supports the findings of this study will be made available by the corresponding author upon request.

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