Pharmaceuticals and illicit drugs in surface water and wild freshwater invertebrates in Luxembourg

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Abstract. Pharmaceutical as well as illicit drugs are massively consumed worldwide and many studies have established that their release, respectively the release of their metabolites, into the environment may have considerable negative effects on the wildlife and general ecosystems functioning. This study deals with the detection and the determination of contamination levels of some commonly used pharmaceuticals and illicit drugs in surface water and in the two freshwater invertebrates *Gammarus pulex* and *Asellus aquaticus* collected in Luxembourg. A method, based on liquid chromatography coupled to a quadrupole time-of flight mass spectrometer (LC-QToF-MS), was developed to investigate eight compounds (alprazolam, bisoprolol, carbamazepine, citalopram, lidocaine, O-desmethyltramadol, MDMA and benzoylecgonine, the major cocaine metabolite). Except for alprazolam all compounds were detected at least once in the surface water. Three of these compounds were detected in biota samples with concentrations ranging from < LOQ to 6.3 ng/g of dry weight. The bioaccumulation (BAF) factors were calculated for two compounds. The results draw attention to the importance of monitoring drugs in freshwater biota to better assess the effects of drugs on non-target organisms.

Keywords: Freshwater invertebrates, Crustacea, pharmaceuticals, illicit drugs, LC-QToF-MS.

1. Introduction

Water pollution with pharmaceuticals and illicit drugs has become a widespread environmental problem during the last decades, particularly in high-income countries. Presence of pharmaceutically active compounds or their metabolites in the environment mainly occurs through incompleted removal in wastewater treatment plants (WWTPs) and also through direct improper disposal via environmentally unfriendly routes such as garbage, toilet or sink (Rogowska et al. 2022) and hydraulic overload of sewer systems after heavy rain for exemple. Their concentration levels in surface waters generally are in the ng/L to µg/L range. For most of the compounds no or only little environmental data such as hydrolysis, bioaccumulation, biodegradation, photodegradation are available (MacCall et al 2016, Bavumiragira, et al. 2022, Sanusi et al. 2023), but it is widely acknowledged that many of them may have chronic or acute negative effects on aquatic organisms (Kock et al. 2023, Nguyen et al. 2024) and on farm and wildlife animals (Vaudin et al. 2022). Even if the associated uncertain-

ties are high, there is no doubt that they may concentrate and accumulate in aquatic food chains (Lagesson et al. 2016, Zhengxin et al. 2017, Gómez-Regalado et al. 2023). The bioconcentration factors (BAF) have been reported to range over several orders of magnitude (Lagesson et al. 2016, Puckowski et al. 2016) and the toxic effects of each drug may be potentialized by additive or synergistic effects of multi drug mixtures often found in surface waters.

Pharmaceutically active compounds may have significant toxic effects on aquatic wildlife. Reported effects are manifold (Burkina et al., 2015, Chen et al., 2021, Miller at al. 2021, Versteegen et al. 2025). They include interferences with enzymes and hormones; impairment of reproduction; growth and development retardment; altered feeding behaviour; mobility and gene expression; impairment of the immune response and, last but not least, DNA damage possibly leading to mutations of the long-term viability. However, the uptake, distribution, degradation and elimination of pharmaceuticals and illicit drugs by invertebrates are still not well studied and potentially have far-reaching implications for ecosystems (Sumpter et al. 2022). Understanding these effects is important for developing strategies to protect biodiversity and ecosystem health. In this context, more studies of wildlife exposure to pharmaceuticals and drugs will help to measure and evaluate the impact of their concentrations in biota.

In the current study, populations of the two freshwater invertebrates *Asellus aquaticus* (Crustacea, Isopoda) and *Gammarus pulex* (Crustacea, Amphipoda) and surface water were collected from several water bodies in Luxembourg and analysed for residues of pharmaceuticals and illicit drugs using a validated LC-QToF-MS method. The goal of this study was to contribute to the knowledge of drug exposure and drug levels in wild freshwater crustaceans.

2. Materials and methods

2.1. Consumables

HPLC-S grade acetonitrile (ACN), methanol (MeOH), water and dichloromethane (DCM) were purchased from Biosolve BV (Valkenswaard, Netherlands). Ammonium acetate a.r. was purchased from ChemLab NV (Zedelgem, Belgium). Emsure® ACS, Reag. Ph Eur formic acid (FA) 98 - 100 % and Emsure® ACS, ISO, Reag. Ph Eur glacial acetic acid 100 % were purchased from Supelco Merck KGaA (Darmstadt, Germany). Solvents for the LC mobile phase, Optima® LC-MS ACN with 1 % FA and Optima® LC-MS water with 0.1 % FA were purchased from Fisher Chemical (Geel, Belgium). Blank dried gammarus were purchased online from www.futter-tiere.de. Cellulose filters were purchased from Macherey-Nagel (Hoerdt, France). Solid phase extraction columns (Oasis HLB, 6 cc, 200 mg) and the HPLC column (Acquity® UPLC BEH Shield RP18 1.7 µm x 2.1 mm x 100 mm) were purchased from Waters (Asse, Belgium).

2.2. Reagents

Citalopram HBr and bisoprolol were purchased from Sigma Aldrich (Overijse, Belgium). Citalopram-d6 HBr was purchased from Lipomed (Arlesheim, Switzerland). Bisoprolol-d5, carbamazepine, carbamazepine-d10, O-desmethyltramadol HCl, O-desmethyltramadol-d6 HCl, lidocaine, lidocaine-d10 HCl, MDMA, MDMA-d5, alprazolam and alprazolam-d5, benzoylecgonine and benzoylecgonine-d3 were all purchased from LGC (Molsheim, France). Stock solutions (c = 1 mg/mL) and working solutions (c = 0.001 mg/mL – 0.1 mg/mL) were prepared in MeOH. 1 mg/mL and 0.1 mg/mL purchased standard solutions were diluted in MeOH to the concentrations of the working solutions. All standard solutions and working solutions were stored in the dark at – 20 °C before use.

2.3. Selection and collection of surface water and biota

The freshwater amphipod *Gammarus pulex* (Linnaeus, 1758) was initially selected as a test species because it was expected to have a high abundance and was already successfully used for the detection of drug residues in several studies (Ogunbanwo et al. 2020).

However, this species was not present in sufficient numbers at all sampling sites. Instead, the water louse *Asellus aquaticus* (Linnaeus, 1758) was found to be more abundant in local running and stagnant waters and was therefore collected at most sites. Freshwater amphipods and water lice are shredders and feed on plant detritus, but coarse particulate organic matter from the surrounding water can be another possible source of pollutant intake (Graça et al 1994).

Sample collection was carried out on 9 sites between November 2023 and April 2024. Predominantly highly populated areas have been selected as, at these spots, higher levels of pharmaceuticals and drug contamination were expected. Sampling sites are shown in Fig. 1. They were located along the Alzette river (sites A, B, F, H, I), the Petrusse river (sites C and D) and Diddelenger creek (site G). A final collection site was a small pond in Luxembourg City (site E). One sampling campaign (defined as a sample collection carried out at a given location and a given day) was carried out at sites B, C, E, H and I and two sampling campaigns were carried out at the sites A, D, F and G.. A total of 13 samples were collected.

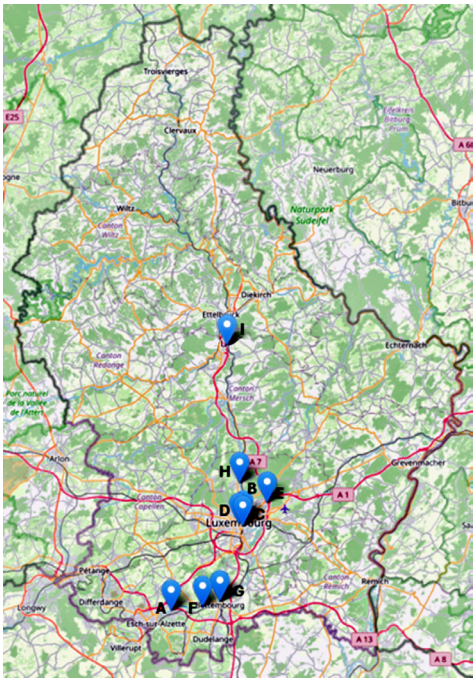


Fig. 1. Locations of sampling sites A-I.

The benthic sediment, including *G. pulex* and *A. aquaticus,* was collected by kick sampling into a landing net. At each sampling site, approximately 100 specimens of the respective target species were collected and directly transferred into a container with freshwater from the collection site. All organisms were collected and analysed regardless of size or gender. At the end of each sampling day, organisms were separated from the water by sieving and transported to the laboratory at 0° C. At the laboratory, the organisms were stored at -20 °C until they were processed. Also, at each sampling site, approximately 350 mL of surface water was collected. The water samples were stored in 1 L glass bottles and kept at -20 °C until further processing.

2.4. Target analytes

The target analytes were selected from pharmaceuticals and illicit drugs known to be commonly consumed in Luxembourg. The selection consisted of alprazolam (tranquilizer and anxiety disorder treatment), bisoprolol (high blood pressure and heart failure treatment), carbamazepine (anticonvulsant), citalopram (antidepressant), lidocaine (local anaesthetic) and O-desmethyltramadol (the active metabolite of the prodrug tramadol, pain medication). Finally, 3,4-methylenedioxy-N-methyl-amphetamine (MDMA, ecstasy) and benzoylecgonine (BZE), the major metabolite of cocaine, were also included in the study.

2.5. Preparation of biota samples

Amphipods and water lice were thawed and rinsed with deionized water. Following, the organisms were counted, all organisms from one sampling site were combined and weighed and the average weight of the organisms from each sampling site was determined.

Before extraction, the biota samples were lyophilized at -100 °C under vacuum for 48 hours using a FreeZone 4.5 Liter Freeze Dryer (Labconca, Kansas City, Missouri, United States) and the residual free water was measured with a LabMaster Neo

(Novasina, Lachen, Switzerland). Pulverization was performed using a Model Mixer Mill (Retsch GmbH, Haan, Germany) at a speed of 25 rotations/sec for 2 min. After pulverisation, a twostep extraction process was applied to concentrate the analytes and reduce as much as possible matrix effects. Samples were extracted by solid/liquid extraction followed by solid phase extraction (SPE) based on a method published by Miller et al (2019). Calibration and blank samples were prepared using purchased dried *G. pulex*. For each sequence, a new set of calibration and blank samples was freshly prepared.

About 100 mg of powdered samples were used for each calibration point, blank and unknown samples. The samples were transferred into a 5 mL flask; reference standards and deuterated internal standards (ISTD) were added to the calibration samples to obtain final concentrations of 0.5, 1, 10, 20, 30 and 40 ng/mL respectively. Samples were then heated at 30 °C for 25 min to evaporate remaining solvent from the ISTD-solution and bind the standards to the matrix. The 5 mL flasks were filled to the mark with ACN followed by ultra-sonication for 15 min. After filtration using a cellulose filter, the solutions were diluted to 100 mL with extraction solvent. The extraction solvent consisted of 1.54 g ammonium acetate dissolved in 2.0 L deionised water, adjusted to pH 6.5 using a 10 % acetic acid solution.

After a second filtering step using a 1 µm syringe filter, SPE was performed to upconcentrate the samples and separate them from the remaining matrix. The extraction was performed under vacuum (900 mbar) using the Oasis HLB 6cc (200 mg) extraction cartridges. Before loading the samples, the columns were activated by applying two times 4 mL MeOH and conditioned by addition of two times 4 mL extraction solvent. After loading, the columns were washed with 1 mL ultrapure water and subsequently dried under vacuum for 40 min. The cartridges were stored at -20 °C until elution of the target compounds. To prepare the samples for LC-QToF analysis, the cartridges were thawed, and the analytes were eluted with 4 mL of elution solvent (50 % DCM / 50 % ACN + 2 % FA).

The solutions were then evaporated to dryness under nitrogen at 40 °C and the residues were reconstituted in 250 µL of a mixture of 2 % ACN and 98 % water, both containing 0.1 % FA. An aliquot of 100 µL was transferred into HPLC-vials for LC-QToF analysis.

2.6. Preparation of water samples

The calibration samples and blanks were prepared in a blank matrix consisting of 50 mL deionised water. ISTD (final concentration 5 ng/mL) and reference standards were added to the calibration samples. The concentration range for all target compounds was 0.5-40 ng/mL. The water samples were diluted to 100 mL with buffer solution (10 mM ammonium acetate pH 6.5). The SPE was carried out as for the biota samples. After evaporation, the residue was reconstituted in 500 µL of a mixture of ACN/H_2O/FA (2 / 98 / 0.1, v/v/v). An aliquot of 100 µL was transferred into HPLC vials for analysis.

2.7. LC-QToF-MS analysis

The instrument was an Agilent 1290 HPLC equipped with an ACQUITY® UPLC BEH Shield RP18 column (1.7 µm x 2.1 mm x 100 mm), coupled to an Agilent 6550 iFunnel Q-ToF MS. Agilent MassHunter Workstation LC/MS Data Acquisition for 6200 series TOF/6500 series Q-TOF (Version 10.1) software was used for data acquisition. Agilent MassHunter Workstation Quantitative Analysis for Q-Tof (Version 10.1) software was used for data analysis. The column temperature was set to 60 °C. The mobile phase consisted of water with 0.1 % FA (solvent A) and ACN with 0.1 % FA (solvent B). A solvent gradient was applied as shown in Table 1 at a flow rate of 0.3 mL/min. The injection volume was 10 µL. The temperature of the autosampler was set to 4 °C. The total run time was 18 minutes.

The mass spectrometer used for the analysis is equipped with a Dual Agilent Jet Stream Technology Electrospray Ionization source. It can operate in both positive and negative ionization mode. The temperature of the

Table 1. Parameters for gradient elution (solvent A: water with 0.1 % FA; Solvent B: ACN with 0.1 % FA).

Time [min]	Solvent A [%]	Solvent B [%]
0	98	2
2	95	5
8	80	20
10	45	55
14	45	55
16	2	98
17	2	98
18	98	2

drying gas (nitrogen) was set to 225 °C with a flow rate of 14 L/min. Nebulizer gas (N_2) pressure was set to 35 psi. The capillary voltage was set to 3000 V and the fragmentor voltage was set to 175 V. The acquisition rate for MS mode was set to 5 spectra/sec with an acquisition time of 200 ms/spectrum. For MS/MS mode, the acquisition rate was 3 spectra/sec with an acquisition time of 333.3 ms/spectrum.

All compounds were analysed in the positive ionization mode. According to their retention times, the analysis was split into five time windows, and scanning for the specific m/z ratios of each analyte was done in each window.

For each substance, the daughter ion with the highest abundance was chosen as the quantifier ion and one or two further fragments were selected as qualifiers. For O-desmethyltramadol, no qualifiers were chosen, as for this compound, no sufficiently abundant fragments were obtained.

2.8. Identification and quantification criteria

In order to confirm the presence of a target analyte, the Rt had to match the Rt of its associated internal standard (± 0.1 min), the parent, qualifier and quantifier ions had to have an *m/z* of ± 100 ppm and the relative response of the analyte had to be three times higher than in the blank sample.

Quantification was performed only when all identification criteria were met. Signals in the blank samples were subtracted from calibration and unknown samples. The concentrations of biomarkers in the samples were determined by plotting the relative response of the analyte to the relative response of the calibration samples. All calibration curves were checked by measuring a control sample containing the reference substances at a concentration of 12 ng/mL. Concentration levels in surface waters were measured in ng/L and concentration levels in biota were measured in ng/mL and converted into ng/g dw (dry weight) for biota using the formula:

$$c\left[\frac{ng}{(g\ \ dw)}\right] = \left[\frac{c\ \ \left[\frac{ng}{mL}\right]\ \ \times 0.250mL}{sample\ \ \ weight\ \ \ [g]}\right]$$

c [ng/g dw]: concentration in the sample/calibration sample; c [ng/mL]: concentration in the vial; 0.250 mL: reconstitution volume after SPE.

2.9. Method validation

The validation criteria included the linearity of the calibration curve (criteria: $R^2 \geq 0.985$), the determination of repeatability of combined extraction and quantification process using a spiked *G. pulex* and tap water samples (c = 20 ng/mL, criteria RSD ≤ 25 %). The limit of detection was defined as S/N > 3; the limit of quantification was defined as the lowest calibration level for each compound. As a new calibration curve was established for each LC/QToF measurement day (approximately 20 samples) the validation was carried out on an intraday basis only.

3. Results

3.1. Characterization of biota

Thirteen collection campaigns were done at nine collection sites. *G. pulex* was sampled 4 times at sites D, G (2 campaigns) and I; *A. aquaticus* was sampled 9 times at sites A (2 campaigns), B, C, D, E, F (2 campaigns)

and H (see fig 1). The average yield was 129 specimens per collection site. *G. pulex* had a wet weight ranging from 11.2 - 43.5 mg/organism; the average weight was 28.5 mg/organism. *A. aquaticus* had a wet weight ranging from 6.27 - 24.8 mg/organism, the average weight was 17.5 mg/organism.

The average weight loss after 48 hours of lyophilisation was 76.2 % for amphipods and 73.6 % for water lice. The dry weight per organism ranged from 2.6 - 10.9 mg for *G. pulex* and 2.4 - 5.6 mg for water lice. Residual free water (a_W) ranged between 0.03 - 0.05 for both species, confirming that the lyophilization process was complete.

3.2. Method validation

Results from test measurements indicated that *G. pulex,* spiked with the target compounds and extracted as described in section 2.5 gave non reproducible results (RSD > 25 %) for alprazolam, benzoylecgonine and carbamazepine when stored for > 7 days at room temperature, 5 °C or -20 °C. Also, for carbamazepine and alprazolam, the r^2 of the calibration curve was < 0.985 for benzoylecgonine and the bias of the control sample was > 25 % after storage for more than one month. For these reasons, all measurements were carried out not more than 3 days after sampling.

Intraday validation results, obtained after spiking blank *G. pulex* and water samples, are summarized in Table 2. In this study, we assumed same matrix effects for *A. aquaticus* and *G. pulex* but this may need confirmation in future studies.

3.3. Drug loads in surface water

Recent studies indicate water contamination with licit and illicit drugs is a major phenomenon in most countries (Colzani et al. 2024). This is confirmed in the present study, as, except for alprazolam, all drugs have been detected (and quantified) at least once at all sampling sites. The main results (Table 3) were:

- samples from location E (small pond in Luxembourg City) were the least contaminated, only desmethyltramadol has been detected in one sampling campaign;
- overall, average loads of desmethyltramadol were the highest (average 63.6 ± 44.4 ng/L); this is in accordance with other reports (Rúa-Gómez et al. 2012, Archer et al. 2017);
- benzoylecogonine levels were second highest (average 34.1 ± 16.7 ng/L) but lower than those reported from other locations (Masemola et al. 2020, Campestrini et al. 2017).

3.4. Drug loads in *Gammarus pulex* and *Asellus aquaticus*

Our results confirm earlier studies reporting drug uptake by amphipods. The main findings are summerized hereunder and in Table 4:

- In 12 out of the 13 sampling campaigns (92.3 %), at least one compound has been detected. Only one *G. pulex* sampling campaign at the Petrusse river was negative for all compounds tested.
- On average 1.8 out of the 8 compounds tested (22.1 %) have been detected in each campaign.
- A total of 104 single measurements have been carried out (13 campaigns x 8 compounds). 81 (77.9 %) of the measurements were negative (no compound detected); in 10 measurements (9.6 %) compounds were detected at levels below the LOQ and in 16 (12.5 %) measurements compounds were detected and have been quantified.
- More positive results were found for *A. aquaticus* (25.0 % of the tests carried) than for *G. pulex* (15.6 %).
- 5 compounds have not been detected in any sample (MDMA, alprazolam, benzoylecgonine, carbamazepine and lidocaine)
- 3 compounds (O-desmethyltramadol, bisoprolol and citalopram) have been detected; all of them several times (range 5 – 10 times); citalopram was the most frequently detected substance (10 positive samples).

Table 2. Validation measurements (intraday) for *Gammarus pulex* and surface water.

Target compound	Linearity calibration (R^2)	Repeatability *G. pulex* (RSD, %)	Drug recovery *G. pulex* (%)	Repeatability water (RSD, %)	Drug recovery water (%)
MDMA	≥ 0.985	7.1	108.5	6.4	79.3
Bisoprolol	≥ 0.985	5.1	87.7	5.3	95.3
Alprazolam	≥ 0.985	8.3	65.9	11.1	79.5
Benzoylecgonine	≥ 0.985	9.4	19.7	5.8	90.7
Carbamazepine	≥ 0.985	15.7	93.4	5.5	96.6
Citalopram	≥ 0.985	2.1	80.1	2.1	88.3
Lidocaine	≥ 0.985	3.5	89.6	4.0	76.5
Desmethyltramadol	≥ 0.985	3.1	85.3	2.5	92.3

Table 3. Drug concentrations in surface water at the 9 sampling sites (all results in ng/L; nd: not detected; < LOQ: detected but result below LOQ; no measurements were carried out for D (2)).

Sampling site	Sample	MDMA	Bisoprolol	Alprazolam	Benzoylecgonine	Carbamazepine	Citalopram	Lidocaine	Desmethyltramadol
A (1)	Surface water	< LOQ	11.1	nd	57.8	17.3	< LOQ	nd	62.8
A (2)	Surface water	< LOQ	10.0	nd	21.9	10.8	< LOQ	nd	47.2
B	Surface water	nd	10.8	nd	< LOQ	37.1	< LOQ	26.5	147.4
C	Surface water	5.6	5.4	nd	41.7	< LOQ	< LOQ	15.6	7.6
D (1)	Surface water	< LOQ	< LOQ	nd	8.6	< LOQ	< LOQ	< LOQ	7.7
D (2)	Surface water	-	-	-	-	-	-	-	-
E	Surface water	nd	nd	nd	nd	nd	nd	nd	21.9
F (1)	Surface water	< LOQ	19.8	nd	45.8	30.9	6.0	18.7	77.3
F (2)	Surface water	nd	23.6	nd	< LOQ	30.9	18.4	nd	81.0
G (1)	Surface water	nd	< LOQ	nd	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
G (2)	Surface water	8.8	< LOQ	nd	nd	< LOQ	< LOQ	< LOQ	nd
H	Surface water	9.5	32.1	nd	38.7	44.8	14.0	42.4	105.3
I	Surface Water	<LOQ	25.1	nd	24.5	37.4	9.0	39.3	78.1

Table 4. Results from drug testing at the 8 sampling sites (all results in ng/g dry weight; nd: not detected, < LOQ: detected but result below LOQ).

Sampling site	Species	MDMA	Bisoprolol	Alprazolam	Benzoylecgonine	Carbamazepine	Citalopram	Lidocaine	O-desmethyl-tramadol
A (1)	*A. aquaticus*	nd	nd	nd	nd	nd	nd	nd	< LOQ
A (2)	*A. aquaticus*	nd	< LOQ	nd	nd	nd	1.8	nd	2.6
B	*A. aquaticus*	nd	nd	nd	nd	nd	4.3	nd	4.8
C	*A. aquaticus*	nd	nd	nd	nd	nd	2.3	nd	nd
D (1)	*A. aquaticus*	nd	nd	nd	nd	nd	< LOQ	nd	nd
D (2)	*G. pulex*	nd	nd	nd	nd	nd	nd	nd	nd
E	*A. aquaticus*	nd	nd	nd	nd	nd	nd	nd	2.6
F (1)	*A. aquaticus*	nd	< LOQ	nd	nd	nd	5.5	nd	1.9
F (2)	*A. aquaticus*	nd	< LOQ	nd	nd	nd	5.4	nd	4.7
G (1)	*G. pulex*	nd	nd	nd	nd	nd	< LOQ	nd	nd
G (2)	*G. pulex*	nd	nd	nd	nd	nd	< LOQ	nd	nd
H	*A. aquaticus*	nd	< LOQ	nd	nd	nd	6.3	nd	2.0
I	*G. pulex*	nd	< LOQ	nd	nd	nd	3.1	nd	< LOQ

Citalopram was found in *G. pulex* and *A. aquaticus* at higher concentrations when compared to other compounds. To the best of our knowledge, citalopram presence in wild *G. pulex* populations has not been reported before. We measured an average concentration of 4.3 ng/g dw (range 1.8 – 6.3 ng/g dw) in six *A. aquaticus* samples and 3.1 ng/g dw in one *G. pulex* sample. Studies carried out on fish reported citalopram concentrations of up to 3.6 ng/g dry weight in liver and up to 33.3 ng/g wet weight in bivalves (Arnnok et al. 2017). Reisinger et al. (2021) have shown that SSRIs such as citalopram may alter food web dynamics and other processes in crayfish, and it induced foot detachment in freshwater snails at environmentally relevant concentrations (Fong et al., 2018).

4. Discussion

Bioaccumulation (BAF), the process by which a substance is accumulated in the tissues of living organisms through ingestion or absorption in the environment, is expressed as the ratio of the substance in biota (Cb) and its total concentration in the water phase (Cw) (Gómez-Regalado et al., 2023):

$$BAF = Cb/Cw$$

BAF is known to be strongly influenced by pH, temperature but also chemical properties (octanol/water partition coefficient, K_{ow}) of the compounds as well as age, size and health of the organisms and duration of exposure. Furthermore, biotransformation of compounds by the amphipods and/or synergistic effects between compounds may take place and add to the overall uncertainty

associated with in-field studies. Despite the many variables and parameters which are impossible to control, it has been shown that the route of exposure of pharmaceuticals through water "may be more important for uptake and bioaccumulation rates than dietary exposure" (Du et al., 2014). Even if we do not have enough results to carry out statistical evaluation, we could estimate the BAF for citalopram in *A. aquaticus* (3 samples) and *G. pulex* (1 sample) as well as for O-desmethyltramadol in six *A. aquaticus* samples. Calculated BAFs are shown in Table 5. The BAF for citalopram in *A. aquaticus* and *G. pulex* were very similar and unfortunately, we had no BAF for O-desmethyltramadol in *G. pulex*.

Table 5. BAFs in *Asellus aquaticus* and *Gammarus pulex* for citalopram and Desmethyltramadol.

Sampling site	Species	Citalopram	Desmethyl-tramadol
A (2)	*A. aquaticus*	/	18.2
B	*A. aquaticus*	/	30.7
E	*A. aquaticus*	/	8.4
F (1)	*A. aquaticus*	1.1	40.7
F (2)	*A. aquaticus*	3.4	17.2
H	*A. aquaticus*	2.2	52.7
I	*G. pulex*	2.9	/

No reference values could be found in the literature for O-desmethyltramadol, BAF for the parent compound tramadol have been reported to vary form 1.2 - 110 in fish and from 1 - several hundreds for citalopram, with most values ranging between 1 – 10 (Gómez-Regalado et al. 2023).

Even if *G. pulex* is routinely used for biomonitoring studies, a recent study by Miller et al. (2021) showed that other species have higher accumulation factors and thus may be better suited as bio-indicators. Also, another possible explanation for the higher positive contamination rate of *A. aquaticus* may be seen in its ecology. *A. aquaticus* is quite indifferent for water perturbation and water temperature and can thus strive well in the upper to lower reaches of water bodies and even in stagnant waters with temporal anaerobe conditions. However, most importantly, water lice prefer but are not restricted to a low to absent water perturbation while *G. pulex* is rheophile, i.e., prefers stronger water currents. This discrepancy in preferred water perturbations, and the ability of *A. aquaticus* to survive in standing waters with even temporarily anaerobe sediments, might partly explain the different uptake rates of pharmaceutical and illicit drugs present in co-inhabited freshwaters.

In conclusion, our results confirm results from other reports and may be helpful in water surveillance projects, especially when considering the decline in water species observed over the last years and decades (Harrison et al. 2018). Continuous monitoring and, if necessary, an efficient clean-up system should be considered for guaranteeing clean surface water. Our results have to be confirmed, for example using composite samples as they more accurately reflect loads of xenobiotics in surface water. Also, more studies need to be carried out to better understand drug uptake and accumulation in freshwater biota as a function of evolving environmental conditions.

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