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HRMS-based suspect screening of pharmaceuticals and their transformation products in multiple environmental compartments: An alternative to target analysis?

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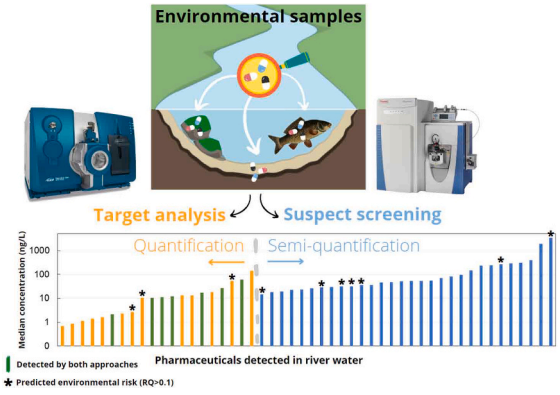
HIGHLIGHTS

- Target method does not account for a variety of PhACs with potential risk.
- Less sensitive suspect screening underestimates the impact of low-level PhACs.
- Semi-quantification shows an acceptable performance in surface waters.
- Tiered approach combining target and suspect methodologies is recommended.

GRAPHICAL ABSTRACT



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ABSTRACT

The comprehensive monitoring of pharmaceutically active compounds (PhACs) in the environment is challenging given the myriad of substances continuously discharged, the increasing number of new compounds being produced (and released), or the variety of the associated human metabolites and transformation products (TPs). Approaches such as high-resolution mass spectrometry (HRMS)-based suspect analysis have emerged to overcome the drawbacks of classical target analytical methods, e.g., restricted chemical coverage. In this study, we assess the readiness of HRMS-based suspect screening to replace or rather complement target methodologies by comparing the performance of both approaches in terms of i) detection of PhACs in various environmental samples (water, sediments, biofilm, fish plasma, muscle and liver) in a field study; ii) PhACs (semi)quantification and iii) prediction of their environmental risks. Our findings revealed that target strategies alone significantly underestimate the variety of PhACs potentially impacting the environment. However, relying solely on suspect

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strategies can misjudge the presence and risk of low-level but potentially risky PhACs. Additionally, semi-quantitative approaches, despite slightly overestimating concentrations, can provide a realistic overview of PhACs concentrations. Hence, it is recommended to adopt a combined strategy that first evaluates suspected threats and subsequently includes the relevant ones in the established target methodologies.

1. Introduction

Pharmaceutically active compounds (PhACs) have raised increasing environmental concern in the last decades, mainly due to its widespread release and detection in natural environments [42]. Their ubiquity in wastewater treatment plant (WWTP) effluents, along with their pseudo-persistence in the environment, have boosted their presence in different biotic and non-biotic environmental compartments [37]. Some of these compounds are human metabolites or transformation products (TPs) of the parent drugs, and they can show a similar (or higher) abundance and toxicity in the environment [20]. The widespread occurrence of PhAC residues in the environment can represent an ecotoxicological hazard, since PhACs are biologically active compounds that can affect organisms even at trace concentrations [9]. In the last years, the intensive use of antibiotics in human and veterinary medicine has raised concerns about the spread of antimicrobial resistance in the environment, and the potential risk that this poses to human health (Singh et al., 2019). Therefore, environmental and human health threats may associate with the exposure to PhACs and their metabolites in contaminated water bodies. Furthermore, a myriad of new active compounds are patented and released to the market each year [6], increasing the number of PhACs of potential concern in receiving ecosystems. This altogether hampers the classical study of PhACs via target analysis strategies since they rely on the pre-selection of PhACs of interest, normally based on their consumption trends, expected environmental occurrence and ecotoxicological effects [12]. Nowadays, low-level monitoring of PhACs (pg/L–ng/L in surface water) is feasible using low resolution mass spectrometers (LRMS) such as triple quadrupole (QqQ) or ion trap instruments. Pre-selecting the compounds of interest is crucial in target approaches. However, additional PhACs cannot be detected, even if they are present at relatively high concentrations.

For a more comprehensive study of PhACs in natural environments, high-resolution mass spectrometry (HRMS)-based suspect analysis strategies are recommended [16]. Suspect screening strategies allow the detection of a large lists of "known unknown" compounds [25] (e.g., above 10,000 PhACs in specific searches [39]), suspected of being present in environmental samples, and without the use of reference standards. However, this significant advantage comes at a cost, since QqQ methodologies used in classical LC-MS/MS screening generally have a higher sensitivity and are more suitable for quantitative analysis than HRMS instrumentation used to date [28,32]. For that reason, PhACs that are present in the environment at levels around LRMS method detection limits (MDLs) may be overlooked in HRMS-based approaches. However, the sensitivity gap between LRMS and HRMS instrumentation has been reduced in the last years, thanks to the latest advances in HRMS. Consequently, MDLs have become more comparable between low and high resolution instruments, as reported in the literature [28,31].

The main advantage of classical target analysis is that, not only it is able to ensure PhACs identity, but it allows quantitative analysis by using reference standards. However, new tools have emerged in the field of suspect and non-target analysis, allowing the semi-quantification of compounds even when all the reference standards are not available. These methods use representative compounds to predict ionization efficiency and concentration [1,13,21,24,38]. Despite accuracy is still limited due to the error between predicted and measured concentrations [21], we hypothesize that semi-quantification with predicted ionization efficiency-based methodologies can be a useful approximation for estimating PhACs abundance and subsequent risk assessment in natural environments.

In this context, the main objective of this work was to assess whether HRMS-based suspect screening is ready to replace or is rather a complementary strategy to classical target methodologies for environmental monitoring. The capabilities of both strategies were explored by analysing PhACs in the same environmental samples, obtained from an extensively studied area (Ebro delta region, north-eastern Spain). Specifically, we compared the number of PhACs and their frequency of detection in surface water, sediments, biofilm, and fish tissue samples. The correspondence between concentrations obtained through target quantification and those obtained by suspect semi-quantification was assessed, as well as the subsequent environmental risks by considering predicted no-effect concentration (PNEC) values.

2. Materials and methods

2.1. Sampling, sample treatment and instrumental analysis

Environmental samples of different biotic and non-biotic compartments (including river water, sediments, seawater, biofilm, and fish tissues (liver and muscle) and plasma) were collected in the Ebro River Delta area in two consecutive weeks during March 2019. The sampling area comprised the lower Ebro River from Miravet (upstream) to Deltebre (downstream), as well as two shallow coastal bays in the Ebro Delta (Fangar and Alfacs). The sampling sites, collected samples, and the number of replicates used for measurement are indicated in Fig. 1.

All sampling procedures, as well as sample preparation are described in detail elsewhere [11]. Briefly, water samples (500 mL of seawater and 100 mL of river water) were extracted as described in Gago-Ferrero et al. [16] using in-house cartridges for solid-phase extraction (SPE) [16]. Freeze-dried sediments (1 g) were extracted by sonication using a digital sonifier (Branson 450 Cell Disruptor). In turn, biological samples (also freeze-dried) were simultaneously homogenised and extracted using zirconium (for fish tissues) and glass beads (for biofilm) through a method adapted from Santos et al. [34] (S[34]). The extraction of sediments and biological samples was followed by a clean-up step using Oasis HLB cartridges (200 mg, 6 cc). Finally, fish plasma samples were deproteinized with acetonitrile following a protocol adapted from Gil-Solsona et al. [17] (G[17]).

The analysis of final sample extracts was performed in two instruments, namely a Waters Acquity Ultra-Performance liquid chromatography system coupled to i) a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (UPLC-Qtrap) for target analysis, and to ii) a Q-Exactive Orbitrap mass analyser (UPLC-Q-Exactive Orbitrap) for suspect screening. Further details about sample analysis and acquisition are provided in the Supplementary Information **(SI-1)**.

2.2. Quality assurance and quality control

Target analysis followed a modification of the LC-MS/MS method of Castaño-Trias et al. [12] and encompassed 68 compounds belonging to 15 different therapeutic groups (listed in Table S1). The method incorporated additional compounds (68 relative to 50 in [12]), such as PhACs with potential relevance in environmental matrices other than water. Two Selected Reaction Monitoring (SRM) transitions were monitored for quantification (most intense transition) and confirmation purposes (second transition). All data was acquired and processed using Analyst 1.6.3 software (AB Sciex). Quality parameters of the analytical method for the different matrices included extraction recoveries (%) and method detection and quantification limits (MDLs and MQLs, respectively).

Recovery samples were spiked at different concentrations before extraction: 10 and 100 ng/L (water), 100 ng/g dw (sediment), 10 and 100 ng/g dw (river biofilm and fish tissues), and 10 ng/mL (fish plasma). The spiked samples were also used to determine MDLs and MQLs based on the minimum amount of analyte with a signal-to-noise ratio of 3 and 10, respectively. Peak area was used for the quantification of samples following the internal standard calibration approach (isotopically labelled standards indicated in Table S1). Eight-point matrix-matched calibration curves (0.1 −100 ng/mL) were injected at the beginning and end of each sequence. The criterion for identification and confirmation relied on Commission Decision 2002/657/CE [15]: i) LC retention time within ± 2% between samples and standards and ii) ratio between SRM transitions within ± 20%.

2.3. Suspect analysis workflow

Based on the acquired HRMS data, a suspect analysis was performed using Compound Discoverer 3.1 software (Thermo Scientific). The suspect analysis workflow was applied to HRMS acquisition data of each environmental matrix, namely water, sediment and biota. After mass range selection (*m/z* 70–1000) and chromatographic alignment, unknown compounds were detected and features were grouped across samples (mass tolerance of ± 3 ppm and retention time tolerance of 0.3 min). The identification of tentative compounds after data filtering was achieved by comparing the acquired MS/MS spectra (when available) with those found in mzCloud database (which contains >20000 individual compounds, including 1370 pharmaceuticals (https://www.mzcloud.org/Stats, last accessed May 2023). A more detailed protocol on data filtering and identification with Compound discoverer can be found in the Supplementary Information **(SI-2)**. Those compounds with a good degree of similarity between acquired spectra and database spectra (>80%) (and confirmed by visual inspection) were treated as positive findings and listed with its corresponding *m/z* and retention time. However, only those belonging to PhACs (including antibiotics) were considered for this work.

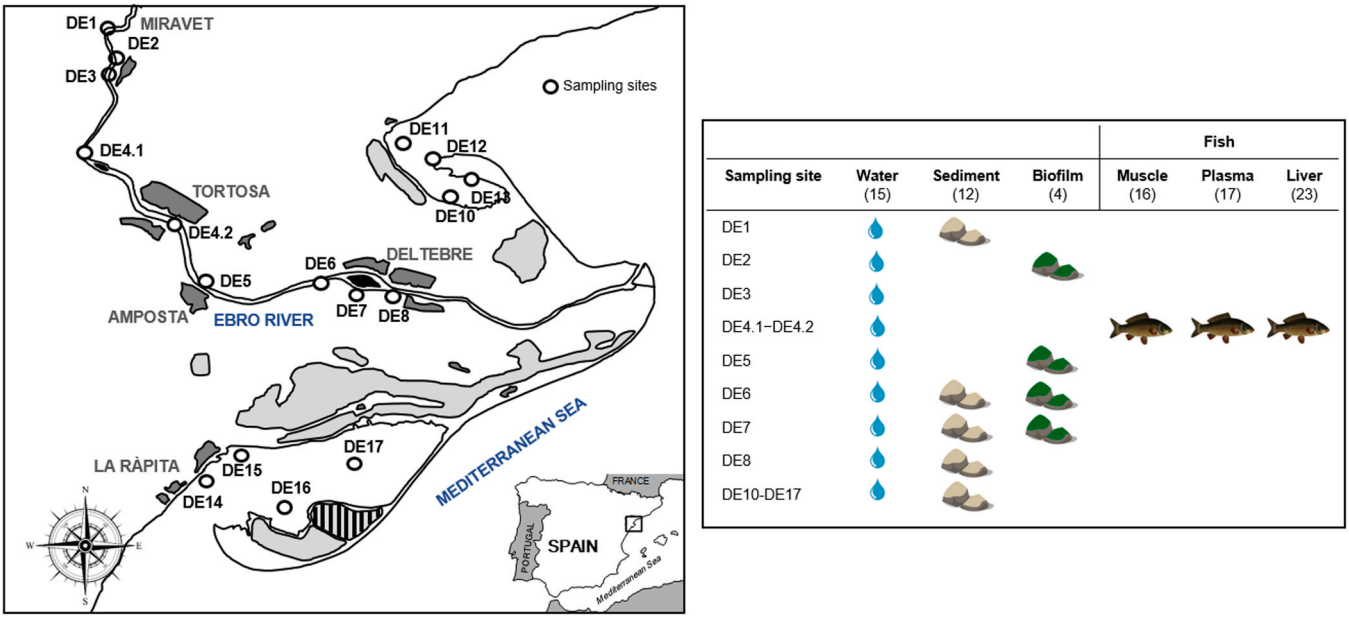
2.4. Semi-quantification and quantification procedure

PhACs found in suspect analysis were semi-quantified using two different strategies adapted from Liigand et al. [24] and Aalizadeh et al. [1]. In the case of the procedure adapted from Liigand et al., ionization efficiency values (logIE) were extracted from their work and used to generate a model to predict the logIE of our suspect PhACs. LogIE values can then be used to derive the semi-quantitative concentration of an unknown compound in environmental samples by means of a calibration curve injected in the same conditions. In the case of the procedure adapted from Aalizadeh et al., their open platform (http://trams.chem.uoa.gr/semiquantification) enables the direct retrieval of semi-quantitative values. A more thorough explanation and discussion of the semi-quantification procedures can be found in the Supplementary Information **(SI-3)**.

These semi-quantification approaches were originally developed for use in pure solvent (methanol/water 10:90, v/v), but they have also been tested to more complex matrices (e.g., cereal extracts) showing acceptable results [24]. Therefore, all our samples were directly semi-quantified with the generated (based on Liigand et al. work) and available models (based on Aalizadeh et al. platform) using a calibration curve created in solvent. In turn, quantification of pre-selected PhACs via target analysis was performed individually with a matrix-matched calibration for sediment and biota samples, as extensively described elsewhere [11]. Quantitative results shown in supplementary tables were retrieved from such previous work (Table S2-**4**). The quotient between median concentrations and method quantification limits (MQLs) for the pre-selected PhACs, or median-to-MQL ratios, was calculated as indicator of the proximity of environmental concentrations to analytical limits (see Section **3.1**). Median concentrations were calculated based on values >MQL, and if <MQL they were replaced by MQL/2 for their use in statistical analyses and data visualization. In addition, the ratio between predicted (semi-quantitative) and observed (quantitative) concentrations was determined to address the accuracy of the semi-quantification approach (see Section **3.2**).

2.5. Environmental risk assessment (ERA)

Environmental risk assessment (ERA) was performed based on the comparison of lowest predicted no-effect concentrations (PNEC) values, retrieved from NORMAN database (https://www.norman-network.com/nds/ecotox, last accessed May 2023), and measured environmental concentrations (MEC). The lowest PNECs are preferably derived from experimental eco-toxicity data (lowest measured for various trophic levels), but in case of no or insufficient data, the lowest value is in-silico predicted by QSAR models (e.g., [2]). For the MECs, maximum



Sampling site	Water (15)	Sediment (12)	Biofilm (4)	Fish: Muscle (16)	Fish: Plasma (17)	Fish: Liver (23)
DE1	●	●				
DE2	●		●			
DE3	●					
DE4.1–DE4.2	●			●	●	●
DE5	●		●			
DE6	●	●	●			
DE7	●	●	●			
DE8	●	●				
DE10-DE17	●	●				

Fig. 1. Map of sampling sites in the lower Ebro River basin (NE Spain). Sample types and number of collected samples are indicated in the right-hand chart.

concentrations in river- and sea-water, as well as sediments, were used. These values were obtained from quantitative data (i.e., target approach to ERA) or semi-quantitative data (i.e., suspect approach to ERA). These maximum MECs alongside lowest PNECs allowed the calculation of risk quotients (RQs) in a worst-case scenario [29]. If necessary, values below the method quantification limit (MQL) were replaced by MQL/2 for the estimation of MECs. Three levels of risk were defined based on the obtained RQs:

- RQ < 0.1: "low risk"
- 0.1< RQ < 1: "moderate risk"
- RQ > 1: "high risk"

3. Results and discussion

3.1. Comparison of detection of pharmaceuticals in the environment using target and suspect strategies

A total of 41 PhACs were detected by target analysis (via LRMS) in the different environmental matrices (water, sediments, biofilm and fish plasma, muscle and liver). Considering all the matrices, eleven of these substances were simultaneously detected by target analysis and suspect screening, and their respective detection frequencies are indicated in Table 1.

Among these, a varying number of PhACs were simultaneously detected by the two strategies in the different matrices analysed (Fig. 2). For example, in river water, seven compounds (out of 20 targets) were detected by both strategies, whereas suspect screening revealed 21 additional PhACs. In the case of fish tissues, among the detected target PhACs (30), five compounds were also elucidated by suspect analysis. Finally, no simultaneous detections by target and suspect strategies were observed for sediment and biofilm samples (Fig. 2).

That difference in compound detection can be attributed to the different sensitivity of each of the analytical strategies, i.e., in general, lower analytical detection limits for LRMS than for HRMS analysis. Therefore, for a given compound, environmental concentrations that are near its corresponding analytical limit may hinder its simultaneous detection by target and suspect strategies. This is illustrated in Fig. 3, where PhACs reported only by target analysis were in general those at lower concentrations than the compounds found by both strategies (e.g., ca. 1 ng/L). This could be explained by their comparatively high MQLs in HRMS, contributing to their non-detection by this less sensitive

Table 1
Heatmap visualization of pre-selected PhACs, by detection frequency (DF), in target analysis (colours) and suspect screening (percentage). Colours: from light (DF<25%) to dark red (DF>75%).

Therapeutic group	Compound	River water	Sea water	River sediment	Biofilm	Fish plasma	Fish liver	Fish muscle
Analgesics/anti-inflammatories	2OH-Ibuprofen							
	Acetaminophen	71%	13%				22%	
	Codeine							
	Ibuprofen							
	Ketoprofen							
	Phenazone	100%	13%				6%	
Antibiotics	Azithromycin							
	Ciprofloxacin							
	Clindamycin							
	Metronidazole							
	Oxytetracyclin							
	Sulfamethoxazole							
	Sulfapyridine							
	Trimethoprim							
Antihelminthic	Levamisol							
Antihypertensives	Irbesartan	100%						
	Losartan	29%						
	Valsartan	100%	13%					
Antiplatelet agent	Clopidogrel							
Calcium channel blockers	Diltiazem							
	Norverapamil							
Diuretics	Furosemide							
	Hydrochlorothiazide							
Lipid regulators	Bezafibrate							
	Gemfibrozil	100%	63%			36%		
Psychiatric drug	Carbamazepine	100%					6%	
	Citalopram					7%	6%	
	Epoxy-carbamazepine							
	Fluoxetine							
	N-desmethylvenlafaxine							
	Norfluoxetine							
	O-desmethylvenlafaxine		63%			21%	11%	
	Paroxetine							
	Sertraline					7%	17%	
	Venlafaxine	71%				14%	11%	
Sedation and muscle relaxation	Azaperone							
X-ray contrast agents	Iopromide							
β-Blocking agents	Atenolol							
	Carazolol							
	Metoprolol							
	Propranolol							

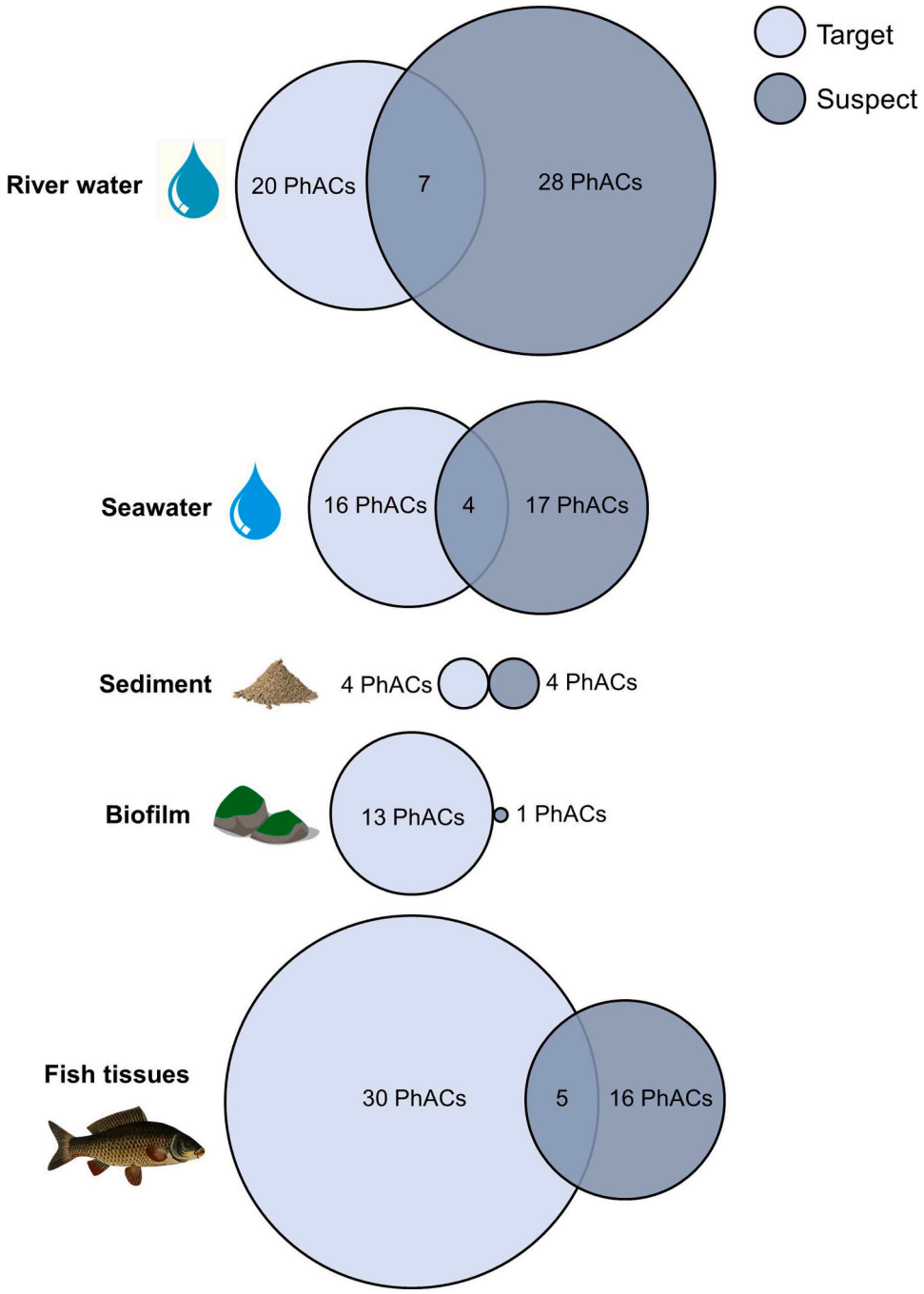


Fig. 2. Venn diagram with the amount of PhACs detected by target and suspect analyses in the different environmental matrices from the Ebro River and Delta. The number in overlapped area represents the simultaneously detected compounds, if any.

approach (suspect strategy). However, there are still some exceptions (compounds only detected by target analysis that were found at 10–100 ng/L). To clarify this pattern, the median-to-MQL ratio was calculated, namely the quotient between the median concentration value (found in our samples) and the MQL (calculated in the target analysis) (Fig. 3C).

It was observed that compounds detected by both methodologies generally had a higher median-to-MQL ratio, compared to those detected by target analysis alone, and that additional PhACs detected by suspect used to occur at high concentrations (no data on their MQL, but virtually high median-to-MQL ratios too). For example, in river water, all PhACs with a median-to-MQL ratios ≥ 15 were detected by both approaches, whereas only 3 out of 15 with levels around the MQL (median-to-MQL ratio < 10) were detected by suspect screening (Fig. 3). Three antihypertensives (irbesartan, losartan and valsartan) and the psychiatric drug venlafaxine were found in a comparable number of river water samples through the two strategies. This pattern was also observed for the compounds with the highest concentrations in fish plasma and liver (citalopram, gemfibrozil, venlafaxine, and sertraline) (Table S2).

Oppositely, PhACs detected at low concentrations (or around their MQLs) such as most antibiotics in surface waters, were only detected by target analysis. Sulfamethoxazole was found at low concentrations (<6 ng/L) in both river- and seawater (DF: 71% and 25%, respectively), but it was not detected by HRMS-based screening. This pattern was also observed for the different compounds detected in biofilm, which were

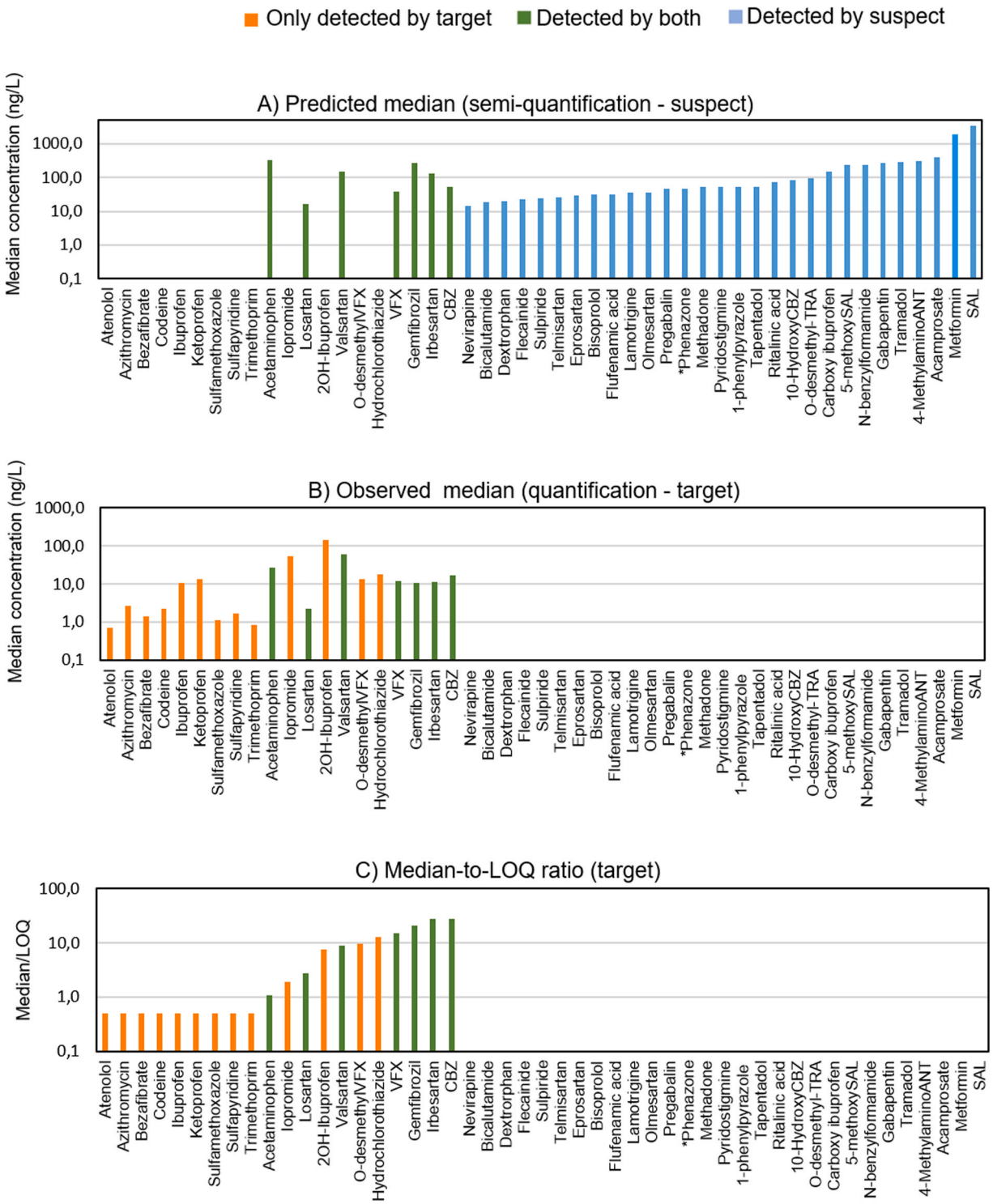


Fig. 3. Predicted (A) and observed (B) median levels in river water (ng/L) for suspect and target pharmaceuticals, respectively. Corresponding Median-to-MQL ratios (target compounds) are shown in the bottom figure (C). Predicted values are based on Liigand et al. semi-quantification approach. ANT: antipyrine; CBZ: carbamazepine; SAL: salicylic acid; TRA: tramadol; VFX: venlafaxine. * : target compound but only detected by suspect.

mostly close to their analytical limits (median concentration-to-MQL ratio <2.5). In fish tissues, besides the most concentrated PhACs mentioned above, other compounds were mostly not identified via suspect screening.

Therefore, the generally lower sensitivity of HRMS instruments may hamper the detection of PhACs at levels close to limits of detection in LRMS (low ng/L range in water) [19]. Based on the results from Fig. 3C, it appears that our LRMS instruments are approximately one order of magnitude more sensitive than our HRMS instrument, although it is possible that more advanced HRMS instruments have narrowed this gap. Nevertheless, it is worth noting that although HRMS-based techniques are not currently as sensitive as LRMS-based target approaches, the constant development and release of new HRMS instrumentation with improved analytical capabilities (higher sensitivity and throughput) might lead to the partial phase-out of LRMS instruments. That being said, we do believe that LRMS instruments (e.g., QqQ) will continue to

be used for target analysis and routine monitoring of regulated mixtures of chemicals, i.e., by regulatory agencies. In this study, the two instruments used belong to the same generation (2009 −2010). Hence, the observed sensitivity gap between LRMS- and HRMS-based approaches might be narrower if comparison is made on the latest instrumentation.

Based on the strategies and instruments compared in this study, although the sensitivity of HRMS-based strategies could be sufficient to assess most PhACs of interest in other matrices (e.g., wastewater samples), it may be challenging to use HRMS-based strategies for the simultaneous detection of low-level compounds across various environmental compartments, considering that most PhACs in the study were present in the low ng/L range (and also near MQLs). This would apply to matrices more complex than water too, such as fish tissues, where median-to-MQL ratios, generally ranging between 1–10 (Table S2) resulted in a limited proportion of compounds detected by both strategies (Fig. 2). Exceptionally, a few PhACs with a low median-to-MQL ratio could be uniformly detected by the two strategies (e.g., acetaminophen in river water, or citalopram in fish liver) (Table S2). The high selectivity of HRMS-based methods, by reducing background noise in the specific m/z of parent ions, may potentially result in higher sensitivity for certain compounds compared to classical target strategies. Indeed, some compounds were detected in more samples with suspect screening compared to target (e.g. O-desmethylvenlafaxine in sea water or fish samples). Conversely, the sensitivity for certain compounds can be particularly low in HRMS-based analysis, as suggested by the non-detection of hydrochlorothiazide in river water despite the high detection frequency and median-to-MQL ratio in target analysis [11]. However, the detection of compounds in complex matrices can be impacted by other factors (e.g., biomatrix effects, chromatographic conditions, mass selectivity of the instrument) which may affect the observed trend in relation to the median-to-MQL ratio. Likewise, the related gap between LRMS- and HRMS-based strategies can be blurred in complex matrices, leading to more compound-specific responses.

The target method measured a range of PhACs, even at low environmental concentrations, but it did not account for up to 28 PhACs out of the 35 revealed by the suspect screening strategy in river water, or 11 out of 16 in fish tissues (Fig. 2). Though not included a priori in the target methodology, these substances were found in river water (28), seawater (17), river sediments (4), biofilm (1), and fish tissues (21). They comprised additional analgesics/anti-inflammatories (e.g., tramadol), antihypertensives (e.g., olmesartan), psychiatric drugs (e.g., lamotrigine), or human metabolites (e.g., O-desmethyl-tramadol). The latter group was particularly well represented in river water (7), followed by analgesics/anti-inflammatories (4), psychiatric drugs (4) and antihypertensives (3). For example, the active metabolite O-desmethyltramadol was found in all samples alongside tramadol.

Among the top-20 most frequently found PhACs in river water worldwide [42], 12 were also found in surface waters from the Ebro Delta (metformin, carbamazepine, gabapentin, sulfamethoxazole, trimethoprim, acetaminophen, atenolol, desmethylvenlafaxine, venlafaxine, citalopram, codeine and ciprofloxacin). Except for metformin and gabapentine (revealed by suspect screening), the detected PhACs are already included in our target methodology. Finally, sitagliptin and diltiazem, both among the top-20 of Wilkinson et al. [42] too, were found in sediment and fish liver, respectively, though not in surface waters.

In sediments, only four additional PhACs were found by suspect screening (cetirizine, O-desmethyl tramadol, pregabalin, and salicylic acid), further indicating a limited role of sediments as sinks of PhACs, as previously suggested by the target analysis [11,44]. A larger diversity of PhACs was observed in fish, which accumulated many of the compounds detected in water samples. However, most of the compounds were restricted to two particularly polluted individuals, as also revealed by target analysis and discussed elsewhere [11]. Among the 21 PhACs detected in fish by suspect screening, 16 were restricted to these two individuals (one or more tissues), although certain compounds were more widespread (e.g., N-benzylformamide and carboxy-ibuprofen).

Noteworthy, since our goal was to obtain a realistic and trustful comparison of the two strategies, only compounds with at least one MS/MS spectra in one data-dependent acquisition (DDA) file were considered as positive assignations in the suspect analytical approach. However, this strategy is clearly conservative and prone to generate false negatives. For example, it led to the non-detection of 2-OH ibuprofen in water, despite a peak could be observed. In such scenario, the use of a less restrictive strategy (e.g. data independent acquisition (DIA) coupled with other suspect screening tools, such as the NORMAN Digital Sample Freezing Platform (DSFP) [4], NORMAN Suspect List Exchange (NORMAN-SLE) [39] or the CompTox Chemicals Dashboard [43], could have allowed the detection of more PhACs. Indeed, in more complex matrices where lots of higher signal endogenous chemicals are present, the possibilities to find a good MSMS for PhACs are reduced compared with cleaner samples such as water. In this context, DIA provides a better tool for digitally storing data (and tentative chemicals present in our samples). Nonetheless, DIA-based strategies may also lead to a lower confidence in the identification than DDA-based ones, as proposed in the latest harmonized identification scoring system in non-target screening [3]) and requires more expertise and time for a correct data treatment to obtain reliable results, in order to avoid possible false negatives. For that reason, we decided to compare target with a DDA-based method, which is currently the most popular way to handle HRMS data, while considering DIA data the number of compounds found in suspect screening strategies may be presumably higher, and the combined use of both strategies when sample volume and time is available, is highly recommended.

3.2. Quantification and semi-quantification approaches

Quantification of target PhACs yielded concentrations in the ng/L range for river and seawater (<MQL–210 ng/L), and in the low ng/g range for sediments and biota, except for fish plasma (<MQL–89 μg/L) and liver (<MQL–170 ng/g dw), as extensively discussed elsewhere [11]. The highest concentrations were associated with 2OH-ibuprofen in river water and with psychiatric drugs (e.g., venlafaxine and sertraline) in fish plasma and liver.

In the case of the PhACs detected by suspect screening a wider range of concentrations were revealed in all the environmental matrices (compared to quantitative results) by the semi-quantification approaches applied (**Section 2.3**). For example, according to the semi-quantification approach of [24], maximum concentrations in river water fluctuated between 18 ng/L (losartan) and salicylic acid (8.5 μg/L) (Table S3). Lower concentrations of PhACs were measured in seawater, with individual levels between 2 ng/L (lamotrigine) and salicylic acid (358 ng/L). The relatively lower number of findings and concentrations of PhACs in seawater (17) might be attributed to environmental attenuation processes, such as dilution in the Ebro Delta bays [11].

Semi-quantitative concentrations in fish liver, which was the most contaminated tissue, generally ranged between 2.4 ng/g (carbamazepine) and 3.4 μg/g dw (carboxy-ibuprofen), except for N-benzylformamide (7.9 μg/g dw). Fish liver is a metabolically active organ which often exhibits a strong accumulation of xenobiotics and metabolites, and it therefore constitutes a useful biomonitoring matrix for the environmental assessment of PhACs [22,40]. Nevertheless, the frequency of detection was relatively low (<20%) for most of the compounds, as mentioned above. The predicted concentration varied slightly between the two semi-quantification approaches [24] and [1], but they generally fell within the same range (Table S3).

Among the most common suspect PhACs, the antidiabetic metformin was found in all Ebro river water samples at a relatively high level (median concentration= 1.9 μg/L, according to Liigand et al. approach), compared to median levels quantified for the other target analytes (<145 ng/L). This compound has been frequently reported in surface

waters [42], occasionally at concentrations above 1 μg/L [10]. Given the increasing use of this compound to treat diabetes, preterm preeclampsia in pregnant women, and obesity, and its ubiquity in the aquatic environment [42], incorporation of metformin in target methodologies is encouraged for routine monitoring of occurrence and adverse ecotoxicological effects.

In addition, some PhACs which are unexplored in the environment, such as acamprosate (used in alcohol dependence treatment) and 4-metylaminoantipyrine (human metabolite of metamizole), were reported at moderate median concentrations in river water of this study (400 and 320 ng/L, respectively). The antihypertensives olmesartan, eprosartan and telmisartan were found at lower levels (ND – 52 ng/L, according to [24] approach), but together with the other three sartans included in the target method: valsartan, irbesartan and losartan (ND – 190 ng/L) (Table S3). The three suspect sartans are traditionally less studied than the target ones [18] but they may be present at comparable concentrations in the environment.

Adopting a suspect screening strategy allows to reveal not only the presence of relevant PhACs but also their semi-quantitative concentrations, which may be unexpectedly high and pose an environmental risk not anticipated by target analysis. This is further discussed in the subsequent section, but it is known that the pre-selection of PhACs for environmental monitoring is biased by prior data ('Matthew effect'), which can lead to perpetually ignore compounds that have not been previously targeted [14]. The data obtained from suspect screening, including semi-quantification, can also be employed to enhance future target methodologies. Lists of pre-selected PhACs can be continuously updated by incorporating the findings of exploratory studies such as suspect and non-target screening approaches, along with prescription data [12].

Semi-quantification using suspect screening data was compared with quantification results (target analysis), focusing on the PhACs that were commonly detected by the two strategies. Most compounds showed comparable concentrations in surface waters, whereas for some others more than one order of magnitude difference between target and suspect approaches was observed. This is shown by ratios of median concentrations (suspect-to-target), generally lying between 1x and 10x (Fig. 4). As shown in the figure, the two semi-quantification methods ([24] and [1]) exhibited a similar performance in respect to observed concentrations in river and seawater. In our study, HRMS semi-quantification resulted in higher predicted than observed (quantitative) levels (ratios $>$ 1x), indicating that semi-quantification could slightly overestimate the concentration of PhACs in water in our case. However, concentration values were generally kept within the same order of magnitude. For example, in river water, median ratios of 7.2x (2.5x – 25x) and 5.3x (4.4x – 32x) were obtained after applying the strategies of Liigand et al. and Aalizadeh et. al., respectively.

A separate study that employed Liigand et al.'s predicted ionization efficiency-based quantification method yielded an average quantification error of 2.1x and 1.8x in spiked and real groundwater samples, respectively, with the highest values reaching 51x and 7x [21]. Before the rise of predicted ionization efficiency-based methods, semi-quantification was typically based on the response factors of structurally related isotopically labelled internal standards (ILISs). This strategy has shown an acceptable accuracy in different studies (e.g., 40 – 160% for most substances), and it has been used for the prioritisation of PhACs and other contaminants [30]. However, it is often limited by the availability of suitable ILIS for the semi-quantification of all the tentatively identified substances, and it has not been validated for a wide range of compounds [26]. These drawbacks may be sorted out by novel approaches based on predicted ionization efficiency, such as the ones available in the literature and applied in this paper.

In fish samples, five simultaneously detected compounds were used to assess the accuracy of PhACs semi-quantification in matrices more

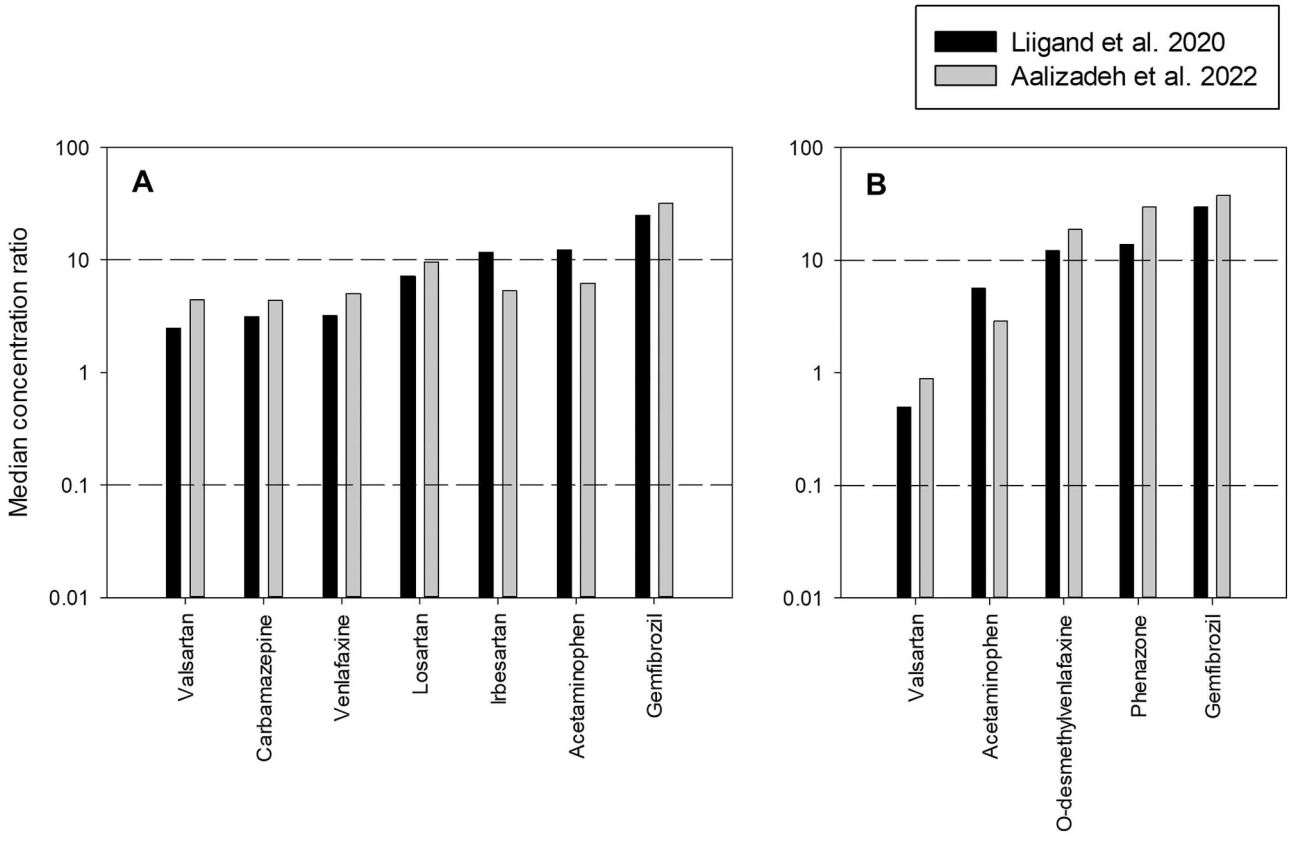


Fig. 4. Ratios of median concentrations predicted by two suspect semi-quantification strategies [1,24] and measured by target quantification (predicted/measured), for simultaneously detected pharmaceuticals in river (A) and sea water (B). Dashed lines indicate one order of magnitude differences between levels.

complex than water. Given the limited detection frequency of most compounds (mostly in 1–2 individuals), maximum concentrations were used to calculate the ratios (to compare the exact same individual), instead of median values. The semi-quantification of PhACs in fish resulted in consistently lower ratios for both plasma (1.2x–5.6x) and liver (0.5x–6.5x), compared with water (2.5x–32x) (Table S3). Based on these results, our hypothesis is that the effect of matrix suppression in fish tissues and biofluids compensated the observed overestimation in water. Aalizadeh et al. pointed out that the lack of harmonization of logIE values into the sample matrix (e.g., through matrix-matched calibration) can result in compensating error and low accuracy of predicted concentrations. However, the influence of various factors that may affect the performance of predicted ionization-based semi-quantification and contribute to uncertainty (including matrix suppression) are still not completely understood, and therefore further experimental testing is needed to confirm this hypothesis.

Furthermore, this overestimation may strongly depend on the compounds used in the calibration curve to harmonize logIE between model and case study. Therefore, it seems necessary to create and/or adapt ionization efficiency prediction in matrix-based models to avoid this matrix effect. In addition, it is also plausible that predicted ionization efficiency values (logIE) in solvent cannot be directly transferred to more complex matrices [1], since matrix effects can affect strongly only a part of the chromatographic run (e.g. strong matrix suppression in the phospholipids zone for plasma/serum samples) (Martin et al., 2023). In this study, the performance of semi-quantification procedures (compared to target) in sediment and biofilm samples could not be assessed due to a lack of simultaneously detected PhACs.

Several factors can influence the accuracy of semi-quantification, including the lack of internal standards to correct matrix effects, the lack of extraction recovery values to counteract chemical losses during sample treatment, the uncertainty associated with predicted ionisation efficiency (logIE), or the possibility to omit in-source fragmentation effects. These factors can make it challenging to obtain accurate estimates of contaminant levels in environmental samples. In addition, matrix-matched calibration curves (e.g., for biota samples), which were not applied for suspected compounds in this study, may improve the performance of semi-quantification methods in complex matrices (such as fish tissues). Nevertheless, our comparison demonstrated the potential of suspect-based semi-quantification for a comprehensive assessment of PhACs occurrence in water.

3.3. Environmental risk assessment

An environmental risk assessment (ERA) was conducted based on both, quantitative and semi-quantitative values for PhACs detected in surface waters and river sediments. (Table S4**)**. Among target compounds, a total of four compounds exceeded risk thresholds in river- and/or seawater, suggesting a moderate (or higher) risk to the environment: two antibiotics (azithromycin and ciprofloxacin), the analgesic/anti-inflammatory ibuprofen, and the X-ray contrast agent iopromide. Among these, ibuprofen, iopromide, and azithromycin posed a high risk to the marine environment (RQ $>$ 1).

These compounds were not detected with the suspect screening methodology most likely due to sensitivity constraints (as discussed in **Section 3.1 .**) This can lead to underestimation of their risk to the environment despite their low PNEC values in freshwater (11–143 ng/L) and marine environments (1–14 ng/L). For example, the antibiotic azithromycin posed a high risk to the marine environment despite its low concentration in seawater (maximum concentration of 2 ng/L based on target analysis) but was not detected by HRMS-based suspect screening and thus considered not risky based on that approach. The PNECs of several antibiotics have been revised lately and lower levels have been generally adopted based on both classical ecotoxicity data and thresholds for antimicrobial resistance development [23]. Antimicrobial resistance poses an emerging threat to humans, animals, and the environment [33]. In that context, since antibiotic residues constitute a pressure for resistance selection even at trace concentrations [8], up-to-date analytical methodologies should enable their detection in the environment at those low levels. In the case of sediments, none of the PhACs detected seem to constitute a risk to benthic organisms (RQ $<$ 0.1) neither by target nor suspect screening approaches.

Additionally, we want to highlight the value of biomonitoring in the assessment of PhAC fate and risks. Bioaccumulative compounds can represent a risk to aquatic organisms despite their trace levels (or even non-detection) in surrounding water. If the MQL of a compound in water is near its PNEC value, its non-detection with current analytical methods may still associate with ecotoxicological effects. For example, the antidepressant sertraline was simultaneously found by target and suspect analyses in fish samples. However, it was not detected in water, probably due to trace levels below the method detection limit (5 ng/L). Actually the MQL of sertraline (17 ng/L) is higher than its PNEC value (9 ng/L). Therefore, water sampling alone may not fully capture environmental exposure and risks. In the last years, toxicity thresholds for measured concentrations in biota have been proposed, generally based on existing freshwater PNECs and predicted bioconcentration factors (BCFs). For example, the NORMAN Ecotoxicology Database incorporated the estimated PNECs for freshwater and marine biota (in ng/g ww) [27] (NORMAN Network, n.d.), in addition to the PNECs for water and sediments (discussed in this study and most commonly used in ERA frameworks). Among compounds that were detected in fish but not in water in this study, the concentrations of sertraline and ciprofloxacin measured in fish liver and/or muscle (Tables S2**-4**) exceeded the corresponding PNECs for freshwater biota (14 and 0.26 ng/g ww, respectively). In addition, the maximum concentration of various antidepressants in fish plasma (88.8, 63.2 and 21.2 ng/mL of venlafaxine, sertraline, and citalopram, respectively (Tables S2**-4**) were similar to their corresponding human therapeutic plasma concentration (HtPCs) [11]. Exposure to antidepressants has been associated with effects on fish behaviour, reproduction, or survival in fish [35]. Therefore, adverse outcomes can be expected in non-target organisms exposed to these PhACs. However, the link between bioaccumulation and toxicity of PhACs remains uncertain [9] and little bioaccumulative compounds may also exert harmful biological effects. Therefore, although bioaccumulation data can be valuable in risk assessment frameworks, these should not omit the possible contribution of little bioaccumulative but toxic PhACs.

In addition to the few target compounds that pose a moderate or high risk to the study area, additional risks may be associated with the suspect compounds tentatively detected in water and sediments (Table S4). Following the aforementioned semi-quantification approaches, at least three suspect PhACs posed a high environmental risk (olmesartan, lamotrigine and flubendazole), whereas five suspect compounds were associated with a moderate risk in river and/or seawater. Most 'risky' compounds were associated with the freshwater environment (Table 2), whereas cetirizine and salicylic acid exceeded the moderate and high risk thresholds for sediments-associated biota. In turn, pregabalin represented a moderate risk to the sediment environment only when using the semi-quantification tool of [1].

Among compounds with the highest predicted environmental risk in this study, olmesartan was widely detected in Japanese and German rivers (up to 2.2 μg/L) [7,36]. However, the environmental occurrence and ecotoxicity of olmesartan remain relatively unexplored [18]. The antihelminthic flubendazole was exclusively found in seawater and its occurrence may arise from its use in aquaculture (e.g., in the Ebro delta bays) for the treatment of parasitic infections. Remarkably, it was detected together with levamisole (target compound), another antihelminthic, in the study area. In Van De Steene et al., 2020, flubendazole represented a negligible risk to Belgian surface waters (RQ=0.08) [41]. The concentration of this compound was in the range of our samples (20.2 ng/L), but its derived PNEC in the freshwater environment [41] is ten times higher than in the marine environment (our findings), which

Table 2
Environmental risk assessment (ERA) of detected PhACs in river water, by target or two suspect strategies: [24] (1) and [1] (2). Only compounds with a moderate (0.1 <RQ<1) or high risk (RQ>1) are shown. MECs (obtained either by target or non-target analysis) and PNECs (retrieved from NORMAN database) are also provided (in ng/L). If necessary, target values below the MQL were replaced by MQL/2 for the calculation of RQs. ND: not detected.

Therapeutic group	Compound	MEC target	MEC suspect	PNEC	RQ target	RQ suspect1	RQ suspect2
Analgesics/anti-inflammatories	Flufenamic acid	ND	51.2	402	-	**0.1**	**0.1**
	Ibuprofen	<MQL	ND	11	**1.0**	-	-
	Salicylic acid	ND	8537.2	18,000	-	**0.5**	**1.9**
Antibiotics	Azithromycin	<MQL	ND	19	**0.1**	-	-
Antihypertensives	Eprosartan	ND	40.6	143	-	**0.3**	**0.3**
	Olmesartan	ND	52.3	4	-	**13.0**	**15.9**
Psychiatric drug	Gabapentin	ND	305.2	1000	-	**0.3**	**0.6**
	Lamotrigine	ND	51.6	8	-	**6.4**	**8.4**
VIH treatment	Nevirapine	ND	159.2	482	-	**0.3**	**0.5**
X-ray contrast agents	Iopromide	58.8	ND	143	**0.4**	-	-

can explain the discrepancy in the derived risk. Likewise, a low environmental risk can be expected for some of the most broadly detected PhACs in this study, such as valsartan, irbesartan and acetaminophen, due to the fact that their lowest PNECs ranged in the μg/L to mg/L levels (Tables S2-**4**), which were not attained in this study.

According to the ERA, when considering the additional suspect PhACs, the cumulative risk to the freshwater environment (i.e., sum of individual RQs for moderate and high risk compounds) would be much higher than solely predicted by target analysis (Table 2). Therefore, this study highlights the potential environmental risks identified through suspect screening, which could help prioritize PhACs, but it is essential to bear in mind that the risk assessment for suspect compounds was based on semi-quantitative data, prone to slightly overestimate PhAC concentrations. Therefore, the associated risks might also be overestimated. For example, when the semi-quantified concentrations in water (for target compounds) were used to predict the environmental risk, four of them (carbamazepine, gemfibrozil, phenazone and o-desmethylvenlafaxine) were wrongly identified as posing an environmental risk (Table S4). However, even considering a general overestimation of 7x in semi-quantification (as previously shown), olmesartan would still be considered a high risk compound, while lamotrigine and flubendazole would be associated with a moderate risk, which evidences the importance of suspect-screening strategies as a complement of target analysis.

Besides the most critical compounds from above highlighted by ERA, other PhACs detected at relatively high concentrations (based on the semi-quantification approach) deserve further consideration. Metformin, for instance, found in all river samples (median: 1.9 μg/L, according to Liigand et al. approach), posed no risk to aquatic life in the study area (RQ>0.1) as it has a high PNEC value of 160 μg/L (https://www.norman-network.com/nds/ecotox, last accessed May 2023). However, recent studies have pointed out that at levels lower than the PNEC, metformin probably poses a risk to aquatic life, supporting the urgent need to revise the current PNEC [5].

4. Conclusions

On one hand, although high-resolution mass spectrometry (HRMS) shows higher performance than low-resolution instruments in many aspects, it lacks the sensitivity to detect potentially hazardous PhACs at low concentrations. For example, although suspect screening revealed 38 additional PhACs, it failed to detect low-level compounds with a potential environmental risk, such as antibiotics (azithromycin and ciprofloxacin). Additionally, semi-quantification is a useful technique that provides a reasonable approximation of actual concentrations, but it has certain limitations that hinder its use as a substitute for conventional target approaches (such as matrix-effect problems and the absence of recovery values).

On the other hand, the main problem of target analysis is its bias towards a pre-selected set of compounds. Indeed, among additional PhACs detected by suspect screening, some can be relatively new to the market, such as vildagliptin (found in fish liver) or constitute an emerging concern to the environment. For example, among other substances, human metabolites (seven found exclusively by HRMS-based suspect strategy in river water) have been largely ignored and traditionally excluded from routine monitoring. Despite the possible overestimation of semi-quantitative results in this study (generally 1–10x), ignoring suspect compounds in risk assessment can largely underestimate risks.

Therefore, from authors' point of view, a tiered approach would be an effective strategy to overcome the current limitations of the methods. This approach would rely on a preliminary semi-quantitative suspect screening to tentatively identify new potential threats to the environment, followed by their inclusion in established target methodologies to precisely determine the environmental occurrence and risk of PhACs. Nevertheless, target analysis should be based on dynamic target lists that are fed not only by suspect data, but by historical assessment of compounds and their risks. The latter will ensure that the approach will not omit certain low-level but relevant compounds, which may be neglected by HRMS-based strategies. The newest more sensitive HRMS instrumentation is foreseen to allow both low-level target and suspect analyses of contaminants, but follow-up studies need to be performed to confirm this scenario.

CRediT authorship contribution statement

J.M. Castaño-Ortiz: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft; **P. Gago-Ferrero**: Formal analysis, Investigation, Writing – review & editing; **D. Barceló**: Conceptualization, Funding acquisition, Writing – review & editing; **S. Rodríguez-Mozaz**: Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing; **R. Gil-Solsona**: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132974.

References

[1] Aalizadeh, R., Nikolopoulou, V., Alygizakis, N., Slobodnik, J., Thomaidis, N.S., 2022. A novel workflow for semi-quantification of emerging contaminants in environmental samples analyzed by LC-HRMS. Anal Bioanal Chem 414, 7435–7450. https://doi.org/10.1007/s00216-022-04084-6.

[2] Aalizadeh, R., von der Ohe, P.C., Thomaidis, N.S., 2017. Prediction of acute toxicity of emerging contaminants on the water flea Daphnia magna by ant colony optimization–support vector machine QSTR models. Environ Sci Process Impacts 19, 438–448.

[3] Alygizakis, N., Lestremau, F., Gago-Ferrero, P., Gil-Solsona, R., Arturi, K., Hollender, J., Schymanski, E.L., Dulio, V., Slobodnik, J., Thomaidis, N.S., 2023. Towards a harmonized identification scoring system in LC-HRMS/MS based non-target screening (NTS) of emerging contaminants. TrAC Trends Anal Chem 159, 116944. https://doi.org/10.1016/J.TRAC.2023.116944.

[4] Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: a European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in “digitally frozen” environmental samples. TrAC - Trends Anal Chem. https://doi.org/10.1016/j.trac.2019.04.008.

[5] Barros, S., Alves, N., Pinheiro, M., Ribeiro, M., Morais, H., Montes, R., Rodil, R., Quintana, J.B., Coimbra, A.M., Santos, M.M., 2022. Are fish populations at risk? metformin disrupts zebrafish development and reproductive processes at chronic environmentally relevant concentrations. Environ Sci Technol.

[6] Batta, A., Kalra, B.S., Khirasaria, R., 2020. Trends in FDA drug approvals over last 2 decades: an observational study. J Fam Med Prim care 9, 105–114. https://doi.org/10.4103/jfmpc.jfmpc_578_19.

[7] Bayer, A., Asner, R., Schüssler, W., Kopf, W., Weiß, K., Sengl, M., Letzel, M., 2014. Behavior of sartans (antihypertensive drugs) in wastewater treatment plants, their occurrence and risk for the aquatic environment. Environ Sci Pollut Res. 21, 10830–10839.

[8] Bengtsson-palme, J., Larsson, D.G.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria. Propos Limits Environ Regul 86, 140–149.

[9] Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ Health Perspect 120, 1221–1229.

[10] Briones, R.M., Sarmah, A.K., Padhye, L.P., 2016. A global perspective on the use, occurrence, fate and effects of anti-diabetic drug metformin in natural and engineered ecosystems. Environ Pollut 219, 1007–1020. https://doi.org/10.1016/j.envpol.2016.07.040.

[11] Castaño-Ortiz, J., Gil-Solsona, R., Ospina-Álvarez, N., Alcaraz-Hernández, J., Farré, M., León, V., Barceló, D., Santos, L., Rodríguez-Mozaz, S., 2024. Fate of pharmaceuticals in the Ebro River Delta region: the combined evaluation of water, sediment, plastic litter, and biomonitoring. Sci Total Environ 906, 167467.

[12] Castaño-Trias, M., Rodríguez-Mozaz, S., Buttiglieri, G., 2023. A decade of water monitoring in a Mediterranean region: pharmaceutical prioritisation for an upgraded analytical methodology. Environ Nanotechnol, Monit Manag 20. https://doi.org/10.1016/j.enmm.2023.100850.

[13] Čelić, M., Jaén-Gil, A., Briceño-Guevara, S., Rodríguez-Mozaz, S., Gros, M., Petrović, M., 2021. Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks. J Hazard Mater 404, 124102. https://doi.org/10.1016/j.jhazmat.2020.124102.

[14] Daughton, C.G., 2014. The Matthew Effect and widely prescribed pharmaceuticals lacking environmental monitoring: case study of an exposure-assessment vulnerability. Sci Total Environ 466, 315–325.

[15] European Commission, 2002. 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044).

[16] Gago-Ferrero, P., Schymanski, E.L., Bletsou, A.A., Aalizadeh, R., Hollender, J., Thomaidis, N.S., 2015. Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS. Environ Sci Technol 49, 12333–12341. https://doi.org/10.1021/acs.est.5b03454.

[17] Gil-Solsona, R., Nácher-Mestre, J., Lacalle-Bergeron, L., Sancho, J.V., Calduch-Giner, J.A., Hernández, F., Pérez-Sánchez, J., 2017. Untargeted metabolomics approach for unraveling robust biomarkers of nutritional status in fasted gilthead sea bream (Sparus aurata). PeerJ 2017. https://doi.org/10.7717/peerj.2920.

[18] Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - a review. Chemosphere 138, 281–291. https://doi.org/10.1016/j.chemosphere.2015.06.024.

[19] González-Mariño, I., Gracia-Lor, E., Bagnati, R., Martins, C.P.B., Zuccato, E., Castiglioni, S., 2016. Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry. Anal Bioanal Chem 408, 4297–4309. https://doi.org/10.1007/s00216-016-9521-0.

[20] Ibáñez, M., Bijlsma, L., Pitarch, E., López, F.J., Hernández, F., 2021. Occurrence of pharmaceutical metabolites and transformation products in the aquatic environment of the Mediterranean area. Trends Environ Anal Chem 29. https://doi.org/10.1016/j.teac.2021.e00118.

[21] Kruve, A., Kiefer, K., Hollender, J., 2021. Benchmarking of the quantification approaches for the non-targeted screening of micropollutants and their transformation products in groundwater. Anal Bioanal Chem 413, 1549–1559. https://doi.org/10.1007/s00216-020-03109-2.

[22] Lajeunesse, A., Gagnon, C., Gagné, F., Louis, S., Čejka, P., Sauvé, S., 2011. Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and after ozone treatment – Evidence of biological effects. Chemosphere 83, 564–571. https://doi.org/10.1016/j.chemosphere.2010.12.026.

[23] Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R., 2017. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environ Int 0–1. https://doi.org/10.1016/j.envint.2017.09.013.

[24] Liigand, J., Wang, T., Kellogg, J., Smedsgaard, J., Cech, N., Kruve, A., 2020. Quantification for non-targeted LC/MS screening without standard substances. Sci Rep 10, 5808. https://doi.org/10.1038/s41598-020-62573-z.

[25] Little, J.L., Cleven, C.D., Brown, S.D., 2011. Identification of “known unknowns” utilizing accurate mass data and chemical abstracts service databases. J Am Soc Mass Spectrom 22, 348–359.

[26] Malm, L., Palm, E., Souihi, A., Plassmann, M., Liigand, J., Kruve, A., 2021. Guide to semi-quantitative non-targeted screening using LC/ESI/HRMS. Molecules 26, 3524.

[27] NORMAN Network, n.d. NORMAN Ecotoxicology Database. ⟨https://www.norman-network.com/nds/ecotox⟩. [WWW Document]. URL ⟨https://www.norman-network.com/nds/ecotox/⟩.

[28] Oesterle, I., Pristner, M., Berger, S., Wang, M., Hernandes, V.V., Rompel, A., Warth, B., 2023. Exposomic biomonitoring of polyphenols by LC-HRMS via non-targeted analysis and suspect screening.

[29] Palma, P., Fialho, S., Lima, A., Catarino, A., Costa, M.J., Barbieri, M.V., Monllor-Alcaraz, L.S., Postigo, C., de Alda, M.L., 2021. Occurrence and risk assessment of pesticides in a Mediterranean Basin with strong agricultural pressure (Guadiana Basin: Southern of Portugal). Sci Total Environ 794, 148703.

[30] Park, N., Choi, Y., Kim, D., Kim, K., Jeon, J., 2018. Prioritization of highly exposable pharmaceuticals via a suspect/non-target screening approach: A case study for Yeongsan River, Korea. Sci Total Environ 639, 570–579. https://doi.org/10.1016/j.scitotenv.2018.05.081.

[31] Picó, Y., Campo, J., 2022. An Overview of the State-of-the-Art: Mass Spectrometry in Food and Environment.

[32] Salgueiro-González, N., Castiglioni, S., Gracia-Lor, E., Bijlsma, L., Celma, A., Bagnati, R., Hernández, F., Zuccato, E., 2019. Flexible high resolution-mass spectrometry approach for screening new psychoactive substances in urban wastewater. Sci Total Environ 689, 679–690. https://doi.org/10.1016/j.scitotenv.2019.06.336.

[33] Sanseverino, I., Navarro, A., Loos, R., Marinov, D., 2018. State of the Art on the Contribution of Water to Antimicrobial Resistance. https://doi.org/10.2760/82376.

[34] Santos, L.H.M.L.M., Freixa, A., Insa, S., Acuña, V., Sanchís, J., Farré, M., Sabater, S., Barceló, D., Rodríguez-Mozaz, S., 2019. Impact of fullerenes in the bioaccumulation and biotransformation of venlafaxine, diuron and triclosan in river biofilms. Environ Res 169, 377–386. https://doi.org/10.1016/j.envres.2018.11.036.

[35] Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., Faggio, C., 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Sci Total Environ 631–632, 789–794. https://doi.org/10.1016/j.scitotenv.2018.03.076.

[36] Suzuki, T., Kosugi, Y., Watanabe, K., Iida, H., Nishimura, T., 2021. Environmental risk assessment of active human pharmaceutical ingredients in urban rivers in Japan. Chem Pharm Bull 69, 840–853.

[37] Świacka, K., Maculewicz, J., Kowalska, D., Caban, M., Smolarz, K., Świeżak, J., 2022. Presence of pharmaceuticals and their metabolites in wild-living aquatic organisms – current state of knowledge. J Hazard Mater 424, 127350. https://doi.org/10.1016/j.jhazmat.2021.127350.

[38] Tadić, Đ., Manasfi, R., Bertrand, M., Sauvêtre, A., Chiron, S., 2022. Use of passive and grab sampling and high-resolution mass spectrometry for non-targeted analysis of emerging contaminants and their semi-quantification in water. Molecules 27, 3167.

[39] Taha, M.H., Aalizadeh, R., Alygizakis, N., Antignac, J.P., Arp, H.P.H., Bade, R., Baker, N., Belova, L., Bijlsma, L., Bolton, E.E., Brack, W., Celma, A., Chen, W.L., Cheng, T., Chirsir, P., Čirka, Ľ., D'Agostino, L.A., Djoumbou Feunang, Y., Dulio, V., Fischer, S., Gago-Ferrero, P., Galani, A., Geueke, B., Głowacka, N., Glüge, J., Groh, K., Grosse, S., Haglund, P., Hakkinen, P.J., Hale, S.E., Hernandez, F., Janssen, E.M.L., Jonkers, T., Kiefer, K., Kirchner, M., Koschorreck, J., Krauss, M., Krier, J., Lamoree, M.H., Letzel, M., Letzel, T., Li, Q., Little, J., Liu, Y., Lunderberg, D.M., Martin, J.W., McEachran, A.D., McLean, J.A., Meier, C., Meijer, J., Menger, F., Merino, C., Muncke, J., Muschket, M., Neumann, M., Neveu, V., Ng, K., Oberacher, H., O'Brien, J., Oswald, P., Oswaldova, M., Picache, J.A., Postigo, C., Ramirez, N., Reemtsma, T., Renaud, J., Rostkowski, P., Rüdel, H., Salek, R.M., Samanipour, S., Scheringer, M., Schliebner, I., Schulz, W., Schulze, T., Sengl, M., Shoemaker, B.A., Sims, K., Singer, H., Singh, R.R., Sumarah, M., Thiessen, P.A., Thomas, K.V., Torres, S., Trier, X., van Wezel, A.P., Vermeulen, R.C.H., Vlaanderen, J.J., von der Ohe, P.C., Wang, Z., Williams, A.J., Willighagen, E.L., Wishart, D.S., Zhang, J., Thomaidis, N.S., Hollender, J., Slobodnik, J., Schymanski, E.L., 2022. The NORMAN suspect list exchange (NORMAN-SLE): facilitating European and worldwide collaboration on suspect screening in high resolution mass spectrometry, 2022 341 Environ Sci Eur 34, 1–26. https://doi.org/10.1186/S12302-022-00680-6.

[40] Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.-W., Isobe, T., Shinohara, R., Kunisue, T., Tanabe, S., 2015. Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-impacted streams. Environ Sci Technol 49, 11649–11658. https://doi.org/10.1021/acs.est.5b02478.

[41] Van De Steene, J.C., Stove, C.P., Lambert, W.E., 2010. A field study on 8 pharmaceuticals and 1 pesticide in Belgium: removal rates in waste water treatment plants and occurrence in surface water. Sci Total Environ 408, 3448–3453.

[42] Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., 2022. Pharmaceutical pollution of the world's rivers. Proc Natl Acad Sci 119 e2113947119.

[43] Williams, A.J., Grulke, C.M., Edwards, J., McEachran, A.D., Mansouri, K., Baker, N. C., Patlewicz, G., Shah, I., Wambaugh, J.F., Judson, R.S., 2017. The CompTox chemistry dashboard: a community data resource for environmental chemistry. J Chemin- 9, 1–27.

[44] Yang, Y., Fu, J., Peng, H., Hou, L., Liu, M., Zhou, J.L., 2011. Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J Hazard Mater 190, 588–596. https://doi.org/10.1016/j.jhazmat.2011.03.092.