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Are preserved coastal water bodies in Spanish Mediterranean basin impacted by human activity? Water quality evaluation using chemical and biological analyses

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ABSTRACT

The Spanish Mediterranean basin is particularly susceptible to climate change and human activities, making it vulnerable to the influence of anthropogenic contaminants. Therefore, conducting comprehensive and exhaustive water quality assessment in relevant water bodies of this basin is pivotal. In this work, surface water samples from coastal lagoons or estuaries were collected across the Spanish Mediterranean coastline and subjected to target and suspect screening of 1,585 organic micropollutants by liquid chromatography coupled to ion mobility separation and high resolution mass spectrometry. In total, 91 organic micropollutants could be confirmed and 5 were tentatively identified, with pharmaceuticals and pesticides being the most prevalent groups of chemicals. Chemical analysis data was compared with data on bioanalysis of those samples (recurrent aryl hydrocarbon receptor (AhR) activation, and estrogenic receptor (ER) inhibition in wetland samples affected by wastewater streams). The number of identified organic contaminants containing aromatic rings could explain the AhR activation observed. For the ER antagonistic effects, predictions on estrogenic inhibition potency for the detected compounds were used to explain the activities observed. The integration of chemical analysis with bioanalytical observations allowed a comprehensive overview of the quality of the water bodies under study.

1. Introduction

Climate change, natural resources overexploitation and human activities are affecting the quality of water ecosystems (Döll and Zhang, 2010). The Spanish Mediterranean basin has been found to be specially affected by these changes and it is particularly vulnerable to the impact of anthropogenic contaminants (Abily et al., 2021; Airoldi and Beck, 2007). A recent study highlighted that the Ebro river (relevant water reservoir in the Northern Spanish Mediterranean area) was affected by elevated concentrations of phosphate originated from agricultural industry reducing the sustainability of the water ecosystem (Diamantini et al., 2018). Similarly, Albufera Natural Park, a typical Mediterranean shallow coastal lagoon located in Valencia, has been affected by many years of negligent management of wastewater streams and a large growing population of surrounding settlements (Martín et al., 2020). As a consequence, hundreds of organic micropollutants have been consistently detected in these water bodies (Barbieri et al., 2021; Borrull et al., 2021; Ccanccapa et al., 2016; Čelić et al., 2019; Sadutto et al., 2021). Therefore, appropriate strategies for the monitoring of water quality should be applied on these and other vulnerable water ecosystems.

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Efforts have been conducted towards the development, application and standardization of comprehensive analytical strategies to monitor the presence of large lists of organic micropollutants (Fabregat-Safont et al., 2021; Fonseca et al., 2020; Gago-Ferrero et al., 2020; Hernández et al., 2015; Krauss et al., 2010; Menger et al., 2020). The utilization of orthogonal analytical techniques such as ion mobility separation coupled to high resolution mass spectrometry (IMS-HRMS) has allowed the improved identification of small organic molecules in complex matrices (Celma et al., 2021a, 2020; Hinnenkamp et al., 2019). Also, the combined application of complementary techniques, such as liquid and gas chromatography coupled to low and high resolution mass spectrometry, has allowed to increase the chemical space and, therefore, the number of contaminants monitored, from non-polar, volatile compounds to (highly) polar, non-volatile compounds, in wide-scope screening strategies (Gago-Ferrero et al., 2020; Hernández et al., 2015; Hollender et al., 2017; Menger et al., 2020; Moschet et al., 2017; Pitarch et al., 2016). More recently, effect-based methodologies have been applied for a wider and more comprehensive quality evaluation of water samples (Hashmi et al., 2018; Jonkers et al., 2020; Lundqvist et al., 2019; Neale et al., 2017; Oskarsson et al., 2021; Tousova et al., 2017). In such approaches, the combination of chemical and bioanalytical analyses permits the measurement of mixed toxicological (cocktail) effects of compounds as well as to potentially relate them with exposure levels of detected organic micropollutants (Brunner et al., 2020; Lundqvist et al., 2019; Tousova et al., 2017). Therefore, by selecting relevant toxicity endpoints based on the nature of the water samples, effect-based methodologies can give insight into other dimensions of the quality of water samples under study (Brunner et al., 2020).

In the present work, a comprehensive strategy combining ultimate analytical and toxicological tools has been applied for the screening of organic micropollutants of a set of 11 relevant and vulnerable water bodies from the Spanish Mediterranean coastline. To this purpose, a large qualitative target screening was performed for 682 micropollutants using both, ultra-high performance liquid chromatography (UHPLC) coupled to IMS-HRMS (556 compounds) and UHPLC coupled to low resolution mass spectrometry with triple quadrupole analyzer (UHPLC-MS/MS QqQ) (183 compounds). 57 compounds were monitored by both instrumental configurations. In addition, a complementary suspect screening of more than 900 substances was applied based on UHPLC-IMS-HRMS. Additionally, a large bioanalytical data set was previously obtained from the same samples over different toxicological endpoints to get a comprehensive overview of the quality of the water bodies under study (Celma et al., 2021b). Briefly, *in vitro* biological analysis of the wetland samples was performed by means of a panel of 8 different toxicity endpoints including aryl hydrocarbon receptor (AhR), (ant)agonistic androgenicity (AR), (ant)agonistic estrogenicity (ER), oxidative stress response (Nrf2) and (ant)agonistic vitamin D receptor (VDR) reporter gene assays. In this work, we aimed to integrate the results from the chemical analyses with the bioanalytical observations to better understand water quality of relevant water bodies from the Mediterranean Spanish coastline.

2. Materials and methods

2.1. Sampling and sample treatment

2.1.1. Sampling locations

In total, 11 water samples were collected from water bodies along the Spanish Mediterranean coastline including both estuaries and coastal lagoon water. Sampling locations were selected based on their inclusion in the list of Wetlands of International Importance (8 out of 11 sites) (Ramsar Convention on Wetlands, 2021) and/or due to their large diversity of aquatic organisms and wildlife. Table 1 depicts the relevance of the samples collected as well as the potential impact of specific activities (*viz.* rice agriculture, citrus-fruit agriculture, touristic activity and/or impact of treated wastewater streams and urban runaways from large cities). Evaluating water quality in these Mediterranean wetlands is a major concern, because of the wide variety of contaminants reported in previous studies (Barbieri et al., 2021; Borrull et al., 2021; Ccanccapa et al., 2016; Čelić et al., 2019; Sadutto et al., 2021), the estimated water loss of 50% over the last century (Perennou et al., 2012), and the dramatic climate changes that might occur in the next years (García-Ruiz et al., 2011; Guiot and Cramer, 2016; Klausmeyer and Shaw, 2009).

Table 1

Sample collection details, relevance and observations.

	Sampling location	Relevance	Observations
#1	Ebro river estuary (40° 43′ 10.20″ N; 00° 51′ 20.88″ E)	Part of 'Delta del Ebro' natural park which is included in list of Wetlands of International Importance (Ramsar Convention, 2021).	Mouth of the largest river in Spain. Upstream infliction of treated urban wastewater.
#2	El Clot de l'Ebre (40° 38′ 35.52″ N; 00° 38′ 24.36″ E)		Rice agriculture irrigation water instream.
#3	L'Estany Peníscola, lagoon (40° 21′ 59.04″ N; 00° 24′ 01.80″ E)	Designated as Place of Community Interest by regional government because of the flora and fauna diversity.	Highly tourist area. Potential impact of human activity.
#4	L'Estany Peníscola, canal (40° 21′ 51.12″ N; 00° 23′ 56.76″ E)		
#5	Prat de Cabanes-Torreblanca (40° 11′ 50.28″ N; 00° 12′ 31.32″ E)	Included in list of Wetlands of International Importance (Ramsar Convention, 2021).	Potential impact of citrus-fruit agriculture.
#6	Marjal Pego-Oliva (38° 52′ 23.52″ N; 00° 02′ 53.88″ W)	Included in list of Wetlands of International Importance (Ramsar Convention, 2021).	Potential impact of citrus-fruit agriculture.
#7	Albufera. Portet Sollana (39° 18′ 37.08″ N; 00° 21′ 25.92″ W)	Part of 'L'Albufera' natural park. Included in list of Wetlands of International Importance (Ramsar Convention, 2021).	Potential impact of rice agriculture.
#8	Albufera. Tancat de la Pipa (39° 22′ 05.52″ N; 00° 20′ 45.60″ W)		Instream of treated wastewater and urban runaways from the city of Valencia (790,000 inh., Spain)
#9	Albufera. Golf de Pujol (39° 20′ 14.04″ N; 00° 11′ 32.64″ W)		Highly tourist area of the natural park. Potential impact of human activity.
#10	L'Estany Almenara (39° 45′ 14.04″ N; 00° 11′ 32.64″ W)	Included in list of Wetlands of International Importance (Ramsar Convention, 2021).	Potential impact of citrus-fruit agriculture.
#11	El Clot de Borriana (39° 52′ 46.92″ N; 00° 03′ 14.04″ W)	Natural park supplied with water from a natural spring.	Potential impact of citrus-fruit agriculture.

Sampling was performed as follows: grab surface water samples (8 L) were collected in polyethylene terephthalate (PET) plastic bottles previously rinsed with surface water from the sampling location in February 2019. After collection, water samples were stored at 4 °C until extraction, which was performed within 24 h. Two procedural blank samples consisting of Milli-Q water were also prepared under the same conditions.

2.1.2. Sample extraction procedure

The extraction procedure was adapted from Celma *et al.* (Celma et al., 2021b). In brief, 2.5 L of water sample was extracted by solid phase extraction (SPE) using Oasis HLB cartridges (20 cc, 1 g, 60 μm) (Waters Corporation, Milford, MA, USA). Cartridges were previously conditioned with 3 × 10 mL of methanol (LC-MS grade, Scharlab, Barcelona, Spain) and 2 × 10 mL Milli-Q water (Millipore, Bedford, MA, USA). During sample loading, silanized glass wool was deployed inside the cartridge to avoid clogging of stationary phase. After sample loading, SPE cartridges were dried under vacuum and then eluted with 3 × 10 mL of methanol. The three eluates were pooled and evaporated at 40 °C under nitrogen (N_2) beam (0.7 bar) using a TurboVap II system (Caliper LifeSciences, Hopkinton, MS, USA) until reaching a final volume of 0.2 mL. Milli-Q water (Millipore, Bedford, MA, USA) was then added up to a final volume of 1 mL (final concentration factor was x2500 in water: methanol 80:20). Sample extracts were then frozen overnight at −20 °C. The following day, extracts were centrifuged at 12,000 rpm for 10 min to remove precipitates and particulate matter. Finally, sample extracts were stored at −20 °C until analysis.

Prior to extraction, raw water samples as well as procedural blanks were spiked with 15 isotopically labelled internal standards (ILIS) for quality control purposes. Details on the spiked ILIS can be found in **Table S1** in Supporting Information (SI).

2.2. *Chemical analysis*

2.2.1. *Instrumentation*

A large screening of organic micropollutants was conducted over the set of 11 water samples from relevant water bodies of the Spanish Mediterranean coastline and the procedural blanks. Compounds under investigation included 1,585 pharmaceuticals and metabolites, pesticides and transformation products, hormones, mycotoxins and psychoactive drugs.

Samples were screened by UHPLC-IMS-HRMS for 556 target compounds as well as 903 suspect compounds including pesticides and transformation products, pharmaceuticals and metabolites, hormones, drugs and mycotoxins. Additionally, a screening by liquid chromatography-tandem mass spectrometry with triple quadrupole (UHPLC-MS/MS QqQ) was also applied for 183 target chemicals (hormones, per- and polyfluoroalkyl substances (PFASs) and other organic micropollutants). 57 compounds out of the 183 targets were also included in the UHPLC-IMS-HRMS screening methodology.

2.2.1.1. UHPLC-IMS-HRMS. A Waters Acquity I-Class UPLC system (Waters, Milford, MA, USA) connected to a VION IMS-QTOF mass spectrometer, using electrospray ionization (ESI) interface operating in both positive and negative ionization mode was used for sample analysis. Compounds were separated using a CORTECS® C18 2.1 × 100 mm, 2.7 μm fused core column (Waters) at a flow rate of 300 μL min^{-1} with a gradient consisting of Milli-Q water from Millipore and methanol as mobile phases, both with 0.01% formic acid. Total run time was 18 min. MS data were acquired using the VION in $HDMS^e$ mode, over the range *m/z* 50–1000, with N_2 as the drift gas, an IMS wave velocity of 250 m s^{-1} and wave height ramp of 20–50 V. Leucine enkephalin (*m/z* 556.27658 and *m/z* 554.26202) was used for mass correction in positive and negative ionization modes, respectively. Two independent scans with different collision energies, 6 eV for low energy (LE) and a ramp of 28–56 eV for high energy (HE), were acquired during the run with a scan time of 0.3 s was set in both LE and HE functions. Nitrogen (≥ 99.999%) was used as collision-induced dissociation (CID) gas. Data were examined using an in-house built accurate mass screening workflow within the UNIFI platform (version 1.9.4) from Waters Corporation.

Compounds included in the IMS-HRMS target database can be found at the freely available online repository Zenodo (https://doi.org/10.5281/zenodo.3966751) (Celma et al., 2019) as well as the Suspect List Exchange platform from the NORMAN network (NORMAN network, 2021). A detailed description of the UHPLC-IMS-HRMS instrumental methodology can be found elsewhere (Celma et al., 2020).

2.2.1.2. UHPLC-MS/MS QqQ. The sample extracts were also analyzed for the qualitative determination of hormones, PFASs and other organic micropollutants by a DIONEX UltiMate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (TSQ QUANTIVA, Thermo Scientific, Waltham, MA, USA).

A Kinetex® Biphenyl column (100 mm × 2.1 mm i.d., 2.6 μm particle size, Phenomenex) was used for chromatographic separation of organic micropollutants and PFASs compounds. The mobile phase consisted of Milli-Q water and methanol, both with 0.1% formic acid. The flow rate was 600 μL min^{-1} and run time was 16 min, with switched positive and negative electrospray ionization modes. Furthermore, an Acquity BEH C18 column (50 mm × 2.1 mm, 1.7 μm, Waters Corporation, Manchester, UK) was used for chromatographic separation of hormones. The mobile phase for hormone analysis consisted of Milli-Q water with 5 mM ammonium acetate and acetonitrile (ACN) at a flow rate of 500 μL min^{-1}. Run time was 15 min using switching positive and negative electrospray ionization modes.

Heated electrospray ionization (H-ESI) was used to ionize the target compounds. The spray voltage was set to static: positive ion 3.5 kV and negative ion 2.5 kV. Nitrogen (purity greater than 99.999%) was used as sheath gas (50 arbitrary units), auxiliary gas (15 arbitrary units), and sweep gas (2 arbitrary units) and collision gas. The vaporizer was heated to 400 °C and the capillary to 325 °C.

A detailed information of the low resolution methodologies followed as well as target compounds evaluated can be found elsewhere (Golovko et al., 2021).

2.2.2. *Criteria for identification*

For UHPLC-IMS-HRMS screening, the identification criteria and confidence levels proposed previously by Celma *et al.* were followed (Celma et al., 2020). In brief, level 1 confirmation (target screening) requires mass accuracy of both precursor and fragment ions < 3 ppm, RT deviation < 0.1 min and CCS deviation < 2% in relation to the reference standard. For levels 2 and 3 (suspect screening) mass accuracy of precursor and fragments ions should be < 3 ppm from the potential molecular formula. For suspect candidates, RT and CCS were predicted using a model developed elsewhere (Bade et al., 2015; Bijlsma et al., 2017) with an accuracy of < ± 2 min for RT, < ± 6% CCS deviation for protonated molecules and < ± 8% CCS deviation for sodium adducts.

For qualitative targeted screening by LC-MS/MS QqQ, RT agreement with the reference standard as well as the observation of the different *m/z* transitions for each analyte (with relative deviation of ratios between monitored transitions below ± 30%) and signal-to-noise (S/N) ratio ≥ 3 were required for the identification of the compound, as suggested elsewhere (European Commission. Directorate General for Health and Food Safety., 2019). For comparison purposes, reference standards were injected alongside the samples in the same chromatographic sequence.

2.3. *Quality assurance for chemical analysis*

Method performance was assessed by spiking a set of 15 ILIS with different psycho-chemical properties in both, samples and procedural blanks, to account for extraction efficiency. Spiked ILIS were chosen based on a set of compounds developed to cover the whole chromatographic run (**Table S1** in SI) (Celma et al., 2018).

ILIS were spiked at 40 ng L^{-1} for individual compound in raw sample yielding an expected final concentration in extract injected into the LC-MS system of 100 μg L^{-1}. The extraction performance was qualitatively evaluated by screening the spiked ILIS by means of UHPLC-IMS-HRMS.

2.4. *Biological analysis of samples*

The whole set of 11 samples and the 2 procedural blanks were biologically analyzed to evaluate water quality with a panel of 8 different toxicity endpoints. Aryl hydrocarbon receptor (AhR), (ant)agonistic androgenicity (AR), (ant)agonistic estrogenicity (ER), oxidative stress response (Nrf2) and (ant)agonistic vitamin D receptor (VDR) reporter gene assays were implemented as they are appropriate indicators of quality of environmental water bodies. Results on bioanalysis are presented elsewhere (Celma et al., 2021b). Extraction for biological analysis was analogue to the one herein performed excepting for the fact that no ILIS was spiked and that the reconstitution solvent was changed to ethanol instead of H_2O to enable its application into alive organisms. Biological analysis performance was evaluated by analyzing specific positive controls for each bioassay alongside the analysis of wetland samples. Quantitative bioassays were carried out to enable the estimate the observed overall toxicity in bio-equivalent concentration of reference standards of well-known toxicity.

A detailed description of the different bioassays applied and the methodology followed is available at Celma *et al.* (Celma et al., 2021b).

3. Results and discussion

3.1. Method performance evaluation

Spiked compounds were investigated by means of UHPLC-IMS-HRMS to qualitatively evaluate the extraction performance. **Table S1** in SI indicates whether compounds could be identified in the samples or they were not detected due to inefficient recovery from sample treatment. Briefly, 60% of the spiked compounds were recovered and detected by means of IMS-HRMS in negative ionization mode and 83% in positive ionization mode. Interestingly, two of the compounds that were systematically not detected in spiked samples were ecgonine-d_3 and di(2-ethylhexyl)phthalate-d_4. Those are the first and last compound eluting from the chromatographic column, denoting high and low polarity, respectively. Despite that they were lost during sample treatment, it is not expected to notably affect the overall performance of the screening as the wide majority of compounds included in this study are not of extreme polarities. Also, ibuprofen-d_3 was not observed in any of the spiked samples which might indicate potential degradation due to its reduced stability in environmental aquatic samples (Jakimska et al., 2014).

3.2. Wide-scope screening of organic micropollutants

3.2.1. Target screening

Target screening permitted the identification of 88 substances in the whole set of surface water bodies. Table 2 summarizes the main findings, including the level of confidence of the identification. Those peaks appearing in procedural blanks at similar intensities than in wetland samples were excluded from further analysis.

From the 57 compounds included in both, IMS-HRMS and QqQ methods, 28 could be identified in the samples by at least one of the methodologies, yielding a total of 160 positives. While 43 were confirmed by both methods, 113 were identified only by MS/MS QqQ and 4 only by IMS-HRMS. Table 2 shows a summary of data obtained in the target screening. It can be seen that several positives could only be found by QqQ, due to its higher sensitivity, illustrating the advantage of using QqQ for detection and identification of target compounds when they are present at very low concentrations. In some cases, slight deviations in RT or mass accuracy were observed in IMS-HRMS screening, and therefore confirmation via MS/MS QqQ gathered was much useful. Besides, 23 more compounds (131 positives) monitored only by QqQ, and 37 compounds (127 positives) monitored only by IMS-HRMS, could also be identified. Independently from the instrumentation used, confirmations with IMS-HRMS or QqQ are considered with the same level of confidence for discussion purposes.

The most prevalent compounds were pharmaceuticals and their metabolites with 43 and 10 substances identified, respectively. 25 pesticides and 6 transformation products, 2 hormones and 2 personal care products were also identified. Considering that the set of surface water samples studied had no direct impact of wastewater streams except for ‘Ebro’ River estuary and Albufera Natural park, it is remarkable that the most dominant group of chemicals were pharmaceuticals in the vast majority of samples (Fig. 1). Yet, the figures of pesticides identified were expected due to agricultural practices in the surrounding areas. Additionally, personal care products and illicit drugs (Fig. 1) could be found in water bodies impacted by wastewater effluents.

Several compounds (*acesulfame, caffeine, nicotine, salicylic acid, theophylline,* and *thiabendazole*) were identified in all samples. Most of them are markers of human activity: *acesulfame* (sweetener), *caffeine* (main stimulant from coffee and tea), *nicotine* (main stimulant from tobacco), *salicylic acid* (important active metabolite of aspirin), and *theophylline* (metabolite from caffeine) (Gracia-Lor et al., 2017). *Thiabendazole*, however, is a pesticide mainly used in fruit and vegetable agriculture due to its antifungal properties although it can also be used as a pharmaceutical against fungal infections (Chitescu et al., 2015; Fonseca et al., 2019). Contrarily, several compounds were only identified once: 10 pharmaceuticals (*clopidogrel, flumequine, iopromide, loratadine, metronidazole, mirtazapine, omeprazole, phenazone, sulfamethazine* and *trimethoprim*) and 7 pesticides (*bromacil, diflufenican, isoproturon, metolachlor, propyzamide, prosulfocarb,* and *terbumeton*). The number of identified chemicals by combination of IMS-QTOF MS and MS/MS QqQ methodologies highlight the importance of a carefully thought out strategy for wide scope screening campaigns. It is also remarkable that while prior studies reported PFASs in surface water bodies using similar extraction procedures (Coggan et al., 2019; Liu et al., 2020), none of them ($n = 10$) was found in any wetland sample herein studied (instrumental limits of detection in sub-ng L^{-1} level (Rostvall et al., 2018)).

From the sample perspective, the sample where most contaminants were identified by target analysis was #1 ‘Ebro’ river estuary (68 compounds) followed by #9 Albufera – ‘Golf de Pujol’ (54 compounds), #7 Albufera – ‘Portet Sollana’ (53 compounds) and #8 Albufera – ‘Tancat de la Pipa’ (49 compounds). This could be expected due to the fact that these locations are affected by urban wastewater effluents. The ‘Ebro’ river collects effluent wastewater from different big cities (e.g. Zaragoza ~ 666,000 inhabitants and Amposta ~ 20,000 inhabitants) and, Albufera Natural Park is affected by wastewater effluents and street run-off streams from Valencia (~790,000 inhabitants). Therefore, finding pharmaceuticals and metabolites in these locations is not surprising. Similarly, the presence of different pesticides due to nearby agricultural activities was predictable. Previous studies conducted in the Ebro river and/or Albufera Natural Park also found similar contamination pattern by pharmaceuticals and pesticides (Ccanccapa et al., 2016; Čelić et al., 2019; Sadutto et al., 2021). Contrarily, samples #5 ‘Prat de Cabanes’ and #6 ‘Marjal’ Pego-Oliva showed the lowest number of identified target chemicals, 13 and 12, respectively. The latter was also in agreement with previous studies (Andreu et al., 2016).

3.2.2. Suspect screening

A suspect screening of more than 900 substances was performed by means of UHPLC-IMS-QTOF MS. During a first data revision process, mass spectrometric features were required to show intensity above 2,000 counts for positive and negative ionization modes. Also, chromatographic peak width < 0.4 min, mass error from candidate structure below 3 ppm ((de)protonated molecule), and the presence of at least one fragment ion was required (Celma et al., 2020). Under these conditions, 77 candidate compounds were indicatively identified to be present in the samples. In a more exhaustive data revision, high collision energy spectra were inspected individually and compared to data reported in scientific literature and mass spectral databases such as MassBank of North America and MassBank Europe (MassBank Consortium; NORMAN network, 2021; MassBank of North America, 2019). In those cases where no previously reported data was available, mass fragmentation was evaluated using MassFragment tool within UNIFI (Yu et al., 2008) for the finding of diagnostic fragments supporting the identification. In order to improve the identification process, chromatographic RT and CCS values were predicted for the suspected structures and compared with the empirical measurements (Bade et al., 2015; Bijlsma et al.,

Table 2
Target compounds identified in water bodies from Spanish Mediterranean coastline. '✓' denotes confirmation at Level 1 by either UHPLC-IMS-HRMS or UHPLC-MS/MS QqQ '✓* (RT)', '✓* (MS frag.)' and '✓* (MS)' denote confirmation at Level 1* via UHPLC-IMS-HRMS followed by an indication of the deviation observed.

Compound	#1 Ebro River	#2 'Clot' Ebro	#3 Peñíscola Lagoon	#4 Peñíscola Channel	#5 'Prat de Cabanes'	#6 'Marjal' Pego-Oliva	#7 Albufera. Portet Sollana	#8 Albufera. 'Tancat Pipa'	#9 Albufera. 'Golf de Pujol'	#10 Almenara	#11 'Clot' Borriana
Compounds only monitored by UHPLC-IMS-HRMS											
2-hydroxy-atrazine	✓* (RT)[a] (MS frag.)[b]	✓* (RT)					✓* (RT) (MS frag.)		✓* (RT) (MS frag.)	✓	
2-hydroxy-terbuthylazine	✓* (RT)								✓* (RT)	✓	✓
4-Acetamidoantipyrin	✓	✓					✓	✓	✓	✓	✓
4-formylaminoantipyrine	✓	✓					✓	✓	✓		
Atrazine	✓									✓	✓* (MS frag.)
Azoxystrobin	✓	✓				✓* (MS)[c]	✓	✓	✓	✓* (MS frag.)	
Bentazone	✓	✓					✓	✓	✓	✓	
Benzophenone-3			✓	✓							
Benzoylecgonine	✓	✓	✓* (MS)					✓			
Bromacil											✓* (MS)
Carbendazim	✓	✓* (RT)					✓* (RT)				✓* (MS)
Chlorfenvinphos			✓* (MS)	✓* (MS frag.)							
Clopidogrel carboxylic acid	✓* (MS)						✓* (MS)				
Deethyl-atrazine	✓* (MS frag.)						✓* (MS)		✓	✓	✓
Deethyl-terbumeton							✓* (RT)		✓* (RT)	✓	✓* (RT)
Deisopropyl-atrazine										✓* (MS frag.)	✓* (MS frag.)
Desethyl terbuthylazine							✓		✓	✓	✓
Diflufenican											✓
Flumequine		✓									
Imazalil	✓* (RT)		✓	✓			✓* (RT)				✓
Iomeprol	✓							✓			
Isoproturon	✓* (MS frag.)										
Lincomycin	✓							✓* (RT)	✓		
Lorazepam	✓							✓* (MS)			
Losartan Carboxylic acid	✓* (MS frag.)						✓	✓* (MS)	✓* (MS frag.)		
MCPA							✓* (MS frag.)	✓* (MS)	✓* (MS frag.)		✓
Metalaxyl	✓						✓		✓	✓	
Metolachlor	✓										
Propamocarb	✓		✓	✓		✓			✓	✓	
Propiconazole	✓	✓	✓				✓* (MS frag.)	✓* (MS frag.)	✓* (MS frag.)		✓* (MS)
Propyzamide	✓										
Prosulfocarb	✓										
Simazine							✓		✓* (MS frag.)	✓	✓* (MS frag.)
Sulfamethazine	✓										
Tebuconazole	✓	✓			✓	✓* (MS)	✓	✓	✓	✓	✓
Terbumeton										✓	
Terbuthylazine	✓									✓	✓
Compounds only monitored by UHPLC-MS/MS QqQ											
10,11-Dihydro-10-hydroxycarbamazepine	✓										
17α-Estradiol		✓			✓						
Acesulfame	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Amidotrizoic acid	✓	✓					✓	✓	✓	✓	✓
Bisoprolol	✓						✓	✓	✓		
Caffeine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cetirizine	✓						✓	✓	✓		
Citalopram			✓	✓			✓	✓	✓		

(*continued on next page*)

Table 2 (*continued*)

Compound	#1 Ebro River	#2 'Clot' Ebro	#3 Peñíscola Lagoon	#4 Peñíscola Channel	#5 'Prat de Cabanes'	#6 'Marjal' Pego-Oliva	#7 Albufera. Portet Sollana	#8 Albufera. 'Tancat Pipa'	#9 Albufera. 'Golf de Pujol'	#10 Almenara	#11 'Clot' Borriana
DEET (N,N-diethyl-m-toluamide)	✓	✓			✓	✓	✓	✓	✓		
Diazepam							✓	✓	✓		
Diltiazem	✓							✓			
Fluconazole		✓					✓	✓	✓		
Hydrochlorothiazide	✓						✓	✓	✓	✓	✓
Loratadine	✓										
Memantine							✓	✓	✓		
Mirtazapine	✓										
Nicotinamide	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nicotine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Niflumic acid							✓	✓	✓		
Oxazepam	✓						✓	✓	✓		
Panthenol			✓	✓							
Theophylline	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Valsartan	✓	✓	✓	✓			✓	✓	✓	✓	✓

Compounds included in both UHPLC-IMS-HRMS and UHPLC-MS/MS QqQ analyses

Compound	#1 Ebro River		#2 'Clot' Ebro		#3 Peñíscola Lagoon		#4 Peñíscola Channel		#5 'Prat de Cabanes'		#6 'Marjal' Pego-Oliva		#7 Albufera. Portet Sollana		#8 Albufera. 'Tancat Pipa'		#9 Albufera. 'Golf de Pujol'		#10 Almenara		#11 'Clot' Borriana	
	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ	HRMS	QqQ
10,11-epoxy-carbamazepine		✓												✓		✓		✓		✓		
Amoxicillin		✓		✓										✓		✓		✓		✓		
Azithromycin		✓				✓		✓		✓				✓		✓		✓		✓		✓
Benzophenone-4	✓	✓											✓	✓	✓	✓	✓	✓				
Carbamazepine	✓	✓		✓		✓		✓						✓	✓	✓	✓* (MS)	✓		✓		✓
Clarithromycin		✓												✓		✓		✓		✓		✓
Clindamycin		✓		✓										✓		✓		✓				
Clopidogrel		✓																				
Cocaine			✓* (MS)				✓* (RT)															
Codeine		✓												✓		✓		✓				
Estrone		✓		✓						✓		✓		✓		✓		✓				✓
Iopromide	✓																					
Irbesartan		✓		✓									✓* (MS frag.)	✓	✓* (MS)	✓		✓		✓		✓
Lidocaine		✓		✓														✓				✓
Losartan	✓	✓											✓* (MS)	✓	✓* (MS)	✓		✓				
Metronidazole		✓																				
O-Desmethyl venlafaxine	✓	✓		✓									✓* (MS frag.)	✓		✓		✓	✓* (MS frag.)	✓		
Ofloxacin		✓				✓		✓						✓		✓		✓				✓
Omeprazole		✓																				
Phenazone															✓							
Primidone		✓		✓										✓		✓		✓		✓		✓
Salicylic acid		✓		✓		✓		✓		✓		✓		✓		✓		✓		✓		✓
Sulfamethoxazole	✓	✓		✓		✓		✓						✓		✓		✓		✓		✓
Telmisartan		✓		✓						✓				✓		✓		✓		✓		
Thiabendazole	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓	✓* (RT)	✓
Tramadol		✓												✓		✓		✓		✓		✓
Trimethoprim		✓																				
Venlafaxine	✓	✓											✓	✓		✓	✓	✓	✓	✓		✓

[a] ✓* (RT): Confirmation at Level 1 with a slight deviation in RT from standard.

[b] ✓* (MS frag.): Confirmation at Level 1 with a slight deviation in mass accuracy of fragment ions.

[c] ✓* (MS frag.): Confirmation at Level 1 with a slight deviation in mass accuracy in (de)protonated molecule.

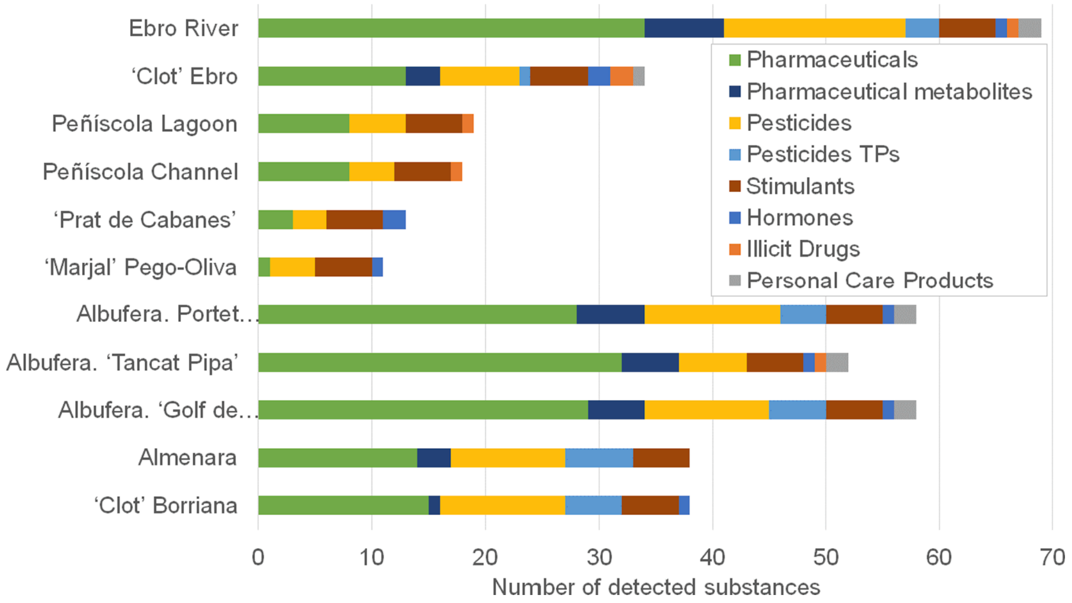

Fig. 1. Summary plot of the compound families detected in targeted screening of water bodies.

2017). Accuracy for predicted values was considered as satisfactory for RT (below ± 2 min) and for CCS (below ± 6% for protonated molecules).

At this point, the number of suspected substances was reduced to 27 candidate compounds. Among them, *17-α-estradiol*, *benzophenone*, *caffeine*, *citalopram*, *nicotine*, *niflumic acid*, *oxazepam* and *valsartan* (Table 2) could be afterwards confirmed by UHPLC-MS/MS QqQ analysis. The remaining 19 substances, tentatively identified at Level 2a or Level 3, are shown in Table 3 (7 pesticides and 2 TPs, 3 pharmaceuticals and 6 metabolites and 1 hormone metabolite). Reference standards could be purchased for 15 substances except for *ethynyl estradiol 3-β-D-glucuronide*, *3-[(4-Carboxy-4-methylpentyl)oxy]-4-methylbenzoic acid*, *nordiazepam* and *simvastatin acyl-β-D-glucuronide*. 3 compounds (*3,4-dichloroaniline, amisulpride and cotinine*) could be, then, confirmed by reference standards and 12 tentative identifications were discarded as also indicated in Table 3. Although the identity of the latter could not be explained in terms of the proposed structures, unidentified substances were detected over the different samples. Further studies could be conducted towards the identification of those particular features.

3.3. Toxicological fingerprint as a complementary tool of chemical analysis for a comprehensive water quality evaluation

Evaluation of water quality only by means of chemical analysis has limitations as only a fraction of all potentially toxic compounds is under the radar. Therefore, recent strategies have been proposed to combine both chemical and bioanalytical analyses to complement the results obtained (Brunner et al., 2020; Lundqvist et al., 2019; Neale et al., 2017). In this work, information gathered from chemical and biological analyses have been combined with the aim of linking observed toxicities with chemicals identified in samples (Celma et al., 2021b) with the aid of *in silico* prediction tools.

Briefly, toxicological evaluation of wetland samples consisted of 8 toxicity endpoints: aryl hydrocarbon receptor (AhR), androgen receptor (AR) activation and inhibition, estrogen receptor (ER) activation and inhibition, oxidative stress response and vitamin D receptor (VDR) activation and inhibition. For the easy access of the reader, bioanalytical results have been extracted from Celma et al. (Celma et al., 2021b) and reproduced in **Figure S1** of **SI**. In general, the vast majority of samples, except for #10 'L'Estany Almenara' and #11 'Clot Borriana', showed a clear activation of the AhR endpoint, mainly triggered by the presence of aromatic hydrocarbon chemicals. Therefore, the AhR activity observed for the majority of samples is most likely explained by the elevated number of organic compounds identified with aromatic hydrocarbon groups within their chemical structure. Particularly, Lundqvist *et al.* (Lundqvist et al., 2019) suggested that the presence in surface water samples of *azoxystrobin*, *carbendazim*, *propioconazole* and *terbuthylazine* (also observed in this study) could be correlated to the activation of AhR receptor, which could help to explain the observed toxicities. However, contributions for the overall AhR activity from undetected compounds could also occur. For the remaining toxicity endpoints evaluated, apart from sporadic detections, quantifiable estrogenic inhibition was observed for samples #8 Albufera – 'Tancat la Pipa' and #9 Albufera – 'Golf de Pujol' which are potentially affected by wastewater and street run-off water streams from Valencia (Spain).

The only compound exclusively detected in samples #8 and #9 was *phenazone*. Potential for inhibition of estrogenic receptor was modelled through the ToxCast Pathway Model within CompTox chemicals dashboard (https://comptox.epa.gov/dashboard) as described by Browne *et al.* (Browne et al., 2015). However, *phenazone* was found to be inactive for the antagonistic ER and, therefore, the inhibition of such toxicity endpoint cannot be explained in terms of this unique compound. Consequently, individual antagonistic estrogenicity for the whole set of chemicals identified in the samples were predicted (either active or inactive) by means of the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) (Mansouri et al., 2016) integrated within OChem website (Online Chemical Database with modelling environment, www.ochem.eu, model 285) (Sushko et al., 2011). In total, 16 substances were found to be estrogenic inhibitors. Several of these compounds were found in samples #8 and/or #9 (*azithromycin, benzophenone-4, bisoprolol, cetirizine, citalopram, clarithromycin, estrone, gemfibrozil M3 (3-[(4-Carboxy-4-methylpentyl)oxy]-4-methylbenzoic acid), o-desmethylvenlafaxine, simvastatin acyl-β-D-glucuronide, tramadol* and *venlafaxine*). Therefore, the observed activity in those samples could be partially explained by the presence of these compounds and also due to a potential synergic mixed effect to enhance the antagonistic estrogenicity of the sample. Nonetheless, thousands of sample components still remain unidentified and might be playing an important role for the toxicity levels observed in samples #8 and #9. Besides, some of these estrogen receptor antagonistic compounds were also detected in non-active samples. Yet, the detected signals were in general lower than that in active samples and, therefore, not concentrated enough to trigger

Table 3

Suspect compounds identified at Level 2a or Level 3 based on different evidences gathered (diagnostic fragments -Diag. frag-, literature review, or data available at Mass Bank of North America -MoNA- or Mass Bank Europe –MBEU-).

Compound	#1 Ebro River	#2 'Clot' Ebro	#3 Peñíscola Lagoon	#4 Peñíscola Channel	#5 'Prat de Cabanes'	#6 'Marjal' Pego-Oliva	#7 Albufera. Portet Sollana	#8 Albufera. 'Tancat Pipa'	#9 Albufera. 'Golf de Pujol'	#10 Almenara	#11 'Clot' Borriana	Evidence	Identity confirmed by reference standard?
3,4-dichloroaniline								Level2a				Literature	Yes, at Level 1
8-hydroxyquinoline		Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	Level 2a	MoNA; MBEU	No
Amisulpride	Level 2a						Level 2a	Level 2a	Level 2a	Level 2a		Literature	Yes, at Level 1
Chlorotoluron	Level 2a											Mass Bank Europe	No
Cotinine	Level 2a	Level 2a					Level 2a	Level 2a	Level 2a		Level 2a	Literature	Yes, at Level 1
Eth-Estr. 3-β-D-Gl.[a]											Level 3	Diag. frag.	*n.a.*
Gemfibrozil Metab.[b]	Level 3							Level 3			Level 3	Diag. frag.	*n.a.*
Metolachlor ESA[c]	Level 2a	Level 2a										Literature	No
Metoxuron	Level 2a											Diag. frag.; Literat.	No
Monuron	Level 2a											Diag. frag.; Literat.	No
Nordiazepam							Level 2a	Level 2a				Literature	*n.a.*
Pirbuterol											Level 3	Diag. frag.	No
Pravastatin Lactone		Level 3			Level 3							Diag. frag.	No
Prometon							Level 2a			Level 2a		MoNA; MBEU	No
Pyrimethanil							Level 2a		Level 2a			Literature	*n.a.*
Simvas. Acyl-β-D-Gl.[d]							Level 3		Level 3			Diag. frag.	*n.a.*
Sitagliptin								Level 2a				Literature	No
Trietazine										Level 3		Diag. frag.	No
Vildagliptin	Level 2a						Level 2a	Level 2a	Level 2a			Literature	No

[a] Eth-Estr. 3-β-D-Gl.: Ethynyl Estradiol 3-β-D-Glucuronide.

[b] Gemfibrozil Metab.: 3-[(4-Carboxy-4-methylpentyl)oxy]-4-methylbenzoic acid.

[c] Metolachlor ESA: Metolachlor ethanesulfonic acid.

[d] Simvas. Acyl-β-D-Gl.: Simvastatin Acyl-β-D-glucuronide.

estrogenic antagonism. Similarly, there were also other predicted antagonistic compounds that were not identified in samples #8 and #9 but in other samples (*17α-estradiol, benzophenone-3, diflufenican and ethynyl estradiol 3-β-D-glucuronide*). Although these compounds were found in non-active samples, their concentration might be not high enough to be detected by the bioanalytical tools applied.

An exhaustive, meaningful and complete chemical explanation of the toxicity figures observed would have required the knowledge of the identity of all compounds present in the sample that can lead to those particular effects. However, this is nearly unachievable due to different implicit limitations of chemical analysis and bioanalytical tools. The chemical space covered in screening strategies is mainly limited by the sample extraction procedure as well as the separation technique selected for analysis. In this study, extracts for chemical and biological analysis were obtained identically to minimize the impact of sample treatment when it comes to comparing chemical and biological analyses. However, the space covered during chemical analysis was limited to LC-amenable compounds analyzed under reverse phase LC and, therefore, some toxicity drivers in the analyzed wetland samples can still remain undiscovered. It is expected that the utilization of additional separation techniques (e.g. gas chromatography or hydrophilic interaction chromatography) as well as the inclusion of larger target and suspect screening databases would yield a higher rate of identified compounds. Therefore, more insight about the actual composition of wetland samples can be gathered and, thus, might be helpful to better explain the observed toxicities. Nonetheless, it is not realistic in terms of resources and time-efforts to seek for the identification of the whole universe of chemicals that can be present in an environmental sample.

Although the number of samples studied in this work is limited, which prevent a comprehensive evaluation, the data herein presented highlight the advantage of applying combined strategies to give a more comprehensive insight in the quality of water. The results provide a snapshot and permit the prioritization of contamination hotspots to be included in more extensive monitoring campaigns in the future. Furthermore, different chemicals were found potentially responsible for the estrogenic toxicities observed although it is noteworthy that unidentified compounds can still be playing an important role. Although the sole presence of the identified chemicals might not arise any concern during chemical analysis, the integration of biological results into the water quality monitoring strategy remarks how combined effects of organic micropollutants might threaten the ecosystem.

4. Conclusions

A comprehensive target and suspect screening of organic micropollutants has been conducted by the combined application of UHPLC-IMS-QTOF MS and UHPLC-QqQ screening. Different benefits from each technique have been gathered *i.e.* cleaner mass spectral and the additional identification parameter provided by IMS-HRMS as well as the increased sensitivity obtained by means of UHPLC-LRMS. As a result, 91 different organic micropollutants were confirmed at the most confident level of identification (Level 1), and 5 were tentatively identified at Levels 2a or 3 in the set of wetland samples studied. Several compounds could only be found by UHPLC-QqQ, due to its higher sensitivity, illustrating the advantage of using QqQ for detection and identification of target compounds when they are present at very low concentrations. Pharmaceuticals and pesticides were the most prevalent groups of chemicals detected. Additionally, a complete picture of the quality of the water bodies has been evaluated by putting into context chemical analysis and bioanalytical quality assessment. 12 organic micropollutants with predicted antagonistic estrogenic activities have been found in active samples. Yet, other still unidentified chemicals can be contributing to the overall activity of the sample. Also, the elevated number of compounds identified with aromatic hydrocarbon groups could likely be an explanation for the AhR activities observed. However, the limited chemical space covered by chemical analysis still stands in the way for a complete and comprehensive chemical explanation of measured biological activities. Although identifying an organic micropollutant in a water sample might not be of concern, the integration of biological results into the water quality monitoring strategy highlighted how mixed effects might really threaten the ecosystem quality. That remarks the importance of combining chemical analysis with bioanalytical tools to provide an improved overview of the actual status of water quality in natural reservoirs.

Environmental Implication

This study presents an evaluation of water quality in several waterbodies in the Spanish Mediterranean coastline, an area which is highly vulnerable to the influence of anthropogenic contaminants. Therefore, we aimed to implement a holistic approach by combining state-of-the-art analytical techniques with toxicological analysis and *in-silico* prediction tools. Assessed waterbodies were investigated for 8 toxicity endpoints and screened for greater than 1,500 organic micropollutants. The presented work does not only show results in specific waterbodies but remarks the importance of combining chemical analysis with bioanalytical tools to provide a comprehensive overview of the actual status of water quality in natural reservoirs.

CRediT authorship contribution statement

Alberto Celma: Investigation, Data curation, Visualization, Writing – original draft. **Pablo Gago-Ferrero:** Resources, Funding acquisition, Methodology, Writing – review & editing. **Oksana Golovko:** Investigation, Data curation, Writing – review & editing. **Félix Hernández:** Resources, Funding acquisition, Writing – review & editing. **Foon Yin Lai:** Investigation, Visualization, Writing – review & editing. **Johan Lundqvist:** Funding acquisition, Project administration, Conceptualization, Writing – review & editing. **Frank Menger:** Investigation, Visualization, Writing – review & editing. **Juan V. Sancho:** Supervision, Conceptualization. **Karin Wiberg:** Resources, Investigation, Writing – review & editing. **Lutz Ahrens:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Lubertus Bijlsma:** Conceptualization, Funding acquisition, Supervision, Visualization, 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.

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Appendix A. Supplementary data

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References

Abily, M., Vicenç, A., Gernjak, W., Rodríguez-Roda, I., Poch, M., Corominas, L., 2021. Climate change impact on EU rivers' dilution capacity and ecological status. Water Res. 199, 117166 https://doi.org/10.1016/j.watres.2021.117166.

Airoldi, L., Beck, M., 2007. Loss, Status and Trends for Coastal Marine Habitats of Europe, in: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.), Journal of Oceanography and Marine Biology. pp. 345–405. 10.1201/9781420050943.ch7.

Andreu, V., Gimeno-García, E., Pascual, J.A., Vazquez-Roig, P., Picó, Y., 2016. Presence of pharmaceuticals and heavy metals in the waters of a Mediterranean coastal wetland: Potential interactions and the influence of the environment. Sci. Total Environ. 540, 278–286. https://doi.org/10.1016/j.scitotenv.2015.08.007.

Bade, R., Bijlsma, L., Miller, T.H., Barron, L.P., Sancho, J.V., Hernández, F., 2015. Suspect screening of large numbers of emerging contaminants in environmental waters using artificial neural networks for chromatographic retention time prediction and high resolution mass spectrometry data analysis. Sci. Total Environ. 538, 934–941. https://doi.org/10.1016/j.scitotenv.2015.08.078.

Barbieri, M.V., Peris, A., Postigo, C., Moya-Garcés, A., Monllor-Alcaraz, L.S., Rambla-Alegre, M., Eljarrat, E., López de Alda, M., 2021. Evaluation of the occurrence and fate of pesticides in a typical Mediterranean delta ecosystem (Ebro River Delta) and risk assessment for aquatic organisms. Environ. Pollut. 274, 115813 https://doi.org/10.1016/j.envpol.2020.115813.

Bijlsma, L., Bade, R., Celma, A., Mullin, L., Cleland, G., Stead, S., Hernandez, F., Sancho, J.V., 2017. Prediction of Collision Cross-Section Values for Small Molecules: Application to Pesticide Residue Analysis. Anal. Chem. 89, 6583–6589. https://doi.org/10.1021/acs.analchem.7b00741.

Borrull, J., Colom, A., Fabregas, J., Borrull, F., Pocurull, E., 2021. Presence, behaviour and removal of selected organic micropollutants through drinking water treatment. Chemosphere 276, 130023. https://doi.org/10.1016/j.chemosphere.2021.130023.

Browne, P., Judson, R.S., Casey, W.M., Kleinstreuer, N.C., Thomas, R.S., 2015. Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. Environ. Sci. Technol. 49, 8804–8814. https://doi.org/10.1021/acs.est.5b02641.

Brunner, A.M., Bertelkamp, C., Dingemans, M.M.L., Kolkman, A., Wols, B., Harmsen, D., Siegers, W., Martijn, B.J., Oorthuizen, W.A., ter Laak, T.L., 2020. Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment. Sci. Total Environ. 705, 135779 https://doi.org/10.1016/j.scitotenv.2019.135779.

Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: Occurrence and risk assessment. Environ. Pollut. 211, 414–424. https://doi.org/10.1016/j.envpol.2015.12.059.

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci. Total Environ. 652, 952–963. https://doi.org/10.1016/j.scitotenv.2018.10.290.

Celma, A., Ahrens, L., Gago-Ferrero, P., Hernández, F., López, F., Lundqvist, J., Pitarch, E., Sancho, J.V., Wiberg, K., Bijlsma, L., 2021a. The relevant role of ion mobility separation in LC-HRMS based screening strategies for contaminants of emerging concern in the aquatic environment. Chemosphere 280, 130799. https://doi.org/10.1016/j.chemosphere.2021.130799.

Celma, A., Bijlsma, L., López, F.J., Sancho, J.V., 2018. Development of a Retention Time Interpolation scale (RTi) for liquid chromatography coupled to mass spectrometry in both positive and negative ionization modes. J. Chromatogr. A 1568, 101–107. https://doi.org/10.1016/j.chroma.2018.07.030.

Celma, A., Fabregat-Safont, D., Ibáñez, M., Bijlsma, L., Hernández, F., Sancho, J. V., 2019. S61 | UJICCSLIB | Collision Cross Section (CCS) Library from UJI (Version NORMAN-SLE S61.0.1.2) . Zenodo. 10.5281/zenodo.3966751.

Celma, A., Mandava, G., Oskarsson, A., Sancho, J.V., Bijlsma, L., Lundqvist, J., 2021b. In vitro bioanalytical assessment of toxicity of wetland samples from Spanish Mediterranean coastline. Environ. Sci. Eur. 33, 70. https://doi.org/10.1186/s12302-021-00510-1.

Celma, A., Sancho, J.V., Schymanski, E.L., Fabregat-Safont, D., Ibáñez, M., Goshawk, J., Barknowitz, G., Hernández, F., Bijlsma, L., 2020. Improving Target and Suspect Screening High-Resolution Mass Spectrometry Workflows in Environmental Analysis by Ion Mobility Separation. Environ. Sci. Technol. 54, 15120–15131. https://doi.org/10.1021/acs.est.0c05713.

Chitescu, C.L., Kaklamanos, G., Nicolau, A.I., Stolker, A.A.M.L., 2015. High sensitive multiresidue analysis of pharmaceuticals and antifungals in surface water using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory. Sci. Total Environ. 532, 501–511. https://doi.org/10.1016/j.scitotenv.2015.06.010.

Coggan, T.L., Anumol, T., Pyke, J., Shimeta, J., Clarke, B.O., 2019. A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices. Anal. Bioanal. Chem. 411, 3507–3520. https://doi.org/10.1007/s00216-019-01829-8.

Diamantini, E., Lutz, S.R., Mallucci, S., Majone, B., Merz, R., Bellin, A., 2018. Driver detection of water quality trends in three large European river basins. Sci. Total Environ. 612, 49–62. https://doi.org/10.1016/j.scitotenv.2017.08.172.

Döll, P., Zhang, J., 2010. Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. Hydrol. Earth Syst. Sci. 14, 783–799. https://doi.org/10.5194/hess-14-783-2010.

European Commission. Directorate General for Health and Food Safety., 2019. Guidance document on Analytical Quality Control and Method Validation for Pesticide Residues Analysis in Food and Feed (SANTE/12682/2019).

Fabregat-Safont, D., Ibáñez, M., Bijlsma, L., Hernández, F., Waichman, A.V., de Oliveira, R., Rico, A., 2021. Wide-scope screening of pharmaceuticals, illicit drugs and their metabolites in the Amazon River. Water Res. 200, 117251 https://doi.org/10.1016/j.watres.2021.117251.

Fonseca, E., Hernández, F., Ibáñez, M., Rico, A., Pitarch, E., Bijlsma, L., 2020. Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. Environ. Int. 144, 106004 https://doi.org/10.1016/j.envint.2020.106004.

Fonseca, E., Renau-Pruñonosa, A., Ibáñez, M., Gracia-Lor, E., Estrela, T., Jiménez, S., Pérez-Martín, M.Á., González, F., Hernández, F., Morell, I., 2019. Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environ. Res. 177, 108570 https://doi.org/10.1016/j.envres.2019.108570.

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H. P., Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of >2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J. Hazard. Mater. 387, 121712 https://doi.org/10.1016/j.jhazmat.2019.121712.

García-Ruiz, J.M., López-Moreno, J.I., Vicente-Serrano, S.M., Lasanta–Martínez, T., Beguería, S., 2011. Mediterranean water resources in a global change scenario. Earth-Science Rev. 105, 121–139. https://doi.org/10.1016/j.earscirev.2011.01.006.

Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122 https://doi.org/10.1016/j.scitotenv.2020.142122.

Gracia-Lor, E., Rousis, N.I., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., Causanilles, A., Hernández, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.K., van Nuijs, A.L.N., Plósz, B.G., Ramin, P., Ryu, Y., Santos, M.M., Thomas, K., de Voogt, P., Yang, Z., Castiglioni, S., 2017. Estimation of caffeine intake from analysis of caffeine metabolites in wastewater. Sci. Total Environ. 609, 1582–1588. https://doi.org/10.1016/j.scitotenv.2017.07.258.

Guiot, J., Cramer, W., 2016. Climate change: The 2015 Paris Agreement thresholds and Mediterranean basin ecosystems. Science (80-.). 354, 465–468. 10.1126/science.aah5015.

Hashmi, M.A.K., Escher, B.I., Krauss, M., Teodorovic, I., Brack, W., 2018. Effect-directed analysis (EDA) of Danube River water sample receiving untreated municipal wastewater from Novi Sad. Serbia. Sci. Total Environ. 624, 1072–1081. https://doi.org/10.1016/j.scitotenv.2017.12.187.

Hernández, F., Ibáñez, M., Portolés, T., Cervera, M.I., Sancho, J.V., López, F.J., 2015. Advancing towards universal screening for organic pollutants in waters. J. Hazard. Mater. 282, 86–95. https://doi.org/10.1016/j.jhazmat.2014.08.006.

Hinnenkamp, V., Balsaa, P., Schmidt, T.C., 2019. Quantitative screening and prioritization based on UPLC-IM-Q-TOF-MS as an alternative water sample monitoring strategy. Anal. Bioanal. Chem. 411, 6101–6110. https://doi.org/10.1007/s00216-019-01994-w.

Hollender, J., Schymanski, E.L., Singer, H.P., Ferguson, P.L., 2017. Nontarget Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go? Environ. Sci. Technol. 51, 11505–11512. https://doi.org/10.1021/acs.est.7b02184.

Jakimska, A., Śliwka-Kaszyńska, M., Reszczyńska, J., Namieśnik, J., Kot-Wasik, A., 2014. Elucidation of transformation pathway of ketoprofen, ibuprofen, and furosemide in surface water and their occurrence in the aqueous environment using UHPLC-QTOF-MS Euroanalysis XVII. Anal. Bioanal. Chem. 406, 3667–3680. https://doi.org/10.1007/s00216-014-7614-1.

Jonkers, T.J.H., Steenhuis, M., Schalkwijk, L., Luirink, J., Bald, D., Houtman, C.J., Kool, J., Lamoree, M.H., Hamers, T., 2020. Development of a high-throughput bioassay for screening of antibiotics in aquatic environmental samples. Sci. Total Environ. 729, 139028 https://doi.org/10.1016/j.scitotenv.2020.139028.

Klausmeyer, K.R., Shaw, M.R., 2009. Climate Change, Habitat Loss, Protected Areas and the Climate Adaptation Potential of Species in Mediterranean Ecosystems Worldwide. PLoS ONE 4, e6392. https://doi.org/10.1371/journal.pone.0006392.

Krauss, M., Singer, H., Hollender, J., 2010. LC-high resolution MS in environmental analysis: From target screening to the identification of unknowns. Anal. Bioanal. Chem. 397, 943–951. https://doi.org/10.1007/s00216-010-3608-9.

Liu, S., Junaid, M., Zhong, W., Zhu, Y., Xu, N., 2020. A sensitive method for simultaneous determination of 12 classes of per- and polyfluoroalkyl substances (PFASs) in groundwater by ultrahigh performance liquid chromatography coupled with quadrupole orbitrap high resolution mass spectrometry. Chemosphere 251, 126327. https://doi.org/10.1016/j.chemosphere.2020.126327.

Lundqvist, J., von Brömssen, C., Rosenmai, A.K., Ohlsson, Å., Le Godec, T., Jonsson, O., Kreuger, J., Oskarsson, A., 2019. Assessment of pesticides in surface water samples from Swedish agricultural areas by integrated bioanalysis and chemical analysis. Environ. Sci. Eur. 31, 53. https://doi.org/10.1186/s12302-019-0241-x.

Mansouri, K., Abdelaziz, A., Rybacka, A., Roncaglioni, A., Tropsha, A., Varnek, A., Zakharov, A., Worth, A., Richard, A.M., Grulke, C.M., Trisciuzzi, D., Fourches, D., Horvath, D., Benfenati, E., Muratov, E., Wedebye, E.B., Grisoni, F., Mangiatordi, G. F., Incisivo, G.M., Hong, H., Ng, H.W., Tetko, I.V., Balabin, I., Kancherla, J., Shen, J., Burton, J., Nicklaus, M., Cassotti, M., Nikolov, N.G., Nicolotti, O., Andersson, P.L., Zang, Q., Politi, R., Beger, R.D., Todeschini, R., Huang, R., Farag, S., Rosenberg, S.A., Slavov, S., Hu, X., Judson, R.S., 2016. CERAPP: Collaborative Estrogen Receptor Activity Prediction Project. Environ. Health Perspect. 124, 1023–1033. https://doi.org/10.1289/ehp.1510267.

Martín, M., Hernández-Crespo, C., Andrés-Doménech, I., Benedito-Durá, V., 2020. Fifty years of eutrophication in the Albufera lake (Valencia, Spain): Causes, evolution and remediation strategies. Ecol. Eng. 155, 105932 https://doi.org/10.1016/j.ecoleng.2020.105932.

MassBank Consortium, NORMAN network, 2021. MassBank Europe (release version 2021.03) [WWW Document]. URL https://massbank.eu/MassBank/ (accessed 4.21.21).

MassBank of North America, 2019. MassBank of North America (MoNA) - Tricyclazole [WWW Document]. URL https://mona.fiehnlab.ucdavis.edu/spectra/display/CCMSLIB00001058767 (accessed 10.18.19).

Menger, F., Gago-Ferrero, P., Wiberg, K., Ahrens, L., 2020. Wide-scope screening of polar contaminants of concern in water: A critical review of liquid chromatography-high resolution mass spectrometry-based strategies. Trends Environ. Anal. Chem. 28, e00102 https://doi.org/10.1016/j.teac.2020.e00102.

Moschet, C., Lew, B.M., Hasenbein, S., Anumol, T., Young, T.M., 2017. LC- and GC-QTOF-MS as Complementary Tools for a Comprehensive Micropollutant Analysis in Aquatic Systems. Environ. Sci. Technol. 51, 1553–1561. https://doi.org/10.1021/acs.est.6b05352.

Neale, P.A., Munz, N.A., Aït-Aïssa, S., Altenburger, R., Brion, F., Busch, W., Escher, B.I., Hilscherová, K., Kienle, C., Novák, J., Seiler, T.-B., Shao, Y., Stamm, C., Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. Sci. Total Environ. 576, 785–795. https://doi.org/10.1016/j.scitotenv.2016.10.141.

NORMAN network, 2021. NORMAN Suspect List Exchange [WWW Document]. NORMAN Suspect List Exch. URL https://www.norman-network.com/nds/SLE/ (accessed 5.13.21).

Oskarsson, A., Rosenmai, A.K., Mandava, G., Johannisson, A., Holmes, A., Tröger, R., Lundqvist, J., 2021. Assessment of source and treated water quality in seven drinking water treatment plants by in vitro bioassays – Oxidative stress and antiandrogenic effects after artificial infiltration. Sci. Total Environ. 758, 144001 https://doi.org/10.1016/j.scitotenv.2020.144001.

Perennou, C., Beltrame, C., Guelmami, A., Tomas-Vives, P., Caessteker, P., 2012. Existing areas and past changes of wetland extent in the Mediterranean region: an overview. ecologia mediterranea.

Pitarch, E., Cervera, M.I., Portolés, T., Ibáñez, M., Barreda, M., Renau-Pruñonosa, A., Morell, I., López, F., Albarrán, F., Hernández, F., 2016. Comprehensive monitoring of organic micro-pollutants in surface and groundwater in the surrounding of a solid-waste treatment plant of Castellón. Spain. Sci. Total Environ. 548–549, 211–220. https://doi.org/10.1016/j.scitotenv.2015.12.166.

Ramsar Convention on Wetlands, 2021. The List of Wetlands of International Importance.

Rostvall, A., Zhang, W., Dürig, W., Renman, G., Wiberg, K., Ahrens, L., Gago-Ferrero, P., 2018. Removal of pharmaceuticals, perfluoroalkyl substances and other micropollutants from wastewater using lignite, Xylit, sand, granular activated carbon (GAC) and GAC+Polonite® in column tests – Role of physicochemical properties. Water Res. 137, 97–106. https://doi.org/10.1016/j.watres.2018.03.008.

Sadutto, D., Andreu, V., Ilo, T., Akkanen, J., Picó, Y., 2021. Pharmaceuticals and personal care products in a Mediterranean coastal wetland: Impact of anthropogenic and spatial factors and environmental risk assessment. Environ. Pollut. 271 https://doi.org/10.1016/j.envpol.2020.116353.

Sushko, I., Novotarskyi, S., Körner, R., Pandey, A.K., Rupp, M., Teetz, W., Brandmaier, S., Abdelaziz, A., Prokopenko, V.V., Tanchuk, V.Y., Todeschini, R., Varnek, A., Marcou, G., Ertl, P., Potemkin, V., Grishina, M., Gasteiger, J., Schwab, C., Baskin, I.I., Palyulin, V.A., Radchenko, E.V., Welsh, W.J., Kholodovych, V., Chekmarev, D., Cherkasov, A., Aires-de-Sousa, J., Zhang, Q.-Y., Bender, A., Nigsch, F., Patiny, L., Williams, A., Tkachenko, V., Tetko, I.V., 2011. Online chemical modeling environment (OCHEM): web platform for data storage, model development and publishing of chemical information. J. Comput. Aided. Mol. Des. 25, 533–554. https://doi.org/10.1007/s10822-011-9440-2.

Tousova, Z., Oswald, P., Slobodnik, J., Blaha, L., Muz, M., Hu, M., Brack, W., Krauss, M., Di Paolo, C., Tarcai, Z., Seiler, T.-B., Hollert, H., Koprivica, S., Ahel, M., Schollée, J. E., Hollender, J., Suter, M.J.F., Hidasi, A.O., Schirmer, K., Sonavane, M., Ait-Aissa, S., Creusot, N., Brion, F., Froment, J., Almeida, A.C., Thomas, K., Tollefsen, K. E., Tufi, S., Ouyang, X., Leonards, P., Lamoree, M., Torrens, V.O., Kolkman, A., Schriks, M., Spirhanzlova, P., Tindall, A., Schulze, T., 2017. European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters. Sci. Total Environ. 601–602, 1849–1868. https://doi.org/10.1016/j.scitotenv.2017.06.032.

Yu, K., Castro-perez, J., Shockcor, J., 2008. MassFragment for structural elucidation in metabolite ID using exact mass MS. Waters white Pap.