Rodrigo Álvarez Ruiz

Emerging pollutants in the aquatic environment: Assessment in biota and influence of microplastics

Tesis Doctoral Internacional

Programa de doctorado 3108 en Contaminación, Toxicología y Sanidad Ambientales

Contaminantes emergentes en el medio acuático: Evaluación en biota e influencia de microplásticos

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Emerging pollutants in the aquatic environment: Assessment in biota and influence of microplastics

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Contaminants emergents en el medi aquàtic: Avaluació en biota i influència de microplàstics

Memoria presentada para optar al título de Doctor por
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I N F O R M A N:

Que el graduado Rodrigo Álvarez-Ruiz ha estado trabajando bajo nuestra dirección durante más de cuatro años en la elaboración de la tesis doctoral que lleva por título **"Contaminantes emergentes en el medio acuático: Evaluación en biota e influencia de microplásticos"** por lo que autorizamos su presentación para optar al grado de Doctor con Mención internacional.

Moncada, 17 de junio de 2021

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I N F O R M A N:

Que la tesis doctoral con mención internacional que lleva por título **"Contaminantes emergentes en el medio acuático: Evaluación en biota e influencia de microplásticos"** se presenta como un compendio de ocho publicaciones indexadas en el JCR o Scopus:

- Picó Y, **Álvarez-Ruiz R**, Wijaya L, Alfarhan A, Alyemeni M, Barceló D. (2018). **Analysis of ibuprofen and its main metabolites in roots, shoots, and seeds of cowpea (Vigna unguiculata L. Walp) using liquid chromatography-quadrupole time-of-flight mass spectrometry: uptake, metabolism, and translocation**. *Analytical and Bioanalytical Chemistry* 410, 1163-1176. [JCR (WOS) IF: 3.286 en el área de Química Analítica 18/84 Q1])
- **Álvarez-Ruiz R**, Pico Y. (2019). **Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography-quadrupole time-of-flight-mass spectrometry**. *Journal of Chromatography A 1595,* 81-90 (JCR (WOS) IF: 4.049, en el área de Química Analítica 14/86 Q1])
- Picó Y, **Álvarez-Ruiz R**, Alfarhan A. H, El-Sheikh M. A, Alshahrani H.O, Barceló D. (2020). **Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes**. *Science of The Total Environment*, 701, 135021 [JCR (WOS) IF: 6.551 en el área de Ciencias Medioambientales 22/265 Q1 (primer decil)]
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- **Álvarez-Ruiz R**, Picó Y, Sadutto D, Campo J. (2021). **Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants**. *Analytical and Bioanalytical Chemistry* (*In press*) [JRC (WOS) IF: 3.637 (2019) en el área de Química Analítica 23/119 Q1]

El doctorando es el primer autor de 5 de ellos, el segundo de 2 de los trabajos presentados [dado que eran parte de un proyecto desarrollado por uno de sus supervisores (Y. Picó) quien firma en primer lugar] y el último de 1 de ellos, ya que realizó las labores de diseño y supervisión. En todos ellos, Rodrigo Álvarez-Ruiz ha realizado la mayor parte o todo el trabajo experimental, vigilando y supervisando estrechamente los experimentos, así como ha procedido al análisis de los resultados y la elaboración de los manuscritos en directa colaboración con nosotros.

Moncada, 17 de junio de 2021

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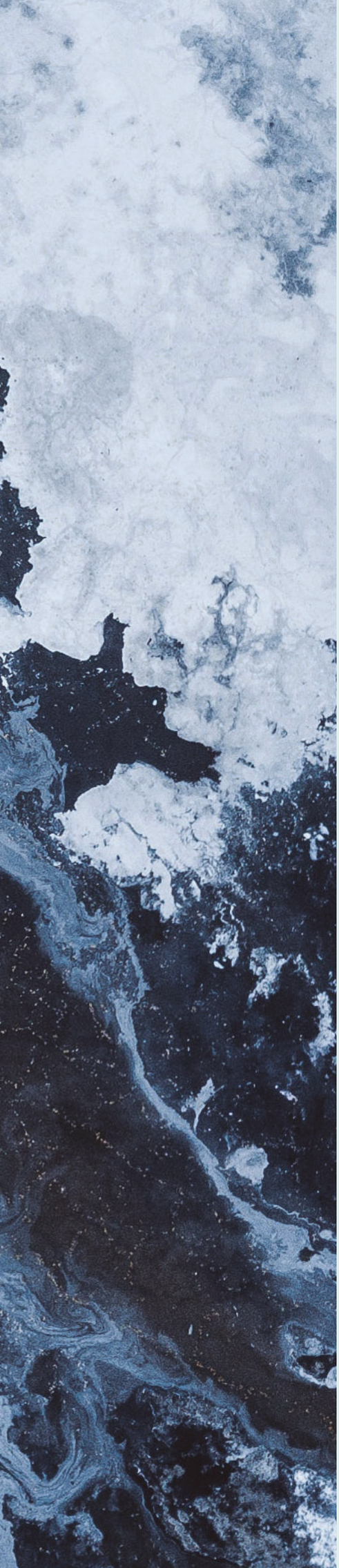
ÍNDICE



PRESENTACIÓN
DE LA MEMORIA

1.

Objetivos y estructura

Objetivos y estructura

El medio ambiente sufre la descarga continua de contaminantes de origen antropogénico. Entre estos se incluyen contaminantes orgánicos tales como fármacos y productos del cuidado personal (PPCPs), plaguicidas, sustancias perfluoroalquiladas (PFASs) o drogas de abuso, y microplásticos. Además, muchos de estos contaminantes están catalogados como contaminantes emergentes o de preocupación emergente [1]. En estas categorías se engloban tanto los contaminantes que han sido descubiertos recientemente y que, por lo tanto, llevan poco tiempo siendo estudiados, como aquellos que, aun habiendo sido ampliamente estudiados, alguno de sus efectos y/o características han sido descubiertos recientemente, reabriendo su estudio y debate.

Una de las principales fuentes de contaminantes al medio ambiente son las estaciones depuradoras de aguas residuales (EDARs), también denominadas por sus siglas en inglés WWTPs (Wastewater treatment plants) [2, 3]. Además, existen otras fuentes de carácter más difuso como la infiltración o las aguas de escorrentía, que pueden transportar plaguicidas y otros contaminantes emergentes empleados en los campos de cultivo [4]. Todos estos vertidos suelen tener un destino en común, el medio acuático, donde numerosos estudios indican una importante presencia de contaminantes antropogénicos [5-7]. Los medios acuáticos son de una gran importancia medioambiental, fuertemente ligados a la riqueza y biodiversidad y en los que se apoyan numerosos ecosistemas. Sin embargo, una de las grandes problemáticas con respecto a la relación entre la contaminación y el agua, es que esta proporciona un medio de rápida dispersión para los contaminantes, pudiendo poner en riesgo la fauna, la flora o el sustrato, entre otros. Por eso el estudio de la contaminación en los ecosistemas acuáticos y como se relacionan estos con otros compartimentos ambientales es de vital importancia.

Uno de los hechos a destacar sobre los estudios de contaminantes antropogénicos en el medio ambiente es que, aunque su presencia y mecanismos en las matrices abióticas (suelo, agua, sedimento) han sido ampliamente investigados [5, 6, 8], los estudios centrados en matrices bióticas (fauna y flora) son mucho más escasos [9, 10]. Por un lado, la presencia de compuestos como lípidos, proteínas o pigmentos dotan a las matrices bióticas de una gran complejidad, lo que supone un desafío a la hora de identificar y determinar correctamente contaminantes orgánicos. Y, por otro lado, los mecanismos relacionados con la exposición a contaminantes tales como, rutas metabólicas, distribución,

biomarcadores o acumulación y depuración de contaminantes son muy complejos y su estudio requiere de la inversión de recursos materiales y tiempo. No obstante, su investigación es crucial para poder evaluar y enmarcar los riesgos de su exposición a dichos contaminantes, así como desarrollar medidas efectivas de remediación y conservación. El estudio de especies estrechamente relacionadas con el agua, tales como las plantas o la biota acuática es especialmente importante. Ya que, como hemos visto anteriormente, el medio acuático es uno de los más afectados por el vertido de contaminantes antropogénicos. Lo que se traduce en que estas especies puedan sufrir una mayor exposición a los mismos.

Por todo lo descrito anteriormente, el **objetivo general** de la presente tesis es la evaluación integral del riesgo ambiental que sufren los medios acuáticos debido a las emisiones de contaminantes orgánicos. Incluyendo la identificación y evaluación de fuentes de emisión, el destino ambiental de los contaminantes, las posibles sinergias entre ellos y como se ven afectadas la fauna acuática y la flora.

Objetivos específicos

1. Desarrollo de técnicas de análisis de cromatografía líquida de alta eficacia acoplada a espectrometría de masas en tándem (HPLC-MS/MS), haciendo uso del triple cuadrupolo (QqQ), para la determinación y cuantificación simultanea de contaminantes orgánicos pertenecientes a diferentes familias.

2. Valoración de las posibles fuentes de emisión de contaminantes orgánicos al medio ambiente y especialmente al medio acuático. Tales como los efluentes de EDARs o el agua de escorrentía proveniente de campos de cultivo.

3. Aplicación de modelos de masas en un ambiente real (estuario del río Brisbane, Australia), utilizando sustancias persistentes como trazadores, para poder estimar las fuentes de emisión y persistencia de contaminantes orgánicos en aguas superficiales, a partir del cálculo de su vida media.

4. Evaluación de la efectividad de técnicas de análisis no dirigidas (non-target) mediante HPLC-MS, haciendo uso del cuadrupolo tiempo de vuelo (QToF), para la determinación de contaminantes orgánicos en matrices ambientales complejas.

5. Optimización de métodos para la extracción simultánea de contaminantes orgánicos pertenecientes a diferentes familias presentes en hemolinfa y masa visceral de mejillón mediterráneo (*Mytilus galloprovincialis*), así como en hígado y músculo de anguila europea (*Anguilla anguilla*).

6. Evaluar la capacidad de *M. galloprovincialis* para la bioacumulación y depuración de compuestos pertenecientes a las familias de los PPCPs, plaguicidas y PFASs

7. Evaluar cómo influye la presencia de microplásticos durante la exposición de *M. galloprovincialis* a los contaminantes mencionados anteriormente, en sus capacidades de bioacumulación y depuración de dichos compuestos.

8. Estudiar la absorción, metabolización y distribución del ibuprofeno y sus metabolitos en *Vigna unguiculata* L. Walp

El **plan de trabajo** que se siguió para el desarrollo de la presente tesis es el siguiente:

En primer lugar, se procedió al estudio de la situación actual del conocimiento relacionado con los objetivos de la presente tesis, investigando los desafíos en torno a la complejidad del análisis de muestras de biota acuática y los contaminantes usualmente determinados en ellas. De esta forma se proporciona una visión general de los estudios realizados hasta ahora y una evaluación de las perspectivas de futuro para este campo de investigación, sobre los que asentara la presente tesis.

A continuación, se pasó a evaluar la presencia de contaminantes antropogénicos en los medios acuáticos. Para ello se realizó el estudio tanto de aguas superficiales, como de aguas pertenecientes a acequias de campos de cultivo. Además, se estudiaron matrices potencialmente contaminadas cuyos compuestos podrían acabar siendo liberados en el medio acuático, como sedimentos. Por último, se consiguió evaluar la persistencia de algunos de estos compuestos en un ambiente real, lo que proporcionó información valiosa sobre su destino ambiental. Con todo ello, se obtuvo una perspectiva de cuáles eran los contaminantes antropogénicos que podían llegar a impactar los medios acuáticos.

El siguiente paso fue la selección de una serie de PPCPs, plaguicidas, PFASs y drogas de abuso. Para su selección se tuvieron en cuenta tanto la frecuencia de su detección en ambientes acuáticos, como el conocimiento previo que se tenía sobre los mismos, favoreciendo la selección de compuestos poco estudiados, como la droga de abuso bufotenina. Entonces, se desarrollaron y validaron tanto métodos de extracción como analíticos para la determinación de estos contaminantes en matrices de mejillón y anguila.

Por último, se realizaron ensayos de laboratorio en un ambiente controlado utilizando *M. galloprovincialis* y *V. unguiculata* L. Walp como objetos de estudio. Esta especie de mejillón tiene un gran valor cultural y gastronómico en el área de Valencia, lugar en el que se desarrolló la presente tesis doctoral y donde el *M. galloprovincialis* es mejor conocido como "clòtxina". En dicho estudio los especímenes fueron expuestos a los contaminantes para los que se desarrollaron los métodos anteriormente mencionados y a microplásticos. Para así poder estudiar los mecanismos de bioacumulación y depuración de los mismos, y la posible influencia de la presencia de microplásticos en estos procesos. Por otro lado, *V. unguiculata* L. Walp fue expuesta a ibuprofeno durante su germinación y crecimiento, para así estudiar sus mecanismos de acumulación y metabolización, además de su distribución en las diferentes partes de la planta.

La presente tesis doctoral está organizada en cinco secciones, donde se incluyen un total de **8 publicaciones** científicas, que recogen todos los hallazgos conseguidos durante su realización.

La **sección 1** es una revisión sobre los desafíos a la hora de analizar contaminantes orgánicos en biota acuática, así como un breve repaso sobre la presencia de contaminantes en estas matrices. Las matrices bióticas mayoritariamente contienen un porcentaje variable de proteínas y lípidos que las dotan de una gran complejidad. Por este motivo, la obtención de extractos con la menor cantidad de compuestos interferentes posible supone un gran reto. En la revisión se presentan las más novedosas metodologías de extracción y detección de compuestos orgánicos en biota, haciendo especial énfasis en aquellas metodologías capaces de eliminar lípidos de forma satisfactoria.

Artículo 1: *Analysis of emerging and related pollutants in aquatic biota*

La **sección 2** reúne un total de dos artículos en los que se ha realizado la detección y/o cuantificación de contaminantes orgánicos en matrices

ambientales acuáticas o estrechamente relacionadas con el medio acuático. Incluyendo el monitoreo de aguas superficiales y provenientes de acequias, sedimento, suelo y especies vegetales; para determinar la presencia de diferentes contaminantes orgánicos y microplásticos. En los estudios también se evalúa el riesgo de estos contaminantes y su destino.

Artículo 2: *Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes*

Artículo 3: *Dataset of pesticides, pharmaceuticals and personal care products occurrence in wetlands of Saudi Arabia*

En la **sección 3** se compilan diferentes métodos de extracción y analíticos de contaminantes orgánicos (incluyendo PPCPs, plaguicidas, PFASs y drogas de abuso), en matrices de mejillón, anguila y sedimento. Por un lado, se encuentran dos estudios enfocados en la extracción de contaminantes en mejillón y un tercero enfocado en anguila. Por otro lado, se incluyen diferentes métodos analíticos, destacando un estudio en el que se realizó una comparación de metodologías non-target utilizando HPLC-QToF-MS/MS.

Artículo 4: *Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants*

Artículo 5: *Multi-residue extraction to determine organic pollutants in mussel hemolymph*

Artículo 6: *Determination of organic pollutants in Anguilla anguilla by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)*

Artículo 7: *Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography quadrupole time-of-flight-mass spectrometry*

La **sección 4** muestra un estudio donde *V. Unguiculata* L. Walp fue expuesta a diferentes concentraciones de ibuprofeno durante su germinación y crecimiento. Para así observar la absorción, metabolización y distribución de ibuprofeno y sus metabolitos en la planta.

Artículo 8: *Analysis of ibuprofen and its main metabolites in roots, shoots and seeds of cowpea (Vigna Unguiculata L. Walp) using liquid chromatography-quadrupole time-of-flight mass spectrometry: uptake, metabolism and translocation*.

En la **sección 5** se reúnen, resumen y discuten los hallazgos obtenidos en las **secciones 2, 3 y 4**. Así como las perspectivas de futuro, las cuales incluyen dos estudios de los que ya se dispone de resultados y que se encuentran en la fase final de su desarrollo. El primero es un estudio de estimación de fuentes de emisión (como las EDARs) y de vida media de contaminantes orgánicos en un estuario de clima subtropical (Este de Australia). El segundo es un estudio de bioacumulación de compuestos orgánicos en mejillones. En dicho estudio los mejillones fueron expuestos a diferentes PPCPs, plaguicidas y PFASs. Además, un grupo fue expuesto también a microplásticos. De esta forma (con la ayuda de las metodologías desarrolladas en la **sección 3**) se pudieron observar los mecanismos de bioacumulación y depuración en *M. galloprovincialis,* para los contaminantes mencionados anteriormente. Así como la influencia de la presencia de microplásticos en estos procesos. En esta sección también se incluyen las conclusiones alcanzadas durante la realización de la presente tesis. Asimismo, se adjunta un apartado de **anexos** donde consta un sumario de las abreviaturas que figuran en la tesis.

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Aim and structure

02

The environment suffers the continue discharge of anthropogenic contaminants. Including organic compounds, such as, pharmaceuticals and personal care products (PPCPs), pesticides, perfluoroalkyl substances (PFASs) or illicit drugs, and microplastics. Furthermore, some of these contaminants are considered emerging contaminants [1], which are both compounds that have been discovered recently, hence they are not well known or those that, even though they are well known, their environmental concern has been raised recently.

Wastewater treatment plants (WWTPs) are one of the main sources of contaminants discharge to the environment [2, 3]. There are also other sources like ground water and run off water, which can spread pesticides and other emerging pollutants employed in farming and agriculture [4]. The discharge of these contaminants usually has a common destination, the aquatic environment, were the occurrence of anthropogenic contaminants has been widely reported [5-7]. Aquatic ecosystems have high environmental value, are crucial for biodiversity and support many other ecosystems. However, this may also pose an environmental problem, since contaminants can be easily spread through water to reach other environments, fauna, and flora. For all these, the study of the occurrence, behaviour, and fate of the contaminants in the aquatic environments is crucial.

A remarkable point related to the study of anthropogenic contaminants in the environment is that, although they have been widely studied in abiotic matrices [5, 6, 8], the studies focused on biotic matrices (fauna and flora) are scarce yet [9, 10]. On the one hand, the presence of interfering compounds in the biotic matrices such as lipids, proteins, and pigments, entails a high degree of complexity and poses a challenge for the correct detection and determination of organic pollutants. On the other hand, the mechanisms related to the exposition of these contaminants such as metabolic pathways, distribution, biomarkers or bioaccumulation and elimination, are very complex and their study requires a great amount of resources and time. However, their study is crucial to understand and assess the risks for the biota (and human health) related to the exposition and to develop preventive and remediation measures. As the aquatic environments are usually the most affected by the discharge of anthropogenic contaminants, the study of aquatic biota should be a priority, since it might be the most affected by these contaminants.

Taking into account all of this, the **general objective** of this thesis is the environmental risk assessment of the aquatic ecosystems due to

the discharge of organic pollutants, including the identification and assessment of sources, transport and fate, possible synergies and effects in the aquatic biota.

Specific objectives

1. Development of analytical techniques employing high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), with a triple quadrupole (QqQ), for the simultaneous determination and quantification of compounds from different families.

2. Assessment of the different sources of pollutants discharge to the aquatic environment, such as WWTPs effluents or the runoff water from crops.

3. Use of a mass balance model in a real environment (Brisbane river estuary, Australia), using persistent compounds as benchmark compounds, to estimate the persistence of organic pollutants in the environment, and their possible discharge sources.

4. Assessment of non-target approaches using HPLC-MS with a quadrupole time of flight (QToF) for the determination of organic contaminants in complex environmental matrices.

5. Procedures optimization for the simultaneous extraction of organic pollutants from different families present in haemolymph and visceral mass of Mediterranean mussel (*Mytilus galloprovincialis*) and muscle and liver of European eel (*Anguilla anguilla*).

6. Assessment of the bioaccumulation and elimination of PPCPs, pesticides, and PFASs in *M. galloprovincialis*

7. Assessing the influence of the presence of microplastics in the accumulation and elimination of the contaminants previously mentioned in *M. galloprovincialis*.

8. Studying the uptake, metabolism and distribution of ibuprofen and its metabolites in *Vigna unguiculata* L. Walp

The **work plan** employed during the thesis is as follows:

First, a research about the current state of knowledge related to the objectives of this thesis was performed. This search was focused on the challenges related to the complexity of the analysis of biota samples and the contaminants usually determined in them. This provided an overview of the studies carried out so far and an assessment of the future research perspectives in this field, laying the foundations of the present thesis.

Next, the presence of anthropogenic pollutants in aquatic environments was assessed. For this purpose, surface waters and waters of irrigation channels and other matrices that could potentially release pollutants to the aquatic environments, such as sediments, were studied. Finally, the persistence of some of these compounds in a real environment, which provided valuable information about their environmental fate, was estimated. All this provided a full picture of the anthropogenic pollutants that could impact aquatic environments.

The next step was the selection of the target PPCPs, pesticides, PFASs and illicit drugs. For this purpose, both the report of their presence in aquatic environments, as well as the previous knowledge about them, were considered, favouring the selection of not well-known compounds, such as the illicit drug bufotenin. Then, both extraction and analytical methods were developed and validated for the determination of these contaminants in mussel and eel matrices.

Finally, laboratory tests under controlled conditions were carried out using *M. galloprovincialis* and *V. unguiculata* L. Walp. This species of mussel has an important cultural and gastronomic value in the area of Valencia, the place where this doctoral thesis was conducted and where *M. galloprovincialis* is better known as “clòtxina”. In this study, the specimens were exposed to microplastics and to the same contaminants for which the analytical methods were developed. To study their bioaccumulation and elimination mechanisms, and the possible influence of the presence of microplastics in these processes. On the other hand, *V. unguiculata* L. Walp was exposed to ibuprofen during its germination and growth, to study the uptake and metabolism of this pharmaceutical, as well as its distribution in the different parts of the plant.

The present thesis is organized into five sections, which include a total of **8 scientific publications**, which collect all the findings obtained during

its completion.

Section 1 is a review about the challenges related to the analysis of organic pollutants in aquatic biota, as well as a brief review of the occurrence of pollutants in these matrices. Biota matrices contain a variable percentage of proteins and lipids that give them great complexity. For this reason, obtaining clean extracts, with as less amount of interfering compounds as possible, is a great challenge. The review presents the newest methodologies for the extraction and detection of organic compounds in biota, with special emphasis on those methodologies focused on lipid removal.

Article 1: *Analysis of emerging and related pollutants in aquatic biota*

Section 2 has a total of two articles in which the detection and/or quantification of organic pollutants in aquatic environmental matrices or matrices closely related to the aquatic environment has been carried out. Including the monitoring of surface waters, irrigation channels, sediment, soil, and plants; to determine the presence of different organic pollutants and microplastic. The studies also assess the risk of these pollutants and their fate.

Article 2: *Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes*

Article 3: *Dataset of pesticides, pharmaceuticals, and personal care products occurrence in wetlands of Saudi Arabia*

Section 3 is a compilation of different extraction and analytical methods for organic contaminants (including PPCPs, pesticides, PFASs, and illicit drugs), in mussel, eel, and sediment matrices. On the one hand, there are two studies focused on the extraction of contaminants in mussels and one focused on eel. On the other hand, there are different analytical methods, highlighting a study in which a comparison of non-target approaches was carried out using HPLC-QToF-MS/MS.

Article 4: *Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants*

Article 5: *Multi-residue extraction to determine organic pollutants in mussel hemolymph*

Article 6: *Determination of organic pollutants in Anguilla anguilla by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)*

Article 7: *Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography quadrupole time-of-flight-mass spectrometry*

Section 4 shows one study where *V. unguiculata* L. Walp was exposed to different concentrations of ibuprofen during its germination and growth. To observe the uptake, metabolism and distribution of ibuprofen and its metabolites in the plant.

Article 8: *Analysis of ibuprofen and its main metabolites in roots, shoots, and seeds of cowpea (Vigna Unguiculata L. Walp) using liquid chromatography-quadrupole time-of-flight mass spectrometry uptake, metabolism and translocation.*

Section 5 summarizes and discusses the findings obtained in **sections 2**, **3** and **4**. It also includes the conclusions raised during the conduction of the present thesis. And the future research perspectives section, which summarizes two studies that are currently in their final stage. One of them assesses contaminant emission sources (such as WWTPs) and estimates the half-live of organic contaminants in a subtropical estuary (East Australia). The second one is a bioaccumulation study of organic compounds in mussels. In this study, the mussels were exposed to different PPCPs, pesticides and PFASs. Furthermore, one group was also exposed to microplastics. In this way (and using the methodologies developed in section 3) it was possible to observe the bioaccumulation and elimination mechanisms in *M. galloprovincialis*, for the selected contaminants. As well as the influence of the presence of microplastics in these processes. Additionally, this section includes an annexes section with a summary of the acronyms employed in the thesis.

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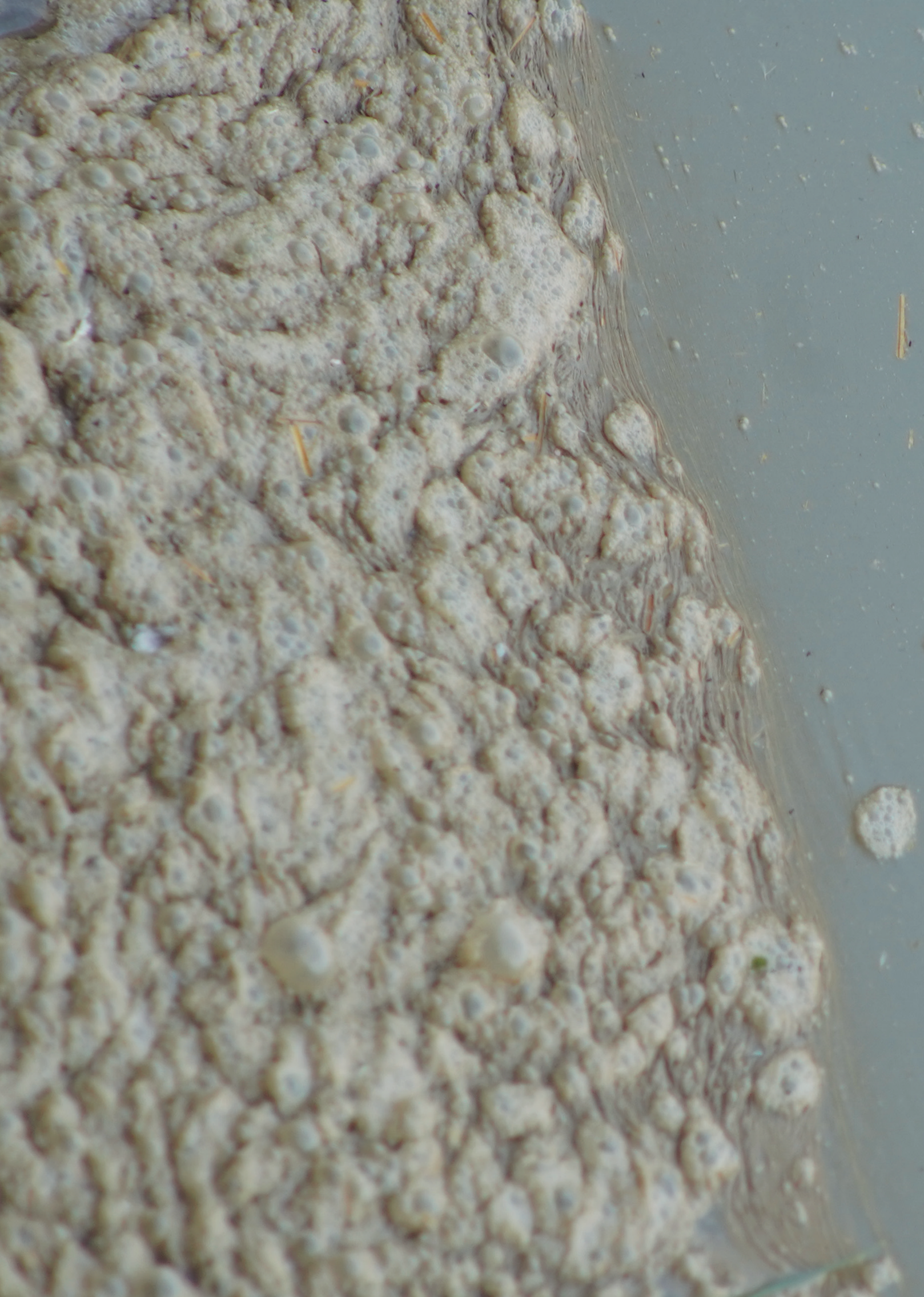

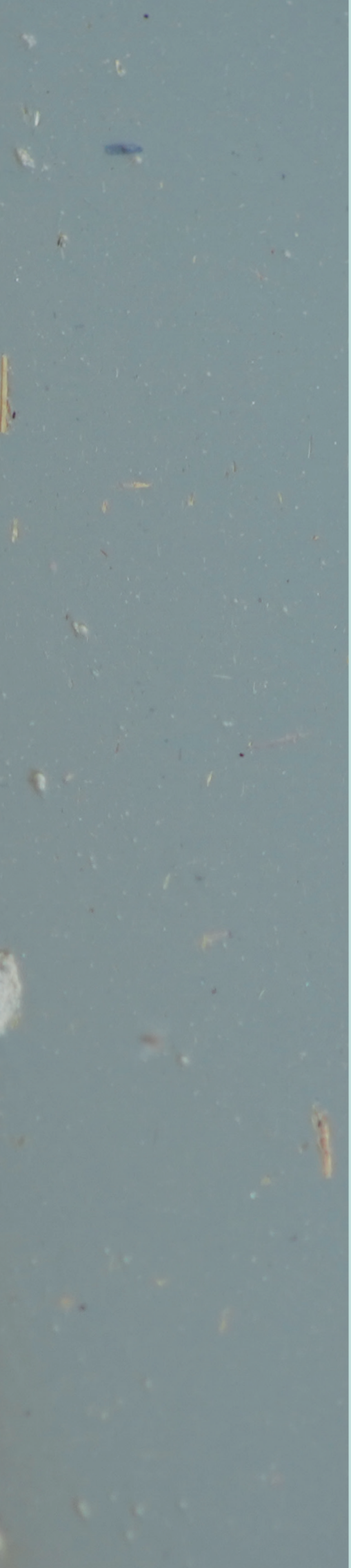

SECCIÓN 1.
INTRODUCCIÓN

10

ARTÍCULO

Analysis of emerging and related pollutants in aquatic biota

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Analysis of emerging and related pollutants in aquatic biota

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ABSTRACT

Water bodies cover approximately 70 % of the earth's surface, making them ecosystems with a high environmental value and the habitat for numerous species of flora and fauna. Emerging pollutants (EPs) are ubiquitous anthropogenic compounds of environmental concern that can be found at different concentration levels in matrices such as sediment, water and aquatic biota. In addition, EPs can be bioaccumulated and biomagnified, inducing adverse effects on biota, and posing a risk to humans when contaminated biota is consumed. Unlike abiotic matrices, the occurrence of EPs in aquatic biota has not been widely studied. This is probably because their complexity, due to the presence of lipids, proteins and other organic compounds, makes the extraction and analysis of EPs difficult. This review gathers the most relevant analytical methods published between 2014 and 2019, comparing them and evaluating their strengths and weaknesses. It is intended to provide a better understanding of the development of new and improved methods, and to be a reference for researchers who are looking for the best methodology for their studies.

1. Introduction

Emerging pollutants (EPs) are ubiquitous in the environment, and found in both continental and oceanic water bodies. Regarding the definition of EP, Sauvé et al. described three scenarios where the word "emergent" can be used: i) new or previously unknown compounds just recently reported, ii) known compounds for which the environmental contamination issues were not fully understood, and iii) "emerging issues" about contaminants where new information is jostling our understanding of their environmental and human health risk [1]. In this study, compounds in any of these three statements are considered EPs, including those that are typically related to "emerging contaminants", such as PAHs (recently related to microplastics [2]) or pesticides (usually extracted with other EPs). Therefore, EPs include a great variety of compounds (Fig. 1), of which pharmaceuticals are probably the most studied because of their environmental relevance as well as their effects [3–5]. Personal care products (PCPs) that commonly reach the aquatic environment together with pharmaceuticals are biologically active and pseudo-persistent [6]. There have also been, several studies about the occurrence of illicit drugs [7,8] and their effects [9]. So-called emerging persistent organic pollutants (ePOPs) refers to EPs that are highly persistent. This group includes pollutants such as flame retardants, perflouroalkyl substances (PFASs) and alkylphenols (APs), among others. Due to their persistence, biota receives long term exposure to these bioaccumulable and biomagnificable compounds [10,11]. Exposure

Abbreviations: AP, alkylphenol; ACN, acetonitrile; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; BFR, brominated flame retardant; BPA, Bisphenol A; DAD, diode array detectors; DCM, dichloromethane; DP, Dechlorane Plus; EDC, endocrine disruptor chemical; ECD, electron capture detector; EP, emerging pollutant; EQS, environmental quality standard; ESI, electrospray ionization; FUSLE, focused ultrasound solid-liquid extraction; GC, gas chromatography; GPC, gel permeation chromatography; HESI, heated electrospray ionization; HFR, halogenated flame retardant; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; HRGC, high resolution gas chromatography; HRPS, high resolution product scan; IC, ionic chromatography; MAC, maximum allowable concentration; MAE, microwave assisted extraction; MeOH, methanol; MRM, multiple reaction monitoring; MS, mass spectrometry; MSPD, matrix solid phase dispersion; LC, liquid chromatography; LLE, liquid liquid extraction; LOD, limit of detection; LOQ, limit of quantification; OCP, organoclorine pesticide; OPP, organophosphorus pesticides; PAH, polycyclic aromatic hydrocarbon; PBDD/F, polybrominated dibenzo-*p*-dioxin/dibenzofuran; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PCDD/F, polychlorinated dibenzo-p-dioxin/dibenzofuran; PCN, polychlorinated naphthalene; PCP, personal care product; PFAS, perfluoroalkyl substance; PFOS, perfluorooctanesulphonic acid; PFR, phosphorus flame retardant; PLE, pressurized liquid extraction; POP, persistent organic pollutant; PuLE, pulverised liquid extraction; QqQ, triple quadrupole; QTOF, quadrupole time of flight; QTRAP, quadrupole ion trap; SCCP, short-chain chlorinated paraffins; SPE, solid phase extraction; SE, solvent extraction; SIM, selected ion monitoring; SIR, single ion recording; SIS, selected ion storage; SRM, selection of monitored reactions; TFC, turbulent flow chromatography; TSI, turbospray ionization source; UAE, ultrasound assisted extraction; UHPLC, ultra-high-performance liquid chromatography; UVAE, vacuum assisted extraction; WWTP, wastewater treatment plant.

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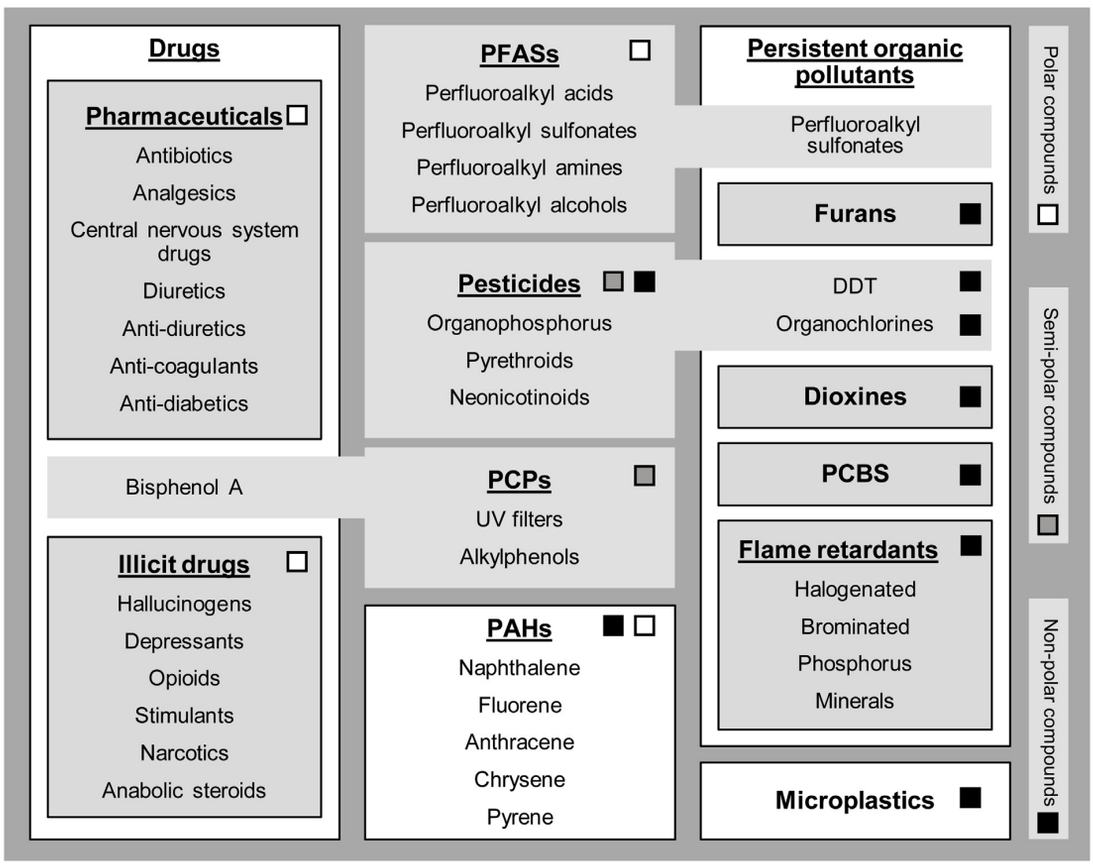

Fig. 1. General overview of the EPs families and their polarity. The indicated polarity is the most common in each family and it does not include exceptions.

occurs via water, sediment, suspended solids, or the intake of biota and microplastics. Microplastics are also considered EPs and their global presence in the aquatic environment and their intake by biota at all trophic levels has recently been proven [12]. Although there is not yet consensus on the issue, numerous studies suggest that these microplastics could efficiently adsorb POPs and other EPs from the surrounding environment and, transfer them to the biota [2,13].

These EPs, mostly of anthropic origin, reach the aquatic environment through its continuous discharge from wastewater treatment plants (WWTPs) effluent and sewage sludges, through run-off (such as PFASs in airports and military bases) [14] and due to the release of precursors (such as secondary microplastics) that form EPs once they are in the water. Their presence in matrices such as water and sediment has been widely studied [15–19], but reports about their occurrence in biota are relatively scarce [20,21]. Aquatic biota is exposed to these compounds, and several studies have already demonstrated their adverse effects on it [9,22,23].

Species in which the occurrence of EPs has been reported include mussel [24], eel [25], seafood and other fish [15] used for human consumption. The health risk from eating seafood containing endocrine disrupting compounds, antibiotics and triclosan, has been established in a few studies [26–28]. Furthermore, the negative effects of eating fish containing pesticides have also been reported [29]. More information about the occurrence of EPs in the biota, would provide a better understanding of human exposure and would help in an assessment of the human health risk related to these compounds.

Aquatic biota samples are environmental matrices with a high protein and lipid content, although their proportion varies depending on the sample (e.g. mussel and eel contain 2 % and 18 % lipids, respectively, and 10 % and 14 % proteins). These matrix constituents can be extracted with organic solvents and, once in the extracts, they can interfere in the analysis of target pollutants. Such complexity poses a great challenge in the development of efficient and reliable extraction procedures. Overcoming this challenge is necessary to obtain quality results that facilitate an understanding of the occurrence, pathways and biodegradation of EPs in aquatic biota, which has particular importance for the environment (including terrestrial animals that consume it) and the human population.

This review discusses methodologies for the analysis of EPs in aquatic biota, and critically examines studies that improve the efficiency of the analytical process, from extraction to determination. The most relevant studies published between 2014 and 2019 involving new or modified methods have been included in this review. Previous reviews on the analysis of EPs from biota samples were focused on one family of compounds, such as pharmaceuticals [3,4], illicit drugs [7], PAHs [30], PCPs [6] or endocrine disruptors (EDCs) as APs and bisphenol A (BPA) [31]. In the last few years, to the best of our knowledge, no review of the analytical methodologies for aquatic biota taking into consideration different types of EPs, has been published. Additionally, the application of these methods for establishing occurrence, transport, pathways, distribution and fate are also discussed in this review. At the end, a short discussion is provided regarding the achievements, limitations, and future directions of the determination of EPs in aquatic biota.

Table 1
Analytical methods for emerging pollutants in aquatic biota.

Pollutants	Sample	Matrix	Pre-treatment	Sample size	Extraction method	Clean up method	Drying	Detection	LODs/LOQs (ng/g)	Recovery (%)	Ref.
Antibiotics	Fish	Bile	–	0.05–0.2 mL	Adjust to pH 3 and SPE SAX/PSA–HLB tandem cartridges	–	–	UHPLC (ESI)-MS/MS in MRM	[a]0.04–9.09* [b]0.13–30.30*	28–121	[49]
		Plasma	–	0.05–0.2 mL	Adjust to pH 3 and SPE HLB cartridges	–	–	UHPLC (ESI)-MS/MS in MRM	[a]0.02–1.58* [b]0.08–5.28*	53–196	[49]
		Muscle and liver	–	0.5–2 g	Double MeOH/sodium acetate UAE	SPE SAX/PSA–HLB tandem cartridges Fat removal with two-layer SAX/PSA cartridge	–	UHPLC (ESI)-MS/MS in MRM	[a]0.04–4.10 [b]0.14–13.70	40–178	[49]
Pharmaceuticals and metabolites	Fish	Plasma	–	10 µL	Dilution in MeOH and acetate buffer pH 4, agitation and centrifugation	–	–	UHPLC (ESI)-MS/MS(QTRAP) in MRM	[a]0.039–0.32*	85.2–105	[68]
		Brain	–	0.014–0.025 g	MeOH/ACN UAE	SPE Oasis HLB cartridges and filtration	–	UHPLC (ESI)-MS/MS(QTRAP) in MRM	[a]0.092–0.73	95.9–103	[68]
Pharmaceuticals	Fish	Plasma	–	0.1 mL	SPE PRiME HLB cartridges	–	Nitrogen	LC(ESI)-MS/MS(QqQ) in MRM	[b]2–200*	94–111	[52]
Pharmaceuticals	Fish	Plasma	–	0.5–1 mL	Dilution with formic acid/water and SPE HLB cartridges	–	–	LC (ESI)-MS/MS (QqQ)	–	–	[63]
Pharmaceuticals	Fish	Liver, brain, gill and muscle	–	–	PLE	Fat removal with low temperature fat precipitation	Rotavapor	LC(ESI)-MS/MS(QqQ)	[b]0.4–1.2	62.4–83.6	[50]
Pharmaceuticals	Fish	Gill, intestine, muscle, liver and brain	Homogenized and freeze-dried	0.010–0.025 g	Triple MeOH ice-bath UAE and ultracentrifugation	SPE HLB cartridges	Nitrogen	HPLC(ESI)-MS/MS (QTRAP) in SRM	[a]0.1–30.3 [b]0.4–100	40–128	[65]
Pharmaceuticals	Fish	Muscle	Homogenized	1 g	MeOH/ACN UAE	Hexane, agitation and centrifugation Fat removal with low temperature fat precipitation	Nitrogen	UHPLC (ESI)-MS/MS in MRM	[a]0.02–2.2 [b]0.04–6.7	–	[67]
Pharmaceuticals	Fish	Liver, brain, gill and muscle		0.5–1 g	MeOH PLE	Decantation Fat removal with low temperature ACN extraction	Rotavapor and nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.08–0.25 [b]0.3–1.0	65.2–85.3	[105]
Pharmaceuticals	Fish	Pool		1 g	Triple HCl/MeOH UAE	SPE Strata-X® polymeric reverse phase cartridges Fat removal with pH adjusting and centrifugation	Rotavapor	HPLC(ESI)-MS(QqTOF)	–	80	[66]
Pharmaceuticals	Fish, plankton, zoobenthos, and shrimp	Muscle for the fish, pool for the rest	Lyophilized	0.25–0.5 g	PLE	SPE HLB cartridges	Rotavapor and nitrogen	UHPLC (ESI)-MS/MS	[b]0.01–1.00	69–126	[116]
Pharmaceuticals and metabolites	Fish and bivalve	Pool for bivalve and muscle for fish	Homogenized and freeze-dried	0.5 g	QuEChERS with formic acid	Centrifugation and filtration	–	UHPLC-MS/MS(QTRAP)	[a]0.01–0.31 [b]0.02-1.03	28–70	[27]
Pharmaceuticals	Fish	Muscle	Homogenized	0.5 g	MeOH SE, agitation, centrifugation and filtration	–	–	LC(ESI)-MS/MS(QTRAP)	[b]0.062–4.6	52–120	[73]
	Invertebrate	Pool	Homogenized	0.5 g	MeOH SE, agitation, centrifugation and filtration	–	–	LC(ESI)-MS/MS(QTRAP)	[b]1.0–4.4	85–150	[73,124]
Pharmaceuticals and metabolites	Bivalve	Pool	Homogenized and freeze-dried	0.5 g	PLE	SPE HLB cartridges Fat quantification with gravimetry and removal with neutral alumina	Nitrogen	UHPLC (ESI)-MS/MS(QTRAP)	[a]0.01–1.11 [b]0.02–3.70	30–110	[88]
Pharmaceuticals		–		–	Triple MeOH SE		Nitrogen		[b]5.3–10.7	71–98	[78]

Table 1 (*Continued*)

Pollutants	Sample	Matrix	Pre-treatment	Sample size	Extraction method	Clean up method	Drying	Detection	LODs/LOQs (ng/g)	Recovery (%)	Ref.
	Bivalve and algae		Freeze-dried and homogenized			SPE HLB cartridges Fat quantification with optical density and chlorophyll removal with hexane LLE (for algae)		LC(ESI)-MS/MS(QqQ)			
Pharmaceuticals	Mussel	Pool	Homogenized	3 g	Homogenization and centrifugation	SPE Discovery DSC-18, filtration and centrifugation	Speedvac	HPLC-FL-DAD	[a]0.5–1	≥98	[39]
Pharmaceuticals	Invertebrate	Pool	Lyophilized and ground	0.05 g	ACN PuLE	SPE HLB cartridges	Nitrogen	LC(ESI)-MS/MS(QqQ) in SRM	–	37–114	[123]
Antineoplastics	Fish	Muscle	Lyophilized and ground	0.05 g	MeOH MAE	SPE Phree Phospholipid Removal cartridges Fat removal with SPE	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.8–536 [b]2.7–1787	53–117	[100]
		Liver	Lyophilized and ground	0.05 g	MeOH MAE	SPE Phree Phospholipid Removal cartridges Fat removal with SPE	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.5–189 [b]1.8–631	59–118	[100]
Diclofenac and metabolites	Fish	Pool	Homogenized and freeze-dried	0.2 g	QuEChERS with heptane	Evaporation with dimethylsulfoxide (DMSO) and filtration Fat removal with heptane	Nitrogen	LC(ESI)-MS/MS(QqQ) in MRM	[a]0.15–0.60 [b]0.77–1.0	80–120	[36]
Fluoroquinolones	Fish	Muscle	Freeze-dried	0.5 g	MeOH FUSLE	SPE HLB cartridges	Nitrogen	HPLC(ESI)-MS/MS (QqQ) in SRM	[b]0.4–2.0	83–162	[64]
		Liver	Freeze-dried	0.5 g	MeOH FUSLE	LLE with hexane and SPE HLB cartridges Fat quantification with and removal with n-hexane and centrifugation	Nitrogen	HPLC(ESI)-MS/MS (QqQ) in SRM	[b]0.4–2.0	14–104	[64]
		Bile and plasma	–	0.1–0.25 mL	pH 7.4 SPE MIP cartridges	–	–	HPLC(ESI)-MS/MS (QqQ) in SRM	[b]0.8–10.0*	70–115	[64]
Triclosan	Fish	Muscle	–	5 g	Acetone an Na_2SO_4 homogenization, filtration, double acetone extraction, double n-hexane extraction	Silica gel columns	Rotavapor	GC(EI)-MS in SIM	[a]0.03 [b]0.09	84	[76]
Triclosan	Fish	Whole body	Homogenized	5 g	QuEChERS	dSPE	–	LC-MS	[b]0.22	–	[82]
Triclosan	Fish	–	Homogenized	5 g	Ethyl acetate Soxhlet	Pre-activated Florisil®, Na_2SO_4 and silica gel in hexane chromatographic column	Rotavapor	HPLC-DAD	[b]10	85	[92]
Pharmaceuticals and PCPs	Fish	Plasma	–	20 μL	Double Acetone LLE-UAE	–	Nitrogen	HPLC(ESI)-MS/MS (QTRAP) in MRM	[a]0.015–8.11* [b]0.05–27.00*	55–137	[61]
Pharmaceuticals and PCPs	Fish	Brain, liver, plasma	–	0.5 g or 1 mL	Enzymatic treatment with β-glucuronidase/arylsulfatase and double ACN UAE	LLE and SPE HLB cartridge Fat removal with GPC and silica gel chromatography	Rotavapor and nitrogen	UHPLC (ESI)-MS/MS(QTRAP) in MRM	[a]0.0092–3.2 [b]0.020–8.7	89.6–135	[58]
Pharmaceuticals and PCPs	Fish and mussel	Muscle and whole body	Freeze-dried and ground	0.2 g	Double ACN UAE and centrifugation	SPE PRiME HLB cartridges and filtration	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.01–1.9 [b]0.03–5.6	43–127	[19]
Pharmaceuticals and PCPs	Invertebrate	Pool	Lyophilized and ground	0.1 g	ACN PuLE	SPE HLB cartridges	Nitrogen	LC(API-ESI)-MS/MS (QqQ) in SRM	[a]<20 [b]4-687	4-89	[109]
	Fish	Blood	–	1 ml			Nitrogen	GC-MS	[b]0.22–2.53*	65–95	[37,125]

Pharmaceuticals, PCPs and EDCs	Bile	–	1 ml	Dilution in MilliQ water pH 2 and filtration Enzymatic treatment with β-glucuronidase, dilution in MilliQ water pH 2 and filtration	SPE HLB cartridges Derivatization with MTBSTFA SPE HLB cartridges Derivatization with MTBSTFA	Nitrogen	GC–MS	[b]0.22–2.35*	60–92	[37,125]
	Muscle	Homogenized	1 g	Triple MeOH UAE	SPE HLB cartridges Derivatization with MTBSTFA	Nitrogen	GC–MS	[b]0.24–2.56	62–91	[37,125]
Pharmaceuticals, PCPs pesticides, PBDEs, PCBs, PAHs and OCPs	Fish Muscle	Homogenized	10 g	QuEChERS	Captiva ND Lipids Cartridges Fat removal with low temperature fat precipitation	Nitrogen	UHPLC (ESI)-MS/MS(QTOF) in IDA	[b]0.08–2	60.9–115.5	[38]
PCPs and pesticides	Fish Muscle	Homogenized	10 g	QuEChERS	$MgSO_4$ and Z-Sep sorbent dSPE Fat removal with low temperature fat precipitation	–	GC(EI)-MS/MS(QqQ) in MRM	[b]0.2–5.4	45–128	[38]
Pharmaceuticals, PCPs, hormones, biocides, and flame retardants	Plant –	–	1 g	Ultrasound-assisted MSPD	Glass column and filtration Derivatization with MTBSTFA:TBDMCS	Personal evaporator	GC(EI)-MS in SIM	[a]0.3–2.2 [b]1.0–4.8	70–120	[44]
Pharmaceuticals, PCPs, pesticides, UV filter, APs, and PFASs	Benthic invertebrate Whole body or pool	Freeze-dried and ground	0.012–0.020 g	QuEChERS with hexane	Centrifugation	Nitrogen	NanoLC (ESI)-MS/MS(QTRAP) in MRM	[b]0.5–260.0	>70	[34]
Pharmaceuticals, metabolites and pesticides	Invertebrate Pool	Freeze-dried	0.2 g	ACN-PuLE	QuEChERS and hexane Fat removal with low temperature fat precipitation	Nitrogen	LC(ESI)-MS/MS(QTRAP) in MRM LC(HESI)-HRMS (Orbitrap)	[b]0.01–2.13	47–127	[119]
Pharmaceuticals, PAHs and flame retardants	Fish, clam and worm –	Homogenized	5–10 g	Triple Hexane/DCM UAE	Filtration Fat removal with hexane/DCM GPC	Rotavapor	LC(ESI)-MS/MS(QqQ) in MRM	[a]1.3–6.9	88–115	[41]
Pharmaceuticals, pesticides PAHs and flame retardants	Fish, clam and worm –	Homogenized	5–10 g	Triple Hexane/DCM UAE	Deactivated Florisil® chromatography Fat removal with hexane/DCM GPC	Rotavapor	GC(EI)-MS/MS(QqQ) in MRM	[a]0.1–22.8	44–117	[41]
Pharmaceuticals, pesticides and BPA	Fish and snail Muscle and pooled (for molluscs)	Freeze-dried and homogenized	0.5 g	QuEChERS	SPE Strata-X® polymeric reversed phase cartridges	Rotavapor and nitrogen	HPLC-MS (QTRAP)	[a]0.02–3.5	64–114	[86]
Pharmaceuticals, pesticides and illicit drugs	Invertebrate Whole organism, pool	Freeze-dried and ground	0.02 g	Acidified ACN UAE	Tandem SPE Strata-X® Alumina-N cartridges and HLB cartridges Fat removal with alumina	Nitrogen	LC(API-HESI)- MS (QqQ) in SRM	[a]0.03–8.3 [b]0.1–25.2	43–100	[57]
Pharmaceuticals, illicit drugs, plasticisers, PFASs and metabolites	Snail and amphipod crustacean Pool	Freeze-dried, powdered	Variable	ACN UAE	SPE Strata-X® polymeric reversed phase cartridges	Rotavapor	HPLC-MS/MS(QqQ) in MRM	–	67	[11,120]
	Plant Pool	Air dry, powdered	0.5 g	ACN UAE	SPE Strata-X® polymeric reversed phase cartridges	Rotavapor	HPLC-MS/MS(QqQ) in MRM	–	81	[11,120]
	Periphyton Biofilm	Air dry, powdered	Variable	ACN UAE	SPE Strata-X® polymeric reversed phase cartridges	Rotavapor	HPLC-MS/MS(QqQ) in MRM	–	62	[11,120]
Pharmaceuticals, PFASs and EDCs	Fish Pool	Freeze-dried and ground	10 g	MeOH/acetone Soxhlet	SPE HLB cartridges and filtration Fat quantification with gravimetry and removal with acidification and centrifugation	Rotavapor and nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.01–0.036 [b]0.0200.114	69.5–103.2	[96]
Pharmaceuticals and EDCs	Bivalve –	Homogenized	1–1.5 g	ACN/MeOH SE, agitation and centrifugation	Filtration	Nitrogen	LC (ESI)-MS/MS in MRM	[a]<1	67–150	[74]

Table 1 (*Continued*)

Pollutants	Sample	Matrix	Pre-treatment	Sample size	Extraction method	Clean up method	Drying	Detection	LODs/LOQs (ng/g)	Recovery (%)	Ref.
Pharmaceuticals and EDCs	Invertebrates	Pool	Homogenized and freeze-dried	0.1 g	MeOH UAE	ACN Ostro™ 96-well plate Fat removal with Ostro™ 96-well plate	–	UHPLC (ESI)-MS/MS(QTRAP) in MRM TFC-LC (ESI)-HRMS (Orbitrap)	[a]0.08–4.3 [b]0.21–14	34–133	[72]
Illicit drugs	Mussel	Pool	Homogenized	10 g	QuEChERS	$MgSO_4$, PSA and C18 dSPE	Nitrogen	HPLC(ESI)-MS/MS (QTRAP) in SRM	[a]0.1–10 [b]0.3-30	77–118	[8]
PCPs	Fish	Muscle	Lyophilized	0.2 g	MSPD	SPE C18 PAH silica as co-column cartridges Fat quantification with gravimetry and removal with silica column	Nitrogen	GC-MS/MS in SIR	[a]4–12 [b]13–40	77–111	[97]
PCPs	Fish, plant and shrimp	Whole plant and seafoods carcasses	Homogenized	1 g	MeOH PLE	SPE mixed-mode cationic exchange cartridges	Nitrogen	LC(ESI)-MS/MS(QTRAP) in MRM	[b]10.0	80.6–107.8	[45]
PCPs	Fish, crab and shrimp	Soft tissue pool	Homogenized	–	Hexane/DCM PLE	DCM GPC and centrifugation Fat removal with PSA	Nitrogen	GC(EI)-HRMS(QTOF) LC(ESI)-HRMS (QTOF)	[b]10–50	46–85	[43]
PCPs and PFASs	Echinoderm	Viscera	Freeze-dried, homogenized and ground	0.5 g	Double ACN SE, agitation, and centrifugation	C18 dSPE	Nitrogen	HPLC(ESI)-MS/MS (QqQ) in MRM	[a]0.008–4.00 [b]0.03–13	65–114	[77]
UV stabilizers	Algae	–	Freeze-dried and ground	0.1 g	MeOH MAE	Filtration	–	UHPLC-DAD	[a]1.79–4.98 [b]5.96–15.3	49.8–92.3	[101]
Glyphosate and metabolites	Fish	Pool	Homogenized	1 g	Acidified MeOH UAE	Centrifugation	–	IC(HESI)-MS/MS (Orbitrap) in DIA	[b]4.08–5.38	80–112	[56]
Pesticides	Fish	Muscle	–	10 g	QuEChERS	$MgSO_4$, PSA and C18 dSPE	–	LC(HESI)-MS/MS (QqQ) in SRM	[a]0.01–50 [b]0.04–100	71–120	[85]
Pesticides	Fish	Muscle	Homogenized	10 g	Double ethyl acetate-ACN UAE	Filtration and methylene dichloride GPC	Rotavapor	LC(ESI)-MS/MS in MRM	[a]0.001 v0.042 [b]0.03v0.247	71v102	[29]
Pesticides	Fish	Pool	Homogenized and lyophilized	2 g	QuEChERS	MgSO4, PSA, C18 and activated charcoal dSPE	–	LC(ESI)-MS/MS(QqQ)	[a]0.01–0.5 [b]0.03–11.25	≈60–150	[81]
Pesticides	Eel and shrimp	Pool	Homogenized	12.5 g	QuEChERS	–	Nitrogen	LC(TSI)-MS/MS(QqQ) in MRM	[b]≤5	<60	[42]
					QuEChERS + EDTA	–	Nitrogen	LC(TSI)-MS/MS(QqQ) in MRM	[b]≤5	55.3–124.6	[42]
					QuEChERS + EDTA	PSA, magnesium sulphate and C18 dSPE	Nitrogen	LC(TSI)-MS/MS(QqQ) in MRM	[b]≤5	62.0–117.9	[42]
Pesticides	Invertebrate	Pool	Homogenized	0.2 g	Micro-QuEChERS	PSA and C18 dSPE	Nitrogen	LC(ESI)-MS/MS(QTRAP) in MRM	[b]1–10	74–132	[87]

								GC(EI)-MS/MS(QqQ) in MRM		
Pesticides	Bivalve	–	Homogenized	5 g	QuEChERS	$MgSO_4$ and PSA dSPE	–	GC-MS/MS (IT) in SIM	[b]0.33–10.3*	78–119	[83]
Pyrethroids	Fish	Pool	Homogenized and freeze-dried	0.3 g	Triple Hexane/DCM UAE	SPE basic alumina and C18 Fat quantification with Gravimetry	Nitrogen	GC-MS/MS (QqQ)	[a]0.03–0.46 [b]0.10–1.54	79	[79]
Fipronil and transformation products	Eel	Liver and muscle	Homogenized and freeze-dried	1–2 g	Triple SE with ACN, agitation and centrifugation	Degreasing and centrifugation Fat quantification with gravimetry and removal with cyclohexane Smedes method	Vaporizer	HPLC(ESI)-MS/MS (QTRAP) in MRM	[b]0.008–0.03 [a]0.003–0.02	99.9–103.2	[53,126]
DDT and flame retardants	Clam	–	Lyophilized	0.5–1 g	n-hexane/acetone Soxhlet	Acid silica gel and Florisil® columns	Nitrogen	GC(EI)-MS/MS(IT)	–	65–112	[28]
APs	Clam	–	Lyophilized	0.67 g	Acetone/n-hexane PLE	–	–	UHPLC-MS/MS	[a]0.8–3.7	65–112	[28]
PCPs and BPA	Clam	–	Lyophilized	1.5 g	MeOH/acetone PLE	–	–	UHPLC-MS/MS	–	65–112	[28]
Pesticides and PCBs	Eel	Muscle, skin and bones pool	Homogenized	–	DCM Soxhlet	GPC Bio-Beads S-X3 Fat quantification with weight the air-dried Soxhlet residue and removal with hexane and GPC	Rotavapor	GC-MS	–	–	[93]
OCPs and PCBs	Eel	Brain, liver and muscle	Freeze-dried and homogenized	–	n-hexane PLE	Sulphuric acid and n-hexane dilution, agitation, centrifugation and SPE Florisil® cartridges Fat removal with n-hexane PLE	Turbovap	HRGC-ECD	[a]0.03 [b]0.4	70–130	[10]
OCPs, PCBs, and PBDE	Eel	Pool	Homogenized	5 g	Glass column with DCM elution	GPC Biobeads SX-3 and silica-alumina column Fat quantification with gravimetry and removal with GPC	–	GC(EI)-MS/MS(IT)	[b]0.0044–10.974	97–110	[35]
Non-ortho PCB and PCDD/F	Eel	Pool	Homogenized	5 g	Glass column with DCM elution	GPC Biobeads SX-3, silica- alumina column and HPLC with Cosmosil 5PYE column Fat quantification with gravimetry and removal with GPC	–	GC-HRMS	[b]0.00019–0.0282	–	[35]
OCPs ,PBDEs, PCBs, PCDD/Fs, and other POPs	Fish	Muscle	Freeze-dried and homogenized	2 g	Homogenization with Na_2SO_4 and DCM/hexane PLE	Acid silica gel column, multi silica gel column, and Florisil column Fat quantification with gravimetry	Nitrogen	HRGC(EI)-HRMS in SIM HRGC (ENCI)-MS in SIM	[a]0.001–0.03	43–120	[91]
OCPs, PAHs, PCBs, PBDEs and OPPs	Bivalve	Pool	Homogenized and freeze-dried	0.5 g	MSPD	Silica SPE	Nitrogen	GC(EI)-MS (QqQ) in SRM and SIM	[a]0.34–13* [b]0.83–29*	55–117	[98]
								GC(EI)-MS/MS(QqQ) in SRM and SIM	[a]0.010–2.7* [b]0.017–3.9*	64v122	[98]
OCPs, PCB and PBDE	Oyster	Pool	Homogenized and freeze-dried	0.5 g	PLE with DCM	Acidic silica gel column	–	GC-ECD	[a]0.1–0.2 [a]0.3–0.6	72–116	[89]
PAHs	Oyster	Pool	Homogenized and freeze-dried	0.5 g	PLE with DCM	Alumina and silica micro-columns	Vacuum evaporation system	GC-MS/MS	[a]0.1–0.2 [a]0.3–0.6	72–116	[89]
PAHs	Fish	Pool	Homogenized	10 g	Double DCM-hexane UAE	Three fractions Florisil® column Fat quantification with double DCM UAE and removal with Freeze removal	Nitrogen	GC(EI)-MS	[a]0.000005–0.109	–	[112]
PAHs	Fish and mussel	Pool and muscle	–	1 g	Stir bar sorptive extraction	Fat removal with alkaline digestion	–		[b]1–50	82–117	[54]

Table 1 (*Continued*)

Pollutants	Sample	Matrix	Pre-treatment	Sample size	Extraction method	Clean up method	Drying	Detection	LODs/LOQs (ng/g)	Recovery (%)	Ref.
								GC(EI)-MS/MS(QqQ) in MRM			
PAHs, PCBs, pyrethroids, DEET	Fish	Muscle, liver, gill, gonad, and stomach	Freeze-dried and ground	0.1–0.5 g	Hexane/DCM and hexane/acetone PLE	Florisil cartridges Fat removal with hexane/isopropanol	Nitrogen	GC(EI)-MS/MS(QqQ) in MRM	[a]0.02–13.6 [b]0.09–33.7	48–203	[110]
Metabolites	Fish	Liver	Freeze-dried and ground	0.1 g	Enzymatic treatment with 4-methylumbelliferyl-β-D-glucuronide	SPE HLB cartridges and filtration	Nitrogen	LC(ESI)-MS/MS(QqQ) in MRM	[a]0.05–62.5 [b]0.074–20	18–264	[110]
PAHs	Oyster	Pool	Freeze-dried, homogenized and ground	0.5 g	QuEChERS	$MgSO_4$, PSA and C18 dSPE	–	GC-MS in SIM	[a]0.15–4.7 [b]0.5–16	72–117	[84]
Benzo(a)pyrene	Mussel	Digestive glands and gills	Homogenized	–	Potassium hydroxide/MeOH MAE and centrifugation	SPE Octadecyl C18 Fat removal with cryostat sections stained by Schmrol reaction	Speedvac	HPLC-FL	–	–	[2,127]
PAH metabolites	Eel	Bile	–	0.025 mL	Enzymatic treatment with β-glucuronidase/arylsulfatase,	Centrifugation Fat quantification with Distell Fish fatmeter	–	HPLC-FL	[a]3.4* [b]22.5*	–	[59]
PAH metabolites, AP and BPA	Eel	Bile	–	0.025 mL	Enzymatic treatment with β-glucuronidase/arylsulfatase	Centrifugation	–	HPLC-FL	[a]0.033–4.03*	47.6–92.4	[60]
Flame retardants	Fish	Pool	Homogenized	1 g	Triple MeOH UAE	SPE Strata-X® reversed polymeric phase cartridges	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in SRM	[a]0.02–0.28 [b]0.04–0.90	47–123	[69]
Flame retardants	Fish	Muscle	Homogenized and freeze-dried	10 g	DCM/n-hexane soxhlet	Double cyclohexane/ethyl acetate SE, Bio-Beads S-X3 GPC and tandem acidified silica gel column with deactivated silica gel column Fat removal withSoxtec™ 2055 Fat Extraction System	Nitrogen and rotavapor	HPLC (HESI)-HRMS (Orbitrap) in PRM	[b]0.001–0.25	84–117	[55]
Flame retardants	Fish and seal	Blubber for seal and pool	Homogenized and freeze-dried	0.5 g	MSPD and n-hexane/DCM SE	Multilayer silica column Fat quantification with gravimetry and removal with n-hexane/DCM	Rotavapor	GC(APCI)-MS/MS in MRM	[a]0.1114–0.01	–	[48]
Flame retardants	Fish, mussel and prawn	Soft tissues	Homogenized and freeze-dried	2.5 g	UVAE	SPE Florisil® cartridges, acidified silica, dSPE, hexane, SPE aminopropyl silica cartridges Fat quantification with gravimetry and removal with Florisil® cartridges, hexane, dSPE and freeze removal	–	GC(EI)-MS in SIM GC(ECNI)-MS in SIM	[b]0.008–3.6	66–135	[40]
Flame retardants	Mussel	–	Freeze-dried	0.5 g	Double hexane/acetone UAE	Centrifugation	–	TFC-LC (HESI)-MS/MS(QqQ) in SRM	[a]0.19–19.3 b0.97–24.8	47–98	[104,128]
Flame retardants and plasticisers	Fish	–	Freeze-dried	0.25 g	Double acetone/hexane UAE	SPE tandem basic alumina and C_{18} cartridges	Nitrogen	LC-MS/MS (QTRAP) in SRM	[a]0.35–51.6 [b]1.12–172	48–113	[71]
Flame retardants and plasticisers	Fish	Liver	Homogenized	1 g	Triple $MgSO_4$, NaCl and hexane/DCM UAE	PSA bonded silica dSPE Fat removal with PSA bonded silica	Nitrogen	UHPLC (APCI)-MS/MS(QqQ) in MRM	[a]0.02–0.14 [b]0.05–0.50	66.3–112.9	[71,129]
Flame retardants	Fish and dolphin	Pool and dolphin blubber	Homogenized and freeze-dried	1 g	Hexane/DCM PLE	SPE alumina cartridges Fat quantification with gravimetry and removal with concentrate sulphuric acid	–	GC(EI)-MS/MS(QqQ) in SRM	[a]0.01–9.66 [b]0.04–32.2	51–87	[47]
Flame retardants	Bivalve	Pool	–	5 g	Triple DCM/hexane UAE	Deactivated silica gel and sulfuric acid silica gel column and Florisil® column	–	LC(ESI)-MS/MS(QqQ)	[a]0.014–0.084	58–96	[70]

PBDEs	Bivalve	Pool	–	5 g	KOH digestion and hexane/methyl terbuthyl ether SE	Fat quantification with gravimetry and removal withFlorisil® column Multilayer silica gel columns andFlorisil® or alumina column Fat quantification with gravimetry and removal with Florisil® or alumina column	–	GC(EI)-HRMS in SIM	[a]0.1–0.5	26–85	[70]
PFRs	Fish, algae, bivalve, invertebrate, crustacean	Pool	Homogenized and freeze-dried	–	DCM/acetone PLE	SPE SPE-NH2 cartridges and centrifugation Fat quantification with Bligh and Dyer determination and removal with SPE-NH2 cartridges	–	HPLC(ESI)-MS/MS (QqQ) in MRM	[a]0.2–29	43–134	[90]
BFRs	Fish, algae, bivalve, invertebrate, crustacean	Pool	Homogenized and freeze-dried	–	Hexane/acetone PLE	Sulphuric acid-treated silica dSPE and hexane and diethyl ether-hexane silica GPC Fat quantification with Bligh and Dyer determination and removal with dSPE and GPC	Nitrogen	GC(ECNI)-MS in SIM HPLC(ESI)-MS/MS (QqQ) in MRM	–	–	[90]
HFRs and MeO-PBDEs	Mussel	–	Freeze-dried	1.5 g	Hexane/DCM PLE	SPE neutral alumina cartridges Fat quantification with gravimetry and removal with sulphuric acid	–	GC(EI)-MS/MS(QqQ) in SRM and SIM	[a]0.002–6.24 [b]0.008–20.8	51–109	[104,130]
PFASs	Fish	Muscle, brain kidney and liver	Freeze-dried and ground	0.25 g	Heptane-ACN-acetic ac. SE, homogenization and centrifugation	PSA and C18 dSPE and centrifugation	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]0.05–17 [b]0.07–30.59	86–94	[51]
		Blood	Agitation	250 µl	ACN SE, agitation, centrifugation and double ACN micro-LLE		Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in MRM	[a]9–13* [b]113–20.16*	104–107	[51]
PFASs	Fish	Pool	Homogenized	2 g	Alkaline digestion and centrifugation	TFC	–	TFC-LC (ESI)-MS/MS(QqQ) in SRM	[b]0.02–2.26*	16–135	[102]
PFASs	Fish	Liver and muscle	–	0.5 g	ACN SE	Centrifugation and filtration	–	LC(HESI)-HRMS (Orbitrap) in HRPS	[b]0.27–5.4 (muscle) [b]1.2–15 (liver)	86–130	[75]
PFASs	Fish	Muscle	–	5 g	Triple ACN UAE	$MgSO_4$ and NaCl dSPE centrifugation, filtration and HybridSPE® Phospholipid Ultra cartridge SPE Fat removal with HybridSPE® Phospholipid Ultra cartridge	Nitrogen	TFC-UHPLC (HESI)-MS/MS(QqQ) in MRM		40–133	[108]
PFASs	Fish and prawn	–	–	0.5 g	ACN FUSLE and filtration	SPE Waters Oasis-WAX cartridges	Nitrogen	HPLC(ESI)-MS/MS (QqQ) in MRM	[a]0.46–2.47* [b]0.73–4.92*	85–117	[115]
PFASs	Eel	Muscle	Homogenized and freeze-dried	1 g	SE with KOH and MeOH and agitation	Double SPE WAX stationary phase and carbon graphitized (Envicarb)	Nitrogen	LC(ESI)-MS/MS(QqQ) in SRM	[b]0.006–1.259	65–125	[60]
PCBs and PBDE	Eel	Muscle	Homogenized and freeze-dried	1 g	Triple PLE, with toluene and acetone	Triple purification with acid silica gel, Florisil® and celite/carbon columns Fat quantification with gravimetry and removal with silica gel column activated with sulphuric acid	Nitrogen	GC(EI)-HRMS in SIM	[a]0.00016–0.0134	60–120	[60]
PCBs and PCDD/F	Fish	Pool	Freeze-dried, homogenized and ground	10 g	Toluene/cyclohexane Soxhlet	Multilayer silica, basic alumina and carbon columns. Fat removal with Silica gel column with sulphuric acid	Rotavapor	GC(EI)-HRMS in SIM	–	60–120	[94]
PCBs	Fish	Pool		1–3 g	Hexane/DCM Soxhlet		Rotavapor		–	60–120	[94]

Table 1 (*Continued*)

Pollutants	Sample	Matrix	Pre-treatment	Sample size	Extraction method	Clean up method	Drying	Detection	LODs/LOQs (ng/g)	Recovery (%)	Ref.
			Freeze-dried, homogenized and ground			Florisil column. Fat removal with Silica gel column with sulphuric acid		GC(EI)-HRMS in SIM			
PBDE	Fish	Pool	Freeze-dried, homogenized and ground	1 g	Double hexane/DCM PLE	SPE with Al-N cartridges	–	GC(EI and CI)-MS/MS (QqQ)	[a]0.004–0.335 [b]0.013–1.113	59–77	[94]
UV filters	Fish	Muscle and whole body	Freeze-dried, ground, and homogenized	2–4 g	Triple MeOH UAE	Ethyl acetate-cyclohexane GPC Fat quantification with gravimetry and removal with GPC	Rotavapor and nitrogen	UHPLC (APCI)-MS/MS(QqQ) in SRM	[b]0.003–10	42–120	[32]
UV filters	Fish	Muscle	Freeze-dried, homogenized and ground	0.5 g	MSPD	SPE C18 cartridges	Nitrogen	GC(EI)-MS (QTRAP) GC(EI)-MS/MS(QTRAP)	[a]0.02–0.03 [b]0.05–0.1	71–102	[99]
UV filters	Fish, mussel, echinoderm and prawn	Whole body and fish muscle	Freeze-dried, homogenized and ground	1 g	DCM/hexane PLE	SPE Supelclean LC-18 cartridges	Nitrogen	UHPLC (ESI)-MS/MS(QqQ) in SRM	[a]0.9–1.9 [b]2.9–6.2	80.4–114.3	[111]
UV filters, UV stabilizers and musks	Mussel	Pool	Freeze-dried, homogenized and ground	2 g	QuEChERS	Na_2SO_4, Bondesil-C18 and PSA dSPE Fat removal with heptane, centrifugation and filtration	Nitrogen	GC(EI)-MS/MS(QqQ) in SRM	[b]0.5–50	91–112	[106]
BPA and APs	Fish	Bile	–	15 μL	Phosphate, β-glucuronidase, sulfatase hydrolysis for 18 h	SPE HLB cartridges	–	HPLC(ESI)-MS/MS (QqQ) in MRM	[a]0.00054–0.00641*	–	[62]
BPA and APs	Fish, mollusc and prawn	–	Homogenized	–	Double ACN UAE with NaCl	SPE ProElut PSA cartridges	Nitrogen	LC(ESI)-MS/MS(QTRAP) in MRM	[a]0.015–2.2	24–120	[114]
PCBs	Clam	Pool	Homogenized	10 g	Saponification and n-hexane soxhlet	Column chromatography	–	GC-MS/MS	[b]<0.001	–	[95]
Parabens	Fish and shrimp	Muscle	Freeze-dried, homogenized and ground	0.5 g	MSPD	SPE silica gel-C18 cartridges Fat removal with ACN elution	Nitrogen	GC(EI)-MS (QTRAP) in SIS	[a]0.06–0.30 [b]0.2–1.0	93–112	[33]
Parabens and metabolites	Fish, marine mammal, invertebrate and algae	Liver, kidney, brain, muscle, blubber and whole body	Homogenized	0.2–0.3 g	Acetone/MeOH/ACN SE, agitation, and centrifugation	Fat removal with ultralow temperature fat precipitation	Nitrogen	LC(ESI)-MS/MS(QqQ) in MRM	[b]0.82–20.5	11–156	[46,131]

[a] Method LODs.
[b] Method LOQs.
* ng/ml.

2. Sample collection and pre-treatment

The analysis of EPs in the aquatic environment has different purposes, including the assessment of human risk from fish and shellfish consumption, the study of the occurrence of pollutants in the aquatic environment, and or the identification of the behaviour and effects of EPs in biota. Samples are obtained in three main ways: a) from providers, such as farms, markets or fisherman [32,33]; b) directly collected or caught in the study area (bivalves and algae were harvested and fish was caught by e.g. fyke and electro-fishing), or c) are inbred laboratory species [34]. Studies based on the in vivo extraction of fluids or focused on laboratory exposure require facilities to keep biota alive until extraction, as well as the materials and compounds for the euthanasia. In these cases, the samples spend a variable amount of time in a controlled environment, just to keep them alive or for exposure to different pollutants, depuration stage, etc.

Table 1 gathers the methods from 93 different studies. A wide variety of biota samples have been used for the analysis of EPs (Fig. 2A), mostly different species of fish (71 % of the studies) [35–38], where the largest amount of EPs (pharmaceuticals, pesticides, PCPs, PAHs, etc.) have been analysed. Moreover, analytical methodologies for molluscs, including snails [11] and different bivalves, such as mussels [8,19,39,40] and clams [28,41], crustaceans [42,43], vegetal samples [44,45] and marine mammal tissues [46–48] have been reported.

The diversity in aquatic biota (Fig. 2A) and the different types of matrices (Fig. 2B) that can be isolated mean that different pre-treatments are required to extract EPs. As Table 1 shows, a few of them involving liquid (bile, plasma, blood) or solid (brain, liver, muscle) matrices do not include any type pre-treatment [49,50]. Excluding these exceptions, a widely used pre-treatment is homogenization. This is especially important when the sample analysed is the full body of the species, due to the potential selective accumulation of these compounds in some tissues. Homogenization ensures the uniform distribution of EPs in the sample. Claws and fish bones are commonly removed. Similarly, when the sample is a pool of several individuals the homogenization of the sample ensures its representativeness because the concentration of pollutants can be different in each individual. Freeze-drying (or lyophilization) is also widely used, in order to eliminate water and determine a dry weight for an analysis of the contaminants. The proportion of water in the solid samples depends on several factors, such as the tissue or the species (e.g. muscle and blood can contain about 70 % and 90 % of water respectively; fish and jellyfish 65–80 % and up to 99 % respectively). The elimination of water provides results that do not depend on this factor and hence it is commonly applied in biota analysis. After the lyophilization, samples are usually ground to homogenize the matrix for the analysis.

Another important pre-treatment, particularly for liquid matrices, is centrifugation, because this treatment eliminates

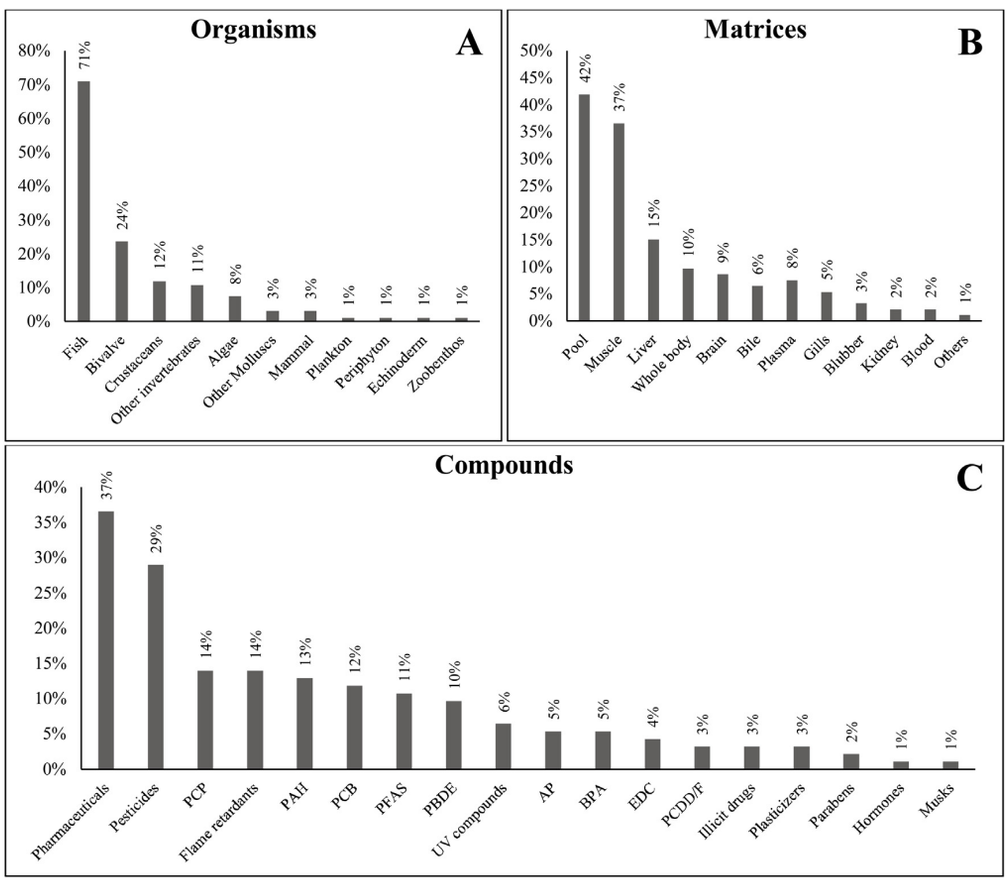

Fig. 2. Distribution of the (A) compounds, (B) organisms and (C) matrices analysed in the studies collected. "Others" includes the matrices analysed just for one study, such us skin, stomach, intestine, bones, biofilm, carcasses, intestine, viscera, gonad and digestive glands. "CC" means "Chromatographic Column" and "CE and or FI" means "Centrifugation and/or Filtration". The sum is >100 % because some studies analyse more than one compound, organism and/or matrix.

suspended solids and other potential interferences. In blood samples, it is used to eliminate the haematocrit, using the plasma or the serum for the analysis. Only two studies of those reported in Table 1 analyse the whole blood [37,51].

3. Extraction

The best extraction procedure to apply depends on the matrix and the EPs of interest (Fig. 2C). Most of the methods have been developed for the extraction of pharmaceuticals (37 %) followed by pesticides (29 %), flame retardants (14 %) and PCPs (14 %). Some specialized procedures focus on a single compound or group of chemically related compounds, like UV filters, or metabolites of a specific compound, with similar characteristics and behaviour. These methods usually have excellent recoveries [45,51–56], but they cannot provide a general overview of the presence of EPs in the matrix analysed. On the other hand, multi-residue extraction methods have been developed to extract the greatest possible variety of compounds and to provide a whole picture of the EPs present, even if they are from different families, saving resources and time. Usually the recoveries present higher variability depending on the compound [11,38,41,57]. Paying attention to the methods compiled in this work, approximately 40 % correspond to multi-residue methods that analyse more than one family of compounds (pharmaceuticals and pesticides, PAHs and flame retardants, etc.), and the remaining 60 % correspond to specific methods (one or a few compounds of the same family). This correlation could show a preference to analyse a few compounds with better accuracy, rather than the preference to get a general overview, or just simply pinpoint how the complexity of the matrix constrains the development of multi-residue methods for biota. The latter involve the challenge of extracting pollutants with a wide polarity range, obtaining clean extracts and good recoveries.

One gap identified by the authors is a lack of standaritation in both nomenclature and reported information among the different articles. Some studies do not provide sample weight, method recoveries, sensitivity, and in some cases, the applied methodology is not even described in detail. In addition, depending on the article, the same procedure can be named in different ways. Montesdeoca-Esponda et al. [6] also highlights the difficulty of comparing articles using different concentration units.

3.1. Liquid samples

Liquid biological samples include blood, bile and plasma. The extraction procedures are usually simpler and with fewer steps than those for solid samples such as muscle or liver (Fig. 3A). In the studies with liquid matrices, the target compounds are mainly pharmaceuticals, followed by PCPs. Many of these compounds have low K_{ow} being easily dissolved in aqueous liquids such as plasma. However, it should be kept in mind that these matrices have a number of binding proteins and lipoproteins that transport from polar to non-polar contaminants. In the case of bile, compounds with a higher K_{ow} value, as BPA, are also analysed. This is due to its lipid and surfactants content that makes it a complex matrix capable of accumulating lipophilic compounds. Enzymatic pre-treatment could be needed for the deconjugation and/or disconnection of the protein-bound or conjugated analytes [37,58–60], ultrasound assisted extraction (UAE) [58] or liquid-liquid extraction (LLE) [51,61] are the main extraction methods used. Just one method to extract BPA and APs in bile applies hydrolysis [62]. In liquid samples the analytes can also be isolated directly using solid phase extraction (SPE) [49,52,63,64] which is an easy and quick alternative to solvent extraction.

3.2. Solid samples

These matrices include different tissues such as muscle, liver or brain, and the whole body of different organisms like fish, algae, bivalves, crustaceans and other invertebrates. They are more frequently analysed than liquid samples (Fig. 2C), and the variety and complexity of the procedures is higher (Fig. 3A). The universal method is solvent extraction (SE) where the compounds are extracted by adding an organic solvent (miscible or immiscible with water) and applying energy (traditionally this was manual agitation, but today other methods are used, including ultrasound, with equal or greater use, and pressure, temperature, microwave or vacuum with a rather sporadic application). The common sources of ultrasound are conventional UAE (ultrasonic bath) and focused ultrasound solid-liquid extraction (FUSLE) [60,64]; which applies ultrasonic waves directly by a micro-tip introduced in the sample. These methods were developed mainly for the extraction of pharmaceuticals [49,65–68], but also for a great variety of other compounds such as flame retardants [69–71] and pesticides [29,56,72].

SE is applied for specific as well as for multi-residue extractions and is commonly performed with organic solvents (methanol (MeOH), acetonitrile (ACN), hexane or acetone depending on the target compounds). Important parameters, for which there is no agreement among the studies, are extraction time, solvent volume and number of extractions performed. Some methods involved only one extraction [60,73–75] while others repeated the extraction several times [76–78] to achieve the extraction of as many analytes as possible at high efficiency from the matrix. The volume of solvent is also variable. The use of higher volumes of solvent commonly improves extraction efficiency but also requires a longer evaporation processes. The extraction time is also important. Longer periods of time provide better recoveries, but, for example, with the ultrasonic bath (in the case of UAE), the extraction time usually does not exceed 20 min [19,65,66,79]. Elevated temperatures (as an additional source of energy) can improve extraction. Dasenaki et al. [67] set the ultrasonic bath to 60 °C, improving the recovery of several pharmaceuticals and drugs. Nevertheless, high temperatures also degrade thermolabile compounds such as carbamazepine [80].

The current trend in the development of extraction methods is to reduce the consumption of organic solvents in order to achieve greener methods that are less polluting. A good example, which deserves a special mention for its wide use is QuEChERS, which has a strong presence in the studies. It is mainly used for the extraction of whole body fish [27,36,42,81,82] matrices and bivalves [27,83,84] and to a lesser extent in invertebrates [34] and fish muscle [38,85,86]. Among the compounds extracted with this method are pharmaceuticals, pesticides, PFASs and UV filters, in both multi-residue or specific compound extraction procedures using several versions, including a miniaturized version called micro-QuEChERS [87]. The miniaturization of the method is within this “greener” trend and adjusts the amount of reagents to the use of smaller samples to generate less waste. This method was originally designed to process samples that are difficult to obtain in large quantities, such as blood or small invertebrates.

Pressurized liquid extraction (PLE) is an automatic process that requires less solvent because high pressure and temperature facilitate extraction. It can achieve better recoveries than QuEChERS [88], but also requires longer extraction times. PLE is applied in several methods, mainly for the extraction of pharmaceuticals, PCPs, brominated and chlorinated compounds in bivalves and different fish tissues [10,45,60,89–91]. Vacuum assisted extraction (UVAE) enhances the penetration of the solvent in the matrix and allows faster extraction than the UAE [40]. As shown in Fig. 3A, a few methods involved Soxhlet [86,92–96],

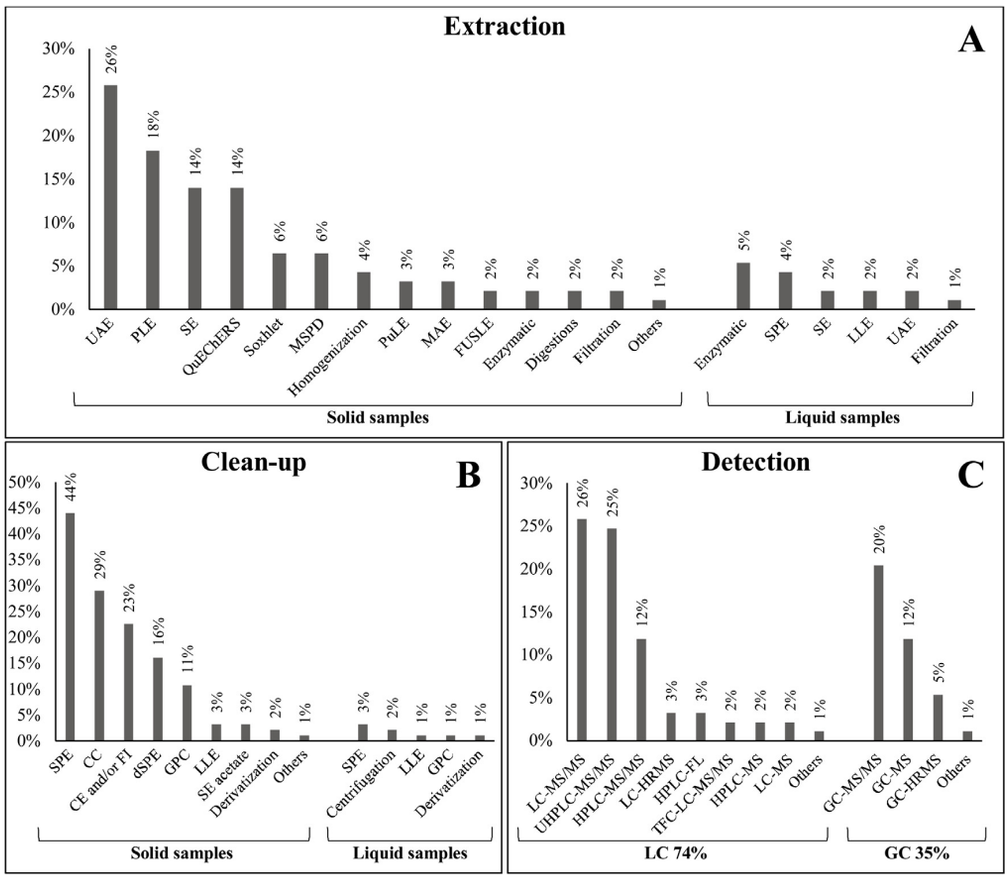

Fig. 3. Distribution of the (A) extraction, (B) clean-up and (C) detection techniques employed in the studies collected. "Others" includes the techniques employed just for one study. In (A) extraction: micro QuEChERS, soxhlet, glass column, stir bar sorptive extraction, UVAE, alkaline digestion and saponification. In (B) clean-up: decantation, Captiva ND lipid, QuEChERS, Ostro, HPLC, alkaline digestion, TFC. In (C) detection LC: HPLC-FL-DAD, HPLC-DAD, nanoLC-MS/MS, HPLC-HRMS, TFC-LC-HRMS, UHPLC-DAD, TFC-UHPLC-MS/MS and IC-MS/MS. In (C) detection GC: HRGC-ECD, HRGC-MS and HRGC-HRMS. The sum is >100 % because some studies use more than one extraction, clean-up and/or detection technique.

matrix solid phase dispersion (MSPD) [33,44,48,97–99], microwave assisted extraction (MAE) [2,100,101] and sorptive extraction [54]. Alkaline digestion is specifically used for the extraction of PFASs in fish and bivalves [102] since these contaminants are resistant to the basic treatment that denatures proteins and breaks their bond to PFASs. This treatment also oxidizes some of the organic matter in the sample providing cleaner extracts.

4. Clean-up

The clean-up of biota tissue extracts is dominated by the need to eliminate matrix components, but their removal is another big challenge. Biota, particularly invertebrates and fish, have a protein content of 10–15 % and a highly variable fat content that can reach 30–35 %. The presence of these proteins and fats generates interferences in the signal when the samples are analysed. Furthermore, tissues with a higher lipid content preferentially accumulate non-polar (lipophilic) compounds (high K_{ow}) [103] and adequate processes are required to extract them (together with the non-polar EPs), and further to reduce their concentration in the extract, keeping the analytes in order to get a clean extract for chromatographic analysis [53]. Such is the importance of lipids that there are methods whose clean-up focuses exclusively on their reduction [32,50,54]. Furthermore, determination of the lipid content of the tissue provides information about the distribution and accumulation of the compounds in the animal.

Even so, not all methods compiled in Table 1 use a fat removal process. Some of them only quantify the amount of fat in the sample to determine its degree of interference. An extended method for fat quantification is gravimetry after non-polar solvent extraction.

The simplest clean-up methods just apply simple physical procedures to the extracts, such as centrifugation [34,59,60,104], filtration [41,74] or both [27], even sporadically, some methods do not use the clean-up step. Cho et al. [42] compared the QuEChERS method with and without the clean-up step, and showed that recoveries obtained for pesticides are similar in eel and shrimp. Other reports combined several physical procedures (some of them specifically for fat removal), such as the method developed by Liu et al. [105] who use decantation combined with low temperature precipitation (the most common method for fat removal among the studies present in the Table 1). The mainstay of this method is the higher solidification point of the lipids compared to the solvents used for the extraction and analytes to be determined, so they precipitate and are easy to remove when they are solid at low temperatures, while the extract containing the analytes remains liquid. Some methods also use centrifugation to facilitate even more the separation of the different layers of the extract.

Liquid-liquid partitioning with organic solvents immiscible with the extract such as hexane, heptane, or DCM, are among the oldest lipid removal clean-up processes [97]. Their efficiency depends on the previous extraction method and the selected target compounds. Tsai et al. [99] showed that n-hexane exhaustively removes lipids from the extract but analytes (salicylates and benzophenone type UV filters) recoveries were very low. On the contrary, the use of ACN does not remove the lipids successfully but analytes are better recovered.

Column chromatography separation with polar sorbents is the most applied technique which, in many cases, has evolved to the cartridges format (Fig. 3B). Currently, both formats coexist (used by 29 % and 44 % of the studies respectively). An important part of the clean-up will depend on the sorbents, silica (neutral or washed with acids and/or bases) and Florisil® (Magnesia silica) being the most characteristic fat and other non-polar compound retainers [97]. These sorbents are utilized mainly to clean-up extracts with PCBs, PAHs, flame retardants or pharmaceuticals [28,40,41,60,92,94]. However, Florisil® cartridges do not achieve recoveries as good as those of the HLB cartridges for moderate to polar analytes such as pharmaceuticals [72]. There are other formats for these sorbents, such as "96–well plates" or filters, whose application is increasing, especially when the volume of the extract to clean-up is low. The dispersive SPE (dSPE) is also widely used [42,71,81,84,85,87,106] (mostly in combination with QuEChERS extraction), but it does not have the same presence in the studies reported in Table 1 as the extraction step and it is mainly used in extracts in which pesticides are intended to be determined. Some of the common sorbents used in dSPE are PSA for removing sugars and fatty acids, C18 to remove remaining lipids and the MgSO4 is to remove water [85].

Two chromatographic separation systems —gel permeation chromatography (GPC) [63] and turbulent flow chromatography (TFC) [53] —were also proposed to separate analytes and matrix co-extractants in complex matrices. GPC separates analytes from the extract co-extractants by differences in their molecular size, allowing the separation of large molecules such as lipids and proteins from small ones (like most of the contaminants considered). The choice of the correct sorbent, present in the column, and the size of its pores, determines the quality of the extract. TFC combines GPC and traditional chromatography principles to separate co-extractants from analytes rapidly according to both size and polarity [107]. It is effective in excluding molecules such as particulate and proteins. In recent years, TFC was applied to environmental samples as an automated clean-up step in the determination of EPs as PFASs [108] and flame retardants [104] combined with solvents such as dichloromethane (DCM) and alumina or silica sorbents.

Despite the general availability and usefulness of the above-mentioned chromatographic systems, the most used approach based on solid sorbents is SPE (44 %). Different cartridges were reported depending on the target compounds, HLB being the most common cartridges [37,64,65,68,78,88,109,110]. SPE is utilized mainly in methods that involve pharmaceuticals and PCPs as target compounds [37,65,68,88,109], but they also provide good recoveries (>80 %) in methods that analyse PFASs [11] or UV filters [111], among others. This makes SPE a clean-up suitable for multi-residue extraction procedures, and, as seen in Table 1, it has been used in almost all of the matrices studied. The last trend within this field is the development of specific sorbent variations for matrices with a high content of lipids that can be used in multi-residue extractions, like the Captiva ND (separates phospholipids) [38], Ostro™ (eliminates phospholipids and proteins) [72] or EMR-Lipids (removes lipids based on a combination of size exclusion and hydrophobic interaction). The application of these new sorbents is still incipient given their recent commercialization, although they offer a very promising solution to the problem of lipids in these types of matrices.

More than one clean-up technique can be applied to the same extract. For example, Xu et al. [40], in order to extract a wide range of flame retardants, developed a method that included several steps involving the partition of the extract in hexane and DCM, SPE using different silica cartridges and several dSPE steps. The main problem of this practice is that the sample preparation step gets longer and more complicated and selective for some analytes, which is counterproductive to rapid extraction and extracting as many compounds as possible.

5. Determination

The most extended techniques for the determination of EPs are liquid chromatography (LC) and gas chromatography (GC), usually used in tandem with mass spectrometry (MS). The first is the most extended method in the studies covered in this review (present in the 74 % of the studies) (Fig. 3C). These techniques enable an accurate qualitative and quantitative identification of the target compounds. Moreover, with the use of high resolution mass spectrometry (HRMS) [38], wide-screening and non-target approaches that could detect unknown or unexpected compounds or just screen a wide range of compounds using a database instead of analytical standards can be implemented.

Mass spectrometry can be performed in MS or MS/MS modes. MS/MS requires the use of two mass analysers and provides information about the precursor and the product ions of a molecule. They are based on quadrupole mass analysers. Triple quadrupole (QqQ) or linear ion traps (QTRAPs) are the most used (Table 1). These systems have the disadvantage of low sensitivity in scan mode (used only rarely). These instruments commonly work using a multiple selection of monitored reactions (SRM). This mode is based on selecting ion precursor→ion product transitions specific to a particular analyte. The technique is therefore very sensitive but demands an "a priori" selection of the EPs to be determined and any other compound will always be invisible to the detection system.

High-resolution mass spectrometry (HRMS) is an alternative that overcomes this problem. The two most used mass spectrometers are the quadrupole time of flight (QqTOF) and the quadrupole orbitrap (QOrbitrap). The orbitrap has better mass resolution (FMHW ca. 60,000–100,000) but longer cycles while the QqTOF (FMHW ca. 30,000) does not reach the resolution of the orbitrap but the cycles are shorter and the number of compounds that can be detected is higher. The high resolution and high mass accuracy can elucidate the most probable empirical formula of the compounds. These instruments also can record MS and MS/MS full mass spectra with high sensitivity. Then, retrospective analysis of the samples is possible together with GC (GC-HRMS) [35,43,60,70] and with LC (LC-HRMS) [43,75].

5.1. GC–MS

Gas chromatography—mass spectrometry (GC–MS) has most commonly been used for the analysis of semi-volatile organic contaminants in environmental and biological samples [41], such as flame retardants, PCPs, PAHs, PCBs or polybrominated diphenyl ethers (PBDEs) [35,38,40,41,48,54,60,89,93,95,97–99,112]. In particular, GC–MS/MS, has proven to offer a favourable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity for the analysis of semi-volatile and volatile organic compounds [98,113]. GC–MS is also used for the detection of lipophilic compounds, such as UV filters [99]. Baduel et al. [38] also analysed pollutants in biota samples by both LC–MS and GC–MS focussing GC–MS on the determination of non-polar and semi-polar compounds such as pesticides, UV filters, PAHs, PCBs and PBDEs that provided better results.

In GC–MS, the most common ionization mode is electron impact (EI) (Table 1). However, other softer ionization modes can improve the determination of some compounds. In an interesting study, Xu et al. [40] developed a multi-residue analytical method to determine a range of flame retardants. The final analysis of phosphorus flame retardants (PFRs) was performed on GC–EI-MS, while PBDEs and emerging flame retardants were measured by GC–electron capture negative ionization (ECNI)-MS [40]. ECNI-MS is a softer ionization method than EI. The main advantage in this example is its higher sensitivity to compounds with brominated atoms, such as PBDEs. The use of GC-atmospheric pressure chemical ionization (APCI)-MS/MS has also been tested successfully in the determination of flame retardants [48].

GC can be also performed without MS. Bonnineau et al. [10] and d Luna Acosta et al. [89] determined organochlorine pesticides (OCPs) and PCBs with high resolution gas chromatography (HRGC), coupled to an electron capture detector (GC-ECD).

Of the various MS detectors, although the most used at present is the QqQ, the single quadrupole is still widely used. This is due to the existence of very elaborated mass libraries that help in identification. The HRMS is being introduced with force in this outlook because it presents additional advantages such as high sensitivity working in full scan, GC-HRMS has already been applied to the determination of UV filters [43] and ePOPs [PBDEs, polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs), polychlorinated naphthalenes (PCNs), OCPs, short-chain chlorinated paraffins (SCCPs) and Dechlorane Plus (DP)] [60,70,91,94]. However, analytes were identified in a traditional way by comparing the GC retention time and the ion-abundance ratio of two exact m/z with the corresponding retention time of an authentic standard and the theoretical or acquired ion-abundance ratio of the two exact m/z's.

5.2. LC–MS

LC is a type of chromatography that can be used for any analyte soluble in a liquid phase, such as MeOH, ACN, water and their different mixtures, among others. LC is one of the most extended techniques in the analysis of EPs and the most used in these studies (74 %) (Fig. 3C). Classic LC–MS/MS is preferred (26 %), being used for the analysis of pharmaceuticals [50,63,73,78], PCPs [34,45], BPA [62,114] flame retardants [41,70,71], pesticides [42], PAHs [41,46] and PFASs [77,102,115] among others. Ultra-high-performance liquid chromatography (UHPLC) is the enhanced version of classic high performance liquid chromatography (HPLC). Both HPLC and UHPLC use high pressure to pump the mobile phase through the column but UHPLC employs shorter columns and with smaller particles (< 2 μm), using less solvent and time for the analysis but requiring special instrumentation able to support high pressures. UHPLC is more expensive but it has better resolution than HPLC, and it is becoming a widely applied technique (Fig. 3C) [27,28,49,58,68,69,77,116]. HPLC-MS and HPLC-MS/MS were applied to determine pharmaceuticals [39,65,66]. Diode array detectors (DAD) enable one to choose the best wavelength for each compound, enhancing FL detection (restricted to few analytes) when coupled as HPLC-FL-DAD [39]. However, DAD has less specificity and sensitivity than MS/MS. In the study by Pacheco-Juárez et al. the limits of detection (LODs) and limits of quantification (LOQs) obtained with UHPLC-DAD were up to three times higher than those obtained with UHPLC-MS/MS [101]. Similarly, Nag et al. also reported low sensitivity: LOQ > 10 ng/g [92]. Ionic Chromatography (IC) uses ion exchange for separation and, coupled as IC-MS/MS, it showed high specificity and good selectivity for very polar compounds [56]. Electrospray ionization (ESI) is the common technique for the identification of EPs although ESI systems are more susceptible to matrix interferences, in comparison with APCI and atmospheric pressure photoionization (APPI) techniques [117]. The three techniques involve a soft (low-energy) ionization resulting in little fragmentation. Most abundant species formed are molecular or quasi-molecular ions, resulting in the subsequent increase in sensitivity and selectivity of MS/MS methods [48,118]. Zacs and Bartkevics [55] tested the effectiveness of APPI, APCI and ESI to determine flame retardants showing that APPI provides the most selective results.

QqQ is the most widely used analyser but, in the field of LC, HRMS mass analysers have been introduced for the analysis of pharmaceuticals [72], pesticides [119], pharmaceuticals [72], UV filters [43], PFASs [75] and flame retardants [55]. The latter mass analysers attain a wide screening against databases enlarging the scope of the analysis and attaining detection of non-target compounds.

The use of just LC–MS or GC–MS is not always enough to identify all of the target compounds, and additional tests can be required. In the studies compiled in Table 1, there are three different strategies:

(a) use several extraction procedures [70,90];
(b) perform one extraction procedure at the beginning, but, at a certain point, take several aliquots and carry out different extraction or clean-up procedures on each one [38,41]; and
(c) separate the analytes along the column clean-up eluting the analytes with different solvents [43,87]. This enables one to obtain different fractions, which are selective for some classes of analytes that are then analysed by GC–MS or LC–MS.

6. Application

The main objectives of the works cited in Table 1 are: (i) the development of extraction methods, (ii) the determination of EPs in biota samples, and (iii) obtaining knowledge in different fields (bioaccumulation, metabolomics, bioremediation, etc.) by carrying out experimental laboratory studies.

6.1. Development of extraction procedures

It is necessary to develop and test new techniques that could achieve better results (higher recoveries, cleaner extracts, etc.), expending less resources and providing easier procedures. Such is its importance that several of the studies collected are focused on this task [27,42,54,72,83,88,119,120]. The methods developed in these studies have been tested in environmental samples to pinpoint their possible application for determining EPs in environmental matrices, such as monitoring, trials, risk assessment, etc. [33,42,64].

6.2. Field studies

The occurrence of EPs in the biota has gained prominence in the last decade [60,111,116]. This information can be used in different types of studies, such as environmental and human health risk assessment, determination of adverse effects of EPs in the biota or even in humans [95]. Typically studied areas are those near known sources of pollutants such as lakes and rivers adjacent to wastewater treatment plants [43,66,105,116] and the coastal areas next to metropolitan areas or estuaries [6,111]. As stated before, the occurrence in biotic samples is not as widely studied as in abiotic samples. The presence of lipids and proteins poses a challenge for the determination and quantification of compounds. The occurrence of EPs in different fish tissues has been one of the most studied aspects [37,60,105,111,114,116], followed by the establishment of their levels in filter-feeding organisms [39,41,106,111] since they have a vital role in water depuration. There are some remarkable studies. Scott et al. reported concentrations of the

pharmaceutical diltiazem in fish plasma that exceeded human therapeutic levels [63]. The concentration of several UV filters in mussels analysed by Picot Groz et al. exceeded the maximum allowable concentration (MAC) established as an environmental quality standard (EQS) under the EU Water Framework Directive (above to 50 μg/kg) [106]. In the studies from Jürgens et al. and Huang et al. the Canadian EQS limits for pesticides and PAHs were exceeded in eel and trout respectively [93,112]. In the study from Casatta et al. on the occurrence of EDCs, the limit for the sum of six PBDE congeners set for the biota in the European Directive (2013/39/EU) to protect human health was exceeded 4–5 times in Manila clams [28].

It is important, when assessing the possible risk of the presence of EPs for the biota, to understand their adverse effects, but there is a lack of knowledge about these effects. Studies that determine the occurrence of EPs can use the same samples to determine if they present potentially adverse effects [35,60]. Furthermore, other levels of the trophic chain can be affected by the presence of EPs [116]. If we identify the occurrence of EPs in human consumption products, their risk for human health can also be assessed. Some studies focus on samples from fish and shellfish farms for human consumption [32,104,111]. Most studies showed that human exposure to EPs from dietary fish poses little or no risk to human health [26,37,112,121,122]. But their occurrence in the environment poses environmental risks, ranging from low [105] to significant [111].

6.3. Laboratory studies

Extraction procedures are usually utilized to analyse samples from trials performed in a controlled environment. There have also been environmental trials such as those performed by Luna Acosta et al. where oysters were transplanted to different areas, and analysed, after a three month period, to determine the environmental presence of PAHs and POPs [89]. In a laboratory trial, after a controlled exposure period, it is possible to determine the uptake, biotransformation and elimination of EPs by the organism [123]. Attending to the transformation products is also possible to establish the metabolic pathways [68] and identify potential exposure biomarkers [39]. Analysis of the different tissues of the organisms provides information about the distribution of pollutants [65] and their bioaccumulation [50], which makes it possible to assess their environmental and human health risk. In the trial carried out by Hedrick-Hopper et al. [82] on the Atlantic croaker (*Micropogonias undulatus*), the treated fish was subjected to a depuration period in order to observe how they recover, after exposure to triclosan. The uptake and elimination by the organisms can be utilized as bioremediation. Ismail et al. [78] studied the potential utility of bivalve augmentation for improving water quality, removing EPs from the water. The objective of the trial could be the study of the synergy and interaction of EPs with other compounds. As an example of this, Pittura et al. [2] exposed aquatic organisms to a known concentration of PAHs with and without the presence of microplastics, confirming that microplastics can transfer adsorbed organic contaminants to tissues of marine organisms. The study of these interactions is important due to the ubiquitous and simultaneous presence of microplastics and EPs in the aquatic environment.

7. Conclusions and further research

The analysis of EPs in aquatic biota samples involves different techniques, procedures and the need for extensive knowledge about the physico-chemical properties of the compounds, the composition of the samples, etc. Therefore, based on the knowledge compiled in this work, three main points can be highlighted: (i) an emphasis on fat removal, (ii) the development of multi-residue vs specific contaminants and (iii) the implementation of a standardised nomenclature.

The lipid content in biota samples still poses a challenge when determining EPs. Its importance will depend on the fat percentage present in the sample, with eel or blubber being the most problematic since they are the fish samples with higher fat content. This becomes even more crucial and difficult when the target compounds are lipophilic. In the studies collected, some do not use a fat removal step, and some even achieve better recoveries when they skip that step. It is necessary to improve fat removal methods; they should be able to remove all, or almost all, of the fatty content without interfering with the compounds' recoveries. Furthermore it should be easy, fast and cheap. Different sorbent manufacturers are currently developing new products capable of eliminating phospholipids, proteins, triglycerides and other endogenous molecules. It is hoped that in the near future, the methods that include these phases in their design will grow.

Analyses of a single family of compounds are easier to perform due to the complexity of the biotic matrices and the different physic chemical properties of EPs belonging to different classes. Even so, EPs are ubiquitous, implying that different families of compounds can occur simultaneously in the same matrix. Research is needed to find new methods capable of extracting as many compounds of as many classes as possible in the same process. They would provide a general perspective on the occurrence of EPs to help evaluate their possible risks and synergies. The multi-residue methods also facilitate laboratory exposure experiments using multiple EPs.

Finally, yet importantly, one of the challenges identified in this review is the standardization of the scientific terminology and information gaps that still exist. In several works, the terminology differs from one to another, e.g. ultrasound assisted extraction as "UAE" [11], "US" [27], or simply "the sample was ... ultrasonicated" [37]. These differences can generate confusion for the reader, even more so if the reader does not have enough background on the topic. The standardisation of terminology could help information exchange in the scientific community and would help scientific dissemination. Moreover, information on sample weight, LODs, LOQs and recoveries is very valuable and should be provided.

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Declaration of Competing Interest

None.

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PROPIEDAD
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SECCIÓN 2.
FUENTES Y EVALUACIÓN DE CONTAMINANTES ORGÁNICOS EN MEDIOS ACUÁTICOS

02

ARTÍCULO

Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes

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Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes

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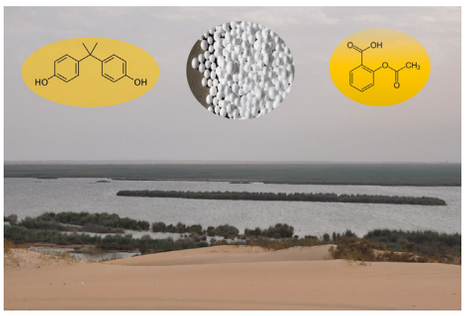
HIGHLIGHTS

- Occurrence of pharmaceuticals, personal care products, pesticides and microplastics.
- Of 107 contaminants, 40 were in water, 27 in sediments, 17 in soils and 21 in plants.
- Microplastics ranged from 0.7 to 9 items/L were distributed in all the area.
- Average of 10 contaminants simultaneously in each sample.
- Chlorpyrifos, diazinon, bifenthrin, caffeine and etoricoxib show risk to biota.

GRAPHICAL ABSTRACT



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ABSTRACT

This study assess the presence of pharmaceutical and personal care products (PPCPs) and pesticides in different environmental compartments and microplastics in water of a characteristic lagoon wetland in Saudi Arabia to establish the transport, accumulation and fate of these pollutants in a water-stressed area under high anthropogenic pressure. In water, diazinon (up to 1016 ng L^{-1}), caffeine (up to 20,663 ng L^{-1}), diclofenac (up to 1390 ng L^{-1}) and paracetamol (up to 3069 ng L^{-1}) were at the highest concentrations. The substances with the highest frequency of detection were carbendazim, atorvastatin, caffeine, etoricoxib, lorazepam, metformin, ofloxacin, paracetamol, salicylic acid and tramadol. Considerably less pesticides and PPCPs at concentrations ranging from 0.01 to 126 ng g^{-1} dry weight (d.w.) were detected in the other matrices (sediment ≫ soil > plants). The concentration of microplastics in water ranged from 0.7 to 7.8 items/L in the Al-Asfar lake and from 1.1 to 9.0 items/L in the Al-Hubail lake. Risk assessment [using hazards quotients (HQ)] was used to highlight pesticides and PPCPs of major ecological concern that should be closely monitored to avoid adverse effects.

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1. Introduction

Wetlands provide countless ecosystem services, but are in a fragile equilibrium easily alterable due to the current anthropogenic pressure accentuated by the prevailing scenario of global change (Eid et al., 2019; Hussain et al., 2019; Reid et al., 2019). Nowadays, wetlands rely more and more on the use of non-conventional water resources to partly alleviate water scarcity receiving an excess or surplus of water used in various human activities (e.g. domestic wastewater, agricultural runoff, etc.), previously treated or not (Elgallal et al., 2016; Hussain et al., 2019; Shifflett and Schubauer-Berigan, 2019). Thus, these areas are affected by emerging contaminants that are constantly being introduced through these non-conventional water resources and characterized by becoming increasingly abundant and being able to affect the biota and, ultimately, the human being (Margenat et al., 2017; Nuel et al., 2018). Although studies on these compounds in wetlands are still scarce and more data are needed, occurrence of pharmaceuticals (Afonso-Olivares et al., 2017; Bayen et al., 2016; Bradley et al., 2017; Cesen et al., 2018; Moeder et al., 2017; Sandoz et al., 2018; Yang et al., 2017), personal care products (Bellver-Domingo et al., 2018; Cesen et al., 2018; Moeder et al., 2017; Yang et al., 2017), pesticides (Moeder et al., 2017; Sofia Plastani et al., 2019), microplastics (Alam et al., 2019; Bayo et al., 2019; Fan et al., 2019) or other compounds (Lorenzo et al., 2019b) have been reported in these areas all around the world. Most of these studies have been performed in water (Bellver-Domingo et al., 2018; Margenat et al., 2017), few also in sediments and/or aquatic biota (Lorenzo et al., 2019b), sparse in soils and up to our knowledge none in wild plants. This highlights the need to correlate the different environmental compartments (Shifflett and Schubauer-Berigan, 2019). Another information gap in these studies is that they individually cover only a few emerging pollutants —usually, of a single type— in a given area, so that a comprehensive overview of the emerging pollutants present is generally lacking (Nuel et al., 2018). The use of these non-conventional water resources could cause serious environmental problems, especially in arid and semi-arid areas, such as the Middle East, a water-stressed zone where wastewater can already be the main water supply for agriculture (Elgallal et al., 2016; Hussain et al., 2019; Maceda-Veiga et al., 2018; Picó et al., 2019). At present, general information on the status and general trends of contamination of wetland resources in Saudi Arabia is virtually non-existent, and it is therefore difficult to predict future changes (Al-Obaid et al., 2017). The preservation of these interesting environments is absolutely needed to ensure that the effects of climate change are mitigated and the knowledge of the whole contamination pattern can be of help (Shifflett and Schubauer-Berigan, 2019).

In accordance with the above mentioned and considering the shortcomings detected, the main objectives of this study were to increase the existing knowledge on the presence, distribution and fate of a large number of pharmaceuticals, personal care products and pesticides, as well as microplastics, in wetlands influenced by treated or untreated wastewater discharges. This study is focus on the fragile zone of the Al-Asfar Lake, (east of Al-Hassa Oasis), one of the most representative shallow wetland lake in Saudi Arabia, which is impacted by water discharges enriched with chemical fertilizers, domestic wastes, and industrial effluents. This is a natural landmark of special significance because its pollution can seriously affect its ecological value and biodiversity, as well as the quality of the water. Few studies were already carried out in this Lake to asses water quality, phytoplankton community, ecological status (Fathi et al., 2009) and occurrence of heavy metals (Al-Sheikh and Fathi, 2010; Fahmy and Fathi, 2011; Hussein et al., 2016) from a limnological and ecological perspective, as well as their effect on the aquatic biota (Abdel-Moneim, 2014). These studies did not provide information on the presence of emerging contaminants crucial to establish the water quality. Thus, these compounds' occurrence and distribution were comprehensively assessed, sampling surface water, sediment and soil samples, and wild plants in different sites along the irrigation channels and the Al-Asfar and Al-Hubail Lakes, to establish the fate of pharmaceuticals, personal care products, pesticides and microplastics (only on water) from the contamination source to receiving Lakes. This study is up to our knowledge the first assessment on the occurrence of emerging contaminants in shallow lakes of Saudi Arabia and the first time that these contaminants are evaluated in wild flora.

2. Experimental

2.1. Chemicals and reagents

High purity standards (98–99.9%) of the 59 currently-used pesticides and 17 acidic and 16 basic or neutral PPCPs were from Sigma-Aldrich (Steinheim, Germany) (listed in Supplementary material Table S1 together with their acronym, CAS number, empirical formula, Log Kow and solubility in water). Ibuprofen-d_3, acetaminophen-d_3, carbamazepine-d_2, diclofenac-d_4. triclosan-d_3, atenolol-d_7, bisphenol A-d_{16}, imazalil-d_5 sulfate and terbuthylazine-d_9 were used as internal standards (IS). Pesticides stock standard and working solutions were prepared in methanol, the PPCPs ones were prepared in a mixture water-methanol (70:30, v/v) and were stored at 4 °C and −20 °C, respectively. Methanol, LiChrosolv®, hypergrade para LC-MS, para HPLC, Supelco® was bought from VWR (Radnor, PA, USA). Ammonium formate and formic acid from AMRESCO (Solon, OH, USA), both at the highest purity grade. Ultra-pure water was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). Citric acid, Na_2HPO_4, Na_2-EDTA were purchased from Alfa Aesar (Karlsruhe, Germany). Strata X 33U polymeric reversed phase (200 mg/6 mL) were from Phenomenex (Torrance, CA, USA).

2.2. Location and sampling

The studied area is located in the eastern province of Saudi Arabia about 60 km of the Arabian Gulf and comprise the drainage channels of the Al-Hufuf (south) and Al-Oyun (north) and the Al-Asfar and Al-Hubail lakes that ultimately receive the water. This area with a population of about 1.3 million people is supplied with water from the Al-Hassa Oasis and its irrigation network that delivers 328,000,000 m^3/y of spring water to about 22,000 farms, with additional water supplied by treated wastewater (ca. 12,780 m^3/y) from Al-Hufuf sewage station. This irrigation network discharges excess water (mostly polluted water from farms, factories and domestic sewage) without any treatment, just drain via an extensive drainage scheme to the Al-Hubail and Al-Asfar Lakes. Furthermore, this area is closed to the Ghawar oil field. Al-Asfar Lake is situated 13 km east of Al-Hassa Oasis and is one of the largest important shallow wetland lakes in the Eastern Province of Saudi Arabia and in the Arabian Gulf region. Al-Asfar Lake has a unique wetland habitats and vegetation, as well as large expanses of open water with hydrophytes and phytoplankton communities. Since February 2019, Al-Asfar Lake considered as protected area from Saudi Ministry Environment, Water & Agriculture because it has a unique and diverse wetland ecosystem of wild vegetation and animals such as turtles, fish as well as native and migratory birds. Al-Hubail Lake is located to the North East of the Al-Asfar Lake, the lake is composed primarily of agricultural drainage water accumulating from Al-Oyun region, and in a lesser

extend of wastewater from nearby industrial plants. The Al-Hubail Lake is visited twice a year by migratory birds. Halophytes, mostly *Phragmites* reeds, grasses and sedges grow around their edges that are used as animal fodders. This region has a severely arid climate that is characterized by average annual rainfall of 68 mm and annual evaporation of 3204 mm. The treated wastewater and the agricultural runoff contribute enough water to ensure this system preservation. However, there is no information on the occurrence of emerging contaminants including microplastics that contaminates its biotic and abiotic components.

The sampling campaign was carried out during the winter 2017–2018. Ten sampling stations were established, 5 in Al Asfar and 5 Al Hubail. Fig. 1 shows the map of the sampling sites that were distributed to represent all variations of the main channels that carries the wastewater to the lakes and the South, Middle and North sections of the two lakes (GPS geo-references are listed in Table S2 and several pictures in Fig S1 shows the characteristics of the sampling points). In each station, water, sediment, soil and plants were sampling according to NEIC (1985). This guideline basically recommends that each sample were taken in triplicate (120 samples in total) for quality control analysis, to avoid contact with rubber and to ensure safety of the operators. Then, following its instructions, after samples were collected with clean glass (washed with acetone and dried) jars that were dipped to collect 1 L of surface water. Jar lids were lined with acetone-washed aluminum foil. Surface soils of the first 10 cm (500 g) were taken with stainless steel scoop, sediment (500 g) with disposable core tubes and plants (500 g) with the help of a clipper and small shovel. All of them were stored in the same glass jars as water. Samples were chilled by crushed ice during transport to laboratory for further analysis.

Microplastics were obtained in duplicate passing each time 20 L of water collected between 0 and 1 m in depth with glass jars and filtered through a nylon plankton net with a circular opening (0.65 m in diameter, 1.55 m in length and 333 μm of diameter in mesh size). The net residue from each site was washed with filtered pure water into a glass bottle. Before laboratory analysis, the samples were fixed in 5% formalin at 4 °C.

2.3. Extraction and determination

2.3.1. PPCPs and pesticides

Once at the laboratory, surface water samples were filtered with glass microfiber filters (90 mm Ø) and stored at −20 °C until the analysis by solid-phase extraction (SPE) with STRATA-X Polymeric Reversed Phase cartridges following a previously described method (Carmona et al., 2014; Carmona et al., 2017; Ccanccapa et al., 2016a; Ccanccapa et al., 2016b). Lyophilized sediments, soil and plant were (sieved, 2 mm Ø) and extracted by ultrasound assisted extraction using methanol distilled water and McIlvaine–EDTA buffer (pH = 4.5) followed by the same SPE clean-up procedure as used for water samples (Carmona et al., 2017; Ccanccapa-Cartagena et al., 2019). Details of extraction methods are shown in Fig S2.

The chromatographic instrument was a 1260 Infinity Ultra-High-Performance Liquid Chromatograph (UHPLC) combined with an Agilent 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS) with an electrospray ionization interface (Agilent Technologies, Santa Clara, CA, USA). Data were processed using a MassHunter Workstatin Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan). Instrumental parameters and selected reaction monitoring (SRM) used for PPCPs and pesticides determination are detailed in Tables S3 and S4.

2.3.2. Microplastics

Once at the laboratory, water samples were filtered through a stain steel sieve of 0.3 mm of diameter of the openings (slightly

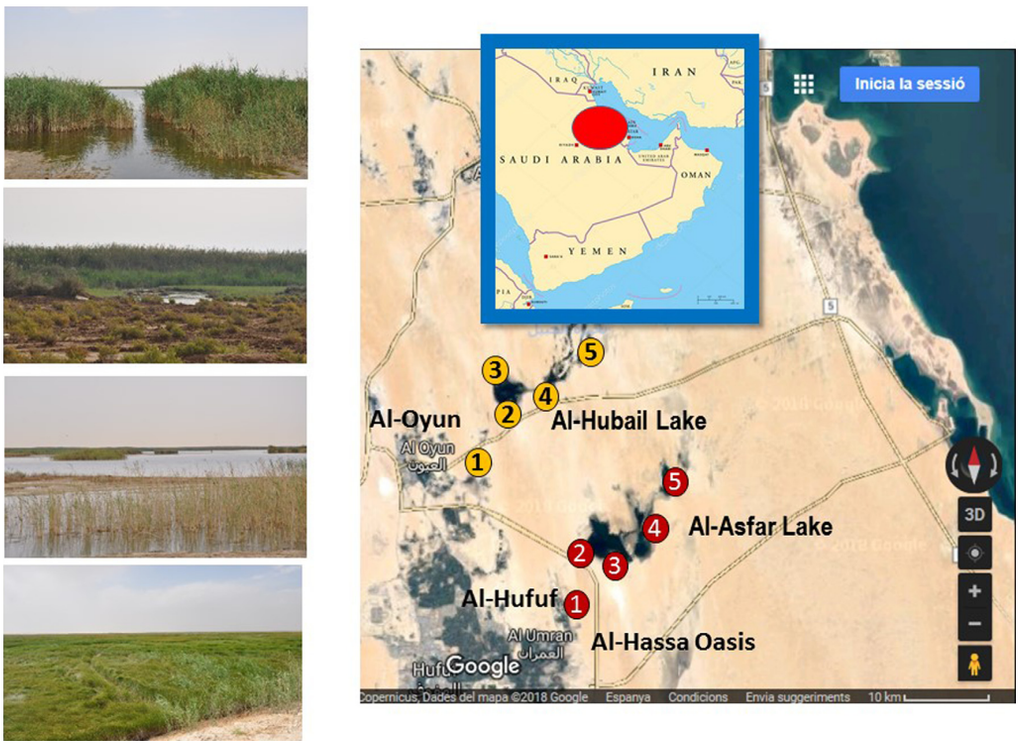

Fig. 1. Location of the sampling sites (indicated by stars) within Al-Asfar and Al-Hubail Lakes area.

lower than that of the plankton net to ensure that all microplastics are retained), the solid collected transferred to 500 mL glass breaker using a spatula and minimum water rising and place in a 90 °C drying oven for 24 h or longer to sample dryness. A wet oxidation method according to the standard protocol proposed by the US National Oceanic and Atmospheric Administration (NOAA) (Masura et al., 2015) was used. Briefly, the stain steel filter was placed in a 500 mL breaker and 20 mL of 0.05 M iron (Fe(II)) solution and 20 mL 30% hydrogen peroxide (Fenton reagent) were added. The beaker was heated to 75 °C for 30 min under continuous agitation, and then, the iron solution and hydrogen peroxide mixture was removed via vacuum filtration on a grid filter.

Materials on the filter papers were inspected visually under a dissecting microscope with a digital camera (M165 FC, Leica, Germany). The suspected microplastics were distinguished chiefly based on classification standards formulated previously (Hidalgo-Ruz et al., 2012). The color, type, size and shape of each microplastic in each sample were recorded and distinguished into two groups: fibers and fragments (any other type of shape). Particles suspected as microplastic looked to have a shape or color that were different from the dominant environment of white/grey color, homogeny colors, and different unique shape such as fibers. This method actually has some limitations, such as misidentification of algae, silica or salt as microplastics. This limitation was handle using two experimented operator to ensure that (i) no structures of biological origin (as cell walls) are presents, (ii) fibers are equally thick and have a three-dimensional bending to exclude a biological origin, (iii) transparent or whitish particles must be examined under high magnification and with the help of fluorescence microscopy to exclude silica or salt particles For microplastic abundance in the water samples, the unit of calculation was the number of microplastics per litre. Each filter was inspected from left to right, then move down one row, and inspected from right to left.

2.4. Validation and quality assurance

For all the contaminants determined in this study (pesticides, PPCPs and microplastics), field or trip and procedural blanks were prepared to check for possible contamination from reagents, tubes or equipment. For PPCPs and pesticides, trip blanks were used for water, created at the laboratory by completely filling the glass jars with lab grade deionized water and sealing the container, there were no background contaminations.

In the case of pesticides and PPCPs analytical blanks (methanol) and control samples (fortified with a known concentration of target compounds) were analyzed every 10 samples. Regression coefficients (R^2) of calibration curves (1–1000 ng mL^{-1}) were $\geq$0.998. Limit of detection (LOD) and limit of quantification (LOQ) values were estimated by injecting in triplicate extracts of samples spiked at low concentration (1 and 5 ng g^{-1}) as the amount that provides a height 3 and 10 times higher than the baseline noise. LOQ values for PPCPs ranged from 0.02 to 0.3 ng g^{-1} dry weight (dw) for sediment, soil and plant and 0.3–2.5 ng L^{-1} for water. For pesticides, the LOQ ranged 0.2–0.9 ng g^{-1} dry weight (dw) for sediment, soil and plants and 0.01–2.0 ng L^{-1} for water. Recoveries were evaluated using 1 g of solid samples or 250 mL of water spiked to obtain a concentration of 50 ng mL^{-1} of each compound in the final extract. Range of recoveries for pesticides and PPCPs in each type of sample was 24–112% for plant, 32–89% for sediment, 20–91% for soil and 50–92% for water with the exception of metformin that present recoveries even lower. Detailed results of the validation process are presented in Table S5.

In the case of microplastics, field blanks were performed by passing at the sampling site 20 L of deionized water. These field blanks are exposed to the atmosphere of the sampling site and to the potential leak of microplastics from the plankton net. No contamination of these blank was observed probably because the size of the openings is important. Furthermore, to avoid airborne and other potential contamination in the laboratory, precautions used by Fan et al. (2019) and Lorenz et al. (2019a) were adopted. The workplace has been kept as clean as possible, staff have only used natural fibre clothings. All instruments and vessels were carefully cleaned before use. The water and solutions used in our study were all filtered through GF/F Whatman glass microfiber filters (pore size 0.45 μm). The filters have been kept covered in glass Petri dishes. Blanks of all the material used were performed periodically and closely monitored. Procedural blanks presents sometimes a few fibers of blue and red color. Then, if similar fibers were found in the samples they were not counted.

3. Results and discussion

3.1. Emerging contaminants

Of the 56 pesticides included in this study, 16 were found in water (Table 1). Average pesticide concentrations ranged from 0.1 ng L^{-1} for fluvalinate to 146 ng L^{-1} for diazinon. The highest concentration was for diazinon 1016 ng L^{-1}. The most frequently occurring pesticide was carbendazim in 100% of the samples followed by diazinon in 80% of the samples. Imidacloprid also appeared in >50% of the samples. The medians indicate that these three pesticides (transportable by runoff due to their water solubility) are relevant in water. Pesticides detected are in good agreement with those reported in previous studies in Saudi Arabia not only in the environment but also in fruits, vegetables and soils (Osman et al., 2010; Picó et al., 2019; Picó et al., 2018). These pesticides were also documented in other areas around the world (Ccanccapa et al., 2016b; Derbalah et al., 2019; Sun et al., 2019).

More than half of the studied PPCPs (24) were in water samples (Table 1). Although the number of selected compounds is lower, higher number of PPCPs than of pesticides were found in water (detailed information in Table S6). This indicates the strong impact of anthropogenic pollution. Atorvastatin, caffeine, etoricoxib, lorazepam, metformine, ofloxacin, paracetamol, salicylic acid and tramadol occurred in the 100% of the samples. Atenolol and bisphenol A were present in 90% of the samples, alprazolam and ibuprofen in 80% and buthylparaben, diclofenac, methylparaben, triclosan and triclocarban in more than 50%. In addition, caffeine, diclofenac, ibuprofen, and paracetamol were at least in some samples at concentrations >1 μg L^{-1}. Alidina et al. (2014) reported that wide range of these compounds were detected in wastewater effluents of four WWTPs in Saudi Arabia. Ali et al. (2017) identified metformin, diclofenac, acetaminophen, and caffeine as the most abundant PPCPs, in the Saudi Arabian coastal waters of the Red Sea. The PPCPs pattern detected are in good agreement with those reported for other countries (Carmona et al., 2014; Carmona et al., 2017; Nkoom et al., 2018).

Special mention deserves the high levels of bisphenol A a basic petrol industry derived-chemical, component of plastics and resin used in the manufacture of many consumer goods. This area is the closest inhabited point to the Ghawar oil field and is surrounded by several industries. Al-Saleh et al. (2017) already reported the widespread presence of this compound in Saudi Arabia treated wastewater at an average concentration of 4.367 μg/L and warned about the concern to Saudi agriculture, as long-term irrigation with treated wastewater could lead to its accumulation in the soil and in the food chain. This compounds has been detected widely throughout the world in surface waters (Bottoni and Caroli, 2018; Carmona et al., 2014).

Other unexpected result was the high concentration of three benzodiazepins alprazolam, lorazepam and tramadol. In Saudi Ara-

Table 1
Minimum, maximum, average and median concentrations and frequency of the detected pesticides and PPCPs in water.

	Concentration (ng/L)				Frequency
	Min	Max	Promedio	Median	No of occurrence
Pesticides					
Acetamiprid	n.d.	12.2	2.9	0.0	3
Bifenthrin	n.d.	45.3	8.7	0.1	5
Carbendazim	2.5	192.9	64.1	32.0	10
Carbofuran-3-hydroxy	n.d.	102.1	10.7	0.0	2
Chlorfenvinphos	n.d.	11.2	3.6	0.0	4
Chlorpyrifos	n.d.	24.3	5.1	0.0	4
Cyhalothrin	n.d.	63.8	7.0	0.0	2
Diazinon	n.d.	1016.0	142.2	38.6	8
Fluvalinate	n.d.	1.3	0.1	0.0	1
Imazalil	n.d.	18.3	3.4	0.0	3
Imidacloprid	n.d.	445.0	74.9	23.7	6
Isoproturon	n.d.	67.4	6.7	0.0	1
Tebuconazole	n.d.	7.8	0.8	0.0	1
Terbuthylazine-2-hydroxy	n.d.	11.6	2.5	0.0	3
Thiabendazole	n.d.	22.4	7.6	4.8	5
Thiametoxan	n.d.	10.8	1.1	0.0	1
PPCPs					
Alprazolan	n.d.	389.4	265.3	317.9	8
Atenolol	n.d.	327.0	124.4	108.0	9
Atorvastatin	23.5	474.7	242.4	225.1	10
Bisphenol A	n.d.	484.9	200.7	190.1	9
Buthylparaben	n.d.	65.2	35.5	56.9	6
Caffeine	230.3	20663.5	4449.2	1639.4	10
Clofibric acid	n.d.	1.5	0.1	0.0	1
Codeina	n.d.	22.5	2.2	0.0	1
Diclofenac	n.d.	1390.0	303.8	32.9	7
Ethylparaben	n.d.	6.2	0.8	0.0	2
Etoricoxib	376.7	474.0	436.2	439.8	10
Ibuprofen	n.d.	2407.0	543.4	157.3	8
Lorazepam	415.2	506.7	467.4	472.7	10
Metformin	2.0	267.0	77.6	33.0	10
Methylparaben	n.d.	27.4	7.5	2.3	6
Naproxen	n.d.	142.9	15.9	0.0	2
Ofloxacin	148.0	610.6	269.0	215.8	10
Paracetamol	105.1	3069.1	539.1	169.4	10
Propylparaben	n.d.	12.5	1.4	0.0	2
Salicylic acid	10.5	129.2	77.0	80.6	10
Tramadol	289.9	353.5	318.5	313.7	10
Triclocarban	n.d.	32.0	10.3	10.2	6
Triclosan	n.d.	33.5	11.6	5.0	5
Trimetroprim	n.d.	586.2	82.3	0.0	2

bia, the prescription of these drugs is restricted to hospitals (surgical services, medicine, psychiatry, etc.) and psychiatrists (Dobia et al., 2019a,b). One of the uses of lorazepan is the treatment of sleep disorders that according to the available data, are increasingly becoming prevalent amongst Saudis (Dobia et al., 2019a). Furthermore, both Al-Hufuf and Al-Oyun are cities with an important number of hospital including several mental health center that can justify the presence of these substances. The concentrations of some of them are higher than those previously reported and even higher than the predicted environmental concentrations (PEC) (Čelić et al., 2019; Cunha et al., 2019). The PECs values depends on the usage and can easily be locally overpassed if these drugs are more extensively used in the area. Unfortunately, there were not data on consumption to be able to estimate the PECs in the studied area. These substances are quite stable in water reporting that alprazolam concentrations in surface water at slightly alkaline pH as that of these lakes (7.4–8.2) remains virtually constant over time (Jimenez et al., 2017).

PPCPs and pesticides were detected in sediments and soils at low ng g^{-1} d.w. concentration. Five pesticides and six PPCPs were detected in soil. Chlorpyrifos (in all samples) and chlorfenvinphos (in 40%) are most frequently detected but at low concentrations <1 ng g^{-1} d.w. Both pesticides tend to accumulate in non-polar matrices (see the log Kow reported in Table S1). Fenitrothion was detected at highest concentration (56.10 ng g^{-1} d.w.) but only in one sample. This pesticide was not found in water probably because is not persistent in soil and does not significantly leach or runoff with water. PPCPs concentrations ranged from 0.7 to 59.7 ng g^{-1} d.w. Bisphenol A (100% of the samples), caffeine (also 100%), and diclofenac (50%) were the most frequently detected at maximum concentrations of each >10 ng g^{-1} d.w. However, the highest concentration found was that of ibuprofen (59.8 ng g^{-1} d.w.) (see Table S1).

Higher number of pesticides and PPCPs were found in sediments than in soils (Table 2). Chlorpyrifos again followed chlorfenvinphos, imidacloprid and terbuthylazine were the most frequent pesticides and imidacloprid (9.1 ng g^{-1} d.w.) was detected at the highest concentration. In the case of terbuthylazine, also its metabolite deethyl-terbuthylazine was found. Regarding pharmaceuticals, atorvastatin, caffeine, etoricoxib, lorazepam, paracetamol, simvastatin and tramadol were found in 100% of the samples, bisphenol A and salicylid acid in 90%, and alprazolam, atenolol and metformin >50%. The concentrations ranged from 0.7 to 126 ng g^{-1} d.w.

The presence of compounds with log Kow <1 that should have little affinity for soil/sediments is more pronounced in the case of sediments where atenolol, atorvastatin, caffeine, metformin, ofloxacin and salicylic acid are frequently detected, even that metformin and caffeine were also detected in soils. The occurrence of these polar compounds can be explained first by their high concentration

Table 2
Minimum, maximum, average and median concentrations and frequency of the detected pesticides and PPCPs in sediment and soil.

	Sediments					Soil				
	Concentration (ng/g dr.w)				Frequency	Concentration (ng/g dr.w)				Frequency
	Min	Max	Average	Median	No of occurrence	Min	Max	Average	Median	No of occurrence
Pesticides										
Atrazine	0.00	0.01	$1 \bullet 10^{-3}$	0.00	1.00	–	–	–	–	–
Carbendazim	–	–	–	–	–	0.00	0.04	$1 \bullet 10^{-3}$	0.00	1.00
Chlorfenvinphos	0.00	1.25	0.78	0.76	9.00	0.00	0.84	0.27	0.00	4.00
Chlorpyrifos	0.00	0.33	0.19	0.21	8.00	0.18	0.84	0.49	0.50	10.00
Cyhalothrin	0.00	0.19	0.02	0.00	1.00	–	–	–	–	–
Diazinon	0.00	0.03	$3 \bullet 10^{-3}$	0.00	1.00	–	–	–	–	–
Fenitrothion	–	–	–	–	–	0.00	56.10	5.61	0.00	1.00
Imazalil	0.00	0.40	0.04	0.00	1.00	–	–	–	–	–
Imidacloprid	0.00	9.10	1.43	0.40	7.00	0.00	0.28	0.03	0.00	1.00
Terbuthylazine	0.00	0.83	0.17	0.03	5.00	–	–	–	–	–
Terbuthylazine-deethyl	0.00	0.05	0.01	0.00	1.00	–	–	–	–	–
Pharmaceuticals										
Alprazolam	0.00	87.00	56.41	78.50	7.00	–	–	–	–	–
Atenolol	0.00	13.51	5.87	6.43	7.00	–	–	–	–	–
Atorvastatin	14.00	84.49	43.67	41.04	10.00	–	–	–	–	–
Bisphenol A	0.00	90.85	34.32	18.87	9.00	3.87	45.25	20.55	19.51	10.00
Buthylparaben	0.00	11.53	2.29	0.00	2.00	–	–	–	–	–
Caffeine	7.07	75.96	41.14	47.51	10.00	1.74	25.44	6.56	3.86	10.00
Diclofenac	0.00	21.73	2.91	0.00	4.00	0.00	12.46	2.49	0.63	5.00
Ethylparaben	–	–	–	–	–	0.00	0.20	0.02	0.00	1.00
Etoricoxib	0.70	63.95	26.21	9.06	10.00	–	–	–	–	–
Ibuprofen	0.00	23.97	2.40	0.00	1.00	0.00	59.57	6.10	0.00	2.00
Lorazepam	100.58	126.46	116.03	117.00	10.00	–	–	–	–	–
Metformin	0.00	0.60	0.18	0.15	6.00	0.00	0.67	0.10	0.00	2.00
Ofloxacin	0.00	17.16	1.72	0.00	1.00	–	–	–	–	–
Paracetamol	11.55	24.98	16.67	15.67	10.00	–	–	–	–	–
Salicylic acid	0.00	17.69	9.26	9.22	9.00	6.17	76.07	17.04	10.11	10.00
Simvastatin	38.36	589.27	389.29	445.98	10.00	0.00	0.00	0.00	0.00	0.00
Tramadol	11.30	107.11	67.85	73.36	10.00	0.00	1.76	0.18	0.00	1.00
Triclocarban	0.00	10.36	1.92	0.00	3.00	0.00	1.91	0.19	0.00	1.00
Triclosan	0.00	0.00	0.00	0.00	0.00	0.00	7.34	1.21	0.00	3.00

in water in comparison to other less polar compounds at lower concentrations. Furthermore, accumulation of some of them, can be explained by the capacity of soil and sediments to retain anions and cations. Contaminants positively charge interact with the negatively charged surface of clays and the negatively charge are sorbed by organic matter and also clays. These results confirm the direct influence of the water contaminants profiles in the sediments and soils when these latter are irrigate with it.

Several authors emphasize that log Kow does not take into account the ionization of the compounds and recommend the use of pH and pKa dependent logD as the most suitable for evaluating the distribution between water and sediments (Carmona et al., 2014; Čelić et al., 2019). For the pesticides detected in water and sediments, sorption coefficients (Kd, in L kg^{-1}) were calculated and values are reported and compared to log D (obtained from the Chemlb) in Table 3. However, the K_D interval obtained at each sampling point has a very wide variability that can reach up 3 orders of magnitude. This variability can be justified if we take into account that K_D must be calculated in equilibrium, a rare situation in natural ecosystems where a number of characteristics, such as the amount of organic matter, pH, exchange capacity or biotic and abiotic degradation influenced the adsorption of these contaminants. In fact, the degradation processes can justify the high presence of salicylic acid, which is product form by degradation of several contaminants (Savun-Hekimoğlu and Ince, 2018; Sennaoui et al., 2019). Concentration levels, frequency and K_D values as well as variability of K_D as a function of the sampling point observed in this study agree for most compounds with those already reported (Ccanccapa et al., 2016b; Čelić et al., 2019).

In the wild plants (Table 4) growing in the channels and lakeshores pesticides found were carbendazim, chlorfenvinphos, chlorpyrifos, diazinon, fenthion sulfone, prochloraz and terbuthylazine-deethyl. The most frequently detected pesticide was chlorpyrifos (80% of the samples). Fenthion sulfone was at the highest concentration (62 ng g^{-1} d.w.). This is the only pesticide not detected in any other matrix. Fenthion sulfone is an ultimate metabolite of Fenthion, and its presence could just indicated residues from an old treatment. Forthteen PPCPs were detected. Bisphenol A and salicylic acid were detected in 100% of the samples, metformin and methylparaben in 90% and caffeine in 60%. The other PPCPs were sporadic. In addition to be a metabolite of several contaminants, salicylic acid occurs naturally in plants (since it is a phenolic phytohormone and precursor of many polyphenols). According to different studies, non-ionizable compounds with a high Kow would tend to be more easily adsorbed in plants. However, compounds such as metformin and caffeine are also detected. These results also agree with several reports (Martínez-Piernas et al., 2019; Picó et al., 2019).

The spatial distribution of pesticide and PPCPs in water is shown in Fig. 2A (detailed data in Table S6). The highest cumulative concentrations of pesticides were in the first sampling point of each channel, which collected drainage wastewater from farms (1303 and 678 ng L^{-1} in the Al-Asfar and Al-Hubail areas, respectively) then, the concentrations of the contaminants decrease. Initial concentrations of detected pesticides are higher in Al-Asfar Lake than in Al-Hubail. However, the attenuation of pesticide concentrations in Al-Asfar is also higher, probably because is larger. The course of PPCPs is very similar, although they are at much higher concentrations. The spatial distribution in sediments showed a different behavior (Fig. 2B, detailed data in Table S6). In Al-Hubail lake, the concentration is higher in the first points and decline gradually as the points move away from the mouth

Table 3
Average water/sediment (Kd, or logKd), calculated for pesticides and PPCPs.

	K_{D1}	K_{D2}	Log D (pH 7.4)	Log K_{D1}	Log K_{D2}
Pesticides					
Chlorfenvinphos	49–1652	219	3.94	1.7–3.2	2.34
Chlorpyrifos	6–12	37	5.00	0.8–1.1	1.57
Cyhalothrin		3	6.00		0.46
Diazinon	0.6	0.02	3.77	−0.2	−1.68
Imazalil	22	12	3.47	1.3	1.07
Imidacloprid	2–467	19	0.46	0.3–2.7	1.28
Terbuthylazine		72	3.05		1.86
Pharmaceuticals					
Alprazolam	239–273	213	1.92	2.4	2.33
Atenolol	25–223	47	−1.76	1.4–2.3	1.67
Atorvastatin	56–2091	180	0.74	1.7–3.3	2.26
Bisphenol A	34–352	171	3.64	1.5–2.5	2.23
Buthylparaben	203	64	3.35	2.3	1.81
Caffeine	0.6–182	9	−0.63	−0.2–2.3	0.97
Diclofenac	1–2120	10	1.44	0.0–3.3	0.98
Etoricoxib	1.5–147.5	60	2.46	0.2–2.2	1.78
Ibuprofen	226	4	0.58	2.4	0.65
Lorazepam	219–263	248	2.38	2.3–2.4	2.39
Metformin	2–303	2	−3.24	0.3–2.5	0.37
Ofloxacin	61.7	6	−0.39	1.8	0.81
Paracetamol	5–166	31	0.47	0.7–2.2	1.49
Salicylic acid	53.5–249	120	−1.14	1.7–2.4	2.08
Tramadol	33–341	213	0.29	1.5–2.5	2.33
Triclocarban	257–636	186	6.07	2.4–2.8	2.27

Table 4
Minimum, maximum, average and median concentrations and frequency of the detected pesticides and PPCPs in plants.

	Concentration (ng/g dr.w)				Frequency
	Min	Max	Average	Median	No of occurrences
Pesticides					
Carbendazim	n.d.	0.35	0.07	0.00	2
Chlorfenvinphos	n.d.	0.90	0.18	0.00	2
Chlorpyrifos	n.d.	0.65	0.35	0.44	8
Diazinon	n.d.	2.67	0.61	0.00	3
Fenthion sulfone	n.d.	62.33	17.18	0.00	3
Prochloraz	n.d.	0.49	0.09	0.00	2
Terbuthylazine-deethyl	n.d.	1.28	0.13	0.00	1
PPCPs					
Atorvastatin	n.d.	16.70	2.24	0.00	2
Benzafibrate	n.d.	62.06	7.96	0.00	2
Bisphenol A	3.18	126.18	53.86	41.98	10
Caffeine	n.d.	5.42	2.26	3.01	6
Diclofenac	n.d.	16.04	1.60	0.00	1
Ibuprofen	n.d.	135.16	13.52	0.00	1
Metformin	n.d.	27.87	3.40	0.73	9
Methylparaben	n.d.	614.34	118.91	69.75	9
Naproxen	n.d.	67.66	6.77	0.00	1
Ofloxacin	n.d.	99.48	9.95	0.00	1
Paracetamol	n.d.	28.34	3.87	0.00	2
Salicylic acid	90.74	1952.00	492.43	293.04	10
Tramadol	n.d.	1.16	0.12	0.00	1
Triclocarban	n.d.	0.21	0.02	0.00	1

of the drainage channel. Instead, in Al-Asfar lake, the concentration is higher in the first and last point being lower in the central part of the lake. Pesticides have a different behavior and their concentration decrease gradually. This may be due to the fact that contamination by PPCPs is more punctual (human settlements) while pesticides constitute a type of diffuse contamination sprayed on large surfaces, and runoff plays a more pivotal role. The spatial distribution of PPCPs and pesticides in soil (Fig. 2C) is more similar to that found in water, particularly for the Al-Hubail lake. Instead, in the case of Al-Asfar Lake, soils located at the end of the channel showed a highest level of contamination. In the case of the plants (Fig. 2D) is difficult to establish a pattern because the contamination is quite variable.

In addition to the number of contaminants that appear in each of the samples, varies between 15 and 28 in water, 12 and 19 in sediments, 4 and 9 in soils and 4 and 10 in plants (Table S7). This shows the high number of substances that coexist even in the least contaminated samples. The high number of contaminants that form a mix has already been remarked in several studies (Carmona et al., 2014; Čelić et al., 2019; Martínez-Piernas et al., 2019).

3.2. Microplastics

In this work, microplastics were observed in all of the surface water samples analyzed (Table 5). The average abundance of

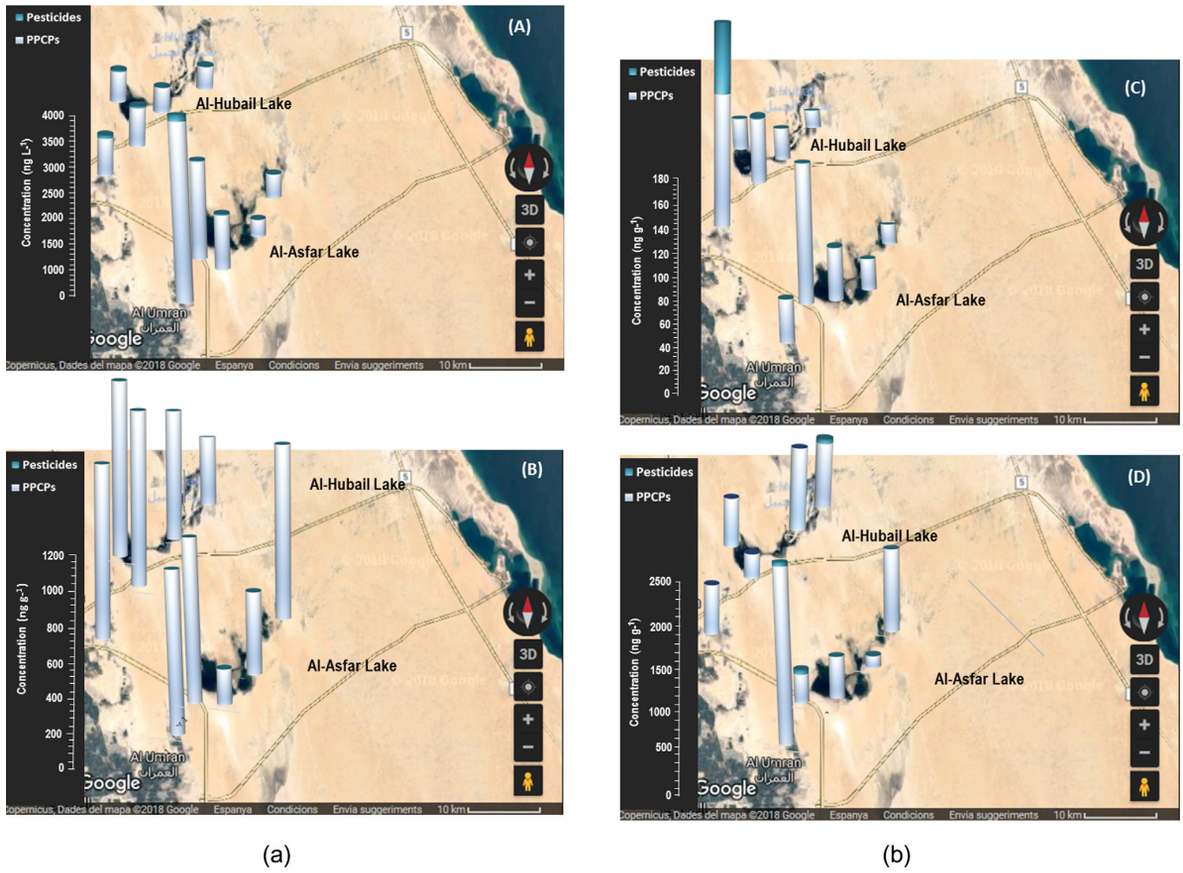

Fig. 2. Spatial distribution of pesticides and PPCPs in (A) water, (B) sediments, (C) soil and (D) plants within the Al-Asfar and Al-Hubail Lakes area.

Table 5
Abundance of microplastics in water.

Compound	Items L^{-1}		
	Fiber	Fragments	Totals
Al-Asfar			
1	7.0 ± 2.5	0.8 ± 0.3	7.8 ± 2.8
2	2.0 ± 0.8	0.7 ± 0.5	2.7 ± 1.3
3	0.7 ± 0.3	0.5 ± 0.3	1.3 ± 0.6
4	0.7 ± 0.4	n.d.	0.7 ± 0.4
5	0.8 ± 0.5	n.d	0.8 ± 0.5
Al-Hubail			
1	8.0 ± 4.2	1.0 ± 0.6	9.0 ± 4.8
2	5.0 ± 2.4	0.8 ± 0.5	5.8 ± 2.9
3	1.0 ± 0.7	0.6 ± 0.3	1.6 ± 1.0
4	0.9 ± 0.5	0.4 ± 0.2	1.3 ± 0.7
5	0.8 ± 0.4	0.3 ± 0.1	1.1 ± 0.5

microplastics in the surface waters was 3.2 items L^{-1}. The concentrations found were comparatively equal as that reported in the Ciwalengke River, Indonesia using a filter with a pore diameter of 1.2 μm (Alam et al., 2019) and in the main stream Pearl River, China using glass filters with pore diameter of 0.7 μm (Fan et al., 2019) but higher than that reported on the surface waters of the North Sea using a neuston net of 100 μm (Lorenz et al., 2019a). These differences in diameter of the net or filters opening makes the result less comparable. However, a careful look to the detailed size distribution, at least in the two first studies reported, show that particles <250 μm represent less that 30% of the total particles whereas particles between 250 and 500 are 70% of the total microplastics. This involves some errors but within the same order of magnitude. Differently, in the Lorenz et al. (2019a) study, the particles <100 μm are 80% of the total particles. This involves an error of one order of magnitude but instead the number of microplastics is lower. An example of particles observation identified as microplastics at the dissection microscope (100 × total magnification) on Whatman GF/C filter paper is shown in Fig. 3. Fiber particles were found more often (83%) than the fragment or other forms (17%). The presence of a greater number of fibers than of more or less circular fragments in surface waters has been noted in a large number of studies (Alam et al., 2019; Lorenz et al., 2019a; Pico et al., 2019). On the size of microplastics, the plankton net used for sampling has a pore of 333 μm, so the size of microplastic detected in this study ranged from 250 to 5000 μm. However, most common range (95% of the reported items) is from 500 to 1000 μm. This range could be conditioned by the size of the net 333 μm and the further sieve 300 μm. These sieves could retain some smaller particles (250–333 μm) due to adsorption but also larger particles (333–500 μm) can go through the sieve due to its shape (e.g. fibers). This could explain why particles are not detected so often.

The average abundance of microplastics was higher in the Al-Hubail Lake (3.7 ± 3.1 items L^{-1}) than in the Al-Asfar Lake (2.7 ± 2.9 items L^{-1}). Spatially, the sites with microplastic abundance higher than 5 items L^{-1} were almost always located near the Al-Hassa and Al-Oyun area, particularly the first point of the Al-Asfar-Lake was located near the sewage treatment plant (Al-Hassa treatment plant), had the highest microplastic abundance.

Fig. 3. Microplastics identified at the dissection microscopy.

The microplastic abundance at the sites located in the lake was generally <5 items L^{-1}. Wastewater from the nearby plant might be a potential source of microplastic pollution in these locations. In the irrigation channels (corresponding to the first sampling point of each channel), the microplastic abundance was comparatively higher (6.3 ± 2.7 items L^{-1}) than in the lake (1.3 ± 0.3 items L^{-1}). The contamination was more severe at the beginning of the main drainage channels, which collect wastewater from farms, factories and domestic sewage from the Al-Hufuf and Al-Oyun regions than from those located at their mouth in the lake (8.4 ± 0.4 items L^{-1} vs 4.2 ± 2.5 items L^{-1}). Thus, the microplastic concentration in the water of the area has a tendency to decrease gradually as the water flows from the irrigation channels to the lake. This spatial correlations between microplastic pollution and wastewater has been extensively reported all over the world (Enfrin et al., 2019; Fan et al., 2019; Lorenz et al., 2019a; Pico et al., 2019; Pico and Barcelo, 2019; Strungaru et al., 2019).

3.3. Risk assessment

Hazard quotients (HQ) were calculated for mean and maximum concentrations in water for algae, Daphnia, and fish (those compounds and values that can be of concern for any of the species are shown in Table S8 together with an explanation of the formula used).

Average concentrations of chlorpyrifos and diazinon provided HQ > 1 for daphnia suggesting a high risk for these organisms. These HQs became much higher when maximum concentrations are used. Average and maximum concentrations of etoricoxib also gave 1 > HQ > 0.1 for daphnia indicating a medium risk.

HQ obtained for fish at average concentrations of bifenthrin, diazinon, alprazolam, and caffeine were 1 > HQ > 0.1 indicating a medium risk. The HQs at the maximum concentrations became >1 for diazinon, bifenthrin and caffeine indicating an important hazard of these compounds. A number of studies has already proven that these compounds produce chronic effects at molecular, biochemical, behavioral and developmental levels in zebrafish embryos at these environmental concentrations (Sposito et al., 2018; Zhou et al., 2019). HQ for cyhalothrin at maximum concentrations in fish is also of medium concern.

For green algae, only bifenthrin and diazinon at the maximum concentrations gave 1 > HQ > 0.1 that could be of concern.

The results demonstrated the interest of these studies to protect aquatic ecosystems, even though the risk assessment carried out — calculation of HQ — is very simplistic and could underestimate real toxic effects has the advantage of rapidity.

In addition to the several compounds that could have a medium or higher risk and therefore, are of concern, a synergistic effect could be produced by the simultaneous presence of different types of compounds which, having similar mechanisms of action could interact. There are very few studies on synergistic interactions (Nilsen et al., 2019), but (Cedergreen, 2014) work compiling existing knowledge on synergies in the case of pesticides shows that cholinesterase inhibitors (organophosphates –diazinon, chlorpyrifos and chlorphenvinphos- and carbamates-carbofuran-3-hydroxy) and azole fungicides (imazalil and carbendazim) are involved in 95% of the cases described. These compounds have been extensively detected in waters and particularly diazinon (up to 1016 ng L^{-1}) and carbendazim (up to 193 ng L^{-1}) at very high concentrations.

In addition of this overview that shows several compounds that individually are of concern for the aquatic fauna and potential synergic effects among several contaminants, the presence of microplastic should not be forgotten. Nowadays, how dangerous microplastics are for living organisms is not totally clear but it is well demonstrated that aquatic organisms and other species, including humans, can absorb microplastic particles (Enfrin et al., 2019; Pico et al., 2019). This alone does not prove toxicity but they are at high amounts expected to increase and even inert and non-toxic substances can have unforeseen effects and interactions with other compounds once they reach a certain level of concentration in the environment.

Other point is the presence of several contaminants in the wild plants (*P. australis*) including in addition to some pesticides, bisphenol A, methyl paraben and salicylic acid at high concentrations. The excessive usage of pesticides and the widespread contamination by PPCPs leads to the soil contamination. Thus, the presence of these substances in plants (with the exception of salicylic acid that can not only be a degradation product of several PPCPs but also an important endogenous compound of plants) is a subject of concern. Although some pesticides and PPCPs are at very low concentration, this could still be detrimental for plant growth. In addition, the presence of these contaminants in the wild plants can be of particular concern when practices such as grazing occur, as it constitutes an entry route for these substances into the food chain. Further studies are required for an in-deep evaluation.

4. Conclusion

The results obtained in the Al-Asfar area showed an important contamination gradient of anthropic origin and the pseudo-persistent character of these contaminants able to impact the whole area. The presence of emerging contaminants in water, sed-

iment, soil and plants at concentrations ranging from n.d. to 20663 ng L^{-1}, n.d. to 126 ng g^{-1} d.w., n.d. to 21 ng g^{-1} d.w. and n.d. to 1952 ng g^{-1} d.w., respectively indicated high contamination levels. Furthermore, carbendazim, atorvastatin, caffeine, etoricoxib, lorazepam, metformin, paracetamol, salicylic acid and tramadol were in 100% of the water samples. This area is also impacted by microplastics (from 0.7 to 9.0 items/L). This density is comparable with that reported all around the world. Fibers (>50%) are the predominant microplastics. The possible interactions between this cocktail of contaminants present in each of the samples needs to be carefully evaluated in order not to underestimate the hazard of this situation.

The spatial distribution of the pollution shows a clear gradient with the maximum concentration at the point closest to the cities of Al-Hofuf and Al-Ouyun and the minimum at the farthest ends of the lake. However, there is a basal contamination in all the area that must be studied and controlled to avoid adverse effects on the environment and the population.

Further studies are needed to obtain a better estimate of the range of chemical contaminants that can be present in crop and wild flora under different condition as well as the public health risk that may arise not only of the presence of contaminants and residues but also of the exposure to mixtures of substances or their metabolites, which could produce different toxic effects.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135021.

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SUPPLEMENTARY MATERIAL

Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes.

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Index

Table S1. Identification, characterization and main properties of the target analytes

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 ºC)	Aquous photolysis DT_{50} (Days)	pKa	Water Solubility (mg/L)	Action	Chemical Family
PESTICIDES									
Acetamiprid	135410-20-7	$C_{10}H_{11}ClN_4$	222.67	0.80 (low)	4.3	0.7[3]	4.2 x 10^3	Insecticide	Neonicotinoid
Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.77	4.14 (high)	14	Not applicable	282	Herbicide	Chloroacetanilide
Acrinathrin	101007-06-1	$C_{26}H_{21}F_6NO_5$	541.40	5.99 (high)	0.0022	Not applicable	6.5	Insecticide	Pyrethroid
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269.77	3.09 (high)	0.5	0.62[3]	240	Herbicide	Chloroacetanilide
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.68	2.7	60	1.7[1]	33	Herbicide	Triazine
Atrazine-deethyl	6190-65-4	$C_6H_{10}ClN_5$	187.63	1.51 (low)	Not reported	Not reported	2700	Metabolite	Triazine
Atrazine-deisopropyl	1007-28-9	$C_5H_8ClN_5$	173.60	1.15 (low)	Not reported	Not reported	980	Metabolite	Triazine
Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$	345.38	3.18 (high)	0.4	Not applicable	4.5	Insecticide. Acaricide	Organophosphorus
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317.32	2.96	3	Not applicable	28	Insecticide. Acaricide	Organophosphorus
Bifenthrin	82657-04-3	C23H22ClF3O2	422.46	6.6 (high)	12	Not applicable	0.001	Insecticide	Pyrethroid
Buprofezin	69327-76-0	$C_{16}H_{23}N_3OS$	305.44	4.93 (high)	33	Not applicable	0.46	Insecticide. Acaricide	Other pesticides
Carbamazepin	10605-21-7	C9H9N3O2	191.21	1.48 (low)	Stable	4.2[6]	8	Fungicide, Metabolite	Benzimidazole
Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$	221.26	1.8 (low)	71	Not applicable	320	Insecticide. Acaricide. Nematicide	Carbamates
Carbofuran-3-hydroxy	16655-82-6	$C_{12}H_{15}NO_4$	237.25	1.45 (low)	Not reported	Not reported	6207	Metabolite	Carbamates
Chlorfenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$	359.60	3.8 (high)	12 – 19	Not applicable	145	Insecticide. Acaricide	Organophosphorus
Chlorpyrifos	5598-13-0	$C_7H_7Cl_3NO_3PS$	322.53	4 (high)	21	Not applicable	8	Insecticide. Acaricide	Organophosphorus
Chlotianidin	210880-92-5	C6H8ClN5O2S	249.70	0.905 (low)	0.1	11.1	340	Insecticide	Neonicotinoid
Coumaphos*	56-72-4	C14H16ClO5PS	362.80	4.13 (high)	--	Not applicable	1.5	Insecticide Acaricide Nematocide	Organophosphorus
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.35	3.69 (high)	50	2.5[3]	60	Insecticide. Acaricide. Repellent	Organophosphorus

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Aquous photolysis DT_{50} (Days)	pKa	Water Solubility (mg/L)	Action	Chemical Family
Diclofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$	315.15	5.14 (high)	Not found	Not applicable	0.24	Insecticide	Organophosphorus
Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$	229.26	0.704 (low)	68	Not applicable	25900	Insecticide. Acaricide	Organophosphorus
2,4-dimethylaniline (DMA)b	95-68-1	C8H11N	121.18	1.68 (low)	43,500	4,89[4]	1 — 5	Metabolite	Amidine
2,4-dimethylphenylformamide (DMF)b	60397-77-5	C9H11NO	149.19	2.07 b (low)	0.166	Not found	959.1	Metabolite	Amidine
2,4-dimethylphenyl-N′-methylformamidine (DMPF)b	33089-74-6	C10H14N2	162.23	2.92 (moderate)	37	Not found	1663	Metabolite	Amidine
Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$	384.48	5.07 (high)	56	Not applicable	2	Insecticide. Acaricide	Organophosphorus
Etofenprox	80844-07-1	C25H28O3	376.49	6.9 (high)	6.3	Not applicable	0.0225	Insecticide	Pyrethroid
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	277.23	3.32 (high)	4 – 54	Not applicable	21	Insecticide	Organophosphorus
Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$	278.33	4.84 (high)	34	Not reported	2	Insecticide	Organophosphorus
Fenthion sulfonec	3761-42-0	$C_{10}H_{15}O_5PS_2$	310.10	2.25 (low)	37.5	Not reported	190.4	Metabolite	Organophosphorus
Fenthion sulfoxide	3761-41-9	$C_{10}H_{15}O_4PS_2$	294.10	1.92 (low)	Not reported	Not reported	3.72	Metabolite	Organophosphorus
Fipronil	424-610-5	C12H4Cl2F6N4OS	437.15	3.75 (high)	0.33	Not applicable	3.78	Insecticide	Phenylpyrazole
Fluvalinate	102851-06-9	C26H22ClF3N2O3	502.90	7.02 (high)	4	Not applicable	0.00103	Insecticide, Acaricide	Pyrethroid
Hexythiazox	78587-05-0	$C_{17}H_{21}ClN_2O_2S$	352.88	2.67 (low)	17	Not applicable	0.5	Acaricide	Other Pesticides
Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	297.18	2.56 (low)	120 to 190	6.25[1]	180	Fungicide	Azol
Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$	255.66	0.57 (low)	40	Not applicable	610	Insecticide	Neonicotinoid
Isoproturon	34123-59-6	C12H18N2O	206.28	2.5 (low)	48	Not applicable	70.2	Herbicide	Urea
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	330.36	2.75 (moderate)	98	Not applicable	145	Insecticide. Acaricide	Organophosphorus
Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$	225.31	3.18 (high)	11	Not applicable	27	Insecticide	Carbamates
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.80	3.4 (high)	39	Not applicable	530	Herbicide	Chloroacetanilide
Molinate	2212-67-1	$C_9H_{17}NOS$	187.30	2.86 (moderate)	21	Not applicable	970	Herbicide	Carbamates

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Aquous photolysis DT_{50} (Days)	pKa	Water Solubility (mg/L)	Action	Chemical Family
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$	213.20	(-) 0.74 (low)	Stable	Not reported	500000	Insecticide. Acaricide	Organophosphorus
Parathion-ethyl	56-38-2	$C_{10}H_{14}NO_5PS$	291.26	3.83 (high)	30	Not applicable	50 - 60	Insecticide. Acaricide	Organophosphorus
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	263.21	3 (moderate)	10 – 60	Not applicable	50 - 60	Insecticide	Organophosphorus
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.70	3.5 (high)	60	3.8[2]	27	Fungicide	Azol
Propanil	709-98-8	$C_9H_9Cl_2NO$	218.08	2.29 (low)	3	19.1[5]	225	Herbicide	Anilide
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	3.95 (high)	35 to 231	1.7[1]	5	Herbicide	Triazine
Pyriproxyphen	95737-68-1	$C_{20}H_{19}NO_3$	321.37	5.37 (high)	3 to 16	6.87[6]	0.36	Insecticide	Juvenile Hormone Mimics
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	2.3 (low)	57	1.62[3]	5	Herbicide	Triazine
Spinosyn A (Spynosad)	168316-95-8	C41H65NO10	731.98	4.1 (high)	0.96	8[7]	7.6	Insecticide	Micro-organism derived
Spinosyn D (Spynosad)	168316-95-8	C42H67NO10	745.98	4.1 (high)	0.84	8[7]	7.6	Insecticide	Micro-organism derived
Tebuconazole	107534-96-3	C16H22ClN3O	307.82	3.7 (high)	Stable	5	36	Fungicide, Plant growth regulator	Triazole
Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$	225.29	3.04 (high)	Not reported	Not applicable	130	Herbicide	Triazine
Terbumeton-deethyl[c]	30125-64-5	$C_8H_{15}N_5O$	197.24	1.93 (low)	60	Not applicable	297	Herbicide	Triazine
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.71	3.4 (high)	18	1.9	6.6	Herbicide. Microbiocide. Algaecide	Triazine
Terbuthylazine-2-hydroxy	66753-07-9	$C_9H_{17}N_5O$	211.33	0.12 (low)	14 to 28	Not reported	7.19	Metabolite	Triazine
Terbuthylazine-deethyl	30125-63-4	$C_7H_{12}ClN_5$	201.68	2.3 (low)	Stable	Not reported	327.1	Metabolite	Triazine
Terbutryn	886-50-0	C10H19N5S	241.36	3.34	14 to 28	4.6[2]	25	Herbicide, Metabolite	Triazine
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.25	2.39 (low)	1.2	4.73	30	Fungicide	Bencimidazole
Thiametoxan	153719-23-4	C8H10ClN5O3S	291.71	–0.13 (low)	2.7	Not applicable	4100	Insecticide	Neonicotinoid
Tolclophos-methyl	57018-04-9	$C_9H_{11}Cl_2O_3PS$	301.13	4.56 (high)	38.3	Not applicable	0.708	Fungicide	Organophosphorus
BASIC AND NETURAL PHARMACEUTICALS AND PPCPs									
Acetaminophen	103-90-2	C8H9NO2	151.20	0.46	1.5	9.38	14000	Analgesic	Nitrofenol
Allopurinol	315-30-0	C5H4N4	136.11	-1.14	360	2.57	800	Prevent gout	Xanthine derivative

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Aquous photolysis DT_{50} (Days)	pKa	Water Solubility (mg/L)	Action	Chemical Family
Alprazolam	28981-97-7	C17H13ClN4	308.77	3.87	37.5	4.42	13.1	Ansiolytic	Benzodiazepine
Amoxicillin	26787-78-0	C16H19N3O5S	365.40	0.87 (low)	37.5	3.23[3]	3430	Antibacterial	Beta-lactams
Atenolol	29122-68-7	C14H22	266.34	0.1 (low)	37.5	Not applicable	685.2	Antihipertensive	Beta-bloquer
Atorvastatin	134523-00-5	C33H35FN2O5	558.64	4.13 (high)	Not reported	4.33[3]	8.7×10^{-5}	Lipid regulator	Statin
Caffeine	58-08-2	C8H10N4O2	194.19	-0.63	15	0.52	2632	Stimulant	Xanthine
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.30	1.19	Not reported	8.21	Moderate	Analgesic	Phenantrene alkaloid
Etoricoxib	202409-33-4	C18H15N2ClO2S	358.84	2.91	Not reported	4.15	0.00328	Antiinflamatory	NSAID
Lorazepam	846-49-1	C15H10Cl2N2O2	321.2	2.39	60	13	3.66	Ansiolytic	Benzodiazepine
Metformin	1115-70-4	C4H11N5	129.16	-1.4	15	3.4	1000000.00	Hypoglycemic agent	Biguanide
Metoprolol	56392-17-7	C15H25NO3	267.36	1.88	37.5	8.34	4777	Antihipertensive	Beta-bloquer
Norfloxacin	70458-96-7	C16H18FN3O3	319.33	-1.03	60	5.77 & 8.68	177900	Antibacterial	Fluoroquinolone
Ofloxacin	82419-36-1	C18H20FN3O4	361.4	-2	60	5.45 & 6.2	67620	Antibacterial	Fluoroquinolone
Simvastatin	79902-63-9	C25H38O5	418,57	4.68	37.5	14.91	0.7653	Lipid regulator	Statin
Telmisartan	144701-48-4	C33H30N4O2	514.617	8.42	Not reported	9.42	0.0035	Antihypertensive	Benzimidazole
Tramadol	27203-92-5	C16H25NO2	263.38	1.34	2.91	9.41	Soluble	Anagelsic	Opiod
Trimethoprim	738-70-5	C24H29N7O6S	543.6	0.91	60	7.68	400	Analgesic	methoxybenzenes
ACIDIC PHARMACEUTICALS AND PPCPs									
Bezafibrate	41859-67-0	C19H20	361.819	4.25	60	4.15	1.224	Antilipidemic drug	Fibrate
Bisphenol A	80-05-7	C15H16O2	228.29	3.64	2	9.78	120	Plasticizers	Biphenol
Butylparaben	94-26-8	C11H14O	194.23	3.47	9	8.47	159	Antifungal agent	Parabens
Clofibric acid	882-09-7	C10H11	214.65	2.84	37.5	3.83	582.5	Anticholesteremic drug metabolite	Fibrate
Chloramphenicol	56-75-7	C11H12Cl2N2O5	323.13	0.92	60	7.49	388.5	Antibacterial	Phenicols
Diclofenac	15307-79-6	C14H11Cl2NO2	296.149	4.02	37.5	3.65	2.37	Antiinflamatory	NSAID
Ethylparaben	120-47-8	C9H10O3	166.17	2.49	15	6.09	1894	Antifungal agent	Parabens

	CAS Number	Chemical Formula	PM (g/mol)	log Kow (pH 7, 20 °C)	Aquous photolysis DT_{50} (Days)	pKa	Water Solubility (mg/L)	Action	Chemical Family
Fenofibrate	49562-28-9	C20H21ClO4	360.831	5.19 (high)	60	3.18	0.195	Antilipidemic drug	Fibrate
Furosemide	54-31-9	C12H11N2ClO5S	330.744	2.03	60	3.9	149.3	Antihipertensive, diuretic	Chlorobenzoic acid
Ibuprofen	15687-27-1	C13H18O2	206,29	3.79	15	3.88	21	Antiinflamatory	NSAID
Indometacin	53-86-1	C19H16ClNO4	357.787	4.23	37.5	4.91	3.114	Antiinflamatory	NSAID
Methylparaben	99-76-3	C8H8O3	152.15	2	15	8.4	5981	Antifungal agent	Parabens
Naproxen	22204-53-1	C14H14O3	230.26	3.1	15	4.5	144.9	Antiinflamatory	NSAID
Propylparaben	94-13-3	C10H12O3	180.2	2.98	15	8.4	592.3	Antifungal agent	Parabens
Salicylic Acid	69-72-7	C7H6O3	138.1	2.24	Not reported	2.97	2000	Analgesic, Skin care	Hydroxybenzoate
Thiamphenicol	15318-45-3	C19H27Cl2N3O9S2	576.5	-0.33	60	3.3	9660	Antibacterials	Fenicol
Triclocarban	101-20-2	C13H9Cl3N2O	315.58	4.9	60	7.65	0.647	Antibacterial	Carbanilide
Triclosan	3380-34-5	C12H7Cl3O2	289.54	4.66	60	12.7	4.62	Antibacterial, antifugic	Polychloro phenoxy phenols

Pesticide data were mostly from PPDB: Pesticide Properties DataBase, University of Herforshire (https://sitem.herts.ac.uk/aeru/footprint/es/Reports/212.htm)

[b]Pubchem (https://pubchem.ncbi.nlm.nih.gov/)

[c]ChemSpider. Predicted EPI Suit (http://www.chemspider.com/Chemical-Structure.18445.html)

PPCPs data were from ChemSpider, Pubchem, drug databank and Chembl.

[1] Very weak base

[2] Weak base

[3] Strong Acid

[4] Conjugated acid

[5] Does not dissociated

[6] Weak acid

[7] Mean of protonated spinosyn A & D

Table S2. Overview of sampling sites with site coordinates

Site # No.	Site ID	Latitude	Longitude	Site description
1	Al-Asfar Channel Site 1	25º 24'3.04"N	49º43'36.20"E	The beginning of the main drainage channel collecting wastewater from farms, factories and domestic sewage at Al-Hufuf region and discharging into Al-Asfar lake. The channel is deep with a high flow of wastewater and common reeds on their edges.
2	Al-Asfar Site 2 (A)	25º 30'43.07"N	49º45'17,57"E	Between the end of the main drainage channel at its mouth into Al-Afar Lake. The channel is also characterized by a deep wastewater stream with common reeds on its edges.
3	Al-Asfar Site 3 (empty)	25º 30'48.30"N	49º47`6.33"E	At the southern section of Al-Asfar Lake, which is characterized by the presence of emerging and submerged hydrophytes, and common reeds, sedges and grasses vegetation in and at the lake shores.
4	Al-Asfar site 4 (B)	25º 30'45.36"N	49º49'58.06"E	Middle section of Al-Asfar Lake typified by emergent and submerged hydrophytes, and common reeds, sedges and grasses vegetation in and at the lake shores.
5	Al-Asfar site 5 (C)	25º 34'41.71"N	49º52'18.55"E	Northern section of Al-Asfar Lake, which is characterized by shallow wastewater with emergent and submerged hydrophytes and common reeds, sedges and grasses vegetation in and at the lake shores.
6	Al-Hubail Channel site 1	25º36'38.30" N	49º38'48.05" E	Beginning of the main drainage channel that collected wastewater from farms and factories at Al-Oyun region and discharged into Al-Hubail lake. The channel has a deep flow of wastewater with common reeds and sedges on their edges
7	Al-Hubail site 2 (M)	25º39'42.30" N	49º39'15.52" E	Between the end of the main drainage channel and its mouth into the Al-Hubail Lake. The channel has a deep wastewater flow with common reeds and sedges on their edges.
8	Al-Hubail site 3 (J)	25º40'41.24" N	49º40'1.69" E	Upper of the southern section of Al-Hubail Lake, which is characterized by deep water with common reeds, sedges and halophytes vegetation in and at the lakeshores.
9	Al-Hubail site 4 (N)	25º40'39.01" N	49º40'58.99" E	Middle section of Al-Hubail Lake, which is characterized by deep water with common reeds, sedges and halophytes vegetation in and at the lakeshores.
10	Al-Hubail site 5 (K)	25º41'42.12" N	49º43'12.49" E	Northern section of Al-Hubail Lake, which is characterized by shallow wastewater with common reeds, sedges and halophytes vegetation in and at the lakeshores.

Table S3. Liquid chromatography-mass spectrometry conditions.

	Pesticides	PPCPs	
		Positive Ionization	Negative ionization
LIQUID CHROMATOGRAPHY			
Analytical column	Luna C18 (15.0 cm × 0.21 cm, 3 µm)(Phenomenex, Torrance, USA)	Kinetex XB-C18 100A (50 × 2.10 mm, 1.7 µm (Phenomenex)	Kinetex XB-C18 100A (50 × 2.10 mm, 1.7 µm (Phenomenex)
Column temperature (ºC)	30	30	30
Volumen injected (µL)	5	5	5
Flow rate (µL/min)	0.3	0.2	0.2
Mobile phase			
Phase A	Water 10 mM ammonium formate	Water 0.1 % formic acid	Water 2.5 mM NH_4F
Phase B	Methanol 10 mM ammonium formate	Methanol 0.1 % formic acid	Water 2.5 mM NH_4F
Gradient	0 min (50 % B), 10 min (83 % B), 12 min (83 % B), 12.5 min (98 % B) and 15.5 min (98 % B)	30% of A to 95% of A in 12 min maintained for 8 min	30% of A to 95% of A in 12 min maintained for 8 min
Equilibrium time	12 min	12 min	12 min
MASS SPECTROMETRY			
Source	ESI	ESI	ESI
Ionization mode	Positive	Positive	Negative
Nebulizer gas (nitrogen) pressure (psi)	15	15	15
Gas flow (L/min)	30	30	30
Capillary voltage (V)	4000	4000	4000
Source temperature (ºC)	300	300	300
Data acquisition	Dynamic MRM	MRM	MRM
MS1	Unit Resolution	Unit Resolution	Unit Resolution
MS2	Unit Resolution	Unit Resolution	Unit Resolution
Accelartion (eV)	7	4	4

Table S4. Retention times of the target compounds and condition of the multiple reaction monitoring used.

Target Pesticide	t_R[a] (min)	Δ t_R [b]	Precursor Ion	SRM_1[c]	Frag[d] (V)	CE[e] (V)	SMR_2[f]	Frag[d] (V)	CE[e] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
PESTICIDES										
Positive Ionization Mode										
Acetamiprid	3.21	2.97	223	126	111	22	56	111	14	37.4 (12)
Acetochlor	14	2	270	224	120	10	148	120	10	46.8 (22)
Acrinathrin $[M+NH_4]^+$	18.4	2	559	208	76	10	181.1	76	30	56.3 (3)
Alachlor	13.63	2	270	238	80	15	162	80	10	50.4 (13)
Atrazine	9.56	2.63	216	132	120	15	174	120	20	17.3 (14)
Atrazine-desethyl	4.06	2.5	188	146	120	15	104	121	24	29.1 (15)
Atrazine-desisopropyl	2.7	2.08	174	96	120	15	132	120	15	78.6 (13)
Azinphos-ethyl	13.8	1.71	346	97	80	20	137	80	32	83.5 (12)
Azinphos-methyl	11.3	1.24	318	125	80	8	132	80	12	85.4 (11)
Bifenthrin	18.38	1.84	440.2	181.1	94	6	166	94	46	35.1 (1)
Buprofezin	17.46	1.1	306	201	120	10	116	120	15	64.6 (13)
Carbendazim	4.16	4.74	192	160	95	17	132	95	25	11.4 (14)
Carbofuran	6.96	2.91	222	123	120	10	165	70	15	98.0 (9,3)
Carbofuran-3-hydroxy	3.87	2.48	255	163	70	5	220	70	15	90.8 (9)
Chlorfenvinphos	14.8	1.61	359	155	120	10	127	120	15	63.8 (11)
Chlorpyriphos	17.3	2.23	350	97	92	13	198	97	13	78.6 (14)
Chlothianidin	2.33	2	250	169	86	9	132	89	5	53.8 (19)
Coumaphos	15.4	2.15	363	335	134	10	307	134	10	24.8 (10)
Diazinon	14.86	1.89	305	169	128	17	153	128	21	66.3 (12)

Target Pesticide	t_R[a] (min)	Δ t_R [b]	Precursor Ion	SRM_1[c]	Frag[d] (V)	CE[e] (V)	SMR_2[f]	Frag[d] (V)	CE[e] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
Dichlofenthion	17.13	2	315	259	120	10	287	120	5	44 (11)
Dimethoate	3.22	2.59	230	199	80	10	171	80	5	45.3 (12)
Diuron	10.7	1.25	233	72	120	20	160	120	20	3.2 (13)
DMA (amitraz)	2.92	2.5	122	107	111	18	77	111	42	3.0 (17)
DMF (amitraz)	5.88	4.5	150	132	111	10	107	111	15	41.6 (16)
DMPF (amitraz)	2.88	4.12	163	122	111	15	107	111	15	0.1 (15)
Ethion	17.63	1.23	385	199	80	5	171	80	15	35.3 (11)
Etofenprox	18.23	3	394.2	359.2	66	10	177.1	66	10	42.2 (3)
Fenitrothion	13.35	1.18	278	125	140	15	109	121	12	95.5 (12)
Fenthion	14.63	1.83	279	247	114	5	169	114	13	76.6 (10)
Fenthion sulfone	8.7	2.3	311	125	146	21	109	146	17	66.7 (11)
Fenthion sulfoxide	7.65	2.68	295	109	136	33	280	136	13	98.1 (14)
Fipronil	14.6	2.9	437	368	150	15	290	150	25	21.8 (11)
Flumethrin (adduct) *	19	2	527.1	267	66	10	239	66	18	59.3 (35)
Fluvalinate	18.3	1.81	503	208	50	10	181	50	26	73.4 (10)
Hexythiazox	17.84	1.15	353	228	120	20	168	120	10	67.4 (9)
Imazalil	15.18	1.71	297	159	120	20	201	120	15	56 (14)
Imidacloprid	2.46	1.96	256	209	80	10	175	80	10	75 (11)
Isoproturon	10.3	2.37	207	72	120	20	165	120	10	16.8 (12)
Lambda-cyhalothrin (adduct) *	18.1	2	467.1	225	66	10	141	66	46	26.1 (32)
Malathion	12.5	1.96	331	99	80	10	127	80	5	98.5 (4)
Methiocarb	11.86	1.93	226	121	80	5	169	80	10	66.6 (11)
Metolachlor	13.67	2.04	284	252	120	15	176	120	10	10 (14)
Molinate	12.64	1.98	188	126	80	20	55	80	10	61.7 (11)

Target Pesticide	t_R[a] (min)	Δ t_R [b]	Precursor Ion	SRM_1[c]	Frag[d] (V)	CE[e] (V)	SMR_2[f]	Frag[d] (V)	CE[e] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
Omethoate	1.69	2.67	214	125	80	5	183	80	20	72.3 (12)
Parathion-ethyl	14.25	1.91	292	236	88	4	264	88	8	45.5 (13)
Parathion-methyl	12.06	1.5	264	125	120	20	232	110	5	34.5 (13)
Prochloraz	15.18	1.91	376	308	80	10	266	80	10	14.3 (9)
Propanil	11.9	2.01	218	162	120	20	127	120	15	92.4 (11)
Propazine	11.61	2	230	146	120	15	188	120	20	93.3 (14)
Pyriproxyfen	17.63	1.33	322	227	120	10	185	120	10	36.1 (12)
Simazine	7.04	1.76	202	124	120	20	132	120	20	93.8 (12)
Spinosyn A	16.85	2.3	732.5	142.1	190	25	98.1	190	65	23.4 (2)
Spinosyn D	17.41	1.74	746.5	142.1	190	25	98.1	190	69	22.9 (3)
Tebuconazole	14.6	2.87	308	125	95	25	70	95	21	6.6 (11)
Terbumeton	11.88	2.89	226	170	95	17	114	95	25	13.8 (14)
Terbumeton-desethyl	7.68	3.76	198	142	90	13	96	95	25	31.7 (12)
Terbuthylazine	11.97	3.01	230	174	95	13	96	95	25	16.4 (13)
Terbuthylazine-2-hydroxy	7.91	3.28	212	156	95	13	86	95	25	28 (13)
Terbuthylazine-desethyl	8	2.81	202	146	95	13	79	95	25	13.2 (14)
Terbutryn	14.1	1.2	242	186	120	20	71	120	15	4.6 (14)
Thiabendazole	5.65	3.5	202	175	95	25	131	95	25	29.1 (18)
Thiamethoxam	3.09	2.58	292	211	78	10	132	78	10	21.3 (11)
Tolclofos-methyl	16.9	1.71	301	125	115	12	269	120	15	73.8 (19)
PPCPs										
Positive Ionization Mode										
Acetaminophen	1.1	—	152	110	88	14	92	88	25	2.9 (17)
Allopurinol	0.82	—	137	54	117	30	110	117	20	35 (18)

Target Pesticide	t_R[a] (min)	Δ t_R [b]	Precursor Ion	SRM_1[c]	Frag[d] (V)	CE[e] (V)	SMR_2[f]	Frag[d] (V)	CE[e] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
Alprazolam	15.2	—	309	205	80	10	281	80	30	70.3 (12)
Amoxicillin	0.77	—	366	114	80	15	134	80	10	18.6 (10)
Atenolol	0.8	—	267	77	91	77	91	91	57	64.2 (14)
Atorvastatin	15.8	—	559	440	167	16	250	167	46	54.4 (12)
Caffeine	1.52	—	195	138	109	18	110	109	22	25.7 (10)
Codeine	1	—	300	215	161	25	119	161	30	64.7 (13)
Etoricoxib	9.3	—	359	280	181	30	279	181	46	78.5 (8)
Lorazepam	14.19	—	321	275	45	16	193	45	60	7.0 (5)
Metformin	0.7	—	130	60	78	10	71	78	22	85.1 (11)
Norfloxacin	1.9	—	320	302	127	21	276	127	15	5.2 (22)
Ofloxacin	1.7	—	362	318	146	20	261	146	25	86.8 (10)
Simvastatin	17	—	419	199	96	10	225	96	10	36.4 (7)
Tramadol	1.4	—	264	58	91	10	—	—	—	—
Trimethoprim	1.3	—	291	230	156	25	123	156	29	76.6 (9)
Negative ionization										
Bezafibrate	13.3	—	360	274	106	10	154	106	22	26.4 (17)
Bisphenol A	13.8	—	227	212	138	14	133	138	25	43.4 (15)
Butylparaben	14.6	—	193	137	122	10	92	122	10	30.2 (13)
Chloramphenicol	8.4	—	321	152	128	10	176	128	10	27.9 (14)
Chlorfibric acid	9.2	—	213	127	76	1	35	76	33	5 (20)
Diclofenac	14.5	—	294	250	88	10	178	88	22	15 (18)
Ethylparaben	11.8	—	165	92	103	10	137	103	22	3.4 (22)
Furosemide	2.3	—	329	285	110	12	—	—	—	—
Ibuprofen	14.8	—	205	161	68	2	—	—	—	—

Target Pesticide	t_R[a] (min)	Δ t_R [b]	Precursor Ion	SRM_1[c]	Frag[d] (V)	CE[e] (V)	SMR_2[f]	Frag[d] (V)	CE[e] (V)	SMR_2/SRM_1 (%) (%RSD)[g]
Indomethacin	15	—	356	297	98	10	282	98	22	44.1 (13)
Methylparaben	9.1	—	151	92	93	10	136	93	18	18.8 (17)
Naproxen	12	—	229	170	88	10	169	88	26	80.4 (8)
Propylparaben	13.4	—	179	92	112	10	137	112	22	5.7 (25)
Salicylic Acid	2.1	—	137	93	86	10	65	86	30	12.5 (21)
Thiamphenicol	2.3	—	354	290	128	10	63	128	74	22.6 (15)
Triclocarban	16.8	—	313	160	86	10	126	86	10	7.5 (19)
Triclosan	16.9	—	287	35	98	14	35	98	7	1.8 (22)

[a] t_R = retention time.

[b] Δ t_R = delta retention time, that is the centered retention time window (only if dynamic MRM is used).

[c] SRM_1 = selected product ion for quantification.

[d] Frag = Fragmentor.

[e] CE = Collision energy.

[f] SRM_2 = selected product ion for qualification.

[g] (%RSD) = relative standard deviation of the ratio SRM_2/SRM_1, calculated from mean values obtained from the matrix-matched calibration curves.

[*] = Adducts of target pesticides [Ion mass + NH_4^+]; non adduct target pesticides [Ion mass + H^+]

Table S5. Method performance parameters for the pesticides and PPCPs in different types of matrices studied.

Target Compound	Water		Sediments		Soil		Vegetables	
	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]
PESTICIDES								
Acetamiprid	81	8	71	11	74	11	75	10
Acetochlor	69	10	40	10	45	14	64	11
Acrinathrin $[M+NH_4]^+$	68	12	55	15	58	12	68	14
Alachlor	72	11	51	10	66	17	54	8
Atrazine	81	10	74	13	62	11	52	7
Atrazine-desethyl	76	10	74	14	77	11	73	13
Atrazine-desisopropyl	82	9	86	11	82	15	75	16
Azinphos-ethyl	70	11	65	13	70	18	71	13
Azinphos-methyl	73	12	62	19	64	11	63	16
Bifenthrin	81	13	71	13	71	11	70	16
Buprofezin	83	14	77	13	70	11	56	11
Carbendazim	79	12	63	13	68	11	87	13
Carbofuran	71	12	66	15	67	11	91	12
Carbofuran-3-hydroxy	85	13	81	16	84	15	92	11
Chlorfenvinphos	89	9	88	13	82	8	89	11
Chlorpyriphos	87	10	83	12	84	15	81	12
Chlothianidin	79	8	73	14	70	14	71	14
Coumaphos	63	8	48	13	54	11	63	11
Diazinon	81	12	71	10	68	11	64	14
Dichlofenthion	82	10	69	11	74	13	83	12
Dimethoate	84	19	70	29	71	15	89	34
Diuron	79	10	74	14	71	11	112	11
DMA (amitraz)	83	9	82	13	20	14	24	12
DMF (amitraz)	85	8	83	12	82	13	82	13
DMPF (amitraz)	87	10	53	11	51	15	53	15
Ethion	88	11	70	12	65	15	68	13
Etofenprox	89	10	82	13	87	13	87	13
Fenitrothion	80	9	71	11	72	12	72	14
Fenthion	81	18	69	22	74	12	70	14
Fenthion sulfone	88	9	82	11	85	16	91	14
Fenthion sulfoxide	84	12	86	19	86	11	96	13
Fipronil	87	9	85	11	82	16	82	15
Flumethrin (adduct) *	88	17	57	23	52	10	63	14
Fluvalinate	85	13	65	17	61	11	69	18
Hexythiazox	88	13	68	16	61	12	83	16
Imazalil	76	10	68	15	63	13	73	15
Imidacloprid	92	10	89	12	80	12	81	12

Target Compound	Water		Sediments		Soil		Vegetables	
	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]
Isoproturon	87	11	78	14	78	12	80	19
Lambda-cyhalothrin (adduct) *	82	8	77	12	78	15	75	13
Malathion	80	8	66	12	73	11	73	13
Methiocarb	78	9	72	12	78	12	82	12
Metolachlor	68	10	55	11	53	13	72	11
Molinate	65	15	48	19	54	11	84	17
Omethoate	64	12	55	16	60	11	65	10
Parathion-ethyl	78	10	70	12	76	11	74	12
Parathion-methyl	77	10	62	13	46	15	50	14
Prochloraz	84	8	73	11	76	11	77	18
Propanil	82	9	72	10	74	11	81	11
Propazine	80	11	77	15	71	11	71	17
Pyriproxyfen	90	9	87	11	55	11	54	22
Simazine	84	10	68	13	60	11	60	22
Spinosyn A	92	10	86	15	80	14	66	11
Spinosyn D	81	12	68	16	64	12	82	11
Tebuconazole	85	22	79	32	85	12	85	16
Terbumeton	65	10	48	16	55	12	54	15
Terbumeton-desethyl	64	10	53	12	52	13	56	17
Terbuthylazine	82	18	74	33	68	13	51	13
Terbuthylazine-2-hydroxy	78	17	66	20	73	14	78	13
Terbuthylazine-desethyl	63	10	51	12	52	14	54	10
Terbutryn	70	11	59	12	49	15	62	10
Thiabendazole	85	11	75	11	28	12	72	11
Thiamethoxam	82	8	78	10	77	11	66	14
Tolclofos-methyl	84	12	72	15	66	10	54	15
PPCPs								
Positive Ionization Mode								
Acetaminophen	62	12	52	14	59	16	58	17
Allopurinol	61	18	32	15	33	16	46	29
Alprazolam	52	14	32	15	37	13	42	16
Amoxicillin	82	11	72	15	70	15	80	17
Atenolol	90	10	88	9	85	11	84	10
Atorvastatin	92	8	85	13	82	11	80	9
Caffeine	69	13	53	6	59	16	57	8
Codeine	84	9	74	10	69	15	70	14
Etoricoxib	90	8	84	8	78	8	76	13
Lorazepam	87	10	82	15	80	9	79	14
Metformin	28	22	16	16	15	9	14	15
Norfloxacin	85	13	72	10	69	10	70	16
Ofloxacin	77	10	47	9	51	9	53	17
Simvastatin	78	12	68	11	73	8	70	16

Target Compound	Water		Sediments		Soil		Vegetables	
	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]	R (%)[a]	RSDs (%)[b]
Tramadol	79	13	50	19	55	9	53	18
Trimethoprim	77	11	57	12	61	10	59	9
Negative ionization								
Bezafibrate	69	14	55	19	59	11	59	10
Bisphenol A	79	12	62	10	69	10	69	13
Butylparaben	70	12	59	11	60	9	60	20
Chloramphenicol	64	12	45	17	54	15	54	16
Chlorfibric acid	81	13	65	16	71	15	71	12
Diclofenac	91	13	78	12	91	10	91	7
Ethylparaben	73	14	55	12	63	10	63	10
Furosemide	86	14	64	10	76	12	76	9
Ibuprofen	92	15	72	13	87	11	87	8
Indomethacin	58	12	48	10	48	10	48	12
Methylparaben	81	10	62	14	71	23	71	11
Naproxen	79	10	68	11	69	10	69	13
Propylparaben	55	22	54	11	45	19	45	18
Salicylic Acid	50	18	45	15	45	10	45	16
Thiamphenicol	60	19	49	15	49	20	49	16
Triclocarban	81	12	76	10	76	10	76	8
Triclosan	84	13	71	5,8	71	0,9	71	10

[a] recoveries (%),

[b] relative standard deviation (±RSD%, n=3).

Table S6. Cumulative concentrations of pesticides and PPCPs at each sampling site.

	Water (ng L^{-1})			Sediment (ng g-1 d.w.)			Soil			Plant		
station	PPCPs	Pesticides	Total	PPCPs	Pesticides	Total	PPCPs	Pesticides	Total	PPCPs	Pesticides	Total
Al-Asfar 1	31,430.4	1,303.7	32,734.1	956.3	2.2	958.5	39.0	0.7	39.7	2,138.2	54.5	2,192.7
Al-Asfar 2	17,432.7	263.5	17,696.3	980.4	1.2	981.6	121.1	0.7	121.8	356.0	62.8	418.8
Al-Asfar 3	9,723.8	294.4	10,018.2	212.3	2.6	214.9	65.3	0.3	65.7	506.6	1.0	507.5
Al-Asfar 4	2,679.5	34.1	2,713.6	478.1	2.2	480.3	13.9	0.6	14.5	142.4	4.9	147.3
Al-Asfar 5	2,581.3	19.5	2,600.8	970.5	0.7	971.2	33.5	0.2	33.7	970.0	0.7	970.8
Al-Hubail 1	5,364.8	677.7	6,042.5	980.7	3.0	983.6	109.7	56.4	166.1	577.5	3.6	581.1
Al-Hubail 2	5,804.7	391.7	6,196.4	1,063.2	2.0	1,065.2	65.1	1.0	66.2	284.9	1.7	286.6
Al-Hubail 3	4,103.2	315.2	4,418.3	992.9	1.2	994.0	35.4	1.3	36.6	533.3	1.3	534.6
Al-Hubail 4	2,840.2	54.1	2,894.3	826.0	1.5	827.5	35.0	1.4	36.3	936.1	-	936.1
Al-Hubail 5	2,626.0	60.8	2,686.8	720.7	9.8	730.5	26.4	1.5	27.9	724.0	55.6	779.7
Total	**84,586.6**	**3,414.6**	**88,001.2**	**8,181.1**	**26.3**	**8,207.4**	**544.4**	**64.1**	**608.4**	**7,169.0**	**186.1**	**7,355.1**

Table S7. Number of contaminants at each sampling site

	Water			Sediment			Soil			Plant		
station	Pesticides	PPCPs	Total	Pesticides	PPCPs	Total	Pesticides	PPCPs	Total	Pesticides	PPCPs	Total
Al-Asfar 1	7	21	28	4	11	15	1	3	4	2	5	7
Al-Asfar 2	7	19	26	4	12	16	2	5	7	2	6	8
Al-Asfar 3	12	16	28	4	10	14	1	7	8	2	6	8
Al-Asfar 4	8	16	24	1	11	12	1	3	4	5	5	10
Al-Asfar 5	5	15	20	1	13	14	1	5	6	2	5	7
Al-Hubail 1	6	18	24	5	14	19	2	5	7	3	7	10
Al-Hubail 2	5	22	27	5	14	19	3	6	9	2	8	10
Al-Hubail 3	4	13	17	3	12	15	2	4	6	2	6	8
Al-Hubail 4	4	12	16	3	11	14	2	3	5		5	5
Al-Hubail 5	3	12	15	3	11	14	2	4	6	1	3	4
Total	**61**	**164**	**225**	**33**	**119**	**152**	**17**	**45**	**62**	**21**	**56**	**77**

Table S8. Data applied and calculation of the HQ

Contaminants	PNEC Green algae (mg L^{-1})	PNEC Daphnia (mg L^{-1})	PNEC Fish (mg L^{-1})	PNEC Green algae (ng L^{-1})	PNEC Daphnia (ng L^{-1})	PNEC Fish (ng L^{-1})	Mean conc. (ng L^{-1})	Max. Conc. (ng L^{-1})	HQ Average Gren Algae	HQ Average Daphnia	HQ Average Fish	HQ maximum Gren Algae	HQ maximum Daphnia	HQ maximum Fish
PESTICIDES														
Acetamiprid	0.6	6.7	27.4	60000	670000	2740000	2.95	12.24	0.00	0	0.00	0.00	0.00	0.00
Bifenthrin	0.0047	0.018	0.0003	470	1800	30	8.74	45.28	0.02	0.00	0.29	0.10	0.03	1.51
Carbendazim	0.67	0.4	0.215	67000	40000	21500	64.1	192.91	0.00	0.00	0.00	0.00	0.00	0.01
Carbofuran-3-hydroxy	0.67	0.4	0.215	67000	40000	21500	10.7	102.09	0.00	0.00	0.00	0.00	0.00	0.00
Chlorfenvinphos	1.13	0.069	0.079	113000	6900	7900	3.56	11.22	0.00	0.00	0.00	0.00	0.00	0.00
Chlorpyrifos	0.0008	0.00005	0.0008	80	5	80	5.1	24.33	0.06	1.02	0.06	0.30	4.87	0.30
Cyhalothrin	0.027	0.019	0.0024	2700	1900	240	6.96	63.87	0.00	0.00	0.03	0.02	0.03	0.27
Diazinon	0.985	0.00008	0.00587	98500	8	587	142.16	1016.04	0.00	17.77	0.24	0.01	127.01	1.73
Fluvalinate	0.031	0.022	0.0029	3100	2200	290	0.13	1.31	0.00	0.00	0.00	0.00	0.00	0.00
Imazalil	2.16	0.305	0.083	216000	30500	8300	3.42	18.32	0.00	0.00	0.00	0.00	0.00	0.00
Imidacloprid	14.9	3.07	42.5	1490000	307000	4250000	74.87	445	0.00	0.00	0.00	0.00	0.00	0.00
Isoproturon	0.051	0.361	0.328	5100	36100	32800	6.74	67.42	0.00	0.00	0.00	0.01	0.00	0.00
Tebuconazole	0.494	0.194	0.017	49400	19400	1700	0.78	7.81	0.00	0.00	0.00	0.00	0.00	0.00
Terbuthylazine-2-hydroxy	2.64	0.81	1.27	264000	81000	127000	2.49	11.57	0.00	0.00	0.00	0.00	0.00	0.00
Thiabendazole	0.458	0.06	0.01	45800	6000	1000	7.58	22.42	0.00	0.00	0.01	0.00	0.00	0.02
Thiametoxan	11.7	2.57	30.8	1170000	257000	3080000	1.08	10.82	0.00	0.00	0.00	0.00	0.00	0.00
PPCPs														
Alprazolan	0.503	0.2	0.018	50300	20000	1800	265.35	389.47	0.01	0.01	0.15	0.01	0.02	0.22
Atenolol	38	6.75	131	3800000	675000	13100000	124.45	326.97	0.00	0.00	0.00	0.00	0.00	0.00
Atorvastatin	1.61	0.257	0.055	161000	25700	5500	242.44	474.69	0.00	0.01	0.04	0.00	0.02	0.09
Bisphenol A	0.227	1.77	0.55	22700	177000	55000	200.73	484.86	0.01	0.00	0.00	0.02	0.00	0.01
Buthylparaben	0.89	2.86	0.199	89000	286000	19900	35.53	65.22	0.00	0.00	0.00	0.00	0.00	0.00

Caffeine	2.63	2.8	0.194	263000	280000	19400	4449.17	20663.48	0.02	0.02	0.23	0.08	0.07	1.07
Clofibric acid	60	22	33	6000000	2200000	3300000	0.15	1.52	0.00	0.00	0.00	0.00	0.00	0.00
Codeine	8	8	12.9	800000	800000	1290000	2.25	22.46	0.00	0.00	0.00	0.00	0.00	0.00
Diclofenac	16.4	4.22	4.58	1640000	422000	458000	303.79	1390	0.00	0.00	0.00	0.00	0.00	0.00
Ethylparaben	2.69	13.5	0.783	269000	1350000	78300	0.78	6.25	0.00	0.00	0.00	0.00	0.00	0.00
Etoricoxib		0.041	0.276	0	4100	27600	436.25	473.96		0.11	0.02		0.12	0.02
Ibuprofen	15.6	4.31	4.94	1560000	431000	494000	543.42	2407	0.00	0.00	0.00	0.00	0.01	0.00
Lorazepam	4.64	7.58	0.673	464000	758000	67300	467.37	506.86	0.00	0.00	0.01	0.00	0.00	0.01
Metformin	1040	93.7	8360	104000000	9370000	8360000	77.65	267.01	0.00	0.00	0.00	0.00	0.00	0.00
Methylparaben	4.63	29.1	1.54	463000	2910000	154000	7.5	27.4	0.00	0.00	0.00	0.00	0.00	0.00
Naproxen	45.3	15.7	21.3	4530000	1570000	2130000	15.89	142.95	0.00	0.00	0.00	0.00	0.00	0.00
Ofloxacin	675	114	2460	67500000	11400000	246000000	268.96	610.58	0.00	0.00	0.00	0.00	0.00	0.00
Paracetamol	37	5.12	26.5	3700000	512000	2650000	539.06	3069.06	0.00	0.00	0.00	0.00	0.01	0.00
Propylparaben	1.55	6.24	0.396	155000	624000	39600	1.38	12.54	0.00	0.00	0.00	0.00	0.00	0.00
Salicylic acid	25.5	7.05	13	2550000	705000	1300000	77.03	129.2	0.00	0.00	0.00	0.00	0.00	0.00
Tramadol	0.347	0.135	0.445	34700	13500	44500	318.53	353.46	0.01	0.02	0.01	0.01	0.03	0.01
Triclocarban	0.013	0.015	0.014	1300	1500	1400	10.31	32	0.01	0.01	0.01	0.02	0.02	0.02
Triclosan	0.227	0.146	0.075	22700	14600	7500	11.62	33.52	0.00	0.00	0.00	0.00	0.00	0.00
Trimetroprim	9.59	0.069	3.59	959000	6900	359000	82.27	586.25	0.00	0.01	0.00	0.00	0.08	0.00

RQ was calculated using the following equation:

RQ = EC/PNEC

where, EC is the mean or maximum concentration of pesticides detected in the water samples and PNEC is the predicted no-effect concentration. PNEC can be calculated for acute or chronic toxicity, dividing the lowest short-term EC50 or long-term NOEC respectively by an assessment factor (AF), in this case 10 (considering that we do not apply to human being and that interspecies variability is already cover). If RQ > 1, harmful effects could be expected due to the

presence of the pollutant in water. On the contrary, if RQ < 0.1, the environmental risk is low. The intermediate situation in which the RQ is between 0.1 and 1 involves medium risk.

PNEC values were obtained using the Ecological Structure Activity Relationships Predictive Model (ECOSAR V2.0) and assessed for long-term exposure and they were estimated using Chronic Toxicity (ChV) data towards fish, mysids (seawater invertebrates), and green algae.

Fig.S1. Overview of the sampling sites

Fig. S2 Details of the extraction methods

WATER SAMPLES

- Put the Phenomenex Strata-X 33u Polymeric Reversed Phase (200 mg/6 mL) cartridges (Phenomenex, Torrance, Ca, USA) into a 12 port vacuum manifold Supelco Visiprep 57030-U of Sigma-Aldrich (St. Louis, MO, EEUU).
- Condition the cartridge with 6 mL methanol and 6 mL of Milli-Q water at 400 mba h^{-1} Pa^{-1} vacuum.
- Pass 250 mL of sample through the cartridge at a flow rate of 10 mL min^{-1} uder vacuum
- Wash the cartridges with 6 mL of Milli-Q water.
- Dry the cartridges under vacuum for 15 min.
- Elute the analytes on a 15 mL Falcon tube with 6 mL of methanol and then 3 mL of a methanol–dichloromethane solution (1:1, v/v) at gravity flow.
- Evaporate the extracts to dryness at 40 °C using a combined sample concentrator model SBHCONC/1 and a heating plate model SBH130D/3 by Stuart® (Stafford, UK).
- Redissolve the residue in 1 mL of water-methanol (70:30, v/v) by agitation and ultrasonication for 1 min and pass the extract to 2 mL amber vials with stoppers 99 mm + Septum Sil/PTFE, both manufactured by Análisis Vínicos S.L. (Tomelloso, Spain).

SEDIMENT AND PLANT SAMPLES

- Prepare the McIlvaine- buffer-EDTA (pH = 4.5) with 100 mL of 0.1 M of citric acid, 62.5 mL 0.2 M Na2HPO4 and 6.05 g of Na2-EDTA.
- Weight 1 g dry weigh of lyophilized soil, sediment and fish samples in a 15 mL Falcon
- Add 5 mL of methanol, 5 mL of distilled water and 5 mL of the McIlvain buffer-EDTA
- Sonicate for 15 min.
- Centrifuge for 6 min at 3000 rpm.
- Collect the supernatant in a 250 mL volumetric flask.
- Perform a SPE purification following the method described for the waters

ARTÍCULO

Dataset of pesticides, pharmaceuticals and personal care products occurrence in wetlands of Saudi Arabia

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Dataset of pesticides, pharmaceuticals and personal care products occurrence in wetlands of Saudi Arabia

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plants

ABSTRACT

The data set presents the occurrence of 59 currently used pesticides (CUPs) and 33 pharmaceuticals and personal care products (PPCPs), from wetland areas, in Saudi Arabia, impacted by wastewater discharge. Wetlands are valuable ecosystems, but are very fragile and easily affected by anthropogenic pressure [1-6]. The occurrence of organic contaminants provides understanding about their fate and possible risk for humans and environment. Up to our knowledge, this is the first report on the occurrence of the mentioned organic pollutants in shallow lakes in Saudi Arabia, and the first time these compounds are analyzed in wild flora. Samples of water, sediment, soil and plants were extracted via ultrasound assisted extraction (UAE) and solid phase extraction (SPE). The compounds determination was performed using ultra-high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Interpretation and discussion of the present dataset can be found in the article entitled "*Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes*" [1].

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Specifications Table

Subject	Pollution
Specific subject area	Organic pollutants occurrence and fate in wetland areas affected by wastewater discharge
Type of data	Table
How data were acquired	The data were acquired via liquid chromatography-mass spectrometry. The instruments were a 1260 Infinity Ultra-High-Performance Liquid Chromatograph (UHPLC) combined with an Agilent 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS), with an electrospray ionizer (ESI) (Agilent Technologies, Santa Clara, CA, USA). Data were processed using a MassHunter Workstatin Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan).
Data format	Raw, Analyzed, Filtered, Tables and graphs. The results presented are average of triplicate sample analysis.
Parameters for data collection	The mobile phases were methanol and water: 10mM ammonium formate for pesticides, 0.1 % formic acid for positive ionization PPCPs and 2.5mM NH_4F for negative ionization PPCPs. The rest of the parameters are specified in the literature [1].
Description of data collection	Concentration of 59 CUPs and 33 PPCPs, were obtained analysing the extracts of environmental samples (water, sediment, soil and plants) collected in the eastern region of Saudi Arabia. The extraction procedures are detailed in the experimental design, materials, and methods section.
Data source location	Institution: Environmental and Food Safety Research Group (SAMA-UV), Desertification Research Centre CIDE (CSIC-UV-GV) City/Town/Region: Moncada, Community of Valencia Country: Spain Latitude and longitude (and GPS coordinates) for collected samples: Al-Asfar Site 1 (25° 24′3.04" N 49°43′36.20" E), Al-Asfar Site 2 (25° 30′43.07" N 49°45′17,57" E), Al-Asfar Site 3 (25° 30′48.30" N 49°47'6.33" E), Al-Asfar site 4 (25° 30′45.36" N 49°49′58.06" E), Al-Asfar site 5 (25° 34′41.71" N 49°52′18.55" E), Al-Hubail site 1 (25°36′38.30" N 49°38′48.05" E), Al-Hubail site 2 (25°39′42.30" N 49°39′15.52" E), Al-Hubail site 3 (25°40′41.24" N 49°40′1.69" E), Al-Hubail site 4 (25°40′39.01" N 49°40′58.99" E), Al-Hubail site 5 (25°41′42.12" N 49°43′12.49" E).
Data accessibility	Data are available with the article
Related research article	Picó, Y., Alvarez-Ruiz R., Alfarhan A. H., El-Sheikh M. A., Alshahrani H. O., Damià Barceló D., *Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes,* Science of The Total Environment, 2020. 701: p. 135021, https://doi.org/10.1016/j.scitotenv.2019.135021

Value of the Data

- The analysis of occurrence of organic pollutants in the environment is needed to assess their risk and fate.
- Concentration values can be used by other researchers and local authorities.
- The occurrence can be useful for supporting further research of the risk and fate of organic compounds, restoration policies and contaminant elimination measures, among others.
- The data of every sampling point provides a better understanding in the distribution of the organic compounds
- The tables offer a comprehensive overview of the occurrence of a wide range of pharmaceuticals and PCPs in water, sediment, soil and plants of a very little studied area.
- These data can be a useful contribution to prioritisation exercises as well as to establish environmental quality standards.

Data Description

The following dataset shows 4 tables with the occurrence of CUPs and PPCPs in the different environmental matrices. For both shallow lakes, Al-Asfar and Al-Hubail, the sites 3, 4 and 5 were located in the shallow lake, while site 1 was located in irrigation channels, which provide wastewater (from farms, factories and/or domestic sewage) to each lake. Site 2 was located between the end of the irrigation channels and the mouth of each lake. Detailed information of each sampling site is provided in the related article [1]. Table 1 shows the occurrence of CUPS and PPCPs in water samples, while tables 2, 3 and 4 show the occurrence in sediments, soil and plants (wild flora *Phragmites australis*) respectively. In order to make the table easier to understand the data has been filtered, eliminating in each table, those compounds that were not detected in the sampling sites. A detailed list of the analyzed compounds is provided in the related article [1]. Furthermore, the CUPs acetochlor, acrinathrin, alachlor, atrazine, atrazine-deethyl, atrazine-deisopropyl, azinphos-ethyl, azinphos-methyl, buprofezin, carbofuran, chlotianidin, coumaphos, diclofenthion, dimethoate, diuron, 2,4-dimethylaniline (DMA), 2,4-dimethylphenylformamide (DMF), 2,4-dimethylphenyl-N′-methylformamidine (DMPF), ethion, etofenprox, fenthion, fenthion sulfoxide, fipronil, flumethrin, hexythiazox, malathion, methiocarb, metolachlor, molinate, omethoate, parathion-ethyl, parathion-methyl, propanil, propazine, pyriproxyphen, simazine, spinosyn A, spinosyn C, spinosyn D, terbumeton, terbumeton-deethyl, terbutryn and tolclophos-methyl and the PPCPs allopurinol, amoxicillin, chloramphenicol, furosemide, indomethacin, norfloxacin and thiamphenicol were not detected in the samples. In addition, the tables also show the total accumulated contamination for each contaminant and matrix, which provides and insight of the overall presence (and use) of each compound in the area.

In the figures are represented the different the actions of the compounds detected in the environmental matrices. Since there are compounds with more than one action, the sum of the percentages of each figure overcomes 100%. Figures 1, 2, 3 and 4 show these actions of the compounds detected in water, sediment, soil and plants respectively. Information about the specific actions of each compound is provided in the related article [1]. These figures provide understanding about population requirements, regardless the compounds used to satisfy them.

Experimental Design, Materials, and Methods

Once at the laboratory, surface water samples were filtered with glass microfiber filters (90 mm Ø) and stored at -20 °C until the analysis by solid-phase extraction (SPE) following a previously described method [2] and this information is also available in the related research article [1]. For the SPE Phenomenex Strata-X33u Polymeric Reversed Phase (200 mg/6 mL) cartridges (Phenomenex, Torrance, California, USA) and a vacuum manifold Supelco Visiprep 57030-U (Sigma-Aldrich, St. Louis, Missouri, USA) were used. The cartridges were conditioned with 6 mL of MeOH and 6 mL of Milli-Q water under vacuum at 400 mba h^{-1} Pa^{-1}. Two-hundred and fifty mL of samples were measured in a volumetric flask, and spiked with the internal standard (IS) to obtain a final concentration in the vial of 20 ng mL^{-1}. Then, each sample was passed through a cartridge at flow rate of 10 mL min^{-1} (wise drop). Then, the cartridges were washed with 6 mL of Milli-Q and dried for 15 min, both steps were performed under vacuum. The analytes were eluted on a 15 mL plastic Falcon tube with 6mL of MeOH and then 3 mL of MeOH-dichloromethane (DCM) solution (1:1, v/v) at gravity flow. Vacuum was just used at the beginning of the elution to break the superficial tension, and at the end, to collect the remaining drops of extract from the cartridges. Extracts were evaporate to dryness at 40°C, under a gentle stream of nitrogen, in a combined sample concentrator model SBHCONC/1 and heating plate model SBH130D/3 (Stuart®UK). The residue was redissolved in 1 mL of MilliQ water-MeOH (70:30, v/v), vortex for 1 min and sonicated for 1 min. Finally, each extract was stored in 2 mL amber vials with stoppers 99mm+Septum Sil/PTFE, (Análisis Vínicos S.L., Tomelloso, España), at -20°C until analysis.

Table 1
Occurrence of CUPs and PPCPs in water of the study area.

	Concentration in water (ng L^{-1})										
	Al-Asfar					Al-Hubail					
CUPs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	**Total per compound**
Acetamiprid	10.10	12.24	7.17	-	-	-	-	-	-	-	30.01
Bifenthrin	-	-	0.32	0.76	0.19	40.82	45.28	-	-	-	87.37
Carbendazim	40.71	25.34	38.52	2.53	4.63	153.73	192.91	141.80	15.29	25.54	641.00
Carbofuran-3-hydroxy	102.09	-	4.93	-	-	-	-	-	-	-	107.02
Chlorfenvinphos	-	11.22	-	7.99	-	-	-	-	5.43	10.93	36.02
Chlorpyrifos	-	-	-	1.11	1.94	-	-	-	23.65	24.33	51.04
Cyhalothrin	-	-	-	5.73	-	-	-	63.87	-	-	69.59
Diazinon	1016.04	131.98	151.95	1.12	1.18	37.15	40.08	42.10	-	-	1421.58
Fluvalinate	-	-	1.31	-	-	-	-	-	-	-	1.31
Imazalil	18.32	8.93	6.98	-	-	-	-	-	-	-	34.22
Imidacloprid	94.03	58.80	42.88	4.63	-	445.00	103.33	-	-	-	748.65
Isoproturon	-	-	-	-	-	-	-	67.42	-	-	67.42
Tebuconazole	-	-	7.81	-	-	-	-	-	-	-	7.81
Terbuthylazine-2-hydroxy	-	-	3.11	10.27	11.57	-	-	-	-	-	24.94
Thiabendazole	22.42	15.05	18.59	-	-	-	10.06	-	9.70	-	75.81
Thiametoxan	-	-	10.82	-	-	-	-	-	-	-	10.82

(*continued on next page*)

Table 1 (*continued*)

	Concentration in water (ng L^{-1})										
	Al-Asfar					Al-Hubail					
CUPs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	**Total per compound**
PPCPs											
Alprazolam	-	343.65	-	287.00	286.00	329.05	317.16	318.65	382.48	389.47	2653.46
Atenolol	-	326.97	219.88	75.05	100.02	60.57	117.67	145.72	82.58	116.06	1244.52
Atorvastatin	474.69	360.36	249.95	203.25	198.00	272.57	23.55	191.79	238.27	211.95	2424.38
Bisphenol A	337.50	484.86	195.05	-	105.09	258.19	185.20	95.82	100.25	140.25	1902.21
Buthylparaben	60.20	56.80	57.06	65.22	-	58.02	58.02	-	-	-	355.32
Caffeine	20663.48	11425.67	5217.00	294.54	269.74	1906.00	2721.26	1372.83	390.83	230.31	44491.66
Clofibric acid	-	-	-	-	-	-	1.52	-	-	-	1.52
Codeine	22.46	-	-	-	-	-	-	-	-	-	22.46
Diclofenac	1390.00	937.40	584.45	10.25	10.25	50.02	45.29	-	-	-	3027.66
Ethylparaben	6.25	-	-	-	-	-	1.52	-	-	-	7.77
Etoricoxib	376.72	462.60	463.11	433.96	433.08	433.55	382.89	473.96	445.61	456.97	4362.45
Ibuprofen	2407.00	685.65	1312.25	72.80	106.25	638.25	3.73	102.05	-	-	5327.98
Lorazepam	480.44	501.25	463.47	496.34	442.78	422.00	415.16	472.50	506.86	472.87	4673.67
Metformin	267.01	173.85	91.86	10.39	9.26	32.61	33.44	152.92	1.98	3.16	776.48
Methylparaben	27.40	22.50	-	10.05	5.25	2.54	2.05	-	-	-	69.79
Naproxen	142.95	-	-	-	-	-	15.98	-	-	-	158.93
Ofloxacin	610.58	393.85	216.36	178.58	152.29	278.33	283.59	215.27	212.77	147.99	2689.61
Paracetamol	3069.06	546.74	196.92	154.83	147.79	175.71	721.98	163.18	109.28	105.06	5390.55
Propylparaben	12.54	-	-	-	-	-	1.25	-	-	-	13.79
Salicylic acid	129.20	120.30	84.45	76.75	5.25	92.23	104.85	45.00	45.00	62.01	765.04
Tramadol	301.17	314.15	346.85	305.25	310.24	313.25	326.74	353.46	324.28	289.89	3185.28
Triclocarban	32.00	18.25	15.09	5.22	-	16.29	16.29	-	-	-	103.14
Triclosan	33.52	21.45	10.08	-	-	25.59	25.59	-	-	-	116.23
Trimetroprim	586.25	236.42	-	-	-	-	-	-	-	-	822.67

-: not detected

Table 2
Occurrence of CUPs and PPCPs in sediment of the study area.

	Concentration in Sediment (ng g^{-1})										
	Al-Asfar					Al-Hubail					
CUPs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	**Total per compound**
Atrazine	-	-	-	-	-	0.01	-	-	-	-	0.01
Chlorfenvinphos	1.25	0.56	1.16	-	0.70	0.82	1.25	0.49	1.06	0.54	7.83
Chlorpyrifos	0.21	0.33	0.23	-	-	0.20	0.21	0.28	0.24	0.20	1.90
Cyhalothrin	-	-	-	-	-	0.19	-	-	-	-	0.19
Diazinon	-	-	-	-	-	-	0.03	-	-	-	0.03
Imazalil	0.40	-	-	-	-	-	-	-	-	-	0.40
Imidacloprid	0.40	-	0.86	2.16	-	0.99	0.40	0.37	-	9.09	14.28
Terbuthylazine	-	0.27	0.32	-	-	0.83	0.06	-	0.17	-	1.65
Terbuthylazine-deethyl	-	0.05	-	-	-	-	-	-	-	-	0.05
PPCPs											
Alprazolam	79.19	82.25	-	75.32	77.80	82.45	80.08	87.00	-	-	564.09
Atenolol	5.78	-	-	-	2.50	13.51	7.08	11.35	11.35	7.10	58.67
Atorvastatin	84.49	68.72	14.00	56.21	21.00	35.08	49.24	28.96	47.00	32.00	436.70
Bisphenol A	65.35	35.33	-	88.41	12.43	90.85	24.86	3.22	9.85	12.87	343.17
Buthylparaben	-	11.53	-	-	11.36	-	-	-	-	-	22.89
Caffeine	13.32	75.96	7.07	11.58	25.53	53.02	54.85	64.02	64.00	42.00	411.35
Diclofenac	-	4.90	0.60	-	21.73	-	1.86	-	-	-	29.09
Etoricoxib	8.22	63.61	0.70	2.37	6.50	63.95	51.43	49.39	9.90	6.00	262.07
Ibuprofen	-	-	-	-	23.97	-	-	-	-	-	23.97
Lorazepam	126.46	120.49	116.00	123.04	115.00	109.68	100.58	118.00	111	120.00	1160.25
Metformin	-	-	-	0.19	-	0.27	0.10	0.32	0.60	0.28	1.76
Ofloxacin	-	-	-	-	-	17.16	-	-	-	-	17.16
Paracetamol	15.35	24.98	14.78	15.98	15.19	12.51	11.55	21.89	17.10	17.41	166.74
Salicylic acid	15.33	6.44	4.83	7.23	-	17.69	11.20	6.62	11.20	12.07	92.61
Simvastatin	472.95	379.11	38.36	63.16	557.00	388.00	589.27	510.00	476.00	419.00	3892.85
Tramadol	69.82	107.11	11.30	34.62	80.52	86.13	76.90	92.08	68.00	52.00	678.48
Triclocarban	-	-	4.67	-	-	10.36	4.19	-	-	-	19.22

-: not detected

Table 3
Occurrence of CUPs and PPCPs in soil of the study area.

	Concentration in soil (ng g^{-1})										
	Al-Asfar					Al-Hubail					
CUPs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	**Total per compound**
Carbendazim	-	-	-	-	-	-	0.04	-	-	-	0.04
Chlorfenvinphos	-	-	-	-	-	-	0.44	0.84	0.74	0.69	2.71
Chlorpyrifos	0.66	0.44	0.34	0.57	0.20	0.28	0.55	0.44	0.62	0.84	4.94
Fenitrothion	-	-	-	-	-	56.10	-	-	-	-	56.10
Imidacloprid	-	0.28	-	-	-	-	-	-	-	-	0.28
PPCPs											
Bisphenol A	18.07	32.23	19.47	3.87	22.77	15.76	45.25	19.55	19.79	8.75	205.51
Caffeine	2.89	12.49	25.44	3.32	1.74	5.15	4.55	2.36	4.39	3.32	65.65
Diclofenac	-	-	2.08	-	1.25	12.46	5.03	4.06	-	-	24.88
Ethylparaben	-	-	-	-	-	-	0.20	-	-	-	0.20
Ibuprofen	-	59.57	1.44	-	-	-	-	-	-	-	61.01
Metformin	-	-	-	-	-	0.28	0.67	-	-	-	0.95
Salicylic acid	18.03	9.45	13.23	6.68	6.17	76.07	9.44	9.38	10.77	11.20	170.42
Tramadol	-	-	1.76	-	-	-	-	-	-	-	1.76
Triclocarban	-	-	1.91	-	-	-	-	-	-	-	1.91
Triclosan	-	7.34	-	-	1.59	-	-	-	-	3.14	12.07

-: not detected

Table 4
Occurrence of CUPs and PPCPs in plants of the study area.

	Concentration in plants (ng g^{-1})										
	Al-Asfar					Al-Hubail					
CUPs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	**Total per compound**
Carbendazim	-	-	-	0.34	0.35	-	-	-	-	-	0.69
Chlorfenvinphos	-	-	-	0.90	-	-	-	0.87	-	-	1.77
Chlorpyrifos	0.65	0.44	0.21	0.47	0.36	0.49	0.44	0.44	-	-	3.50
Diazinon	-	-	0.84	2.67	-	2.63	-	-	-	-	6.14
Fenthion sulfone	53.88	62.33	-	-	-	-	-	-	-	55.62	171.83
Prochloraz	-	-	-	0.49	-	0.41	-	-	-	-	0.9
Terbuthylazine-deethyl	-	-	-	-	-	-	1.28	-	-	-	1.28
PPCPs											
Atorvastatin	-	-	-	-	-	5.67	16.70	-	-	-	22.37
Bezafibrate	-	-	-	-	-	62.06	17.52	-	-	-	79.58
Bisphenol A	96.72	45.13	51.28	36.97	38.83	126.18	3.18	96.72	28.19	15.38	538.58
Caffeine	-	-	5.42	3.36	3.05	-	-	3.01	3.01	-	22.61
Diclofenac	-	-	-	-	-	-	-	16.04	-	-	16.04
Ibuprofen	-	-	-	-	-	135.16	-	-	-	-	135.16
Metformin	1.14	1.40	0.75	0.29	0.29	1.28	-	0.71	0.26	27.87	33.99
Methylparaben	59.97	79.52	32.08	11.07	614.34	95.48	27.56	144.25	124.78	-	1189.05
Naproxen	-	-	-	-	-	-	67.66	-	-	-	67.66
Ofloxacin	-	-	99.48	-	-	-	-	-	-	-	99.48
Paracetamol	28.34	10.40	-	-	-	-	-	-	-	-	38.74
Salicylic acid	1952.00	218.37	317.55	90.74	313.54	151.67	147.26	272.54	779.86	680.79	4924.32
Tramadol	-	1.16	-	-	-	-	-	-	-	-	1.16
Triclocarban	-	-	-	-	-	-	0.21	-	-	-	0.21

-: not detected

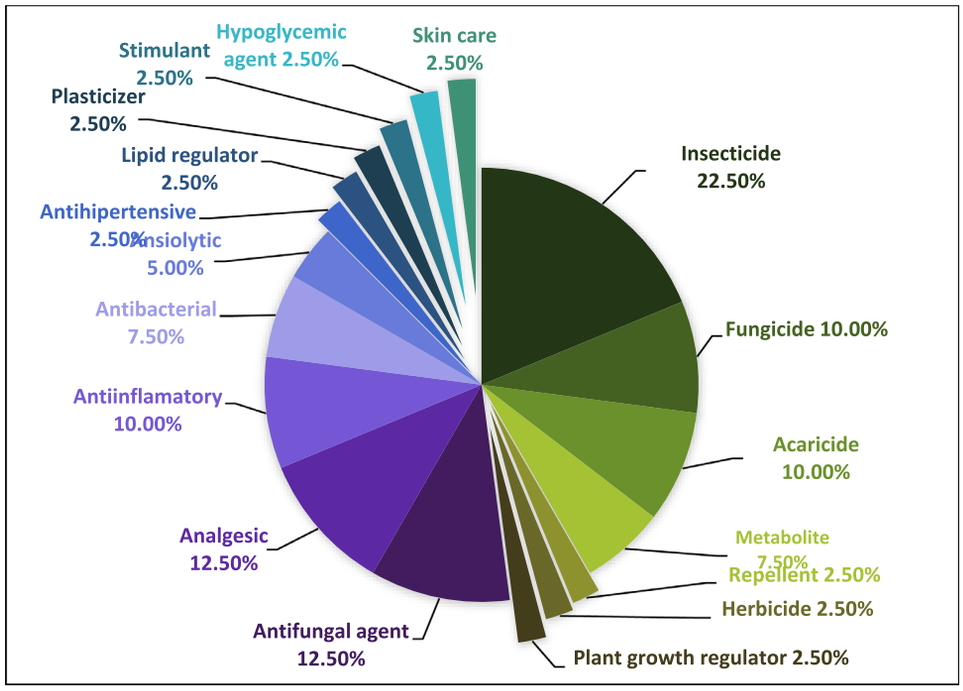

Fig. 1. Actions of the 40 compounds detected in the water samples of the study area.

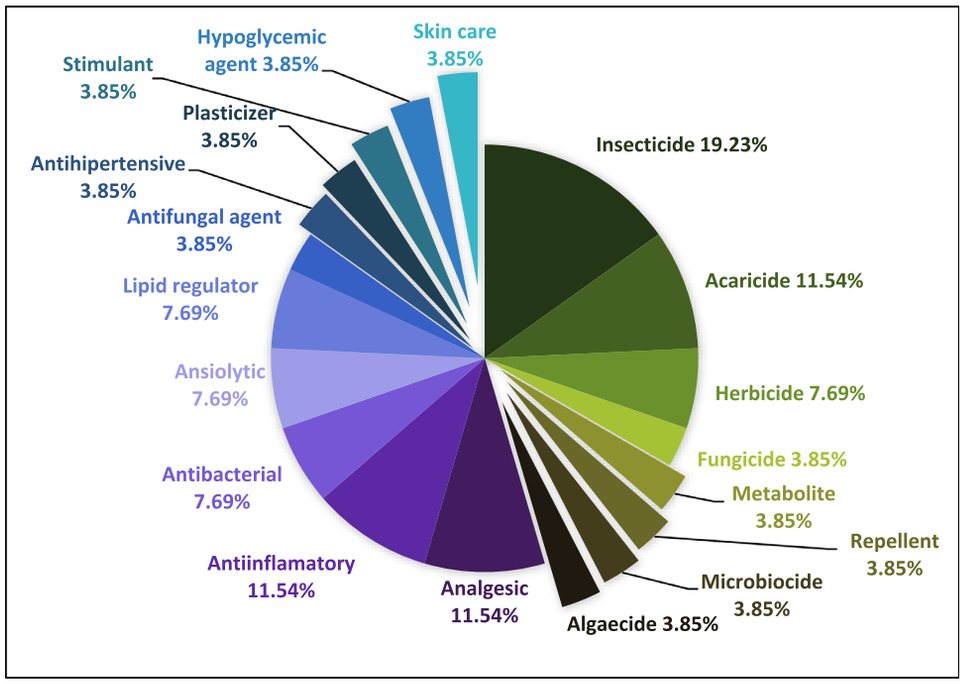

Fig. 2. Actions of the 26 compounds detected in the sediment samples of the study area.

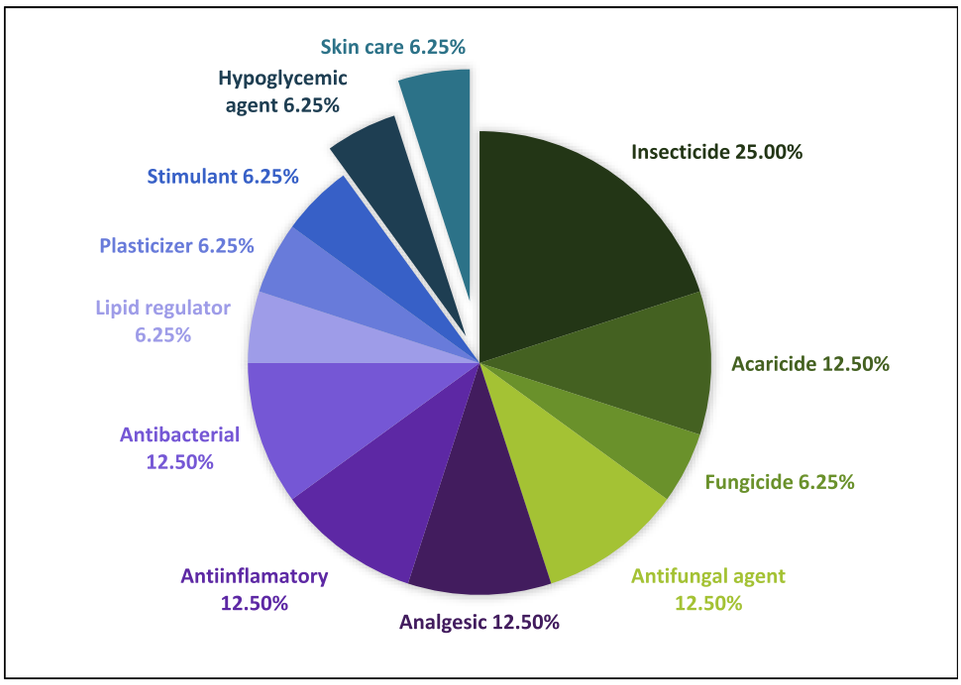

Fig. 3. Actions of the 16 compounds detected in the soil samples of the study area.

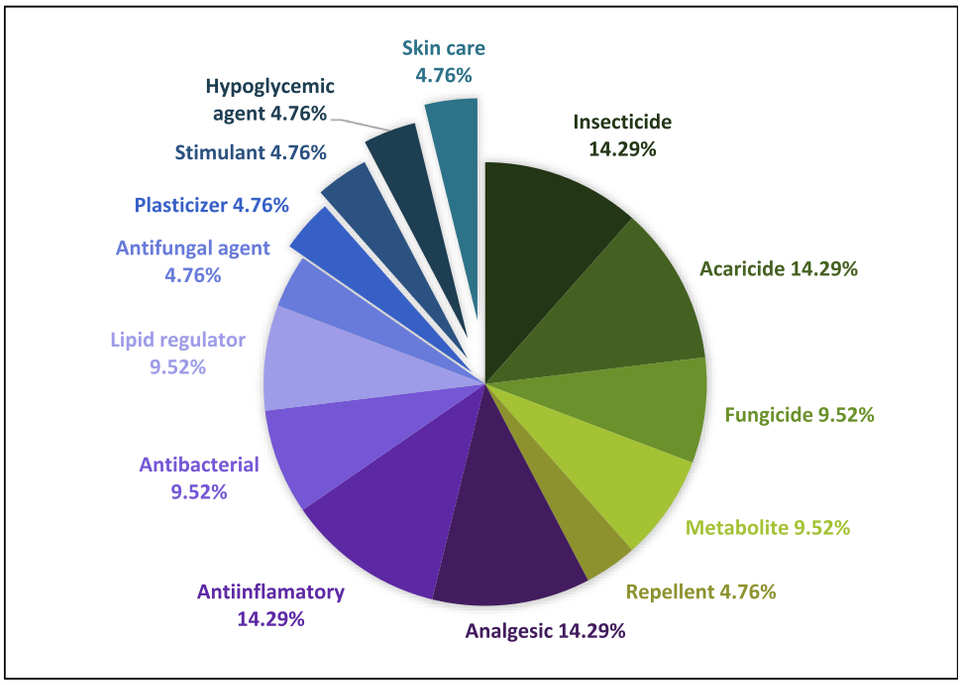

Fig. 4. Actions of the 21 compounds detected in the plant samples of the study area.

Lyophilized sediment, soil and plant were sieved (2 mm Ø) and extracted by ultrasound assisted extraction (UAE) using the McIlvaine–EDTA method, followed by the same SPE clean-up procedure as used for water samples [3]. To perform the UAE McIlvaine-EDTA buffer was prepared mixing 100 mL of 0.1 M citric ac. solution, 62.5 mL of 0.2 M Na_2HPO_4 solution and 6.05 g of Na_2-EDTA. Using MilliQ water as solvent. Then 1 g of sample was placed in a 50 mL Falcon plastic tube and spiked with the IS as described before. Then 5 mL of MeOH, 5 mL of MilliQ water and 5 mL of the MCIlvaine-EDTA buffer were added. The mix was vortex for 3 min, sonicated for 15 min and centrifuged for 6 min at 1811 rcf. The supernatant was collected in a 250 mL volumetric flask, filled with MilliQ water. Then the SPE was applied as described before.

The conditions used for the LC-MS/MS are exhaustively detailed in the related article, as well as the identification, characterization and main properties of the target analytes [1].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships, which have, or could be perceived to have, influenced the work reported in this article.

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DESARROLLO DE METODOLOGÍA ANALÍTICA

ARTÍCULO

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RESEARCH PAPER

Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants in mussel

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Abstract
This study aimed to develop multi-residue methods for the extraction of organic pollutants in mussels (*Mytilus galloprovincialis*), including 11 pharmaceuticals, 5 pesticides, 5 perfluoroalkyl substances (PFASs) and 2 illicit drugs. The combination of 4 different QuEChERS methods and 12 clean-ups (a total of 44 combinations) was tested. QuEChERS included acidified (AQ), non-acidified (SQ) and their miniaturized versions. The clean-ups included 6 different conventional dispersive solid phase extraction (dSPE) plus 2 enhanced matrix removal (EMR-Lipid) and 4 SPE procedures (including sorbents focused on phospholipid removal and polymer-based). After sample analysis via HPLC-MS/MS, the three methods that provided the best results were validated in terms of linearity, accuracy, precision, sensitivity and matrix effect. The methods selected were the combination of (i) SQ and EMR-Lipid, (ii) AQ and Z-sep+ bulk-based dSPE and (iii) AQ and graphitized carbon black (GCB)-based dSPE. Recoveries at two concentration levels (50 and 500 ng/g) ranged 54–124%, 59–124% and 60–127%, respectively, and limits of quantification (LOQs) were < 30 ng/g for most analytes using any of the methods. The three methods were tested in non-spiked mussel samples purchased in local markets, but organic pollutants were not detected in any sample. However, the methods probed to successfully extract a wide range of organic pollutants families in mussel samples from the market and from bioaccumulation trials.

Keywords Lipid removal · Clean-up · Pesticides · Pharmaceuticals · PFASs · Illicit drugs

Introduction

Emerging pollutants (EPs) (e.g. agrochemical and pharmaceutical residues, personal care products (PCPs), drugs of abuse and pollutants of industrial origin) [1] are commonly of anthropic origin and can reach the aquatic environment through sources such as the discharges from wastewater treatment plants (WWTPs) or uncontrolled release of residues. They can also proceed from the environmental degradation of precursors [1]. EPs have been widely reported in matrices such as water and sediment [2–4]. Although aquatic biota is exposed to these compounds and their adverse effects have been already demonstrated [5], studies about their occurrence in biota are less abundant [5, 6]. Interest in the analysis of EPs in aquatic biota is not only based on the negative effects on ecosystems' biodiversity but also in health risk assessment related to human consumption [7]. Accordingly, most studies have been carried out in fish [8–11] and other seafood [12], even though one of the strong constraints found is the lack of appropriate analytical methods due to the matrix complexity.

Aquatic biota samples are difficult matrices with high protein contents (from 15 to 22% depending on the species) and a highly variable lipid content ranging from <2% for crustaceans and mollusc to up to 25% for oily fish [13]. Such complexity poses a great challenge in the development of efficient and reliable extraction procedures [6]. The lipid content in Mediterranean mussels (*Mytilus galloprovincialis*) is usually between 2 and 4% w.w. and proteins around 15% [14].

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However, lipid content in mussels may vary depending on the geographical site, season, animal's life cycle, sex and spawning [15]. As an example, Prato et al. reported a total lipid content between 3.5 and 24.7% d.w. depending on the season [16].

Multi-residue methods, which analyse more than one family or type of compounds, are intended to show the whole picture of EPs present in the samples employing a single procedure, saving resources and time. These methods are still scarce because of the involved challenge to extract EPs with a wide polarity range eliminating lipid, proteins and other matrix components to obtain clean extracts and appropriate recoveries. Additionally, new extraction methods seek to reduce the consumption of organic solvents in order to achieve greener methods that are cheaper and less polluting. One of the extraction methods more widely used for its versatility and good results is the QuEChERS [6, 11, 17] and its miniaturized version called micro-QuEChERS [18].

After QuEChERS, the resulting extracts need clean-up to remove matrix components. Lipids and proteins are the most difficult to eliminate. In fact, such is the importance of lipids that most methods traditionally focused clean-up exclusively on their reduction [6, 19]. Column chromatography (now widely applied in the cartridge format) using polar sorbents such as silica (neutral or washed with acids and/or bases) and Florisil (used to retain fat and other non-polar compounds) and gel permeation chromatography (GPC) are most classical approaches. The dispersive solid phase extraction (dSPE) is also common with sorbents as primary secondary amine (PSA), to remove sugars and fatty acids, C_{18} for the lipids and $MgSO_4$ to sorb water [20]. However, the most used clean-up based on solid sorbents is the solid phase extraction (SPE), being HLB and Strata™ the more popular cartridges in several multi-residue extraction procedures [6]. Last developments in these clean-up techniques include specific sorbent modifications for matrices with high lipid and protein contents applicable in multi-residue extractions like the Captiva ND [21] and Phree™ to separate phospholipids, Ostro™ to remove phospholipids and proteins [22], and enhanced matrix removal (EMR-Lipid) to eliminate mostly triglycerides. The use of these new sorbents is still incipient given their recent commercialization. Although they offer a very promising solution for lipid- and protein-rich matrices [6], there is still a lack of systematic analysis and comparison with other more classical approaches.

The aim of the present study was to develop and validate multi-residue extraction procedures for twenty-three compounds in mussel matrix. The covered compounds belong to four chemical groups (pesticides, pharmaceuticals, new psychoactive substances and perfluoroalkyl substances (PFASs)) considered as high incidence EPs [1]. The individual compounds were selected taking into account those reported in aquatic environments by previous studies [3, 6, 7, 11, 23, 24]. Furthermore, as these contaminants include a wide variety of chemical structures and classes as well as polarities, the scope of the method could be easily extendable [8]. To achieve this overall purpose, two partial objectives were established: (i) to study the extraction efficiency of four types of QuEChERS and (ii) to examine the purification capacity for each type of QuEChERS by twelve clean-up procedures that involved dSPE, SPE and the use of new sorbents, as EMR-Lipid, Phree™ and Strata X-PRO for clean-up. This research provides up to our knowledge the first assessment of different clean-up methods including the new lipid removal phases.

Materials and methods

Reagents and materials

The HPLC grade methanol (MeOH) ≥ 99.8% purity, acetonitrile (ACN) ≥ 99.9% purity, trisodium citrate dehydrate (TSC), ammonium chloride (NH_4Cl) and ammonium hydroxide (NH_4OH) were from VWR Chemicals® (Radnor, PA, USA). Formic acid (CH_2O_2) was provided by ACROS ORGANICS (Geel, Belgium). Ammonium formate (NH_4HCO_2), magnesium sulfate ($MgSO_4$) and disodium hydrogen citrate sesquihydrate (DSC) were from Alfa Aesar (Karlsruhe, Germany). Sodium acetate 3-hydrate was from Panreac AppliChem (Barcelona, Spain). Sodium chloride (NaCl) was from Sigma-Aldrich (Steinheim, Germany). PSA and C18 sorbents were from Análisis Vínicos S.L. (Tomelloso, Spain). GCB dSPE was purchased from Agilent Technologies (Madrid, Spain). Z-sep+ bulk was from Supelco (Bellefonte, PA, USA).

EMR-Lipid clean-up dSPE was from Agilent Technologies. Strata™–X 33 μm Polymeric reversed phase, 200 mg/6 mL cartridges, Phree™ Phospholipid Removal Solutions 1-mL tubes and Strata™–X PRO Polymeric reversed phase, 60 mg/3 mL cartridges, were from Phenomenex® (Torrance, CA, USA). The VISIPREP™ manifold was distributed by Supelco. High purity water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). The 15-mL and 50-mL polypropylene centrifuge Falcon tubes were from VWR International Eurolab (Barcelona, Spain). Nylon 0.22-μm filters were purchased from Membrane Solutions (Plano, TX, USA) and polypropylene/polyethylene syringes were manufactured by BRAUN and distributed by Scharlab S.L. (Barcelona, Spain). The 2-mL amber glass vials with stoppers 99 mm + Septum Sil/PTFE used to inject the samples were also from Análisis Vínicos S.L. and the 250-μL polypropylene inserts were from Agilent Technologies.

The analytical standards of pharmaceuticals and personal care products (acetaminophen, atenolol, caffeine, diclofenac sodium, etoricoxib, ibuprofen, metformin, naproxen, salicylic

acid, triclosan, vildagliptin), pesticides (bentazone, chlorfenvinphos, chlorpyrifos, imazalil, terbuthylazine) and the PFASs perfluoropentanoic acid (PFPeA) and perfluorobutanesulfonate (PFBS) were from Sigma-Aldrich (Steinheim, Germany). Perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA) and perfluorooctane sulfonate (PFOS) were from Wellington (Ontario, Canada) and the illicit drugs bufotenine and 4-methoxyphencyclidine (4-MeO-PCP) were from LGC Standards (Ontario, Canada).

The internal standards caffeine-d_9, chlorfenvinphos-d_{10} (diethyl D_5), chlorpyrifos-d_{10} (diethyl D_{10}) and vildagliptin-d_3 were purchased from LGC Standards. Acetaminophen-d_3 and atenolol-d_7 were from Sigma-Aldrich. Triclosan-d_3 was acquired from Toronto Chemicals Research (Toronto, Canada). PFOA-d_4 (MPFOA), PFOS-d_4 (MPFOS) and PFDA-d_4 (MPFDA) were from Wellington.

Sampling

The samples analysed were Mediterranean mussels harvested in the Mediterranean Sea next to the city of Valencia, Spain. Mussels harvested in this area are cultivated using rafts and commonly known as "clòtxina"; they are available between March and July and are a very popular food for locals and tourists. Mussels were purchased from 3 different local markets, and then the shells were removed and the visceral mass was pooled in four different groups. The first group contained mussels from 3 supermarkets and was employed to test the different extraction procedures. The other 3 groups contained mussels from each supermarket, in order to test the selected method in real non-spiked samples. Then, the samples were placed in 50-mL Falcon tubes and frozen at −20 °C until analysis.

Sample extraction and clean-up

Four different QuEChERS procedures were tested: (i) the buffered version of the QuEChERS was a modified version of the UNE-EN 15662 [25], coined as "Standard QuEChERS" (SQ) from now on; (ii) a modified version of the official AOAC method [26], "Acid QuEChERS" (AQ) from now on; and (iii and iv) to minimize the waste of resources, miniaturized versions of both procedures were also tested (mini standard QuEChERS" (mSQ) and "mini acid QuEChERS" (mAQ)). These miniaturized QuEChERSs used the same steps and amount of sample but half the amount of reagents and sorbents.

The buffered QuEChERS was as follows: pooled mussel (1 g) was placed in 50-mL Falcon tubes. Samples were spiked with 200 μL of internal standard mix at a concentration of 1000 ng/mL. Then, 7.5 mL of MilliQ water and 10 mL of ACN were added followed by vortexing for 3 min to ensure homogenization. Next, 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of DSC and 1 g of TSC were added and the tube was immediately vigorously shaken for 3 min to avoid salt agglomeration. After 5 min of centrifugation at 3500 rpm (2465 rcf), the extract was separated in layers, with ACN at the top, and ready for clean-up.

Acid QuEChERS presented difference in the solvents and salts employed. The solvent added along with 7.5 mL MilliQ water was 10 mL of ACN-acetic acid 1%, and salts were 4 g of $MgSO_4$ and 1 g of sodium acetate. The other steps of the procedure were exactly the same as described above.

After the QuEChERS extraction, a total of 12 clean-ups were tested with each of the four QuEChERS extracts. These clean-ups included dispersive solid phase extraction (dSPE), lipid removal extraction and SPE.

Every dSPE was carried out in the same global procedure. That is, 1.5 mL of the supernatant was added to a 15-mL Falcon tube containing a mixture of salts. The tubes were vortexed for 30 s and centrifuged for 5 min at 3500 rpm. The supernatant was filtered using Nylon 0.22-μm filters and polypropylene/polyethylene syringes, and then, stored in vials with inserts ready for analysis. Six different salt mixtures were employed in the dSPE:

- Traditional (Tr): 150 mg of $MgSO_4$, 50 mg of PSA and 50 mg of C18.
- Carbon (Cb): 150 mg of $MgSO_4$, 25 mg of PSA and 7.5 mg of GCB.
- Z-sep+ 1 (Z+1): 50 mg of Z-sep+, 50 mg of PSA and 50 mg of C18.
- Z-sep+ 2 (Z+2): 25 mg of Z-sep+, 25 mg of PSA and 25 mg of C18.
- Z-sep+ 3 (Z+3): 150 mg of Z-sep+.
- Z-sep+ 4 (Z+4): 150 mg of Z-sep+ and 150 mg $MgSO_4$.

For the lipid removal clean-up, EMR-Lipid clean-up dSPE was used as follows:

– EMR-Lipid (EM): the EMR-Lipid mixture was placed in a 15-mL Falcon tube and activated by adding 5 mL of MilliQ water and vortexing for 30 s. Then, 5 mL of the supernatant was added and the tube was vortexed for 30 s more and centrifuged for 5 min at 3500 rpm. Next, 5 mL of the supernatant was added to another 15-mL Falcon tube containing the partitioning phase (so-called polish phase by Agilent) consisting of a mixture of 1600 mg of $MgSO_4$ and 400 mg of NaCl. The tube was then shaken vigorously for 30 s to avoid salt agglomeration and later centrifuged for 5 min at 3500 rpm. The extract was filtered and stored as described above until analysis.
– Traditional + EMR-Lipid (TEM): other versions of this procedure were tested adding a previous dSPE step. A mixture similar to that employed in the traditional dSPE consisting of 500 mg of $MgSO_4$, 165 mg of PSA and 165 mg of C18 was placed in a 15-mL Falcon tube.

Then, 5 mL of the supernatant was added and the tube was vortexed for 30 s and centrifuged for 5 min at 3500 rpm. Then, the procedure continued as described above, first with the EMR-Lipid dSPE and after with the partitioning dSPE, followed by filtration and storing.

The 4 SPE clean-up procedures were carried out in a VISIPREP™ manifold as follows:

- Strata-X (SPE): for this procedure, 4 mL of the supernatant was placed in a 100-mL volumetric flask which was filled with 100 mL of MilliQ water. Strata™–X 33 μm Polymeric reversed phase, 200 mg/6 mL cartridges were conditioned using 6 mL of ACN and 6 mL of MilliQ water. Then, 100 mL of the solution was passed through the cartridges drop wise using vacuum. Once all the samples passed, the cartridges were washed with 6 mL of MilliQ water and dried under vacuum for 15 min. Analytes were eluted using 2 mL of ACN followed by other 2 mL of the same solvent at gravity flow. The extract was then filtered and stored as described above, ready for analysis.
- Phree (Ph): 1 mL of the supernatant was loaded directly on the Phree™ Phospholipid Removal Solutions 1-mL cartridge and then passed through it drop wise under vacuum. The extract was then filtered and stored.
- Strata-X PRO basic (XB): the procedure used the 3-step RAPID method described by Phenomenex® [27]. Firstly, a pH 9 basic buffer solution of ammonium chloride and ammonium hydroxide was made. Then, 500 μL of the supernatant-buffer (1:1) solution was loaded directly into the Strata™–X PRO Polymeric reversed phase, 60 mg/3 mL cartridge and passed through drop wisely using vacuum. Next, the cartridge was washed with 600 μL of 5% MeOH in MilliQ water. The elution was carried out using 600 μL of 0.1% formic acid ACN-MeOH 9:1 under gravity flow. The extract was then filtered and stored.
- Strata-X PRO acid (XA): this procedure follows exactly the same steps of Strata-X PRO basic, the only difference is the buffer used. It was a pH 3.5 acid buffer solution of formic acid and ammonium formate.

Both Strata-X PRO clean-up procedures were the only ones that were not tested using the supernatant of the 4 QuEChERS. Only the supernatants from the non-miniaturized versions were used. This allowed a total of 44 combinations, which are summarized in Fig. 1.

LC-MS/MS analysis

Analysis was performed via LC-MS/MS, using an ExionLC AD coupled to a Sciex QTRAP 6500$^+$ mass spectrometer (both from Sciex, Concord, Ontario, Canada) with electrospray ionization (ESI) in Turbo Spray IonDrive mode (curtain gas 30 psi, ion-spray voltage 4.5 kV, temperature 350 °C and the ion source gases 1 and 2 at 50 and 65 psi, respectively). The QTRAP operated in multiple reaction monitoring (MRM) mode. The column used was an ACQUITY UPLC BEH C_{18} column (1.7 μm 130 Å, 50 × 2.1 mm, Waters). When operated in negative ionization mode, the mobile phases employed were (A) H_2O 2.5 mM NH_4F and (B) MeOH 2.5 mM NH_4F. For the positive ionization mode, the mobile phases employed were (A) H_2O 0.1% formic acid and (B) MeOH 0.1% formic acid. The linear gradient was as follows: 0 min (70% A), 12 min (5% A), 22 min (5% A), 23 min (70% A) and 30 min (70% A). The injection volume was 2 μL and column temperature was held at 45 °C. MS information of the compounds analysed is available in Tables S1 and S2 in the Supplementary Information (ESM).

Method validation and quality assurance

The chromatographic performance was stablished according to the US EPA criteria, each HPLC-MS/MS chromatographic peak should have a minimum of 10 data points [28]. Each combination of QuEChERS and clean-up (44 in total) included a procedural blank (non-spiked mussel pool). An 8-point calibration standard at 5, 10, 25, 50, 75, 100, 200 and 500 ng/mL in ACN was injected at the beginning and the end of each analytical sequence. The compounds in the calibration solution that deviated more than 20% from the theoretical concentration were excluded of the calibration curve. A standard was also injected every 15 samples to check the instrumental variation and to avoid false negatives as well solvent and procedural blank were injected to avoid false positive. Only regression coefficients (R^2) >0.99 were accepted in the calibration curve.

Recoveries were calculated in pooled mussels fortified at 50 and 500 ng/g w.w. (equivalent of 5 ng/mL and 50 ng/mL in the final extract) in triplicate. These concentrations are in the range of those found in the environment [2, 3, 22]. For caffeine, chlorfenvinphos, chlorpyrifos, vildagliptin, acetaminophen, atenolol, triclosan, PFOA, PFOS and PFDA, the reported recoveries were relative recoveries (RR%), were the matrix effect (ME), and other potential errors due to sample manipulation, were corrected thanks to the internal standard. For the other compounds, quantified with external calibration, the reported recoveries were really the efficiency (E%) (when affected by recovery and ME) or absolute recoveries (R%) (if the ME is corrected using matrix-matched standards). Both were calculated following Eq. 1, where "EC" is the expected concentration in the final extract assuming a recovery of 100%:

$$\text{RR\%or E\%} = \left(\frac{\text{Final concentration of the spiked sample}}{\text{EC}}\right) \cdot 100 \quad (1)$$

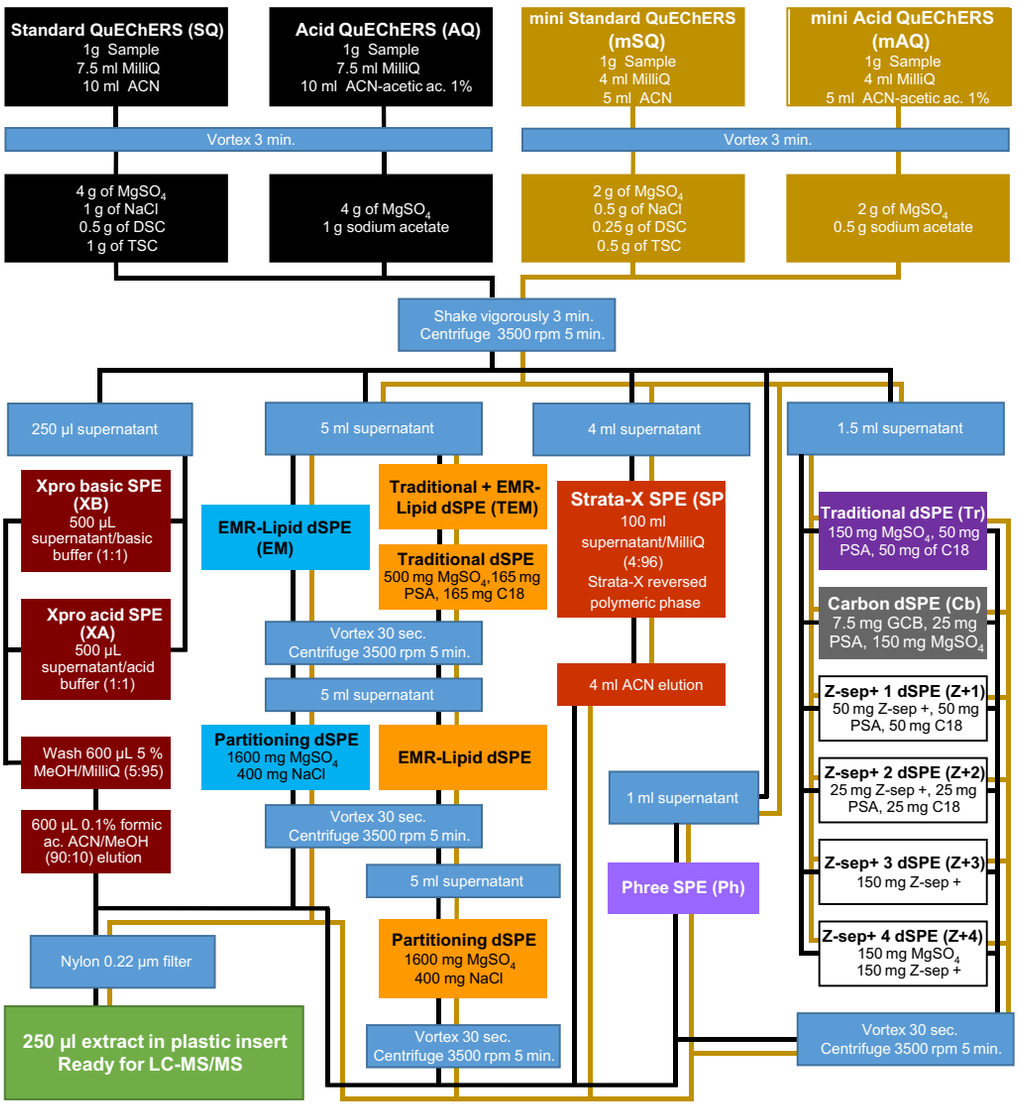

Fig. 1 QuEChERS and clean-up procedures tested for the development of a multi-residue extraction procedure in mussel (*Mytilus galloprovincialis*)

Method limits of detection (LODs), method limits of quantification (LOQs) and precision were determined analysing in triplicate mussels spiked at 50 ng/g, to obtain a concentration of 5 ng/mL in the final extract. Each extract was injected twice. The LOD and LOQ for each analyte were estimated as the concentration that provides a response 3 × and 10 ×, respectively, the signal-to-noise ratio. Precision was evaluated in terms of intra-day repeatability (Intra-R) and inter-day reproducibility (Inter-R). Intra-R was calculated as the SD of the signal divided by mean (% RSDs) of the same six injections used for the determination of LODs and LOQs. For the determination of Inter-R, one replicate at 50 ng/mL used for the recoveries described above was injected in three different days ($n = 3$) and Inter-R was calculated as the SD of the signal divided by mean (% RSDs).

For the determination of ME, a batch of the mix containing the external standards of the target analytes at the same concentration that of the calibration curve (5, 10, 25, 50, 75, 100, 200 and 500 ng/mL) was prepared in ACN. Next, 300 µL of this mix was introduced in different 15-mL Falcon tubes, along with one blank per batch, and evaporated to dryness under a gentle stream of nitrogen in a STUART Sample Concentrator SBHCONC/1 using a STUART Block Heater SBH200D/3. Then, SQ-EM, AQ-Z+2 and AQ-Cb extraction procedures were performed as described above and 300 µL of the mussel extract was used to redissolve the content of the Falcon tubes followed by 30-s vortexing, 3-min sonication and storing. After LC-MS/MS analysis using external calibration, ME was calculated comparing the slope of the calibration

curve in matrix and the slope of the calibration curve in ACN [21] (Eq. 2).

$$\mathrm{ME} = \left(\frac{\text{Slope of calibration curve in matrix}}{\text{Slope of calibration curve in ACN}} \right) \cdot 100 - 100 \quad (2)$$

Additional recovery tests were performed for the methods selected for validation. In this case, the samples were fortified at 50 and 500 ng/g w.w. and the procedure was the same as that described above.

Results and discussion

The combination of the 4 QuEChERS and the 12 clean-ups resulted in 44 different extraction procedures (combinations of mSQ and mQA with the clean-ups XA and XB were not tested). Table S3 and Fig. S1 (see ESM) show the RR% and E% of these tests. However, all of them will be named recoveries in order to simplify the discussion.

The QuEChERS extraction

In accordance with the European Commission Guidelines, recoveries within the range 70–120% are considered acceptable [29]. However, since E% might be affected by strong ME, the comparison of the different methods prior to validation was carried out contemplating recoveries 50–140% as "satisfactory" since, also according to the European Commission Guidelines, this practical default range may be used for individual recoveries in routine analysis if they are consistent (RSD ≤ 20%) [29]. Some of the compounds are not recovered properly. The worst is metformin, which in no case is recovered substantially (< 20%). This behaviour was attributed to its physico-chemical properties (basic character with pKa = 12.4, positively ionized at environmental pH, high solubility in water and poor in acetonitrile) [30]. Metformin will not be considered further.

Salicylic acid was tested in this study but was also discarded. The results showed concentrations up to thirty times higher than the spiked one (around 15,000 ng/g). This compound was also present in the blanks and its concentrations were two times higher in the miniaturized QuEChERS. This could mean that mussels were highly contaminated with salicylic acid; indeed, the study of Nuñez et al. [31] attributed significant levels (103 ng/g) of salicylic acid in their blanks to environmental contamination. However, a more likely possibility is that a compound naturally present in mussels, such as 3,4-dihydroxybenzaldehyde—used by mussels for adhering to surfaces—may have interfered with the salicylic acid signal since both compounds have the same empirical formula (isobaric interference) and MS/MS fragments in common [32]. Further research is needed to clarify it.

The original QuEChERS method does not recommend water addition in samples with moisture >80% [17] as is the case of mussels, but it commonly uses 10 g of sample (equivalent to 8.2 mL of water). In this case, 1 g of sample is used which means that without water addition, the water content is just 0.8 mL. Without water addition, the separation of ACN layer becomes complicated. It should be kept in mind that mussels are much complex matrix that those for which this method was designed. Both, lipids (2–4%) and proteins (ca. 15%) are prone to forming emulsions. The addition of water (to make it equivalent to what would be obtained with 10 g of sample) in the QuEChERS for aquatic biota has been also widely reported without significant loss in the recoveries of most polar compounds [9, 20]. The water volume selected in this study was within that selected in those studies.

In this way, SQ extraction allowed the extraction from 11 to 19 compounds, out of the total of 21 compounds analysed after the exclusion of metformin and salicylic acid, with recoveries ranging 50–140% depending on the clean-up (except for both XA and XB). Depending on the clean-up employed, very low recoveries or even total loss of the compound was also found for atenolol, bufotenine and 4-MeOPCP while high recoveries (130–180%) were observed for triclosan, PBS and PFPeA. The latter also has a high variability and recoveries can vary from unrecovered to 180%. This can be due to a strong negative or positive matrix effect or to some of the physico-chemical properties of the analytes. Atenolol at any value above pH 3.0 is a neutral compound but quite soluble in water and difficult to solubilize in acetonitrile. There is an important lack of bufotenine and 4-MeO-PCP data but both substances are ionic at any of the pH tested and probably they could form easily complexes with other molecules. This could explain the lack of recoveries in some cases. It is also well-known that bufotenine degrades rapidly 5-hydroxyindoleacetic acid, which can also justify low recoveries.

Some differences were observed for the compounds analysed in the negative ionization mode depending on the QuEChERS employed (ESM Table S3 and Fig. S1). Since they are neutral at acidic pH, PFPeA, naproxen and ibuprofen showed higher recoveries and lower variability with AQ than with SQ. Differences in the retention times were also observed for salicylic acid, PFPeA and PFBS, which showed an increase of 1 min in their retention times with the acidified version. Naproxen also showed higher retention times, but shift was usually less than 30 s. Finally, PFPeA, PFBS, diclofenac and naproxen showed less background noise when extracted using the acidified version. PFPeA and PFBS were often over recovered when both acidified versions of QuEChERS were employed. Few studies employing QuEChERS acidified with formic acid for PFPeA and PFBS were found [33, 34]. Previous studies did not report unusual recoveries for these compounds. However, they were tested in other matrices (fruits, vegetables or

milk) and not compared with non-acidified versions. PFOA, PFDA and PFOS results were corrected by the IS, but their chromatographic response showed a similar behaviour. Altogether, the results showed that AQ was generally better for compounds analysed in negative ionization mode. Non-remarkable differences between QuEChERS methods (SQ and AQ) were noticed for compounds analysed in positive ionization mode.

On the other hand, mSQ, which saves solvents and internal standard, provided similar recoveries to those of SQ with values from 40 to 130% for 10 to 19 analytes depending on the clean-up, and the behavioural pattern of the studied compounds was the same. Since the proportion solvent/sample was the double using miniaturized QuEChERS, the extracts were dirtier. Although the signal of the analytes was generally enhanced compared to non-miniaturized QuEChERS, the absolute intensity of the background noise was also the double, interfering with those compounds that presented poor recoveries and providing higher detection limits.

Clean-up procedures

This section is focused in the clean-ups of the three procedures that obtained the best recoveries (SQ-EM, AQ-Z+2 and AQ-Cb) and therefore were validated. The results of all the combinations and discussion about the other clean-ups can be found in ESM in Table S3, Fig. S1 and Text S1, respectively. The clean-up methods tested were prepared to eliminate (or to retain in different sorbents) matrix interferences, such as proteins, carbohydrates or lipids. However, specific matrix components and analytes can also interact by the formation of complexes, even after the clean-up step. For example, the 4-MeO-PCP case showed poor recoveries (generally below 20%) except for SPE (95–130%) or Cb (64–80%) as clean-up. This could be explained by specific interaction between sorbents and some interferents. In the case of GCB, its chemical structure contributes to the formation of the positively charged oxonium group that can interact by anion exchange and the hydrophobic interactions with specific non-polar pigments or sterols.

Using Tr clean-up, a higher number of compounds were recovered after the AQ (20) than the SQ (18). More compounds were within the 50–140% practical range using the SQ-Tr (13, 62%) than the AQ-Tr (10, 48%). In mSQ and mAQ, 13 and 11 analytes, respectively, were within this range. This clean-up was used as a reference for comparison.

GCB in combination with QuEChERS has been widely employed for green vegetables since it removes pigments [35]. This ability is due to the GCB capacity of retaining planar compounds (such as sterol-derived molecules). However, this sorbent can retain many other types of molecules such as non-polar compounds and other structures susceptible of (π-π) interactions. Hence, Cb clean-up was tested in order to eliminate mostly slight orange-yellow pigmentation observed in the ACN supernatant of the different QuEChERSs. Its use along PSA (which removes sugars and fatty acids) was expected to provide a comprehensive clean-up of the sample. Compared with the traditional clean-up procedure, higher recoveries were obtained for 4-MeO-PCP, acetaminophen, atenolol, bufotenine, caffeine, chlorfenvinphos, chlorpyrifos, imazalil and vidagliptin. This pointed out that low recoveries can be due to the matrix effects, interactions between analytes and matrix components or even adsorption of the compounds to the sorbent [9, 19, 21, 36]. Furthermore, Cb clean-up provides cleaner extract than Tr as could be observed by the reduction of the baseline noise.

The use of Z-sep+ is recommended for matrices with >18% of lipid content and has shown to eliminate lipid interferences better than PSA and C18 [10]. Furthermore, not in the field of sorbent clean-up but in other aspects of protein research, zirconium has shown ability to retain proteins [37]. Regarding the different procedures involving Z-sep+, all of them enable recoveries ranging 50–140% of 11–15 and 10–19 compounds for SQ and AQ, respectively. Among them, Z+2 provided the best results improving recoveries of acetaminophen, bufotenine, caffeine, chlorpyrifos, imazalil and vidagliptin in comparison with the Tr clean-up.

EM clean-up is divided in two steps: first the EMR-Lipid dSPE, which is specially focused in lipid removal, then the partitioning step removes the excess water and improves analyte partitioning [38]. Results for EM showed high recoveries for acetaminophen, bentazone, bufotenine, chlorfenvinphos, chlorpyrifos, dichlofenac, imazalil and vidagliptin. Furthermore, acid compounds (ibuprofen, naproxen and PFPeA) also improve their recoveries using SQ. Using this clean-up, only 4-MeO-PCP was not recovered. This method provides the cleanest extract comparing the baseline noise reduction.

Method validation

Method validation was performed for the three methods that showed the best recoveries for the higher number of compounds. The selected methods were SQ-EM, AQ-Z+2 and AQ-Cb which respectively extracted 20, 19 and 20 compounds within the 50–140% practical range. The GCB and zirconium sorbents thanks to their physico-chemical properties (such as surface pores with high surface area, important hydrophobic interaction) contribute to reduce the amount of lipids and pigments that could damage the detection of the studied compounds. Furthermore, the acid condition could contribute to reduce ionization phenomena for acid substances, improving their recoveries.

Sensitivity and precision

Intra-R (Table 1) was acceptable (≤20%) [29] in all the cases except ibuprofen (25%) in SQ-EM and atenolol (32%) in

Table 1 Validation of the selected methods in terms of absolute and relative (for compounds with internal standard) recoveries (Rec.) at two different concentrations, 50 ng/g ($n = 6$) and 500 ng/g ($n = 3$), LOQs ($n = 6$), Intra-R ($n = 6$) and Inter-R ($n = 3$)

	SQ-EM					AQ-Z+2					AQ-Cb				
	Rec. (%) 50 ng/g	Rec. (%) 500 ng/g	Intra-R (% RSDs)	Inter-R (% RSDs)	LOQ (ng/g w.w.)	Rec. (%) 50 ng/g	Rec. (%) 500 ng/g	Intra-R (% RSDs)	Inter-R (% RSDs)	LOQ (ng/g w.w.)	Rec. (%) 50 ng/g	Rec. (%) 500 ng/g	Intra-R (% RSDs)	Inter-R (% RSDs)	LOQ (ng/g w.w.)
4-MeO-PCP	16	5	4	**	16	30	28	1	**	2.3	99	96	4	26	10
Acetaminophen	91	84	4	13	9.7	45	107	4	26	5.1	92	94	8	6	42
Atenolol*	-	112	7	24	63	-	99	7	26	63	105	103	32	35	86
Bentazone*	-	88	5	25	46	-	85	5	35	46	-	76	5	19	46
Bufotenine	57	111	6	32	5.8	38	68	4	17	2.5	37	90	1	13	0.65
Caffeine	41	53	11	19	13	59	63	9	18	16	40	68	2	17	8.4
Chlorfenvinphos	101	76	4	26	19	102	73	0	18	0.39	101	81	3	29	18
Chlorpyrifos	105	96	0	22	0.78	102	67	1	17	2.4	105	67	6	11	24
Diclofenac	134	103	4	10	29	137	120	1	21	5.5	119	118	2	3	13
Etoricoxib	106	79	3	28	11	93	74	8	18	27	109	60	7	28	22
Ibuprofen	116	106	25	9	68	106	93	8	17	32	114	126	2	12	14
Imazalil	107	124	1	26	4.4	76	115	4	27	10	91	102	4	24	21
Naproxen	114	97	3	12	11	103	105	10	17	30	111	96	1	19	5.5
PFBS	109	123	2	12	17	94	112	2	9	13	105	124	7	15	32
PFDA	107	95	2	12	9.9	127	100	6	8	35	107	104	7	9	33
PFOA	73	95	3	13	3.3	95	113	1	13	1.1	108	117	3	10	16
PFOS	74	91	2	9	8.5	78	93	3	6	9.6	74	97	4	3	19
PFPeA	119	111	2	17	13	127	120	1	18	6.1	120	123	6	11	28
Terbuthylazine	120	105	1	12	6.1	124	119	3	3	16	87	127	3	3	16
Triclosan	72	97	9	16	37	86	121	4	10	18	72	90	17	26	49
Vildagliptin	91	57	1	23	4.8	91	61	4	19	16	91	94	3	11	11

*LOQs were calculated using the lowest point of the linearity (5 ng/ml). Except atenolol for AQ-Cb

**Low recoveries avoided the determination of a reliable Inter-R

Bold files represent RR%; the other compounds are represented in R%

AQ-Cb. Due to the low recoveries of 4-MeO-PCP, a reliable Inter-R cannot be calculated (except for AQ-Cb). For the rest of the compounds, the Inter-R was ≤30%, except for bufotenine in SQ-EM, bentazone in AQ-Z+2 and atenolol in AQ-Cb. The background noise was too high to calculate the LOQs of bentazone and atenolol (except atenolol for AQ-Cb); therefore, the lowest point of the linearity (5 ng/mL) was injected 6 times to calculate the LOQs for these compounds. LOQs ranged 0.78–19 ng/g (average 11 ng/g) for most of the compounds extracted by SQ-EM; some exceptions were the pharmaceuticals ibuprofen and triclosan of which LOQs were 68 and 37 ng/g, respectively. For AQ-Z+2, LOQs ranged 0.39–30 ng/g (average 11 ng/g) except for ibuprofen and PFDA with LOQs of 32 and 38 ng/g, respectively. In the case of AQ-Cb, LOQs ranged 0.66–28 ng/g (average 15 ng/g), except for acetaminophen, PFBS, PFDA and triclosan with LOQs of 42, 32, 33 and 48 ng/g, respectively. Atenolol and bentazone were also among the highest LOQs, but since they were calculated using the linearity, their LOQs are related to the analytical method rather than to the extraction procedure. As can be seen in Table 1, the lowest LOQs were usually achieved by SQ-EM and AQ-Z+2, while AQ-Cb presented higher values for more compounds. This can also be seen in the chromatograms (ESM Fig. S2), where SQ-EM generally shows the best ion-noise ratio, closely followed by AQ-Z+2. However, the LOQs may vary substantially from one method to another depending on the compound, such is the case of chlorfenvinphos and diclofenac sodium of which LOQs were respectively 50 and 5 times lower with AQ-Z+2 than with SQ-EM. Bufotenine, chlorpyrifos and PFOA were usually among the compounds with lower LOQs for all the three extraction procedures, while ibuprofen, triclosan, and PFDA were among the highest. The reported sensitivity is in accordance with previous studies [12, 36], although it varies significantly depending on the compound.

However, the present LOQs are higher than those achieved by some other works, especially those including a sample concentration step [11, 21]. Evaporation step was not applied in order to avoid possible concentration of remaining residues (lipids, proteins, etc.) that might result in chromatographic interferences.

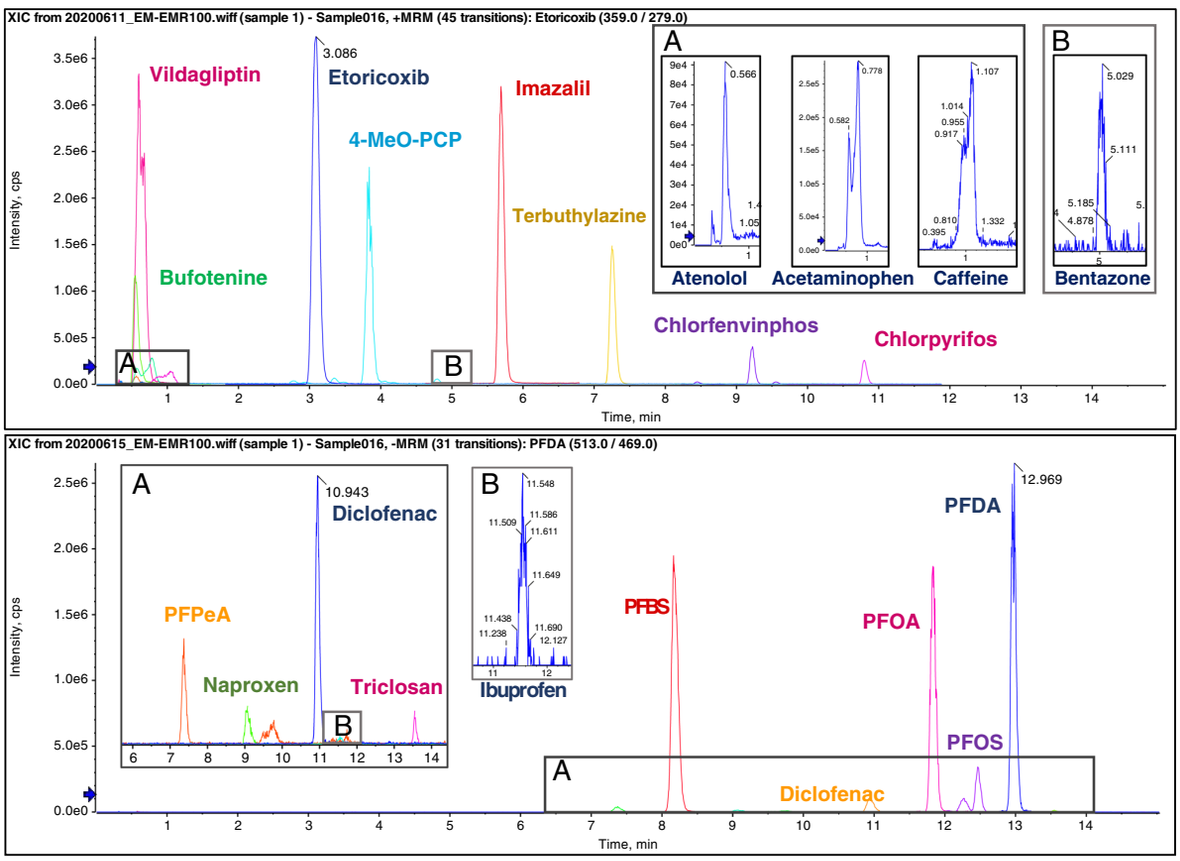

Fig. 2 Chromatograms of the target compounds from the SQ-EM matrix effect sample spiked at 100 ng/mL. *Up*: compounds with positive ionization. *Down*: compounds with negative ionization

Matrix effect

ME was categorized as low (0–10%), moderate (10–30%) and strong (>30%). Although the analytical method provided a good separation for most of the compounds (Fig. 2), Table S4 (see ESM) and Fig. 3 show strong ME for several compounds. SQ-EM and AQ-Z+2 presented low to moderate matrix effect for the 59% of the compounds, while in AQ-Cb, for the 55%. The signal enhancement or suppression for each compound was similar for the three methods, especially between SQ-EM and AQ-Z+2 (Fig. 3). The compounds showed predominant signal suppression except for most of the PFASs and triclosan, which generally presented from low to strong positive ME. These were significantly weaker in QA-Cb. On the other hand, acetaminophen, bufotenine, atenolol, vildagliptin and caffeine presented strong negative effect matrix for the three methods, generally ≤ −50%. Results show similarities in the ME of pesticides for AQ-Z+2 (−33 to −19%) and AQ-Cb (−25 to −23%), while the study of Kaczyński et al. showed slightly weaker ME for pesticides when using GCB than for zirconium-based dSPE [10]. However, they were tested in different fish matrices: liver and muscle respectively.

Predominance of signal suppression in biological samples is fairly known, particularly, for positive ionization mode using ESI [9, 19, 21]. Sapozhnikova and Lehotay [36] also showed predominant positive ME in pesticides when using QuEChERS along with zirconium-based dSPE in fish. Peña et al. employed a procedure similar to SQ-EM to extract pharmaceuticals for two types of fish [19]. They observed a predominance of strong signal suppression (among 32–92% for the 72% of their compounds) in samples of *Leuciscus cephalos*, and the ME obtained from *Salmo salar* was generally weaker, as an example, acetaminophen (7%) and atenolol (19%). They suggested that the higher fat content reduced the ME. This is in concordance with the results, since mussels have significant lower fat content and showed significant higher ME.

Recoveries

After the correction of the ME, SQ-EM, AQ-Z+2 and AQ-Cb were able to recover 16, 15 and 15 compounds, respectively, within the acceptable ranges (70–120%) [29] at 500 ng/g with recoveries ranging 53–123% and 61–121% for SQ-EM and AQ-Z+2, respectively. On the other hand, AQ-Cb showed generally higher recoveries ranging 60–127% for all the compounds. There were some discrepancies between recoveries at the two concentration levels spiked, such is the case of bufotenine, which presented remarkable better recoveries at 50 ng/mL, or bentazone, which was not detected at 5 ng/mL. The opposite behaviour can be seen for vildagliptin with better recoveries at 5 ng/mL (Table 1). Altogether, the other

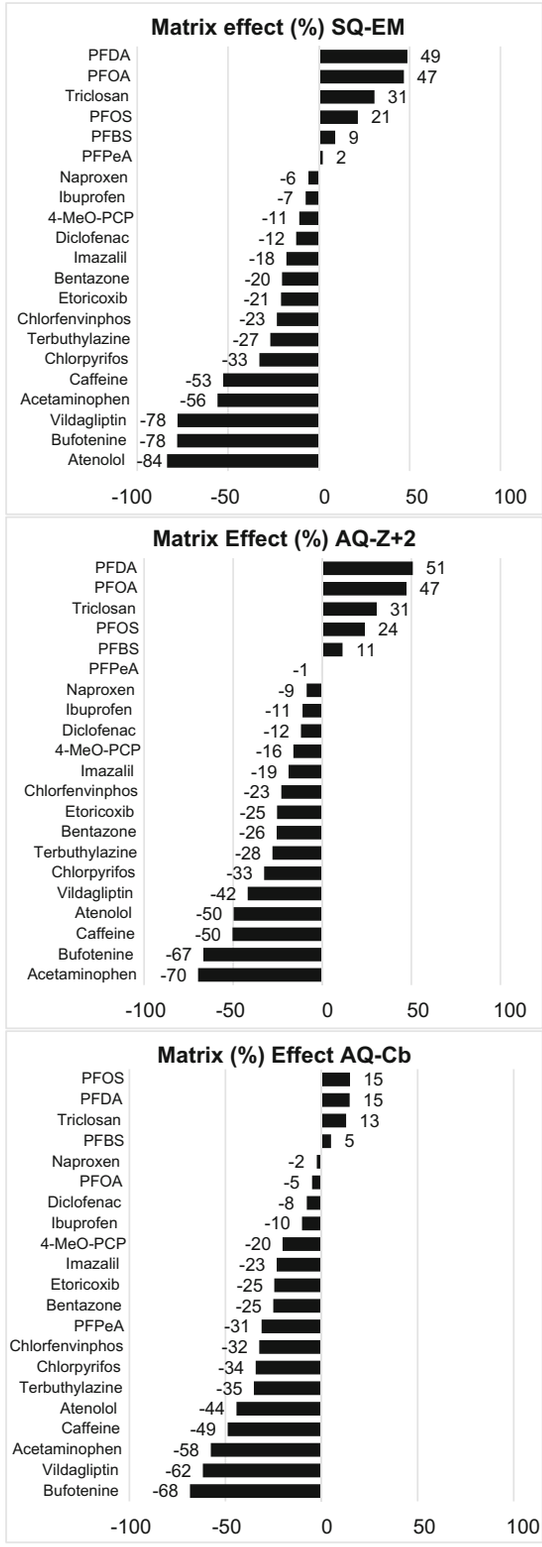

Fig. 3 ME (%) of the validated methods for each compound

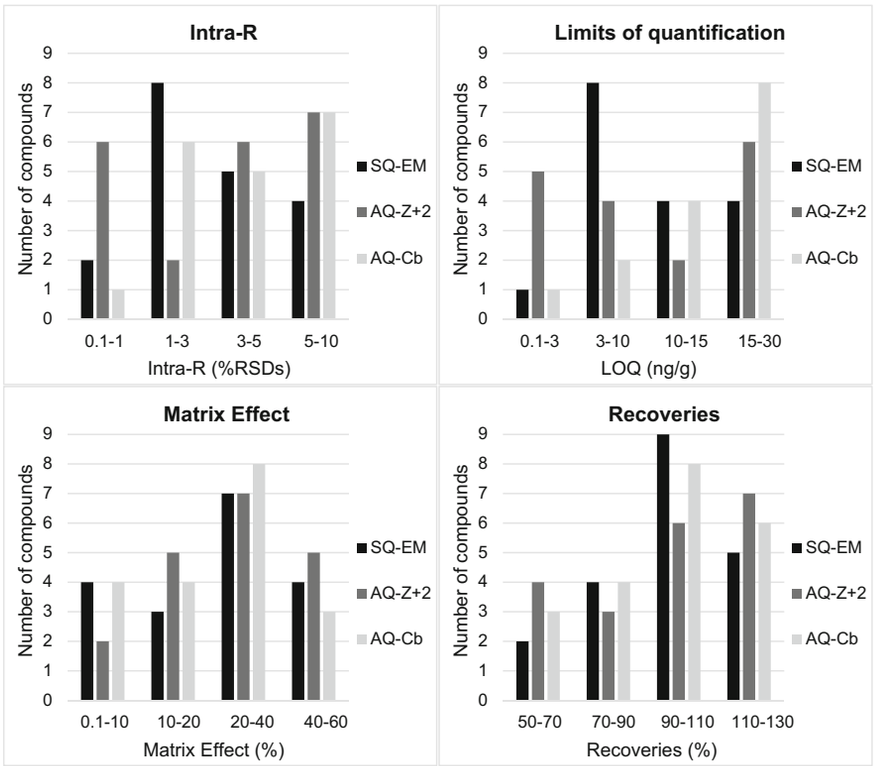

Fig. 4 Comparison of the parameters: Intra-R, LOQs, ME and recoveries of the three selected methods. The graphics show the number of compounds that fulfil the different ranges of values for the different parameters

compounds have a general good agreement between both fortified concentrations.

QuEChERS is highly used for pesticide extraction. The results of the present study are in concordance with previous studies, which show QuEChERS (which is usually followed by a dSPE containing $MgSO_4$, PSA and C18) as a suitable methodology for pesticide extraction in aquatic biota [6], although QuEChERS is not a common method for pharmaceuticals, illicit drugs and PFASs extraction. Previous multi-residue methods using QuEChERS showed recoveries ranging 40–118% for pharmaceuticals [12, 39], 84–114% for PFASs [40, 41] or 77–118% for illicit drugs [12]. The recoveries of the present study ranged 60–120% for most of the compounds. Along with the previously cited studies, the present work shows that QuEChERS (along with a proper clean-up) is a promising extraction procedure for multi-residue approaches.

Previous works employing QuEChERS in combination with Z-sep+, GCB or EMR-Lipid in aquatic biota are scarce (especially those regarding the use of GCB). Recoveries present in Table 1 were slightly better than those obtained by Peña et al. [19], using QuEChERS and EMR-Lipid in fish samples for pharmaceutical extraction. Baduel et al. also tested different multi-residue procedures in fish using Captiva ND lipid cartridges, and results showed similar recoveries for acetaminophen (78%), naproxen (91%) and terbuthylazine (80%); better recoveries for caffeine (85%); and poorer recoveries for diclofenac (63%) and atenolol (45%) [21]. Han et al. evaluated EMR-Lipid for the extraction of pesticides from salmon and showed variable recoveries (11–151%) [42]. Their results for imazalil (85%) and chlorpyrifos (66%) were lower than those obtained in the present work (124% and 105% respectively). Kaczyński et al. and Sapozhnikova showed recoveries ranging 70–120% for most of the pesticides analysed using zirconium-based dSPE in fish muscle [10, 36]. Furthermore, Kaczyński et al. obtained similar results using GCB in fish liver [10], while Zhang et al. recovered 70–119% for the majority of pesticides extracted in fish using GCB along with PSA [43]. In the case of PFASs, the studies of Gao et al. and Chiesa et al. showed recoveries ranging 90–105% in fish using QuEChERS [11] and 82–114% in bivalves using SPE [40], respectively, which overall is in concordance with the results of this study. Unluckily, and up to our knowledge, studies on QuEChERS extraction of bufotenine and 4-MeO-PCP from mussels or aquatic biota were not reported. Nevertheless, López-Garcia et al. developed a method for other illicit drugs with recoveries ranging 80–115% [12].

Comparing the results, the three methods showed similarities in the ME and recovery pattern (Fig. 4). SQ-EM and AQ-Cb showed better recoveries than AQ-Z+2; it is remarkable that AQ-Cb was the only method among the validated which recovered 4-MeO-PCP satisfactorily, so it was chosen as the

best option. However, SQ-EM and AQ-Z+2 presented similar recoveries with lower LOQs and Intra-R (Fig. 4). It is also remarkable that SQ-EM showed the lowest background noise levels in the chromatograms and might be a very interesting option for samples with higher lipid content [19].

Application to non-spiked samples

Samples of mussels from 3 different local markets were extracted in order to test the efficacy of the methods in non-spiked samples. Five mussels of each market were pooled and extracted in triplicate. The results showed that no compounds were detected in any sample with any method. However, in the city of Valencia, commercial mussels usually spend time in a purification plant before they are sold. In these plants, the mussels spend several weeks in clean water in order to remove impurities and pollutants. This purification process likely removed part of the target pollutants, since the chromatograms of some samples showed distinctive peaks for PFOA with signals below the LOQs (ESM Fig. S3). Further research is needed to elucidate the occurrence of EPs in mussels from coasts next to Valencia.

As alternative, the three methods were tested in samples from a bioaccumulation study with mussels. In this study, the mussels were exposed to different EPs dissolved in water at a concentration of 10 ng/mL during 28 days. The samples analysed corresponded to the 7th day of exposure. The compounds naproxen, diclofenac, triclosan, etoricoxib, imazalil, terbuthylazine, chlorfenvinphos, chlorpyrifos PFOA, PFDA and PFOS were successfully detected (ESM Fig. S3) and quantified in these samples with the three methods, showing concentrations ranging 16.8–172 ng/g for most of them and higher ranging 370–826 ng/g for chlorpyrifos, imazalil and PFDA.

Conclusions

The study of several extraction and clean-up platforms provides insight into the best procedures for extracting each type of compounds. The three methods validated are able to extract a wide range of types of organic pollutants within the acceptable ranges. Predominant signal suppression was determined for the three methods as part of the ME. AQ-Cb was the method with the best recoveries and ME for the compounds selected in the present study. On the other hand, SQ-EM achieved lower LOQs and better signal-noise ratio, making it a promising option for samples with high fat content. Metformin was not satisfactorily extracted by any procedure, while 4-MeO-PCP was satisfactorily extracted just by procedures using SPE and Cb as clean-up. Based on the results of this study, we concluded that the simple changes made to the classic QuEChERS method can improve the overall recoveries for many types of different contaminants without sacrificing the performance of a multi-residue method. However, the results of this study also pointed out the need to explore alternative methods for some compounds that are not properly extracted using QuEChERS.

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Data availability All data generated or analysed during this study are included in this published article.

Code availability Not applicable

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

Source of biological material Local supermarkets from the city of Valencia, Spain.

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Supplementary Information

Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants in mussel

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Table of contents

Text S1. Clean-up procedures

Regarding the rest of the clean-ups employed and using again Tr as a reference:

Among the other Z-sep+ clean ups, Z+3 was the next that provided the best results (after Z+2), performing better for acetaminophen, atenolol (using AQ), bentazone, bufotenine and imazalil showing less variation in the results independently of the QuEChERS used (**Fig. S1**) followed by Z+1 and Z+4 that only improve recoveries for acetaminophen and bentazone. It also could be noted that 4-MeO-PCP is worse recovered using Z+3 than Tr clean-up. Furthermore, this clean-up also provides a reduction of the baseline noise in comparison to Tr, even though not so high as the reported for Cb.

Although recoveries were similar to EM when using TEM, some differences could be observed. Thus, as can be seen in **Table S3**, when using TEM recoveries tend to be similar to those obtained using Tr clean up and lower that when using EM. On the contrary, when using mSQ-TEM and mAQ-TEM results tend to be similar to EM. Recoveries decrease for 4-MeO-PCP, caffeine and vildagliptin and increase for imazalil and PFBS. Other values are within the range obtained for Tr clean-up. The reduction of the noise of this method was similar to the others.

In relation to the SPE clean-up, recoveries from SQ were slightly higher than those of AQ contrary to what was observed with the miniaturized versions of them (mAQ-Tr>mSQ-Tr). Atenolol, bufotenine and PFPeA were not recovered or showed very low recoveries (2-9%). Contrary to these results, Chiesa et al. showed recoveries ranging 84-114% for PFPeA and other PFAS when using multi-residue extraction combining QuEChERS and SPE [1]. Along with Cb, this was the only method that recovered 4-MeO-PCP. For the other compounds, SPE clean-up provides similar recoveries to Tr clean-up except for caffeine that were worst, PFPeA that was not recovered and for ibuprofen and naproxen that were higher for the SQ extraction.

Ph clean-up is designed for plasma extractions, however its capability for phospholipid removal was considered interesting for mussel matrix. Recoveries were also similar to the traditional clean-up except for 4-MeO-PCP, that were lower, and atenolol, that were higher (**Fig. S1**).

Clean-ups XA and XB were the only ones that were not tested using the miniaturized versions of QuEChERS. The cartridges employed are an enhanced version of the cartridges employed for the SPE clean-up. A total of 9 and 11 compounds provided recoveries within 50-140% when XA was used for SQ and AQ respectively, but only 5 compounds fulfilled the range for XB using both QuEChERS. These were the clean-ups that showed poorer recoveries in general, bentazone, etoricoxib and naproxen were not recovered for XB, as well as atenolol for XA. While 4-MeO-PCP and PFPeA were not recovered by any method. Since the procedure used was the "RAPID" method [2], which is designed for fast extraction with few steps, the results might be better using procedures with more steps. As an exception PFOA and PFOS presented unusual high values (124-211%) using XA and XB clean-up. The blanks showed that the cartridges did not yield these substances. A detailed observation of the chromatograms corresponding to these extractions showed that the response of the internal standard was abnormally low. However, further studies would be needed to reach a firm conclusion. Globally, all the clean-up methods based on SPE provide an important reduction of the baseline noise comparable to that of Cb. However, the recoveries were not the best ones.

Table S1: External standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Mass labelled
POSITIVE COMPOUNDS				
Etoricoxib 1	359	280	3	
Etoricoxib 2	359	279	3	
Chlorfenvinphos 1	359	155	9.2	Chlorfenvinphos-d10
Chlorfenvinphos 2	359	127	9.2	Chlorfenvinphos-d10
Chlorpyrifos 1	350	198	10.9	Chlorpyrifos-d10
Chlorpyrifos 2	350	97	10.9	Chlorpyrifos-d10
Vildagliptin 1	304	154	0.7	Vildagliptin-d3
Vildagliptin 2	304	91	0.7	Vildagliptin-d3
Imazalil 1	297	201	5.8	
Imazalil 2	297	159	5.8	
Atenolol 1	267	91	0.6	Atenolol-d7
Atenolol 2	267	77	0.6	Atenolol-d7
Terbuthylazine 1	230	174	7.3	
Terbuthylazine 2	230	96	7.3	
Bufotenine 1	205	160	0.6	
Bufotenine 2	205	58	0.6	
Caffeine 1	195	138	0.6	Caffeine-d9
Caffeine 2	195	110	0.6	Caffeine-d9
Acetaminophen 1	152	110	0.8	Acetominophen-d3
Acetaminophen 2	152	92	0.8	Acetominophen-d3
Metformin 1	130	71	0.6	
Metformin 2	130	60	0.6	
4-MEO-PCP 1	274	189	3.9	
4-MEO-PCP 2	274	121	3.9	
Bentazone 1	241	107	6.5	
Bentazone 1	241	199	6.5	
NEGATIVE COMPOUNDS				
Salicylic acid	137	93	1	
PFDA 1	513	469	13	MPFDA
PFDA 2	513	269	13	MPFDA
PFOS 1	499	99	12.5	MPFOS
PFOS 2	499	80	12.5	MPFOS
PFOA 1	413	369	11.9	MPFOA
PFOA 2	413	169	11.9	MPFOA
PFBS 1	299	99	8.2	
PFBS 2	299	80	8.2	
Diclofenac sodium 1	294	250	10.9	
Diclofenac sodium 2	294	178	10.9	
Triclosan 1	289	35	13.5	Triclosan-d3
Triclosan 2	287	35	13.5	Triclosan-d3
PFPeA 1	263	219	7.3	
Naproxen 1	229	185	8.9	
Naproxen 2	229	170	8.9	
Ibuprofen 1	205	159	11.5	

Table S2: Mass labelled standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)
POSITIVE COMPOUNDS			
Chlorfenvinphos-d10 1	369	101	9.2
Chlorfenvinphos-d10 2	369	170	9.2
Chlorpyrifos-d10 1	360	199	10.9
Chlorpyrifos-d10 2	360	99	10.9
Vildagliptin-d3 1	307	157	0.7
Vildagliptin-d3 2	307	93	0.7
Atenolol-d7 1	274	145	0.6
Atenolol-d7 2	274	79	0.6
Caffeine-d9 1	204	144	0.6
Caffeine-d9 2	204	116	0.6
Acetominophen-d3 1	155	111	0.7
Acetominophen-d3 2	155	65	0.7
NEGATIVE COMPOUNDS			
MPFDA 1	515	270	13
MPFDA 2	515	470	13
MPFOS 1	503	99	12.5
MPFOS 2	503	80	12.5
MPFOA 1	417	372	11.9
MPFOA 2	417	169	11.9
Triclosan-d3 1	290	35	13.5

Table S3: Recoveries of the 44 extraction procedures tested. Expressed as relative recoveries (RR%) for those compounds with internal standard, while the rest of compounds are expressed as efficiency (E%).

	Traditional clean-up				Carbon clean-up			
	SQ	AQ	mSQ	mAQ	SQ	AQ	mSQ	mAQ
4-MeO-PCP	41	32	56	44	80	80	79	64
Acetaminophen	70	45	54	54	80	94	76	109
Atenolol	-	17	4	16	84	103	104	144
Bentazone	37	38	53	42	52	60	70	67
Bufotenine	15	17	16	15	51	53	50	48
Caffeine	37	43	40	41	83	68	74	70
Chlorfenvinphos	54	51	53	46	82	81	89	64
Chlorpyrifos	65	42	45	38	103	67	83	53
Diclofenac	74	132	73	113	72	110	80	103
Etoricoxib	67	45	48	51	30	48	47	49
Ibuprofen	-	79	24	88	-	114	43	83
Imazalil	58	37	82	64	110	86	108	109
Naproxen	-	76	-	74	15	94	24	93
PFBS	76	129	76	158	83	130	72	115
PFDA	94	98	104	96	118	104	109	99
PFOA	96	101	94	117	96	117	113	136
PFOS	101	107	101	92	107	97	113	80
PFPeA	52	-	-	165	97	93	-	95
Terbuthylazine	94	110	115	117	76	94	90	97
Triclosan	114	148	134	123	96	90	116	100
Vildagliptin	38	13	21	15	74	94	80	99

	Z-sep+1 clean-up				Z-sep+2 clean-up			
	SQ	AQ	mSQ	mAQ	SQ	AQ	mSQ	mAQ
4-MeO-PCP	29	34	31	39	31	24	28	39
Acetaminophen	72	57	85	61	73	107	70	82
Atenolol	-	25	4	16	-	99	6	25
Bentazone	49	54	46	50	65	68	66	63
Bufotenine	14	26	8	24	37	41	37	28
Caffeine	38	40	43	41	64	63	65	60
Chlorfenvinphos	50	48	48	46	69	73	69	62
Chlorpyrifos	58	39	54	38	89	67	75	55
Diclofenac	85	157	69	149	84	107	118	133
Etoricoxib	44	51	45	58	51	59	37	54
Ibuprofen	-	76	-	83	25	84	-	89
Imazalil	47	49	67	56	70	97	96	62
Naproxen	-	86	-	86	7	96	31	88
PFBS	114	162	87	164	84	127	73	115
PFDA	99	98	108	103	96	100	105	97
PFOA	85	104	103	110	88	113	96	116
PFOS	100	100	117	91	107	93	107	87
PFPeA	91	186	-	284	46	119	41	140
Terbuthylazine	92	107	106	119	82	93	84	99
Triclosan	87	113	109	111	103	121	92	114
Vildagliptin	27	22	22	24	54	61	57	61

	Z-sep+3 clean-up				Z-sep+4 clean-up			
	SQ	**AQ**	**mSQ**	**mAQ**	**SQ**	**AQ**	**mSQ**	**mAQ**
4-MeO-PCP	-	18	2	7	36	61	37	52
Acetaminophen	86	116	88	118	102	84	96	88
Atenolol	15	66	37	66	-	4	-	14
Bentazone	65	71	64	63	76	63	20	67
Bufotenine	69	54	42	30	4	10	0	6
Caffeine	52	49	46	45	53	53	53	49
Chlorfenvinphos	59	60	54	53	58	58	60	58
Chlorpyrifos	78	57	58	43	84	64	72	47
Diclofenac	82	150	115	146	68	162	93	160
Etoricoxib	31	30	21	31	28	16	14	31
Ibuprofen	-	94	50	81	-	78	-	68
Imazalil	52	113	112	118	14	47	28	50
Naproxen	6	99	33	67	1	65	10	73
PFBS	107	177	103	146	97	184	86	188
PFDA	90	93	99	91	99	97	134	97
PFOA	95	96	90	99	124	90	107	112
PFOS	105	109	104	100	102	102	101	98
PFPeA	67	148	-	163	-	68	-	71
Terbuthylazine	80	105	97	118	82	103	88	112
Triclosan	77	102	110	76	63	95	95	133
Vildagliptin	19	34	20	32	5	18	5	19

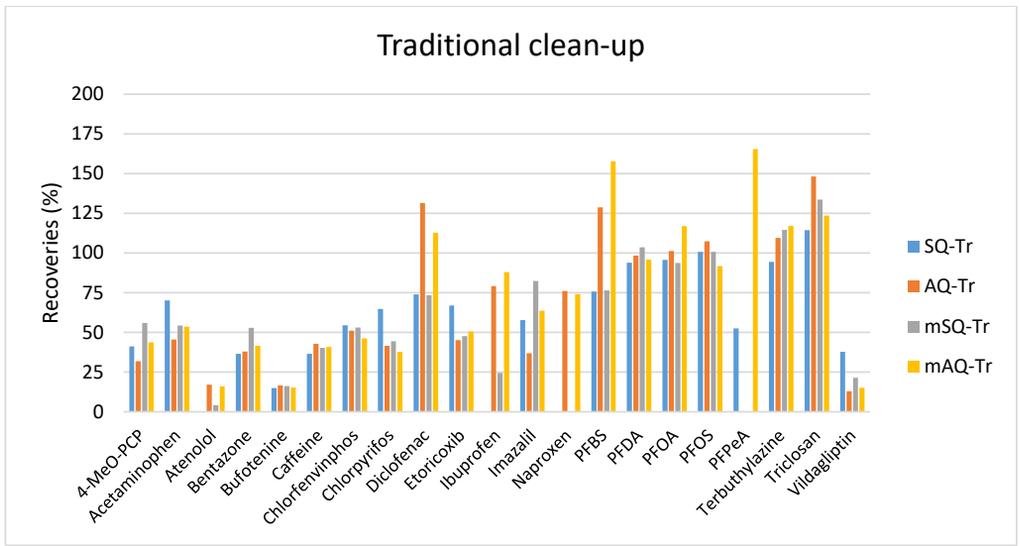
	EMR-lipid clean-up				Traditional + EMR-lipid clean-up			
	SQ	**AQ**	**mSQ**	**mAQ**	**SQ**	**AQ**	**mSQ**	**mAQ**
4-MeO-PCP	5	4	2	3	0	-	-	-
Acetaminophen	83	72	80	78	71	41	39	45
Atenolol	112	103	38	48	2	28	5	8
Bentazone	73	67	66	64	36	43	55	62
Bufotenine	62	28	54	65	25	7	10	20
Caffeine	53	51	46	54	23	23	25	26
Chlorfenvinphos	76	74	73	71	52	56	52	46
Chlorpyrifos	96	82	72	64	69	68	50	46
Diclofenac	92	112	114	118	72	129	124	166
Etoricoxib	65	50	77	69	51	55	77	72
Ibuprofen	99	108	113	91	-	103	94	92
Imazalil	124	120	128	108	131	132	173	149
Naproxen	92	93	98	92	4	84	86	107
PFBS	135	132	130	123	128	176	170	202
PFDA	95	95	98	107	104	94	94	97
PFOA	95	99	105	110	95	103	106	109
PFOS	91	98	95	100	94	108	102	93
PFPeA	113	194	80	183	-	181	261	372
Terbuthylazine	83	85	93	127	106	119	127	131
Triclosan	97	131	102	87	88	92	116	100
Vildagliptin	57	53	47	60	8	4	3	6

	SPE clean-up				Phree clean-up			
	SQ	AQ	mSQ	mAQ	SQ	AQ	mSQ	mAQ
4-MeO-PCP	98	95	108	131	-	18	6	10
Acetaminophen	67	70	63	82	57	96	53	184
Atenolol	-	-	9	2	101	98	137	165
Bentazone	70	65	62	73	57	62	59	59
Bufotenine	7	4	7	5	58	38	11	29
Caffeine	18	21	19	20	33	37	32	38
Chlorfenvinphos	61	61	55	62	54	51	54	48
Chlorpyrifos	51	45	28	29	59	55	35	37
Diclofenac	145	143	151	159	147	139	133	166
Etoricoxib	33	24	47	56	58	45	56	65
Ibuprofen	117	80	97	115	95	90	62	58
Imazalil	87	77	89	83	150	52	138	18
Naproxen	92	75	96	84	77	91	63	80
PFBS	98	85	88	101	125	148	101	132
PFDA	90	93	73	96	92	106	87	113
PFOA	98	97	91	92	90	115	92	110
PFOS	95	86	82	86	91	109	85	104
PFPeA	-	-	-	-	157	299	131	266
Terbuthylazine	103	105	131	133	102	99	107	115
Triclosan	81	77	73	82	104	82	75	70
Vildagliptin	13	8	13	7	25	40	24	42

	Strata-X PRO basic clean-up		Strata-X PRO acid clean-up	
	SQ	AQ	SQ	AQ
4-MeO-PCP	-	-	-	-
Acetaminophen	90	-	-	125
Atenolol	39	7	-	-
Bentazone	-	-	22	23
Bufotenine	26	26	2	2
Caffeine	8	8	-	8
Chlorfenvinphos	22	28	59	38
Chlorpyrifos	49	70	64	67
Diclofenac	88	78	99	99
Etoricoxib	-	-	14	12
Ibuprofen	17	-	123	91
Imazalil	57	65	48	61
Naproxen	-	-	72	62
PFBS	1	0	12	1
PFDA	88	108	101	98
PFOA	177	174	114	111
PFOS	211	175	124	75
PFPeA	-	-	-	-
Terbuthylazine	53	80	85	86
Triclosan	-	38	47	113
Vildagliptin	19	20	4	3

Table S4: Matrix effect of the three extraction procedures validated.

Matrix effect (%)	SQ-EM	AQ-Z+2	AQ-Cb
4-MeO-PCP	-11	-16	-20
Acetaminophen	-56	-70	-58
Atenolol	-84	-50	-44
Bentazone	-20	-26	-25
Bufotenine	-78	-67	-68
Caffeine	-53	-50	-49
Chlorfenvinphos	-23	-23	-32
Chlorpyrifos	-33	-33	-34
Diclofenac	-13	-12	-8
Etoricoxib	-21	-25	-25
Ibuprofen	-7	-11	-10
Imazalil	-18	-19	-23
Naproxen	-6	-9	-3
PFBS	9	11	5
PFDA	49	51	15
PFOA	47	47	-5
PFOS	22	24	15
PFPeA	2	-1	-31
Terbuthylazine	-27	-28	-35
Triclosan	31	31	13
Vildagliptin	-78	-42	-62


Traditional clean-up
Recoveries (%)
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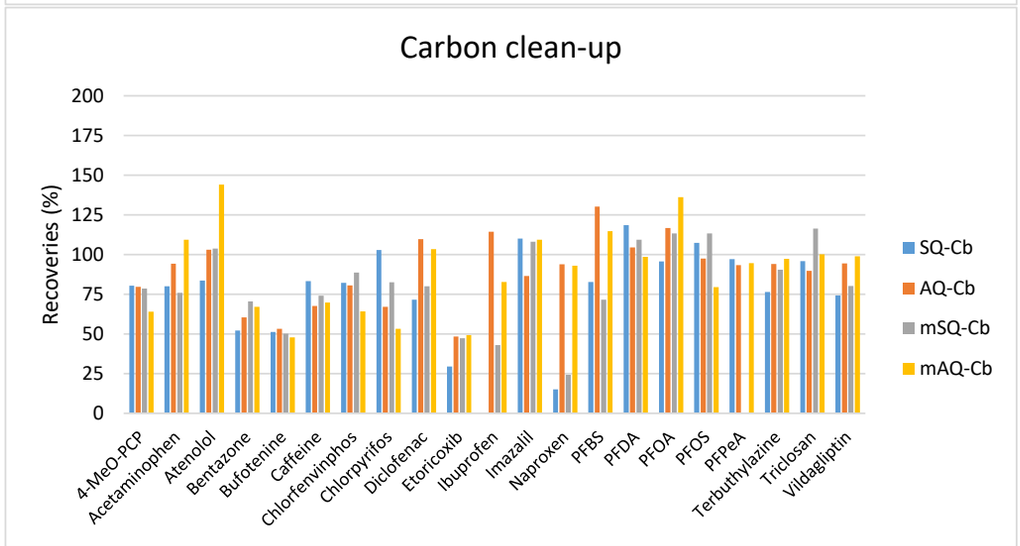
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
PFOS
PFPeA
Terbuthylazine
Triclosan
Vildagliptin
SQ-Tr
AQ-Tr
mSQ-Tr
mAQ-Tr


Carbon clean-up
Recoveries (%)
200
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
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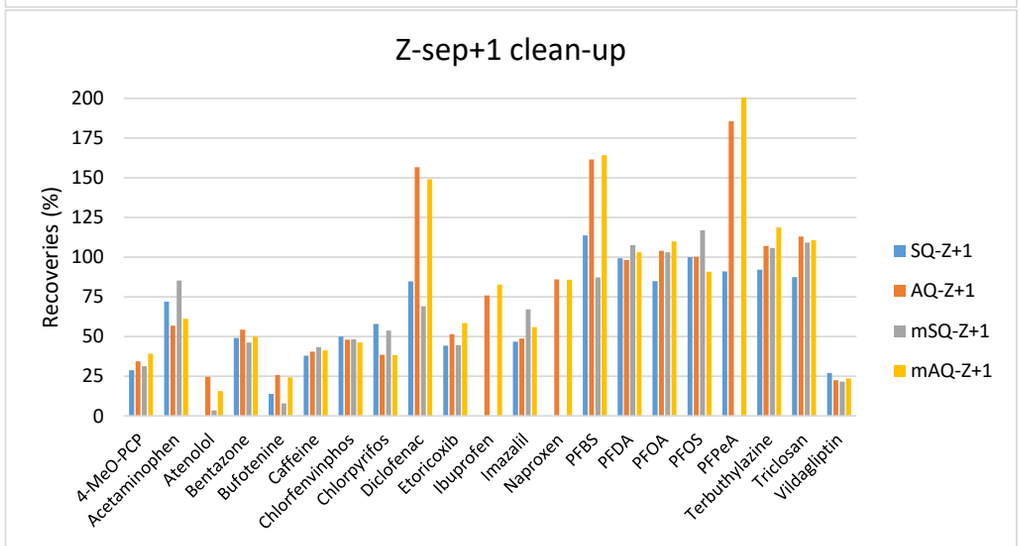
Terbuthylazine
Triclosan
Vildagliptin
SQ-Cb
AQ-Cb
mSQ-Cb
mAQ-Cb


Z-sep+1 clean-up
Recoveries (%)
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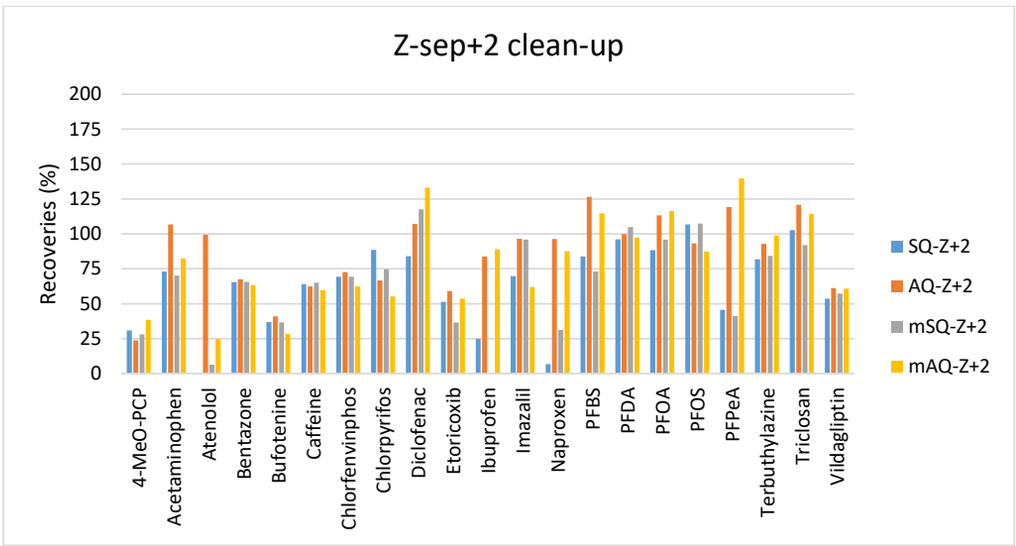
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
PFOS
PFPeA
Terbuthylazine
Triclosan
Vildagliptin
SQ-Z+1
AQ-Z+1
mSQ-Z+1
mAQ-Z+1

S-11


Z-sep+2 clean-up
Recoveries (%)
200
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
PFOS
PFPeA
Terbuthylazine
Triclosan
Vildagliptin
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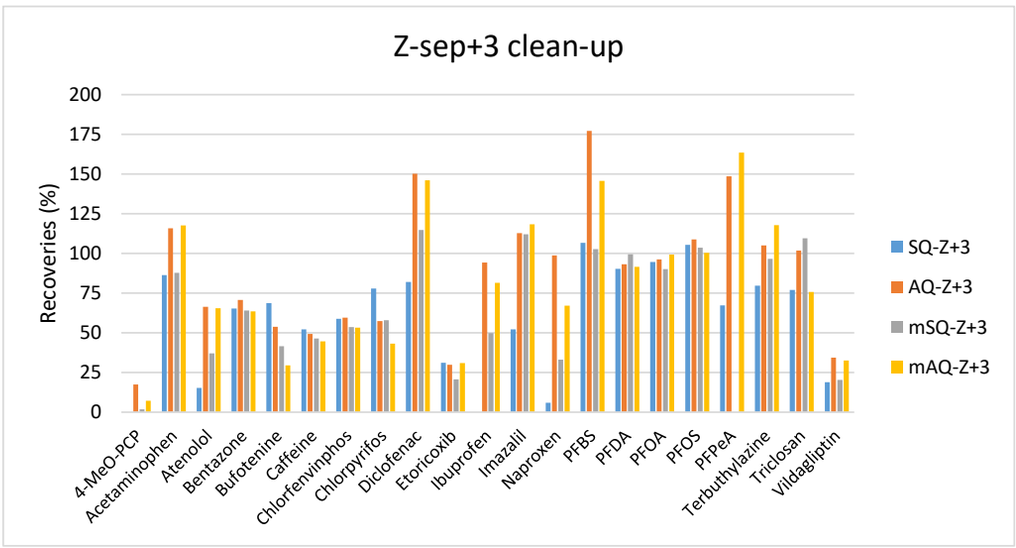
AQ-Z+2
mSQ-Z+2
mAQ-Z+2


Z-sep+3 clean-up
Recoveries (%)
200
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
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Triclosan
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AQ-Z+3
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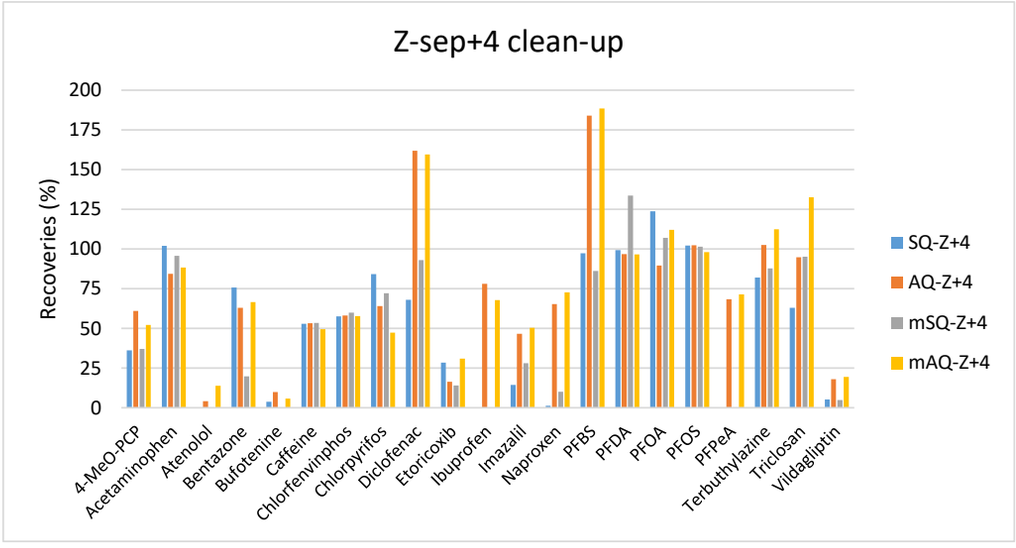
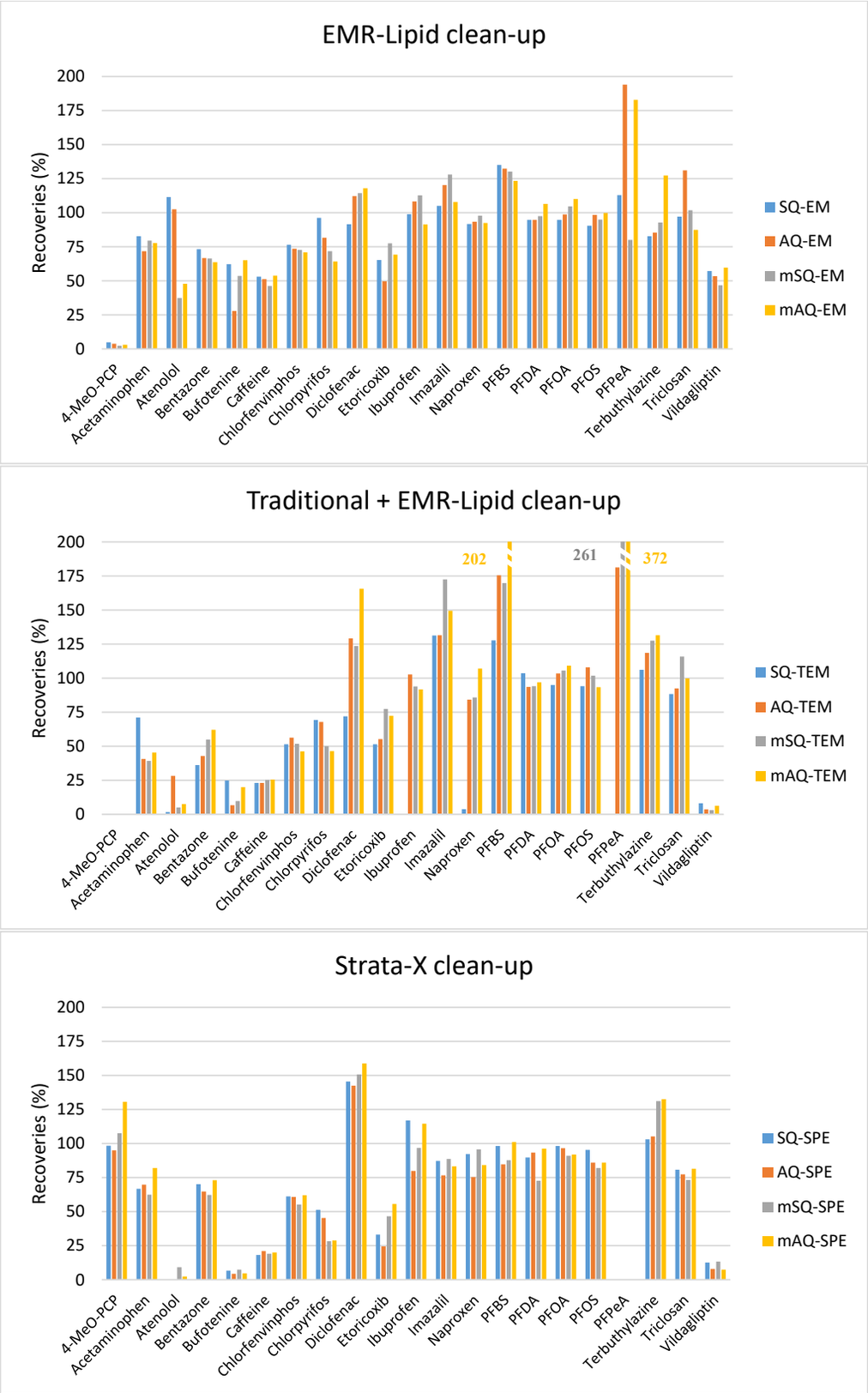

Z-sep+4 clean-up
Recoveries (%)
200
175
150
125
100
75
50
25
0
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
PFOS
PFPeA
Terbuthylazine
Triclosan
Vildagliptin
SQ-Z+4
AQ-Z+4
mSQ-Z+4
mAQ-Z+4

S-12


EMR-Lipid clean-up
Recoveries (%)
SQ-EM
AQ-EM
mSQ-EM
mAQ-EM
Traditional + EMR-Lipid clean-up
Recoveries (%)
202
261
372
SQ-TEM
AQ-TEM
mSQ-TEM
mAQ-TEM
Strata-X clean-up
Recoveries (%)
SQ-SPE
AQ-SPE
mSQ-SPE
mAQ-SPE
4-MeO-PCP
Acetaminophen
Atenolol
Bentazone
Bufotenine
Caffeine
Chlorfenvinphos
Chlorpyrifos
Diclofenac
Etoricoxib
Ibuprofen
Imazalil
Naproxen
PFBS
PFDA
PFOA
PFOS
PFPeA
Terbuthylazine
Triclosan
Vildagliptin

S-13

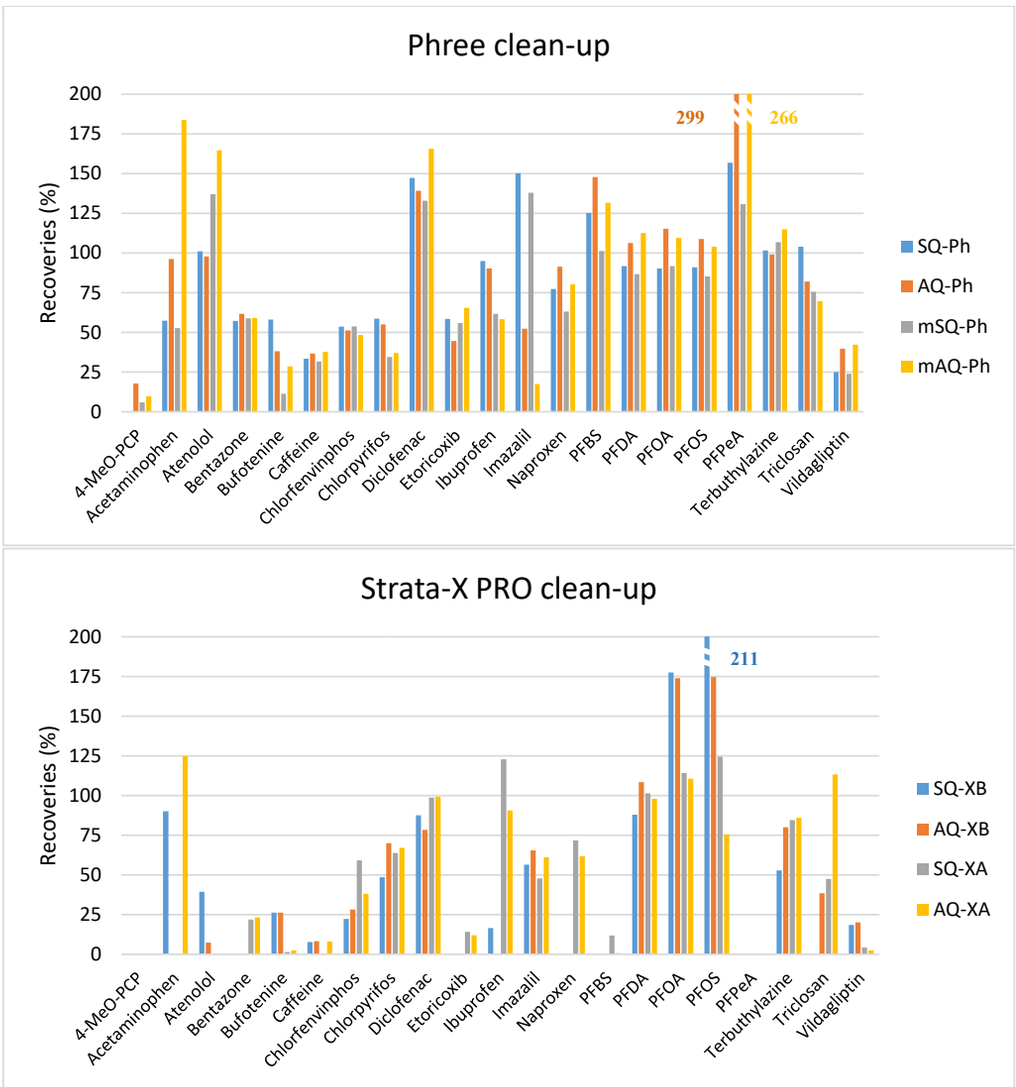


Fig. S1: *Recoveries of the 44 extraction procedures tested. Expressed as relative recoveries (RR%) for those compounds with internal standard, while the rest of compounds are expressed as efficiency (E%).*

S-14

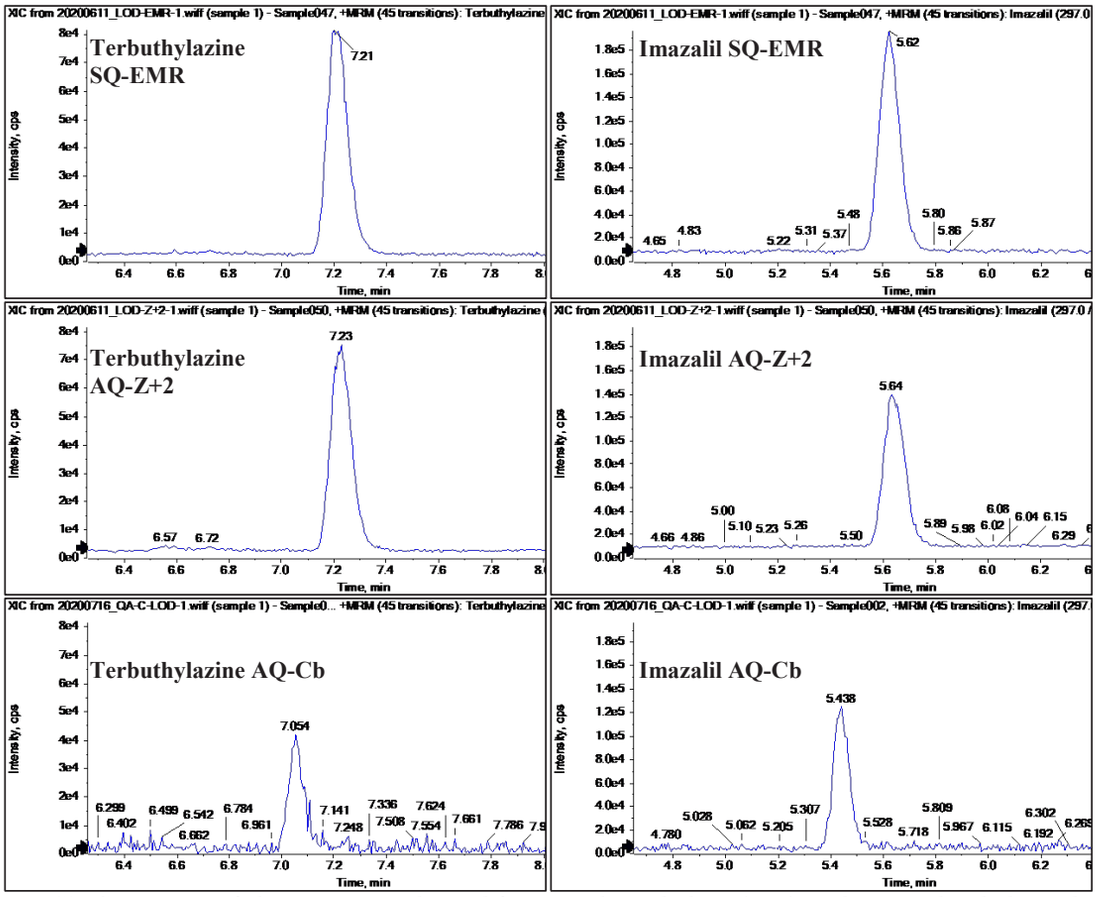


Fig. S2: *Comparison of chromatograms obtained from samples spiked at 50 ng/g and extracted with the methods SQ-EMR, AQ-Z+2 and AQ-Cb.*

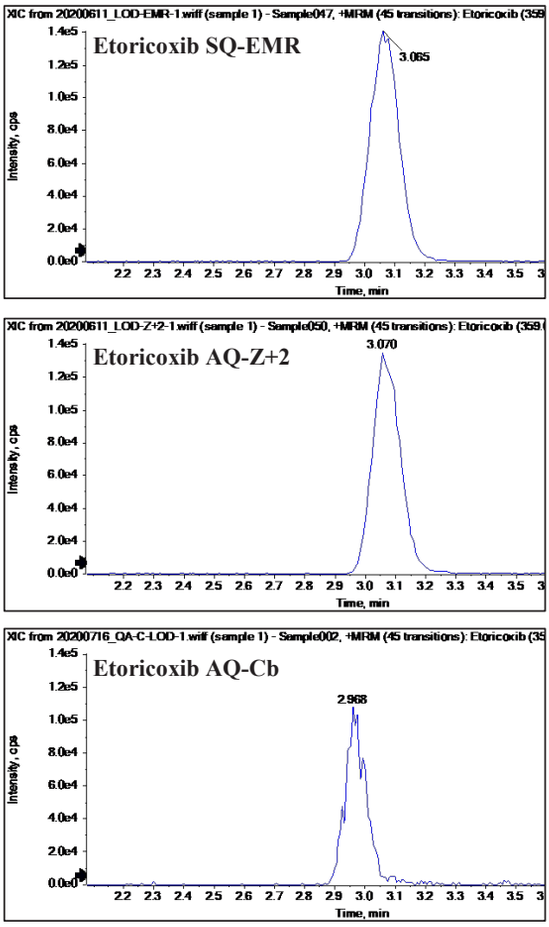


Fig. S2 (continuation): *Comparison of chromatograms obtained from samples spiked at 50 ng/g and extracted with the methods SQ-EMR, AQ-Z+2 and AQ-Cb.*

S-16

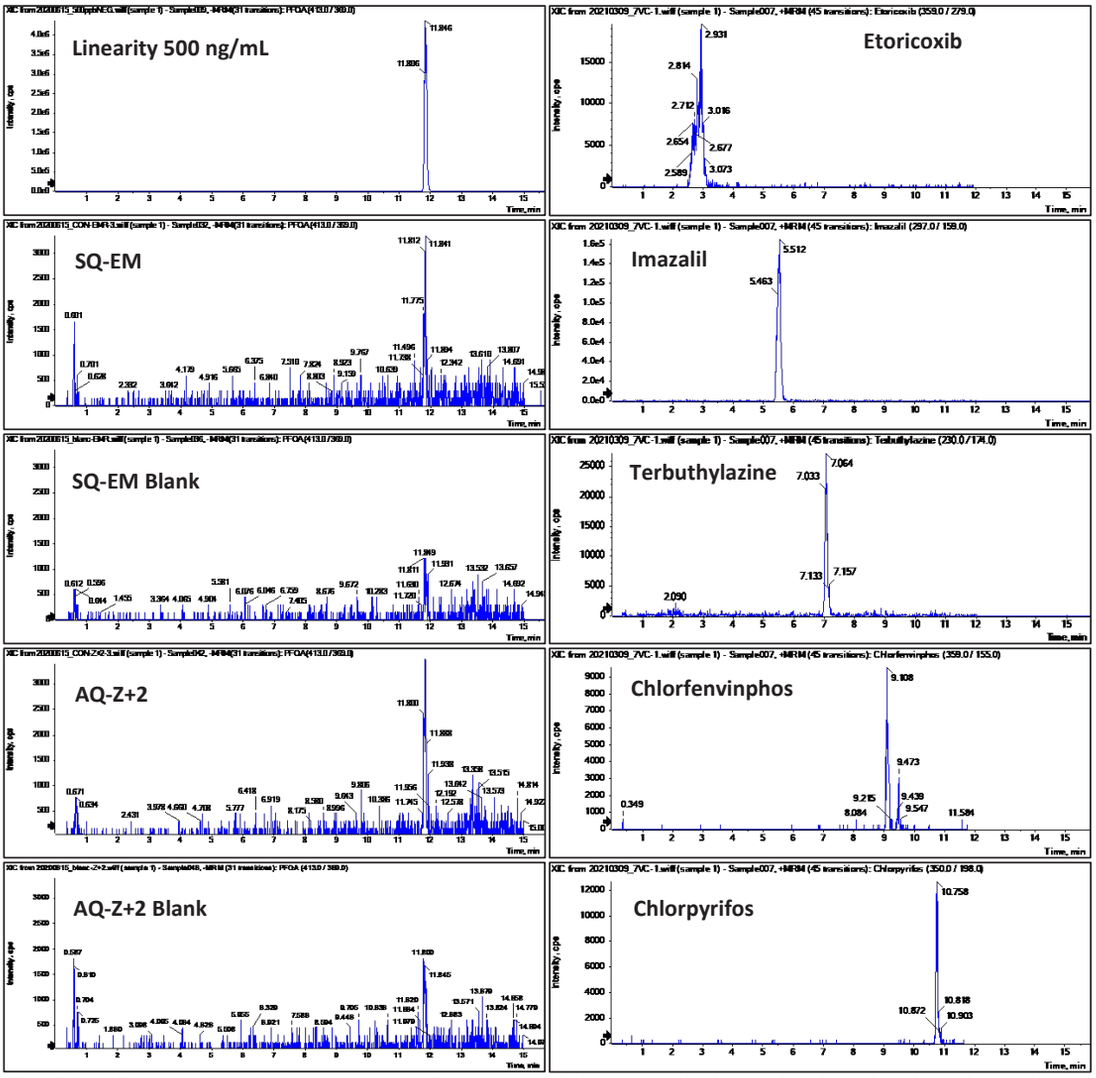


Fig. S3 *Left: Example of chromatograms from some of the non-spiked samples from local markets, showing distinctive peaks for PFOA. Right: examples of chromatograms from compounds detected in the bioaccumulation samples extracted with SQ-EM.*

References

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ARTÍCULO

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RESEARCH ARTICLE

Multi-residue extraction to determine organic pollutants in mussel hemolymph

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This study assesses the extraction of eleven pharmaceuticals, five pesticides, five perfluoroalkyl substances, and two illicit drugs in hemolymph from *(Mytilus Galloprovincialis*). Four extraction procedures using Phree™ Phospholipid Removal cartridges were tested using different volumes of methanol (400 and 600 μL) and acetonitrile (300 and 450 μL). The pollutants were determined by high-performance liquid chromatography–tandem mass spectrometry. The use of methanol gave several problems during the extraction procedure, such as longer times and sample loss. Three methods (acetonitrile 300 and 450 μL; and methanol 600 μL) were validated. Recoveries at three concentration levels (5, 50, and 100 ng/mL) ranged 35.1–129.0 and 29.3–133.0% for acetonitrile 300 and 450 μL, respectively, while recoveries for methanol 600 μL ranged 52.2–166.0%. Limits of detection were < 10 ng/mL for most analytes using any of the methods. Methanol 600 μL was the only method capable to extract the illicit drug 4-methoxyphencyclidine and provided a better peak shape and higher signal-noise ratio. When applied to non-spiked samples from local markets salicylic acid and diclofenac were detected at 33.50-97.79 and 28.30-30.31 ng/mL respectively. To our knowledge, there are no methods to determine organic contaminants in hemolymph and this is the first application of Phree™ cartridges for mussel hemolymph extraction.

KEYWORDS
illicit drugs, perfluoroalkyl substances, pesticides, pharmaceuticals, solid-phase extraction

1 | INTRODUCTION

Emerging pollutants (EPs) are toxic, persistent, and ubiquitous in the aquatic environment, because of uncontrolled discharges, wastewater treatment plant effluents, and/or the environmental transformation of several precursors into EPs [1–3]. Due to their persistence and/or continuous release, aquatic biota is long-term exposed to these bioaccumulable and biomagnificable compounds [4, 5]. Exposure of biota occurs via water, sediment, suspended solids, or the intake of other biota (food chains)

Article Related Abbreviations: 4-MeO-PCP, 4-methoxyphencyclidine; A-3, ACN extraction 3:1 ACN:hemolymph; A-4.5, ACN extraction 4.5:1 ACN:hemolymph; E%, efficiency; EP, emerging pollutant; Inter-R, reproducibility; Intra-R, repeatability; M-4, methanol extraction 4:1 MeOH:hemolymph; M-6, methanol extraction 6:1 MeOH:hemolymph; ME, matrix effect; MeOH, methanol; MRM, multiple reaction monitoring mode; PBS, perfluorobutanesulfonate; PCP, personal care product; PFAS, perfluoroalkyl substance; PFDA, perfluorodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; PFPeA, perfluoropentanoic acid; R%, absolute recoveries; RR%, relative recoveries

and/or microplastics as reported in different studies [6], several adverse effects have been confirmed [7]. The occurrence of EPs has been reported in mussel [8], eel [6], seafood and other fish [9] used for human consumption. The health risk from eating this kind of food has been estimated in several studies [10, 11].

Biota studies analysed the species as a whole [12] or divided into their different organs and tissues [13, 14]. This latter offers additional information on absorption, distribution, metabolism, target organs, accumulation, and excretion. The presence and concentrations of EPs in fish tissues and filter-feeding organisms [15] still need further study since they play a vital role in biodiversity and water depuration.

Multi-residue extraction methods have been developed to save resources and time by extracting the greatest possible variety of compounds in order to provide a whole picture of the EPs present in the sample, even if they are from different families [4]. However, the complexity of biota matrices constrains the development of such methods, since they involve the challenge of extracting pollutants with a wide polarity range, obtaining clean extracts and good recoveries. The universal method for tissues is solvent extraction where the compounds are extracted by adding an organic solvent and applying energy (i.e. manual agitation, ultrasound, pressure, temperature, microwave, or vacuum). For aqueous matrices and for the clean-up of the tissue extracts the most used approach is solid-phase extraction (SPE). Different cartridges have been reported depending on the target compounds, but the common is the HLB (Hydrophilic-Lipophilic-Balanced) [14].

SPE clean-up has been used in methods screening pharmaceuticals [14], personal care product (PCPs) [16], perfluoroalkyl substances (PFASs) [9], organophosphate flame retardants [17] or illicit drugs [18] in biota matrices. This makes SPE a clean-up suitable for multi-residue extraction procedures but sometimes, SPE sorbents fail to eliminate interferences from the matrix. The last trend within this field is the development of specific sorbent variations, like the Phree™ and Captiva ND (to separate phospholipids and proteins that favor the elimination of lipids) [19], Ostro™ (to eliminate phospholipids and proteins) [20] or EMR-Lipids (to remove lipids). The application of these new sorbents is still incipient given their recent placement on the market, even though they offer a very promising solution to problems as the high content of lipids in biota matrices. Phree™ cartridges have been tested in the extraction of blood and/or human serum [21], and recently as a clean-up of fish muscle [22] and mussels showing promising results in liquid samples.

Among the bivalve mollusks, mussels have been used as sentinel organisms in biomonitoring programs, such as Med Pol, UNEP Mediterranean Biomonitoring Program or the OSPAR Convention [23, 24]. The hemolymph of these bivalve mollusks (the invertebrate equivalent of mammalian blood) is an attractive bio-fluid in contact with different tissues to assess biomarker responses to contamination, because it can report about the functional status of the organs which are perfused but lacks the molecular complexity of whole organ tissues [25]. Mussel hemolymph has a very variable proportion of minerals, proteins and cells depending on the tissue and the specimen [26, 27], which make it a complex matrix. However, up to our knowledge, analytical methods to determine contaminants in hemolymph have not been reported yet.

The aim of this study was the development and validation of a method based on the use of Phree™ cartridges followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine eleven pharmaceuticals, five pesticides, five perfluoroalkyl substances, and two illicit drugs in hemolymph from *(M. Galloprovincialis)*. Then, this method was successfully employed for evaluating the presence of these contaminants in hemolymph of several commercial samples and of mussels that were exposed to several emerging contaminants. This study improves our knowledge of the presence, distribution, and biodegradation of EPs in aquatic biota, which has particular importance for the environment and the human population.

2 | MATERIALS AND METHODS

2.1 | Reagents and materials

The LC-grade methanol (MeOH) and acetonitrile (ACN) of a purity ≥99.8%, were from VWR Chemicals® (Radnor, Pennsylvania). Formic acid (CH_2O_2) was provided by ACROS ORGANICS (Geel, Belgium). Ammonium formate (NH_4HCO_2) was from Alfa Aesar (Karlsruhe, Germany).

Phree™ Phospholipid Removal Solutions 1 mL tubes were from Phenomenex® (Torrance, CA, USA). The 1 mL polypropylene syringes BD Plastipak™ and the needles 25G × 5/8″ 0.5 × 16 mm BD Microlance™ were from BD (Madrid, Spain). The VISIPREP™ manifold was distributed by Supelco. High purity water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). The 15 mL polypropylene centrifuge falcon tubes were from VWR International Eurolab (Barcelona, Spain). The 2 mL amber glass vials with stoppers 99 mm + Septum Sil/PTFE used to inject the samples were from Análisis Vínicos S.L. (Tomelloso, Spain), and the 250 μL polypropylene inserts were from Agilent Technologies (Santa Clara, CA, USA). The STUART Sample Concentrator SBHCONC/1 with a STUART Block Heater SBH200D/3 set to 39°C was from Stuart® (Staffordshire, UK).

The analytical standards of pharmaceuticals (acetaminophen, atenolol, caffeine, diclofenac, etoricoxib, ibuprofen, metformin, naproxen, salicylic acid, triclosan, vildagliptin), pesticides (bentazone, chlorfenvinphos, chlorpyrifos, imazalil, terbutylazine), and PFASs (perfluoropentanoic acid [PFPeA] and perfluorobutanesulfonate [PFBS]) were from Sigma–Aldrich (Steinheim, Germany). While perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), and perfluorooctanesulfonate (PFOS) were from Wellington (Ontario, Canada). Finally, illicit drugs: bufotenine, and 4-methoxyphencyclidine (4-MeO-PCP) were from LGC Standards (Ontario, Canada).

The surrogate (internal) standards acetaminophen-d3, atenolol-d7, and ibuprofen-d3 were from Sigma–Aldrich. Caffeine-d9, chlorfenvinphos-d10 (diethyl D5), chlorpyrifos-d10 (diethyl D10), and vildagliptin-d3 were from LGC Standards. Diclofenac-d4 and triclosan-d3 were purchased in Toronto Chemicals Research (Toronto Canada). And PFOA-d4 (MPFOA), PFOS-d4 (MPFOS), and PFDA-d4 (MPFDA) were from Wellington.

2.2 | Sampling

The sample analysed was hemolymph from Mediterranean mussels (*M. galloprovincialis*) harvested in the Mediterranean Sea next to the city of Valencia, Spain. These mussels —cultivated using raft can be in contact with anthropogenic contaminants due to the proximity of Valencia city— are available just between March-July and are an emblematic ingredient for the local gastronomy, commonly known as "clótxinas." Mussels were purchased from three different local markets and processed when they were still alive, the shells were filed with a steel file next to the posterior adductor muscle until opening a hole big enough to introduce the syringe needle. Then the hemolymph was extracted directly from the posterior adductor mussel using a 1 mL syringe. Hemolymph composition is different depending on the tissue and it is not clear where the fluids come from when it is extracted from the posterior adductor mussel. However, Eggermont et al. [27] suggest that this hemolymph could be from small spaces and fissures between the muscle fibers that are connected to the posterior gastro-intestinal artery. The volume extracted was between 0.2-0.5 mL depending on the specimen. For the method optimization, hemolymph from the three different markets was pooled. Then hemolymph from mussels of the same supermarket was collected and stored separately in order to test the selected method in real non-spiked samples. All the samples were stored in 15 mL falcon tubes and frozen at −20°C until analysis.

2.3 | Sample extraction

The Phree™ cartridges were placed in a vacuum manifold, and loaded with 100 μL of hemolymph. A solution of ACN 1% formic acid spiked with the IS (300 μL) was added directly in the sample placed in the cartridge (the so called direct addition). This step is crucial to ensure a proper mixing and complete precipitation [28]. Addition of the solvent sliding down the walls of the cartridge would not provide satisfactory results. After 2 min to assure complete precipitation (Figure S-1), vacuum (254-381 mmHg) was applied to elute the remaining mix of solvent and hemolymph dropwise in 15 mL falcon tubes. The extracts were stored in vials with 250 μL polypropylene (PP) inserts and frozen at −20°C until analysis. Four variations of this procedure were tested.

Two procedures employed ACN 1% formic acid as solvent. In this case, the procedures were exactly as described above but one employed 300 μL (A-3) of solvent and the other 450 μL (A-4.5), obtaining a final proportion hemolymph:ACN of 1:3 and 1:4.5, respectively. The concentration of the IS in the solvent was adjusted for each method, ensuring a final concentration of 20 ng/mL in the final extracts (assuming recovery of the 100%). The other two procedures employed MeOH 1% formic acid as solvent. The procedure was very similar as the described above, but in this case, the vacuum pressure applied during the SPE was higher (508-635 mmHg) and the amount of solvent employed was 400 μL in M-4 and 600 μL in M-6 obtaining a proportion hemolymph:MeOH in the extracts of 1:4 and 1:6, respectively. With both ACN and MeOH, the lowest amount of solvent corresponds to that recommended by the manufacturers, and as they indicate that a higher proportion of solvents can improve sometimes results, solvents with 50% more organic component were tested.

2.4 | LC–MS/MS analysis

Analysis was performed via LC–MS/MS, using an Agilent 1260 UHPLC from Agilent technologies coupled to an Agilent 6410 Mass Spectrometer QQQ also from Agilent technologies, with electrospray ionization (ESI) in both negative and positive ionization modes (nebulizer gas 15 psi, gas flow 11 L/min. ion-spray voltage 4 kV and temperature 300°C) operated in multiple reaction monitoring mode (MRM). The column used for the detection pesticides and etoricoxib was Luna® 3 μm C18(2) 100 Å 150 × 2 mm and the column employed for PFAS, illicit drugs and the rest of PPCPs was a Kinetex 1.7 μm XB-C18 100 Å 50 × 2.1 mm, both from Phenomenex. Yielding

a total of three LC methods, one in negative ionization mode using Kinetecs column and two in positive mode using Kinetecs and Luna® columns, respectively. When operated in positive ionization mode, the mobile phases employed were (A) H_2O 0.1% formic acid and (B) MeOH 0.1% formic acid. For negative ionization mode, the mobile phases employed were (A) H_2O 2.5 mM NH_4F and (B) MeOH 2.5 mM NH_4F. The linear gradient was as follows: 0 min (70% A), 12 min (5% A), 25 min (5% A), 26 min (70% A), and 30 min (70% A) either in positive or negative ionization mode (only the mobile phases were different). The injection volume was 5 μL and column temperature 30°C. MS detailed information is available in Supporting Information Tables S1 and S2.

2.5 | Validation

Every batch of samples extracted included a procedural blank (non-spiked hemolymph pool). At the beginning and at the end of each analytical sequence, a seven points calibration standard set (5, 10, 25, 50, 100, 200, and 500 ng/mL) was injected. This calibration was prepared in MeOH:MilliQ 4:1 or ACN:MilliQ 3:1 depending on the solvent used to extract the samples. A 100 ng/mL spiked sample extracts was also injected every 15 samples to check possible instrumental variation. Only regression coefficients (R^2) > 0.99 were accepted in the calibration curve.

Recoveries were calculated in hemolymph fortified at three different concentrations: 5, 50, and 100 ng/mL in triplicate. After LC–MS/MS, recoveries were calculated comparing the peak area of the spiked samples with the area of the seven points of the calibration curve. For the compounds acetaminophen, atenolol, caffeine, chlorfenvinphos, chlorpyrifos, diclofenac, ibuprofen PFDA, PFOA, PFOS, triclosan, and vildagliptin, the results obtained were relative recoveries (RR%) where the matrix effect (ME), and other potential inaccuracy during sample handling, were corrected using the internal standards. The other compounds were quantified with external calibration. Hence, the results were represented as efficiency (E%), if the results are affected by either recovery and matrix effects, or absolute recoveries (R%), if the matrix effect is corrected using matrix-matched standards. Both were calculated following Eq. 1.

$$RR\%\ or\ E\% = \left(\frac{Final\ concentration\ of\ the\ spiked\ sample}{EC}\right) \cdot 100 \quad (1)$$

where *EC* is the expected concentration in the final extract assuming a recovery of 100%.

For the determination of ME, a batch of ACN or MeOH (depending on the extraction solvent) with the compounds mix at the same concentration as the calibration curve (5, 10, 25, 50, 100, 200, and 500 ng/mL) was prepared. For each concentration, 300 μL of its mix was placed in 15 mL falcon tubes, and blown down to dryness under a gentle stream of nitrogen, then, 300 μL of hemolymph extract were added to the falcon tubes. This extract was then vortexed 30 s, sonicated 3 min and injected. A procedural blank was also included in each batch. After LC–MS/MS analysis using external calibration, ME was calculated comparing the slope of the calibration curve in matrix and the slope of the calibration curve in CAN [19] (Eq. 2).

$$ME = \left(\frac{Slope\ of\ calibration\ curve\ in\ matrix}{Slope\ of\ calibration\ curve\ in\ solvent}\right) \cdot 100 - 100 \quad (2)$$

The E% of the compounds without internal standard was corrected using the ME to obtain R% using Eq. 3.

$$R\% = \frac{EE\% \cdot (100 - ME)}{100} \quad (3)$$

Sensitivity was established as method limits of detection (LODs) and method limits of quantification (LOQs) (Table 1) by analysing the extractions fortified at 5 ng/mL used for the recoveries described above. The extracts (performed in triplicate) were injected in duplicate (n = 6). LODs were set as three times the standard deviation (SD) of their signal and LOQs were set as 10 times the SD. Precision was evaluated in terms of repeatability (Intra-R) and reproducibility (Inter-R). Intra-R was calculated as the SD of the signal divided by its mean (% RSDs) of the six injections used for the determination of LODs and LOQs injected in a row. Inter-R was determined injecting one replicate of the extracts fortified at 50 ng/mL also used for the recoveries described above in three different days (n = 3). Then Inter-R was also calculated as the SD of the signal divided by its mean (% RSDs).

3 | RESULTS AND DISCUSSION

3.1 | Extraction procedure and analysis considerations

The solvents (ACN and MeOH) employed for the extraction were those recommended by the manufacturer, who also recommended vacuum negative pressure ranges of 127–254 and 381–508 mmHg for ACN and MeOH, respectively, even though it is also suggested that higher pressures may be required [28]. Vacuum pressures of 254–381 and 508–635 mmHg for ACN and MeOH, respectively, were needed in the present work.

TABLE 1 Validation of the selected methods in terms of sensitivity (LODs, LOQs), Intra-R and Inter-R

	A-3				A-4.5				M-6			
	Intra-R (% RSDs)	Inter-R (% RSDs)	LOD (ng/mL)	LOQ (ng/mL)	Intra-R (% RSDs)	Inter-R (% RSDs)	LOD (ng/mL)	LOQ (ng/mL)	Intra-R (% RSDs)	Inter-R (% RSDs)	LOD (ng/mL)	LOQ (ng/mL)
4-MEOPCP*	12.30	-**	4.51	13.50	4.08	-**	3.83	11.50	7.16	20.90	7.87	23.60
Acetaminophen	2.28	29.50	1.75	5.26	4.00	4.04	3.41	10.20	5.32	9.22	4.10	12.30
Atenolol	5.43	25.60	1.56	4.67	2.02	22.80	0.79	2.37	3.82	20.90	3.63	10.90
Bentazone*	16.40	25.20	8.95	26.90	16.40	26.30	12.30	36.90	18.20	9.12	19.10	57.30
Bufotenine	11.50	35.70	1.60	4.81	11.50	39.30	2.21	6.62	3.63	25.30	0.91	2.73
Caffeine	1.29	29.60	1.20	3.60	2.13	21.50	2.33	6.99	3.45	18.70	3.30	9.89
Chlorfenvinphos	3.46	9.13	2.90	8.69	3.48	12.90	4.15	12.50	2.07	25.00	2.93	8.80
Chlorpyrifos	2.09	8.14	0.92	2.75	1.96	8.65	1.40	4.19	0.73	15.50	0.94	2.81
Diclofenac	6.41	14.70	5.34	16.00	1.02	16.90	1.28	3.83	3.61	25.20	5.58	16.70
Etoricoxib	29.00	8.61	5.26	15.80	11.10	4.07	8.90	26.70	4.08	16.20	3.59	10.80
Ibuprofen	9.13	17.40	4.80	14.40	7.91	3.04	7.36	22.10	2.81	18.10	3.51	10.50
Imazalil	4.98	21.50	3.09	9.26	2.00	29.90	2.39	7.18	2.89	26.20	2.73	8.20
Metformin	11.20	10.20	9.28	27.80	16.00	12.40	8.33	25.00	7.51	33.40	16.30	48.80
Naproxen	2.48	2.11	5.35	16.10	2.17	20.20	8.12	24.40	0.86	7.82	2.72	8.17
PFBS	0.82	24.00	1.84	5.51	0.97	21.60	3.28	9.85	2.51	4.72	10.70	32.20
PFDA	3.78	4.54	2.27	6.81	8.83	5.00	10.60	31.70	5.38	6.75	6.43	19.30
PFOA	0.85	12.90	0.44	1.33	3.49	16.10	2.83	8.48	3.96	2.92	3.62	10.90
PFOS	8.08	18.50	4.28	12.90	2.72	22.20	1.83	5.49	0.41	6.29	0.30	0.89
PFPeA	3.80	26.70	3.55	10.60	4.86	4.74	5.74	17.20	2.65	10.20	4.30	12.90
Salicylic acid	6.65	10.60	15.30	46.00	4.20	13.50	11.60	34.90	1.22	5.83	2.82	8.46
Terbuthylazine	1.31	27.80	2.60	7.80	0.49	9.15	1.53	4.59	1.03	5.53	3.07	9.22
Triclosan	1.11	18.70	5.55	16.70	0.91	25.50	7.21	21.60	4.19	20.30	22.00	66.00
Vildagliptin	8.28	10.20	4.34	13.00	2.20	18.30	1.78	5.34	1.98	5.94	1.71	5.12

**LODs, LOQs and Intra-R were calculated using the lowest point of the linearity (5 ng/ml). Except 4-MeO-PCP for M-6.*
***Low recoveries avoided the determination of a reliable Inter-R.*

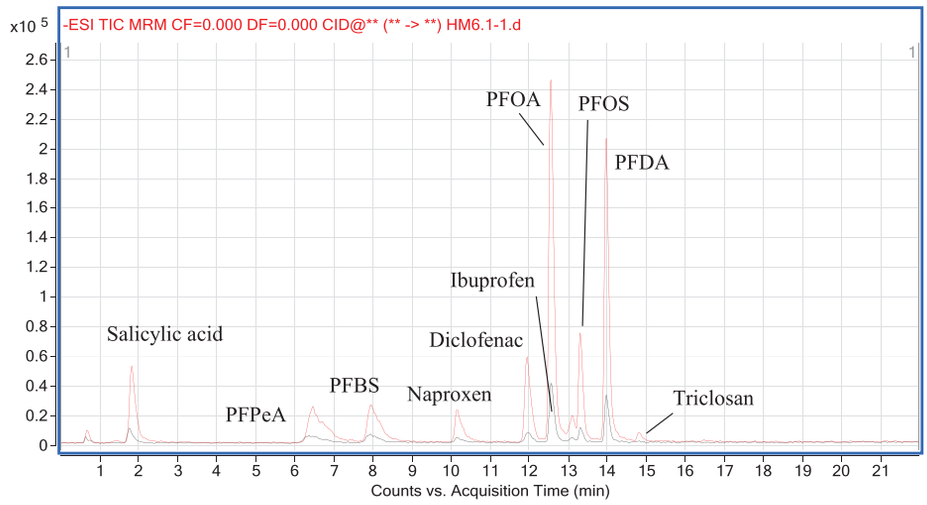

FIGURE 1 Chromatograms of the compounds analysed in negative mode from the samples spiked at 100 ng/mL after extraction with A-4.5 (black) and M-6 (red). Ibuprofen and PFOA peaks are overlaped

The manufacturer did not specify the time required for precipitation when using SPE cartridges. After 30 s of adding the solvent the precipitation was apparently complete (Figure S1). However, the manufacturer recommends 2 min for complete precipitation when using Phree™ in 96-well plate format [28], which have higher bed volume. Then, the cartridges were left 2 min to ensure complete precipitation.

When employing MeOH as solvent, the cartridge elution was very slow even using the highest pressures that the manifold achieves (close to 762 mmHg). Due to the volatility of the solvent and the high vacuum used, the sample is below vapour pressure of the solvent and this favour solvent evaporation during the procedure, achieving low volumes of extract (around 100–150 and 200–250 μL for M-4 and M-6 respectively). Occasionally, it was not possible to pass or percolate the samples through the cartridges and/or the process was such slow that obtaining extracts was not possible because of the complete solvent evaporation. This was especially problematic with M-4, where no extract was obtained in the 50% of the attempts (n = 10) and low volumes were obtained with the other attempts. For this reason, M-4 was discarded as a valid extraction procedure.

Recovery tests at 100 μg/L for M-4 commonly showed recoveries higher than 100%. This was probably due to the evaporation of solvent during the extraction and subsequent concentration of analytes in the extract. This was not so marked when using M-6.

Regarding LC-MS/MS analysis, the signal provided by the compounds was generally enhanced when MeOH was used in the analysis. Figure S1 shows the chromatograms of the 500 ng/mL calibration point and extracts obtained with A-4.5 and M-6 with signal-noise remarkably higher when MeOH is employed. This difference was especially remarkable when working in negative mode (Figure 1). Furthermore, the signal enhanced using M-6 was generally followed by a lower background noise and better peak shape, especially for compounds such as diclofenac, ibuprofen, triclosan, caffeine, bentazone, or vildagliptin (Figure S-2). Obviously, the background noise was also related to the proportion sample:solvent, being A-3 the method with less dilution factor hence the one that presented higher background noise. Up to our knowledge, Phree™ cartridges have not been employed for hemolymph before. As Phree™ cartridges were designed for plasma analysis there are several studies that employed them for this purpose [21, 29], which mainly use ACN as solvent. Hence, previous works that assess the differences regarding the use of MeOH and ACN have not been found.

3.2 | Method validation

Method validation was performed for the methods A-3, A-4.5 and M-6. M-4 was discarded due to extraction issues with the pressure explained before.

3.2.1 | Sensitivity and precision

Regarding precision and following the European Commission Guidelines [30], Intra-R (Table 1) was satisfactory (< 20%) except for etoricoxib when using A-3. Inter-R was satisfactory (< 30%) except for metformin for M-6 and bufotenine for both A-3 and A-4.5. In general, precision

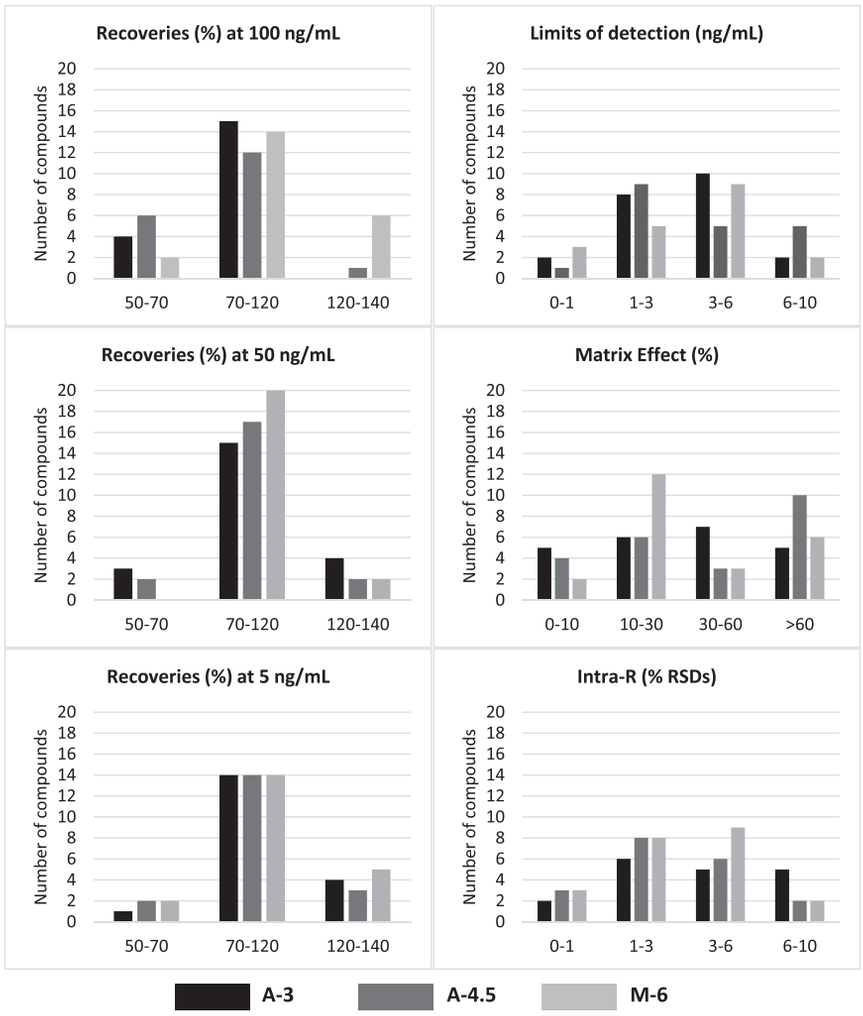

FIGURE 2 Comparison of the parameters: Intra-R, LODs, ME and recoveries between the methods. The graphics show the number of compounds that fulfill the different ranges of values for the different parameters

results were slightly better for M-6 than both ACN extractions (Figure 2).

LODs showed a range of 0.44-9.28, 0.79-12.30, and 0.30-10.70 ng/mL for A-3, A-4.5, and M-6, respectively, except for salicylic acid. (15.30 ng/mL) in A-3 and bentazone (19.10 ng/mL), metformin (16.30 ng/mL), and triclosan (22.00 ng/mL) in M-6 (Table 1). LODs of M-6 had ranges similar to A-3 and A-4.5 ACN despite the dilution factor of the sample (7, 4, and 5.5, respectively). This is due to the higher signal-noise ratio, as described before. However, as shown in Figure 2, A-3 showed slightly higher sensitivity when compared with the other methods.

The background noise was too high for the proper calculation of LODs, LOQs, and Intra-R of bentazone and 4-MeO-PCP using the samples spiked at 5 ng/mL (except for 4-MeO-PCP in M-6). Therefore, they were calculated using the lowest point of the linearity (5 ng/mL in solvent). In a similar way, low recoveries for 4-MeO-PCP in the samples spiked at 50 ng/mL avoided the correct calculation of Inter-R for A-3 and A-4.5.

3.2.2 | Matrix effect and recoveries

Several compounds presented strong ME (Figure 3 and Table S-3), the 52, 61 and 39 of compounds had a ME $\geq \pm 30\%$ for the methods A-3, A-4.5 and M-6, respectively. Signal suppression was predominant for M-6. While signal enhancement was predominant for A-4.5, including remarkably strong signal enhance for salicylic acid (+200.0%), diclofenac (+160.0%), PFBS (+117.0%) and imazalil (+107.0%). On the other hand, A-3 presented

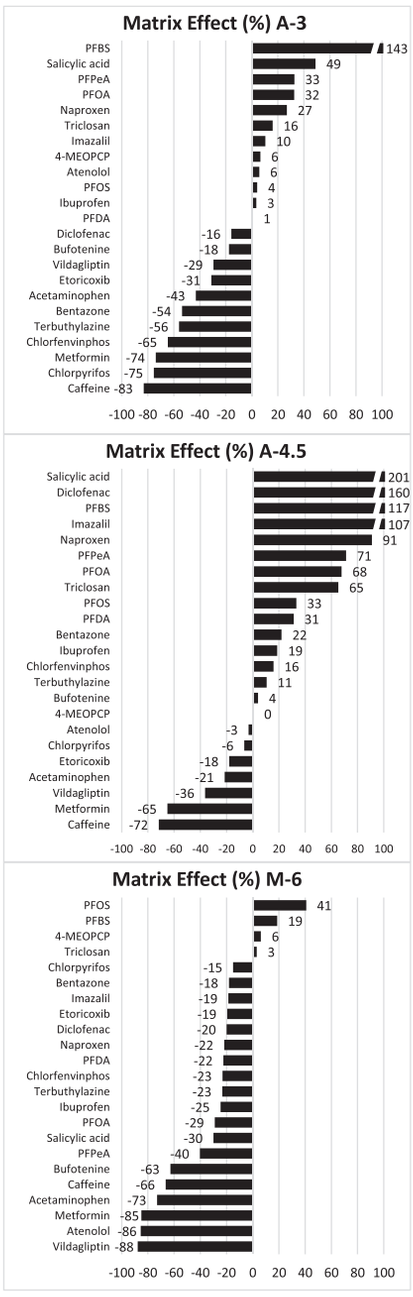

FIGURE 3 ME of the validated methods for each compound

mixed results including very strong signal enhanced for PFBS (+143.0%). Despite the results in Figure 3 are heterogeneous, overall A-3 and M-6 were the methods with weakest matrix effects.

Most of the compounds with signal enhance were acids while the opposite behaviour was predominant for basic compounds (especially in A-4.5). This is in accordance with previous studies, where basic compounds commonly showed signal suppression in biological samples [31, 32]. However, other studies suggest that signal enhancement or suppression due to matrix effect is unpredictable and unique for each analysis [33, 34].

Recoveries were determined at three concentration levels: 5, 50, and 100 ng/mL (Table 2). Recoveries ranging 70–120% were considered acceptable (following the European Commission Guidelines [30]). A-3 was able to satisfactory recover 15 at 50 and 100 ng/mL, while A-4.5 was able to recover 17 and 12 compounds, respectively. M-6 was the only method that recovered properly the dissociative anaesthetic drug 4-MeO-PCP, with 20 and 14 compounds within the accepted range at 50 and 100 ng/mL, respectively. And the three methods were able to extract 14 compounds at 5 ng/mL, where bentazone was not recovered in any of the three methods.

Summarizing, the three methods provided recoveries within the acceptable range (70–120 %) for the majority of the compounds. As can be seen in Figure 2 and Table 2, an average of 15 compounds (corresponding to 65%) using A3, 16 compounds (69 %) using A4.5 and M6 showed recoveries within that range. If a wider and practical range of 60–140% is considered, and average > 20 compounds (85 %) for any of the three methods is within the acceptable range.

Hemolymph analyses in bivalves are usually employed to assess the effects of the organic compounds, such as DNA damage [31], alteration of the immune parameters [32], or the analysis of other pollutants related biomarkers [35, 36]. However, up to our knowledge, studies about methodology development or occurrence of organic pollutants in mussel hemolymph have not been found. Attending to other aquatic biota, the occurrence of the pharmaceutical fluoxetine has been determined in crab hemolymph [18, 25, 35], however it is not a target compound of the present study and the extraction procedure was substantially different. The only study found using Phree™ cartridges in aquatic biota, employed them as a purification step (after a ACN solvent extraction) for the extraction of 41 antibiotics from fish muscle, with recoveries ranging 99.8–112% and providing a remarkable improvement of the sensitivity [22].

3.3 | Application to nonspiked samples

Hemolymph samples from three different local markets were analysed in order to test the efficacy of the methods in real samples. The hemolymph from five mussels of each market was pulled, and then extracted by triplicate using the procedures A-4.5 and M-6. When extracted with A-4.5, results showed concentrations ranging 33.50–97.80 ng/mL for salicylic acid in two of the markets. On the other hand, results of extractions using M-6 showed con-

TABLE 2 Absolute and relative (for compounds with internal standard) recoveries for the three methods at spiked concentrations of 100, 50 and 5 ng/mL

	A-3			A-4.5			M-6		
Recoveries (%)	**100 ng/mL**	**50 ng/mL**	**5 ng/mL**	**100 ng/mL**	**50 ng/mL**	**5 ng/mL**	**100 ng/mL**	**50 ng/mL**	**5 ng/mL**
4-MEOPCP	2.4	0.5	–	1.8	2.6	–	102.0	109.0	98.6
Acetaminophen	75.6	87.0	128	78.4	84.1	104.0	71.4	73.6	73.4
Atenolol	86.7	119.0	47.8	85.6	118.0	48.9	136.0	96.2	90.5
Bentazone	72.2	126.0	–	105.0	117.0	–	117.0	111.0	–
Bufotenine	46.7	58.8	35.5	39.9	40.8	41.3	166.0	146.0	64.2
Caffeine	106.0	84.8	125.0	118.0	114.0	112.0	52.2	78.2	91.0
Chlorfenvinphos	65.6	85.1	120.0	60.6	62.3	115.0	87.9	81.2	105.0
Chlorpyrifos	68.9	110.0	73.1	66.5	102.0	86.4	77.9	94.7	123.0
Diclofenac	108.0	110.0	119.0	102.0	103.0	58.6	84.4	109.0	147.0
Etoricoxib	108.0	131.0	58.5	127.0	116.0	118.0	93.4	94.7	104.0
Ibuprofen	95.4	118.0	87.6	105.0	129.0	113.0	109.0	114.0	119.0
Imazalil	43.5	128.0	93.6	64.4	104.0	69.9	103.0	113.0	111.0
Metformin	85.4	104.0	108.0	81.6	96.2	95.5	115.0	125.0	134.0
Naproxen	110.0	89.1	126.0	43.1	78.2	133.0	127.0	89.0	130.0
PFBS	93.0	60.7	125.0	59.2	86.5	130.0	109.0	84.9	124.0
PFDA	87.8	98.0	99.9	100.0	95.6	110.0	72.8	92.0	114.0
PFOA	57.8	95.5	86.8	69.2	105.0	98.2	57.0	77.0	87.1
PFOS	65.9	102.0	88.3	76.7	91.6	81.7	71.9	96.6	69.0
PFPeA	87.1	103.0	117.0	65.9	77.9	83.5	83.1	80.1	86.6
Salicylic acid	35.1	62.5	117.0	29.3	62.3	114.0	122.0	111.0	129.0
Terbuthylazine	92.9	104.0	70.6	72.7	108.0	71.8	127.0	106.0	108.0
Triclosan	109.0	108.0	118.0	89.5	118.0	124.0	120.0	114.0	100.0
Vildagliptin	110.0	129.0	87.3	101.0	125.0	97.9	122.0	133.0	90.2

a) *Bold files represent RR%, the other compounds are represented in R%.*

centrations ranging 45.29–66.82 ng/mL for salicylic acid, and 28.30–30.31 ng/mL for diclofenac in two and three of the markets samples, respectively.

The methods A-4.5 and M-6 were also tested using samples from a bioaccumulation study were *M. Gallo-provincialis* were exposed to different emerging pollutants during 28 days at a concentration of 10 ng/mL in water. The hemolymph samples analysed correspond to the 14th day of exposure. When extracted using A-4.5 results showed concentrations of 8.04-85.60 ng/mL for acetaminophen, diclofenac, metformin, naproxen, PFOA, and terbuthylazine, while chlorfenvinphos, etoricoxib, naproxen, PFPeA, PFDA, PFBS, and PFOS showed values below the LODs or LOQs. On the other hand, the extracts of M-6 showed concentrations of 1.14-96.30 ng/mL for diclofenac, etoricoxib, ibuprofen, imazalil, metformin, PFPeA, PFOS, salicylic acid, and terbutylazine, while chlorfenvinphos, chlorpyrifos, naproxen, PFOA PFDA, PFBS, and vildagliptin presented values below the LODs or LOQs. It is important to mention that M-6 generally detected more compounds at higher concentrations as is the case of chlorpyrifos, ibuprofen, imazalil, salicylic acid, and vildagliptin not detected using A-4.5. However, acetaminophen was only detected when using A-4.5.

4 | CONCLUSIONS

The methods assessed are able to satisfactorily extract a wide range of organic compounds from mussel hemolymph. The method M-6 extracted all the target compounds with 20 of them ranging recoveries between 73.6 and 114.0%. M-6 also achieved the best precision and overall recoveries. On the other hand, A-3 provided the weakest ME and lowest LODs. Since strong ME were noticed, the use of ISs for every compound in future studies will likely improve these results. The use of MeOH as extract solvent involved improvements in the chromatographic signal-noise ratio. However, the use of MeOH also entailed slower extraction procedures and, in some cases, the clogging of the cartridges. Further research is needed to solve this extraction issues.

The proposed methods allowed the determination of organic pollutants in hemolymph from mussels purchased

in local markets. The pharmaceuticals salicylic acid and diclofenac were detected with concentrations of 33.50-97.79 and 28.30–30.31 ng/mL, respectively. Furthermore, the methods were tested using samples from a bioaccumulation study. Where M-6 detected nine compounds with concentrations ranging 1.14–96.30 ng/mL and A-4.5 detected only six compounds with concentrations ranging 8.04–85.60 ng/mL.

The results of the present study show that, despite the procedural issues, M-6 was the best method for the multi-residue extraction of organic pollutants in hemolymph.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary Information

Multi-residue extraction to determine organic pollutants in mussel haemolymph

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Table of contents

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Table S-1: External standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Mass labelled
POSITIVE COMPOUNDS				
Etoricoxib 1	359	280	14	
Etoricoxib 2	359	279	14	
Chlorfenvinphos 1	359	155	18.4	Chlorfenvinphos-d10
Chlorfenvinphos 2	359	127	18.4	Chlorfenvinphos-d10
Chlorpyrifos 1	350	198	20.1	Chlorpyrifos-d10
Chlorpyrifos 2	350	97	20.1	Chlorpyrifos-d10
Vildagliptin 1	304	154	0.7	Vildagliptin-d3
Vildagliptin 2	304	91	0.7	Vildagliptin-d3
Imazalil 1	297	201	14.3	
Imazalil 2	297	159	14.3	
Atenolol 1	267	91	1.0	Atenolol-d7
Atenolol 2	267	77	1.0	Atenolol-d7
Terbuthylazine 1	230	174	17.6	
Terbuthylazine 2	230	96	17.6	
Bufotenine 1	205	160	0.7	
Bufotenine 2	205	58	0.7	
Caffeine 1	195	138	1.6	Caffeine-d9
Caffeine 2	195	110	1.6	Caffeine-d9
Acetaminophen 1	152	110	0.8	Acetominophen-d3
Acetaminophen 2	152	92	0.8	Acetominophen-d3
Metformin 1	130	71	0.6	
Metformin 2	130	60	0.6	
4-MEOPCP 1	274	189	1.0	
4-MEOPCP 2	274	121	1.0	
Bentazone 1	241	92	17.7	
Bentazone 1	241.1	196	17.7	
NEGATIVE COMPOUNDS				
Salicylic acid	137	93	1	
PFDA 1	513	469	14	MPFDA
PFDA 2	513	269	14	MPFDA
PFOS 1	499	99	13.5	MPFOS
PFOS 2	499	80	13.5	MPFOS
PFOA 1	413	369	12.9	MPFOA
PFOA 2	413	169	12.9	MPFOA
PFBS 1	299	99	7.5	
PFBS 2	299	80	7.5	
Diclofenac sodium 1	294	250	11.9	Diclofenac-d4
Diclofenac sodium 2	294	178	11.9	Diclofenac-d4
Triclosan 1	289	35	14.8	Triclosan-d3
Triclosan 2	287	35	14.8	Triclosan-d3
PFPeA 1	263	219	5.8	
Naproxen 1	229	185	10	
Naproxen 2	229	170	10	
Ibuprofen 1	205	159	12.8	Ibuprofen-d3

S-2

Table S-2: Mass labelled standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)
POSITIVE COMPOUNDS			
Chlorfenvinphos-d10 1	369	101	18.4
Chlorfenvinphos-d10 2	369	170	18.4
Chlorpyrifos-d10 1	360	199	20.1
Chlorpyrifos-d10 2	360	99	20.1
Vildagliptin-d3 1	307	157	0.7
Vildagliptin-d3 2	307	93	0.7
Atenolol-d7 1	274	145	0.7
Atenolol-d7 2	274	79	0.7
Caffeine-d9 1	204	144	1.3
Caffeine-d9 2	204	116	1.3
Acetominophen-d3 1	155	111	0.9
Acetominophen-d3 2	155	65	0.9
NEGATIVE COMPOUNDS			
MPFDA 1	515	270	14
MPFDA 2	515	470	14
MPFOS 1	503	99	13.5
MPFOS 2	503	80	13.5
MPFOA 1	417	372	12.9
MPFOA 2	417	169	12.9
Diclofenac-d4 1	298	254	11.9
Triclosan-d3 1	290	35	14.8
Ibuprofen-d3 1	208	164	12.8

Table S-3: Matrix effect of the three extraction procedures.

Matrix effect (%)	**A-3**	**A-4.5**	**M-6**
4MeO-PCP	6.5	0.4	6.2
Acetaminophen	-43.0	-21.4	-73.0
Atenolol	5.7	-3.2	-85.6
Bentazone	-53.7	22.0	-18.2
Bufotenine	-17.6	4.1	-62.8
Caffeine	-83.1	-71.6	-66.5
Chlorfenvinphos	-64.6	15.9	-23.0
Chlorpyrifos	-75.3	-6.4	-15.0
Diclofenac	-15.8	160.0	-19.9
Etoricoxib	-31.2	-17.9	-19.5
Ibuprofen	3.5	18.7	-24.5
Imazalil	10.3	107.0	-18.6
Metformin	-73.9	-65.1	-85.0
Naproxen	26.8	90.9	-21.8
PFBS	143.0	117.0	18.6
PFDA	0.5	31.3	-22.3
PFOA	32.4	67.8	-29.0
PFOS	4.1	33.3	40.8
PFPeA	32.7	71.3	-40.3
Salicylic acid	48.9	200.0	-30.0
Terbuthylazine	-56.0	10.7	-23.2
Triclosan	16.0	65.4	3.1
Vildagliptin	-29.5	-36.4	-87.8

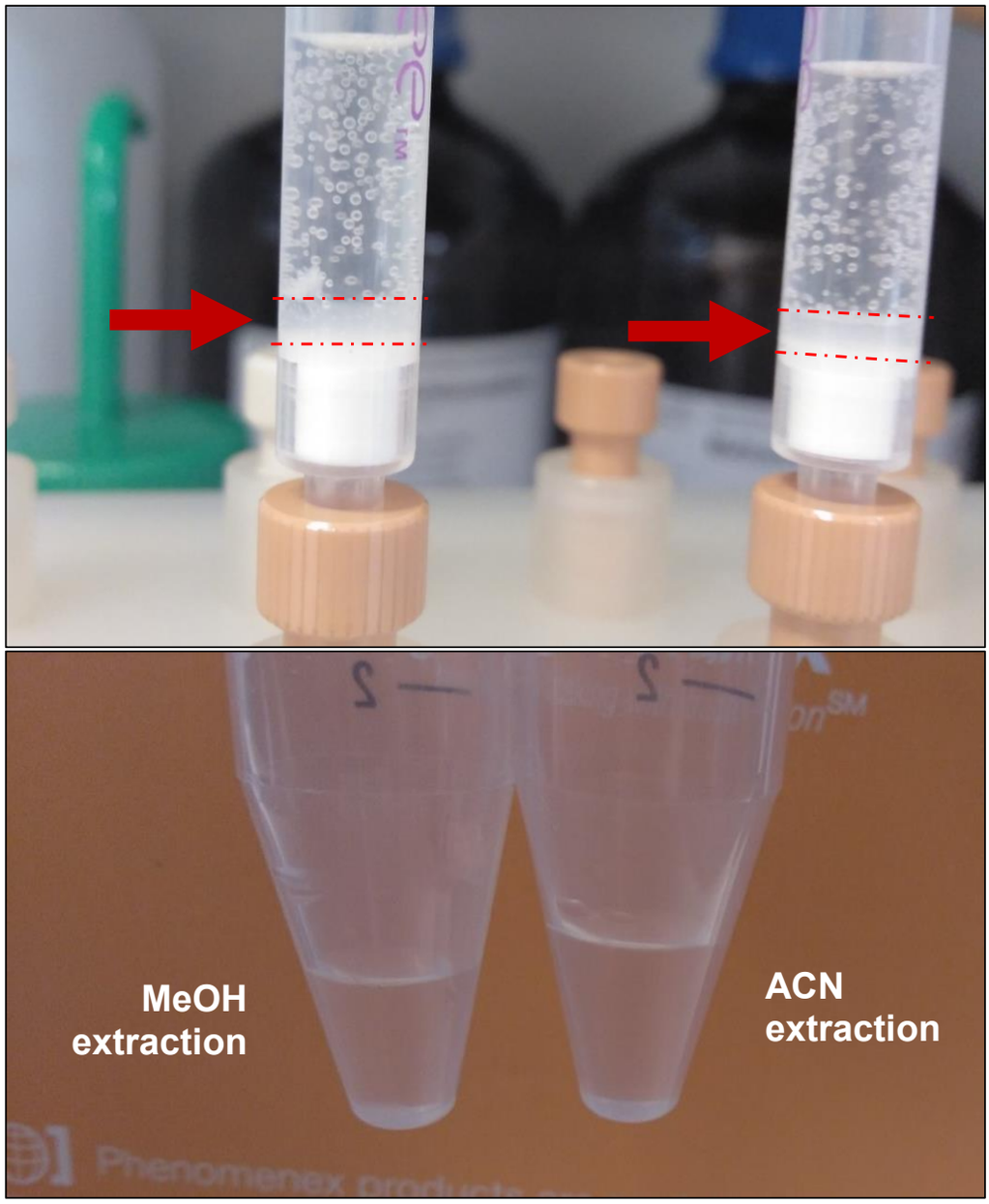
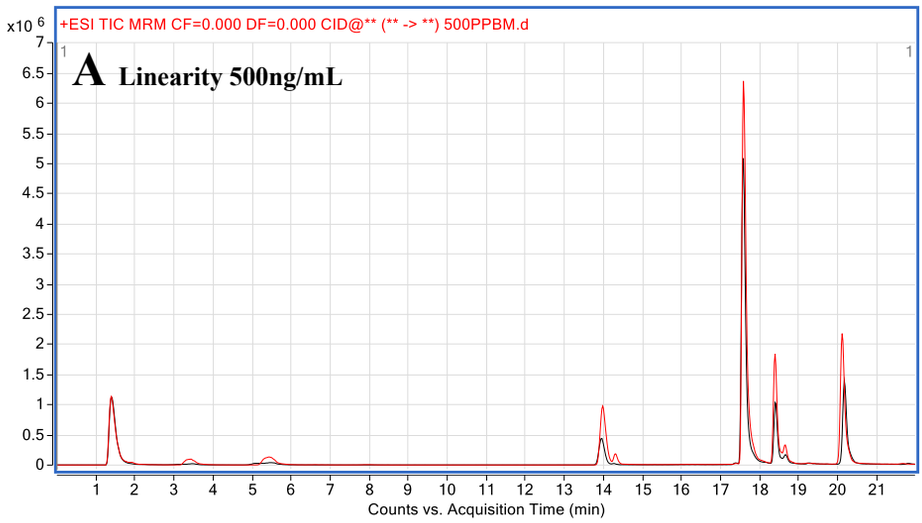
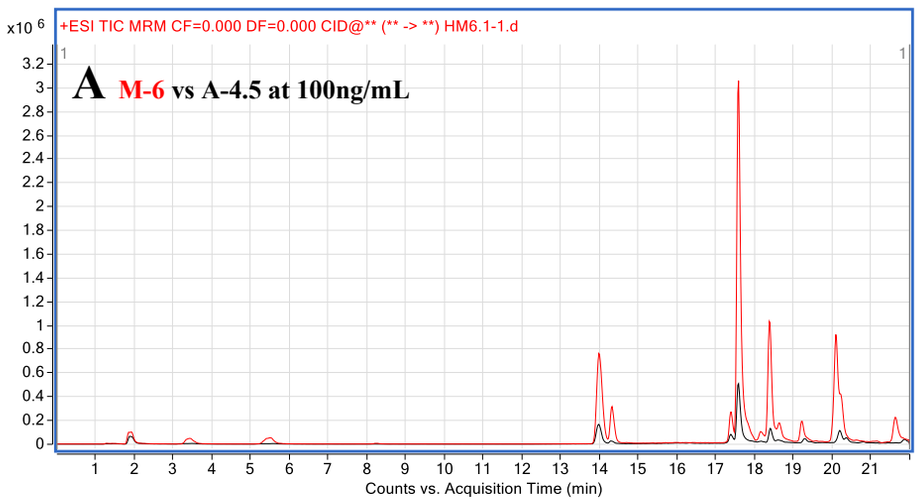
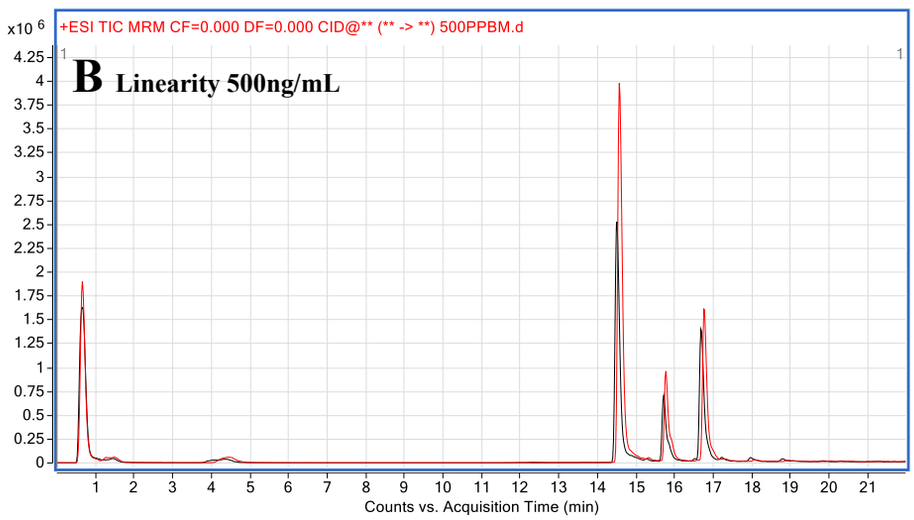


***Figure S-1**: Upper panels: phospholipids precipitate at the bottom of the cartridges after direct addition of the solvent, where they formed a cloudy layer. Lower panel, haemolymph extracts after using the validated methods M-6 (left) and A-4.5 (right). The extracts are clean, not presenting any cloudiness or turbidity, as a result of a proper precipitation of the phospholipids.*

S-5


x10 6
+ESI TIC MRM CF=0.000 DF=0.000 CID@** (** -> **) 500PPBM.d
A Linearity 500ng/mL
Counts vs. Acquisition Time (min)


x10 6
+ESI TIC MRM CF=0.000 DF=0.000 CID@** (** -> **) HM6.1-1.d
A M-6 vs A-4.5 at 100ng/mL
Counts vs. Acquisition Time (min)


x10 6
+ESI TIC MRM CF=0.000 DF=0.000 CID@** (** -> **) 500PPBM.d
B Linearity 500ng/mL
Counts vs. Acquisition Time (min)

S-6

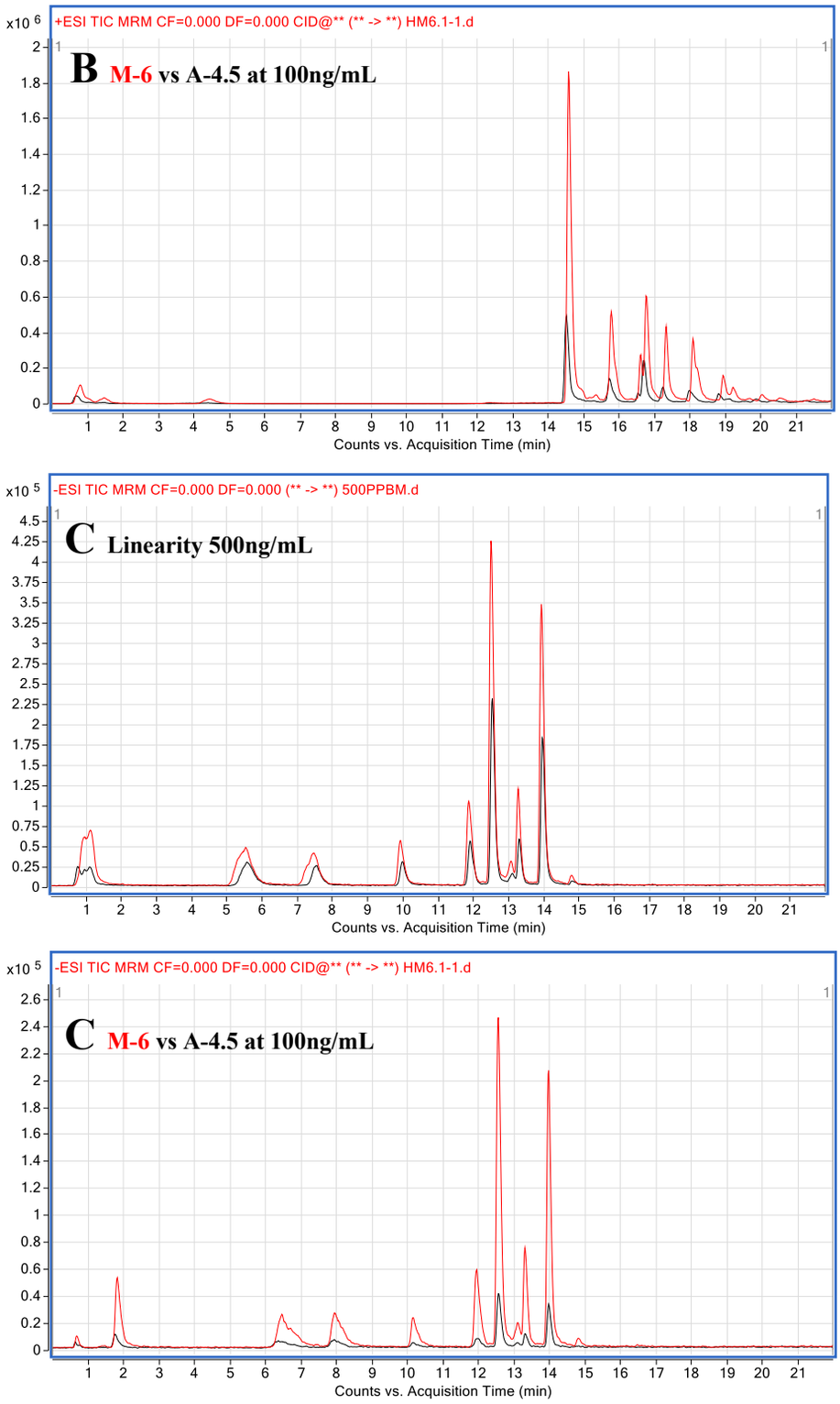
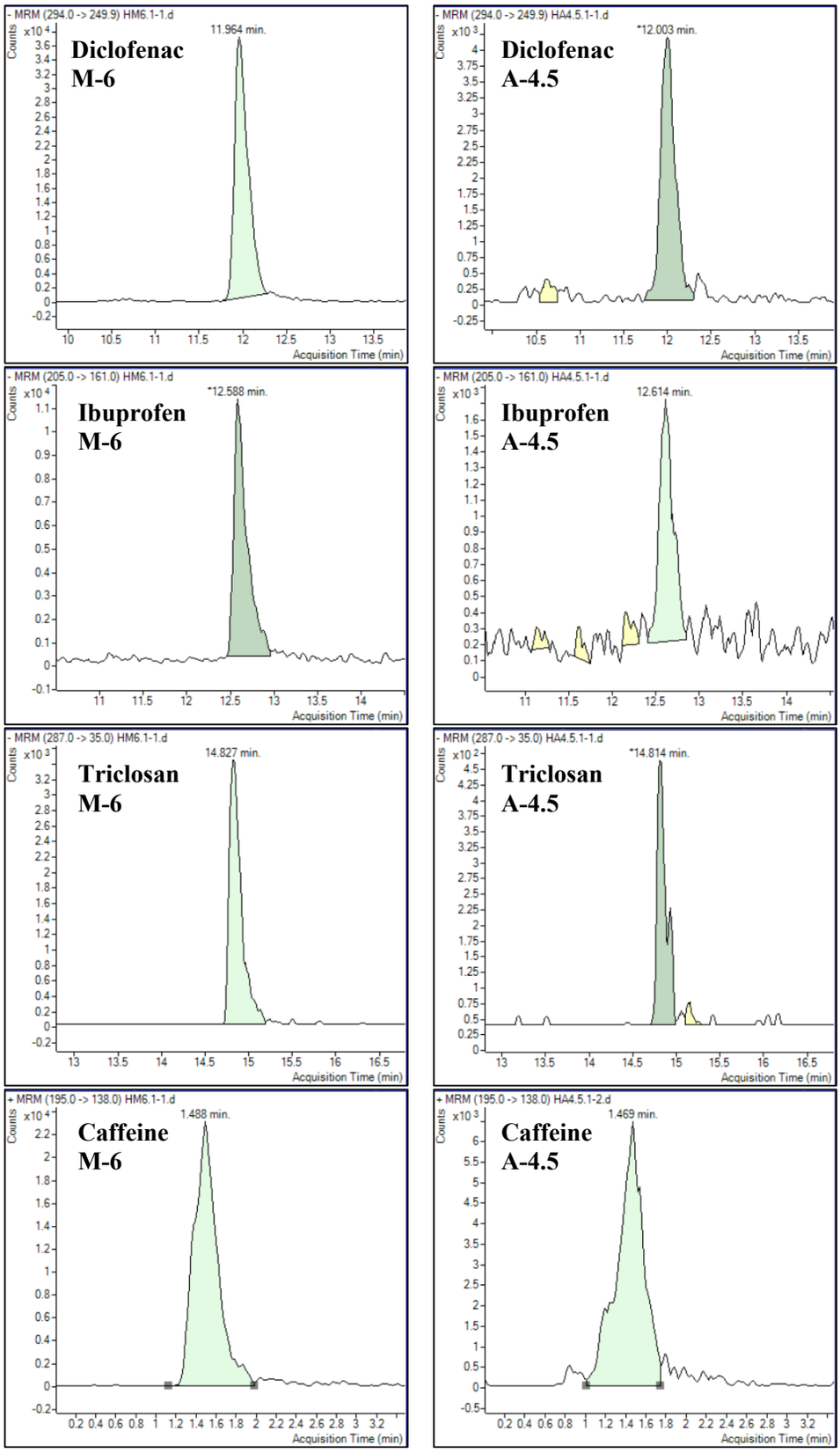


Figure S-2: *Chromatogram comparison when employing MeOH (red) and ACN (black) as solvent for the 500ng/mL linarity. Also chromatograms from A-4.5 and M-6 extractions spiked at 100 ng/mL. A: chromatograms of the compounds analysed in positive mode employing Luna® 3µm C18(2) column. B: chromatograms of the compounds analysed in positive mode employing Kinetex 1.7µm XB-C18 column. C: chromatograms of the compounds analysed in negative mode.*

S-7


- MRM (294.0 -> 249.9) HM6.1-1.d
11.964 min.
Diclofenac
M-6
- MRM (294.0 -> 249.9) HA4.5.1-1.d
*12.003 min.
Diclofenac
A-4.5
- MRM (205.0 -> 161.0) HM6.1-1.d
*12.588 min.
Ibuprofen
M-6
- MRM (205.0 -> 161.0) HA4.5.1-1.d
12.614 min.
Ibuprofen
A-4.5
- MRM (287.0 -> 35.0) HM6.1-1.d
14.827 min.
Triclosan
M-6
- MRM (287.0 -> 35.0) HA4.5.1-1.d
*14.814 min.
Triclosan
A-4.5
+ MRM (195.0 -> 138.0) HM6.1-1.d
1.488 min.
Caffeine
M-6
+ MRM (195.0 -> 138.0) HA4.5.1-2.d
1.469 min.
Caffeine
A-4.5
Counts
Acquisition Time (min)

S-8

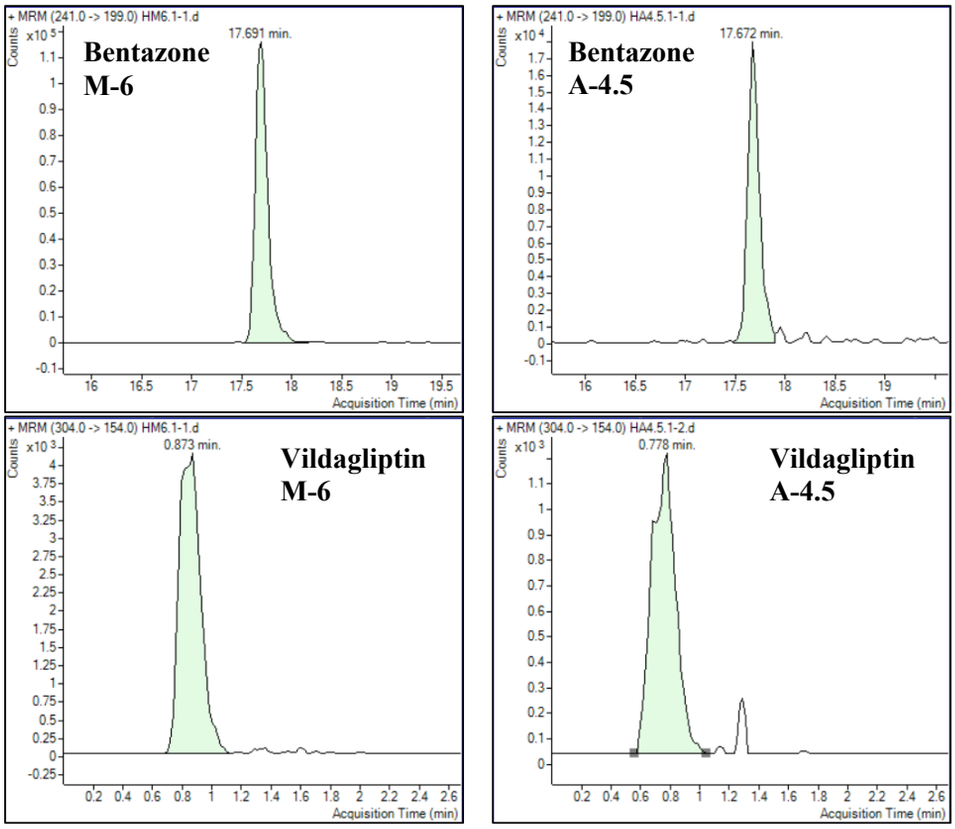


Figure S-3: *Comparison of chromatograms from samples spiked at 100 ng/mL and extracted with the methods M-6 (left) and A-4.5 (right).*

S-9

Determination of organic pollutants in *Anguilla anguilla* by liquid chromatography coupled with tandem mass spectrometry

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Method Article

Determination of organic pollutants in *Anguilla anguilla* by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)☆

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A B S T R A C T

One of the aspects considered about the presence of contaminants in the aquatic ecosystems is their possible effect on critically endangered species, as the case of European eel, *Anguilla anguilla*. However, there is a lack of analytical methods to determine these contaminants due to the complexity of eel matrix (contains 5–20 % of lipids and 5–15 % of proteins). Thus, a multi-residue method using QuEChERS extraction a clean-up based on new specific sorbents (to eliminate lipids) and liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed to determine a mix of 21 contaminants. Compared to the previously reported methods (Degani et al., 1986), which were developed for mussels, in this study, one of the proposed extraction methods were adapted to different fish tissues of higher complexity, such as liver and muscle of *A. anguilla*.

- The effectivity of dispersive solid phase extraction (dSPE) using new specific Enhanced Matrix Removal (EMR-lipid) as clean-up for lipid removal was tested.
- Clean extracts of matrices with high protein (5–15 %) and lipid (5–20 %) content were obtained ensuring robustness and durability of the analytical systems.
- Emerging contaminants extractable by this procedure comprise four different families (pesticides, perfluoroalkyl substances (PFASs), pharmaceuticals and drugs of abuse). Then, it could be further applied to wide scope screening strategies.

A R T I C L E I N F O

Method name: Multi-residue method based on quick easy cheap effective rugged and safe (QuEChERS) procedure and Enhanced Matrix Removal (EMR-lipid) clean-up
Keywords: QueChERs, EMR-lipid, Emerging pollutants, Liver, Muscle, Pesticides, Pharmaceuticals, PFASs, Illicit drugs
Article history: Available online 15 April 2021

☆ Direct Submission or Co-Submission: *Direct Submission*

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Specifications Table

Subject Area	Environmental Science
More specific subject area	Advanced mass spectrometric analysis for environmental and food safety
Method name	Multi-residue method based on quick easy cheap effective rugged and safe (QuEChERS) procedure and Enhanced Matrix Removal (EMR-lipid) clean-up.
Name and reference of original method	Development of multi-residue extraction procedures using QuEChERS and liquid chromatography tandem mass spectrometry for the determination of different types of organic pollutants in mussel
Resource availability	Under review at Analytical and Bioanalytical chemistry

Background

In Europe, *Anguilla anguilla* is critically endangered because it is highly affected by several anthropic pressures, such as habitat modification, overfishing and contamination. This latter has been widely reported in its habitats. This together with a complicated live cycle that involves migration (> 5000 km), the success of which depends on nutritional and sanitary state of the eel has caused the decline of its population. Organic pollutants have already been profusely described in any type fish [6]). The evaluation of the presence of organic pollutants, in eels is crucial to assess their possible influence in the decline of this endangered species. Eels are rich in proteins (5–15% w/w) and highly unsaturated lipids (5–20% w/w) [1]. Therefore, their analysis is also crucial to assess any risk for human health. For these tasks, the development of analytical methods capable of dealing with complex matrices (high content in proteins and lipids) is needed. In this way, multi-residue methods allow the analysis of compounds from different families at the same time, saving time and resources.

Method details

The Multi-residue extraction and clean-up selected for this study, was originally reported in Álvarez-Ruiz et al. [4] for mussel matrix as one of the three best methods among 44 different combinations of QuEChERS (including acidified QuEChERS) and clean-ups. The application of a novel sorbent (EMR-Lipid) based on size exclusion and hydrophobic interaction. Offers a very promising solution for the removal of the high lipid content of eel tissues. Therefore, in the present work the method was employed for the extraction of organic pollutants from eelś muscle and liver. Furthermore, since the amount of water employed during the QuEChERS procedure could influence the method performance ([4]), five variations of water addition have been tested. The compounds analyzed were 5 pesticides, 5 PFASs, 10 pharmaceuticals and 1 illicit drug, as in the original work, they were selected due to their presence in aquatic environments [4].

Materials and reagents

The high-performance liquid chromatography (HPLC) grade acetonitrile (ACN) ≥ 99.9% purity and trisodium citrate dehydrate (TCD) were purchased from VWR Chemicals® (Radnor, Pennsylvania). Magnesium sulphate and disodium hydrogen citrate sesquihydrate (DCS) were from Alfa Aesar (Karlsruhe, Germany). Sodium chloride from Sigma-Aldrich (Steinheim, Germany). EMR-Lipid clean-up dSPE was from Agilent Technologies. Polypropylene centrifuge falcon tubes (either 15 mL or 50 mL) were purchased from VWR International Eurolab (Barcelona, Spain). Polypropylene/polyethylene syringes manufactured by BRAUN and distributed by Scharlab S.L., Barcelona, Spain. Nylon 0.22 µm filters were purchased from Membrane Solutions (Plano, TX, USA). The 2 mL amber glass vials with stoppers 99 mm + Septum Sil /PTFE used to inject the samples were from Análisis Vínicos S.L. (Tomelloso, Spain) and the 250 µL polypropylene inserts were from Agilent Technologies (Santa Clara, CA, United States).

The analytical standards of pharmaceuticals (acetaminophen, atenolol, caffeine, diclofenac, etoricoxib, ibuprofen, naproxen, salicylic acid, triclosan, vildagliptin), pesticides (bentazone, chlorfenvinphos, chlorpyrifos, imazalil, terbutylazine), and PFASs (perfluoropentanoic acid [PFPeA] and perfluorobutanesulfonate [PFBS]) were from Sigma-Aldrich (Steinheim, Germany). While

perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), and perfluorooctanesulfonate (PFOS) were from Wellington (Ontario, Canada) and the illicit drug bufotenine was purchased from LGC Standards (Ontario, Canada). The surrogate (internal) standards acetaminophen-d3 and atenolol-d7 were from Sigma– Aldrich. Chlorfenvinphos-d10 (diethyl D5), chlorpyrifos-d10 (diethyl D10), and vildagliptin-d3 were purchased from LGC Standards. Diclofenac-d4 was purchased in Toronto Chemicals Research (Toronto Canada). PFOA-d4 (MPFOA), PFOS-d4 (MPFOS), and PFDA-d4 (MPFDA) were from Wellington. Both the internal and external standard mix were created using ACN as solvent.

Sampling

For this study, approximately 25 *A. anguilla* specimens were obtained from a fish local market and supermarket at, Valencia (Spain), then refrigerated and transferred to the laboratory of Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE, UV-CSIC-GV), University of Valencia, Spain. Once in the laboratory, samples of liver and muscle tissue were pooled. Muscle was chopped in small pieces and then homogenize using a pestle and placed in 50 mL Falcon tubes. Since the livers are soft and a very scarce and valuable resource (1,2 g for specimen), they were chopped, placed in a 50 mL falcon tube (to avoid any loss of sample) and then homogenized using metal tweezers. The tubes were then stored at -20 °C until analysis.

Extraction procedure (QuEChERS)

An aliquot of 1 g w.w. (wet weight) of pooled eel liver or muscle placed in 50 mL falcon tubes was spiked with 200 µL of an internal standard mix at 1 mg/mL to achieve a final concentration in the extracts of 20 ng/mL (assuming a recovery of the 100%). Also 50 µL of the external standard mix at 10 mg/mL (what is translated in to 500 ng/g) were added and the sample was left until the solvent evaporated. Water was added and to ensure the optimal partitioning 0, 3, 5, 7.5, and 10 mL were tested. Then, ACN (10 mL) was also added. The mix was vortexed for 3 min to ensure homogenization. Next, 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of DCS and 1 g of TCD were added and, immediately, the tube was vigorously shaken by hand for 3 min to avoid salt agglomeration and then, centrifuged for 5 min at 3500 rpm (2465 rcf).

For the clean-up, EMR-Lipid dSPE phase was placed in 15 mL Falcon tubes and activated by adding 5 mL of MilliQ water and vortexed for 30 s. Then, 5 mL of QuEChERS supernatant were added, the tube was vortexed for 30 s more and centrifuged for 5 min at 3500 rpm. Next, 5 mL of this supernatant were added to another 15 mL Falcon tube containing the polish phase consisting of a mixture of 1600 mg of $MgSO_4$ and 400 mg of NaCl. The tube was manually shaken for 30 s to avoid salt agglomeration and then, centrifuged for 5 min at 3500 rpm. The supernatant was filtered using Nylon 0.22 µm filters and polypropylene/polyetilene syringes, and then, stored in vials with inserts ready for analysis.

Recovery tests were performed in triplicate for each variation in the water amount. Furthermore, a procedural blank containing non-spiked sample was included at least every 9 samples.

LC-MS/MS analysis

Analysis was performed via LC-MS/MS as described by Álvarez-Ruiz et al. [5], an Agilent 1260 UHPLC from Agilent technologies coupled to an Agilent 6410 Mass Spectrometer triple quadrupole (QqQ) also from Agilent technologies were employed. With electrospray source ionization (ESI) in both negative and positive ionization modes (nebulizer gas 15 psi, gas flow 11 L/min. ion-spray voltage 4 kV and temperature 300 °C) operated in multiple reaction monitoring mode (MRM). The column used for the detection of pesticides and etoricoxib was Luna® 3 µm C18(2) 100 Å 150 × 2 mm and the column employed for PFAS, illicit drug and the rest of PPCPs was a Kinetex 1.7 µm XB-C18 100 Å 50 × 2.1 mm, both from Phenomenex. Yielding a total of three LC methods, one in negative ionization mode using Kinetex column and two in positive mode using Kinetex and Luna® columns, respectively. When operated in positive ionization mode, the mobile phases employed were (A) H_2O 0.1% formic acid and (B) MeOH 0.1% formic acid. For negative ionization mode, the mobile phases employed were

(A) H_2O 2.5 mM ammonium fluoride and (B) MeOH 2.5 mM ammonium fluoride. The linear gradient was as follows: 0 min (70% A), 12 min (5% A), 25 min (5% A), 26 min (70% A), and 30 min (70% A) either in positive or negative ionization mode (only the mobile phases were different). The column temperature was 30 °C. The injection volume was 5 µL and the ACN extract were directly injected. As the injection volume is low, no modification of the retention times was observed for pesticides and PFASs. However, the pharmaceuticals and illicit drugs some retention times are shorter but not double peaks were observed. Detailed information on the retention times, the selected transitions (precursor ion → product ion) for each compound, and those compounds that were determined using internal standards as well as by external standard is available in the Supplementary material (Table S1). Information on the transitions used to determine the internal standards are also reported in Supplementary material (Table S2). The confirmation of the presence of a target compounds in the sample was carried out considering the presence of the two transitions (precursor ion → product ion) and relative intensity of the two product ions (if possible), and retention time. As can be observed except for PFPeA and Ibuprofen that only gave one transition with enough intensity, for the other compounds two transitions were selected in order to ensure a proper identification. Data were obtained using the Mass Hunter software, qualitative (for the identification of substances) and quantitative (for the quantity of the substances obtained on time). Each batch of samples included a procedural blank (non-spiked tissues pool). At the beginning and at the end of each analytical sequence, a seven-points calibration standard was injected. A 100 ng/mL standard was also injected every 15 samples to check the instrumental variation and to avoid false negatives as well solvent and procedural blanks were injected to avoid false positive.

Method optimization

The addition of different volumes of water was tested in order to optimize the partition between water and ACN that takes place in QuEChERS after salting out. Results showed not significant differences in the recoveries using different water volumes even for the most polar compounds (bufotenine, salycilic acid and caffeine) (Fig. 1). Then, the volume of 7.5 mL was chosen in order to keep proportion used in the original QuEChER.

Regarding the LC-MS/MS analysis it is possible to determine the compounds using just the Kinetex column. However, the pesticides and etoricoxib usually presented chromatograms remarkably with better signal-noise ratio and better shaped peaks when Luna® was employed. Hence both were employed, as described above, in order to obtain better LC-MS/MS information.

Method validation

The selected protocol was validated for specificity, accuracy, precision, linearity, limits of detection (LOD), limit of quantification (LOQ), and matrix effects (ME), as described in Álvarez-Ruiz et al. [5]. The linearity was prepared in ACN, established through seven-points calibration standards (5, 10, 25, 50, 100, 200 and 500 ng/mL) and only regression coefficients (R^2) > 0.99 were accepted in the calibration curve. Recoveries were calculated in eel liver and muscle tissues fortified at 50, 250 and 500 ng/g in triplicate (5, 25 and 50 ng/mL the final extract). A 7-points calibration curve that considers peak areas or if the internal standard is available the ratio of the peak area and the area of the internal standards (y-axis) vs. contaminant concentrations (x-axis), Then, the peak area or its ratio with the internal standard of the sample is interpolated in the calibration curve to quantify them. For the compounds chlorfenvinphos, chlorpyrifos, PFDA, PFOA, PFOS, acetaminophen, atenolol, diclofenac and vildagliptin, the results obtained were relative recoveries (RR%) where the ME and other potential inaccuracy during sample handling, were corrected using the internal standards. The other compounds: imazalil, bentazone, terbuthylazine, PFBS, PFPeA, bufotenine, caffeine, etoricoxib, ibuprofen, naproxen, salicylic ac. and triclosan were quantified with external calibration. Hence, the results were represented as efficiency (E%), if the results are affected by either recovery and ME, or absolute recoveries (AR%), if the ME is corrected using matrix-matched standards. Both were

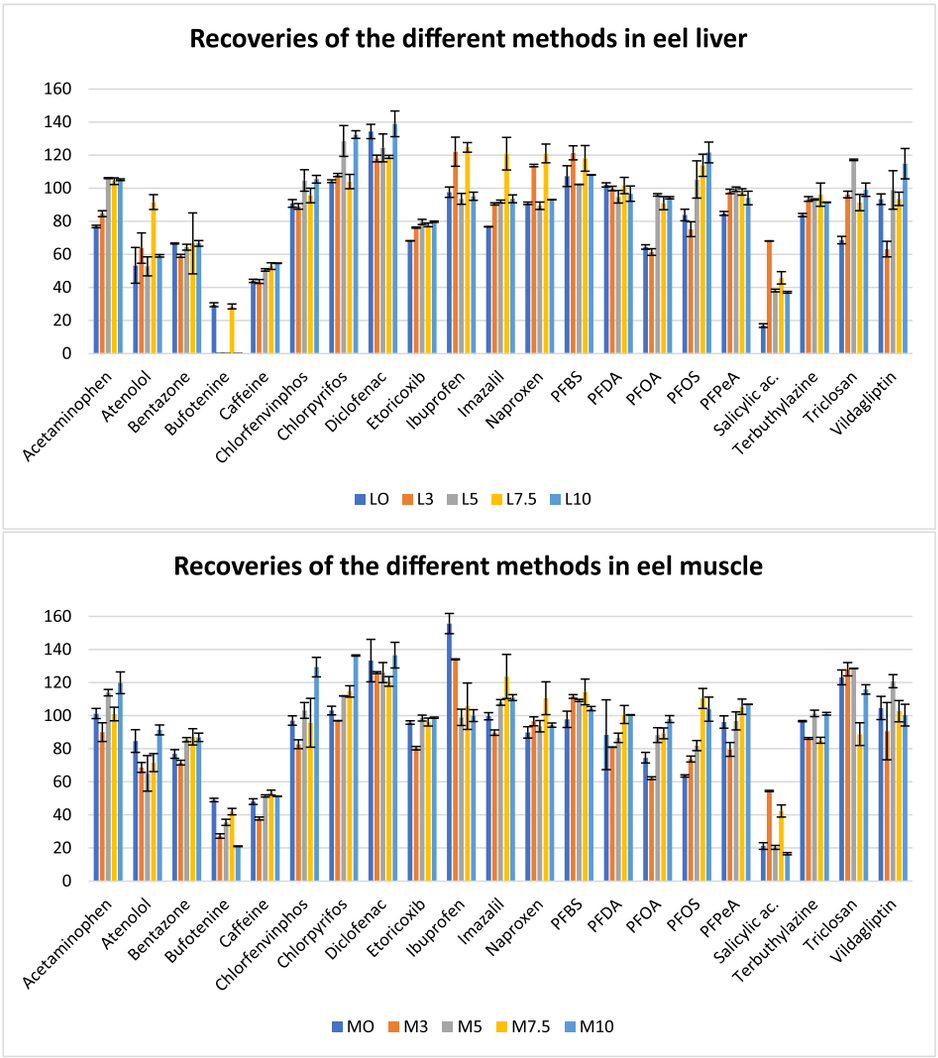

Fig. 1. Recoveries of the 5 methods with different addition of water tested ("L" for liver tissue and "M" for muscle with 0;3;5;7,5:10 ml of water). The compounds with internal standard are represented in RR%, while the rest of compounds are represented in E%. Error bars are set as ± SD.

calculated following Eq. (1).

$$RR\% \text{ or } E\% = \left(\frac{Final\ concentration\ of\ the\ spiked\ sample}{EC} \right) \cdot 100 \quad (1)$$

where EC is the expected concentration in the final extract assuming a recovery of 100%. For the determination of ME, 7 mixes with the calibration curve concentration levels (5, 10, 25, 50, 100, 200, and 500 ng/mL) were prepared in ACN. For each concentration, 300 µL of mix was placed in 15 mL falcon tubes and blown down to dryness under a gentle stream of nitrogen and they were redissolved adding 300 µL of extract (muscle or liver) from a non-spiked sample. This extract was then vortexed 30 s, sonicated 3 min and injected. After LC-MS/MS analysis using external calibration, ME

was calculated comparing the slope of the calibration curve in matrix and the slope of the calibration curve in ACN [9] (Eq. (2)).

$$ME = \left(\frac{Slope\ of\ calibration\ curve\ in\ matrix}{Slope\ of\ calibration\ curve\ in\ ACN} \right) \cdot 100 - 100 \quad (2)$$

The E% of the compounds without internal standard was corrected using the ME to obtain AR% using Eq. (3).

$$AR\% = \frac{E\%}{(100 + \mathrm{ME}\%)} \cdot 100 \quad (3)$$

Sensitivity was established as method limits of detection (LODs) and method limits of quantification (LOQs) (Table 2) by analysing the extractions fortified at 50 ng/g used for the recoveries described above. The extracts (performed in triplicate) were injected in duplicate (n = 6). LODs were set as three times the standard deviation (SD) of their signal and LOQs were set as 10 times the SD.

Due to the complexity of the matrices de ME was categorized as low ($\leq \pm$ 20%), moderate ($\pm$ 20–50%) and strong ($\geq \pm$ 50%). Results showed from low to moderate ME for most of the compounds, while just 6 and 5 compounds showed strong ME in liver and muscle, respectively (Fig. 2). Strong suppression of the signal was found for atenolol, salicylic acid, bufotenine, acetaminophen and vildagliptin in both matrices. On the other hand, imazalil presented strong signal enhancement, also in both matrices. The use of isotopically labelled internal standards helps to compensate any interference, such as those from ME (signal suppression/enhancement), hence improving accuracy and precision.

Precision was evaluated in terms of repeatability (Intra-*R*) and reproducibility (Inter-*R*). Intra-*R* was calculated as the SD of the signal divided by its mean (% RSDs) of the six injections used for the determination of LODs and LOQs injected in a row. Inter-R was determined injecting one replicate of the extracts fortified at 50 ng/mL also used for the recoveries described above in three different days (n = 3). Then Inter-R was also calculated as the SD of the signal divided by its mean (% RSDs).

Intra-R was satisfactory ($<$ 20%) except for bufotenine in liver (Table 2). Inter-*R* was also satisfactory ($<$ 30%) except for ibuprofen and, again, bufotenine in liver. In fact, bufotenine showed to be the compound with poorer recoveries and reproducibility. LODs for liver were in the range of 1.4–9.2 ng/g except for bufotenine (11.0 ng/g), while for muscle ranged 1.5-9.2 ng/g, except for triclosan (10 ng/g). In both cases, PFPeA presented LODs of 12.00 ng/g, however, since it was not detected in the 50 ng/g spiked sample it was calculated with 3 consecutive injections of the lowest point (5 ng/mL) of the calibration curve. As well as the LODs of atenolol and bufotenine (just for muscle samples), since they were not detected in the 50 ng/g samples either.

Examples of the chromatographic peaks obtained for extracts of spiked Eels at 100 ng g^{-1} of each compound are presented in Fig. 3.

In accordance with the European Commission Guidelines, the recoveries range within 70–120% are considered acceptable [2]. However, since E% might be affected by strong ME, according to the European Commission Guidelines, also the recoveries of 50–140% were considered "satisfactory", comparing the different methods prior validation for a (RSD $\leq$ 20%) of individual recoveries in routine analysis [2]. In this way, for the samples spiked at 500 ng/g, 18 and 20 compounds were recovered in the acceptable range (70–120%) for liver and muscle, respectively (Table 1). Both matrices presented 20 compounds in the satisfactory (50–140%) range. Samples spiked at 50 and 250 ng/g showed similar results to those from the samples spiked at 500 ng/g for most of the compounds, with slightly poor recoveries. Bufotenine, atenolol and caffeine were the compounds with poorer recoveries (Table 1).

Previous studies employing QuEChERS and EMR-Lipid dSPE in liver and muscle from *A. anguilla* have not been found. A similar method was developed by [7] for the determination of niclosamide in fish, including *A. Anguilla*. The study employed HPLC and LC-MS/MS and the QuEChERS method, combining extraction and cleanup in one step. Fat content of *A. anguilla* in that study was reported to be 20.86% . This high lipid content made niclosamide more difficult to extract from eel than the other fish, the fat content also contributed to strong ME. Another study by Peña-Herrera et al.

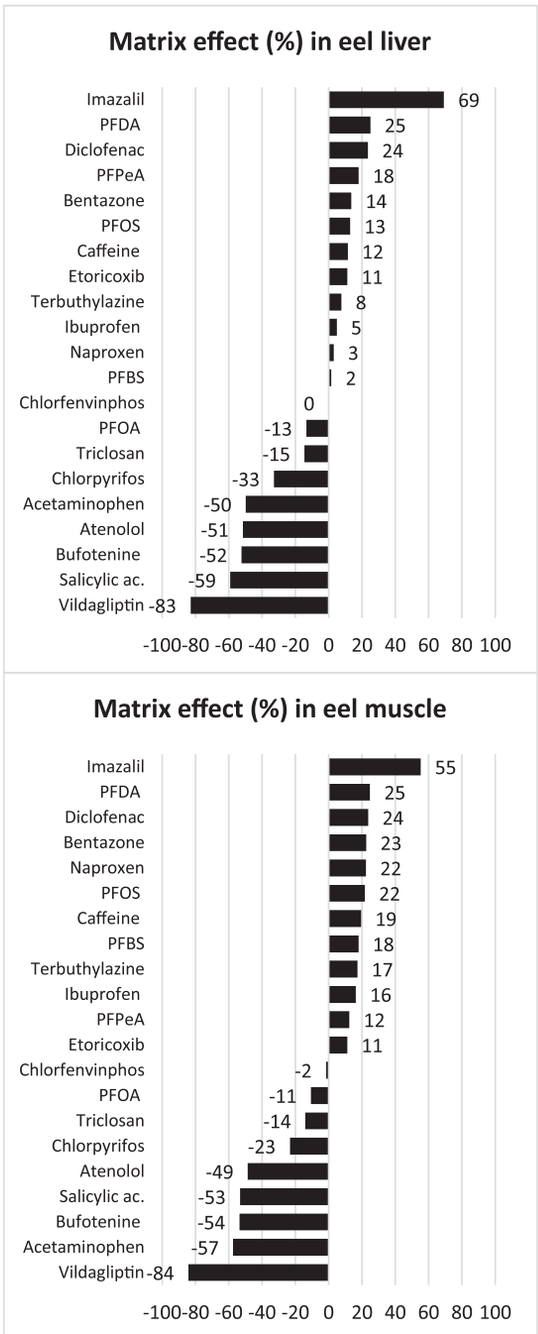

Fig. 2. Matrix effects of the validated method in eel liver and muscle.

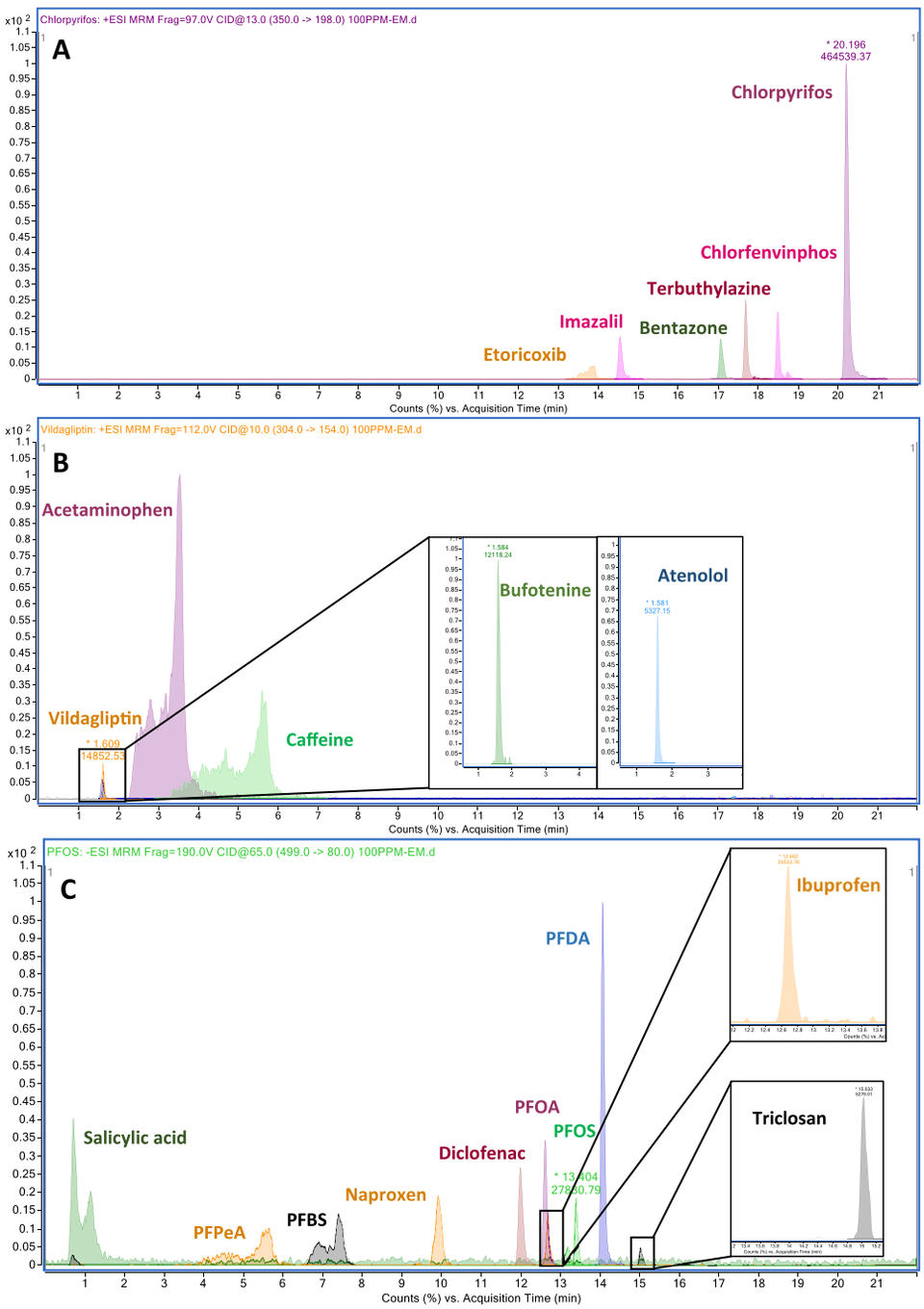

Fig. 3. Chromatograms of the compounds analysed in the present study in an extract employed for the calculation of matrix effect in eel muscle spiked at 100 ng/mL. (A) pesticides and etoricoxib analysed in positive mode employing Luna® column. (B) Rest of the compounds analysed in positive mode employing Kinetex column. (C) Compounds analysed in negative mode.

Table 1
Recoveries of the validated method in both eel liver and muscle matrix.

Recoveries (%)	Eel liver			Eel muscle		
	50 ng/g	250 ng/g	500 ng/g	50 ng/g	250 ng/g	500 ng/g
Acetaminophen	120	97	100	95	96	100
Atenolol	-	58	92	-	55	72
Bentazone	42	120	59	74	110	71
Bufotenine	69	23	60	-	-	90
Caffeine	46	64	47	50	51	45
Chlorfenvinphos	81	67	96	94	82	96
Chlorpyrifos	85	95	100	97	97	120
Diclofenac	93	96	120	110	96	120
Etoricoxib	90	88	70	90	89	86
Ibuprofen	140	120	120	61	81	91
Imazalil	71	77	71	55	77	80
Naproxen	100	140	120	77	79	90
PFBS	99	120	110	80	85	95
PFDA	89	92	100	100	98	100
PFOA	79	74	91	91	73	89
PFOS	110	110	110	110	90	110
PFPeA	-	120	83	-	110	94
Salicylic ac.	140	130	110	140	72	91
Terbuthylazine	91	97	89	75	88	73
Triclosan	79	120	110	110	130	100
Vildagliptin	110	89	94	81	98	100

[a] Bold files represent RR%, the other compounds are represented in AR%.
[b] "-" indicates that the compound was not recovered.

clean-up methods, where the EMR-Lipid dSPE yielded the best recoveries for 21 of 27 analytes and for the majority of the analytes recoveries were > 70%. The validated method was applied to natural riverine fish from the Evrotas river (Greece) and the Adige river (Italy) with positive findings for acetaminophen, propranolol, and venlafaxine reaching concentrations as high as 80 ng/g in muscle.

The validated method was tested in non-spiked samples from three different local markets, extracted by triplicate. The results showed that triclosan was detected in muscle of eels from one of the supermarkets with concentrations below the LOQs. While PFOA concentrations were also below the LOQs in muscle of eels from two markets.

In conclusion, the method validated in the present study successfully extracted a wide variety of compounds in eel liver and muscle. In the case of muscle, 20 target compounds were extracted in the range of 70–120, while liver presented 18 compounds in that range. This study in *A. anguilla species* showed to be a promising tool for future studies also in other organisms related to toxicology, metabolomics and occurrence monitoring of organic pollutants.

Further considerations and future perspectives

The study of complex matrices sometimes implies unusual behaviors; such was the case of the chromatograms of PFOS when extracted from liver. Next to the characteristic peak of PFOS (RT 13.4) a secondary peak (RT 14) was present in every liver sample, the shape of this peak was more or less constant in all the liver samples (Fig. S1). Despite both peaks were very close, they were not overlapped, allowing the correct determination of PFOS. The formation of branched isomers in biota samples, especially in the liver due to the presence of several enzymatic pathways, has already been reported [8]. Although further study would be required, this is the most probably explanation.

The QuEChERS method involves a partition between water and ACN by salting out. The proper partition of the contaminants will depend on the water volume. Originally, the QuEChERS method was developed for pesticides (from moderate to non-polar) in vegetables. Then, vegetables have a water content ranging from 75 to 82 % and the original amount of samples was 10 g. In this case, the addition of water to the QuEChERS was not recommended. In this study, as the eel amount is 1 g, the

Table 2
Validation of the selected method in terms of sensitivity (LODs, LOQs), Intra-*R* and Inter-*R*.

	Eel liver				Eel muscle			
	LOD (ng/g w.w.)	LOQ (ng/g w.w.)	Intra-R (% RSDs)	Inter-R (% RSDs)	LOD (ng/g w.w.)	LOQ (ng/g w.w.)	Intra-R (% RSDs)	Inter-R (% RSDs)
Acetaminophen	3.4	10.0	1.9	3.4	2.5	7.6	1.8	7.8
Atenolol*	9.2	28.0	5.8	3.6	9.2	28.0	5.8	17.0
Bentazone	4.7	14.0	6.5	25.0	8.9	27.0	6.5	11.0
Bufotenine*	11.0	34.0	31.0	30.0	5.9	18.0	4.2	8.4
Caffeine	1.6	4.7	2.1	7.3	1.5	4.6	1.7	5.3
Chlorfenvinphos	3.3	9.9	2.7	15.0	2.4	7.2	1.7	23.0
Chlorpyrifos	1.4	4.3	1.1	8.0	3.4	10.0	2.3	6.5
Diclofenac	3.2	9.7	2.3	1.5	2.3	6.9	1.3	4.7
Etoricoxib	5.2	16.0	3.4	2.1	5.3	16.0	3.5	5.7
Ibuprofen	4.0	12.0	1.8	32.0	5.2	16.0	4.8	27.0
Imazalil	3.4	10.0	1.9	24.0	4.8	14.0	3.7	26.0
Naproxen	3.0	9.0	1.9	20.0	7.6	23.0	5.4	18.0
PFBS	4.9	15.0	3.1	17.0	6.6	20.0	4.5	14.0
PFDA	2.9	8.8	2.2	9.6	2.8	8.3	2.1	11.0
PFOA	2.9	8.6	2.4	9.7	2.0	5.9	1.5	8.2
PFOS	4.5	13.0	2.8	21.0	5.6	17.0	3.4	11.0
PFPeA*	12.0	36.0	4.7	4.0	12.0	36.0	4.7	8.8
Salicylic ac.	7.6	23.0	8.5	16.0	6.7	20.0	6.6	18.0
Terbuthylazine	1.8	5.4	1.2	12.0	3.9	12.0	2.9	4.3
Triclosan	9.1	27.0	9.0	20.0	10.0	31.0	7.3	16.0
Vildagliptin	2.8	8.3	1.6	8.9	3.2	9.6	2.6	12.0

(a) *: LODs, LOQs and Intra-*R* were calculated using the lowest point of the linearity (5 ng/ml). Except bufotenine in eel liver.

amount of water would be 10 times lower than that present in the original method, so the addition of water is need for a proper salt partition. However, an excess of water could be disadvantaging for compounds highly soluble in water, such as pharmaceuticals, that are that might get dissolved in the water layer rather than in the can, such was likely the case of metformin in Álvarez-Ruiz et al. [4]. For this reason, different additions of water were tested in the present work, however no significant improvement was observed for the compounds tested.

A solution to improve the LOQs might be the concentration of the extract's prior injection. However, it may imply the concentration of possible residues (protein and lipids) present in the matrix, generating important matrix interferences and also reducing the life of the columns. For this reason, no concentration step was applied in this study.

Multi-residue extraction procedures for biota are relatively scarce. The development and optimization of new methods is crucial to move forward in the analysis of these complex matrices and the protection of the biosphere. The insight and questions provided by this study shows the need to keep researching to develop more efficient and sensitive methodologies.

Supplementary material *and/or* Additional information:

Supplementary material associated with this article can be found separately.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2021.101342.

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Supplementary Information

Determination of organic pollutants in *Anguilla anguilla* by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

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Table of contents

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Table S-1: External standards LC-MS/MS characteristics (transitions, retention time, and internal standard).

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Mass labelled	Fragmentor	Collision Energy
POSITIVE COMPOUNDS						
Etoricoxib 1	359	280	14		181	30
Etoricoxib 2	359	279	14		181	46
Chlorfenvinphos 1	359	155	18.4	Chlorfenvinphos-d10	120	10
Chlorfenvinphos 2	359	127	18.4	Chlorfenvinphos-d10	120	15
Chlorpyrifos 1	350	198	20.1	Chlorpyrifos-d10	97	13
Chlorpyrifos 2	350	97	20.1	Chlorpyrifos-d10	92	13
Vildagliptin 1	304	154	1.6	Vildagliptin-d3	112	10
Vildagliptin 2	304	91	1.6	Vildagliptin-d3	112	30
Imazalil 1	297	201	14.5		120	20
Imazalil 2	297	159	14.5		120	20
Atenolol 1	267	91	1.5	Atenolol-d7	91	57
Atenolol 2	267	77	1.5	Atenolol-d7	91	77
Terbuthylazine 1	230	174	17.6		97	13
Terbuthylazine 2	230	96	17.6		95	25
Bufotenine 1	205	160	1.5		98	14
Bufotenine 2	205	58	1.5		98	10
Caffeine 1	195	138	5.6		109	18
Caffeine 2	195	110	5.6		109	22
Acetaminophen 1	152	110	3.5	Acetominophen-d3	88	14
Acetaminophen 2	152	92	3.5	Acetominophen-d3	88	25
Bentazone 1	241	107	17		76	0
Bentazone 1	241	199	17		76	16
NEGATIVE COMPOUNDS						
Salicylic acid	137	93	0.7		86	10
PFDA 1	513	469	14	MPFDA[a]	89	5
PFDA 2	513	269	14	MPFDA	89	13
PFOS 1	499	99	13.4	MPFOS[b]	190	41
PFOS 2	499	80	13.4	MPFOS	190	65
PFOA 1	413	369	12.6	MPFOA[c]	87	5
PFOA 2	413	169	12.6	MPFOA	87	5
PFBS 1	299	99	7.4		142	38
PFBS 2	299	80	7.4		142	26
Diclofenac sodium 1	294	250	12	Diclofenac-d4	88	10
Diclofenac sodium 2	294	178	12	Diclofenac-d4	88	22
Triclosan 1	289	35	15		98	14
Triclosan 2	287	35	15		98	14

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Mass labelled	Fragmentor	Collision Energy
PFPeA 1	263	219	5.6		66	5
Naproxen 1	229	185	10		76	6
Naproxen 2	229	170	10		76	15
Ibuprofen 1	205	159	12.7		68	2

[a]MPFDA: Perfluoro-n-[1,2-^{13}C2]decanoic acid
[b]MPFOS: Sodium perfluoro-1-[1,2,3,4-^{13}C4]octanesulfonate
[c]MPFOA: Perfluoro-n-[1,2,3,4-^{13}C4]octanoic acid

Table S-2: Mass labelled standards LC-MS/MS characteristics.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Fragmentor	Collision Energy
POSITIVE COMPOUNDS					
Chlorfenvinphos-d10 1	369	101	18.4	109	32
Chlorfenvinphos-d10 2	369	170	18.4	109	56
Chlorpyrifos-d10 1	360	199	20.2	114	32
Chlorpyrifos-d10 2	360	99	20.2	114	32
Vildagliptin-d3 1	307	157	1.7	119	12
Vildagliptin-d3 2	307	93	1.7	119	32
Atenolol-d7 1	274	145	1.5	45	28
Atenolol-d7 2	274	79	1.5	45	24
Acetominophen-d3 1	155	111	3.1	96	14
Acetominophen-d3 2	155	65	3.1	96	34
NEGATIVE COMPOUNDS					
MPFDA 1	515	270	14	92	12
MPFDA 2	515	470	14	92	5
MPFOS 1	503	99	13.5	180	41
MPFOS 2	503	80	13.5	180	61
MPFOA 1	417	372	12.6	82	5
MPFOA 2	417	169	12.6	82	13
Diclofenac-d_4 1	298	254	12	15	10

S-3

Table S-3: Recoveries obtained for the two matrices (L: liver and M: muscle) adding different amounts of water.

Recoveries (%)																				
	Liver										Muscle									
	L0	*SD n=3*	*L3*	*SD n=3*	*L5*	*SD n=3*	*L7.5*	*SD n=3*	*L10*	*SD n=3*	*M0*	*SD n=3*	*M3*	*SD n=3*	*M5*	*SD n=3*	*M7.5*	*SD n=3*	*M10*	*SD n=3*
Acetaminophen	77	0.8	85	1.8	110	0.3	100	2.0	110	0.5	100	3.1	90	5.7	110	2.0	100	4.4	120	6.6
Atenolol	53	11.0	64	9.1	53	5.7	92	4.5	59	0.8	85	6.9	69	3.0	65	11.0	72	0.8	91	3.0
Bentazone	67	0.3	59	0.8	64	1.8	67	18.0	67	1.8	77	2.7	72	1.4	85	1.3	87	4.9	87	2.6
Bufotenine	30	1.2	-	-	-	-	29	1.5	-	-	49	1.1	27	1.4	36	1.8	42	10.0	21	0.2
Caffeine	44	0.9	44	1.2	51	0.7	53	2.0	55	0.1	48	1.7	38	0.9	51	0.7	54	1.4	51	0.2
Chlorfenvinphos	91	2.3	89	1.7	110	6.5	96	4.3	110	2.3	97	2.8	83	2.7	100	4.7	96	15.0	130	5.9
Chlorpyrifos	100	0.8	110	1.0	130	9.3	104	4.4	130	2.3	100	2.6	97	0.1	110	0.0	120	3.4	140	0.4
Diclofenac	130	4.4	120	2.0	120	8.4	119	0.9	140	7.8	130	13.0	130	0.6	130	6.1	120	2.9	140	7.8
Etoricoxib	68	0.1	76	0.4	80	1.6	78	1.1	80	0.4	96	1.1	80	1.0	99	1.8	96	2.4	99	0.4
Ibuprofen	98	3.2	120	8.8	94	3.2	120	2.9	95	2.5	160	6.1	130	0.3	99	4.9	110	0.1	100	3.6
Imazalil	77	0.2	91	0.7	92	0.9	120	9.9	94	2.4	100	2.1	90	1.7	110	1.7	120	13.0	110	1.7
Naproxen	91	0.7	110	0.8	89	2.2	120	5.7	93	0.1	90	3.5	97	2.7	94	3.4	110	9.9	94	1.3
PFBS	110	6.3	120	4.2	100	0.1	120	7.8	110	0.1	98	4.9	110	1.1	110	0.7	110	5.9	100	1.3
PFDA	100	1.3	100	1.3	95	3.8	100	4.9	97	4.6	88	21.0	81	0.0	87	2.9	100	5.5	100	0.1
PFOA	64	1.4	61	2.0	96	0.8	91	3.9	94	0.7	75	3.2	62	0.8	88	4.5	89	3.2	98	2.1
PFOS	84	3.5	75	4.5	110	11.0	110	6.7	120	6.3	64	0.7	74	1.9	82	3.0	110	6.0	100	7.3
PFPeA	85	1.3	98	1.3	99	1.4	98	2.0	94	4.1	96	3.7	80	4.2	97	5.5	110	4.6	110	0.1
Salicylic ac.	17	1.1	68	0.1	38	0.9	46	3.7	37	0.5	21	2.0	54	0.4	20	1.2	42	3.7	17	0.7
Terbuthylazine	84	0.9	93	1.4	93	0.4	96	7.1	91	0.2	97	0.4	86	0.5	100	1.9	85	1.8	100	0.8
Triclosan	69	2.2	96	2.0	120	0.4	91	4.9	99	4.1	120	4.5	130	4.0	130	0.2	89	6.4	120	2.8
Vildagliptin	93	3.2	63	4.7	99	11.6	94	4.1	120	9.2	110	7.0	91	17.0	120	4.0	100	6.3	100	6.5

a) Bold files represent RR%, the other compounds are represented in E%.

b) "-" indicates that the compound was not recovered

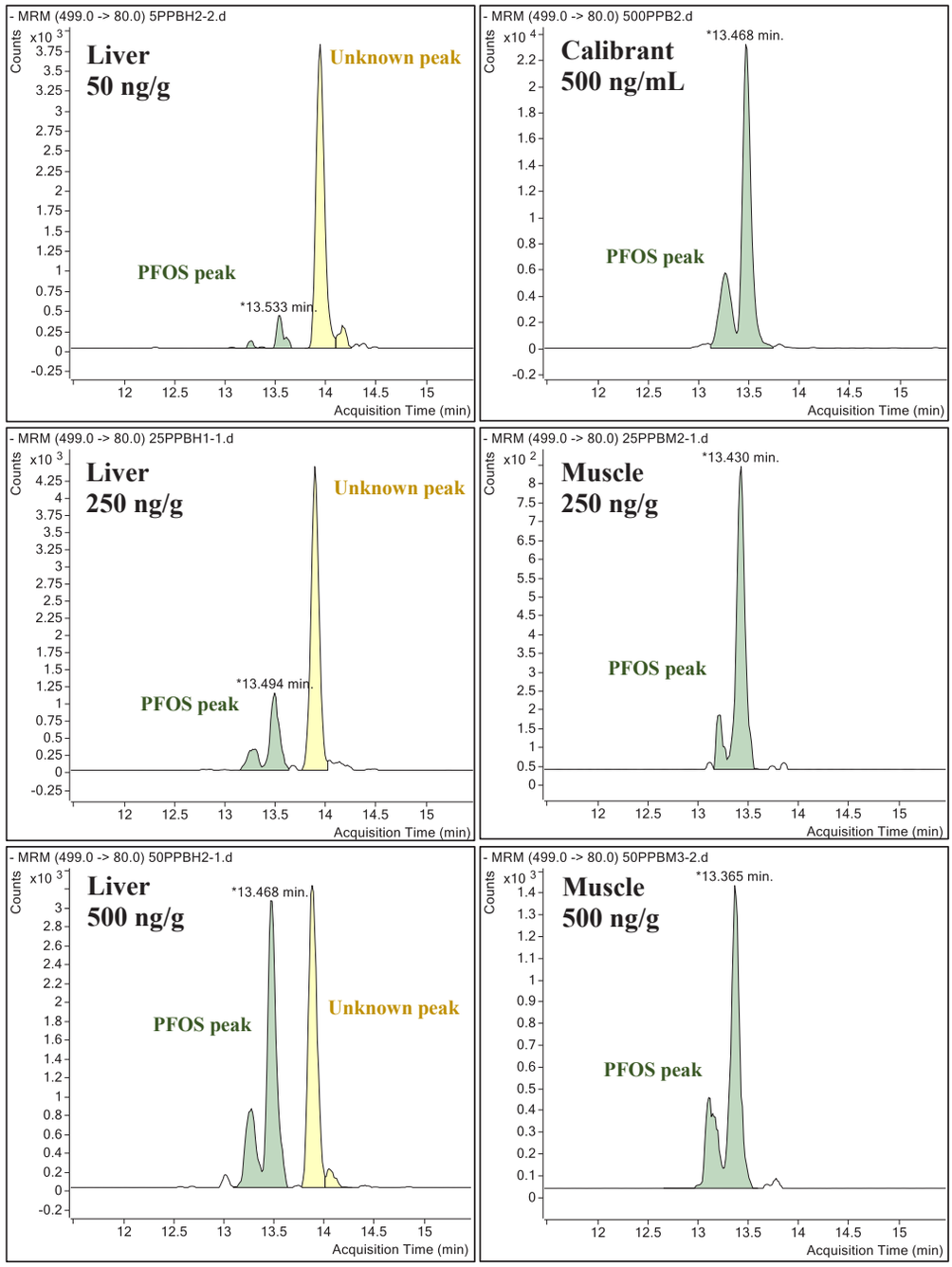

Figure S-1: *PFOS chromatograms in spiked eel liver and muscle, and the 500 ng/mL point of the calibration curve. Liver samples present a secondary peak at T ≈ 13.8 min. which is not a characteristic peak of the PFOS.*

S-5

Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography quadrupole time-of-flight-mass spectrometry

07

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Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography-quadrupole time-of-flight-mass spectrometry

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ABSTRACT

The aquatic ecosystems are dynamic environments often affected directly or indirectly by a myriad of anthropogenic contaminants that need to be properly identified. In this study, liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QqTOF-MS) suspected-screening was applied to mussels and riverine sediment both, non-spiked and spiked with a mixture of 32 pharmaceuticals. Three data acquisition methods —sequential window acquisition of all theoretical fragment-ion spectra (SWATH), in fix (FSWATH) and variable (VSWATH) window modes and Information Dependent Acquisition (IDA)— were compared to determine the most suitable acquisition technique. The results obtained in the spiked samples showed that the two SWATH modes enable to obtain the MS/MS spectrum of a higher number of compounds (up to 27 with FSWATH and 25 with VSWATH) than IDA (up to 19) in sediment and mussel. The different data acquisition modes were also tested in non-spiked samples to verify the results obtained in the spiked ones. Importantly, all the methods are able to detect the MS/MS spectrum of several contaminants in the samples when analysed against a database of >600 compounds. Up to 7 contaminants were tentatively detected with IDA, 15 with FSWATH and 17 with VSWATH. Most pollutants were pesticides and pharmaceuticals, being of particular interest the presence of ibuprofen and acetaminophen in mussels.

1. Introduction

The profiling of environmental pollutants has reached a new dimension through modern high-resolution mass spectrometry (HRMS). Quadrupole time-of-flight (QqTOF) and several combinations of the orbitrap with other mass analysers have achieved remarkable target, suspected-screening and non-target working strategies [1–3]. The suspected-screening expands the knowledge on the occurrence of pollutants in comparison to target analysis [1,4] by preliminary identifying a large number of them against a database. With the non-target screening, the MS and MS/MS spectra contribute information to establish the most probable elemental composition and to elucidate the structure through the study of the MS/MS fragmentation, enabling to discover unknown substances, including metabolites or degradation products [5,6]. In recent years, the traditional data acquisition in HRMS —necessarily complex— has evolved towards a wide range of modes to identify and quantify compounds that could be distinguished into Data (or Information) Dependent Acquisition (DDA or IDA) and Data Independent Acquisition (DIA), whose features have not been fully exploited yet. The former fix some characteristics of the precursor ion in the first quadrupole (Q1) of the QqTOF (e.g. given *m/z* value, minimum intensity threshold, etc.) to fragment it providing a TOF MS/MS (also known as MS/MS or product ion). Information obtained in the MS/MS is from specific precursor ions and this reduces the possible background noise and signal interferences because the other precursor ions are dismissed. This approach already showed prospects to identify and quantify environmental contaminants [6,7]. However, the disadvantage is the long cycle times required. Not all the relevant ions reach the isolation criteria along the cycle time, and they are not detected. This particularly occurs when complex matrices with a large number of other com-

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pounds are analysed. Contrarily, in DIA mode, no precursor ion is isolated, and information on the fragmentation of all ions present in the extract could be obtained. There are several DIA strategies, the most well-known is MS^E that simultaneously acquire low-energy and high-energy collision induced dissociation (CID) mass spectra. There are hundreds of examples using MS^E to determine contaminants in environmental matrices, such as water, sediments or fish [8–10]. The most important disadvantage is the loss of the direct relation between a precursor ion and its fragment ions, so resulting fragment ion spectra is chimeric and lack specificity. A more specific alternative strategy is the sequential window acquisition of all theoretical fragment-ion spectra (SWATH) [11,12], in which sequential MS/MS of multiple smaller acquisition windows is performed. This product ion spectrum is known as MS^{All} because the obtained spectrum can correspond to any of the precursors captured in the window or to all together if there are several ions and they fragment [13]. SWATH acquisition can be performed using fix windows (FSWATH) or variable windows (VSWATH) [14]. In the first one, the size of the *m/z* Q1 windows is uniform along all the LC–MS run, but in the second one, the *m/z* windows is variable depending on the density of precursor ions (narrower if ion density is high and wider if it is low) [15]. The VSWATH should improve the quality of the data acquired because it adjusts the isolating window to the number of precursor ions within the window [16] and the relationship between the mass window range and the results obtained has been verified [17]. However, not all the MS/MS data acquired with SWATH is useful since it can be from several precursor ions not all of them of interest, consequently, some fragments of other precursors could interfere with the signal of the analyte of interest, being the MS/MS data of inferior quality than IDA, as seen in previous works [18,19]. However, both, F and V SWATH are not commonly implemented yet and the few papers already published are mostly focus on proteins [20–24]. Few applications are focused on small molecules as peptides or metabolites or even hormones in the environment [25,26]. Furthermore, little information is available on its effectiveness in comparison to IDA and even less on the comparison between F and V SWATH [19].

In this study, three different acquisition methods IDA, FSWATH, VSWATH have been compared in complex environmental samples to establish the technique that is able to identify more contaminants (providing MS and MS/MS data). As model compounds, 32 pharmaceutical and personal care products selected for their incidence in the environment [27–29] and two complex environmental matrices —riverine sediment and mussel— were selected to study the ability of different modes (IDA as well as F and V SWATH) working in both, positive and negative ionization. Furthermore, the three acquisition modes were also tested in non-spiked samples in order to ascertain their ability to identify a wide range of compounds. Up to our knowledge, this is the first report comparing the suitability of these three modes, which are widely used in the detection of emerging pollutants in environmental matrices, as shown before. This workflow helps to establish the best acquisition method to obtain as much information as possible about the emerging pollutants present in environmental matrices, providing important knowledge for future research.

2. Material and methods

2.1. Reagents, materials and sample extraction

The reagents and the analytical standards were of high purity. Methanol (MeOH) LC–MS PAI ≥ 99.8% purity, acetonitrile ≥ 99.9% purity, dichloromethane 99.8% purity and trisodium citrate dihydrate ($C_6H_9Na_3O_9$) were distributed by VWR Chemicals® (Radnor, Pennsylvania). Citric acid ($C_6H_8O_7$) and Na_2EDTA (EDTA) were distributed by PanReac AppliChem (Barcelona, Spain). Disodium monohydrogen phosphate (Na_2HPO_4) and ammonium fluoride (NH_4F) were from Scharlab S.L. (Barcelona, Spain), magnesium sulphate ($MgSO_4$) and disodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5H_2O$) from Alfa Aesar (Karlsruhe, Germany), and sodium chloride (NaCl) from Sigma-Aldrich (Steinheim, Germany). Formic acid (CH_2O_2) was provided from ACROS ORGANICS (Geel, Belgium). Finally PSA and C18 sorbents, were from Análisis Vínicos S.L. (Tomelloso, Spain). High purity water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). The McIlvaine-EDTA buffer was prepared mixing 100 mL of citric acid 0.1 M solution and 62.5 mL of disodium hydrogen phosphate 0.2 M solution, then, adjusting the pH of the solution to 4 with HCl and, finally, adding 6.05 g of EDTA.

The high purity grade (>95%) analytical standards acetaminophen, salicylic acid, alprazolam, atenolol, atorvastatin, bezafibrate, bisphenol A, butylparaben, caffeine, chloramphenicol, clofibric acid, codeine, enalapril, diclofenac sodium, ethylparaben, flufenamic acid, furosemide, hydroxyibuprofen, ibuprofen, imazalil, indomethacin, lorazepam, methylparaben, metformin, nitenpyram, norfloxacin, omeprazole, propylparaben, simvastatin, thiamphenicol, tramadol, triclocarban, triclosan and warfarin, from Sigma-Aldrich (Steinheim, Germany).

The lyophilizer used to dry the sediments was a 4KBTXL-75 by VirTis SP Scientific of SP Industries (Philadelphia, USA). The 15 mL and 50 mL falcon plastic tubes were purchased at VWR® International Eurolab (Barcelona, Spain). The SPE equipment was a VISIPREP™ distributed by Supelco (Madrid, Spain). The ultrasound system used was an Elmasonic S120H manufactured by Elma® (Germany). The SPE was carried with Strata-X Polymeric Reversed phase 200 mg/mL cartridges by Phenomenex (Torrance, CA, USA). The evaporation of the samples was made using a combined sample concentrator and heating plate, the concentrator model was SBH-CONC/1 and heating plate model was SBH130D/3 both provided by Stuart® (Stafford, United Kingdom). The 2 mL amber glass vials with stoppers 99 mm + Septum Sil/PTFE used to inject the samples were manufactured by Análisis Vínicos S.L. (Tomelloso, Spain).

The fragmentation or not as well as differences in the MS/MS data related to their concentrations in the samples were assessed analysing spiked samples at four different concentration levels 10, 25, 50 and 100 ng/g (d.w. for sediment and w.w. for mussels), spiked with 10, 25, 50 and 100 μL of the standard mixture at 1 μg/mL. These samples were identified as S10, S25, S50 and S100 for sediment and M10, M25, M50 and M100 for mussels. In the spiked samples, the sediment was the same for all concentration levels as well as the mussels were all bought the same day in the same store. All the spiked samples were analysed in quintuplicate.

2.2. Sample extraction

The sediment samples were from the Turia River basin in Valencia (Spain), lyophilized and store at -20 °C until the extraction. The extraction method was an ultrasound assisted extraction (UAE) with McIlvaine-EDTA buffer flow SPE already described as a suitable extraction method for pharmaceuticals in solid environmental matrices [27]. Briefly, 1 g of sample was placed in a 50 mL falcon tube and spiked with the appropriate volume of the external standard depending on its group (10, 25, 50 or 100), and then 5 mL of MilliQ water, 5 mL of MeOH and 5 mL of McIlvaine-EDTA buffer were added. The solution was shacked in a vortex for 5 min and sonicated for 10 min, then was centrifuged 6 min at 1811 rcf. The supernatant was placed in a 200 mL volumetric flask and filled with MiliQ water. The 200 mL extract was passed through the previously conditioned (6 mL MeOH + 6 mL water) Strata-X cartridges (10 mL/min) under vacuum. Cartridges were washed with 6 mL of MilliQ water and dried for 15 min under vacuum. Then,

analytes were eluted with 6 mL of MeOH followed by 3 mL of MeOH-dichloromethane solution (1:1, v/v) at gravity flow. This eluent was evaporated to dryness with a gentle constant N_2 stream at 42 °C, reconstituted in 1 mL of water- MeOH (7:3, v/v) by sonication for 3 min, placed in 2 mL vial and stored at -20 °C until the analysis.

The Mediterranean mussels (*Mytilus galloprovincialis*) from the Mediterranean Sea were obtained in a commercial store in Valencia (Spain) and stored at -20 °C in the laboratory until the extraction. Mussels were extracted using QuEChERS [30–32], this procedure was slightly modified as follows. The shells of the mussels were removed and 10 g of their visceral mass were placed in a 50 mL falcon tube, then 10 mL of acetonitrile were added and the tube was vigorously shaken for 3 min. A mixture of 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of $C_6H_6Na_2O_7 \cdot 1.5H_2O$ and 1 g of $C_6H_9Na_3O_9$ was added to the tube, then it was vigorously shaken for 3 min and centrifuged at 2465 rcf for 3 min. A 15 mL falcon tube was prepared with 150 mg of $MgSO_4$, 50 mg of PSA and 50 mg of C_{18}. Then, 1.5 mL of the supernatant was added to the tube that was vigorously shaken for 30 s and centrifuged for 1 min at 2465 rcf. The supernatant was placed in 2 mL amber glass vial and store at -20 °C until analysis.

To verify the results obtained with the spiked samples, non-spiked samples were also analysed. These samples were analysed by triplicate unlike the spiked ones.

2.3. Liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QqTOF-MS)

The samples were analysed with a ultra high pressure liquid chromatograph (UHPLC) model Agilent 1290 Infinity, manufactured by Agilent (Santa Clara, USA), in tandem with a TripleTOF™ 5600 LC/MS/MS System manufactured by AB SCIEX (Madrid, Spain). The analytical column used was an Acquity UPLC BEH C18, 130 Å, 1.7 μm (2.1 x 5 mm), distributed by Waters (Massachusetts, USA). The mobile phase used in the positive ionization mode was H_2O 0.1% formic acid (A) and MeOH 0.1% formic acid (B) at flow rate 0.4 mL/min. The linear gradient was as follows: 0 min (70% A), 10 min (15% A), 15 min (2% A), 15.5 min (70% A) and 25 min (70% A). The mobile phase for the negative ionization mode was H_2O 2.5 mM NH_4F (A) and MeOH 2.5 mM NH_4F (B) at a flow rate 0.2 mL/min. The linear gradient was as follows: 0 min (70% A), 12 min (5% A), 20 min (5% A) and 32 min (70% A). In both cases the injection volume was 10 μL.

MS (TripleTOF 5600, ABSciex) operated in positive and negative ionization modes with a DuoSpray ion source at a resolving power [full width at half-maximum (FWHM) at *m/z* 400] of 30,000 in MS and 30,000 in MS/MS (high-resolution mode). The automated calibration device system (CDS) was set to perform an external calibration every six samples. The source conditions were as follows: temperature, 450 °C; curtain gas (CUR), 30 psi; ion source gas (GS) 1 and 2 at 45 psi; and the ion-spray voltage floating (ISVF) at 5.5 kV in positive ionization and -4.5 kV in negative (as recommended by the manufacturer). All MS parameters were controlled by Analyst™ Software 1.6 (ABSciex). Data were processed with PeakView® 1.2 and Multi Quant 2.1 software (ABSciex).

2.4. IDA and SWATH settings

The acquisition using IDA consisted of a full scan and information-dependent trigger events. The accumulation time for full scan was 100 ms for scanning a mass range from *m/z* 100 to *m/z* 750 at CE of 10 eV. The accumulation time for each IDA experiment was 100 ms, and the CE was set to 45 eV with a CE spread of 15 eV in high resolution mode. IDA criteria were as follows: 6 most intense ions (number of IDA experiments) with an intensity threshold above 100 cps, isotope exclusion was switched off, and an exclusion time of 6 s (half peak width) was set. Dynamic background subtraction was switched on. Each cycle was 750 ms long.

The acquisition using FSWATH consisted of the same full scan as IDA but with an accumulation time of 50 ms, followed by several MS/MS windows that covered a mass range of *m/z* 100 – 750 with a 25 Da window width for Q1 isolation (overlap 1 Da). Each SWATH window had an accumulation time of 40 ms, using a CE value of 45 eV with a CE spread of 15 eV in high resolution mode. The FSWATH has 26 widows and each cycle takes 1400 ms.

The acquisition using VSWATH consisted also of a full scan as for FSWATH, and several MS/MS windows that cover a mass range of *m/z* 100–750 with variable window width for Q1 isolation (overlap 1 Da). The different windows were obtained with the Variable Window Calculator tool that enables to build an optimized variable window SWATH™ acquisition. The calculator scales the window size across the *m/z* range depending on the number of precursors. That is, Q1 window widths are varied over the *m/z* range so, each one contains a constant density of precursor from an intensity weighted histogram of the survey scan data vs *m/z*. The maximum number of windows was established in 30 and the minimum *m/z* in 5 Da with an overlap of 1 Da. Each SWATH window had an accumulation time of 40 ms, using a CE value of 45 eV with a collision energy spread of 15 eV in high-resolution mode. The variable window was optimized for both sediment and mussels.

2.5. Compound identification

The obtained data were analysed using the PeakView® 1.2 software. A special database within the extracted ion chromatogram (XIC) manager with only the information of the 32 spiked compounds was used to identify the pharmaceuticals present in the spiked samples and process them more rapidly. A more extensive database containing information on >600 compounds was used to identify other compounds present in non-spiked samples. The more extensive databases take more time to process the samples.

The linearity of the 32 compounds prepared with analytical standards in MeOH was injected using LC-QqTOF-MS and the three different acquisition techniques in order to compare the chromatograms with those obtained from spiked sediment and mussels. The compounds were identified by the retention time (t_r), MS (error < 5 ppm) and MS/MS (match of at least 2 ions). However, to compare the different acquisition modes, the ability to obtain the MS/MS spectrum was considered as the most relevant parameter and the quality of the MS/MS spectrum was evaluated. For positive identification, a criteria similar to that proposed by Roemmelt et al. [19] was used: at least two matching fragments must be present with an abundance above 10% and mass shift to the exact mass must be < 5 ppm.

The MS/MS spectra of the non-target compounds detected in the non-spiked samples by the acquisition software was compared visually with the experimental spectra present in the METLIN database, available online, to establish their tentative identity. Identity always that possible was confirmed using the analytical standards. Otherwise, it was always considered only as tentative.

3. Results and discussion

3.1. Pollutants detected

The data obtained with the different acquisition methods were compared to a self-made library that includes the 32 pharmaceuticals (Table S-1). The MS TOF used as survey scan gave information on the accurate *m/z* of analytes in the spiked samples. However, the analytes are considered as tentatively detected (TD) only in those cases in which the characteristic MS/MS of the compound

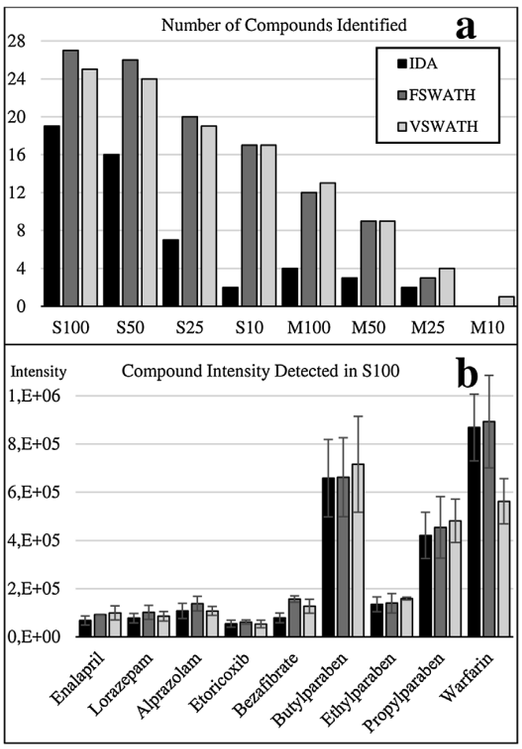


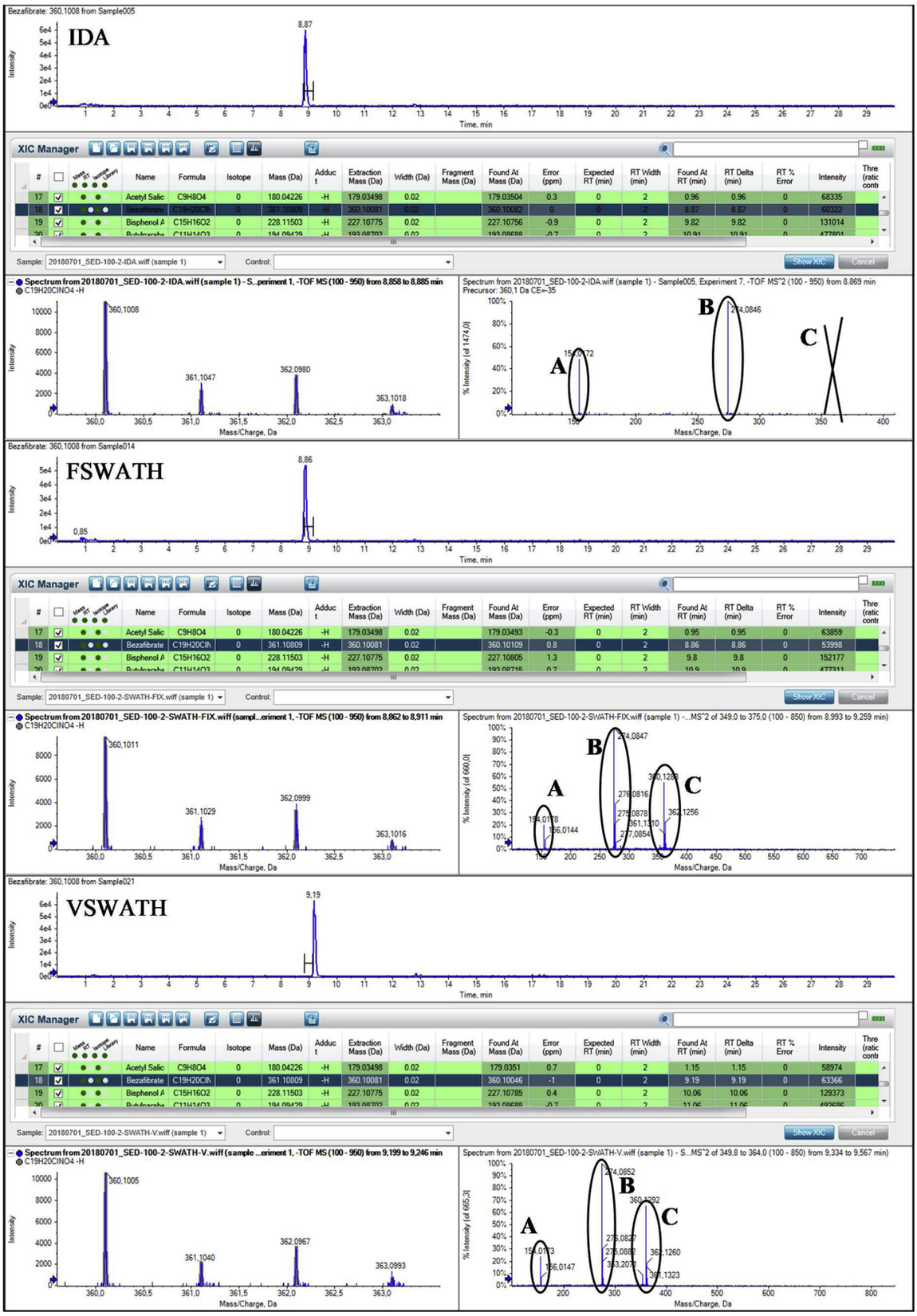
Fig. 1. a. Compounds detected and confirmed in the spiked samples with IDA, FSWATH and VSWATH acquisition. b. Average intensity of several of the detected compounds in the S100 spiked sample.

was obtained. The number of TD compounds were the same in every one of the 5 replicates. Up to 19 of the 32 pharmaceuticals were TD in sediment and up to 4 in mussels with the IDA acquisition (Tables S-3and S-4). In the case of the FSWATH acquisition, up to 27 pharmaceuticals were TD in sediment and up to 12 in mussels (Tables S-5 and S-6). Finally, with the VSWATH acquisition, up to 25 compounds were TD in sediment and 13 in mussels (Tables S-7 and S-8). The compounds, metformin, norfloxacin, omeprazole and salicylic acid were not TD in samples by any acquisition method. However, all the analytes were TD by the three data acquisition modes in the standards prepared in MeOH that do not have matrix interferences. As is shown in Table S-2, more compounds were TD in all the samples using the SWATH acquisition techniques, but even SWATH was not able to TD all the compounds in complex matrices. In the case of mussels, two compounds —acetaminophen and caffeine— were detected with IDA but not with SWATH. This could be due to the high intensity of precursor ion but low intensity of the fragments that are hindered by those of the other compounds in SWATH. In sediment samples, the compounds TD with IDA were also detected with both SWATH acquisition techniques, excepting tramadol that was TD only with FSWATH in one sample. The difference in detection capability between IDA and SWATH is more significant for the compounds with negative ionization, ranging from almost double number of analytes TD by SWATH in some sediment samples to five fold more analytes in some mussel samples.

According to the overall results (Fig. 1), the SWATH acquisition techniques are suitable to TD more compounds than IDA. In some samples analysed with IDA, the MS/MS spectrum of a number of compounds was not obtained, even through the chromatographic peak of the compound was clearly distinguishable, the t_r matched with the standard, the precursor ion *m/z* had errors < 5 ppm and the background noise was practically imperceptible. The lack of MS/MS spectrum could be due to the IDA acquisition workflow that provides only relevant information of the precursor ions isolated (restricted to the six most intense signals along each cycle). Then, although dynamic exclusion was used, the process could end up without TD some relevant ions because they are not included within the 6 most intense and are discarded for fragmentation. Contrarily, SWATH gets information on the MS/MS fragmentation of all ions present in a narrow *m/z* window. These results in higher number of analytes TD but also in a higher number of fragments that could be from other precursor ions co-existing with that of the analyte of interest, thus, some of the information provided by SWATH could not be useful and the user has to discriminate the relevant information. Overall, the quantity of relevant information provided with both SWATH techniques is higher than with IDA because it is able to TD more contaminants in the studied matrices. As can be observed in Fig. 1a, equal number of compounds provided MS/MS in all the replicates performed at the same concentration. Then, there is not variability in the number of compounds detected. This result is logical since the same matrix and the same concentration of analyte are used. Fig. 1b shows the variation of the intensity of several peaks among the replicates for the three techniques (as standard deviation), values are always < 30% of the average intensity showing a narrow range of variability that indicates appropriate reproducibility of the method. These results are in accordance with other studies, carried out with complex matrices demonstrating that IDA missed some analytes comparing to the SWATH [18,19]. The best accuracy of SWATH would reduce the number of false positive or negative results in the emerging pollutants analysis [17].

The results obtained with FSWATH and VSWATH were similar, but with slight differences between them. The FSWATH results were better than the VSWATH for basic compounds in sediment samples (Fig. S-1), but for the acidic ones, the number of TD compounds was the same. In mussels is difficult to establish the best SWATH acquisition method, being FSWATH better only to TD basic compounds in the M100 sample. For the other samples, the VSWATH gave equal or better results (Fig. S-1). It was expected that the VSWATH gave better results in mussels, because the variable window workflow was design to optimize sample acquisition with complex matrices, but not big differences were observed for the samples and compounds analysed.

Several previous studies have determined pharmaceuticals in mussels using HRMS (either LC-QqTOF-MS [33,34] or orbitrap-[35–37]). The data acquisition in those methods was full scan and MS^E-based which enables an additional confirmation of the compound to be detected but only using a pseudo MS/MS. Most of these methods are restricted to search of metabolites and/or degradation products that keep some structural similarity with the parent compounds that make them easily identifiables. Few of them also identify compounds extracting peaks with the help of large database using system in Full MS/IDA MS/MS (full-scan data-dependent MS/MS) mode [36] or in full MS/DIA (full scan data independent acquisition) [37]. These studies do not report the ability of each data acquisition mode to get information on the contaminants present vs the matrix co-extractants. Other studies also developed methods to confirm the presences of pharmaceuticals in sediments by TOF [38] or orbitrap [39] using IDA. Neither, the study of Jelić et al. [40] or that of Nanou et al. [39] showed the results of the non-target IDA analysis. Consequently, the number of compounds that are able to detect by the MS/MS spectra and the number of compounds that could remain undetected and how this can be modified testing other data acquisition methods cannot be compared. Then, our study provides complementary information to improve the knowledge about the capabilities of the different acquisition methods and better fix the


Bezafibrate: 360,1008 from Sample005
IDA
8,87
Time, min
XIC Manager
Sample: 20180701_SED-100-2-IDA.wiff (sample 1)
Control:
Show XIC
Cancel
Spectrum from 20180701_SED-100-2-IDA.wiff (sample 1) - S...periment 1, -TOF MS (100 - 950) from 8,858 to 8,885 min
C19H20ClNO4 -H
360,1008
361,1047
362,0980
363,1018
Mass/Charge, Da
Spectrum from 20180701_SED-100-2-IDA.wiff (sample 1) - Sample005, Experiment 7, -TOF MS^2 (100 - 950) from 8,869 min
Precursor: 360,1 Da CE=-35
% Intensity (of 1474,0)
A
B
C
274,0846
Mass/Charge, Da
Bezafibrate: 360,1008 from Sample014
FSWATH
8,86
0,85
Time, min
XIC Manager
Sample: 20180701_SED-100-2-SWATH-FIX.wiff (sample 1)
Control:
Show XIC
Cancel
Spectrum from 20180701_SED-100-2-SWATH-FIX.wiff (sampl...eriment 1, -TOF MS (100 - 950) from 8,862 to 8,911 min
C19H20ClNO4 -H
360,1011
361,1029
362,0999
363,1016
Mass/Charge, Da
Spectrum from 20180701_SED-100-2-SWATH-FIX.wiff (sample 1) -...MS^2 of 349,0 to 375,0 (100 - 850) from 8,993 to 9,259 min
% Intensity (of 660,0)
A
B
C
274,0847
276,0816
275,0878
277,0854
361,1310
362,1256
Mass/Charge, Da
Bezafibrate: 360,1008 from Sample021
VSWATH
9,19
Time, min
XIC Manager
Sample: 20180701_SED-100-2-SWATH-V.wiff (sample 1)
Control:
Show XIC
Cancel
Spectrum from 20180701_SED-100-2-SWATH-V.wiff (sample ...eriment 1, -TOF MS (100 - 950) from 9,199 to 9,246 min
C19H20ClNO4 -H
360,1005
361,1040
362,0967
363,0993
Mass/Charge, Da
Spectrum from 20180701_SED-100-2-SWATH-V.wiff (sample 1) - S...MS^2 of 349,8 to 364,0 (100 - 850) from 9,334 to 9,567 min
% Intensity (of 665,3)
A
B
C
274,0852
276,0822
275,0883
277,0857
361,1260
362,1292
363,1323
Mass/Charge, Da

scope of these techniques when applied to highly complex matrices.

3.2. MS and MS/MS spectra differences

As an example, Fig. 2 shows the results using IDA, FSWATH and VSWATH for bezafibrate in the 100 ng/g d.w. spiked sediment where the MS/MS spectra showed the two clearly distinguishable fragments characteristics of bezafibrate (the match to the analytical standard as well as the MS/MS spectra of the Metlin database was checked). This compound presented almost the same behaviour by the three fragmentation modes. However, the MS/MS data for SWATH acquisition provided more products ions due to SWATH ability to fragment all the isotopes of the precursor ion [bezafibrate has one chlorine atom then ^{35}Cl (100% intensity) and ^{37}Cl (33%)] because not only one ion but a short *m/z* window is selected. Information on isotopic pattern helps to make a better identification. For many compounds, the MS^{All} spectra presents these isotopic fragments. In the IDA acquisition, only one precursor ion is selected and fragmented, then the MS/MS data loses the isotopic abundance.

For some analytes signal intensity and, thus, sensitivity also changes between the IDA and SWATH methods. Most compounds gave better sensitivity with SWATH than with IDA, particularly in the cases of enalapril, etoricoxib, chloramphenicol, flufenamic acid, ethylparaben and warfarin. This could be due to the use of a wider window.

One of the problems of SWATH acquisition is that frequently some fragments do not match the spectra of the compound obtained with individual analytical standards, as shown in Fig. 3. To determine how the quality of the spectra is affected by the presence of these non-matching fragments, a slightly modified version of the quality index of the MS/MS spectra (QLS) established by Zhu et al. [18], that attain the comparison between MS/MS spectra of a compound obtained using different acquisition methods.

The QLS compares the quality of the MS/MS spectrum obtained by any of the three data acquisition methods for each compound, each matrix and each concentration level. The MS/MS spectrum of each analyte spiked at a given concentration in the sample by three different acquisition methods is compared to the reference standard counting the number of products ions that match those of reference standard: k, m or n for IDA, FSWATH or VSWATH, respectively. This index determines the ratio between the lowest number of products ions (X) that match those of the analytical standard in any of three MS/MS spectra (IDA, FSWATH or VSWATH) and how many of these characteristics products ions are between the X most intense of each mass spectrum (Y). Then, the next formula is applied:

$$QLS = Yif(X \geq 10)or(Y/X)x10if(X < 10)$$

Where X is the smaller number among k, m or n and Y is the number of fragments between the top 10 ($X \geq 10$), or top X ($X < 10$), of higher intensity ions of the spectrum matching the reference standard. With this formula a QLS for each compound at a given concentration by each acquisition method is obtained. The QLS of each method at a given concentration will be the average of the QLS values of the compounds detected.

This index is a number between 0 and 10 that do not have units of measurement. A spectrum with a QLS of 10 would match all its fragments (or the 10 more intense if there are more than 10 fragments) with those of the reference spectrum (that of the standard prepared in MeOH that do not have significant interferences). While a lower value would mean the presence of fragments (among the most intense) that do not match with the reference spectrum. The lower the QLS is, the higher the number of interferences present. A QLS of 0 means the 10 most intense fragments of the mass spectrum obtained do not match with any fragment of the references spectrum.

Table 1
QLS of the different acquisition methods.

		Sediment	Mussel
100 μl	IDA	9.26	–
	FSWATH	6.65	7.69
	VSWATH	6.82	7.69
50 μl	IDA	9.17	–
	FSWATH	6.47	6.56
	VSWATH	6.47	7.19
25 μl	IDA	8.00	–
	FSWATH	7.67	–
	VSWATH	5.67	–

Because the QLS compares different methods, in sediment, data analysed were only of the compounds detected with the three acquisition methods (IDA, FSWATH and VSWATH) at any concentration. In order to obtain a representative average, those concentration levels that provided the MS/MS spectra for less than 5 compounds for the three acquisition modes (as the 10 ng/g level in sediment) were discarded. In mussels, data were not enough (MS/MS < 5 compounds) to apply the QLS to the IDA acquisition method, so only FSWATH and VSWATH were compared as in sediment. Table 1 shows that the QLS is better in IDA than in SWATH, which agrees with previous works [18,19]. There are not significant differences between both SWATH methods at high concentrations, but the results of the 25 μg/g could indicate that FSWATH has better performance at low concentrations than VSWATH.

Summarizing, the spectra obtained in IDA has better quality than the obtained in SWATH, but the SWATH method provides more fragments for the identification of the compounds.

3.3. Application to environmental samples

Several non-spiked samples were also analysed by IDA, FSWATH and VSWATH acquisition to verify the applicability of the method. Contaminants were identified using a self-made library with more than 600 compounds including pharmaceuticals and pesticides. All the compounds with analytical standard available in the laboratory were confirmed and quantified, all the confirmed compounds had a LOQ of 10 ng/g and a relative standard deviation (RSD) <20%, showing a good repeatability. The results in Table 2 show that higher number of compounds were detected with SWATH than with IDA. This supports the results obtained with the spiked samples. Again, there are slightly differences between the FSWATH and VSWATH, but not enough to establish the best acquisition method.

The Fig. 3 shows the differences in the spectra of the three data acquisition methods for imazalil TD in sediment samples. IDA shows less fragments that match with the reference spectrum (because this compound has two chlorine atoms and only one isotope is fragmented) than FSWATH (in which all chlorine isotopes are fragmented without selection). However, FSWATH has several fragments with high intensity that do not match with the reference spectrum. In this case, contrarily, VSWATH shows only a single matching fragment of imazalil. There are several potential explanations to this anomalous result. One of them is the presence of co-extractives that fragment in the same window providing

Fig. 2. 100 μL sediment sample Bezafibrate data obtained with IDA, FSWATH and VSWATH acquisition. "A", "B" and "C" are the respective fragments present (or not) in the spectra, that match with the reference spectra.

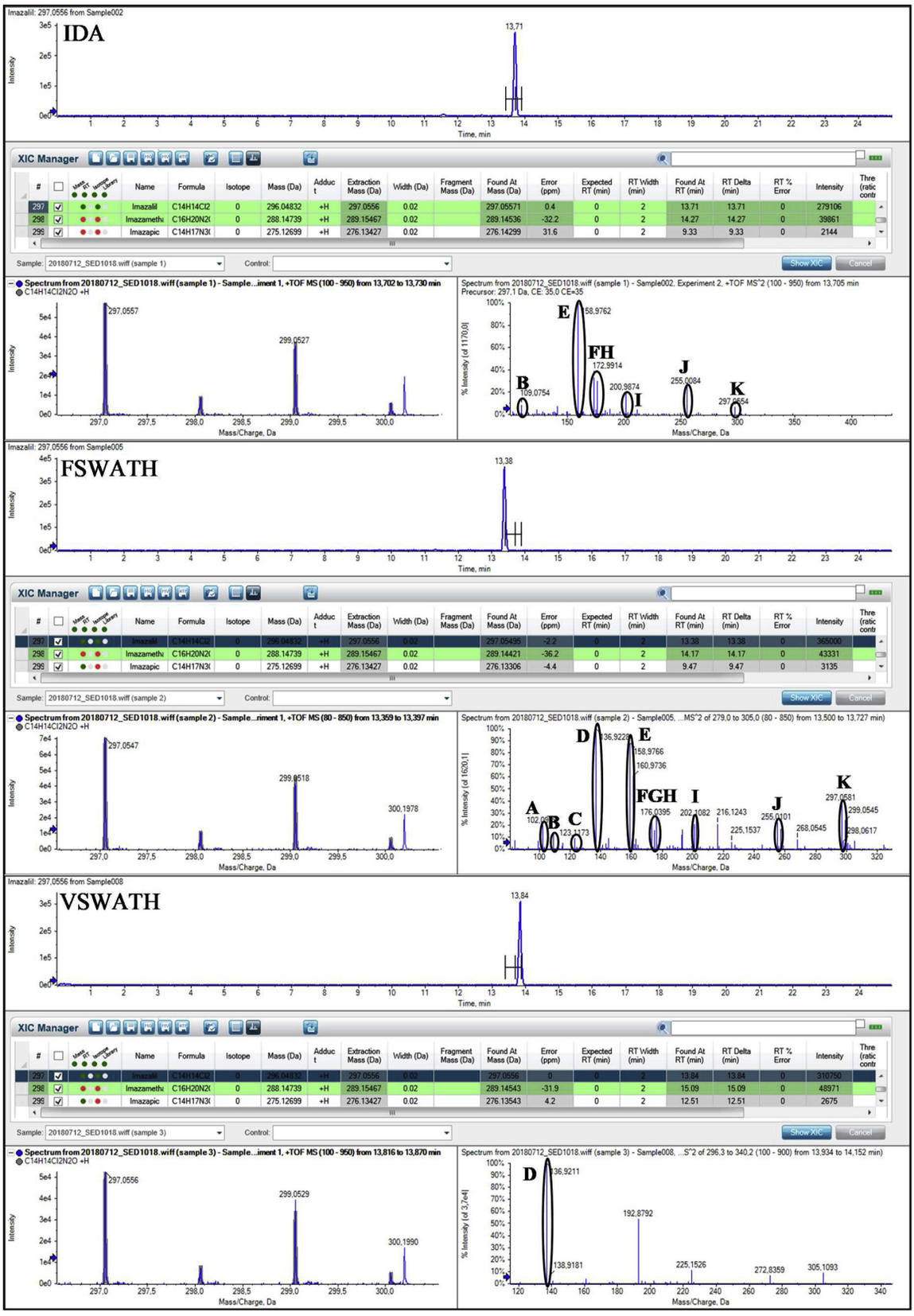

Imazalil: 297,0556 from Sample002
IDA
13,71
Intensity
Time, min
XIC Manager
Name Formula Isotope Mass (Da) Adduct Extraction Mass (Da) Width (Da) Fragment Mass (Da) Found At Mass (Da) Error (ppm) Expected RT (min) RT Width (min) Found At RT (min) RT Delta (min) RT % Error Intensity
297 Imazalil C14H14Cl2 0 296.04832 +H 297.0556 0.02 297.05571 0.4 0 2 13.71 13.71 0 279106
298 Imazameth C16H20N2(0 288.14739 +H 289.15467 0.02 289.14536 -32.2 0 2 14.27 14.27 0 39861
299 Imazapic C14H17N3(0 275.12699 +H 276.13427 0.02 276.14299 31.6 0 2 9.33 9.33 0 2144
Sample: 20180712_SED1018.wiff (sample 1)
Control:
Show XIC
Cancel
Spectrum from 20180712_SED1018.wiff (sample 1) - Sample...iment 1, +TOF MS (100 - 950) from 13,702 to 13,730 min
C14H14ClN2O +H
297,0557
299,0527
Mass/Charge, Da
Spectrum from 20180712_SED1018.wiff (sample 1) - Sample002, Experiment 2, +TOF MS^2 (100 - 950) from 13,705 min
Precursor: 297,1 Da, CE: 35,0 CE=35
% Intensity (of 1170,0)
E
158,9762
FH
172,9914
200,9874
I
J
255,0084
K
297,0654
B
109,0754
Mass/Charge, Da
Imazalil: 297,0556 from Sample005
FSWATH
13,38
Intensity
Time, min
XIC Manager
297 Imazalil C14H14Cl2 0 296.04832 +H 297.0556 0.02 297.05495 -2.2 0 2 13.38 13.38 0 365000
298 Imazameth C16H20N2(0 288.14739 +H 289.15467 0.02 289.14421 -36.2 0 2 14.17 14.17 0 43331
299 Imazapic C14H17N3(0 275.12699 +H 276.13427 0.02 276.13306 -4.4 0 2 9.47 9.47 0 3135
Sample: 20180712_SED1018.wiff (sample 2)
Control:
Show XIC
Cancel
Spectrum from 20180712_SED1018.wiff (sample 2) - Sample...riment 1, +TOF MS (80 - 850) from 13,359 to 13,397 min
C14H14ClN2O +H
297,0547
299,0518
300,1978
Mass/Charge, Da
Spectrum from 20180712_SED1018.wiff (sample 2) - Sample005, ...MS^2 of 279,0 to 305,0 (80 - 850) from 13,500 to 13,727 min)
% Intensity (of 1620,1)
D
136,9229
E
158,9766
160,9736
FGH
176,0395
I
202,1082
216,1243
225,1537
A
102,0913
B
C
123,1173
J
255,0101
268,0545
K
297,0581
299,0545
298,0617
Mass/Charge, Da
Imazalil: 297,0556 from Sample008
VSWATH
13,84
Intensity
Time, min
XIC Manager
297 Imazalil C14H14Cl2 0 296.04832 +H 297.0556 0.02 297.0556 0 0 2 13.84 13.84 0 310750
298 Imazameth C16H20N2(0 288.14739 +H 289.15467 0.02 289.14543 -31.9 0 2 15.09 15.09 0 48971
299 Imazapic C14H17N3(0 275.12699 +H 276.13427 0.02 276.13543 4.2 0 2 12.51 12.51 0 2675
Sample: 20180712_SED1018.wiff (sample 3)
Control:
Show XIC
Cancel
Spectrum from 20180712_SED1018.wiff (sample 3) - Sample...iment 1, +TOF MS (100 - 950) from 13,816 to 13,870 min
C14H14ClN2O +H
297,0556
299,0529
300,1990
Mass/Charge, Da
Spectrum from 20180712_SED1018.wiff (sample 3) - Sample008, ...S^2 of 296,3 to 340,2 (100 - 900) from 13,934 to 14,152 min)
% Intensity (of 3,7e4)
D
136,9211
192,8792
138,9181
225,1526
272,8359
305,1093
Mass/Charge, Da

Table 2
Compounds present in the sediment and mussel not spiked samples.

		Concentration (ng/g)					
		IDA		FSWATH		VSWATH	
		SED	MUSSEL	SED	MUSSEL	SED	MUSSEL
PESTICIDES	2,3,5 trimethacarb	–	–	T.D.	–	T.D.	–
	Azoxystrobin	T.D.	–	T.D.	T.D.	T.D.	T.D.
	Benomyl	T.D.	–	–	–	–	–
	Diazinon	–	–	T.D.	–	–	–
	Imazalil	27.91	–	36.00	–	31.07	–
	Nitenpyram	22.27	–	26.75	–	–	–
	Propiconazole	T.D.	–	T.D.	–	T.D.	–
	Tricyclazole	–	–	–	–	T.D.	–
PHARMACEUTICALS AND PCPS	3-Cresotinic acid	–	–	T.D.	T.D.	T.D.	T.D.
	24-Hydroxy-3-methylbenzoic acid	–	–	T.D.	T.D.	T.D.	T.D.
	Acetaminophen	–	–	–	17.41	–	10.99
	Simvastatin	–	–	–	–	14.52	–
	Bisphenol A	–	–	10.02	–	10.01	–
	Butamben	–	–	<LOQ	–	–	–
	Ethylparaben	–	–	11.26	–	<LOQ	–
	Hydroxyibuprofen	–	–	–	45.65	–	39.03
	Ibuprofen	–	–	–	85.56	–	86.80
	Methylparaben	–	–	58.95	–	48.79	–
	Norfloxacin	–	–	–	–	<LOQ	–
	Propylparaben	–	–	–	–	<LOQ	–
	R-(-)-Mandelic acid	–	–	T.D.	T.D.	T.D.	T.D.
	Salsoline	T.D.	–	T.D.	–	T.D.	–
BIOLOGICAL COMPOUNDS	6α-methylprednisolone	–	T.D.	–	T.D.	–	T.D.
	16-phenyl tetranor PGF2α	–	T.D.	–	T.D.	–	T.D.
	16-phenyl tetranor Prostaglandin E1	–	T.D.	–	T.D.	–	T.D.
	Cicaprost	–	T.D.	–	T.D.	–	–
	Megesterol acetate	–	–	–	T.D.	–	–
	Oleamide	–	–	–	–	–	T.D.
	Phenylalanine	T.D.	–	–	–	T.D.	–
	Uracil	–	–	–	T.D.	–	–
CHEMICALS	Racemic mix Hydroxyphenylacetic acid	–	–	T.D.	T.D.	T.D.	T.D.
	TOTAL	**7**	**4**	**15**	**14**	**17**	**13**

T.D. (Tentative Detected). < LOQ (Concentration detected below the LOQ).

product ions of higher intensity than imazalil, so its other characteristic fragments have abundances < 10% and are not detected. The imazalil fragment detected present a much higher intensity, $3.7{\cdot}10^4$, compared to that of $1.17{\cdot}10^3$ and $1.62{\cdot}10^3$ obtained for IDA and FSWATH respectively. This could be due to isobaric interferences, the presence of several precursor ions that fragment to the same product ion or an unexpected matrix effects (due to the co-extractives) in the fragmentation pattern of imazalil (less fragmentation). Any of these causes would explain why most of the fragments of imazalil are not visible in the MS/MS spectrum.

In the mussel samples, pharmaceuticals as ibuprofen and acetaminophen were detected. Some known metabolites of these compounds were added to the library in order to perform retrospective screening to verify their possible presence, but just hydroxyibuprofen was detected and confirmed using the analytical standard. The presence of some pharmaceuticals in mussels has been reported in previous works in Pacific Ocean [41], Atlantic Ocean and Mediterranean sea [42], but up to our knowledge, the presence of ibuprofen and acetaminophen was not reported in mussels from the Mediterranean sea yet. These compounds not only could provide negative effects in the mussels [43,44] but also could suppose a risk for the humans that consume them.

In sediments, several pharmaceuticals and personal care products occur, but agrochemicals, such as imazalil, propiconazol or diazinon were the most relevant. The presence of pesticides in sediments has been widely studied [45–47] and also their potential risk [45,46]. The source of these compounds could be the orchards placed close to the river. The presence of pharmaceuticals indicates that other anthropogenic discharges should be taken into account in order to develop future research in the area. Although the existing software has facilitated compounds identification, the difficulties of this operation also deserve mention. For example, some MS/MS spectra obtained can correspond to several compounds (with the same empirical formula but different and quite similar structures) according to the Metlin database (e.g. MS/MS spectra that could correspond to three variations of hydroxyfenilatyc acid or two different types of 16-phenyl tetranor were detected). We cannot go ahead in these identifications due to the lack of analytical standards, consequently was not possible for us to determine exactly which of these compounds were in the sample. A further research is needed to clarify this point.

4. Conclusions

The ability to offer information on the MS/MS spectrum of all the compounds present in the sample is crucial in environmental screening. FSWATH and the VSWATH acquisition modes TD more compounds than IDA acquisition. This is an unquestionable advantage in profiling environmental contamination. The counterpart is that the IDA MS/MS spectra is of better quality than those obtained by both SWATH methods, as shown in the QLS values. Although, in all the experiments carried out in this study, contaminants TD

Fig. 3. Imazalil data obtained with IDA, FSWATH and VSWATH acquisition from a not spiked sediment sample. From "A" to "K" are the respective fragments present (or not) in the spectra, that match with the reference spectra.

were identified by their MS/MS spectra, the identification with the SWATH acquisition requires more time, due to the presence of more fragments including those that do not match with the reference spectra, which should be discarded manually. There were not important differences between FSWATH and VSWATH for the compounds and matrices analysed.

The efficacy of the SWATH compared to the IDA has been also verified in non-spiked samples. The three acquisition methods demonstrated their reliability to analyse environmental samples and to TD and quantify environmental contaminants. The occurrence of emerging pollutants in the analysed samples needs further research to assess their potential environmental risk, specially, in the case of the mussels that could also suppose a human health risk. Results obtained in non-spiked samples pointed out that the workflow developed can help to make important advances in this field since SWATH combines advantages of IDA and traditional MS^E avoiding some of their main disadvantages.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chroma.2019.02.041.

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Supplementary Information

Sequential window acquisition of all theoretical fragments versus information dependent acquisition for suspected-screening of pharmaceuticals in sediments and mussels by ultra-high pressure liquid chromatography-quadrupole time-of-flight-mass spectrometry

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Table of contents

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Table S-1. Spiked pharmaceuticals information

	Empirical formula	Adduct	Mass (Da)	Extraction mass (Da)	Intensity at LOD	LOQ (ng/g)
Acetaminophen	$C_8H_9NO_2$	H+	151.0633	152.0706	8026	10
Alprazolam	$C_{17}H_{13}ClN_4$	H+	308.0828	309.0901	16819	10
Atenolol	$C_{14}H_{22}N2O_3$	H+	266.1630	267.1703	1330	10
Atorvastatin	$C_{33}H_{35}N_2FO_5$	H+	558.2530	559.2602	6484	10
Caffeine	$C_8H_{10}N_4O_2$	H+	194.0803	195.0876	8978	10
Codeine	$C_{18}H_{21}NO_3$	H+	299.1521	300.1594	5723	10
Enalapril	$C_{20}H_{28}N_2O_5$	H+	376.1998	377.2071	12454	10
Etoricoxib	$C_{18}H_{15}ClN_2O_2S$	H+	358.0542	359.0615	8356	10
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	H+	320.0119	321.0192	12372	10
Metformin	$C_4H_{11}N_5$	H+	129.1014	130.1087	4025	10
Norfloxacin	$C_{16}H_{18}N_3O_3$	H+	300.1348	301.1420	38490	10
Omeprazole	$C_{17}H_{19}N_3O_3S$	H+	345.1147	346.1219	6075	10
Simvastatin	$C_{25}H_{38}O_5$	H+	418.2719	419.2792	24132	10
Tramadol	$C_{16}H_{25}NO_2$	H+	263.1885	264.1958	12160	10
Salicylic Acid	$C_9H_8O_4$	H-	180.0422	179.0349	86634	10
Bezafibrate	$C_{19}H_{20}ClNO_4$	H-	361.1080	360.1008	20033	10
Bisphenol A	$C_{15}H_{16}O_2$	H-	228.1150	227.1077	27633	10
Butylparaben	$C_{11}H_{14}O_3$	H-	194.0942	193.0870	89325	10
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	H-	322.0123	321.0050	17724	10
Clofibric Acid	$C_{10}H_{11}ClO_3$	H-	214..0396	213.0324	6116	25
Diclofenac Sodium	$C_{14}H_{11}Cl_2NO_2$	H-	295.0166	294.0094	7123	10
Ethylparaben	$C_9H_{10}O_3$	H-	166.0629	165.0557	2222	10
Flufenamic Acid	$C_{14}H_{10}F_3NO_2$	H-	281.0663	280.0590	55754	10
Furosemide	$C_{12}H_{11}ClN_2O_5S$	H-	330.0077	329.0004	5127	10
Ibuprofen	$C_{13}H_{18}O_2$	H-	206.1306	205.1234	4475	25
Indomethacin	$C_{19}H_{16}ClNO_4$	H-	357.0767	356.0695	2957	10
Methylparaben	$C_8H_8O_3$	H-	152.0473	151.0400	38124	10
Propylparaben	$C_{10}H_{12}O_3$	H-	180.0786	179.0713	71973	10
Thiamphenicol	$C_{12}H_{15}Cl_2NO_5S$	H-	355.0048	353.9975	80584	10
Triclocarban	$C_{13}H_9Cl_3N_2O$	H-	313.9780	312.9707	11242	10
Triclosan	$C_{12}H7Cl_3O_2$	H-	287.9511	286.9438	9062	10
Warfarin	$C_{19}H_{16}O_4$	H-	308.1048	307.0975	99762	10

Table S-2: A- Positive ionization compounds detected on the experiment. B-Negative ionization compounds detected on the experiment.

A	IS 100	IS 50	IS 25	IS 10	IM 100	IM 50	IM 25	IM 10	SFS 100	SFS 50	SFS 25	SFS 10	SFM 100	SFM 50	SFM 25	SFM 10	SVS 100	SVS 50	SVS 25	SVS 10	SVM 100	SVM 50	SVM 25	SVM 10
Acetaminophen	-	-	-	-	X	-	-	-	X	X	X	X	-	-	-	-	X	X	-	-	-	-	-	-
Alprazolam	X	X	X	X	-	-	-	-	X	X	X	X	X	X	-	-	X	X	X	X	X	-	-	-
Atenolol	-	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Atorvastatin	X	-	-	-	-	-	-	-	X	X	X	-	-	-	-	-	X	X	X	-	-	X	X	-
Caffeine	X	-	-	-	X	X	X	-	X	X	X	X	-	-	-	-	X	X	X	X	-	-	-	-
Codeine	X	X	X	-	-	-	-	-	X	X	X	-	-	-	-	-	X	X	X	-	-	-	-	-
Enalapril	X	X	X	-	-	-	-	-	X	X	X	X	-	-	-	-	X	X	X	X	-	-	-	-
Etoricoxib	X	X	X	X	-	-	-	-	X	X	X	X	X	-	-	-	X	X	X	X	X	X	-	-
Lorazepam	X	X	-	-	-	-	-	-	X	X	X	X	X	X	-	-	X	X	X	X	X	X	-	-
Metformin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Norfloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Omeprazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simvastatin	X	X	-	-	-	-	-	-	X	X	X	X	X	X	-	-	-	-	X	X	-	-	-	-
Tramadol	X	X	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	9	7	4	2	2	1	1	0	11	10	9	7	4	3	0	0	9	8	8	6	3	3	1	0

B	IS 100	IS 50	IS 25	IS 10	IM 100	IM 50	IM 25	IM 10	SFS 100	SFS 50	SFS 25	SFS 10	SFM 100	SFM 50	SFM 25	SFM 10	SVS 100	SVS 50	SVS 25	SVS 10	SVM 100	SVM 50	SVM 25	SVM 10
Salicylic Acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bezafibrate	X	X	-	-	-	-	-	-	X	X	X	X	-	-	-	-	X	X	X	X	-	-	-	-
Bisphenol A	X	X	-	-	-	X	X	-	X	X	X	X	X	X	X	-	X	X	X	X	X	X	X	-
Butylparaben	X	X	X	-	X	-	-	-	X	X	X	X	X	X	-	-	X	X	X	X	X	X	X	-
Chloramphenicol	X	X	-	-	-	-	-	-	X	X	X	X	-	-	-	-	X	X	X	X	X	-	-	-
Clofibric Acid	-	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	X	-	-	-	-	-	-
Diclofenac Sodium	-	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	X	-	X	X	-	-	-
Ethylparaben	X	X	-	-	-	-	-	-	X	X	X	X	X	-	-	-	X	X	X	-	-	-	-	-
Flufenamic Acid	X	X	-	-	-	-	-	-	X	X	X	X	X	X	-	-	X	X	X	X	X	-	-	-
Furosemide	-	-	-	-	-	-	-	-	X	X	X	-	-	-	-	-	X	X	X	-	-	-	-	-
Ibuprofen	-	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	X	-	-	X	-	-	-
Indomethacin	-	-	-	-	-	-	-	-	X	X	X	X	-	-	-	-	X	X	X	-	-	-	-	-
Methylparaben	X	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	X	-	X	-	-	-	-
Propylparaben	X	X	X	-	-	-	-	-	X	X	X	X	X	X	-		X	X	X	X	X	X	-	-
Thiamphenicol	X	X	-	-	X	X	-	-	X	X	X	X	X	-	-	-	X	X	X	X	X	X	-	-
Triclocarban	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	-	-	-	-	-	X	X	X	X
Triclosan	-	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	X	X	-	X	-	-	-	-
Warfarin	X	X	X	-	-	-	-	-	X	X	X	X	X	X	X	-	X	X	X	X	X	X	-	-
TOTAL	10	9	3	0	2	2	1	0	16	16	11	10	8	6	3	0	16	16	11	11	10	6	3	1

"I" refers to IDA acquisition, "SF" refers to SWATH fix window acquisition, "SV" refers to SWATH variable window acquisition, "S" refers to sediment sample, "M" refers to mussel sample. In the total line is the total number of detected compounds in each sample.

Table S-3: Data obtained from the sediment samples analysed with IDA acquisition.

	IS 100		IS 50		IS 25		IS 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Alprazolam	0.7	6.72	-0.1	6.67	2.3	7.32	0.8	7.59
Atorvastatin	0.5	9.51	-	-	-	-	-	-
Caffeine	4.8	1.77	-	-	-	-	-	-
Codeine	2.7	0.48	0.5	0.48	-0.5	0.49	-	-
Enalapril	-0.8	4.22	0.2	4.18	-0.5	4.14	-	-
Etoricoxib	-0.9	2.80	-0.9	2.78	-0.7	2.76	0.3	2.72
Lorazepam	0.5	6.87	0.4	6.82	-	-	-	-
Simvastatin	-0.9	11.47	0.7	11.89	-	-	-	-
Tramadol	0.1	1.32	0.6	1.32	-	-	-	-
Bezafibrate	0.0	8.87	-2.9	9.21	-	-	-	-
Bisphenol A	-0.9	9.82	-0.1	9.84	-	-	-	-
Butylparaben	-0.7	10.91	-0.6	10.94	0.2	10.92	-	-
Chloramphenicol	-2.4	3.01	-0.4	2.70	-	-	-	-
Ethylparaben	0.0	6.56	0.0	6.57	-	-	-	-
Flufenamic Acid	-1.4	11.94	-0.2	11.96	-	-	-	-
Methylparaben	-0.5	10.29	-	-	-	-	-	-
Propylparaben	0.4	9.14	0.1	9.16	-0.3	9.15	-	-
Thiamphenicol	-0.7	1.03	-1.6	1.03	-	-	-	-
Warfarin	-0.7	9.83	-0.6	9.83	-0.6	9.78	-	-

Table S-4: Data obtained from the mussel samples analysed with IDA acquisition.

	IM 100		IM 50		IM 25		IM 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Acetaminophen	0.7	0.53	-	-	-	-	-	-
Caffeine	57.4	0.5	52.3	0.5	57.2	0.49	-	-
Bisphenol A	-	-	0.3	9.52	-0.4	9.59	-	-
Butylparaben	0.2	10.56	-	-	-	-	-	-
Thiamphenicol	-0.5	1.02	0.1	1.01	-	-	-	-

Table S-5: Data obtained from the sediment samples analysed with FSWATH acquisition.

	FSS 100		FSS 50		FSS 25		FSS 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Acetaminophen	-1.1	0.80	-0.4	0.81	-1.8	0.82	0.5	0.84
Alprazolam	0.0	9.99	-0.2	9.74	-1.1	9.52	0.1	9.13
Atenolol	0.1	0.58	4.0	12.86	-	-	-	-
Atorvastatin	-3.5	12.13	0.7	11.91	0.3	11.76	-	-
Caffeine	-0.6	1.59	0.4	1.60	-0.8	1.60	-0.5	1.70
Codeine	-0.8	0.74	-0.2	0.74	1.0	0.74	-	-
Enalapril	0.2	7.87	-0.1	7.64	0.2	7.39	0.3	8.10
Etoricoxib	-2.4	6.18	-0.3	6.00	0.1	5.83	0.3	5.59
Lorazepam	0.4	10.11	-0.7	9.87	1.3	9.65	0.6	9.26
Simvastatin	-1.5	13.82	-1.1	13.61	0.2	14.00	-3.8	13.77
Tramadol	-0.9	3.55	-	-	-	-	-	-
Bezafibrate	0.8	8.86	0.5	8.90	0.5	9.26	1.1	8.86
Bisphenol A	1.3	9.80	0.7	9.82	-1.7	10.10	0.4	9.79
Butylparaben	0.7	10.9	-0.2	10.91	-1.0	11.10	-1.8	11.04
Chloramphenicol	0.5	2.69	-0.8	2.74	-0.7	4.38	0.5	2.74
Clofibric Acid	0.0	6.21	0.7	6.55	-	-	-	-
Diclofenac Sodium	-0.3	11.05	-0.2	11.07	-	-	-	-
Ethylparaben	-0.1	6.52	-0.7	6.58	-0.4	7.43	0.2	7.12
Flufenamic Acid	2.0	11.93	-0.3	11.94	-0.4	12.01	0.9	11.94
Furosemide	0.5	1.97	0.9	2.01	-0.2	4.39	-	-
Ibuprofen	4.7	22.93	-0.1	24.18	-	-	-	-
Indomethacin	-1.2	11.42	1.0	11.45	1.1	11.99	0.9	12.11
Methylparaben	-0.5	10.63	0.1	9.66	-	-	-	-
Propylparaben	0.6	9.12	-0.5	9.15	0	9.56	0.3	9.01
Thiamphenicol	-0.2	1.03	-0.6	1.03	3.3	0.67	4.3	0.69
Triclosan	4.8	13.82	1.5	13.82	-	-	-	-
Warfarin	0.8	9.83	-0.6	9.87	-0.6	10.07	-0.3	9.83

Table S-6: Data obtained from the mussel samples analysed with FSWATH acquisition.

	FSM 100		FSM 50		FSM 25		FSM 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Alprazolam	0.0	10.73	-0.2	10.59	-	-	-	-
Etoricoxib	0.9	6.54	-	-	-	-	-	-
Lorazepam	0.3	10.84	-0.6	10.70	-	-	-	-
Simvastatin	1.8	14.36	-2.6	14.22	-	-	-	-
Bisphenol A	-0.5	9.76	-2.7	9.80	0.9	9.81	-	-
Butylparaben	0.2	10.86	-2.2	11.27	-	-	-	-
Ethylparaben	-0.6	7.53	-	-	-	-	-	-
Flufenamic Acid	-0.8	11.94	-1.8	11.55	-	-	-	-
Propylparaben	0.5	9.09	0.6	9.85	-	-	-	-
Thiamphenicol	0.0	1.03	-	-	-	-	-	-
Triclocarban	-1.0	13.68	0.0	13.69	-0.4	13.66	-	-
Warfarin	0.1	9.82	-0.5	9.48	-0.3	9.92	-	-

Table S-7: Data obtained from the sediment samples analysed with VSWATH acquisition.

	VSS 100		VSS 50		VSS 25		VSS 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Acetaminophen	1.5	1.19	0.8	1.21	-	-	-	-
Alprazolam	0.6	11.29	-0.8	11.20	-0.5	11.06	0.7	10.92
Atenolol	0.6	0.61	-	-	-	-	-	-
Atorvastatin	-2.2	13.11	-1.9	13.03	-1.5	12.93	-	-
Caffeine	-0.7	1.63	0.0	1.71	-1.1	1.62	0.8	2.04
Codeine	0.6	0.75	-0.2	0.78	0.8	0.75	-	-
Enalapril	0.2	9.10	-0.6	9.09	-1.1	8.90	-0.1	9.33
Etoricoxib	-0.7	6.69	-0.5	6.86	0.1	6.63	-1.6	6.53
Lorazepam	0.1	11.41	-0.8	11.29	-0.4	11.90	-0.1	11.03
Simvastatin	-	-	-	-	1.1	15.21	-1.4	15.08
Bezafibrate	-1.0	9.19	0.0	8.89	-0.7	8.90	-0.9	8.89
Bisphenol A	0.4	10.06	-1.5	9.83	-0.9	9.83	-0.7	9.83
Butylparaben	-0.7	11.06	0.0	10.91	0.6	11.37	-1.4	10.91
Chloramphenicol	-1.7	4.35	-0.6	2.73	-0.2	2.71	0.6	2.75
Clofibric Acid	-1.1	6.98	-0.2	6.27	-	-	-	-
Diclofenac Sodium	-2.0	11.15	0.5	11.06	-	-	1.0	11.11
Ethylparaben	-0.5	7.36	0.5	6.58	0.9	7.15	-	-
Flufenamic Acid	0.3	11.98	-0.6	11.94	-0.1	11.95	-0.9	11.95
Furosemide	0.0	4.38	0.3	2.00	-1.6	4.59	-	-
Ibuprofen	-0.5	21.96	-1.8	21.88	-	-	-	-
Indomethacin	-1.9	11.51	-3.3	11.42	-0.2	11.41	-	-
Methylparaben	-0.5	8.85	-0.9	9.34	-	-	0.3	-1.20
Propylparaben	-0.8	9.49	-0.7	9.15	-0.4	9.15	-1.4	9.15
Thiamphenicol	-0.5	1.38	0.8	1.03	-0.8	1.03	0.4	0.68
Triclosan	-0.7	13.83	3.0	13.81	-	-	-4.7	13.81
Warfarin	-0.8	10.01	-0.8	9.87	0.6	9.88	0.5	9.86

Table S-8: Data obtained from the mussel samples analysed with VSWATH acquisition.

	VSM 100		VSM 50		VSM 25		VSM 10	
	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)	Error (ppm)	Retention time (min)
Alprazolam	-0.5	11.79	-	-	-	-	-	-
Atorvastatin	-	-	0.1	17.25	0.7	16.97	-	-
Etoricoxib	0.6	6.97	1.6	6.77	-	-	-	-
Lorazepam	0.6	11.91	2.5	11.37	-	-	-	-
Bisphenol A	-0.6	10.28	0.9	10.26	0.1	10.18	-	-
Butylparaben	-1.0	10.89	-0.9	10.5	-3.5	10.87	-	-
Chloramphenicol	-0.9	8.20	-	-	-	-	-	-
Diclofenac Sodium	-1.9	11.31	-	-	-	-	-	-
Flufenamic Acid	0.2	11.94	-	-	-	-	-	-
Ibuprofen	-0.6	22.48	-	-	-	-	-	-
Propylparaben	0.2	9.08	-0.5	9.10	-	-	-	-
Thiamphenicol	-0.6	1.03	1.0	1.01	-	-	-	-
Triclocarban	0.6	13.69	-0.5	13.64	-1.6	13.66	2.1	13.70
Warfarin	0.7	9.8	-0.4	9.83	-	-	-	-

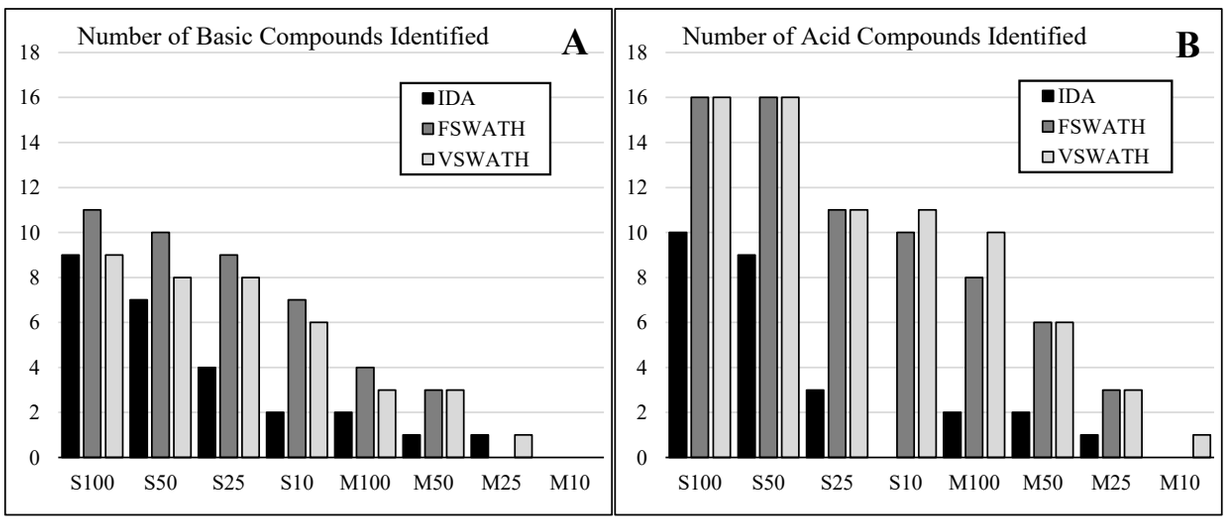

Figure S-1: Number of compounds detected in the spiked samples. A-Positive ionization compounds. B-Negative ionization compounds

SECCIÓN 4.

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08

ARTÍCULO

Analysis of ibuprofen and its main metabolites in roots, shoots and seeds of cowpea (*Vigna unguiculata* L. Walp) using liquid chromatography-quadrupole time-of-flight mass spectrometry: uptake, metabolism and translocation.

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RESEARCH PAPER

Analysis of ibuprofen and its main metabolites in roots, shoots, and seeds of cowpea (*Vigna unguiculata* L. Walp) using liquid chromatography-quadrupole time-of-flight mass spectrometry: uptake, metabolism, and translocation

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Abstract

A liquid chromatography quadruple time-of-flight mass spectrometry (LC–QqTOF-MS/MS) method was developed for simultaneous quantitative analysis of ibuprofen (IBU), 1- and 2-hydroxyibuprofen (1-OH IBU and 2-OH IBU), and carboxyibuprofen (CBX IBU) while preserving the ability of the instrument to get precursor and product ion mass spectra of non-target compounds. The trigger was the precursor ions reaching 100 cps intensity. Sample preparation was carried out by ultrasound solid-liquid extraction with methanol as extraction solvent at pH < 2 followed by solid-phase extraction (SPE) clean-up using STRATA-X cartridges and methanol as an eluent. Linearity was obtained in the range 50–10,000 ng mL^{-1} for IBU, each OH IBU and CBX IBU ($r \geq 0.99$). The proposed method was satisfactorily validated showing absolute recoveries of > 70% for all target analytes at low and high concentration levels. The lowest limit of quantification was < 50 ng g^{-1} in plant. This method was applied to investigate IBP behavior in cowpea (*Vigna unguiculata* (L.) Walp) treated at high IBU concentrations and its presence in vegetables irrigated with treated water. Up to 46 metabolites, mostly hydroxylated metabolites and conjugates with hexosides and amino acids, were identified. The most abundant metabolites were also identified in an eggplant sample.

Keywords Ibuprofen · High-resolution mass spectrometry · Plant metabolites · Shoots · Roots · Seeds

Introduction

Contaminants of emerging concern (CECs) are widespread in the environment and well documented in various aquatic compartments, because of their incomplete removal in wastewater treatment plants (WWTPs) [1–4]. The reuse of treated wastewater or wastewater-impacted river water is growing, particularly in arid areas due to the climate change [5–7]. This practice together with the implementation of the sludge disposal as soil amendments is recognized as the main source of CECs for agricultural crops [8]. The CEC accumulation in soil and their subsequent uptake pathway, bioaccumulation, translocation, and metabolism by plants are not well understood yet [9–11]. Furthermore, discrepancies between studies are frequent because the data are diverse, incomplete, or collected for different purposes [12–14].

Ibuprofen (IBU) [a non-steroidal anti-inflammatory drug (NSAID)] is one of the most frequently detected

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pharmaceuticals in WWTPs, at microgram per liter concentrations, and even effluents seem to contain it quite frequently at concentrations up to 100 μg L^{-1} [15]. The IBU metabolism has been widely studied in mammals, fungi, and microbes [16–20]. The oxidative biotransformation to carboxyibuprofen (CBX IBU) and 2-hydroxyibuprofen (2-OH IBU) is the common metabolic pathway either in animals or microorganisms. However, little is known on how IBU is metabolized by plants, mostly due to analytical methodology gaps for determining these compounds at low concentration in complex matrices with a number of potential interferences, such as amino acids, complex carbohydrates, and chlorophylls [21–24].

Several reviews already cover current analytical techniques, instruments, and methodologies used to determine CECs in general and IBU in particular in plants [11, 25, 26]. The extraction is mostly carried out by solid-liquid procedures aided by ultrasounds, microwaves, or pressures followed by isolation and concentration by solid-phase extraction (SPE) clean-up in order to achieve and exhaustive procedure. Determination of CECs in plants has been performed preferably by liquid chromatography (LC) with fluorescence (FLD) or mass spectrometry (MS) to exploit all features to enhance sensitivity and obtain accurate quantification. The former was proposed to quantify IBU and its two main metabolites in *Lemna gibba* L. and, therefore, to establish their potential uptake, metabolism, removal, and biodegradation capacity [27]. In the same way, LC-MS with a triple quadrupole was used to establish that metabolites of IBP, such as CBX IBP, 2-OH IBP, and 1-OH IBP, accumulated in the leaves of *Typha angustifolia*, indicating the phytotransformation of IBP in the plant tissues [28]. However, these studies are always based on the determination of already known metabolites of the compounds commonly characteristics of the human metabolic routes. High-resolution mass spectrometry (HRMS) presents an exciting opportunity to address the significant challenges associated with the transformation and metabolism of CECs in the plant [29]. The uptake and metabolism of IBU by plants at the cellular level was already investigated by HRMS using a suspension culture of *Arabidopsis thaliana* [21]. More than 300 metabolites, many of them for the first time, were determined. Hydroxylated derivatives were the main first-step products of IBU degradation, but conjugates of these products with simple carbohydrates, amino acids, or methyl groups were the dominant metabolites in the culture. This information is highly valuable but far away of what really occurs in plants. IBU and potential intermediates were already measured at environmental levels in *Phragmites australis* by HRMS using an Orbitrap. Four intermediates were detected in the plant tissues: OH IBP, 1,2-dihydroxy (DiOH)-IBP, CBX IBP, and OH IBP hexoside [30]. Other application of the orbitrap in *L. gibba* treated at slightly higher concentrations reported the identification of more than 11 transformation products of IBU [31]. Differences involved the absence of CBX IBU and the detection of IBU hexoside, IBU acetyl hexoside, OH IBU acetyl hexoside, and IBU malonyl hexoside. However, there are no more real-life studies yet, and IBU metabolite detection is in its infancy.

This study aims to evaluate the metabolism, uptake, and translocation of IBU in a series of experiments using cowpea (*Vigna unguiculata* L.), which is a drought-tolerant and warm-weather crop and well-adapted to the drier regions of the tropics, where other food legumes do not perform well. Furthermore, several crops irrigated with treated wastewater were taken as an example of the situation of natural exposure. To perform this study, the development and validation of a liquid chromatography–high resolution-tandem mass spectrometric (LC–HR–MS/MS) method for determining IBU, 1 hydroxyibuprofen (1-OH IBU), 2-OH IBU, and CBX IBU in plants was required. Our goal was to develop the method without losing the HRMS ability to obtain the precursor and product ion mass spectrum of any non-target compound that reach an intensity threshold to identify the metabolites produced during the detoxification process in roots, tales, and seeds as well as establish its uptake accumulation and translocation. To our knowledge, this is the first study on the identification of IBU and its main metabolites in roots, shoot, and seeds of cowpea. In addition, the method was tested in samples irrigated with treated wastewater.

Experimental

Chemicals and reagents

IBU (CAS No. 15687-27-1, ≥ 98%), CBX IBU (mixture of diastereomers; CAS No. 15935-54-3, ≥ 98%), 1-OH IBU (mixture of diastereomers; CAS No. 53949-53-4, ≥ 98%), and 2-OH IBU (mixture of diastereomers) (CAS No. 51146-55-5, ≥ 98%) were from Santa Cruz Biotechnology obtained through Epica (Zaragoza, Spain). The deuterated standard [internal standard (IS)] IBU-d3 was purchased from Sigma-Aldrich. LC-grade "suprasolv" methanol was purchased from VWR chemicals (Darmstadt, Germany). Deionized water (< 18 MΩ cm resistivity) was from a Milli-Q SP reagent water system (Millipore, Bedford, MA, USA). Ammonium fluoride for analysis (CAS No. 12125-01-8, ≥ 99.9%) were from Sigma-Aldrich. STRATA-X 33 μm polymeric reversed-phase cartridges (200 mg sorbent/6 mL) of 800 m^2/g surface area were from Phenomenex (Torrance, CA, USA). Stock solutions were prepared with methanol and stored in a freezer at − 20 °C. Germination was monitored every day and roots were collected at the end of the treatment to measure the length, fresh weight, and dry weight.

Uptake experiments

Seeds of *V. unguiculata* from Gizan area (Saudi Arabia) were pretreated with sodium hypochlorite 0.5% (to kill the microbes) and germinated in Petri plates using a graduated treatment regimen (control, 400, 800, 1200, 1600, and 2000 mg L^{-1} of IBU). Fifteen seeds per petri plate were used, three replicates for each treatment. Treatments seeds were incubated in a growth chamber (Sanyo, Japan) in the dark at 26 °C for 5 days with the addition of 1 mL of Hoagland nutrient solutions every day. Germination was monitored every day. Roots were collected at the end of the treatment to measure the length, fresh weight, and dry weight.

The surface sterilized seeds (as done in the first experiment) of *V. unguiculata* L. were sown in polyvinyl chloride pots (diameter = 15 cm and depth = 15 cm) filled with Perlites. One seedling per pot (with three replicates for each treatment) along with the control was maintained. These seedlings were irrigated three times weekly (40 mL) with graduated treatment regimen (control, 400, 800, 1200, 1600, and 2000 mg L^{-1} of IBU) throughout the whole experiment. Additional watering was done on alternate days with 200 mL of full-strength Hoagland nutrient solution to ensure adequate nutrition. In the growth chamber, photosynthetically active radiation (PAR) = 170 μmol m^{-2} s^{-1}; day and night temperatures = 26/20 °C; day length = 14 h day, 10 h night; and relative humidity ± 45% were maintained throughout the experiment. The plants were sampled at 25 and 50 days after transplantation to assess the various growth and photosynthetic parameters. Length, fresh and dry weight of shoots and roots, and leaf chlorophyll SPAD were measured at the sampling time. The total leaf area (LA) for each plant was determined using an automatic leaf area meter (CI-202 area meter, CID, Inc., WA, USA).

The chlorophyll (Chl) and carotenoid (Car) contents of leaves were measured by the method described by [32] and [33]. The pigments of 0.1 g of fresh leaf were extracted by acetone 80% in mortar and pestle. Extracts were filtered by a filter paper and the absorbance of samples was measured at 645, 663, 652, 480, and 510 nm by UV-visible spectrophotometer (Pharmacia Ultrospec III). Chlorophyll a and b, total chlorophyll, and carotenoid contents were calculated.

Field samples

Twenty-five samples of eggplants, cabbages, chili peppers, and courgettes were taken before and after the influence of the Riyadh wastewater treatment plant.

Extraction procedure

The vegetal materials were separately frozen at − 20 °C. The samples (ca. 1 g) were chopped, triturated, placed in a polypropylene tube of 15 mL, spiked with 100 μL of the IS at 1000 ng mL^{-1} (final concentration 100 ng g^{-1}), and treated with 0.5 mL of HCl 0.1 M and 4 mL of methanol. Then, the tube was shaken, sonicated for 10 min, and centrifuged for 15 min at 4000 rpm. This process was repeated three times, and the extracts were mixed obtaining a volume of approximately 12 mL that was evaporated to 4 mL diluted to 200 mL with Milli-Q water in a volumetric flask and passed under vacuum through STRATA-X cartridges, previously preconditioned with 5 mL of methanol and 5 mL of H_2O. Once the sample passed, the cartridges were air-dried for 15 min and, then, the analytes eluted with 10 mL of methanol first using vacuum and then under gravity. Extracts were evaporated to dryness and reconstituted with 1 mL of methanol-water (10:90).

Liquid chromatography–tandem mass spectrometry

IBU and its metabolites were separated by ultra-high performance liquid chromatography (Agilent 1260 Infinity, Waldbronn, Germany). Separations were carried out using an Agilent Poroshell 12 D EC-C18 (50 × 30 mm i.d., 2.7 μm) then eluted with water (A) and methanol (B), both with 2.5 mM of ammonium fluoride at 0.4 mL min^{-1}. The gradient was 70% A at 0 min, 70–15% A from 0 to 10 min, 15–3% A from 10 to 15 min, then 3–70% A from 15 to 16 min, and held at 70% A for 15 min before injection. Column temperature was 30 °C, and the injection volume was 5 μL.

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) analysis were accomplished by using an AB SCIEX TripleTOF™ 5600 mass spectrometer (AB SCIEX, Foster City, CA, USA) equipped with a Turbo V ion source in electrospray (ESI) mode. Data acquisition processing and instrument control were performed using Analyst, Peak View 1.0, and MultiQuant 2.0. software. Mass spectra were obtained in negative mode. Electrospray voltage was set to 4.5 kV. Source temperature and nitrogen gas flows (GS1 and GS2) were set to 400 °C and 50 psi, respectively. Collision cell energy was optimized as 35 eV. Dwell time and scan rate were set to 200 ms and 10 kDa s^{-1}, respectively, for the entire duration of the experiment. The HRMS method file comprised of two experiments in a single period. The first and second experiments in the MS method file were programmed to perform full scan (mass range from 100 to 950) and information dependent acquisition (IDA) MS/MS (mass range from 100 to 950) data acquisition, respectively. Accumulation times for MS and IDA-MS/MS experiments were 100 and 150 ms, respectively. By applying this HRMS method programming strategy, for example, an MS/MS scan will be triggered when the precursor ion reach an intensity of 100 cps.

Table 1 Recovery (*R* %), precision (RSD %), and matrix effect (ME %) for the determination of IBU and its metabolites in soybean germinate samples ($n = 6$)

Compounds	Concentration (ng g^{-1})	Absolute values			Relative values		
		R %	RSDs %	ME %	*R* %	RSDs %	ME %
IBU	50 (LLOQ)	90	12	−25	98	6	−4.6
	200	92	10	−15	97	5	−3.8
	10,000	95	8	−10	99	6	−3.1
1-OH IBU	50 (LLOQ)	87	17	−30	92	8	−12
	200	89	16	−28	95	7	−9.2
	10,000	91	12	−19	99	6	−11
2-OH IBU	50 (LLOQ)	84	18	−20	95	5	5
	200	92	15	−17	94	4	7
	10,000	89	12	−10	93	5	3
CBX IBU	50 (LLOQ)	75	13	−45	89	9	−20
	200	82	15	−50	92	5	−23
	10,000	72	10	−52	85	8	−27

Absolute values obtained using external calibration (analyte response=area under the peak). Relative values obtained using internal calibration [analyte response = ratio analyte/internal standard (ibuprofen-d3)]

$$\text{ME } \% = \left(1 - \frac{\textit{Analyte response in matrix}}{\textit{Analyte response in solvent calibration}}\right) \times 100$$

Validation of the analytical procedure

The analytical method was validated in compliance with the requirements in standard guidelines [34]. Linearity of the method was determined with an 8-point calibration curve in the range of 50 to 10,000 ng mL^{-1}. Precision was determined by injecting a 100-ng mL^{-1} standard solution for eight times. The lower limit of quantification (LLOQ) was considered the lowest concentration of analyte in a sample, which can be quantified reliably, with an acceptable accuracy and precision (between 70 and 120%, RSDs < 20%). Furthermore, the analyte signal of the LLOQ sample should be at least five times the signal of a blank sample. The LLOQ were used as the lowest calibration standard.

The recovery and matrix effects were studied in germinated soya beans brought from an organic supermarket in Valencia (Spain). The experiment for each matrix included 35 blank samples of 1 g each, i.e., matrix sample, which was expected not to contain the analytes of interest. First five samples were spiked with 50 μL of 1 μg mL^{-1}, second five with 200 μL of 1 μg mL^{-1}, and third five samples with 100 μL of 100 mg mL^{-1} of IBU, CBX IBU, and 1 and 2-OH IBU. These 15 samples were also spiked with 100 μL of 1 μg mL^{-1} IBU-d3. All additions were carried out before the extraction. The subsequent 15 samples were post-spiked (before the LC determination) with the same solutions of IBU and its metabolites and with 100 μL of 1 μg mL^{-1} IBU-d3. The last five samples were pre-spiked only with the IS. Recovery and precision as well as matrix effects were calculated both as absolute values using an external calibration and as relative values using the internal standard calibration.

The concentration of IBU and its degradation products in the samples was determined on calibration curves constructed for each individual analyte. Quality control samples were run in parallel during the quantification process. Positive controls consisting of matrix spiked (fortified) with IBU and its metabolites and internal standard were used, whereas negative controls consisting of matrix and internal standard were used to exclude possible procedural contaminations.

The stability studies showed that the samples were stable ($p > 0.05$) during freeze and thaw cycles, short-term exposure to room temperature, storage at −20 °C, and biotransformation conditions.

Results and discussion

Method optimization

The chromatographic conditions and mass spectrometry parameters were optimized for IBU, 1 and 2-OH IBU, and CBX IBU. Both the positive and negative ionization modes were tested. For all analyzed standards, the signal intensity was higher in the negative ionization mode than in the positive one. Mass spectrometric source conditions (curtain gas, collision gas, spray voltage, source temperature, and source gases) were then optimized for IBU and its metabolites. A suitable collision energy (CE) was selected by finding the maximum

Table 2 Metabolites of IBU tentatively identified in roots, shoots, and seeds of *Vigna unguiculata* L. Walp (the number of marks gives a qualitative idea of the relative abundance in the matrix)

Name	Empirical Formula	Roots	Shoot	Seeds
IBU	$C_{13}H_{18}O_2$	✓✓✓✓	✓✓✓✓	✓✓✓✓
Phase I				
1-OH IBU	$C_{13}H_{18}O_3$	✓✓✓✓	✓✓✓✓	✓✓✓✓
2-OH IBU	$C_{13}H_{18}O_3$	✓✓✓✓	✓✓✓✓	✓✓✓✓
3-OH IBU	$C_{13}H_{18}O_3$	✓✓✓	✓	
Di-OH IBU	$C_{13}H_{18}O_4$	✓✓✓	✓✓✓	✓✓
Tri-OH IBU	$C_{13}H_{18}O_5$			✓✓
O deethyl IBU	$C_{11}H_{12}O_3$		✓✓	✓
O demethyl IBU	$C_{12}H_{14}O_3$			✓✓
O,OH IBU	$C_{13}H_{16}O_4$	✓✓	✓	
Phase II				
IBU Acetyl	$C_{15}H_{20}O_3$	✓	✓✓	✓
OH IBU Methyl	$C_{14}H_{20}O_3$	✓	✓	✓✓
OH, O IBU methyl	$C_{14}H_{18}O_4$	✓	✓✓✓✓	✓
DiOH IBU methyl	$C_{14}H_{20}O_4$		✓✓	✓
CBX IBU methyl	$C_{15}H_{20}O_4$	✓	✓	
OH IBU dihexoside	$C_{25}H_{38}O_{12}$		✓✓✓✓	✓✓✓
IBU hexoside	$C_{19}H_{28}O_7$	✓✓✓✓	✓✓	✓✓✓✓
IBU methyl hexoside	$C_{20}H_{30}O_8$	✓	✓✓	
OH IBU hexoside	$C_{19}H_{28}O_8$	✓	✓✓✓✓	✓✓✓✓
OH IBU glucuronide	$C_{19}H_{26}O_9$	✓	✓✓✓✓	✓
Di OH IBU hexoside	$C_{19}H_{28}O_9$		✓	✓✓✓
O IBU hexoside	$C_{19}H_{26}O_8$		✓✓✓	✓✓✓
IBU acetyl hexoside	$C_{21}H_{30}O_8$		✓	
OH IBU acetyl hexoside	$C_{21}H_{30}O_9$	✓	✓	✓
IBU malonyl hexoside	$C_{22}H_{30}O_{10}$		✓	
Di OH IBU methyl hexoside	$C_{20}H_{30}O_9$	✓	✓	✓
OH IBU malonyl hexoside	$C_{22}H_{30}O_{11}$		✓	✓
IBU dihexoside	$C_{25}H_{38}O_{11}$		✓	✓✓✓
O,OH IBU hexoside	$C_{19}H_{26}O_9$		✓	✓✓✓
IBU glutathione conjugate	$C_{23}H_{33}N_3O_8S$			✓✓
IBU-Ser	$C_{16}H_{23}O_4N$	✓		✓✓✓
IBU-Leu	$C_{19}H_{29}O_3N$			✓✓
IBU-Ala	$C_{16}H_{23}O_3N$	✓		✓✓
IBU-Lys	$C_{19}H_{30}N_2O_3$			✓✓
IBU-Arg	$C_{19}H_{30}N_4O_3$	✓	✓	✓✓✓
IBU-Trp	$C_{24}H_{28}N_2O_3$			✓✓
IBU-Val	$C_{18}H_{27}NO_3$			✓✓
OH IBU-Tyr	$C_{22}H_{27}O_5N$			✓✓
OH IBU-Thr	$C_{17}H_{25}O_5N$	✓		✓✓
OH IBU-Ser	$C_{16}H_{23}O_5N$		✓	✓✓
OH IBU-Phe	$C_{22}H_{27}O_4N$	✓		✓✓✓✓
OH IBU-Leu	$C_{19}H_{29}O_4N$	✓		✓✓
OH IBU-Ala	$C_{16}H_{23}O_4N$	✓		✓✓
OH IBU-His	$C_{19}H_{25}O_4N_3$			✓✓
OH IBU-Arg	$C_{19}H_{30}N_4O_4$			✓✓
OH IBU-Lys	$C_{19}H_{30}N_2O_4$			✓✓
OH IBU-Trp	$C_{24}H_{28}N_2O_4$		✓	✓✓
OH IBU-Val	$C_{18}H_{27}NO_4$	✓		✓✓

Table 3 IBU metabolites in plant according to several studies

Plant specie	Metabolites common to this study	Other metabolites found	Metabolites studied but not found	Ref.
Plant cells *A. thaliana*	OH IBU, DiOH IBU, TriOH IBU, O deethyl IBU, O dimethyl IBU, O, OH IBU, OH IBU methyl, OH,O methyl, DiOH methyl, CBX IBU methyl, IBU hexoside, OH IBU hexoside, DiOH IBU hexoside, OH IBU dihexoside, OH IBU malonyl hexoside, O,OH IBU hexoside, OH IBU-Ala, OH IBU-Tyr, OH IBU-Thr, OH IBU-Ser, OH-IBU-Phe, OH-IBU-Leu, OH IBU-His	CBX IBU, O IBU, OH deethyl IBU, O DiOH IBU, DiOH methyl, O IBU methyl hexoside, OH IBU-Gln, OH IBU-Glu Sulfonylated metabolites of oxidized IBU	IBP-glutathione conjugate	[21]
L. gibba hydroponic (exposed to f 1 mg L^{-1} IBU)	OH IBU, DiOH IBU, IBU acetyl, IBU acetyl hexoside, IBU malonyl hexoside, IBU desoxyhesoxyl hexoside, OH IBU glucuronide, OH IBU acetyl hexoside, OH IBU dihexoside, IBU malonyl hexoside, OH IBU hexoside	IBU acetyl dihexoside	–	[39]
P. australis green house conditions (exposed to 50 µg of IBU)	OH IBU, OH IBU hexoside	CBX IBU	IBP-glutathione conjugate CBX IBU hexoside, IBU hexoside	[30]

response of the product ion. As an IDA method, in which a minimum peak intensity was the trigger to get its product, ion mass spectrum which used the selected CE is applied to all the precursor ions. Then, an agreement between the optimal CEs for the different compounds was 35 V. Using these conditions, IBU, 1-OH IBU, and 2-OH IBU provided intense characteristic MS and MS/MS fragments, whereas CBX IBU does not (see Electronic Supplementary Material (ESM) Fig. S1). The lack of CBX IBU fragmentation using a QqTOF mass spectrometer was already reported [30].

The UHPLC mobile phase composition was investigated after the optimization of MS/MS parameters, achieving symmetric and narrow peak shapes. Methanol and acetonitrile were compared for the choice of a strong mobile phase solvent. Analytes' responses were almost the same with both solvents (retention times slightly lowers with acetonitrile). However, the peak shape was not optimal and then, considering that further addition of salts and acids to improve peak shape would be required, methanol was selected since most of the salts are more soluble in it. Pure water and methanol, as well as the addition of acetic acid, formic acid, ammonium acetate, ammonium formate, and ammonium fluoride to both solvents, were tested. The analyzed compounds and IS reached the highest responses and the best peak shapes in a mobile phase containing 2.5 mM of ammonium fluoride. Hence, the optimal chromatographic conditions for the elution of IBU and its metabolites were achieved using water and methanol, both with 2.5 mM of ammonium fluoride as mobile phase in the gradient profile (see ESM Fig. S2). The suitability of this mobile phase additive to determine acidic compounds in the negative ionization, with better sensitivity than that obtained by other additives, was already reported [1, 35].

In order to obtain high levels of sensitivity and precision, the effects of ultrasound extraction time, ionic strength, pH, centrifugation time, and speed of the extraction method were studied. All determinations were performed in triplicate for each optimized extraction parameter in three independent experiments. Extraction recovery was measured as a response to the processed spiked soybean germinated sample, which was expressed as peak area and finally calculated as the mean of the replicates. As solvents, both methanol and acetonitrile were tested providing similar recoveries. Then, methanol was selected to use the same solvent through the whole procedure. However, recoveries were relatively low. The pH of the sample solution may influence the extraction efficiency because it can affect the ionization of the analyte. Since IBU and its metabolites are weakly acidic compounds (pK about 4.4), the extraction medium should be acidic enough in order to keep the analytes in their neutral form and, consequently, improve the extraction from the plant matrix to solvent. The influence of sample pH on extraction efficiency was also studied over the range 2.0–6.0. The pH was adjusted with the addition of sodium hydroxide or hydrochloric acid.

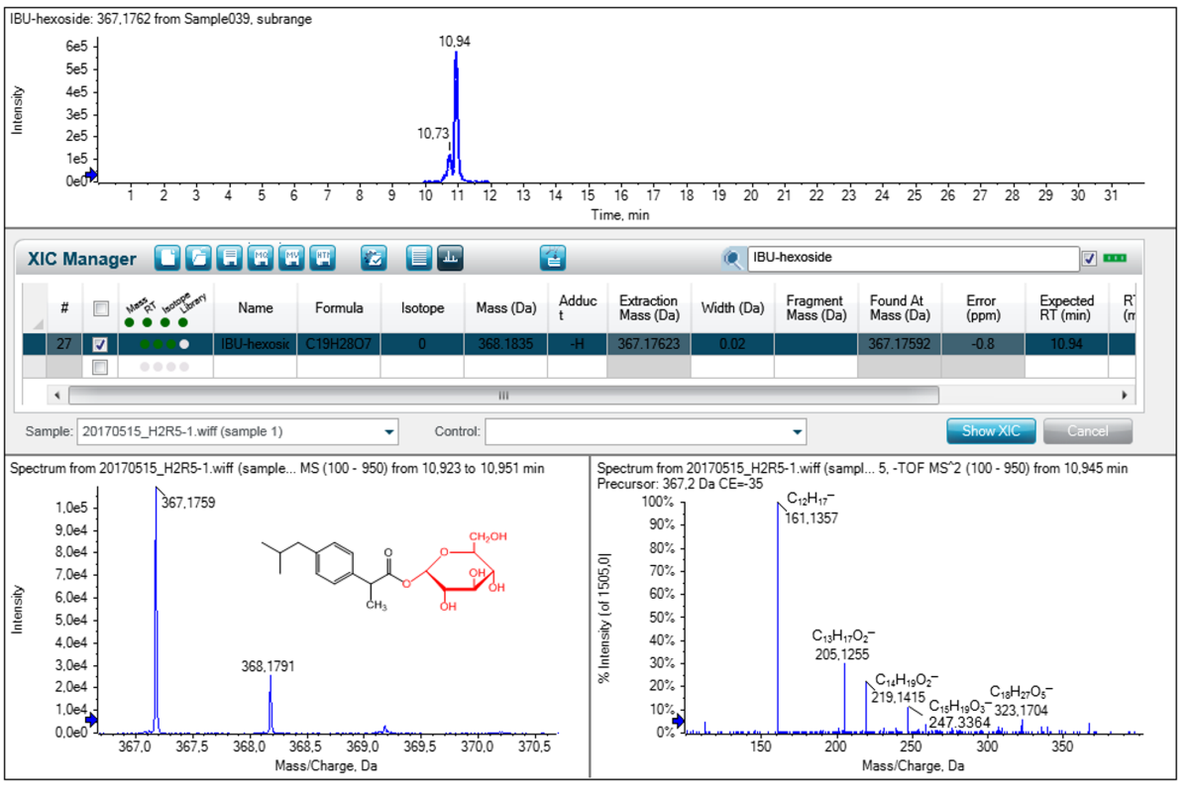

Fig. 1 Identification of IBU hexoside on a root taken at 25 days treated with 400 mg L^{-1} solution. The picture shows the XIC chromatogram, mass error (< 5 ppm), and MS and MS/MS mass spectra

Experimental results showed that the extraction efficiency of IBU and its metabolites remained unchanged at pH 2 and any further increase in pH resulted in a decrease in extraction efficiency (see ESM Fig. S3).

The effect of extraction time on the extraction efficiency of IBU and its metabolites was studied in the range of 2–20 min. Based on the experimental results, by increasing the extraction time, the peak signal was increased up to 5 min, which was sufficient for obtaining maximum extraction recovery. At longer times, an increase in extraction efficiency was not observed. Hence, an ultrasonication time of 10 min was selected for further studies. The effect of repeating the extraction procedure on the recovery was also checked up to five extraction steps. Only in the second one, about an additional 15% of IBU and its metabolites was extracted. Then, to ensure that extraction is exhaustive, this extraction procedure was repeated three times. The centrifugation time and speed were studied in the range of 2–15 min and 2500–6500 rpm, respectively. Similar results were achieved using centrifugation times between 5 and 15 min. The centrifugation step does not affect recoveries, but the optimum separation of the extract was obtained after 10 min centrifugation at 4000 rpm.

Method validation

The selectivity of the method was determined by comparing the chromatograms of IBU, its metabolites and IS obtained from a blank soybean germinated samples and the corresponding spiked soybean germinated ones. No significant interferences from endogenous substances were observed in the chromatograms of IBU-free soybean germinates at the retention times of the analytes and IS. All of the peaks for analytes and IS were detected with good peak shapes. These data show that the methodology was highly selective, and there were no endogenous substances or contaminants interfering with the quantification.

The calibration curves in the soybean germinates matrix were linear in the concentration range of 50–10,000 ng/mL for IBU and its metabolites. The correlation coefficients were 0.9996, 0.9998, 0.9994, 0.9998, and 0.9996 for IBU, 1-OH IBU, 2-OH IBU, and CBX IBU, respectively. The intraday and interday precisions of the standard solution were < 12%. The matrix effects for analytes, calculated as outlined in Table 1, ranged from − 10 to −52% considering absolute values and between 7 and − 27% considering those relative to IBU-d3. The lowest concentration with an RSD < 20% was considered as LLOQs and those for IBU and its metabolites

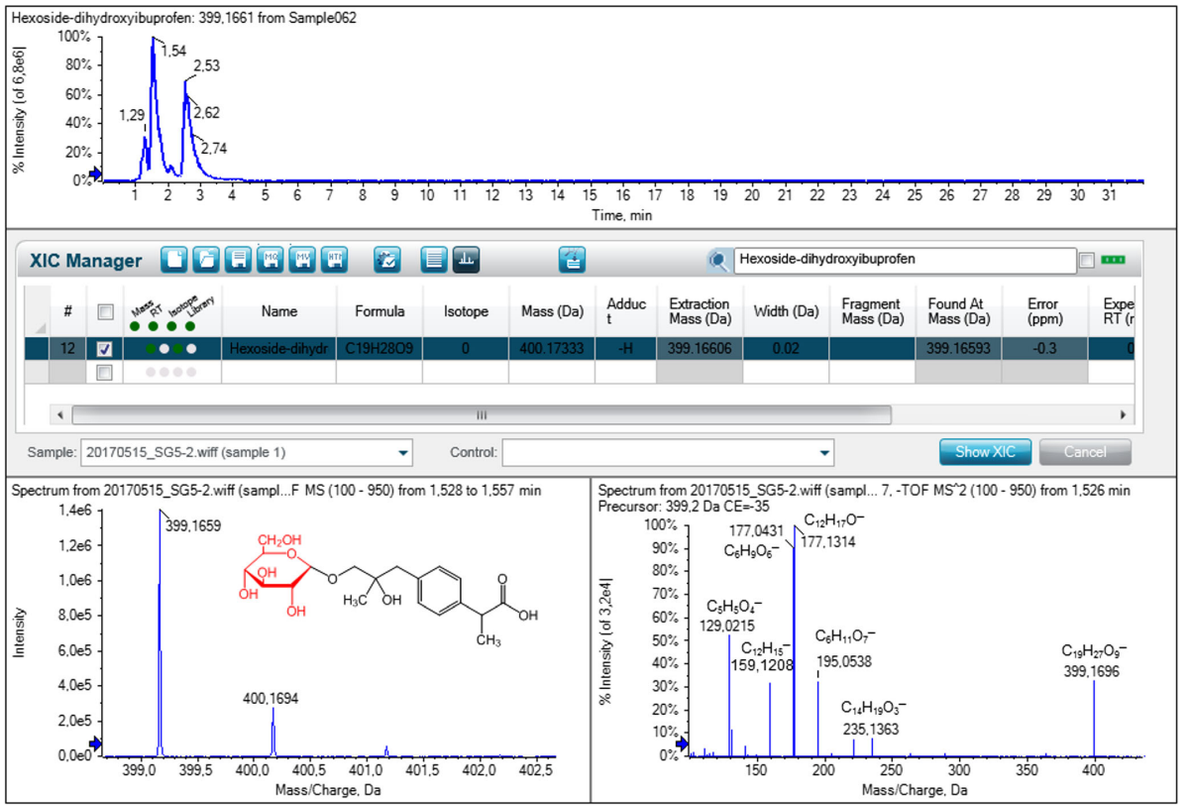

Fig. 2 Identification of DiOH IBU hexoside on a germinated seeds treated with 1800 mg L^{-1} solution. The picture shows the XIC chromatogram, mass error (< 5 ppm), and MS and MS/MS mass spectra

were found to be 50 ng mL^{-1} in the extract or 50 ng g^{-1} in the plant. This level of sensitivity is unlikely ever to be required in this application (IBU in plant materials spiked at high concentrations), but it may be useful for the studies in non-spiked crop just irrigated with treated water.

The extraction recovery of analytes from plant materials was determined by comparing the peak responses of germinated soybean samples spiked before extraction with those of germinated soybean samples spiked after extraction. As shown in Table 1, recoveries ranged from 72 to 95% with a RSD < 18% in absolute values and from 85 to 99% with a RSD < 9% in relative ones. These results indicate that the method developed could offer good extraction recovery for these analytes in germinated soybean matrices and, consequently, in any other type of legume germinates at low and high concentrations.

Ibuprofen metabolite identification

Actually, numerous strategies as well as analytical tools have been proposed to in-depth search metabolites in complex matrices, such as mass defect filtering, diagnostic fragment ion filtering, and so forth [36, 37]. In particular, multivariate statistical analysis-assisted metabolite seeking has offered a promising choice to characterize the metabolism profile [38]. However, in this study, the samples were analyzed in a more classical way. The IBU metabolite identification in plant tissues was carried out in two different ways. First, chromatograms of control and treated samples were compared, and any peak identified in the chromatogram of the treated samples that was not present in the non-treated ones was processed with the formula finder. Second, potential metabolites were identified building a database (see ESM Table S1) that compiled all the information reported in previous studies on the IBU metabolism in plants [30, 27, 21], as well as those obtained previously with the formula finder.

The visual comparison between control and treated samples pointed out the presence of IBU, 2- and 3-OH IBU, IBU hexoside, and OH IBU hexoside in most samples. However, the search against a database was able to identify 46 metabolites in the samples. Table 2 shows the identified metabolites, as well as their relevance in the three different matrices studied, and Table 3 summarizes metabolites of IBU already reported in other studies to facilitate comparison.

These metabolites can be classified into phase I metabolites that undergo several chemical reactions, and phase II

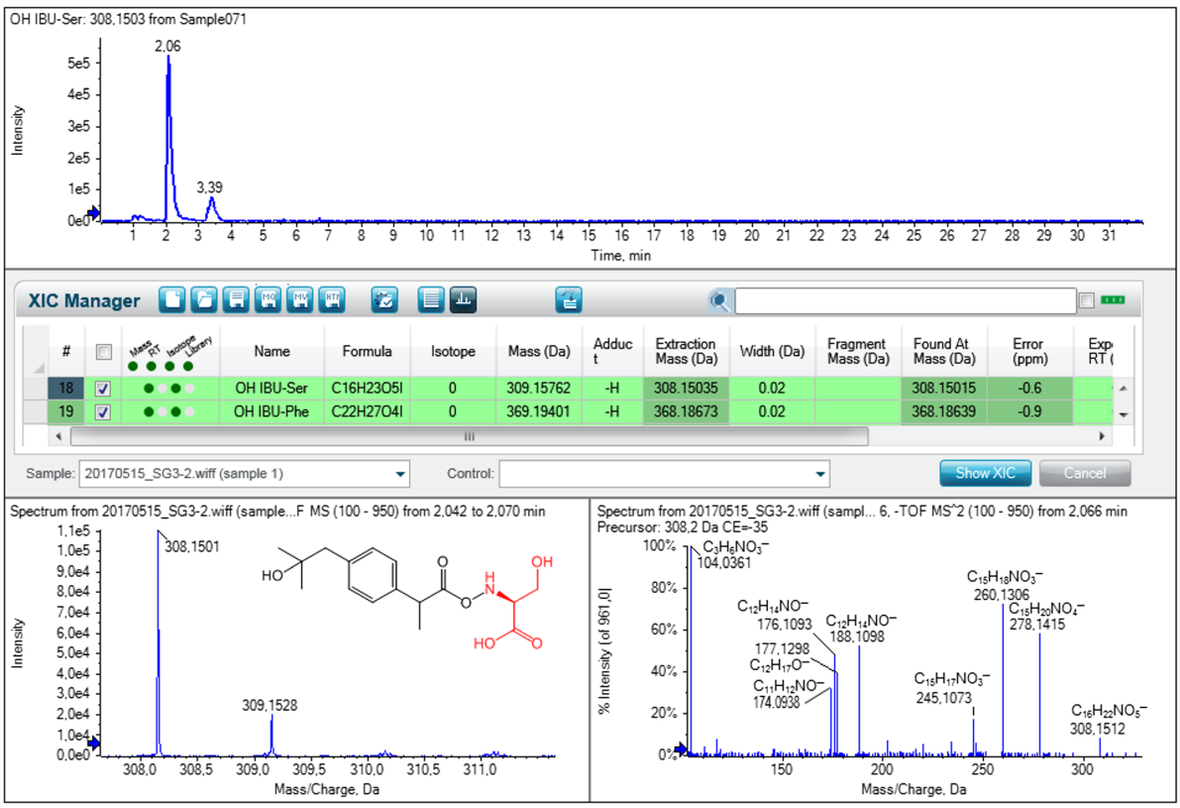

Fig. 3 Identification of OH IBU-ser on a germinated seeds treated with 1200 mg L^{-1} solution. The picture shows the XIC chromatogram, mass error (< 5 ppm), and MS and MS/MS mass spectra

metabolites mostly conjugated to other molecules to be detoxified and incorporated to plant structures or eliminated. Eight phase I metabolites mostly derived from oxidation were detected including as major compounds 1, 2, and 3OH IBU and DiOH IBU. Oxohydroxy (O, OH) IBU, trihydroxy (TriOH) IBU, oxo demethyl (O dimethyl) IBU, and oxo deethyl (O deethyl) IBU were detected at low amounts in not all the parts of the plant. CBX IBU was not detected in any of them. Cowpea possesses several intracellular or extracellular enzymes (e.g., cytochrome oxidases, peroxidases, etc.) able to oxidize compounds as one of the most important phase I detoxification steps in plants. The representative metabolic reaction in mammalian liver (and in a wide range of other organisms) is the IBU oxidation at the isobutyl chain to CBX IBU by P450 cytochrome oxidases. The results obtained here agree with those reported in the aquatic plant *L. gibba* [31] that identified OH IBP, and 1,2-DiOH IBP as intermediates, but no CBX IBP. They suggested that the metabolic pathway of IBP in duckweed was different from mammals and microbes but similar to fungi. In contrast, CBX IBP was detected in *P. australis* roots as well as *A. thaliana* cell suspension culture but at trace levels [21, 30]. These results altogether are not contradictory and clearly pointed out that CBX IBU is not a main metabolite in plants. It should be also taken into account that the OH IBP and 1,2-DiOH IBP found in plants could be from the liquid IBU phase and be uptaken by plants. Therefore, whether their presence in tissue was a result of transport from the liquid phase or production in the plant could not be distinguished.

The phase II metabolism in plants involved the conjugation of IBU to endogenous compounds (proteins, peptides, amino acids, carbohydrates) by formation of peptide, ether, esther, thioether, or other covalent bonds. Glycosylation is one of the most well-known mechanisms of detoxification in higher plants. This study confirmed the existence of IBP hexoside (main metabolite in root and seeds, Fig. 1), OH IBP hexoside (main metabolite in shoots and seeds), and OH IBP glucuronide in shoots. Figure 1 also shows as the most representative ions of IBU at *m/z* 159, 161, and 205 could be observed in the MS/MS. The hexoside conjugates were also reported in the studies carried out with *A. thaliana* cell suspension and *L. gibba* and *P. australis* plants [21, 30, 31]. Glycosyl conjugates of IBU and its derivatives were the largest and heterogeneous group of metabolites found in *V. unguiculata*, including their malonyl and acetyl derivatives. Fourteen different compounds, including OH IBU DiOH IBU and IBU

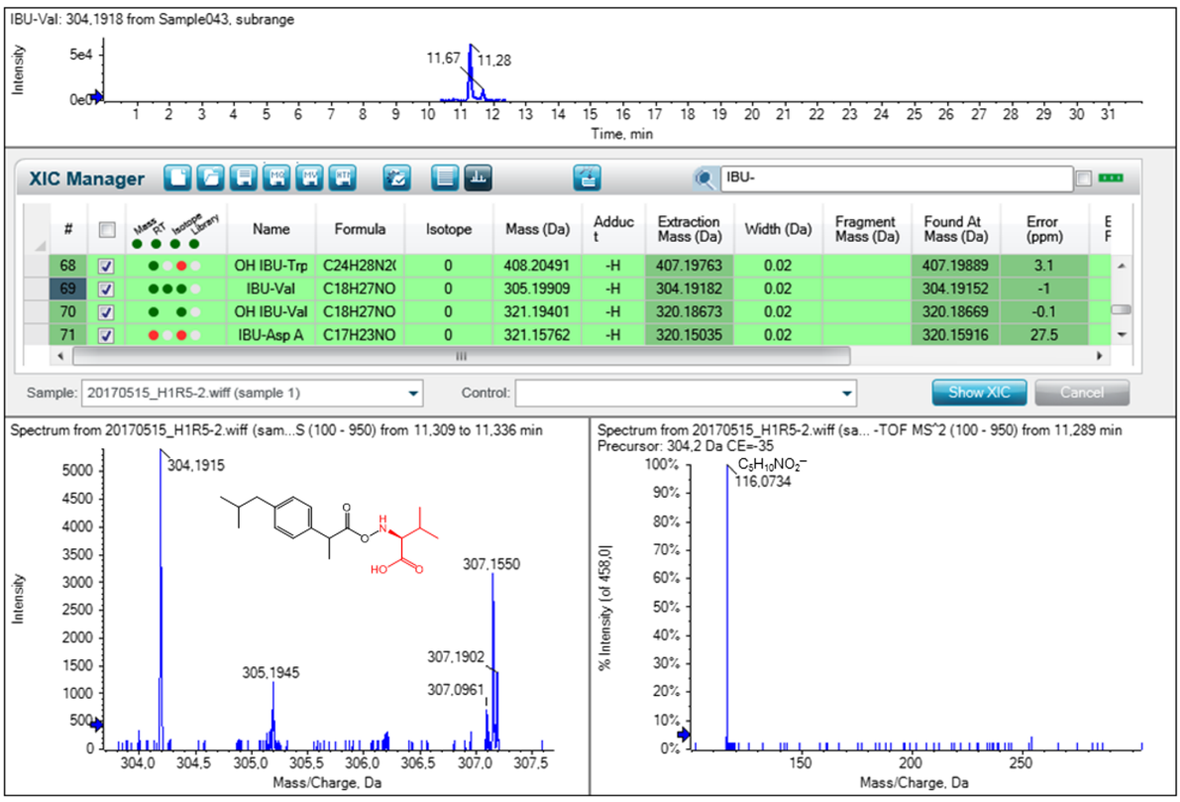

Fig. 4 Identification of IBU-Val on roots taken at 25 days treated with 1200 mg L^{-1} solution. The picture shows the XIC chromatogram, mass error (< 5 ppm), and MS and MS/MS mass spectra

derivatives with hexosides, methyl hexosides, malonyl hexosides, acetyl hexosides, and dihexosides, were found mostly in shoots. As other example of a less abundant metabolite identification, Fig. 2 shows the diOH IBU hexosides MS/MS with the two characteristic ions of OH IBU *m/z* 159.1181 and 177.0431 are present (for additional examples, see ESM Figs. S4-S6). Characteristic ions of IBU, OH IBU, DiOH IBU, and of hexosides are present in MS/MS allowing their identification. Those typical of hexose-type carbohydrates moieties were *m/z* 163.0601, *m/z* 145.0945, *m/z* 147.0652, and *m/z* 129.0546 as already described [30]. The same compound was also identified in *L. gibba* together with IBU acetyl hexoside, IBU malonyl hexoside, IBU desoxyhesoxyl hexoside, OH IBU glucuronide, OH IBU acetyl hexoside, OH IBU dihexoside, IBU malonyl hexoside, and IBU acetyl desoxyhexosyl hexoside [31]. Only the latter was not found in *V. unguiculata*. Most of these hexosides were also found in the study carried out in cell plants of *A. thaliana* [21].

Methylation and acetylation through methyl and acetyl transferases are also possible detoxification mechanisms in plants. In this study, both types of metabolites were found, particularly O,OH IBU methyl was a major metabolite in shoots and IBU acetyl is present in roots, shoots, and seeds. These esthers can be further conjugated with hexoses, given a number of the previously reported compounds. Methyl esters of IBU and its metabolites were identified as the second most abundant group of products in the cell culture of *A. thaliana* [21]. Data of IBU acetyl obtained in this study are similar to those obtained for *L. gibba* [31].

Conjugates with amino acids were reported as the third-largest group of IBU metabolites in cell cultures of *A. thaliana* [21]. Almost all were derived from monohydroxyl IBU with 16 coding amino acids. These amino acid derivatives have never been described in studies carried out in plants [30, 31]. These amino acid derivates were found in *V. unguiculata* mostly in seeds, less in roots, and insignificantly in shoots. Eighteen amino acids derivatives were found, nine in common with the previous study in *A. thaliana* cells but other nine reported for first time in plants. Combination of IBU with 7 different amino acids and of OH IBU with 11 were found. The interpretation of the mass spectrum was difficult due to the lack of previous information on the fragmentation. The Metlin Database was used to identify main fragments of amino acids in negative ionization mode [39]. For most of the proposed structures, the characteristics fragments of the amino acids were in MS/MS spectra of the tentatively identified compounds. Figures 3 and 4 show the identification of OH IBU-Ser and IBU-Val with ion characteristics of the amino acid

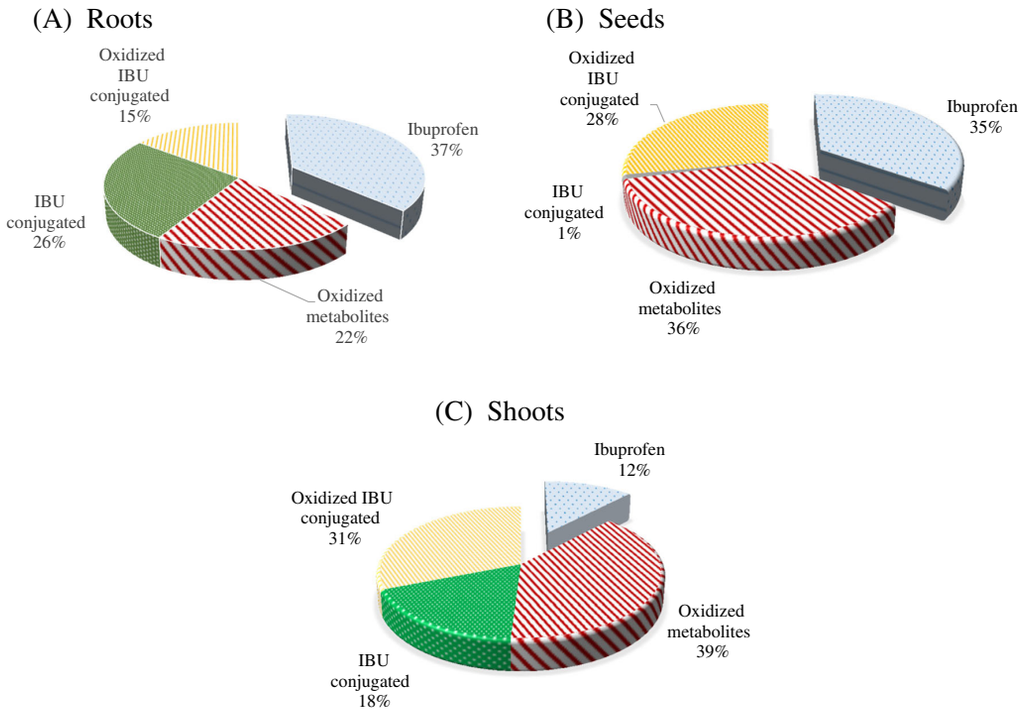

Fig. 5 Relative abundance of unaltered IBU, oxidized IBU metabolites (phase I), IBU conjugates (phase II), and oxidized IBU conjugates (phase II) in (**A**) roots, (**B**) seeds, and (**C**) shoots

and/or IBU (for additional examples see ESM Figs. S8-S9). This could be explained because legume seeds represent primary source of protein for animal feedings and human nutrition. Cowpea has a content in protein that varies between 22 and 28% depending on the genotype and total free amino acid content ranged between 1.7 and 3.7 mg/100 g seed flour [40, 41]. The high protein and amino acid content of cowpea seeds could explain why they are reported for first time in plants in this study. Legumes have also been used in intercropping systems because their capacity of fixing N_2, which could explain how even through in low amounts amino acid derivatives still appear [41].

Glutathionyl conjugates were very minor metabolites probably because glutathione-*S*-transferase (GST) mostly catalyze conjugation at electrophilic double bonds or halogen functions, which clearly explains why the IBP-glutathione conjugate was only detected in seeds treated at highest concentrations. The lack of sulfur conjugates to detoxify IBU or any of its metabolites was also pointed out in several studies [21, 30].

Uptake, bioaccumulation, and biotransformation

Different treatments at high concentrations were performed in this study in order to identify both major and minor metabolic routes. The amount of IBU in the pots, taken into account that plants were irrigated with 40 mL, was 0, 400, 800, 1200, 1600, and 2000 mg. This concentration is high compared to environmental ones but ensures identification of all potential metabolites. The IBU concentration probably was constant since several studies established a rapid removal of IBU in plant solutions following a pseudo-first-order reaction with a half-life of $t_{1/2} = 2.1$ days ($R^2 = 0.97$) [30]. There was no difference in the metabolites profile between 25- and 50-day treatment. The amount of bioaccumulated IBU is variable, but it represents a small percentage that varies between 5 and 15%. Degradation of IBU in solutions has been widely reported. The IBP relative concentrations in root, shoot, and seed tissue of treated plants is summarized in Fig. 5. The parent IBP was present in all tissues. However, its percentage was higher in seeds and roots directly in contact with the solution than in shoots. Oxidized metabolites percentage was higher in roots and shoots than in seeds. Conjugated metabolites (of parent IBU or of oxidated metabolites) are much higher in shoots. This indicates that IBP was transported upward from medium to root and seeds and further translocated through roots to shoot. As plants lack excretory pathways for xenobiotics, they store those compounds in vacuoles or cell walls or metabolize xenobiotics into nontoxic forms. Thus, *V. unguiculata* could take up, translocate, and possibly degrade IBP.

To establish potential negative effects of these treatments in *V. unguiculata* growth, several parameters responsible of germination for seeds and growing for plants harvested at 25 and

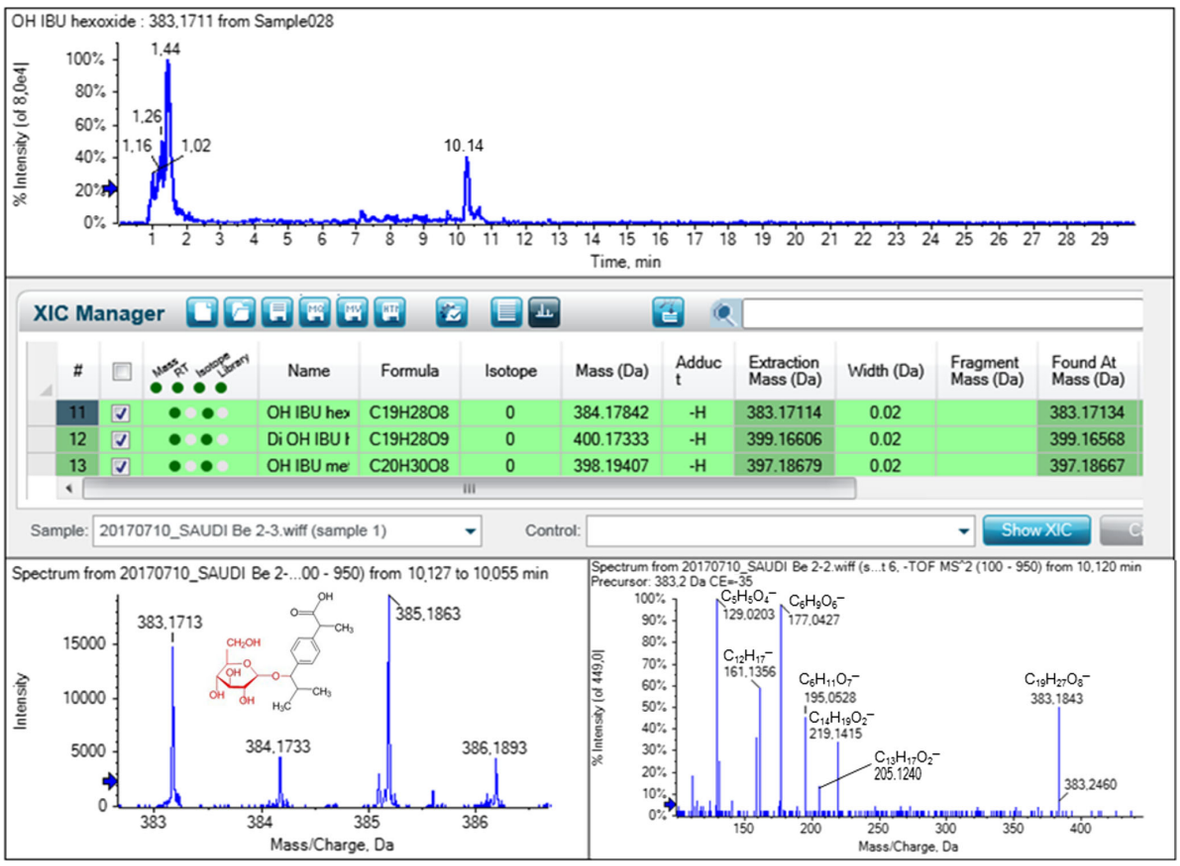

Fig. 6 Identification of OH IBU hexoside at low concentrations in an eggplant sample

50 days were analyzed (see ESM Tables S2-S5). High concentrations of IBU have 50% of inhibition in seed germination and germinate growth and provide less weight as well. On plant growth at 25 and 50 days, the most remarkable effect observed was a decrease in pigment production. The application of one-way ANOVA points out that plants were negatively affected by these treatments.

Field samples

The analysis of vegetal samples irrigated with treated water from the Riyadh WWTPs showed little incidence of IBU or its metabolites in plants. Only one eggplant showed low levels of OH IBU 52 ng g^{-1} close to the LLOQ as well as the presence of OH IBU hexoside. The latter could not be quantified since the analytical standards were not available. However, the retention time, MS, and MS/MS fit exactly the metabolite identified in the laboratory study (Fig. 6). No additional hypothesis can be established since information on the contaminants in the wastewater or irrigation treatment was not available. However, these results confirmed the applicability of the method for field samples treated with wastewater.

Conclusions

In this paper, a SLE extraction-SPE clean-up method coupled with UHPLC–QqTOF-MS/MS was developed for the determination of IBU and its metabolites in legumes. For this purpose, the extraction procedure was optimized by evaluating the influence of different parameters on the recoveries of the target compounds. Then, the analytical performance of the optimized method was evaluated achieving satisfactory linearity and precision and LLOQ of the target analytes. The proposed procedure was an efficient, simple, rapid, sensitive, and cost-effective method for the determination of IBU and its metabolites. The results showed good applicability of the proposed method for the determination of selected compounds in soya bean germinates extensible to other legume germinates.

In the present study, there are 46 metabolites of IBU in *V. unguiculata*. The 1-OH and 2-OH IBU were confirmed and quantified with the analytical standards. The structures of the other metabolites have been proposed using HRMS and HRMS/MS data. In particular, the combination of mass accuracy and the fragmentation patterns of metabolites and parent compounds allowed proposing plausible structures for

each metabolite. Six hexosides were already reported in study on *P. australis* and *L. gibba*. Thirty-eight of the identified metabolites were already reported in a study on cell cultures of *A. thaliana* and nine of them (conjugates of IBU or OH IBU with amino acids) are, up to our knowledge, reported for first time in plants. Two of the most abundant metabolites were also identified in an eggplant irrigated with treated wastewater. Then, advantages of this method are as follows: (i) attains higher identification confidence; (ii) achieves identification, for the first time, of a number of metabolites never reported plants (it should be noted that cellular cultures are not so complex matrices as the whole plant); and (iii) could be applicable to determine ibuprofen and its metabolites at low concentrations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate Not applicable.

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Analytical & Bioanalytical Chemistry

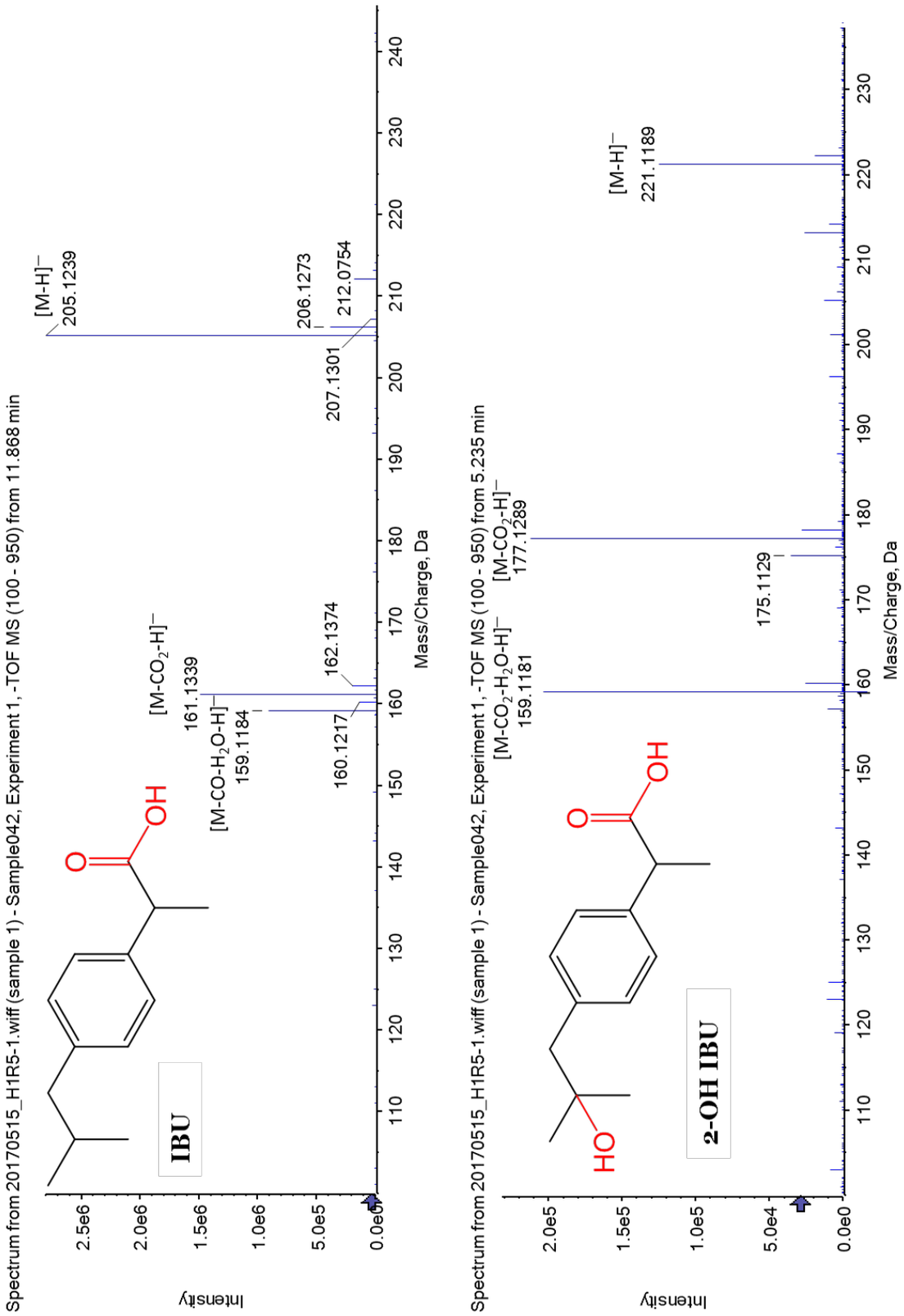
Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Analysis of ibuprofen and its main metabolites in roots, shoots, and seeds of cowpea (*Vigna unguiculata L.Walp*) using liquid chromatography-quadrupole time-of-flight mass spectrometry: uptake, metabolism, and translocation

Yolanda Picó, Rodrigo Alvarez-Ruiz, Leonard Wijaya, Ahmed Alfarhan, Mohammed Alyemeni, Damià Barceló

Figure S1. Mass spectra of parent ibuprofen and related metabolites



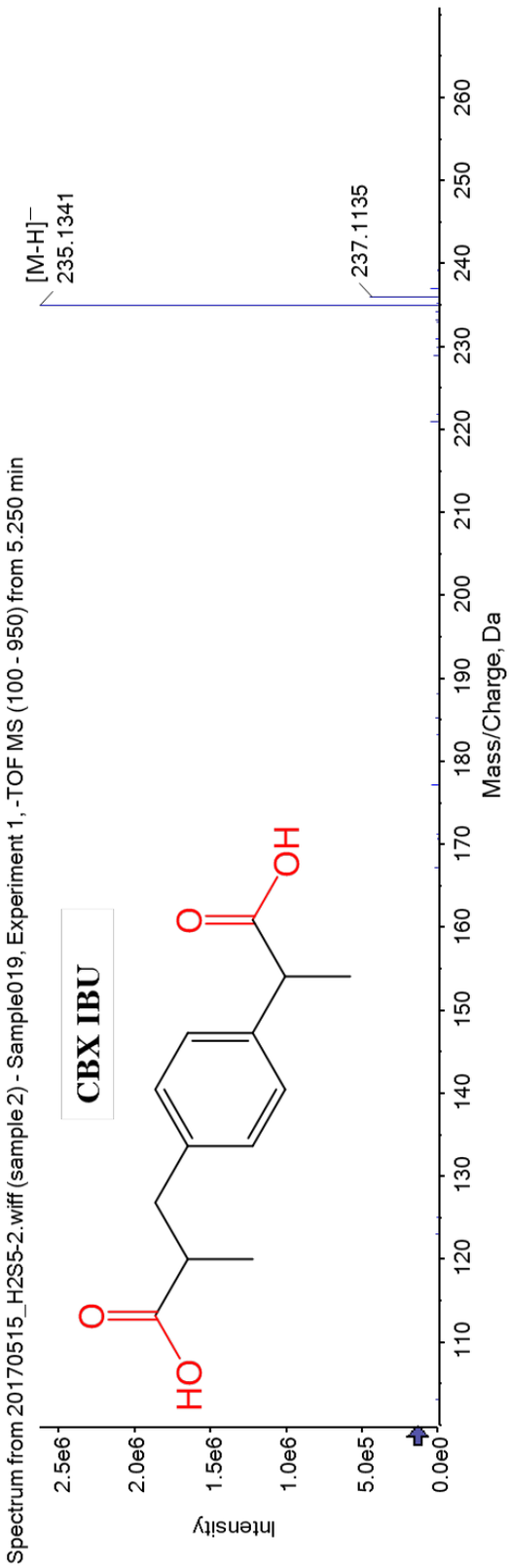
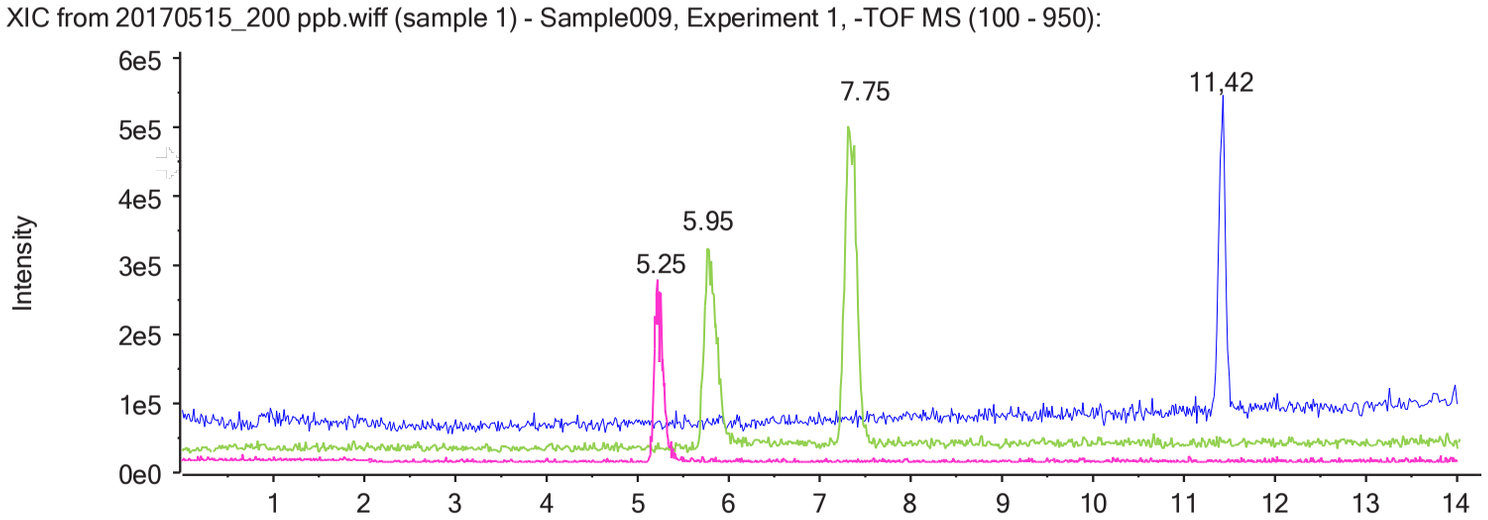

Spectrum from 20170515_H2S5-2.wiff (sample 2) - Sample019, Experiment 1, -TOF MS (100 - 950) from 5.250 min
[M-H]⁻
235.1341
237.1135
CBX IBU
HO
O
O
OH
Intensity
2.5e6
2.0e6
1.5e6
1.0e6
5.0e5
0.0e0
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
Mass/Charge, Da

Figure S2. Extract ion chromatograms (XIC) of a standard mixture of target compounds at 50 ng mL^{-1}. Peak identification t_R= 5.25 CBX IBU; t_R= 5.95 2-OH IBU; t_R= 7.75 1-OH IBU; t_R= 11.42 IBU.



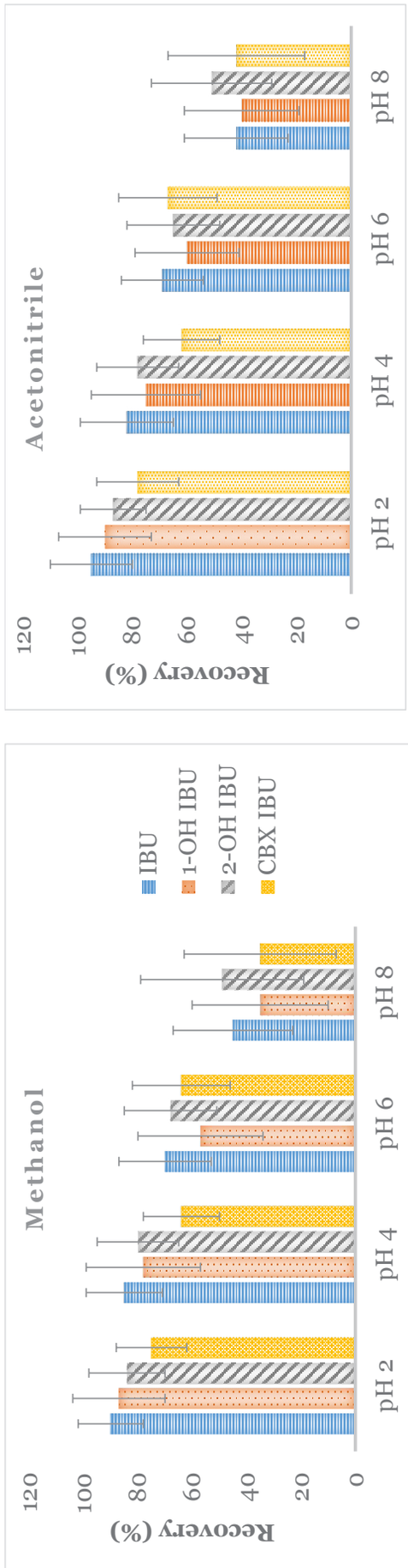


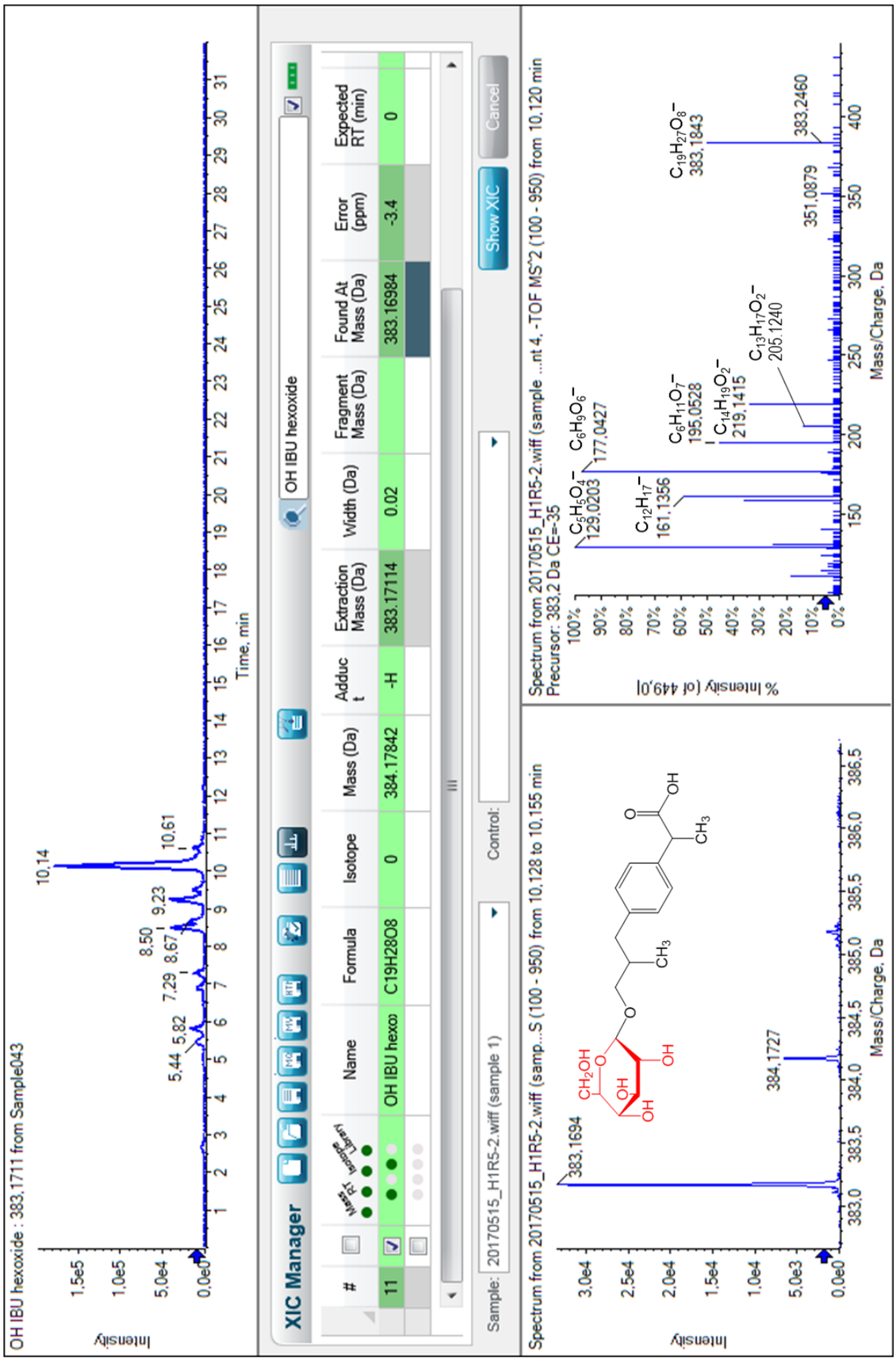
Figure S3. Optimization of the extraction solvent and sample pH

Table S1. Database of IBU metabolites used with the XIC Manager feature of the peakview 2.0

Compound name	Empirical Formula	Ion	Exact Mass	Retention time
IBU	C13H18O2	-H	205.12340	11.95
1-OH IBU	C13H18O3	-H	221.11832	7.78
2-OH IBU	C13H18O3	-H	221.11832	5.68
3-OH IBU	C13H18O3	-H	221.11832	2.45
CBX IBU	C13H16O4	-H	235.09758	1.13
IBU-d3	2H3C13H15O2	-H	208.14223	11.95
Tri OH IBU	C13H18O5	-H	253.10815	1.14
Di OH IBU	C13H18O4	-H	237.11323	1.35
glucopyranosyloxy-hydroxy-IBP	C19H27O8	-H	382.16332	4.62
oxoddemethylIBU	C12H14O3	-H	205.08702	1.04
OH IBU Methyl	C14H20O3	-H	235.13397	4.74
OH IBU hexoxide	C19H28O8	-H	383.17114	1.73
Di OH IBU hexoside	C19H28O9	-H	399.16606	1.67
OH IBU methyl hexoside	C20H30O8	-H	397.18679	11.05
OH IBU Dihexoside	C25H38O12	-H	529.22905	10.85
OH IBU Malhexoside	C22H30O11	-H	469.17154	1.16
OH IBU-Tyr	C22H27O5N	-H	384.18165	1.76
OH IBU-Thre	C17H25O5N	-H	322.16600	1.44
OH IBU-Ser	C16H23O5N	-H	308.15035	1.26
OH IBU-Phe	C22H27O4N	-H	368.18673	7.01
OH IBU-Leu	C19H29O4N	-H	334.20238	6.81
OH IBU-Ala	C16H23O4N	-H	292.15543	1.56
OH IBU-His	C19H25O4N3	-H	358.17723	1.63
OH IBU-Glu	C18H25O6N	-H	350.16091	1.29
OH IBU-Gln	C18H26O5N2	-H	349.17690	1.21
OH IBU glucuronide	C19H26O9	-H	397.15041	1.59
IBU Acetyl	C15H20O3	-H	247.13397	11.22
IBU-hexoside	C19H28O7	-H	367.17623	6.05
IBU acetyl hexoside	C21H30O8	-H	409.18679	1.24
OH IBU acetyl hexoside	C21H30O9	-H	425.18171	1.31
IBU malonyl hexoside	C22H30O10	-H	453.17662	1.05
IBU desoxyhexosyl hexoside	C25H38O11	-H	513.23414	10.38
IBU acetyl desoxyhexosyl hexoside	C27H39O12	-H	554.23688	1.27
OH IBU	C13H18O3	-H	221.11832	9.55
IBU Glutathion	C23H33N3O8S	-H	510.19156	0.97
O,OH IBU	C13H16O4	-H	235.09758	1.13
O, 2OH IBU	C13H16O5	-H	251.09250	1.17
O Deethyl IBU	C11H12O3	-H	191.07137	1.73
O demethyl IBU	C12H14O3	-H	205.08702	1.04
OH deethyl IBU	C11H14O3	-H	193.08702	1.2
OH IBU Mal Di hexoside	C28H40O16	-H	631.22436	1.05
OH(-2H) IBU methyl hexoside	C20H28O8	-H	395.17114	1.69
Di OH IBU methyl hexoside	C20H30O9	-H	413.18171	3.12
Di OH IBU methyl	C14H20O4	-H	251.12888	1.13
O IBU hexoside/OH IBU (-2H) hexoside	C19H26O8	-H	381.15549	4.62
CBX IBU methyl	C15H20O4	-H	263.12888	2.74
O,OH IBU methyl hexoside	C20H28O9	-H	411.16606	1.5
O.OH IBU hexoside/CBX IBU hexoside	C19H26O9	-H	397.15041	1.59

O,OH IBU methyl	$C_{14}H_{18}O_4$	-H	249.11323	8.11
O IBU methy/OH(.2H) IBU methyl	$C_{14}H_{18}O_3$	-H	233.11832	2.64
IBU-Meth	$C_{18}H_{27}NO_3S$	-H	336.16389	1.2
OH IBU-Arg	$C_{19}H_{30}N_4O_4$	-H	377.21943	1.57
OH IBU-Pro	$C_{18}H_{25}NO_4$	-H	318.17108	1.22
OH IBU-Lys	$C_{19}H_{30}N_2O_4$	-H	349.21328	1.51
IBU-Thre	$C_{17}H_{25}O_4N$	-H	306.17108	9.45
IBU-Ser	$C_{16}H_{23}O_4N$	-H	292.15543	8.44
IBU-Phe	$C_{22}H_{27}O_3N$	-H	352.19182	12.25
IBU-Leu	$C_{19}H_{29}O_3N$	-H	318.20747	12.23
IBU-Ala	$C_{16}H_{23}O_3N$	-H	276.16052	9.88
IBU-His	$C_{19}H_{25}O_3N_3$	-H	342.18232	8.52
IBU-Glu	$C_{18}H_{25}O_5N$	-H	334.16600	4.18
IBU-Gln	$C_{18}H_{26}O_4N_2$	-H	333.18198	8.04
IBU-Lys	$C_{19}H_{30}N_2O_3$	-H	333.21837	8.98
IBU-Pro	$C_{18}H_{25}NO_3$	-H	302.17617	1.72
IBU-Arg	$C_{19}H_{30}N_4O_3$	-H	361.22451	2.46
OH IBU-Meth	$C_{18}H_{27}NO_4S$	-H	352.15880	1.2
IBU-Trp	$C_{24}H_{28}N_2O_3$	-H	391.20272	11.8
OH IBU-Trp	$C_{24}H_{28}N_2O_4$	-H	407.19763	7.06
IBU-Val	$C_{18}H_{27}NO_3$	-H	304.19182	11.53
OH IBU-Val	$C_{18}H_{27}NO_4$	-H	320.18673	4.06
IBU-Asp A	$C_{17}H_{23}NO_5$	-H	320.15035	8.26
OH IBU-Asp A	$C_{17}H_{23}NO_6$	-H	336.14526	1.18
IBU-Asp	$C_{17}H_{23}N_2O_4$	-H	318.15851	1.31
OH IBU-Asp	$C_{17}H_{23}N_2O_5$	-H	334.15342	4.18
IBU-Gly	$C_{20}H_{21}NO_3$	-H	322.14487	10.77
OH IBU-Gly	$C_{20}H_{23}NO_4$	-H	340.15543	12
IBU-Cys	$C_{16}H_{23}NO_3S$	-H	308.13259	1.25
OH IBU-Cys	$C_{16}H_{23}NO_4S$	-H	324.12750	1.28

Figure S4. Identification of **OH IBU hexoside** in a root sample treated with 400 mg L^{-1} of IBU



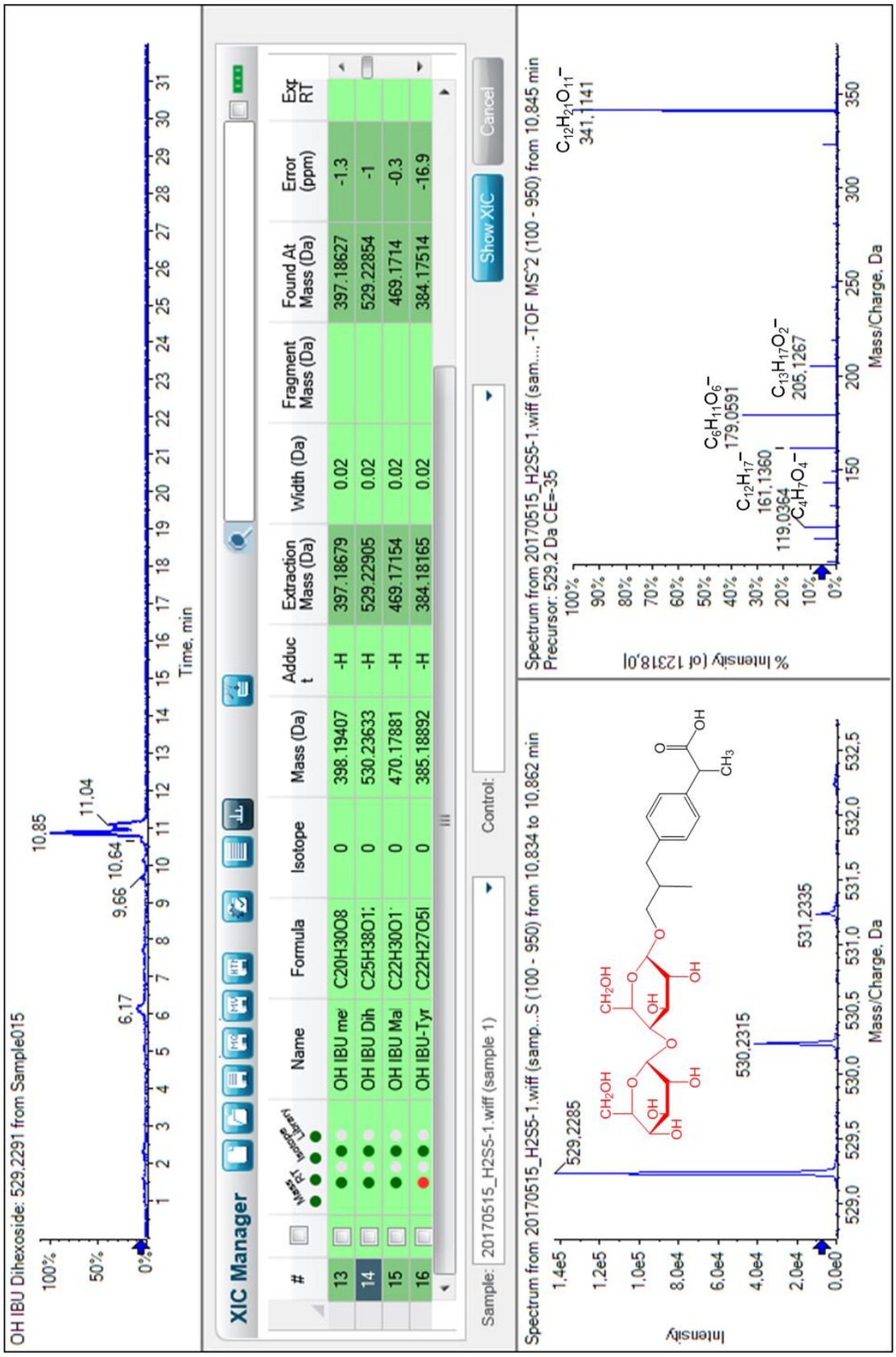
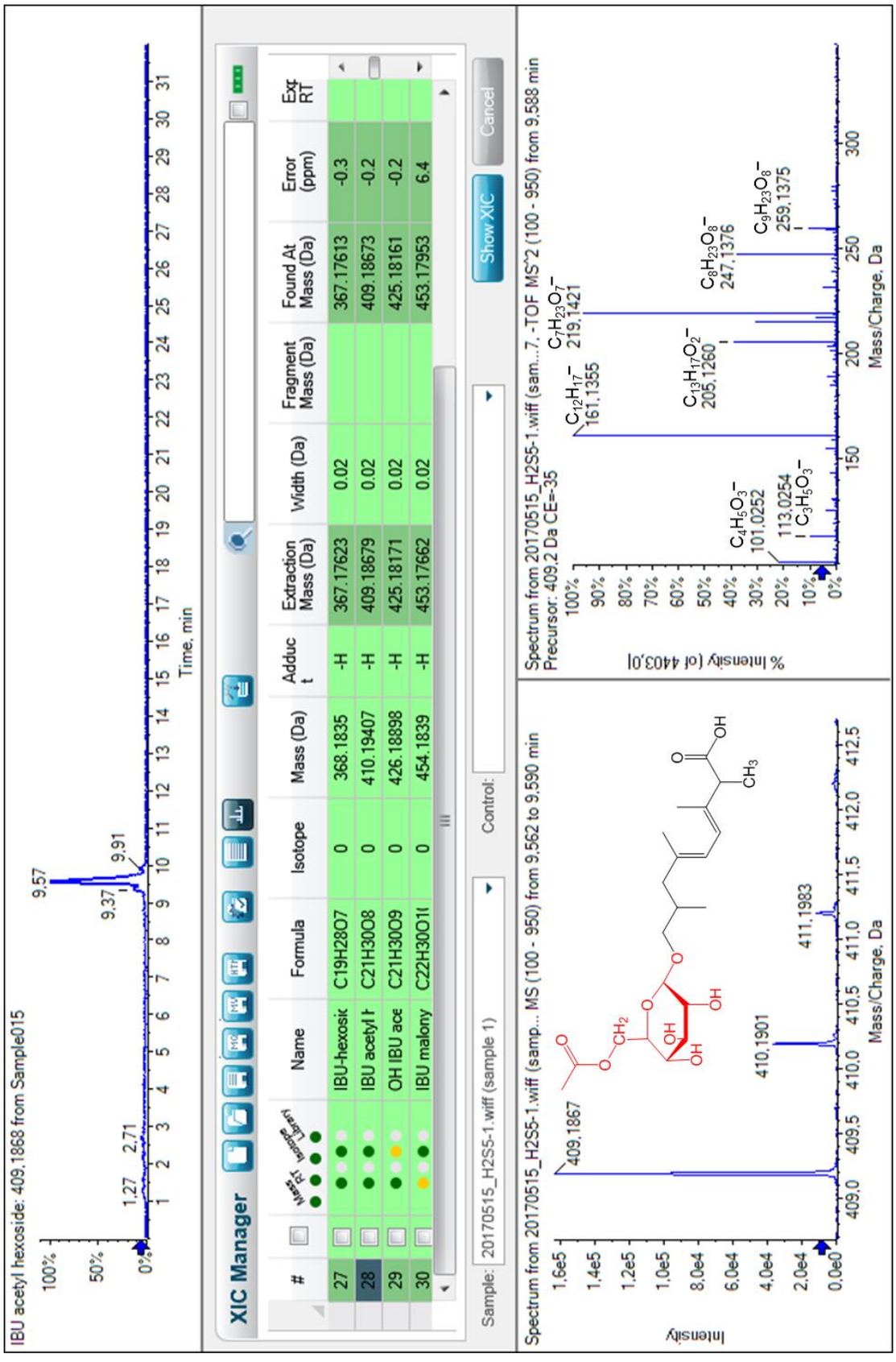


Figure S5. Identification of **OH IBU di hexoside** in a root sample treated with 400 mg L^{-1} of IBU



#	Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Fragment Mass (Da)	Found At Mass (Da)	Error (ppm)	Exp RT
27	IBU-hexosic	C19H28O7	0	368.1835	-H	367.17623	0.02		367.17613	-0.3	
28	IBU acetyl h	C21H30O8	0	410.19407	-H	409.18679	0.02		409.18673	-0.2	
29	OH IBU ace	C21H30O9	0	426.18898	-H	425.18171	0.02		425.18161	-0.2	
30	IBU malony	C22H30O11	0	454.1839	-H	453.17662	0.02		453.17953	6.4	

Figure S6. Identification of **IBU acetyl hexoside** in a root sample treated with 400 mg L^{-1} of IBU

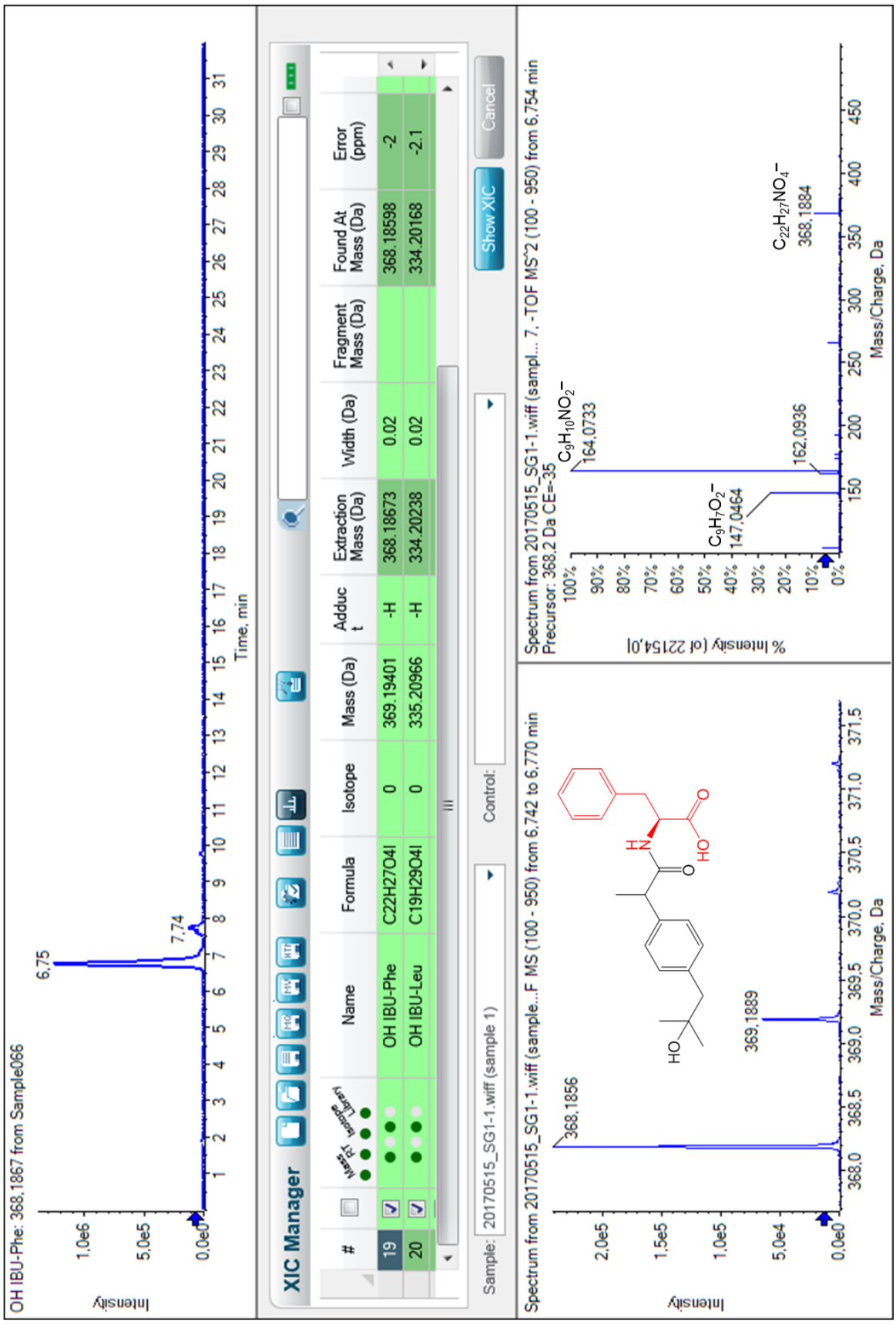


Figure S7. Identification of of **OH IBU-Phe** in a seed sample treated with 400 mg L^{-1} of IBU

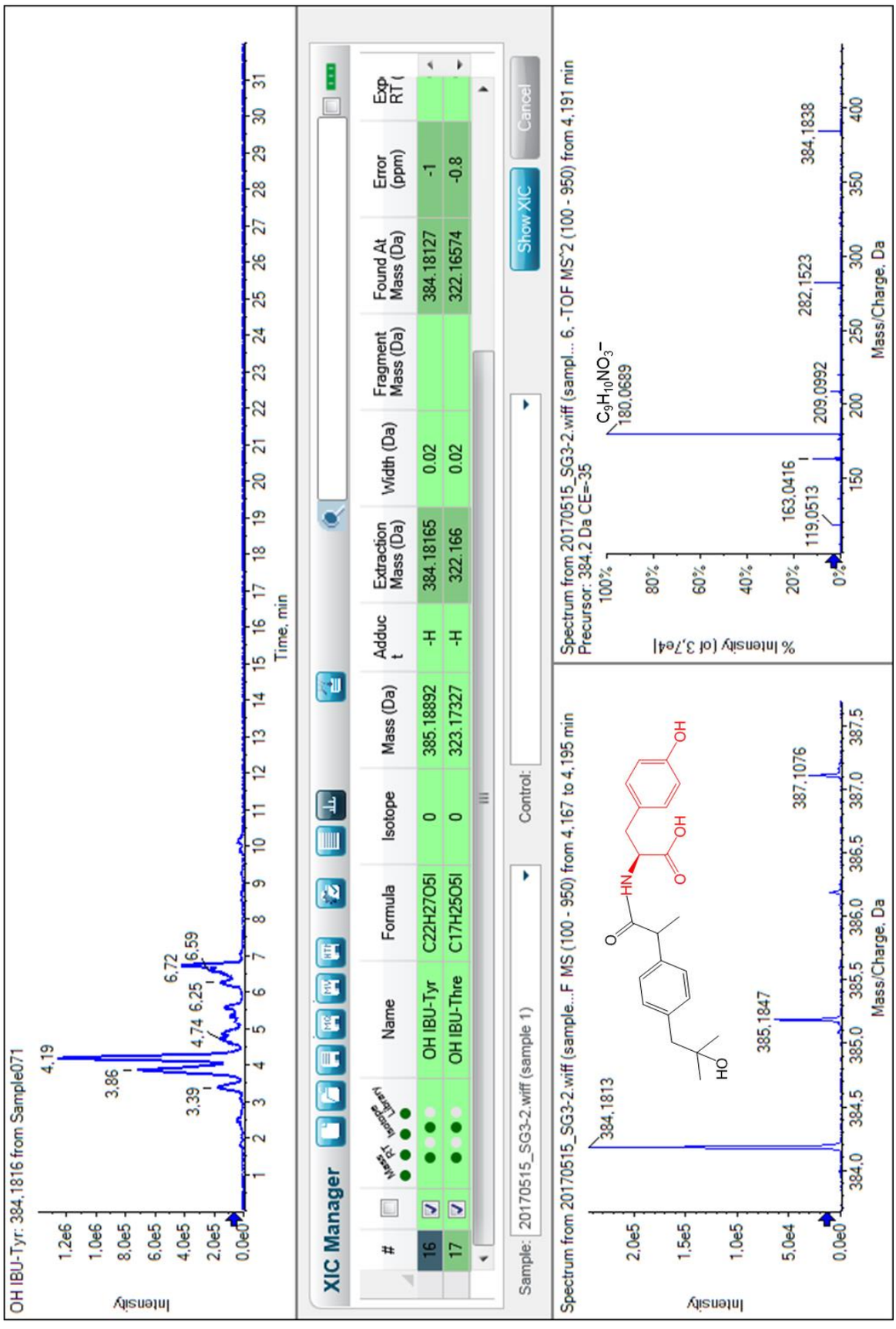


Figure S8. Identification of OH IBU-Val in a germinated seed treated with 1200 mg L^{-1} of IBU

Figure S9. Identification of OH IBU-Tyr in a germinated seed treated with 1200 mg L^{-1} of IBU

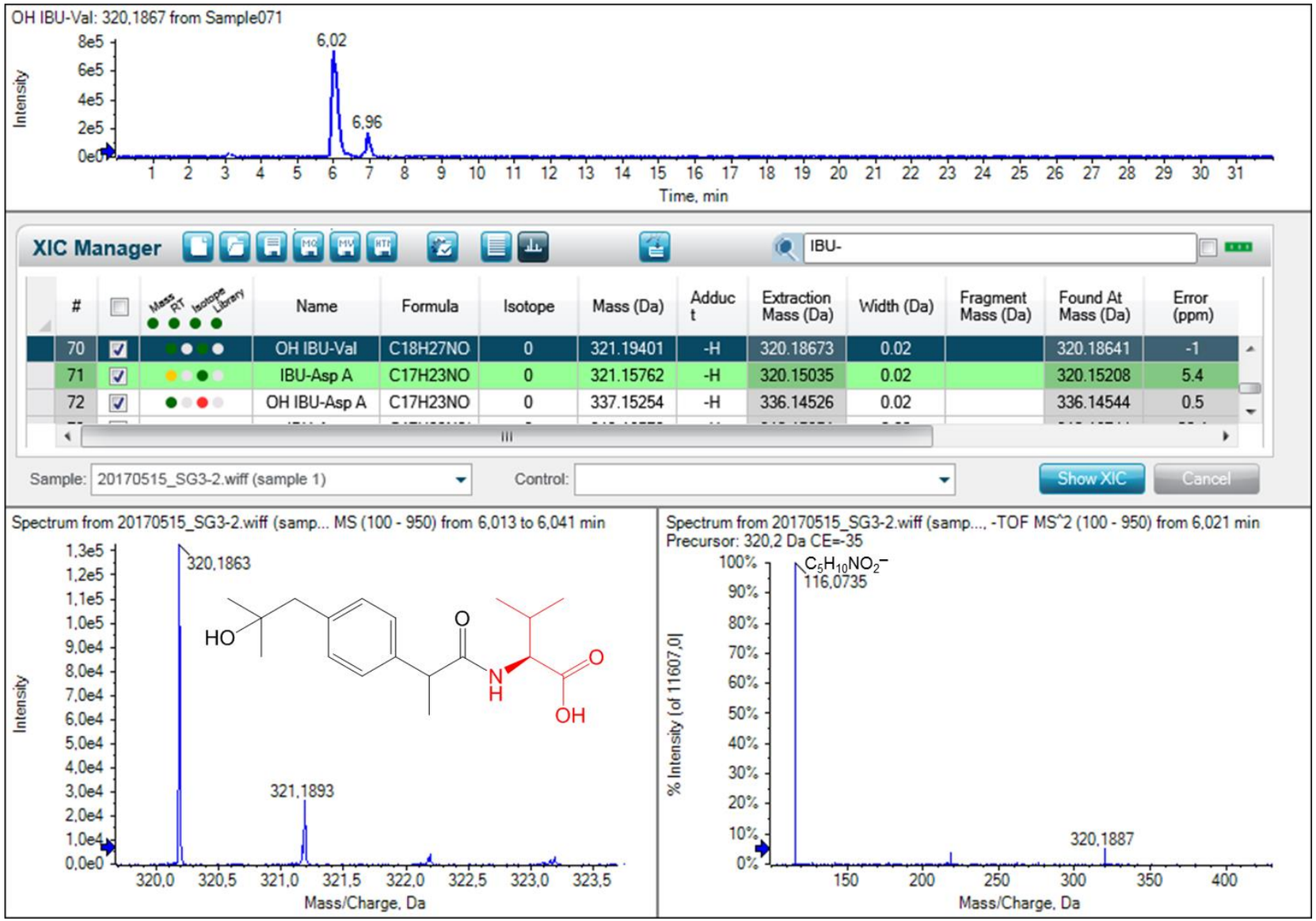


Table S2 Parameters affected seed germination according to IBU concentration including percentage of germination/day, length and weight

Factor	Control (0)	400 mg/L	800 mg/L	1200 mg/L	1600 mg/L	2000 mg/L	Total Average	F-Value	P-Value
Day1	66.67 ± 5.78	70.00 ± 10.00	76.67 ± 5.78	70.00 ± 10.00	60.00 ± 10.00	53.34 ± 5.78	66.12 ± 10.37	3.08	0.05
Day2	100.00 ± 0.00	90.00 ± 0.00	86.67 ± 5.78	83.34 ± 11.55	70.00 ± 10.00	63.34 ± 11.55	82.23 ± 14.38	8.13	0.00
Day3	100.00 ± 0.00	96.67 ± 5.78	93.34 ± 11.55	86.67 ± 15.28	70.00 ± 10.00	70.00 ± 10.00	86.12 ± 15.01	5.26	0.01
Day4	100.00 ± 0.00	96.67 ± 5.78	93.34 ± 11.55	86.67 ± 15.28	73.34 ± 5.78	70.00 ± 10.00	86.67 ± 14.15	5.25	0.01
Day5	100.00 ± 0.00	96.67 ± 5.78	93.34 ± 11.55	86.67 ± 15.28	73.34 ± 5.78	70.00 ± 10.00	86.67 ± 14.15	5.25	0.01
Hlength	6.60 ± 0.37	5.67 ± 0.26	5.07 ± 0.16	4.50 ± 0.20	3.87 ± 0.33	2.74 ± 0.64	4.74 ± 1.32	43.73	0.00
Elength	2.34 ± 0.16	2.47 ± 0.16	2.34 ± 0.21	2.27 ± 0.31	2.47 ± 0.06	1.90 ± 0.18	2.30 ± 0.26	3.63	0.03
HFW	4.20 ± 0.06	3.84 ± 0.07	3.57 ± 0.10	3.33 ± 0.05	3.13 ± 0.07	2.94 ± 0.07	3.50 ± 0.45	145.19	0.00
EFW	1.11 ± 0.07	1.17 ± 0.09	1.13 ± 0.12	1.12 ± 0.11	1.22 ± 0.07	1.25 ± 0.22	1.17 ± 0.12	0.73	0.62
HDW	0.77 ± 0.03	0.73 ± 0.02	0.66 ± 0.02	0.65 ± 0.01	0.59 ± 0.02	0.55 ± 0.02	0.66 ± 0.08	93.00	0.00
EDW	0.22 ± 0.03	0.23 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.24 ± 0.01	0.23 ± 0.04	0.23 ± 0.03	0.22	0.94

Legends

Hlength = Hypocotyl length (cm)
Elength = Epicotyl length (cm)
HFW=Hypocotyl Fresh Weight (10 seedling in g)
EFW=Epicotyl Fresh Weight (10 seedling in g)
HDW=Hypocotyl Dry Weight (10 seedling in g)
EDW=Epicotyl Dry Weight (10 seedling in g)
Day 1 = Germination percentage at Day 1

Table S3 Parameters affected plant growth according to IBU concentration for 25 days plants

Factor	Control (0)	400 mg/L	800 mg/L	1200 mg/L	1600 mg/L	2000 mg/L	Total Average	F-Value	P-Value
SPAD	53.40 ± 1.30	47.60 ± 0.66	48.97 ± 3.01	52.17 ± 4.35	48.70 ± 0.85	46.77 ± 1.37	49.60 ± 3.15	3.77	0.03
SL	13.40 ± 1.73	10.40 ± 1.37	10.63 ± 1.17	11.90 ± 1.41	10.63 ± 0.59	9.47 ± 1.29	11.07 ± 1.70	3.34	0.04
LA	352.00 ± 26.96	302.00 ± 13.00	306.33 ± 6.66	320.00 ± 35.37	281.00 ± 32.91	233.00 ± 44.51	299.06 ± 45.04	5.48	0.01
FLW	7.36 ± 0.60	6.17 ± 0.59	6.57 ± 0.80	7.07 ± 0.50	5.85 ± 0.77	4.93 ± 1.12	6.32 ± 1.04	4.05	0.02
FSW	3.05 ± 0.38	2.19 ± 0.40	2.31 ± 0.52	2.60 ± 0.50	1.78 ± 0.27	1.69 ± 0.41	2.27 ± 0.60	4.39	0.02
RL	28.83 ± 3.14	27.73 ± 4.84	36.97 ± 1.67	29.03 ± 0.59	30.13 ± 2.24	30.20 ± 3.55	30.48 ± 4.00	3.64	0.03
FRW	6.75 ± 1.06	6.20 ± 1.91	7.36 ± 0.99	8.22 ± 1.06	5.67 ± 1.25	6.00 ± 2.24	6.70 ± 1.55	1.21	0.36
DLW	0.75 ± 0.04	0.65 ± 0.02	0.88 ± 0.12	0.88 ± 0.07	0.71 ± 0.09	0.60 ± 0.14	0.75 ± 0.13	5.09	0.01
DSW	0.26 ± 0.04	0.19 ± 0.04	0.30 ± 0.07	0.32 ± 0.03	0.23 ± 0.03	0.21 ± 0.05	0.25 ± 0.06	4.11	0.02
DRW	0.31 ± 0.04	0.29 ± 0.09	0.41 ± 0.02	0.37 ± 0.04	0.32 ± 0.07	0.32 ± 0.12	0.34 ± 0.07	1.27	0.34
Chla	0.86 ± 0.05	0.52 ± 0.04	0.55 ± 0.02	0.49 ± 0.02	0.49 ± 0.00	0.44 ± 0.00	0.56 ± 0.14	86.03	0.00
Chlb	0.80 ± 0.02	0.51 ± 0.03	0.52 ± 0.03	0.55 ± 0.01	0.61 ± 0.03	0.53 ± 0.02	0.59 ± 0.10	60.32	0.00
Car	0.30 ± 0.02	0.20 ± 0.01	0.22 ± 0.03	0.27 ± 0.00	0.27 ± 0.00	0.26 ± 0.01	0.25 ± 0.04	11.86	0.00
TotChl	1.35 ± 0.04	0.87 ± 0.01	0.85 ± 0.06	0.85 ± 0.03	0.87 ± 0.02	0.82 ± 0.01	0.94 ± 0.19	110.40	0.00

SPAD =Total Chlorophyll in SPAD unit
SL = Shoot Length (cm)
LA = Leaf Area in cm2
FLW = Fresh Leaf Weight (g)
RL = Root Length (cm)
FSW = Fresh Stem Weight (g)
FRW = Fresh Root Weight (g)
DLW = Dry Leaf Weight (g)
DSW = Dry Stem Weight (g)
DRW = Dry Root Weight (g)
Chl a = Chlorophyll a (mg/g)
Chl b = Chlorophyll b (mg/g)
CAR = Carotenoid (mg/g)
Tot Chl = Total Chlorophyll (mg/g)

Table S4 Parameters affected plant growth according to IBU concentration for 50 days plants

Factor	Control (0)	400 mg/L	800 mg/L	1200 mg/L	1600 mg/L	2000 mg/L	Total Average	F-Value	P-Value
SPAD	61.70 ± 2.69	55.53 ± 1.85	50.03 ± 3.10	40.27 ± 0.21	38.97 ± 1.88	36.33 ± 7.25	47.14 ± 10.05	24.57	0.00
SL	109.83 ± 7.29	93.00 ± 6.08	80.73 ± 1.78	65.50 ± 2.81	55.83 ± 4.08	44.13 ± 4.16	74.84 ± 23.28	79.42	0.00
LA	883.75 ± 69.42	774.63 ± 45.42	555.83 ± 21.53	431.92 ± 2.13	386.91 ± 2.53	264.19 ± 11.50	549.54 ± 225.87	136.59	0.00
FLW	24.12 ± 0.66	19.73 ± 0.53	14.36 ± 1.14	8.43 ± 0.81	6.33 ± 0.37	5.52 ± 0.26	13.08 ± 7.20	367.02	0.00
FSW	11.77 ± 0.82	9.08 ± 0.61	7.91 ± 0.12	6.92 ± 0.03	5.49 ± 0.06	4.49 ± 0.41	7.61 ± 2.49	98.82	0.00
RL	32.30 ± 2.01	39.93 ± 3.67	38.00 ± 2.26	33.53 ± 1.50	32.33 ± 1.10	37.67 ± 2.59	35.63 ± 3.67	6.00	0.01
FRW	10.01 ± 0.42	11.95 ± 0.59	7.56 ± 0.47	6.33 ± 0.44	5.73 ± 0.16	5.49 ± 0.37	7.85 ± 2.47	110.51	0.00
DLW	3.40 ± 0.05	2.38 ± 0.15	1.93 ± 0.07	1.48 ± 0.08	1.31 ± 0.05	1.23 ± 0.02	1.95 ± 0.78	319.30	0.00
DSW	2.75 ± 0.06	1.89 ± 0.09	1.77 ± 0.01	1.30 ± 0.05	1.15 ± 0.07	0.98 ± 0.10	1.64 ± 0.61	260.51	0.00
DRW	0.88 ± 0.02	1.05 ± 0.02	0.75 ± 0.01	0.67 ± 0.01	0.57 ± 0.02	0.57 ± 0.02	0.75 ± 0.18	428.39	0.00
Chla	0.66 ± 0.01	0.58 ± 0.00	0.48 ± 0.02	0.41 ± 0.02	0.37 ± 0.01	0.33 ± 0.01	0.47 ± 0.12	251.18	0.00
Chlb	0.76 ± 0.11	0.70 ± 0.02	0.64 ± 0.05	0.56 ± 0.02	0.56 ± 0.01	0.54 ± 0.00	0.63 ± 0.09	8.99	0.00
Car	0.34 ± 0.01	0.32 ± 0.00	0.29 ± 0.01	0.26 ± 0.01	0.24 ± 0.00	0.23 ± 0.01	0.28 ± 0.04	92.78	0.00
TotChl	1.13 ± 0.07	1.06 ± 0.01	0.91 ± 0.02	0.85 ± 0.01	0.83 ± 0.01	0.78 ± 0.01	0.93 ± 0.13	67.96	0.00

SPAD =Total Chlorophyll in SPAD unit
SL = Shoot Length (cm)
LA = Leaf Area in cm2
FLW = Fresh Leaf Weight (g)
RL = Root Length (cm)
FSW = Fresh Stem Weight (g)
FRW = Fresh Root Weight (g)
DLW = Dry Leaf Weight (g)
DSW = Dry Stem Weight (g)
DRW = Dry Root Weight (g)
Chl a = Chlorophyll a (mg/g)
Chl b = Chlorophyll b (mg/g)
CAR = Carotenoid (mg/g)
Tot Chl = Total Chlorophyll (mg/g)

SECCIÓN 5.

RESUMEN Y CONCLUSIONES

01

Resumen

1. Fuentes de vertido de contaminantes orgánicos

A pesar de la mejora continua de los sistemas de eliminación y depuración, las estaciones depuradoras de aguas residuales (EDARs), siguen siendo una de las principales fuentes de vertido de contaminantes antropogénicos en los medios acuáticos [1-3]. Entre ellos se encuentran contaminantes orgánicos ampliamente estudiados, como los hidrocarburos aromáticos policíclicos (PAHs), y contaminantes que son considerados de preocupación emergente, tales como fármacos y productos del cuidado personal (PPCPs), drogas de abuso, retardantes de llama o sustancias perfluoroalquiladas (PFASs) [4]. Asimismo, hay contaminantes entre los que destacan ciertos PFASs, los plaguicidas o los microplásticos que, aunque también se detectan en efluentes de EDARs [2, 3, 5], tienen vías alternativas de vertido al medio ambiente. Como las aguas de escorrentía y subterráneas, provenientes de aeropuertos en el caso de los PFASs [6] y de cultivos en el caso plaguicidas [7], o la gestión inapropiada de residuos en el caso de los microplásticos [8].

Los estudios en las zonas de Al-Asfar y Al-Hubail, en Arabia Saudita, no solo confirmaron que las EDARs son potenciales fuentes de vertido de contaminantes, si no que el vertido de aguas sin tratar (de origen doméstico, industrial, ganadero, etc.) al medio ambiente es una problemática todavía presente en muchos países, con las consecuencias ambientales que ello implica. La combinación de vertidos sin tratar y procedentes de EDARs, dio lugar a una a fuerte presión antrópica en estas áreas. Donde se observó la presencia de 21 plaguicidas, 26 PPCPs y microplásticos; siendo atorvastatin, carbendazima, cafeína, etoricoxib, lorazepam, metformina, paracetamol, ácido salicílico y tramadol los compuestos más frecuentemente detectados.

Lo que tienen en común todas estas fuentes de vertido es que suelen acabar desembocando en el medio acuático [9-12], el cual no solo se ve afectado directamente por estos contaminantes, si no que sirve como vector para la propagación de los mismos a otros ambientes acuáticos (ríos, mar, lagos, humedales, etc.) y terrestres (campos de regadío, zonas costeras, etc.). Lo cual acaba afectando a su vez a todos los seres vivos en general, que dependen del agua para su supervivencia.

2. Presencia de contaminantes orgánicos y microplásticos en el medio ambiente

2.1. Agua

Se analizaron muestras de agua provenientes de los lagos Al-Asfar y Al-Hubail (Arabia Saudita) y de los canales de drenaje que los suplen de agua. Estos canales sufren la descarga de aguas residuales sin tratar de uso doméstico, fábricas y explotaciones ganaderas y agrícolas. Las aguas se extrajeron utilizando extracción en fase solida (SPE) y se analizaron mediante cromatografía líquida acoplada a espectrometría de masas (LC-MS/MS) [10, 13].

Se detectaron un total de 16 plaguicidas con concentraciones medias de 0.1-146 ng/L. Diazinón fue el plaguicida que mostró la concentración más alta (1016 ng/L) y la segunda frecuencia más alta (80% de las muestras), solo superado por la carbendazima, la cual estuvo presente en el 100% de las muestras. En el caso de los PPCPs, se detectaron un total de 24 compuestos con concentraciones medias de 0.1-4449 ng/L. Y hasta nueve compuestos detectados en el 100% de las muestras: atorvastatina, cafeína, etoricoxib, lorazepam, metformina, ofloxacina, paracetamol, ácido salicílico y tramadol. Tanto en el caso de los plaguicidas, como los PPCPs, las concentraciones más elevadas fueron detectadas en las aguas procedentes de los canales de drenaje. La elevada frecuencia, junto a las elevadas concentraciones (diazinón, cafeína, diclofenaco, ibuprofeno y paracetamol mostraron concentraciones >1000 ng/L en algunos puntos de muestreo), pusieron de reflejo la importante presión antrópica que sufre la zona.

2.2. Suelo y sedimento

Se analizaron suelos y sedimentos procedentes de las áreas de Al-Asfar y Al-Hubail, en Arabia Saudita, zonas cuyas características se han descrito anteriormente. Los cuales fueron liofilizados y extraídos usando una extracción asistida por ultrasonidos (UAE) con EDTA McIlvaine y analizadas mediante LC-MS/MS [13, 14]. En las muestras de suelo se detectaron 5 plaguicidas y 10 PPCPs, con concentraciones medias siempre por debajo de 21 ng/g. Y siendo clorpirifós, bisfenol A, cafeína y ácido salicílico, los compuestos detectados en todas las muestras. En sedimentos se detectó una cantidad significativamente superior de compuestos, con un total de 9 plaguicidas y 17 PPCPs. Las concentraciones también fueron más elevadas, llegando a los 389 ng/g de media en el caso de la simvastatina. Y la atorvastatina, la cafeína, el

etoricoxib, el lorazepam, el paracetamol, la simvastatina y el tramadol fueron detectados en todas las muestras analizadas. Estos resultados podrían ser debido al hecho de que los sedimentos están continuamente en contacto con el agua, la cual, como se ha explicado anteriormente, presentó frecuencias y concentraciones elevadas de PPCPs.

También se analizaron muestras de sedimento procedentes del río Turia en Valencia (España) utilizando métodos no dirigidos (o non-target) comparándolos con una biblioteca de más de 600 compuestos. Las metodologías se explican detalladamente en su sección correspondiente. El espectro de referencia para las identificaciones tentativas se tomó de la base de datos METLIN. En total se detectaron hasta 21 compuestos, entre los que destacó la presencia de plaguicidas (como el imazalil). De estos compuestos 13 fueron detectados tentativamente y 8 se confirmaron con patrones de referencia.

2.3. Biota

Se analizaron muestras de vegetación silvestre en las zonas colindantes a los lagos de Al-Asfar y Al-Hubail, y sus canales de drenaje. Las muestras se extrajeron y analizaron con los mismos protocolos que suelo y sedimentos [13, 14]. Se detectaron 7 plaguicidas siendo clorpirifós el más frecuente (80% de las muestras). Las concentraciones medias fueron bajas (<0.7 ng/g), excepto para fentión-sulfona (17 ng/g), el cuál no fue detectado en agua, suelo ni sedimento de la misma zona. También se detectaron 14 PPCPs, con bisfenol A y ácido salicílico siendo detectados en todas las muestras. Las concentraciones medias fueron más elevadas que para los plaguicidas, con un rango de 0.02-118 ng/g, excepto para el ácido salicílico, que tuvo una concentración media de 492 ng/g y máxima de 1952 ng/g. Sin embargo, el ácido salicílico se puede encontrar de forma natural en las plantas, como precursor de múltiples polifenoles, lo que podría interferir a la hora de querer determinar la concentración de ácido salicílico antropogénico en plantas.

También se detectaron metabolitos de ibuprofeno en berenjenas de cultivos influenciados por la descarga de aguas residuales de la EDAR de Riad en Arabia Saudita. La metodología utilizada fue la misma que la empleada para la judía de careta y se explica en su sección correspondiente.

Tal y como se hizo para sedimentos procedentes del río Turia, se emplearon técnicas non-target en mejillones, recolectados en el área de Valencia (España), empleando una biblioteca de más de 600

compuestos y METLIN. Se detectaron un total de 15 compuestos, siendo los fármacos el grupo más abundante. 12 compuestos fueron detectados de forma tentativa, mientras que los fármacos paracetamol, ibuprofeno y el metabolito 1-hidroxi-ibuprofeno fueron confirmados utilizando patrones de referencia.

2.4. Microplásticos

Previo a su análisis, las aguas superficiales provenientes de los canales de drenaje y lagos de Al-Asfar y Al-Hubail fueron filtradas con filtros metálicos con 300 µm de diámetro de poro. Entonces se llevó a cabo un método de oxidación en húmedo [15] y el resultado se filtró por vacío con filtros de papel. Estos filtros se examinaron visualmente con ayuda de un estereomicroscopio y los microplásticos se clasificaron en fragmentos o fibras siguiendo la clasificación de Hidalgo-Ruz et al. [16].

Se detectaron microplásticos en todas las muestras, con una frecuencia media aproximada de 3.2 objetos/L. En general, las muestras procedentes del área de Al-Hubail mostraron una mayor frecuencia de microplásticos que las de Al-Asfar. Las fibras fueron las más comunes, suponiendo un 83% de los microplásticos identificados. El tamaño de los microplásticos identificados estuvo entre 250-5000 µm, aunque el 95% de los microplásticos identificados se encontró en el rango de 500-1000 µm: esto pudo ser debido al tamaño de poro del filtro (300 µm) que pudo haber evitado que microplásticos más pequeños quedasen retenidos. Esto también explicaría por qué las partículas (que normalmente son de un menor tamaño que las fibras) se identificaron con tan poca frecuencia. Los canales de drenaje presentaron una concentración de microplásticos significativamente más elevada que los lagos, con frecuencias de entre 2.7-7.8 items/L en Al-Asfar y 5.8-9.0 items/L en Al-Hubail. De hecho, los puntos con más concentración de microplásticos fueron aquellos situados a la entrada de los canales de drenaje (7.8 items/L en Al-Asfar y 9.0 items/L en Al-Hubail), los cuales sufren la descarga de aguas residuales de uso ganadero, industrial y doméstico, así como efluentes de EDARs. Estos resultados señalaron a las EDARs, y otros tipos de vertidos de origen antropogénico, como la fuente más probable de microplásticos en la zona de estudio.

3. Desarrollo de la metodología

3,1. Complejidad de las matrices de biota

Las matrices provenientes de biota son muy complejas y poseen una cantidad variable de interferentes, como proteínas, lípidos o pigmentos. Su presencia puede dar lugar a interferencias tales como, el aumento de ruido de fondo o fuertes efectos de matriz, entre otras. Su eliminación durante el proceso de extracción es de vital importancia para la correcta determinación de los compuestos de interés. En esta tesis se lidió principalmente con la presencia de pigmentos en muestras vegetales y la presencia de lípidos y proteínas (y en menor medida pigmentos), presentes en mejillones y anguilas. En el caso de la masa visceral de mejillón, los lípidos y las proteínas suponen un 2-4% y 15% (w.w.), respectivamente [17], y en el caso de las anguilas un 7-15% y 5-20% (w.w.), respectivamente [18]. En la hemolinfa la proporción de proteínas, minerales y células es muy variable dependiendo del espécimen [19, 20], lo que hace muy complicado establecer unos valores de referencia.

La determinación de compuestos orgánicos puede entrañar otras dificultades, a parte del ruido de fondo o los efectos de matriz provocados por elementos tales como pigmentos, proteínas o lípidos. Por ejemplo, la posible existencia de compuestos naturalmente presentes en las matrices, con una estructura similar a los compuestos de interés. O la formación de isómeros ramificados, especialmente en matrices como el hígado, donde tienen lugar diversos procesos enzimáticos [21]. Estas dificultades contribuyen a hacer que el proceso de determinación, y más concretamente la cuantificación, sean mucho más complejos.

3.2. Métodos de extracción

Se desarrollaron métodos denominados multi-residuo para matrices de mejillón del Mediterráneo (*Mytilus galloprovincialis*) y anguila europea (*Anguilla anguilla*), con la intención de extraer fármacos, PFASs, plaguicidas y drogas de abuso simultáneamente, para así poder ahorrar recursos materiales y tiempo. Se hizo especial énfasis en la eliminación de lípidos, proteínas y pigmentos. Por otro lado, también se optimizó un método enfocado exclusivamente en la extracción de ibuprofeno y sus metabolitos en la judía de careta (*Vigna unguiculata* L. Walp). El resto de métodos de extracción empleados (agua, sedimentos, etc.) fueron desarrollados previamente en otros estudios y se encuentran referenciados en los capítulos correspondientes.

3.2.1. Matrices de mejillón y anguila sólidas – QuEChERS

En primer lugar, se empleó masa visceral de mejillón para poner a prueba 4 tipos de extracción por QuEChERS y 12 sistemas de purificación (incluyendo SPE y extracciones en fase sólida dispersiva [dSPE]), dando lugar a un total de 44 combinaciones. El objetivo era conseguir la extracción de los plaguicidas: bentazona, clorfenvinfos, clorpirifós, imazalil y terbutilazina; los PPCPs: acetaminofén, atenolol, cafeína, diclofenaco, etoricoxib, ibuprofeno, metformina, naproxeno, ácido salicílico, triclosan y vildagliptina; los PFASs: PFPeA, PFOA, PFDA, PFBS y PFOS; y las drogas de abuso: bufotenina y 4-MeO-PCP.

El método QuEChERS se suele emplear para la extracción de muestras orgánicas y se basa en la interacción entre la fracción acuosa de la muestra (o adicionada), el solvente (acetonitrilo) y una mezcla de sales. Las variantes de QuEChERS empleadas fueron el descrito en la UNE-EN 15662 [22] y el QuEChERS en medio ácido (acetonitrilo-ácido acético 1%) oficial de la AOAC [23], denominados SQ y AQ respectivamente. Además, se desarrollaron dos versiones “mini” de los mismos llamados mSQ y mAQ, respectivamente. Estas versiones utilizaban la mitad de los reactivos que las originales, lo que permite ahorrar recursos y minimizar los residuos generados. Todos los QuEChERS emplearon 1 g de masa visceral y se realizaron tal y como se describen en las referencias [22, 23].

Entre los clean-ups empleados destacaron los que utilizaron a) Z-sep+, indicado para matrices con alto contenido en grasa; b) carbón, empleado para la eliminación de pigmentos; y c) el Enhanced Matrix Removal (EMR)-Lipid, creado específicamente para la eliminación de lípidos por Agilent Technologies. De entre todas las combinaciones fueron finalmente validados los tres métodos que obtuvieron las mejores recuperaciones: SQ-EMR, AQ-Carbón y AQ-Z+2 (uno de los cuatro clean-ups que utilizaban Z-sep+). Cuyos procedimientos se detallan a continuación:

a) EMR: la mezcla de sales EMR-Lipid se introduce en un tubo Falcon, se le añaden 5 mL de agua MilliQ y la mezcla se agita durante 30 s. Entonces se le añaden 5 mL del sobrenadante de la extracción QuEChERS se añaden, se vuelve a agitar todo durante 30 s. y se centrifuga a 3500 rpm durante 5 min. 5 mL del sobrenadante resultante se pasan a un tubo que contiene la fase de pulido, consistente en 1600 mg de $MgSO_4$ y 400 mg de NaCl. El tubo se agita durante 30 s. y se centrifuga a 3500 rpm durante 5 min. El extracto resultante se filtra con filtros de Nylon de 0.22 µm y se almacena a -20 °C hasta el análisis.

b) Carbón: 1.5 mL de sobrenadante de la extracción QuEChERS se introducen en un tubo Falcon de 15 mL, que contiene 150 mg de $MgSO_4$, 25 mg de PSA y 7.5 mg de carbono negro grafitizado (GCB). El tubo se agita durante 30 s. y se centrifuga a 3500 rpm durante 5 min. El extracto resultante se filtra y almacena tal y como se ha descrito anteriormente.

c) Z+2: el proceso es el mismo que el descrito para "Carbón", excepto por la mezcla de sales utilizada, que en este caso es 25 mg de Z-sep+, 25 mg de PSA y 25 mg of C18.

Tras la validación y la corrección de efectos de matriz (en su mayoría negativos y bastante pronunciados), SQ-EMR, AQ-Carbón y AQ-Z+2 proporcionaron recuperaciones entre 54-124%, 60-127% y 59-124% para la mayoría de compuestos, respectivamente.

Cabe destacar que la metformina no se extrajo apropiadamente con ningún método, debido a su gran solubilidad en agua y su poca solubilidad en acetonitrilo (Solvente empleado para el QuEChERS). Por otro lado, los resultados sugirieron que un compuesto con una estructura similar a la del ácido salicílico podía estar presente de forma natural en los mejillones, interfiriendo en su cuantificación. Por lo tanto, estos dos compuestos no fueron tenidos en cuenta en futuros análisis de masa visceral.

Los métodos incluyendo el clean-up EMR-Lipid fueron los que presentaron los resultados más estables. Por lo tanto, se decidió validar el método SQ-EMR también en matrices de músculo e hígado de anguila (que poseen un alto contenido en materia grasa) para los compuestos descritos anteriormente. Como parte del procedimiento se decidió hacer extracción QuEChERS variando la cantidad de agua añadida (0, 3, 5, 7.5 ó 10 mL) para comprobar si esto mejoraba la recuperación de aquellos compuestos muy solubles en agua. Finalmente, no se observaron variaciones entre las diferentes cantidades de agua. Por lo que se procedió con la validación del mismo método que el empleado para mejillones, obteniendo recuperaciones de entre 45-120% y 47-120% para la mayoría de compuestos en músculo e hígado respectivamente.

3.2.2. Hemolinfa – SPE

Para la determinación de los compuestos indicados anteriormente en hemolinfa se utilizó la técnica SPE con los cartuchos Phree™ Phospholipid Removal, diseñados por Phenomenex para realizar extracciones en matrices de plasma sanguíneo. Para el procedimiento

se ponen cartuchos Phree™ en un manifold donde se introducen 100 µl de hemolinfa. A continuación, se añaden 600 µl de metanol (MeOH) con ácido fórmico al 1%. La adición debe realizarse directamente sobre la muestra, con cuidado de evitar las paredes del cartucho, de esta forma se asegura que muestra y solvente se mezclan correctamente. Tras esperar 2 min. (para asegurar una correcta precipitación de los compuestos interferentes), se aplica vacío para que la muestra pase a través del cartucho. El extracto se almacena a -20 °C hasta el análisis.

Para la puesta a punto del método se emplearon 4 procedimientos diferentes cuya variación se encontraba en el tipo y volumen de solvente añadido. De esta forma, a parte del descrito anteriormente, se emplearon 3 métodos que añadían 300 µl y 450 µl de acetonitrilo y 400 µl de MeOH, respectivamente. Los resultados mostraron que, a la hora de la detección, la sensibilidad mejoraba sustancialmente cuando se empleaba MeOH como solvente. Además, la droga de abuso 4-MeO-PCP solo se extrajo cuando se empleó MeOH como solvente. No obstante, el proceso de extracción se volvía más lento al utilizar MeOH y requería de mayores presiones de vacío. Esto fue especialmente significativo cuando se empleó el método con 400 µl de MeOH, ya que en el 50% de los casos no fue posible hacer pasar a través del cartucho la muestra en su totalidad, por lo que fue descartado. Por otro lado, los efectos de matriz fueron menores cuando se empleó acetonitrilo.

Con todo ello, y tras la validación de los tres métodos restantes, el método que empleó 600 µl de MeOH fue el elegido. Esto se debió a la mejora sustancial de la sensibilidad y al hecho de que fue el único método capaz de extraer satisfactoriamente los 23 compuestos, con 20 de ellos presentando recuperaciones entre 73-114%.

3.2.3. Muestras vegetales – UAE

Para la extracción de ibuprofeno y sus metabolitos de las diferentes muestras de judía de careta (germinado, raíces y tallo) se empleó una UAE. Se colocó un gramo de muestra triturada en tubos Falcon de 15 mL, al que se añadió el patrón interno, junto con 0.5 mL de HCl y 4 mL de MeOH. Entonces los tubos se agitaron y se sometieron a un baño de ultrasonidos durante 10 min., para, a continuación, ser centrifugados y pasar el sobrenadante a otro tubo. Este proceso se realizó un total de 3 veces por muestra, obteniendo un volumen final de sobrenadante de aproximadamente 12 mL. El sobrenadante se evaporó hasta alcanzar un volumen aproximado de 4 mL, los cuales se llevaron a 200 mL utilizando agua MilliQ. Estos 200 mL se sometieron a SPE utilizando cartuchos

Strata-X de fase polimérica reversa. Los analitos fueron eluidos con 10 mL de MeOH y evaporados a sequedad, para terminar siendo reconstituidos en 1 mL de MeOH-agua MilliQ 10:90 y almacenados a -20°C hasta su análisis.

La optimización del método se llevó a cabo para ibuprofeno y los metabolitos 1-hidroxi-iobuprofeno, 2-hidroxi-ibuprofeno y carboxi-ibuprofeno. Como parte de la optimización se probaron dos disolventes (acetonitrilo y MeOH), se comprobó la eficacia que tenía repetir la UAE hasta 5 veces, la duración del baño de ultrasonidos (2-20 min.) y se probó la eficacia de 4 niveles de pH diferentes (2, 4, 6 y 8). Se comprobó que no había diferencias significativas en la extracción independientemente del disolvente utilizado, ni tras repetir la UAE más de 3 veces, ni con baños de ultrasonidos superiores a 10 min. Por último, debido a la naturaleza ácida del ibuprofeno y sus metabolitos, se comprobó que se obtenían mejores recuperaciones a pH 2. Las cuales empeoraban a medida que se aumentaba el pH. Con todo ello, el método seleccionado presentó recuperaciones de 85-99 % para los 4 compuestos.

3.3. Métodos de determinación dirigidos

Todos los métodos de determinación empleados hicieron uso de LC-MS. Como parte de la presente tesis se desarrollaron métodos de determinación dirigidos (o target en inglés), para la determinación de los compuestos descritos anteriormente en matrices de mejillón y anguila.

La determinación de PFASs, plaguicidas, fármacos y drogas de abuso en matrices de anguila y mejillón se realizó mediante HPLC-MS/MS, utilizando tanto un triple cuadrupolo (QqQ) como un QTRAP. El resto de métodos de determinación empleados fueron desarrollados previamente en otros estudios y se encuentran referenciados en las secciones correspondientes.

En el primer caso se utilizó un Agilent 1260 UHPLC acoplado a un espectrómetro de masas Agilent 6410 (QqQ) con ionización mediante electrospray (ESI) en modo de ionización negativo y positivo (gas del nebulizador 15 psi, flujo de gas 11 L/min. Voltaje del ion-spray 4 kV y temperatura 300 °C), operado en modo de monitoreo de reacción múltiple (MRM). La columna empleada para la determinación de plaguicidas y etoricoxib fue una Luna® 3 µm C18(2) 100 Å 150x2 mm y la columna empleada para la detección de PFASs, drogas de abuso y el resto de fármacos, fue una Kinetex 1.7 µm XB-C18 100 Å 50x2.1 mm, ambas de Phenomenex. Lo que dio lugar a un total de 3 métodos,

uno con ionización negativa usando la columna Kinetex, para PFASs y fármacos. Y otros dos en ionización positiva con la columna Luna y la Kinetex, para la detección de etoricoxib y plaguicidas y la de drogas de abuso y fármacos, respectivamente. La fase móvil empleada en modo de ionización negativo fue (A) H2O 2.5 mM NH_4F y (B) MeOH 2.5 mM NH_4F. Y la fase móvil para el modo de ionización positivo fue (A) H2O 0.1% ácido fórmico y (B) MeOH 0.1% ácido fórmico. El gradiente utilizado en ambos casos fue: 0 min. (70% A), 12 min. (5% A), 22 min. (5% A), 23 min. (70% A) y 30 min. (70% A). El volumen de inyección fue 5 µl y la temperatura de la columna se mantuvo a 30 °C.

La determinación en masa visceral de mejillón también se realizó mediante HPLC-MS/MS utilizando un cromatógrafo ExionLC AD acoplado a un espectrómetro de masas Sciex QTRAP 6500+ con ESI en modo Turbo Spray IonDrive (Curtain gas 30 psi, voltaje ion-spray 4.5 kV, temperatura 350°C, y fuente iónica de gas 1 y 2 a 50 y 65 psi respectivamente) operado en modo MRM. La columna empleada fue una ACQUITY UPLC BEH C18 (1.7 µm 130 Å, 50 x 2.1 mm, Waters). La fase móvil empleada en ambos modos y el gradiente fueron los mismos que los indicados en el párrafo anterior. El volumen de inyección fue 2 µl y la temperatura de la columna se mantuvo a 45 °C.

Todos estos métodos fueron capaces de separar y detectar correctamente los compuestos objetivo.

3.4. Métodos de determinación no dirigidos

La detección de contaminantes orgánicos en mejillones y sedimento con métodos no dirigidos (o non-target) se realizó mediante UHPLC-MS/MS e incluyó La obtención de datos via “Information dependant acquisition” (IDA) seleccionando como información la intensidad del ion y “information independent acquisition” (IIA) utilizando sequential window acquisition of all theoretical fragment-ion spectra (SWATH) con ventanas fijas “FSWATH” y con ventanas variables “VSWATH”.

3.4.1. Configuración de los equipos

Se empleó un cromatógrafo Agilent 1290 Infinity acoplado a un espectrómetro TripleTOF™ 5600 de SCIEX. Operado tanto en modo de ionización positiva como negativa, con una fuente de ionización DuoSpray a un poder de resolución (“full width at half-maximum” (FWHM) a m/z 400) de 30,000 en MS y 30,000 en MS/MS (modo de alta resolución). El sistema te calibración automatizado se fijó para que

realizase una calibración externa cada 6 muestras. Las condiciones de la fuente se ajustaron a 450°C cortina de gas 30 psi, gas de la fuente de ionización 1 y 2 a 45 psi y el voltaje variable del ion-spray a 5.5 kV en ionización positiva y -4.5 kV en ionización negativa. La columna empleada fue una Acquity UPLC BEH C18, 130 Å, 1.7 µm (2.1 x 5 mm) de Waters. La fase móvil empleada en el modo de ionización positivo fue H2O 0.1% ácido fórmico and (B) MeOH 0.1% ácido fórmico, con un flujo de 0.4 mL/min. y el siguiente gradiente: 0 min. (70% A), 10 min. (15% A), 15 min. (2% A), 15.5 min. (70% A) y 25 min. (70% A). La fase móvil empleada en modo de ionización negativo fue (A) H2O 2.5 mM NH_4F and (B) MeOH 2.5 mM NH_4F con un flujo de 0.2 mL/min. y el siguiente gradiente: 0 min. (70% A), 12 min. (5% A), 20 min. (5% A) y 32 min. (70% A).

El IDA consistió en un escaneo completo y eventos activados por información dependiente. El tiempo de acumulación de un escaneo completo fue 100 ms para escanear un rango de masas de m/z 100 a m/z 750 a una energía de colisión (CE) de 10 eV. El tiempo de acumulación para cada experimento IDA fue 100 ms con una CE de 45 eV y una CE de propagación de 15 eV en modo de alta resolución. Como criterio de selección se escogieron los 6 iones más intensos siempre que estuvieran sobre los 100 cps, con la exclusión isotópica desactivada, una exclusión temporal de 6 s y substracción de fondo dinámica activada. Cada ciclo duró 750 ms.

En el caso del FSWATH se realizó un escaneo completo como el del IDA, pero con un tiempo de acumulación de 50 ms, seguido por varias ventanas MS/MS que cubrieron un rango de m/z 100 – 750 con una anchura por ventana de 25 Da para aislar el Q1 (superposición de 1 Da). Cada ventana tuvo un tiempo de acumulación de 40 ms, con una CE de 45 eV y una CE de propagación de 15 eV en modo de alta resolución. En total, cada ciclo de FSWATH duró 1400 ms y se compuso de 26 ventanas.

VSWATH también constó de un escaneo completo con ventanas MS/MS en un rango de m/z 100 – 750 con una anchura de 25 Da (superposición de 1 Da). La diferencia es que las ventanas se obtuvieron con la herramienta “Varieble Window Calculator” que permite optimizar el tamaño de las ventanas. De esta forma, el software escala el tamaño de las ventanas dependiendo del número de iones precursores detectados, haciendo que sean más estrechas o más anchas en los tiempos con mayor y menor densidad de iones precursores, respectivamente. El número máximo de ventanas fue fijado a 30 y el m/z mínimo a 5 Da (superposición de 1 Da). Cada ventana tuvo un tiempo de acumulación de 40 ms, con una CE

de 45 eV y una CE de propagación de 15 eV en modo de alta resolución. Las ventanas variables se optimizaron para ambas matrices, mejillón y sedimento.

3.4.2. Comparación de IDA, FSWATH y VSWATH

Para la comparación de los tres métodos se emplearon muestras de sedimento y mejillón que fueron fortificadas con 32 fármacos. Los sedimentos fueron extraídos utilizando el protocolo de Carmona et al. [13] y los mejillones con el método de Chiesa et al. [24].

Para la identificación de los compuestos se tuvieron en cuenta el RT, MS (error <5 ppm) y MS/MS. No obstante, se decidió que la concordancia de los iones MS/MS con el espectro de referencia fuese el parámetro más relevante para la identificación, siguiendo un criterio similar a Roemmelt et al. [25] (identificación positiva cuando al menos 2 iones concuerden).

Los resultados mostraron que, en ambas matrices, los dos métodos que emplearon SWATH consiguieron detectar un mayor número de compuestos (27 con FSWATH y 25 con VSWATH) que el que utilizó IDA (19). Siendo el FSWATH ligeramente mejor que el VSWATH en los sedimentos. Al contrario que ocurrió en las muestras de mejillón, donde VSWATH obtuvo resultados ligeramente mejores. No obstante, los métodos que emplearon SWATH proporcionaban mucha información MS/MS que no correspondía con los compuestos objetivo, haciendo que el análisis de la información fuese muy laborioso. Aunque la información proporcionada por IDA fuese más escasa, a menudo correspondía con los iones de referencia. Por lo tanto, se hizo un índice de calidad del espectro MS/MS similar al de Zhu et al. [26]. Que establece un valor de 0 a 10 en función de la proporción entre iones obtenidos e iones que corresponden con los de referencia. Utilizando este índice IDA obtuvo resultados significativamente mejores (8.0-9.3) que FSWATH (6.7-7.8) y VSWATH (5.7-6.8).

4. Absorción y metabolismo de ibuprofeno en plantas

Para estudiar la absorción y metabolismo del ibuprofeno en plantas se eligió la especie *V. unguiculata* L. Walp, mejor conocida como “judía de careta”. Una leguminosa resistente a las sequías y adaptada al clima cálido propio de las regiones situadas en los trópicos. El objetivo fue observar tanto la absorción como la formación de metabolitos de ibuprofeno durante diferentes etapas de crecimiento de la planta (germen, 25 días tras el germinado 50 días tras el germinado), además

de su distribución en la planta adulta (raíces y tallo).

Las semillas de *V. unguiculata* fueron desinfectadas con hipoclorito de sodio al 0.5% y se germinaron en placas Petri divididas en seis grupos expuestos a diferentes concentraciones de ibuprofeno a través del agua: control, 400, 800, 1200, 1600 y 2000 mg/L. Una vez germinadas, dos tercios de las semillas se trasplantaron a macetas con Perlite, donde se regaron tres veces por semana con 40 mL de las concentraciones de ibuprofeno descritas anteriormente. Las plantas fueron muestreadas a los 25 y 50 días desde el trasplante y se separaron en raíces y tallos. Cada nivel de exposición (control, 400, 800, 1200, 1600 y 2000 mg/L de ibuprofeno) y de etapa vital (germen, 25 días y 50 días) tuvieron un total de 3 réplicas. Durante todo el proceso se hizo tanto un seguimiento del germinado como del crecimiento de las plantas.

Las muestras fueron extraídas mediante la triple UAE con MeOH descrita anteriormente. Para después ser analizados mediante LC-MS/MS utilizando un TripleTOF™ 5600 de SCIEX con adquisición IDA, configurado tal y como se ha descrito en la sección correspondiente. Para la identificación de los metabolitos, se creó una base de datos compuesta por lo metabolitos encontrados en la literatura previa [27, 28], y se realizó una comparación visual de los espectros. Lo metabolitos se dividieron en fase I (aquellos resultados de reacciones químicas) y fase II (los resultados de la conjugación con otras moléculas para ser eliminados o asimilados por el organismo).

Se detectaron un total de 8 metabolitos de fase I incluyendo 1-hidroxi-ibuprofeno, 2-hidroxi-ibuprofeno y 3-hidroxi-ibuprofeno. Sin embargo, no se detectó carboxi-ibuprofeno en ninguna de las muestras, que es uno de los principales metabolitos presentes en animales debido a la oxidación por P450 citocromo oxidasas. Indicando que las plantas podrían tener rutas metabólicas diferentes a las de los animales para el ibuprofeno, algo que ya sugerían resultados anteriores [29]. Se llegaron a detectar hasta 38 metabolitos de fase II, entre los que destacaron el ibuprofeno hexóxido (frecuentemente detectado en germinados y raíces), el hidroxi-ibuprofeno hexóxido (en tallo y germinados) y el hidroxi-ibuprofeno glucurónido (en tallo). Estos resultados coincidieron con los de otros estudios[27-29], que mostraron una abundancia de hexóxidos como consecuencia del metabolismo del ibuprofeno en plantas. También fueron habituales los conjugados con aminoácidos (especialmente en los germinados). Esto es posible que sea debido al alto contenido en proteínas en las semillas de *V. unguiculata* [30], en

comparación con su tallo y raices. Además, esta fue la primera vez que se reportaron 9 de estos conjugados con aminoácidos en plantas.

En general, se observó más abundancia de ibuprofeno inalterado en germinados y raíces, seguramente debido a que están en contacto directo con el fármaco. Mientras que la presencia de conjugados parentales del ibuprofeno solo se detectaron en raíces y tallos, indicando que estos mecanismos de conjugación no se dan hasta la etapa adulta de la planta. Por otro lado, no se encontraron diferencias significativas en el número de metabolitos detectados entre los distintos niveles de exposición.

Los resultados también mostraron que altas concentraciones de ibuprofeno produjeron una inhibición del 50% en la germinación de las semillas, así como una disminución de la pigmentación en plantas adultas.

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Perspectivas futuras

02

1. Fuentes de vertido de contaminantes y su presencia en agua superficial

El análisis de efluentes provenientes de las EDARs de Bundamba y Goodna, situadas en la ciudad de Brisbane, Queensland, Australia; mostró que las aguas tratadas contenían una amplia variedad de compuestos que estaban siendo vertidos al río Brisbane. Entre los que se incluían 9 PFASs, 32 plaguicidas y 24 fármacos y productos del cuidado personal (PPCPs) con emisiones de entre 0.10-354 mg/día, 0.01-5700 mg/día y 0.03-1.28x10^6 mg/día, respectivamente. También se determinó que las aguas de escorrentía provenientes de campos de cultivo fueron la principal fuente de contaminación de plaguicidas como metomilo, tebuthiuron o prometryn, entre otros. Por último, los resultados indicaron que el transporte a través de las aguas subterráneas desde un aeropuerto cercano podría haber sido la principal fuente de contaminación de algunos PFASs, como PFOS o PFHxS. Todos estos resultados coincidieron con lo descrito en el primer párrafo del resumen.

También se evaluó la presencia de contaminantes en el agua del río Brisbane. Para ello se realizaron tomas de muestras de forma periódica entre marzo y diciembre de 2017 a la altura del barrio de Goodna, zona influenciada por las descargas de la estación depuradora de aguas residuales (EDAR) de Goodna, situada a tan solo 2 km aguas arriba del punto de muestreo. Parte del muestreo se realizó coincidiendo con un periodo de lluvias torrenciales, por lo que el área también se vio influenciada por la llegada de aguas de escorrentía, algunas de ellas provenientes de zonas agrícolas cercanas; así como de un aumento del flujo de aguas subterráneas, que pueden transportar contaminantes desde otras zonas colindantes [1]. Las muestras se extrajeron por extracción en fase sólida (SPE) y analizaron mediante cromatografía líquida acoplada a espectrometría de masas en tándem (LC-MS/MS) siguiendo protocolos previamente publicados [1-4]

En total se detectaron 29 PFASs con concentraciones que fueron desde por debajo de los límites de cuantificación (LOQ) hasta los 54 ng/L. Destacando PFBA, PFPeA, PFHxA PFOA, PFDA, PFBS, PFPeS, PFHxS, PFHpS y PFOS, como compuestos que estuvieron presentes en todas las muestras a lo largo del periodo de muestreo. También se detectaron 31 plaguicidas y metabolitos con concentraciones que llegaron a alcanzar los 468 ng/L (DEET), con un gran número de compuestos estando presentes en todas las muestras, de entre los que se incluyeron, metomil,

simazina, atrazina y sus metabolitos, metolacloro e imidacloprid, y otros. En el caso de los PPCPs, 20 compuestos y metabolitos fueron detectados, alcanzando concentraciones de hasta 750 ng/L. Estando cotinina, cafeína, tramadol, atenolol, venlafaxina e iopromide presentes en todas las muestras.

2. Persistencia ambiental de los contaminantes orgánicos

La persistencia de un compuesto determina el tiempo que tardará en ser transformado por procesos de biodegradación, fotólisis, hidrólisis, etc. Cuanto más persistente sea un compuesto, mayor será el tiempo que este permanezca en el medio ambiente y, por lo tanto, es más posible que este sea transportado a otros ambientes, lo que aumenta su ubicuidad. Además, una mayor persistencia también implica una mayor probabilidad de exposición por parte de la fauna, la flora o el ser humano a estos contaminantes. Además de que dicha exposición puede tener lugar durante periodos más prolongados. Por otro lado, hay compuestos cuyos productos de degradación suponen un mayor riesgo ambiental que el compuesto de partida [5]. Por todos estos motivos, conocer la persistencia de los contaminantes es crucial para hacer una correcta evaluación del riesgo que suponen para el medio ambiente y la salud humana.

Para determinar la persistencia de contaminantes orgánicos se realizó un estudio en el estuario del río Brisbane, Queensland (Australia). Se trata de un estuario con un flujo de agua muy lento [6], en una zona donde es común que ocurran lluvias torrenciales durante la estación húmeda [6]. La hipótesis plantea que debido al flujo particularmente lento en la zona, los contaminantes se acumulan en el estuario, como si de un lago se tratase, y la llegada de lluvias torrenciales (junto con la consiguiente crecida del río) podría arrastrar estos contaminantes, disminuyendo su concentración en el estuario de forma drástica. Esto posibilitaría observar cómo los contaminantes vuelven a acumularse en el río y así estimar su persistencia.

En marzo de 2017, la llegada del ciclón Debbie a la ciudad de Brisbane vino acompañada de lluvias torrenciales, que fueron seguidas de 7 meses sin precipitaciones significativas. Se realizó un muestreo de aguas superficiales el día antes de que las lluvias tuviesen lugar y de forma periódica durante los siguientes meses, hasta mediados de diciembre de ese mismo año. Con el fin de comprobar si la hipótesis era cierta. Las

muestras se extrajeron utilizando SPE y analizaron mediante LC-MS/MS, para la detección de plaguicidas, PPCPs y PFASs [1-4].

Los resultados mostraron una disminución drástica de la concentración de la mayoría de los compuestos con la llegada de las lluvias y volvió a aumentar de forma gradual ante la ausencia de precipitaciones. Lo que permitía estimar la persistencia de los compuestos mediante el uso de un modelo de balance de masas. Para poder definir el mayor número de variables del balance de masas, se propuso la utilización de compuestos de referencia (benchmarking). Debido a que los PFASs son conocidos por su persistencia [7, 8], fueron propuestos como los mejores candidatos a compuestos de referencia. Se determinó que entre los PFASs detectados, el PFHxA reunía las características necesarias para ser el compuesto de referencia más fiable y se utilizó para definir el volumen del sistema y las fuentes de emisión de los contaminantes. Una variación de este modelo de balance de masas se utilizó para algunos compuestos (todos plaguicidas) que mostraron un comportamiento opuesto al resto de compuestos: un aumento drástico de la concentración coincidiendo con la llegada de las lluvias, seguido de una disminución exponencial de la concentración. Además, en ambos modelos de balance de masas, se introdujeron variables de radiación UV y temperatura, lo que permitió determinar si la fotólisis o la biodegradación eran los procesos más significativos en la transformación de los diferentes compuestos.

Se consiguió determinar la persistencia de 7 plaguicidas, con vidas medias de entre 11 y 66 días, excepto para terbutiurón (140 días) y diazinón (280 días). Y 10 PPCPs, con vidas medias de entre 18 y 140 días, excepto para el metabolito de la nicotina, cotinina (210 días). La mayoría de los PPCPs mostraron una tendencia a ser sensibles a la fotólisis, mientras que entre los plaguicidas 3 mostraron una mayor tendencia a ser biodegradados, 2 a ser fotolizados y otros 2 no mostraron tendencia a ser transformados por ninguno de estos dos procesos. En general, más de la mitad de los plaguicidas y PPCPs modelados (11 de 18) mostraron vidas medias superiores a los 2 meses, establecidos como criterio de la Convención de Estocolmo sobre la clasificación de contaminantes persistentes en agua [9]. Los resultados de este estudio proporcionaron información crucial para evaluar el riesgo ambiental de estos compuestos en ambientes acuáticos y su posible propagación a otros ambientes.

3. Bioacumulación de contaminantes orgánicos en mejillones

Los mejillones tienen una amplia distribución en los ambientes acuáticos y son de un elevado valor ambiental debido a su función de animales filtradores. Además, el mejillón Mediterráneo (*M. galloprovincialis*), es de un gran valor gastronómico y cultural en la Comunidad Valenciana (España) donde es mejor conocido como "clòtxina". Por estos motivos fue la especie seleccionada para la realización de los ensayos de bioacumulación. Una veintena de compuestos de las familias de PPCPs, PFASs y plaguicidas, además de microplásticos, fueron los elegidos como contaminantes de exposición, ya que fueron detectados previamente (apartados 1 y 2 del resumen y 1 de las perspectivas de futuro) y/o ampliamente reportados en ecosistemas acuáticos [10, 11]. Además, se ha reportado que algunos contaminantes orgánicos podrían establecer sinergias con ciertos tipos de microplásticos [12]. El objetivo de estos ensayos fue, por un lado, evaluar la bioacumulación de los contaminantes emergentes anteriormente mencionados en *M. galloprovincialis*, así como su capacidad de depuración. Y por otro lado comprobar la influencia de los microplásticos en estos procesos. Siendo la primera vez que se estudia la bioacumulación en fauna acuática de varios de estos compuestos, así como sus posibles sinergias con microplásticos.

Los ensayos se llevaron a cabo en acuarios, bajo un ambiente controlado. Tuvieron dos fases, una fase de exposición a los contaminantes a través de agua o comida (dependiendo de la solubilidad de los contaminantes), que duró de los días 0 al 28. En la que se pretendía observar la bioacumulación de los diferentes compuestos en el organismo de los especímenes. Y una fase de depuración que duró de los días 29 a 58, al comienzo de la cual los acuarios se limpiaron minuciosamente y se reemplazó el agua. Esta sirvió para observar el proceso de eliminación de los compuestos acumulados. Los mejillones se obtuvieron de mercados locales y tras la aclimatación se dividieron de forma aleatoria en 3 grupos: un grupo control (B), un grupo expuesto a contaminantes (C) y un grupo expuesto a contaminantes y microplásticos de polietileno (C+M). Los compuestos disueltos en agua estuvieron a una concentración de 10 µg/L, en la comida se inocularon 10 ng de compuestos por espécimen y día y en el caso de C+M, junto a la comida también se añadió 1 mg de microplásticos de polietileno por espécimen y día.

Se muestrearon 5 mejillones de cada grupo los días 0, 2, 4, 7, 14, 28,

29, 30, 32, 35, 42, y 58. A los cuales se les extrajo la hemolinfa desde el músculo abductor posterior. Se reservó un mejillón por grupo y día para someterlo a una digestión con potasa [13] y, posteriormente, examinar sus restos mediante un estereoscopio, en busca de microplásticos. La masa visceral del resto de especímenes se trituró para su homogeneización y se realizaron pools tanto de hemolinfa como de masa visceral de cada grupo y día. Los cuales fueron extraídos y analizados mediante LC-MS/MS con un Agilent 1260 UHPLC acoplado a un espectrómetro de masas Agilent 6410 (QqQ), tal y como se describe en secciones anteriores.

La digestión de los mejillones con potasa mostró que, efectivamente, los tejidos de los mejillones del grupo C+M presentaban microplásticos, desde el día 2 hasta el último día del ensayo. Mientras que en los grupos B y C no se observaron estos microplásticos en ninguna de las muestras. Además, en la mayoría de muestras se observó la presencia de fibras plásticas, algo común en estos moluscos [14], y que podrían haber entrado en su organismo cuando estos se hallaban en el mar, durante su transporte o puesta a la venta en el mercado.

Se observó la acumulación de un total de 4 plaguicidas (clorfenvinfos, clorpirifós, imazalil y terbutilazina), 3 PFASs (PFDA, PFOA y PFOS) y 3 PPCPs (diclofenaco, etoricoxib y triclosan) en la masa visceral, durante la fase de exposición. Sin embargo, solo clorfenvinfos, clorpirifós, imazalil, terbutilazina, PFDA y PFOS fueron detectados durante la fase de depuración. En el caso de la hemolinfa, se observó la acumulación de 2 plaguicidas (imazalil y terbutilazina), 1 PFASs (PFPeA) y 2 PPCPs (cafeína y etoricoxib). Mientras que no se detectaron compuestos en la fase de depuración. El hecho de que PFPeA y cafeína solo fuesen detectados en hemolinfa, podría indicar su acumulación específica en este tejido.

La acumulación y eliminación de los compuestos se analizaron empleando modelos cinéticos [15], que permitieron calcular su factor de bioconcentración (BCF) y vida media de eliminación. De esta forma, los BCFs en masa visceral fueron desde 6.4 hasta 120 L/kg, excepto en el caso de clorpirifós (3800-15000 L/kg). Y las vidas medias de eliminación para los compuestos que fueron detectados estuvieron en el rango de 2.3-32 días. En el caso de la hemolinfa, los BCFs fueron significativamente inferiores con un rango de 0.9-3.3 L/kg. En general, los BCFs obtenidos en este estudio y el de Vidal Liñán et al. [15] (que también utilizó modelos cinéticos), fueron significativamente inferiores a los obtenidos en estudios que emplearon modelos de estado de equilibrio [16, 17]. Lo que indica que estos últimos podrían proporcionar BCFs sobreestimados,

con respecto de los modelos cinéticos. Por otro lado, la mayor parte de la acumulación observada en el presente estudio tuvo lugar en las primeras 48 horas de la fase de exposición, por lo que no fue posible modelar un aumento paulatino de la concentración y podría haber dado lugar a una infraestimación de los BCFs. Vidal Liñán et al. [15] también observó un aumento importante de la concentración en las primeras 24 horas que *M. galloprovincialis* estuvo expuesto a filtros UV. Por lo que, para mejorar el modelado de los BCFs en futuros ensayos de bioacumulación, se recomienda realizar muestreos continuados dentro de las primeras 24-48 horas de la fase de exposición.

En cuanto a la influencia de los microplásticos en estos procesos, se observaron diferencias entre los resultados de los grupos C y C+M, especialmente en plaguicidas y PFASs. Con comportamientos similares tanto en masa visceral como hemolinfa. Los plaguicidas clorpirifós y terbutilazina mostraron menores BCFs, así como una mayor velocidad de eliminación en el grupo C+M. Esto podría ser debido a la capacidad que tienen los microplásticos para reducir la biodisponibilidad de algunos plaguicidas [18, 19]. El caso de imazalil fue particular, ya que mostró una mayor bioacumulación, pero, al mismo tiempo, una mayor velocidad de eliminación en presencia de microplásticos. Por desgracia no se encontró información que ayudase a dilucidar las causas de este comportamiento. En el caso de los PFASs PFDA, PFOA, PFOS y PFPeA, estos mostraron mayores BCFs en el grupo C+M. Mientras que, PFDA y PFOS, los únicos detectados en la fase de depuración, también mostraron una menor velocidad de eliminación en presencia de microplásticos. Esto podría ser debido a la capacidad de los PFASs de ser adsorbidos por diferentes materiales plásticos [20], que además funcionarían como vectores para la acumulación de PFASs [21]. Los microplásticos en el interior de los mejillones podrían contribuir a una mayor acumulación de PFASs en los mismos durante la fase de exposición. Mientras que, en la fase de depuración, estos mismos microplásticos podrían seguir liberando los PFASs que tienen retenidos, acumulándose en los mejillones.

La escasez de información disponible sobre la bioacumulación de contaminantes orgánicos en mejillones y sobre las sinergias que estos tienen con los microplásticos, supuso un gran reto a la hora de interpretar los resultados de este estudio.

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Ampliación de las perspectivas futuras

3.0

Ampliación 1. Persistence of PPCPs and pesticides in a subtropical river estuary

In collaboration with Darryl W. Hawker, Jochen F. Mueller, Michael Gallen, Sarit Kaserzon, Yolanda Picó and Michael S. McLachlan

A revised and improved version of this study has been already published:

Rodrigo Álvarez-Ruiz, Darryl W. Hawker, Jochen F. Mueller, Michael Gallen, Sarit Kaserzon, Yolanda Picó, and Michael S. McLachlan, Postflood Monitoring in a Subtropical Estuary and Benchmarking with PFASs Allows Measurement of Chemical Persistence on the Scale of Months. Environmental Science & Technology 2021. https://doi.org/10.1021/acs.est.1c02263

Abstract

Measurements of chemical persistence in natural environments can provide insight into behaviour not easily replicated in laboratory studies. However, it is difficult to find environmental situations suitable for such measurements, particularly for substances with half-lives exceeding several weeks. The objective of this study was to estimate organic contaminant persistence in an estuary from concentration trends following a major flushing event (cyclone). Water samples were collected in the upper Brisbane River estuary on 36 occasions over 37 weeks and analysed for 127 pharmaceuticals and personal care products (PPCPs), pesticides and perfluoroalkyl substances (PFASs). High quality time trend data were obtained for 41 substances. For many of these, data on wastewater treatment plants input to the upper estuary were also obtained. A mass balance model of the estuary stretch was formulated and parameterized using PFASs as persistent benchmarking chemicals. Transformation half-life estimates were obtained for 10 PPCPs and 7 pesticides ranging from 17-250 days. Furthermore, insight was obtained into dominant transformation processes as well as the magnitude of chemical inputs to the estuary and their sources. Results show that under certain conditions, estuaries can be used to quantify the persistence of organic contaminants with half-lives of the order of several months.

1. Introduction

Pharmaceuticals and personal care products (PPCPs), pesticides and perfluoroalkyl substances (PFASs) are widespread in the environment and may pose a threat to environmental health and human safety [1, 2]. Aquatic ecosystems are particularly vulnerable to these compounds because they are continually discharged into these environments via wastewater treatment plants (WWTPs) [3] and human activities such as agriculture [4]. However, there is relatively little knowledge on the in-situ persistence and fate of these compounds, even though this is crucial for assessing the risk that they represent. Persistence affects how long a chemical remains in the environment, and hence the possibility of it reaching distant areas and their resident organisms before being transformed.

Currently, most studies of persistence are conducted in the laboratory under controlled conditions [5-8]. Frequently, it is not clear for which cases extrapolation of the results of such studies to the environment is possible. As an example, biotransformation depends on the evolution over time of the microbial community and this may undergo substantial variations between experiments even if they are conducted under similar conditions [9]. Hence, even if the characteristics of an environment are meticulously reproduced, there is no guarantee that the microbial community will evolve in the same way as in an actual environment and, therefore, nor will biotransformation. Laboratory studies usually focus on one transformation process (predominantly photolysis and/or hydrolysis) [5-8]. However, in the field, different transformation processes may take place simultaneously and transferring the information from diverse laboratory studies to the environment to provide a more complete characterization can be difficult and introduce more uncertainty. Furthermore, natural environments are heterogeneous, possessing variable and unique characteristic that are hard (or even impossible) to reproduce or capture in a laboratory study. Hence, it can be difficult to characterize persistence in a natural environment without actually measuring it in-situ. Moreover, field measurements of the spatial and temporal variability of transformation are essential to build the fundamental understanding needed to extrapolate persistence from one environment to another [10].

The large number of environmental processes that can affect chemical fate complicates field measurement of persistence. It is difficult to find field situations where the processes are constrained enough to allow persistence measurement. For this reason, such measurements in

natural environments are scarce. To help overcome these constraints, mass balance modelling can be used to quantify the influence of some variables in a field experiment. Other processes can be controlled by studying the behaviour of chemicals that respond sensitively to a particular process.

In this study, a mass balance model approach was employed to determine the half-lives of several emerging contaminants in the Brisbane River estuary. This estuary is a recipient of organic pollutants [11] and has relatively little flow during dry periods [12], making it susceptible to accumulation of some pollutants. In such an environment with long water residence times, persistence measurement becomes feasible at time scales that are relevant for regulation (half-lives from weeks to months). In addition, this estuary is subject to periodic significant rainfall events, which flush the accumulated contaminants out of the estuary and return it to a pre-accumulation condition. To exploit this, the evolution of the concentrations of a range of PPCPs, pesticides and PFASs was measured in the estuary during a flood event and the subsequent eight month dry period.

2. Theory, material and methods

2.1. Study area

The Brisbane River is the longest river in South-East Queensland, Australia, rising near Mount Stanley and discharging after 344 km into Moreton Bay. The Brisbane River has several tributaries and flows through the city of Brisbane forming an approximately 90 km long estuary. The climate is subtropical with rainfall occurring predominantly in summer and early autumn. Between 2002-2011, the average flow in the lower estuary was 3.2 m^3/s between June and November (dry season) and 7.7 m^3/s between December and May (wet season), excluding flood events [12]. These low flow rates compared to the size of the drainage basin (13600 km^2) are a consequence of upstream flow regulation and drinking water abstraction. During the wet season, tropical cyclones and low-pressure systems can provide brief periods of intense rainfall that greatly increase estuary flow [13]. If the rainfall is sufficiently intense, the subsequent flood can flush some contaminants out of the estuary, while there may be a pulse input of other contaminants such as pesticides. We hypothesize that the change in contaminant concentrations in the estuary during a dry period following a flood can be used for persistence estimation.

The sampling site was adjacent to the suburb of Goodna in the upper reaches of the estuary (27°36'11.5"S 152°54'02.7"E) (**Fig. 1**) where the salinity is approximately 0.7 PSU [11] and the maximum tidal amplitude approximately 3 m (**Fig. S-1**). This section of the estuary is influenced by effluent from Goodna WWTP, located 2 km upstream of the sampling point. The sampling site was also 9 km downstream of the mouth of the Bremer River, one of the main tributaries of the estuarine portion of the Brisbane River. The Bremer River runs by a military airbase, and it is also impacted by effluent from the Bundamba WWTP (14 km upstream of the sampling point), both of which are probable sources of contaminants.

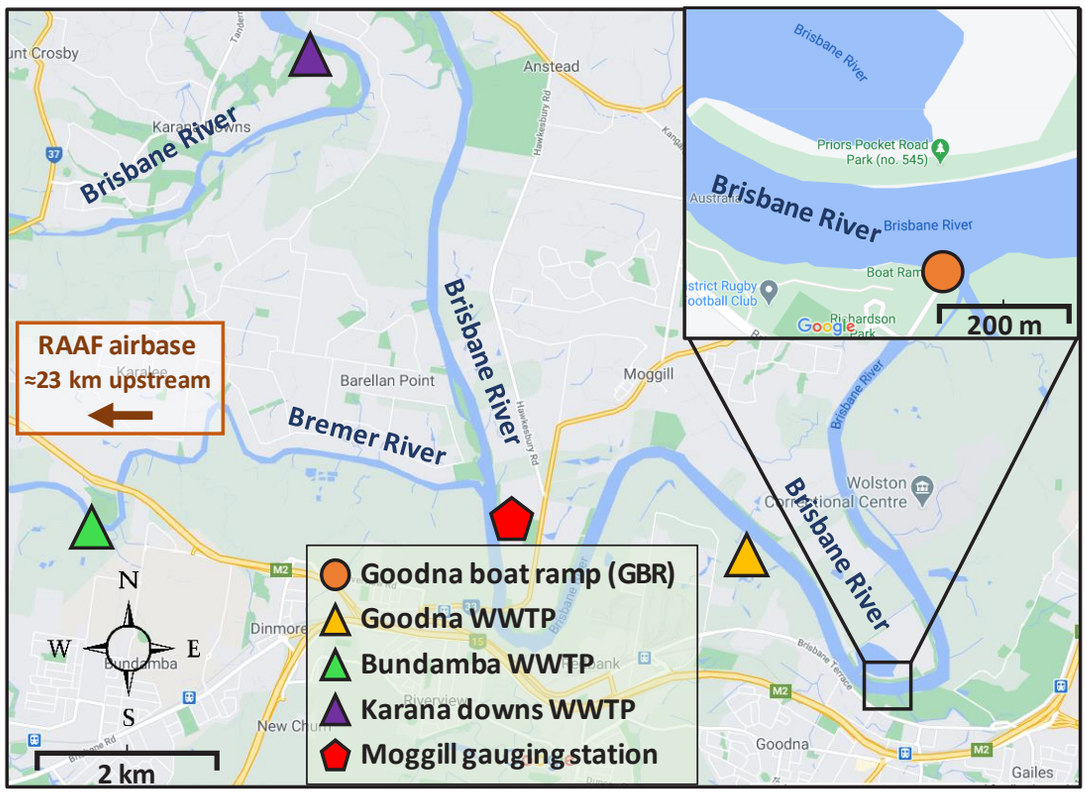

Figure 1: *Map of the study area.*

2.2. Sampling

Grab samples of estuary surface water were collected between late March and December of 2017 from the Goodna boat ramp (GBR) (**Fig. 1**). The river is approximately 135 m wide at this point. Sampling started the day before the rainfall associated with Cyclone Debbie, which was downgraded to a tropical storm on March 29 and reached Brisbane on March 30 [14]. Samples were taken with an aluminium bucket thrown 5-10 meters from the shore attached to

a rope. The bucket was allowed to sink to a depth of up to 1 m and then retrieved. The water samples were immediately placed in 500 mL polyethylene bottles, transported in a container with ice and stored at -20 ºC until analysis. Samples were taken almost daily during April and with a lower frequency thereafter (**Table S-1**).

Effluent samples from the Goodna and Bundamba WWTPs were available for 6-12 August 2017 to help quantify the contaminant input from these sources. The samples were 24 h time proportional composites, with sub-samples collected every 15 minutes and stored at 4 ºC. Immediately after sampling was completed, the samples were acidified to pH 5 with hydrochloric acid and stored at -20ºC until analysis.

All sample storage bottles were rinsed twice with methanol (MeOH) and MilliQ water before use.

2.3. Reagents

All reagents and analytical standards were of high purity. A total of 40 PFASs were analysed for, including perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs), together with 87 PPCPs and pesticides, including metabolites. Further details are presented in **Text S-1** in the Supplementary Material.

2.4. Extraction and analysis

Surface water (150 mL) and wastewater effluent (50 mL) were spiked with surrogate internal standards and extracted using solid phase extraction (SPE) following the standard operating procedures developed by the Queensland Alliance for Environmental Health Sciences. After volume reduction and filtration, instrument performance standards were added. PPCPs and pesticides were analysed via LC-MS/MS using a previously published method [3, 15] with minor modifications, while PFASs were analysed by UHPLC-MS/MS. Further details are provided in **Text S-2** and **Tables S-2** to **S-7**.

2.5. Quality assurance

The quality assurance protocols were similar to those described in Taylor et al. [16], blanks and an internal reference material in each batch of samples. Further details are provided in **Text S-3** and **Tables S-2**, **S-5** and **S-6**.

2.6. Mass balance model

A mass balance model of the study area for the post-flood dry time period was assembled. All chemical inputs to the estuary section were subsumed in an emissions term E (mol/d). Due to upstream dam regulations, freshwater inflow from the Brisbane River was negligible. Water inflow to the estuary section was essentially limited to groundwater inflow, WWTP discharge, and inflow of downstream water due to tidal action. Together these contributed to the water outflow G_{out} (m^3/d) from the estuary section, which when multiplied by the chemical concentration in water C_w (mol/m^3) gave the advective loss term. The other loss process considered was chemical transformation which was modelled as a first order process, the loss term being equal to the product of a first order transformation rate constant k_r (1/d), the volume of water in the estuary section V (m^3, treated as a constant) and C_w. All of the chemicals modelled had a Henry´s Law constant <1x10^{-6} atm·m^3/mol, and hence volatilization was not considered as a loss process. In addition, the modelled chemicals had a log K_{OW} <4 and thus a low tendency to sorb to sediment. Sequestration to sediment was therefore also not included. Given the tidal influence, it was further assumed that the water was well mixed in this segment of the river. The chemical mass balance for the estuary section can then be expressed as:

$$\frac{d(VC_w)}{dt} = E - G_{out}C_w - k_rVC_w \quad (1)$$

Three solutions to this differential equation were initially employed. In all cases it was assumed that E, G_{out}, and k_r are either constant with time or negligible. If E is not insignificant, then **Eq. 1** can be solved as follows:

$$C_w = C_i + \left(\frac{E}{G_{out} + k_rV} - C_i\right)\left(1 - e^{-\frac{(G_{out}+k_rV)}{V}t}\right) \quad (2)$$

where C_i is the concentration in water at the beginning of the post-flood period. This equation describes how one would expect the concentrations in water to change over time in a situation of constant ongoing emissions. For a persistent compound the term k_rV is negligible and if advective loss is also negligible, integration of **Eq. 1** affords:

$$C_w = \frac{E}{V}(t - t_i) + C_i \quad (3)$$

where t_i is the time at the beginning of the post-flood period, allowing calculation of the volume of the estuary segment (V) when the terms E and C_w are known. If, on the other hand, E is negligible, then the solution to **Eq. 1** becomes:

$$C_w = C_i e^{-\frac{(G_{out}+k_r V)}{V}t} \quad (4)$$

and the concentration decreases exponentially over time from the initial post-flood value.

Further equations assuming negligible advective losses and variable k_r were employed to fit the observed data for some chemicals. One approach, applicable to chemicals removed predominantly via phototransformation, assumes k_r is proportional to UV radiation intensity. We employed the hourly UV Index readings for Brisbane as a measure of UV radiation intensity to derive the following equation for chemicals for which E can be taken as constant with time:

$$\frac{dC_w}{dt} = \frac{E}{V} - k_{rp}(1.563 - 0.831\sin(t - 81.6))C_w \quad (5)$$

where k_{rp} is a pseudo first order rate constant for phototransformation on a day with an average UV index of 1 over a 24 h period and t is calendar day.

For chemicals primarily released during the flood and for which subsequent emissions are negligible, **Eq. 5** simplifies to:

$$\frac{dC_w}{dt} = -k_{rp}(1.563 - 0.831\sin(t - 81.6))C_w \quad (6)$$

The other fitting approach with variable k_r accounts for temperature-dependent transformation using an Arrhenius-type expression. Employing data for water temperature in the Brisbane River at Savage´s Crossing, the following equations for chemicals for which E can be taken as constant with time and those primarily released during the flood with negligible subsequent emissions were derived:

$$\frac{dC_w}{dt} = \frac{E}{V} - k_{rT} e^{\left(-\frac{U}{R}\left(\frac{1}{\left[\left(23.36\left(1-0.236\sin\left(t-111\right)\right)\right)+273\right]}-\frac{1}{298}\right)\right)} C_w \quad (7)$$

$$\frac{dC_w}{dt} = -k_{rT}e^{\left(-\frac{U}{R}\left(\frac{1}{\left[\left(23.36\left(1-0.236\sin\left(t-111\right)\right)\right)+273\right]}-\frac{1}{298}\right)\right)}C_w \quad (8)$$

where k_{rT} is a pseudo first order reaction rate constant at a reference state of 298 K, U is the activation energy (J/mol) and R is the gas constant (8.314 J/K mol). More information on the derivation of these fitting equations is provided in **Text S-4**.

Analytical solutions to **Eq. 5**, **Eq. 7** and **Eq. 8** were inaccessible and so numerical integration was undertaken. Concentration trends of chemicals were modelled with GraphPad Prism v8.4.3 software (GraphPad, San Diego, CA, USA). Each numerical integration run using this software consisted of a maximum of 1000 iterations with convergence to a solution defined as being when 5 iterations in a row changed the sum-of-squares by less than 0.0001%. The un-weighted nonlinear regression fit of models was assessed using Akaikes Information Criterion (AIC) that calculates and reports the probability that the data supports a model, taking into account both the goodness-of-fit (sum-of-squares) and the number of parameters in the model [17].

For some chemicals the monitoring data were fit to three models depending on concentration profile trends: either **Eq. 2**, **Eq. 5** and **Eq. 7** assuming E is constant and G_{out} negligible or **Eq. 4**, **Eq. 6** and **Eq. 8** if both E and G_{out} can be taken as negligible. In choosing between the models, the simplest model (constant k_r, **Eq. 2** or **Eq. 4**) was chosen unless the comparative model fit index indicated that one of the more complex models was clearly better (AIC >90%). In choosing between the more complex models, if the AIC threshold was not exceeded then the choice was based on information on transformation processes in the literature.

3. Results and discussion

3.1. Hydrological and meteorological considerations

The influence of the major rainfall event on the flow in the estuary began on March 30 and ended on April 7 according to the flow data at the Moggill gauging station (**Fig. 1**) (further information can be found in **Text S-5**). For the next six months there was very little rainfall. Mid-October marked the beginning of a wetter period (**Fig. S-1)**.

3.2. Concentration in WWTP effluents

Goodna WWTP showed markedly higher emissions of all PFASs, PPCPs and pesticides quantified than Bundamba WWTP (**Tables S-8** and **S-9**). This is mainly due to the higher daily volume of effluent discharge during the sampling period (an average of $1.3x10^7$ and $1.5x10^4$ L/day, for Goodna and Bundamba respectively). The emission flux was quite stable throughout the 7-day sampling period for most of the PFASs and several PPCPs and pesticides. The concentrations in the available samples (6-12 August 2017) were assumed to be representative of the emissions during April-December 2017.

3.3. Concentration time trends

To ensure the quality of the time trend data, only compounds quantifiable in at least 23 consecutive samples (62% of all samples collected) were selected. A total of 10 PFASs, 13 PPCPs and 18 pesticides fulfilled this criterion (**Fig. S-2** to **S-5**). Four types of time trends were observed:

Trend Type A (T-A): The concentration decreased markedly during the flood and thereafter the concentration increased linearly during the post-flood period (e.g. PFHxA **Fig. 2a**).

Trend Type B (T-B): As in the case of T-A, the concentration decreased markedly during the flood and then increased. However, the increase was not linear over time but approached a plateau or reached a maximum and then decreased (e.g. hydrochlorothiazide **Fig. 2b**).

Trend Type C (T-C): The concentration increased markedly during the flood and thereafter decreased (e.g. methomyl **Fig. 2c**).

Inconsistent trends: Some compounds showed trends that were erratic or different from those described above and were not modelled because they did not fulfil the model assumptions. This was the case for PFBA, MCPA, haloxyfop, desethyl atrazine, DEET, caffeine, ametryn hydroxy, atrazine, imidacloprid, atenolol, metalaxyl, metolachlor and imazethapyr. The time trends of these substances are presented in **Fig. S-5** and discussed in **Text S-6** of the Supplementary Material.

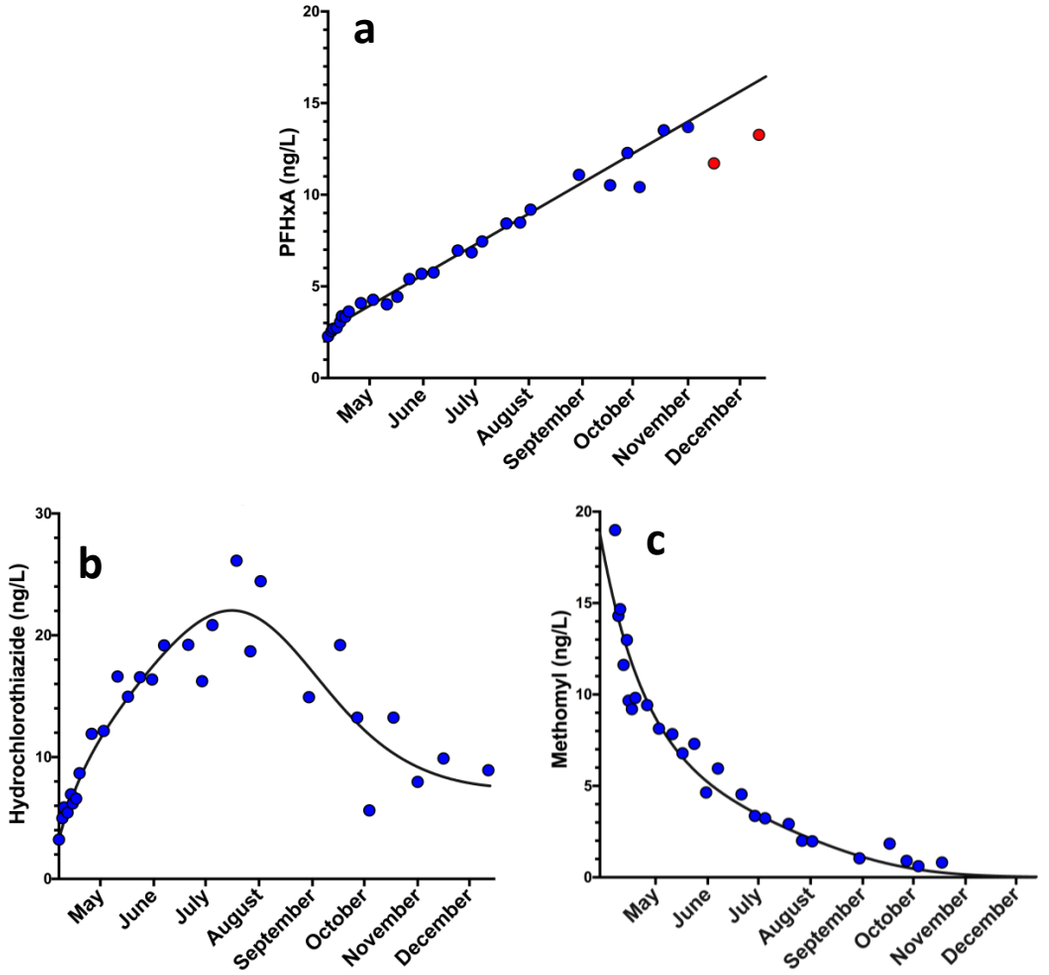

Figure 2: *Examples of the three types of consistent time trends in contaminant concentration observed: a) PFHxA (T-A); b) hydrochlorothiazide (T-B); c) methomyl (T-C). The deviation for the last two points for PFHxA (in red) from the model was used to correct the concentrations of the other substance for dilution that arose with the onset of wet season,*

3.4. Type A chemicals: Quantifying the advective loss term and volume of the system

Compounds belonging to this group were mainly PFASs (PFPeA, PFHxA PFOA, PFDA, PFBS, PFPeS, PFHxS, PFHpS and PFOS) but also included the pesticide simazine and the pesticide metabolite desisopropyl atrazine (**Fig. S-2**). PFASs are very persistent chemicals that are not known to degrade in water at all [18, 19].

PFHxA shows an extraordinarily linear increase in C_W for 7 months during the dry period (**Fig. 2**); a linear regression of the data gave an R^2 of 0.99. This indicates that the rain event flushed PFHxA out of the estuary and returned it to a pre-accumulation condition. Furthermore, the linearity indicates that transformation was insignificant and the model assumptions of constant

emissions and good mixing were fulfilled for this substance. Regarding the constancy of emissions, WWTP effluent is expected to be the major source of PFHxA to the estuary since there are no other known sources. This is supported by the studies of Anim et al. [11, 20], who measured the concentration profile of several PFASs and PPCPs along the Brisbane River estuary and found the maximum concentration of PFHxA downstream of Goodna WWTP.

The fact that C_W increased linearly over the 7-month period also indicated that loss of chemical due to advection out of the estuary stretch was negligible. Consequently, **Eq. 3** was applicable and could be used to solve for V, the volume of the estuary. Populating **Eq. 3** with the emissions of PFHxA from the Goodna WWTP together with the measured time trend data of C_W yielded $V = 4.1\times10^6$ m^3. Assuming a width of 135 meters and a mean depth of 2.5 meters (at Moggill), this is equivalent to an estuarine segment of approximately 12 km. For comparison, the estuary extends 20 km upstream from the sampling point.

The concentrations on the two last sampling days, November 16th and December 12th, were lower than expected from the time trend during the previous 7 months (**Fig. 2**). This was attributed to dilution caused by inflowing water as a result of the onset of wetter weather in mid-October (**Fig. S-1**). The quotient of the measured PFHxA concentration at these time points and the concentrations predicted from the linear regression gave dilution factors, which were used to correct November and December concentrations of the remaining T-A, T-B and T-C compounds for modelling purposes.

The concentrations of the remaining PFCAs were fit to **Eq. 2** using V as estimated above. The 95% confidence interval of the exponential term ($G_{out}/V + k_r$) intersected 0 for all the PFCAs except PFOA, for which it was very small (**Table 1**). This is consistent with expectations given the persistence of the PFCAs, and it provides further support for the assumptions of constant emissions and good mixing as well as the conclusion that advective losses of chemical from the estuary were negligible. The emissions E determined by fitting **Eq. 2** agreed well with the emissions estimated (mean of the emissions measured on 6-12 August 2017) at the Goodna and Bundamba WWTPs for all PFCAs (**Table 1**). This lends support to the WWTPs being the dominant source of PFCAs in the estuarine segment of interest.

The PFSAs and the pesticides desisopropyl atrazine and simazine show some elements of T-A behaviour, and we choose to address them here together with the PFCAs. PFBS, PFPeS, PFHpS, PFOS and the two pesticides were not present in the WWTP effluents above the LOQ

(**Tables S-8** and **S-9**), and even though PFHxS was detected, WWTP emissions were insufficient to explain the concentrations of PFSAs detected in estuarine water. Concentration profiles of several PFSAs were not as linear as those of PFCAs and in addition some exhibited a sharp concentration increase in November and December (the latter feature also shown by desisopropyl atrazine and simazine). The primary source of PFSAs to the estuary may be contaminated surface and ground water from use of aqueous film forming firefighting foam at the Amberley Air Force Base, located adjacent to the Bremer River. This is consistent with the reports of PFASs in the Brisbane River and surrounding areas by Baddiley et al. [21], who suggest Amberley Air Force Base as a possible intermittent source of PFSAs. Furthermore, their results show PFSAs concentrations were higher during the wet season. A close connection between precipitation and PFSAs and pesticides transport via ground and surface water to the estuary could then explain the observed time trend in C_W, with decreasing rates of increase of C_W as the dry period progresses due to diminishing groundwater inflow and higher rates of increase in C_W with the onset of the wet period in mid-October. Due to the evidence for temporal variability in PFSA emissions, the model was not applied to these chemicals. However, desisopropyl atrazine and simazine trends were sufficiently linear during the dry period and therefore they were modelled (**Table 1**). Further discussion can be found in **Text S-6.**

Table 1. Rate constant for chemical loss in the estuarine segment estimated by fitting the measured water concentrations for Type A compounds to Eq. 2, and emission flux to the estuarine section as estimated from a) concentrations estimated in WWTP effluents; and b) fitting the estuarine water concentrations to the model (Eq. 3).

	k_r (1/day)			Chemical input E (mg/day)		
	Median	**95% CI**	**R^2**	**Estimated from WWTP**	**Modelled**	**Esti/Mod**
PFPeA	$1.3x10^{-3}$	??? to $4.4x10^{-3}$*	0.96	126	138	0.94
PFHxA	$2.1x10^{-8}$	??? to $1.6x10^{-3}$*	0.99	224	§	-
PFOA	$4.1x10^{-3}$	$9.0\cdot10^{-4}$ to $7.7x10^{-3}$	0.94	329	359	0.91
PFDA	$2.8x10^{-3}$	??? to $8.5x10^{-3}$*	0.87	31	39	0.79
Desisopropyl atrazine	$6.1x10^{-11}$	??? to $2.5x10^{-3}$*	0.71	<3	37	<0.08
Simazine	$1.5x10^{-10}$	??? to $4.3x10^{-3}$*	0.76	<16	310	<0.05

**: Denotes that the software was unable to calculate the lower boundary.*
§: For PFHxA the estimated E was used to estimate V, so no independent estimate of modelled E was obtained.
<: The concentrations in the WWTPs effluent were below the LOD and LOQ for desisopropyl atrazine and simazine, respectively. Maximum possible emissions were calculated using the LOD and LOQ for desisopropyl atrazine and simazine, respectively.

3.5. Type B chemicals: Quantifying transformation rates of chemicals emitted post-flood

Hydrochlorothiazide, gabapentin, cotinine, hydroxycotinine, diuron, carbamazepine, tramadol, desmethyl diazepam, venlafaxine, temazepam and iopromide concentrations showed a T-B time trend. The mass balance model described by **Eq. 2** assumes that emissions *E* and the rate constant for removal k_r are constant. Concentration time trends indicated that these assumptions are plausible for some of the chemicals throughout the post-flood sampling period (**Fig. S-3**). For diuron, abrupt concentration changes suggested variable emission fluxes and so model fitting was applied only to that part of the sampling period where a smooth concentration profile was observed. For other chemicals, concentrations reached a maximum and then attenuated, a profile inconsistent with the asymptote to a plateau described by **Eq. 2** but possibly due to a temporally varying transformation rate constant.

Chemical loss was dominated by environmental transformation because advection was negligible (see above). Environmental transformation processes can be purely chemical (e.g. hydrolysis), due to solar radiation (phototransformation) or mediated by microorganisms (biotransformation). They are influenced by environmental variables and can be expected to exhibit seasonal variability: phototransformation due to the influence of solar radiation intensity, and both bio- and chemical transformation due to the influence of ambient temperature. Therefore, in addition to **Eq. 2**, which assumes that k_r is constant, we fitted the monitoring data with two further models, one assuming that k_r varies with UV intensity (**Eq. 5**) and the other assuming that it varies with temperature (**Eq. 7**).

At least one of the models provided a good fit of the data for each chemical. **Table 2** shows the half-lives and estimated WWTP emission fluxes (E) from the best fitting model for each chemical. **Table 2** also compares these values to half-lives reported in the literature and estimated emission fluxes from the WWTPs. The k_r and half-lives derived from the UV- and temperature-dependent models (Tables S-12 to S-15) are based on chosen standard conditions (298 K and a UV index of 1 [25 mW/m^2 of UV radiation] averaged over a day).

The mass balance modelling approach employed provides information on compound persistence, possible sources and dominant transformation mechanisms. Of the ten Type B compounds for which measured WWTP chemical input data exist, seven including carbamazepine, desmethyl diazepam, diuron, iopromide and temazepam have modelled input

rates within a factor of two of measured input rates from WWTPs, with the latter ranging from <100 to >50000 mg/day. The good agreement for chemicals for which we expect the WWTP to be the dominant source indicates that the methodology employed works well.

Poor agreement between estimated and modelled emissions was observed for cotinine (ratio 1:3), venlafaxine (4:1) and gabapentin (260:1) (**Table 2**). This may indicate the presence of other sources, errors in the determination of emissions, problems in the measurement of concentrations in water, or an inadequate mass balance model. Cotinine is a transformation product of nicotine [22], and formation from precursors in the estuary is one possible explanation for the higher modelled emissions. Aliphatic amines including venlafaxine and tramadol are sequestered into acid vesicles in protozoa via ion trapping [23], and this pool may not have been accessed by our sampling and analytical protocol in the estuary, resulting in lower modelled emissions. Gabapentin showed a particularly bad agreement, which may suggest poor performance of the analytical method for WWTP effluent. Some of the possible causes of poor agreement between modelled and estimated emissions can also influence the estimated half-life, and hence the latter is associated with greater uncertainty until the causes have been identified.

In regard to dominant transformation processes, carbamazepine and gabapentin were the only compounds that showed a better fit for the constant k_r model. UV- and temperature-dependant models performed significantly better for the remaining compounds, with the former usually providing the best fit, indicating that phototransformation was a significant transformation process (**Table 2**). However, since UV- and T-dependent transformation show a similar seasonal dependence (distinguished mostly by an approximately 1 month time shift) (**Fig. S-6**), the performance of UV- and T-dependent models was similar for cotinine, desmethyl diazepam, hydroxycotinine, iopromide, and temazepam. Therefore, the dominant transformation process for these compounds could not be inferred.

Table 2. Transformation half-life (days) for T-B compounds estimated by fitting the measured concentrations in estuarine water to **Eq. 2** (C), **Eq. 5** (UV) or **Eq. 7** (T) and for T-C chemicals estimated by fitting the measured concentrations to **Eq. 4** (C), **Eq. 6** (UV) or **Eq. 8** (T) in comparison to literature values. The results shown are for the model giving the best fit. Also shown is modelled chemical emission flux (E, mg/day) to the estuary segment compared with the flux estimated from the WWTP data.

	Chemical input E (mg/day)			$k_{(r, rp \text{ or } rT)}$ (1/day)			Half-life (days)				
T-B compounds	**Estimated from WWTP**	**Modelled**	**Esti/Mod**	**Median**	**95% CI**	**R^2**	**Median**	**95% CI**	**Model**	**Literature**	**Ref.**
Carbamazepine	4100	4000	1.03	$1.1 \cdot 10^{-2}$	$7.5 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}$	0.94	69	51 to 97	C^+	>69	[24]R
Cotinine	78	250	0.31	$3.3 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$ to $5.0 \cdot 10^{-3}$	0.91	210	140 to 380	UV^+	n.f.	-
Desmethyl diazepam	87	66	1.32	$4.9 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$ to $1.1 \cdot 10^{-2}$	0.91	140	63 to 210	UV^+	2-8	[7]P
Diuron	620	640	0.97	$1.0 \cdot 10^{-2}$	$7.1 \cdot 10^{-3}$ to $1.4 \cdot 10^{-2}$	0.98	66	48 to 98	UV	9-101	[9]RS
Gabapentin	920000	3500	260	$1.8 \cdot 10^{-2}$	??? to $2.7 \cdot 10^{-2}$*	0.93	40§	27 to 58	C^+	33-52	[9]RS
Hydrochlorothiazide	5100	2900	1.76	$3.9 \cdot 10^{-2}$	$2.8 \cdot 10^{-2}$ to $5.9 \cdot 10^{-2}$	0.85	18	12 to 25	UV	2-49	[25]R
Hydroxycotinine	460	230	2.0	$6.6 \cdot 10^{-3}$	$4.6 \cdot 10^{-3}$ to $8.9 \cdot 10^{-3}$	0.89	110	78 to 150	UV^+	n.f.	-
Iopromide	51000	32000	1.59	$1.1 \cdot 10^{-2}$	$8.0 \cdot 10^{-3}$ to $1.6 \cdot 10^{-2}$	0.80	61	44 to 87	UV^+	8-69	[26]RS
Temazepam	830	1100	0.75	$7.0 \cdot 10^{-3}$	$6.2 \cdot 10^{-3}$ to $7.8 \cdot 10^{-3}$	0.98	99	89 to 112	UV^+	2-3	[7]P
Tramadol	-	3200	-	$1.0 \cdot 10^{-2}$	$8.1 \cdot 10^{-3}$ to $1.2 \cdot 10^{-2}$	0.93	69	56 to 85	UV	2-10	[27, 28]R
Venlafaxine	9200	2100	4.4	$9.6 \cdot 10^{-3}$	$7.8 \cdot 10^{-3}$ to $1.2 \cdot 10^{-2}$	0.94	72	59 to 88	UV	1	[27]R
T-C compounds											
2,4-D	-	-	-	$2.3 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$	0.99	30	28 to 33	UV^+	12	[29]R
Diazinon	-	-	-	$2.5 \cdot 10^{-3}$	$1.7 \cdot 10^{-3}$ to $3.4 \cdot 10^{-3}$	0.65	280	200 to 410	UV^+	138	[6]H
Dicamba	-	-	-	$6.2 \cdot 10^{-2}$	$1.8 \cdot 10^{-2}$ to $1.8 \cdot 10^{-1}$	0.82	11	4 to 39	T^+	10-14	[29]R
Methomyl	-	-	-	$3.8 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$ to $6.3 \cdot 10^{-2}$	0.94	18	11 to 32	T^+	0.6-9	[30]B
Prometryn	-	-	-	$2.2 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$ to $3.8 \cdot 10^{-2}$	0.89	32	18 to 59	T	<1	[31, 32]B
Tebuthiuron	-	-	-	$5.1 \cdot 10^{-3}$	$4.2 \cdot 10^{-3}$ to $6.0 \cdot 10^{-3}$	0.89	140	110 to 160	C^+	944	[33]RS

§: *Half-lives are uncertain due to analytical issues, see **Text S-6** for more details.*
+: Denotes that, although the chosen model gave the best fit, at least one of the other models also gave a good fit.
R: Half-life derived from measurements in a real environment (river, wetland or lake).
RS: Half-life derived from a simulation experiment.
P: Half-life derived from a phototransformation experiment.
H: Half-life derived from a hydrolysis experiment.
B: Half-life derived from a biotransformation experiment.
n.f.: Not found.

The in-situ half-lives estimated in the present work were generally within the range or higher than those from the literature although relevant studies providing half-lives from field studies in aquatic environments were scarce and their magnitudes reflected site specific characteristics [24, 25, 27, 28]. Since several of the compounds showed susceptibility to phototransformation, the turbidity (40 to 100 NTU) and depth of this stretch of the Brisbane River [12] may have reduced phototransformation rates compared to other field studies in less turbid water or laboratory derived data typically obtained using clean water and short light pathlengths.

More detailed compound by compound discussion of the estimated half-lives, model fits, transformation processes and emissions of the T-B chemicals is provided in **Text S-6.**

3.6. Type C chemicals: Quantifying transformation rates of chemicals released during the flood

The general decrease in concentrations of this group of compounds after the flood suggests that emissions to the estuary were greatest during the flood. The group included 2,4-D, diazinon, dicamba, methomyl, tebuthiuron and prometryn. Assuming post-flood emissions were negligible and neglecting G_{out} as discussed above, **Eq. 4**, (constant k_r approach), **Eq. 6** (UV intensity dependent k_r approach) and **Eq. 8** (temperature dependent k_r approach) were fit to the time trend data to estimate k_r, k_{rp} and k_{rT} (**Table S-15**). Since all the compounds were pesticides, runoff from nearby land during and after the storm was the most likely source. Increasing levels of pesticides in surface water attributed to runoff after a rainfall event have been reported previously [34, 35].

Some T-C chemicals such as 2,4-D and dicamba did not show an exponential decrease during the whole dry period, which may suggest that emissions were significant at certain times. Emissions pulses are not unexpected for these substances, since all the T-C compounds are pesticides that typically have seasonal use. For these compounds just the part of the period showing an exponential decrease was fit to the models (**Suplementary 2**).

As with the T-B chemicals, the UV- and temperature-dependant models showed a better fit than the constant k_r approach for most of the compounds except tebuthiuron (**Table 2**). For some compounds such as dicamba and methomyl, the variable k_r models performed similarly.

The estimated half-lives were significantly higher than those from the reference studies for all T-C compounds except tebuthiuron (**Table 2**). As for T-B compounds, the turbidity and depth of water in the estuary stretch of interest may have reduced phototransformation rates compared to other studies. The example of prometryn, with a half-life estimated in laboratory biotransformation studies of <1 d [31, 32] and a half-life in the Brisbane River of 32 d, illustrates that biotransformation conditions can also differ greatly between the laboratory and the field. We note that ongoing significant emissions cannot be ruled out in our study, and this would cause us to overestimate the half-life.

Further discussion of the estimated half-lives, model fits, dominant transformation processes and possible on-going emissions of the T-C chemicals can be found in **Text S-6**

3.7. Utility of the method

An estuary with significant contaminant inputs that had been initially washed out by a flood event proved to be a useful scenario for measuring contaminant persistence in a real environment. The relatively long water residence time of the estuary meant that it was possible to quantify chemicals' half-lives that are longer than those that can be measured in flowing rivers and that lie in the range of regulatory thresholds for persistence. By using chemicals known to be fully persistent and with measurable input rates to the estuary (PFHxA and several of its homologues) as benchmarks, it was possible to estimate contaminant input rates for other studied chemicals and gain insight into their sources. It also created possibilities to quality assure the observations and the interpretive model used to quantify half-lives, and this led to the flagging of four pharmaceuticals with uncertain half-lives (cotinine, venlafaxine, tramadol and gabapentin). The use of benchmarks could conceivably be further expanded, for instance by identifying a chemical with constant inputs that is removed primarily by a specific mechanism, such as phototransformation. If the rate constant of that mechanism is known, the identified chemical could then be used as a benchmark for the transformation potential by that mechanism of other contaminants in the system. Further development of the analytical methods would allow the environmental persistence of a broader spectrum of contaminants to be assessed.

This measurement of transformation half-lives in natural environments can provide information for assessing chemicals against regulatory thresholds for persistence. Of the PPCPs and pesticides studied here, the median half-life in the Brisbane River exceeded the Stockholm

Convention criterion for persistence in water, 60 d [36], for carbamazepine, cotinine, desmethyl diazepam, diuron, hydroxycotinine, iopromide, temazepam, venlafaxine, tramadol, diazinon and tebuthiuron (the half-lives for cotinine, tramadol and venlafaxine are more uncertain as noted above). Persistence assessment is typically performed using simple laboratory protocols, but these cannot capture the full complexity of environmental transformations. Measurements in real environments can provide a useful reality check.

Field measurements are also useful for developing and evaluating methods to extrapolate laboratory test results to the environment. A thorough exploration of this subject was beyond the scope of this work. However, the comparison of our half-lives with values from the literature does provide a few insights, even if we selected other field studies or more environmentally realistic simulation experiments over standard laboratory test protocols where possible. For example, compounds for which phototransformation is believed to be an important loss process (e.g., desmethyl diazepam and temazepam) showed markedly longer half-lives in the Brisbane River compared with laboratory studies. Environmental factors such as depth, turbidity and the composition and quantity of dissolved organic matter play an important in modulating phototransformation in the environment [37], and here field measurements can help in developing our understanding. There were also large differences in laboratory and Brisbane River half-lives for chemicals subject to biotransformation (e.g., prometryn). Of all transformation processes, biotransformation is most difficult to extrapolate from the laboratory to field due to the difficulty in reproducing the composition and activity of the microbial community in the laboratory. It is therefore likely that our understanding of this process could benefit most from field measurements of transformation.

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Supplementary Information

Persistence of PPCPs and pesticides in a subtropical river estuary

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Table of contents

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Text S-1. Reagents

All reagents and analytical standards were of high purity. MeOH (LiChrosolv® LC grade ≥99.8%) was purchased from MERCK (Bayswater, VIC, Australia). Ammonium hydroxide solution 28.0-30.0% was from Sigma Aldrich (Bayswater, VIC, Australia).

The PFASs analysed for were: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulphonate (PFBS), perfluoropentanesulsulphonate (PFPeS), perfluorohexanesulsulphonate (PFHxS), perfluoroheptanesulphonate (PFHpS), perfluorooctanesulsulphonate (PFOS), perfluorononanesulfonate (PFNS), perfluorodecanesulfonic acid (PFDS), perfluordodecanesulphonate (PFDoDS), perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamide acetic acid (FOSAA), N-ethyl perfluorooctanesulfonamide (N-Et-FOSA), N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA), N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE), N-methyl fluorooctanesulfonamide (N-MeFOSA), N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA), N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE), perfluoroethylcyclohexanesulfonate (PFECHS), the fluorotelomer sulfonates (FTS): 10:2 FTS, 8:2 FTS, 6:2 FTS, and 4:2 FTS and the perfluoroalkyl phosphate esters (PAP): 8:2 PAP, 6:2 PAP, SAmPAP, 8:2 DiPAP, 6:2 DiPAP and 6:2 8:2 DiPAP. A mixture of ^{13}C and ^{18}O labelled PFASs (purchased from Wellington Laboratories, Guelph, Ontario, Canada) served as surrogate internal standards (ISs) to correct for method recovery (**Table S-3**). $^{13}C_8$-PFOA and $^{13}C_8$-PFOS were used as instrument performance standards to check for correct functioning of the instruments (**Table S-4**).

The PPCPs and pesticides analysed for were: salicylic acid, acesulfame K, (4-Chloro-2-methylphenoxy) acetic acid (MCPA), ibuprofen, 2,4-Dichlorophenoxyacetic acid (2,4-D), dicamba, 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), triclopyr, bromoxynil, triclosan hydrochlorothiazide, furosemide, diketonitrile, paracetamol, 3,4 dichloroaniline, methomyl, nicotine, gabapentin, desisopropyl atrazine, cotinine, paraxanthine, simazine hydroxyl, desethyl atrazine, clopyralid, 5-hydroxyindoleacetic acid (5HIAA), N,N-Diethyl-meta-toluamide (DEET), hydroxycotinine, caffeine, ametryn hydroxyl, pyrimethanil, terbuthylazine desethyl, simazine, desdimethyl diuron (DCPU), propoxur, metribuzin, atrazine, desmethyl diuron (DCPMU), dichlorvos, carbofuran, methiocarb, ametryn, tebuthiuron, propazine, terbuthylazine, asulam, naproxen, diuron, fluometuron, carbamazepine, prometryn, terbutryn, picloram, hexazinone, fluroxypyr, imidacloprid, bromacil, tramadol, atenolol, desmethyldiazepam,

imazapic, venlafaxine, metalaxyl, pendimethalin, metolachlor, imazethapyr, codeine, temazepam, fenamiphos, diazinon, tebuconazole, fluoxetine, desmethyl citalopram, citalopram, fluazifop, malathion, propiconazole, prothioconazole, chlorpyriphos, haloxyfop, metsulfuron-methyl, tadalafil, verapamil, sildenafil, atorvastatin, iopromide and haloxyfop-methyl. A total of 48 ISs were used for their quantification (**Table S-7**): 2,4-D-13C6, 5HIAA-D2, acesulfame-D4, atenolol-D7, atorvastatin-D5, atrazine-D5, atrazine desisopropyl-D5, bromacil-D3, caffeine-13C3, carbamazepine-D10, carbofuran-D3, citalopram-D6, codeine-D3, cotinine-D3, DEET-D7, desethyl atrazine-D6, diazinon-D10, diuron-D6, fluazifop-D4, fluoxetine-D6, gabapentin-D10, haloxyfop-D4, hexazinone-D6, hydrochlorothiazide-13CD2, hydroxycotinine-D3, ibuprofen-D3, imidacloprid-D4, iopromide-D3, MCPA-D6, methiocarb-D3, methomyl-D3, metolachlor-D6, metribuzin-D3, metsulfuron-methyl-D3, nicotine-D4, paracetamol-D4, paraxanthine-D3, prometryn-D6, propazine-D6, propiconazole-D5, propoxur-D3, simazine-D10, tebuconazole-D6, tebuthylazine desethyl-D9, temazepam-D5, terbuthylazine-D5, tramadol-D6 and venlafaxine-D6.

For the PPCPs, pesticides and PFASs for which IS analogues were not available, quantification was done using external calibration or the IS with the most similar structure (**Tables S-2, S-5 and S-6**).

Text S-2. Extraction and analysis

Extraction procedure for PPCPs and pesticides

Samples were extracted using solid phase extraction (SPE) in a RESTEK manifold. Prior to extraction, 150 mL of surface water sample (50 mL for wastewater) was spiked with 10 µL of a 1 µg/mL IS mix and the pH recorded. The SPE cartridges used were Strata^{TM}–X 33µm Polymeric reversed phase, 200 mg/6 mL, from Phenomenex®, conditioned with 4 mL of MeOH followed by 4 mL of MilliQ water. Samples were first shaken to ensure homogenization, and then passed through the cartridges dropwise. The cartridges were washed with 4 mL of MilliQ water, dried under vacuum for 30 min and then eluted using 2 mL of methanol dripped through the cartridge by gravity flow. This elution procedure was repeated, obtaining in total 4 mL of extract, which was collected in glass Falcon tubes and blown down to approximately 1 mL using a 40 ºC heating plate and a gentle stream of nitrogen in a RATEK blowing down unit. The extracts were then filtered using PTFE (0.2 µm) Captiva filters (from Agilent technologies, USA), preconditioned with 1 mL of MeOH and blown down again to a final volume of 50 µL. Finally, 200 µL of MilliQ water was added to obtain a final solvent mixtureof 20: 80 MeOH: H_2O. The final extracts were stored on amber vials and refrigerated until analysis.

Extraction procedure for PFASs

This extraction procedure was based in previous literature [3, 4], with some modifications. Briefly, the samples were also extracted using SPE. The sample volume was the same as for PPCPs and pesticides, and they were also spiked with 10 µL of a 0.2 µg/mL IS mix. The SPE cartridges (Phenomenex® Strata™ X-AW 33 µm Polymeric weak anion exchange, 100 mg/6 mL) were conditioned using 4 mL of 0.2% ammonium hydroxide: MeOH, followed by 4 mL of MeOH and 4 mL of MilliQ water. The samples were passed through the cartridge and then washed as for PPCPs and pesticides. Then, they were dried with vacuum for at least 3 hours, until they were absolutely dry. The cartridge was eluted with 4 mL of 0.2% ammonia: MeOH, dripped through the cartridge by gravity flow. The extract was placed in Falcon tubes and blown down to a final volume of 80 µL, then 120 µL of aqueous mobile phase (A) was added, affording a final solvent mixture of 40: 60 MeOH: H_2O. Then, 10 µL of 0.2 µg/mL Recovery Standards (**Table S-4**) mixture was added. Finally, the extract was stored in a plastic vial and refrigerated until analysis.

The extraction protocol for wastewater samples was as described above, except that the blowdown process was interrupted when the extracts volume was approximately 1mL when the extract was passed through a Bond Elut Carbon, 100mg, 1mL 100/pk cartridge, from Agilent technologies (USA), previously conditioned with 1 mL of MeOH. The resulting extract was then blown down to 80 µL and the process continued as described above.

To avoid possible adsorption of PFASs to in the glassware materials, the material used for the storage was polyethylene, and the material for manipulation and extraction procedures was made of polystyrene.

LC-MS/MS

PPCPs and pesticides were analysed via LC-MS/MS, using a Shimadzu Nexera X2 HPLC system (Shimadzu Corp., Kyoto, Japan), equipped with a Kinetex® EVO C18 precolumn (5 µm 100 Å, LC Column 50 x 4.6 mm, Phenomenex) and a Kinetex® Biphenyl column (2.6 µm 100 Å, LC Column 50 x 2.1 mm, Phenomenex). The HPLC was coupled to a Sciex QTRAP 6500^+ mass spectrometer (Sciex, Concord, Ontario, Canada) equipped with electrospray ionization (ESI) (TurboV) interface (Curtain gas 30 psi, ion-spray voltage 5.4kV, temperature 480ºC, and the ion source gases 1 and 2 at 60 psi). It was operated in negative and positive modes, with fast polarity switching, and multiple reaction monitoring mode (MRM), with a turbo Spray IonDrive. The mobile phases employed were (A) H_2O 1% MeOH, 0.1% acetic acid and (B) MeOH 5% H_2O, 0.1% Acetic acid. The linear gradient was as follows: 0 min (95% A), 0.5 min (90% A), 5.2 min (0% A), 9.6 min (0% A) and 9.61 min (95% A). The injection volume was 5 µl and column temperature was held at 45 ºC.

PFASs were analysed by UHPLC-MS/MS, using a Sciex ExionLC UHPLC system (Sciex, Concord, Ontario, Canada), equipped with a Kinetex® EVO C18 precolumn (5 µm 100 Å, LC Column 50 x 4.6 mm, Phenomenex) and a Kinetex® EVO C18 column (2.6 µm 100 Å, LC Column 100 x 2.1 mm, Phenomenex). The mass spectrometer was a Sciex TRIPLE QUAD 6500^+ (Sciex, Concord, Ontario, Canada) equipped with electrospray ionization (ESI) (TurboV), operated in negative mode and MRM, with a turbo Spray IonDrive (curtain gas 30 psi, ion-spray voltage floating at -4.5 kV, temperature 360 °C, and the ion source gas 1 and 2 at 55 psi). The mobile phases were (A) H_2O 1% MeOH, 8 mM ammonium acetate, 5 mM N-methyl piperidine and (B) MeOH 5% H_2O, 8 mM ammonium acetate, 5 mM N-methyl piperidine. The linear gradient was as follows: 0 min (85% A), 0.5 min (60% A), 6 min (0% A), 9 min (0% A) and 9.10 min (85% A). Injection volume was 5 µl and column temperature was held at 50 ºC.

The identification and confirmation of compounds was made by comparing their retention times (RT) and mass spectral transitions with those from reference standards (**Tables S-2**, **S-5** and **S-6**). The software used was MultiQuant 3.0.2 from Sciex.

Text S-3. Quality assurance

Each batch of a maximum of 10 samples included a procedural blank (MilliQ water). In addition, a "side spike" was included in every batch, made by repeating every pippeting applied to the samples, but into an empty vial. Later a MeOH: MilliQ water solution was added to obtain the same final proportion as in the extracted samples. In other words, this vial contained all the reagents present in the extracts but omitting the extraction procedure. This was made in order to check if the pipetting protocol and practice during the extraction procedure was correct. A full set of calibration standards (8 for PPCPs and pesticides, and 10 for PFASs) was injected at the beginning and the end of each analytical sequence. Also, a standard was injected every 10 samples to check for instrumental variation. Only regression coefficients (R^2) > 0.99 were accepted for the calibration curve. Instrumental limits of detection (ILODs) were determined as three times the standard deviation (SD) of the signal from repeated injection (7×) of a low-level standard (0.4 ng/mL for PFASs and 0.5 ng/mL for PPCPs and pesticides). Instrumental limits of quantification (ILOQs) were set as three times the ILODs. Limits of reporting (LORs) were set to three times the average concentration in the MilliQ blanks. Any concentration below the LORs or ILODs was reported as not detected (n.d.). Repeatability was calculated as the SD divided by mean (%CV) of seven injections of the low-level standard.

Text S-4. Fitting models with variable reaction rate constants

Two models with variable reaction rate constants k_r were applied to fit the observed data for some T-B chemicals. One, applicable to chemicals removed predominantly via phototransformation, assumes a rate constant proportional to UV radiation intensity. We summed the hourly UV Index readings for Brisbane for each day during 2017 (denoted UV_{24}) and fitted them with a sin function (**Fig. S-6**), obtaining the equation:

$$UV_{24} = 37.503(1 - 0.532\sin(t - 81.6)) \quad \text{(S-1)}$$

where t is calendar day. We then defined a pseudo first order rate constant for phototransformation, k_{rp}, with a reference state of $UV_{24} = 24$ (or an average UV index of 1 over a 24 h period):

$$k_r = k_{rp}\frac{37.503}{24}(1 - 0.532\sin(t - 81.6)) \quad \text{(S-2)}$$

Substituting this into **Eq. S-3**,

$$\frac{d(VC_w)}{dt} = E - G_{out}C_w - k_rVC_w \quad \text{(S-3)}$$

and neglecting loss by advection yields:

$$\frac{dC_w}{dt} = \frac{E}{V} - k_{rp}(1.563 - 0.831\sin(t - 81.6))C_w \quad \text{(S-4)}$$

The second model with a variable reaction rate constant k_r accounts for temperature dependent transformation using an Arrhenius-type approach. First, water temperature in the Brisbane River at Savage´s Crossing during 2017 was plotted and fitted with a sin function (**Fig. S-6**), yielding the equation:

$$T_C = 23.36(1 - 0.236\sin(t - 111)) + 273 \quad \text{(S-5)}$$

where T_C is water temperature (K). We then defined a pseudo first order reaction rate constant at a reference state of 298 K (k_{rT}) and described the temperature dependence using the Arrhenius equation:

$$k_r = k_{rT} \cdot e^{\left(-\frac{U}{R}\left(\frac{1}{[(23.36(1-0.236\sin(t-111)))+273]} - \frac{1}{298}\right)\right)} \quad \text{(S-6)}$$

where U is the activation energy (J/mol) and R is the gas constant (8.314 J/K mol). Substituting this into **Eq. S-1**, and neglecting loss by advection yields:

S-8

$$\frac{dC_w}{dt} = \frac{E}{V} - k_{rT} e^{\left(-\frac{U}{R}\left(\frac{1}{[(23.36(1-0.236\sin(t-111)))+273]}-\frac{1}{298}\right)\right)} C_w \quad \text{(S-7)}$$

For T-C chemicals that were primarily released during the flood and whose concentration tended to decrease with time thereafter, an analogous approach was employed. Substituting **Eq. S-2** and **Eq. S-6** into **Eq. S-8** that describes transformation loss but no advective loss and no emissions post-flood.

$$\frac{dC_w}{dt} = -k_r C_w \quad \text{(S-8)}$$

affords

$$\frac{dC_w}{dt} = -k_{rp}(1.563 - 0.831\sin(t - 81.6))C_w \quad \text{(S-9)}$$

$$\frac{dC_w}{dt} = -k_{rT} e^{\left(-\frac{U}{R}\left(\frac{1}{[(23.36(1-0.236\sin(t-111)))+273]}-\frac{1}{298}\right)\right)} C_w \quad \text{(S-10)}$$

Text S-5. Hydrological and meteorological considerations

The Savage´s Crossing alert weather station, located approximately 60 km upstream of the sampling point, registered 183 mm of precipitation the 30th of March [5], while stations in southern areas registered from 242 to 297 mm the next day [6]. Major flood levels were recorded in Ipswich, while downstream at the Brisbane river the levels remained below the threshold for a minor flood [6]. The water level at Moggill increased by about 2.5 meters during March 31. This suggests that most of the water from this event came from Ipswich through the Bremer River and then discharged into the Brisbane River just upstream of the Moggill gauging station.

Text S-6. Interpretation of temporal concentration profiles

T-A chemicals

Compounds belonging to this group were mainly PFASs (PFPeA, PFHxA PFOA, PFDA, PFBS, PFPeS, PFHxS, PFHpS and PFOS) but also included the pesticide simazine and the pesticide metabolite desisopropyl atrazine (**Fig. S-2**). The concentration profiles of perfluorinated carboxylates (PFCAs) PFPeA, PFHxA PFOA and PFDA have already been discussed in the manuscript.

In contrast to the PFCAs, the perfluorosulfonates (PFSAs) PFBS, PFPeS, PFHpS and PFOS were not present in the WWTP effluents above the LOQ (**Tables S-8** and **S-9**), and while PFHxS was detected in the WWTP effluent, these emissions were insufficient to explain the concentrations detected in the estuarine water. This indicates that there must have been other major sources of the PFSAs. Furthermore, the curvilinear time trend in C_W exhibited by several PFSAs during the dry season followed by a sharp increase in November and December contrasts with the linear C_W time trends shown by most PFCAs and suggests that there must have been variable emissions. High levels of PFOS+PFHxS have been detected in water and fish in the Bremer River [7] (**Fig. 1**). Based on their transect of PFASs contamination along the Brisbane River, Anim et al. [8, 9] suggest that the Bremer River is an important source of several PFSAs to the estuary. A governmental investigation of the consequences of the use of aqueous film forming firefighting foam at the Royal Australian Air Force Base Amberley, which is located adjacent to the Bremer River, has indicated widespread off-base contamination of surface and ground water with PFASs [10]. This may be the primary source of PFSAs to the estuary. As mentioned in the manuscript, a close connection between precipitation and PFSA transport via ground and surface water to the Bremer River could explain the observed time trend in C_W, with decreasing rates of increase of C_W as the dry period progresses due to diminishing groundwater inflow and higher rates of increase in C_W with the onset of the wet period in mid-October.

Modelling of the concentration trends of desisopropyl atrazine and simazine showed that like most of the PFCAs, the 95% confidence interval of the exponential term ($G_{out}/V + k_r$) also intersected zero (**Table 1**), indicating that these two substances were very persistent in the estuary. Unlike the PFCAs, they were not present in the WWTP effluents above the LOQ (**Tables S-8** and **S-9**). They are pesticides that could be entering the estuary through atmospheric deposition [11], drainage channels from agricultural fields [12], or groundwater discharge. The apparent constancy of the inputs during this dry period would seem to exclude the first two possibilities, making groundwater discharge the most likely source. Desisopropyl atrazine is an environmental transformation product of atrazine, sebuthylazine, and simazine, and could have been formed in the estuary after discharge of atrazine herbicides. However, the levels of atrazine and simazine in the WWTP effluents (0.05 mg/d and <16 mg/d, respectively) themselves are insufficient to explain the levels

of desisopropyl atrazine in the estuary and there are likely to be other sources for atrazine or the other potential precursors.

T-B chemicals

Hydrochlorothiazide, gabapentin, cotinine, hydroxycotinine, diuron, carbamazepine, tramadol, desmethyl diazepam, venlafaxine, temazepam and iopromide concentrations showed a T-B time trend.

Diuron is a broad-spectrum residual herbicide. The very good agreement between estimated input flux and estimated WWTP emissions (ratio of 0.97) indicates that the WWTPs were the main source of diuron to the estuarine segment. Since the modelled data were collected during a dry period, inputs from surface runoff are expected to have been negligible. In contrast to simazine and desisopropyl atrazine, diuron inputs from groundwater appear to have been insignificant. Indeed, Prichard et al. suggest that diuron is more likely to remain in the upper soil layers rather than infiltrate [13]. Our estimated half-life (66 d) was in the range of those measured in a laboratory biotransformation experiment using sediment-water suspensions (9-101 d) [14]. Relatively slow phototransformation (half-life of approximately 4 months) of diuron in demineralized water has been reported during exposure to natural sunlight at higher latitudes (57° N) in summer [15]. Hence both biotransformation and phototransformation of diuron are possible under environmental conditions. Although our results suggested that phototransformation was the dominant transformation process, this conclusion is speculative because the model´s fit was strongly influenced by the last data point. Diuron transformation in the Brisbane River may well be due to a combination of processes.

Desmethyl diazepam, also known as nordazepam, is a pharmaceutical used to treat anxiety disorders as well as a diazepam metabolite. It also shows good agreement between the modelled inputs and estimated WWTP emissions (ratio of 1.30) (**Table 2 and S-13**). The modelled half-life (140 d) is among the longest of the chemicals studied. The UV- and temperature-dependant models showed AIC scores. The UV-dependent model was chosen since desmethyl diazepam is known to be susceptible to phototransformation, with half-lives in the laboratory of 2-8 d [8]. The much longer half-lives in our study may be a consequence of the turbidity (40 to 100 NTU) and depth of this stretch of the Brisbane River [16], which would reduce the rate of phototransformation compared to that from the clean water and short light path length used in the laboratory study.

Hydrochlorothiazide, a diuretic, shows somewhat poorer agreement between the modelled and estimated emissions (ratio of 1.8 (1.2-2.3, 95% CI)). Given that the day-to-day variation in estimated emissions was low and use is not expected to vary from week-to-week, we have no explanation for this. The half-life was the shortest of all T-B chemicals (18 d), and the fit to the phototransformation model was clearly the

best (**Table 2 and S-13**). This is in accordance with the study of Zou et al [17], which reported a significant seasonality in the persistence of hydrochlorothiazide in a Swedish lake (2-49 d) and attributed this to the seasonal variation of solar radiation.

The antidepressant carbamazepine and the benzodiazepine temazepam, used for sleep disorders, both displayed good agreement between modelled and estimated emissions from the WWTP (ratios of 1.00 and 0.75 respectively). The constant k_r model provided a comparatively good fit for carbamazepine (69 d), while the UV-dependent model gave a better fit for temazepam (99 d). Temazepam and carbamazepine have both been shown to be susceptible to phototransformation in the laboratory [18, 19]. However, the attenuation of carbamazepine in natural water bodies has been reported to be very slow [20], and it has been used as a conservative tracer [17, 21].

The estimated emissions of the contrast medium iopromide were 60% higher than the modelled emissions (**Table 2 and S-13**). This may be due to higher uncertainty in the estimated emissions. There was relatively high day-to-day variation in estimated emissions and usage of iopromide is expected to vary more than for many of the other PPCPs studied. The UV-dependent model provided the best fit of the data. Iopromide is susceptible to phototransformation under environmental conditions [22] and has also been shown to biotransform in simulation tests with river water [23]. Therefore, its transformation may be the consequence of multiple processes. The estimated half-life in the Brisbane River (61 d) lies in the range of that observed in these simulation tests (8-69 d).

Tramadol and venlafaxine are used as an analgesic and antidepressant respectively, but are structurally similar [24]. The estimated input rate of venlafaxine was over 4 times smaller than the actual emission rate from the Goodna WWTP, while no comparison was possible for tramadol because it was not detected in the WWTP effluent. Gulde et al. [25] showed protozoa sequestered aliphatic amines (including venlafaxine and tramadol) into acid vesicles in protozoa via ion trapping. This pool may not have been accessed by our protocol, either because the sequestering organisms settle in the estuary (being not in the samples), or because the organisms are not completely extracted in the SPE column. This could explain part of the lower modelled input rate of venlafaxine compared to the WWTP emission rate. An alternative explanation is that the venlafaxine measured in water consisted primarily of ion-trapped residues, and that the freely dissolved fraction had already been largely degraded. Due to the uncertainty surrounding the nature of the residues of these weak bases in the water samples, an accurate interpretation of the observed half-lives of tramadol (69 d) and venlafaxine (72 d) is not possible.

The modelled input rate of gabapentin to the estuarine section was 260 times smaller than the estimated emission rate from the WWTP (**Table 2 and S-13**). Further assessment showed that the estimated emission rate of this analgesic and anticonvulsant was higher than could be expected from therapeutic use by the

population serviced by the WWTP. Furthermore, there were very large fluctuations in the estimated emissions over the 7-day sampling period (**Table S-9**) that are inconsistent with the expected use of the compound. The constant k_r model provided a good fit of the observed concentrations in the Brisbane River, yielding a half-life of 40 d. This relatively long half-life is difficult to reconcile with the much lower than expected concentrations in the estuary. One possible explanation for these inconsistencies is poor performance of the analytical method. More investigation is required before conclusions can be drawn about the behaviour of gabapentin in this system.

Cotinine and hydroxycotinine are nicotine metabolites. The modelled input of cotinine is 3 times greater than the estimated emissions from the WWTP. This suggests that cotinine is being formed in the estuary or there are other sources. The effective absence of nicotine in the estuarine water and WWTP effluent samples (**Table S-9**) suggests that this chemical is not being formed from nicotine discharged by the WWTP. On the other hand, hydroxycotinine showed the opposite behaviour, with estimated emissions 2 times higher than modelled. Although a previous study conducted in southeast Queensland, Australia between 2010-2017, showed inputs of hydroxycotinine to WWTPs significantly higher than for cotinine (as in the present study), it also showed high variability of hydroxycotinine inputs [26]. Furthermore, humans excrete a large fraction of cotinine and hydroxycotinine as conjugates [27], which may uncouple once in the estuary. These could help explain the disagreement. With estimated half-lives of 210 and 110 d respectively, cotinine and hydroxycotinine were the most and third most persistent T-B compounds. The UV-dependent model best fit the temporal concentration profile of hydroxycotinine, while UV- and temperature-dependent models performed comparably for cotinine. Relevant literature information about the transformation processes in water was not found for hydroxycotinine and scarce for cotinine. Neither photolysis nor hydrolysis are expected to be important transformation processes for cotinine [28], while it can be biotransformed in sediment [29].

T-C chemicals

This group comprised the pesticides 2,4-D, diazinon, dicamba, methomyl, tebuthiuron and prometryn.

Methomyl displayed a highly significant exponential decrease in concentration up until mid-October when a rainy period began ($r^2 = 0.94$) (**Fig. 2**). The models using UV-radiation and temperature dependent k_r performed similarly, and they were clearly better than the model assuming constant k_r. Methomyl has been reported to be susceptible to biotransformation and indirect photolysis, whereby the former is believed to be more rapid [30]. However, the estimated half-life (18 d) from this work was longer than that reported in laboratory biotransformation experiments using river-derived microbial biofilms (0.6-9 d) [31].

Tebuthiuron, prometryn and diazinon levels also showed a decrease over the whole course of the sampling period. The constant k_r model gave the best fit to the data for tebuthiuron, and the half-life was 140 d.

Tebuthiuron is reported to undergo slow volatilization, hydrolysis, biotransformation and phototransformation in water [32], which is consistent with the relatively long half-life we derived. In a partially shaded outdoor experiment with seawater and intertidal sediments conducted in Australia, a dissipation half-life of 944 d was measured [33]. It remains unclear what the primary transformation mechanisms are for tebuthiuron.

The concentration of prometryn also decreased relatively slowly over the study period. The temperature dependant k_r model clearly provided the best fit to the observations, yielding an estimated half-life of 32 d. Biotransformation of prometryn has been widely studied [34, 35]. Liu et al. reported half-lives of few hours in wastewater [34], which is much more rapid than what we derived or the Brisbane estuary. This provides an indication of the limitations in applying biotransformation rates from laboratory studies with wastewater to the natural environment.

Diazinon is subject to hydrolysis with a reported half-life of 138 d at pH 7 [36, 37]. This compares well with the half-life estimated for the Brisbane River, especially considering the length of the half-life and the uncertainty in our determination (95% CI, 200-410 d). Although the UV-dependent model provided the best fit, the data are relatively scattered and both of the other models also provided reasonable fits.

The concentrations of 2,4-D and dicamba decreased only during part of the sampling period (**Fig. S-4**), and just a portion of the overall time trend data was used for the model fitting. The concentration of dicamba decreased until early June, after which it fluctuated between 55-130 ng/L. This indicates that there were ongoing inputs of this compound. No model provided a fit that was clearly better than the other models. The half-lives obtained from the period up to early June were 46 d for the UV-radiation dependent model and 11 d for the temperature dependant model. The later agrees the half-life of 10-14 d reported by Degenhardt et al. [38] for dicamba in 2 wetlands. However, the authors in that study attributed an indeterminant portion of the attenuation in the wetlands to water infiltration, in which case the half-lives due to dicamba transformation would have been longer and therefore might have a better agreement with the half-life provided by the UV-dependant model.

In contrast to Dicamba, the time trend for 2,4-D was characterized by an abrupt increase by a factor of eight in mid-September followed by an equally abrupt decrease at the end of the month (**Fig. S-4**). This concentration pulse could be due to new inputs from seasonal use of the pesticide. The existence of such inputs is also suggested by a more gradual increase in concentrations during June. The abrupt decrease in September would suggest rapid transformation with a half-life <2 d. This decrease is incompatible with the half-life of 30 d suggested by the exponential decrease in concentration between early April and late May. An attenuation half-life of 12 d was reported for 2,4-D in the same study as mentioned above for dicamba [38]. This 12 d half-life is more consistent with the 30 d half-life derived from the first 6 weeks of our

observations, suggesting that the two high concentrations measured in September may have been the result of some anomaly. This example of 2,4-D illustrates some of the limitations to interpreting field data on chemical attenuation.

Inconsistent Trends

The compounds PFBA, haloxyfop, caffeine, imidacloprid, metalaxyl, metolachlor, imazethapyr, MCPA, desethyl atrazine, DEET, ametryn hydroxy, atrazine and atenolol displayed unstable or inconsistent temporal concentration trends. The observed trends could be due to, for example, fluctuating emissions from the WWTPs, as observed for imidacloprid and metolachlor (**Table S-9**), or contributions from other sources. Metalaxyl and imazethapyr were not detected in WWTP effluents or were below the LOQ (**Table S-9**). In addition, their concentrations in the surface water were also very low, close to the LOQ (**Table S-11**). Hence, source attribution is not possible.

PFBA showed a remarkable concentration increase during the day just after the rain event (**Table S-10**). Its profile during the dry period was also quite inconsistent compared with those from the other PFASs, especially in early April. Baddiley et al. [39] also reported concentration variation along time for PFBA in the Bremer River, entering the Brisbane River estuary. However, the reasons of this inconsistency remain unknown. On the other hand, its WWTPs effluent discharge were more or less stable (**Table S-8**). The development of a single method that works for the extraction of all PFASs is challenging, and PFBA is known to be a problematic compound when SPE is applied. The inconsistent results may have also been a result of poorer method precision or accuracy.

Caffeine, a broadly consumed stimulant, showed relatively constant WWTP emissions (**Table S-9**). However, there was no consistent temporal trend in the estuarine water concentration. Further research is needed to elucidate caffeine's behaviour.

Atenolol, a beta blocker, would also not be expected to exhibit variable inputs on a time scale of weeks or months. The WWTP effluent data show stable emissions on a daily time scale (**Table S-9**). This expected low variability in inputs to the estuary is not reflected in the time trend in water concentrations (**Fig. S-2**). Marked increases in early and late May are notable. Further research is needed to elucidate the reasons of this behaviour.

The herbicide metolachlor showed a very erratic time trend during early April. The concentrations during the week following the end of the flood show a significant increase followed by a rapid decrease (**Table S-11**). There was no clear time trend during the dry period, with concentrations ranging from 50-150 ng/L. Consequently, half-life estimation was not possible.

The pesticides MCPA, DEET, imidacloprid, haloxyfop and atrazine showed more or less defined profiles (**Fig. S-2**), but these were not consistent with the mass balance model assumptions. These compounds show a significant increase during spring, which is consistent with their seasonal use as pesticides. Furthermore, they showed fluctuations in their concentration before this season (specially atrazine), which suggests that emissions were not constant.

The pesticide metabolites desethyl atrazine and ametryn hydroxy displayed a similar behaviour, especially during early April. After the rainfall event their concentrations rose rapidly, reaching an apparent steady state in less than a week (**Fig. S-5**). Since this transition occurred during the transition from a wet to a dry period, it is unclear if it is the result of a transition from high to negligible emissions with little transformation or significant constant emissions with rapid transformation. These two substances are metabolites, so their rate of input might be related to the concentration of their precursor compounds. However, while the concentration of atrazine did show an increase during rainfall, ametryn was not detected (**Table S-11**).

The concentrations of the pesticides imazethapyr and metalaxyl increased during the flood, which could indicate input via runoff (**Figure S-5**). These substances were not detected in the WWTP effluent. Imazethapyr concentrations fluctuated strongly from July onwards in a manner that we could not interpret. The metalaxyl concentration decreased sharply just after the rainfall period. However, most of the decrease in the concentration happened before April 7, i.e., during the time period when the estuary was still influenced by the flood. Hence this decrease could have been influenced by dilution. Afterwards the concentration remained more or less stable around 0.25-1.00 ng/mL. It is unclear whether this is the result of very low inputs and slow transformation or relatively constant ongoing inputs accompanied by relatively rapid transformation.

Table S-1. Sample collection dates and times.

Sampling Day	Sampling time	notes
29-Mar-20	18:00	pre-rain
31-Mar-17	9:00	-
02-Apr-17	11:00	-
03-Apr-17	9:00	-
04-Apr-17	9:00	-
05-Apr-17	9:00	-
06-Apr-17	9:00	-
07-Apr-17	9:00	-
09-Apr-17	9:00	-
10-Apr-17	9:00	-
12-Apr-17	9:00	-
14-Apr-17	11:00	-
15-Apr-17	10:00	-
17-Apr-17	8:00	-
19-Apr-17	9:00	-
26-Apr-17	9:00	-
03-May-17	9:00	-
11-May-17	10:00	-
17-May-17	9:00	-
24-May-17	9:00	-
31-May-17	1:30	-
07-Jun-17	-	-
21-Jun-17	9:00	-
29-Jun-17	17:00	-
05-Jul-17	9:00	-
19-Jul-17	9:00	-
27-Jul-17	17:00	-
02-Aug-17	17:00	-
30-Aug-17	9:00	-
17-Sep-17	17:00	-
27-Sep-17	17:00	-
04-Oct-17	9:00	-
18-Oct-17	9:00	-
01-Nov-17	9:00	-
16-Nov-17	17:00	-
12-Dec-17	9:00	-

Table S-2. MS and quality assurance information for PFASs.

Analyte ID	Retention Time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
PFBA 1	1.6	212.8	169	MPFBA	0.03	0.10	0.16	0.07	3
PFPeA 1	3	262.8	219	MPFPeA	0.04	0.11	0.10	0.05	3
PFPeA 2	3	262.8	69	MPFPeA					
PFHxA 1	3.7	312.8	269	MPFHxA	0.06	0.19	0.12	0.05	5
PFHxA 2	3.7	312.8	119	MPFHxA					
PFHpA 1	4.27	362.8	319	MPFHpA	0.08	0.23	6.59	2.93	7
PFHpA 2	4.27	362.8	169	MPFHpA					
PFOA 1	4.8	412.8	369	MPFOA	0.09	0.27	0.28	0.12	6
PFOA 2	4.8	412.8	169	MPFOA					
PFOA 3	4.8	412.8	219	MPFOA					
PFNA 1	5.24	462.8	419	MPFNA	0.08	0.23	0.51	0.23	5
PFNA 2	5.24	462.8	169	MPFNA					
PFDA 1	5.7	512.8	469	MPFDA	0.06	0.18	0.10	0.04	5
PFDA 2	5.7	512.8	269	MPFDA					
PFUnDA 1	6	562.8	519	MPFUnDA	0.06	0.17	0.10	0.05	4
PFUnDA 2	6	562.8	269	MPFUnDA					
PFDoDA 1	6.3	612.8	569	MPFDoDA	0.13	0.39	0.07	0.03	9
PFDoDA 2	6.3	612.8	169	MPFDoDA					
PFTrDA 1	6.6	662.8	619	M2 PFTeDA	0.31	0.93	0.03	0.01	19
PFTrDA 2	6.6	662.8	169	M2 PFTeDA					
PFTeDA 1	6.71	712.8	669	M2 PFTeDA	0.23	0.70	0.03	0.01	16
PFTeDA 2	6.71	712.8	169	M2 PFTeDA					
PFHxDA 1	7.2	812.8	769	M2 PFHxDA	1.33	3.98	1.13	0.50	72
PFHxDA 2	7.2	812.8	169	M2 PFHxDA					
PFODA 1	7.5	912.8	869	M2 PFHxDA	0.42	1.25	0.12	0.05	19
PFODA 2	7.5	912.8	169	M2 PFHxDA					
PFBS 1	3.1	298.9	80	M3 PFBS	0.03	0.08	0.40	0.18	2
PFBS 2	3.1	298.9	99	M3 PFBS					
PFPeS 1	3.8	349	80	M3 PFBS	0.06	0.19	0.03	0.01	5
PFPeS 2	3.8	349	99	M3 PFBS					
PFHxS 1	4.4	398.8	80	MPFHxS	0.05	0.14	0.16	0.07	4
PFHxS 2	4.4	398.8	99	MPFHxS					
PFHxS 3	4.4	398.8	119	MPFHxS					
PFHxS 4	4.4	398.8	130	MPFHxS					
PFHps 1	4.9	448.8	80	MPFHxS	0.04	0.13	0.02	0.01	4
PFHps 2	4.9	448.8	99	MPFHxS					
PFOS 1	5.3	498.8	80	MPFOS	0.04	0.13	0.54	0.24	4
PFOS 2	5.3	498.8	99	MPFOS					
PFOS 3	5.3	498.8	169	MPFOS					
PFOS 4	5.3	498.8	230	MPFOS					
PFNS 1	5.7	548.8	80	MPFOS	0.09	0.26	0.00	n.d.	8
PFNS 2	5.7	548.8	99	MPFOS					
PFDS 1	6	598.8	80	MPFOS	0.10	0.30	0.04	0.02	9
PFDS 2	6	598.8	99	MPFOS					
PFDoDS 1	6.5	698.8	80	MPFOS	0.12	0.35	0.03	0.01	17
PFDoDS 2	6.5	698.8	99	MPFOS					
FOSA 1	6.1	497.8	78	M8 FOSA	0.15	0.44	0.09	0.04	12

Analyte ID	Retention Time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
FOSA 2	6.1	497.8	64	M8 FOSA					
FOSAA 1	5.5	556	169		0.22	0.65	0.02	0.01	21
FOSAA 2	5.5	556	78						
N-Et FOSAA 1	6	583.8	419	D5 N-Et FOSAA	0.09	0.28	0.00	n.d.	7
N-Et FOSAA 2	6	583.8	219	D5 N-Et FOSAA					
N-Me FOSAA 1	5.9	569.8	419	D3 N-Me FOSAA	0.10	0.30	0.01	0.00	8
N-Me FOSAA 2	5.9	569.8	512	D3 N-Me FOSAA					
N-Me FOSA 1	6.6	511.8	219	D3 N-Me FOSA	0.11	0.33	0.00	n.d.	10
N-Me FOSA 2	6.6	511.8	169	D3 N-Me FOSA					
N-Et FOSA 1	6.8	525.8	169	D5 N-Et FOSA	0.40	1.19	0.00	n.d.	40
N-Et FOSA 2	6.8	525.8	219	D5 N-Et FOSA					
10:2 FTS 1	6.2	626.9	607	M2 8:2 FTS	0.15	0.44	0.02	0.01	13
10:2 FTS 2	6.2	626.9	81	M2 8:2 FTS					
8:2 FTS 1	5.7	526.8	507	M2 8:2 FTS	0.10	0.29	0.02	0.01	7
8:2 FTS 2	5.7	526.8	81	M2 8:2 FTS					
6:2 FTS 1	4.8	426.8	407	M2 6:2 FTS	0.07	0.22	0.05	0.02	5
6:2 FTS 2	4.8	426.8	81	M2 6:2 FTS					
4:2 FTS 1	3.6	326.8	307	M2 4:2FTS	0.05	0.14	0.01	0.01	4
4:2 FTS 2	3.6	326.8	81	M2 4:2FTS					
N-Me FOSE	6.6	616	59	D7 N-Me FOSE	0.62	1.85	0.59	0.26	45
N-Et FOSE	6.8	630	59	D9 N-Et FOSE	0.20	0.59	0.38	0.17	12
PFECHS 1	4.8	460.9	381	MPFOS	0.04	0.12	0.01	n.d.	3
PFECHS 2	4.8	460.9	99	MPFOS					
6:2 PAP 1	4.1	442.9	97		0.10	0.30	1930.49	858.00	8
6:2 PAP 2	4.1	442.9	79		0.05	0.18			
8:2 PAP 1	5.3	542.9	79		0.48	1.43	0.00	n.d.	41
8:2 PAP 2	5.3	542.9	97		0.72	2.39			
SamPAP 1	5.9	649.9	525.9		0.19	0.58	0.00	n.d.	17
SamPAP 2	5.9	649.9	79		0.80	2.68			
6:2 DiPAP 1	6.7	788.8	442.9		0.12	0.35	12.44	5.53	31
6:2 DiPAP 2	6.7	788.8	97		0.03	0.12			
8:2 DiPAP 1	7.3	988.7	542.9		0.44	1.48	0.00	n.d.	
8:2 DiPAP 2	7.3	988.7	97		0.39	1.30			
6:2 8:2 DiPAP 1	7.1	888.8	97		0.10	0.32	0.00	n.d.	
6:2 8:2 DiPAP 2	7.1	888.8	542.9		0.09	0.31			

Table S-3. MS information for PFAS labelled standards.

Analyte ID	Retention Time (Min)	Q1 Mass (Da)	Q3 Mass (Da)
MPFBA	1.6	216.8	172
MPFPeA	3	265.9	222
MPFHxA	3.7	314.9	270
MPFHpA	4.27	321.8	322
MPFOA1	4.79	416.9	372
MPFOA2	4.79	416.9	169
MPFNA	5.3	467.8	423
MPFDA	5.7	514.8	470
MPFUdA	6	564.8	520
MPFDoA	6.3	614.8	570
M2PFTeDA 1	6.72	714.8	670
M2PFTeDA 2	6.72	714.8	169
M2PFHxDA 1	7.2	814.8	770
M2PFHxDA 2	7.2	814.8	169
M3 PFBS 1	3.1	301.8	80
M3 PFBS 2	3.1	301.8	99
MPFHxS 1	4.4	402.8	103
MPFHxS 2	4.4	402.9	84
MPFOS1	5.25	502.9	80
MPFOS2	5.25	502.9	99
M8 FOSA 1	6	505.9	78
M8 FOSA 2	6	505.9	64
D5 N-Et FOSAA 1	6	588.9	419
D5 N-Et FOSAA 2	6	588.9	483
D3 N-Me FOSAA 1	5.78	572.9	419
D3 N-Me FOSAA 2	5.78	572.9	219
D3 N-Me FOSA 1	6.38	514.9	169
D3 N-Me FOSA 2	6.38	514.9	219
D5 N-Et FOSA 1	6.67	530.9	169
D5 N-Et FOSA 2	6.67	530.9	219
M2 8:2 FTS 1	5.7	528.9	509
M2 8:2 FTS 2	5.7	528.9	80.9
M2 6:2 FTS 1	4.8	428.9	409
M2 6:2 FTS 2	4.8	428.9	81
M2 4:2FTS 1	3.6	328.9	309
M2 4:2FTS 2	3.6	328.9	80.9
D7 N-Me FOSE 1	6.6	623	59
D7 N-Me FOSE 2	6.6	609	45
D9 N-Et FOSE 1	6.8	639	59
D9 N-Et FOSE 2	6.8	625	45
M2 6:2 PAP 1	4.01	444.9	79
M2 6:2 PAP 2	4.01	444.9	97
M2 8:2 PAP 1	5.22	544.9	79
M2 8:2 PAP 2	5.22	544.9	97
M4 6:2 DiPAP 1	6.7	792.8	445
M4 6:2 DiPAP 2	6.7	792.8	97
M4 8:2 DiPAP 1	7.3	992.8	544.9
M4 8:2 DiPAP 2	7.3	992.8	97

Table S-4. MS information for PFAS Recovery standards.

Analyte ID	Retention Time (Min)	Q1 Mass (Da)	Q3 Mass (Da)
M3PFBA	1.6	215.9	172
M5PFPeA 1	3	267.9	223
M5PFPeA 2	3	267.9	70
M8PFOA1	4.9	420.9	172
M8PFOA 2	4.9	420.9	376
M3MPHxS 1	4.33	401.9	80
M3MPHxS 2	4.33	401.9	99
M8PFOS1	5.3	506.8	80
M8PFOS2	5.3	506.8	99

Table S-5. MS and quality assurance information for PPCPs and pesticides analysed in ESI positive mode.

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
Paracetamol 1	1.56	152.1	110	Paracetamol D4	0.37	1.10	3.04	1.35	15
Paracetamol 2	1.56	152.1	65.1	Paracetamol D4					
3,4 DiCl Aniline 1	5.63	162	127		0.08	0.24	0.51	0.23	5
3,4 DiCl Aniline 2	5.63	162	74						
Methomyl 1	4.39	163.1	88.1	Methomyl D3	0.05	0.14	0.00	0.00	3
Methomyl 2	4.39	163.1	106	Methomyl D3					
Nicotine 1	0.94	163.1	132	Nicotine D4			0.93	0.41	
Nicotine 2	0.94	163.1	106.1	Nicotine D4					
Gabapentin 1	3.14	172.1	154	Gabapentin D10	0.02	0.05	0.00	n.d.	1
Gabapentin 2	3.14	172.1	137	Gabapentin D10					
Desisopropyl Atrazine 1	4.07	174	104	Desisopropyl Atrazine D5	0.05	0.16	0.00	n.d.	4
Desisopropyl Atrazine 2	4.07	174	96	Desisopropyl Atrazine D5					
Cotinine 1	3.38	177.1	80	Cotinine D3	0.02	0.05	0.16	0.07	1
Cotinine 2	3.38	177.1	98	Cotinine D3					
Paraxanthine 1	3.99	181	124	Paraxanthine D3			0.23	0.10	
Paraxanthine 2	3.99	181.1	96	Paraxanthine D3					
Simazine hydroxy 1	3.57	184.1	114		0.28	0.85	0.00	n.d.	32
Simazine hydroxy 2	3.57	184.1	69						
Desethyl Atrazine 1	4.81	188	146	Desethyl Atrazine D6	0.01	0.04	0.08	0.04	1
Desethyl Atrazine 2	4.81	188	104	Desethyl Atrazine D6					
Clopyralid 1	2.9	192	110		0.05	0.15	0.00	n.d.	3
Clopyralid 2	2.9	192	146						
5HIAA 1	0.1	192.1	146.1	5HIAA D2			0.00	n.d.	
5HIAA 2	0.1	192.1	117.1	5HIAA D2					
DEET 1	6.26	192.1	119	DEET D7	0.04	0.13	3.04	1.35	3
DEET 2	6.26	192.1	91	DEET D7					
Hydroxycotinine 1	1.4	193.1	134.1	Hydroxycotinine D3	0.06	0.18	0.95	0.42	3
Hydroxycotinine 2	1.4	193.1	80.1	Hydroxycotinine D3					
Caffeine 1	4.75	195.1	138.1	Caffeine 13C3	0.04	0.13	0.85	0.38	3
Caffeine 2	4.75	195.1	110.1	Caffeine 13C3					
Ametryn hydroxy 1	4.2	198.11	156		0.32	0.96	0.05	0.02	33
Ametryn hydroxy 2	4.2	198.11	86						
Pyrimethanil 1	6.31	200.1	107		0.15	0.45	0.10	0.04	10
Pyrimethanil 2	6.31	200.1	183						
Terbuthylazine desethyl 1	5.54	202	146	Tebuthylazine desethyl D9	0.03	0.10	0.10	0.05	2
Terbuthylazine desethyl 2	5.54	202	104	Tebuthylazine desethyl D9					
Terbuthylazine desethyl 3	5.54	202.1	110	Tebuthylazine desethyl D9					
Simazine 1	5.55	202.1	132	Simazine D10	0.10	0.29	0.03	0.01	7
Simazine 2	5.55	202.1	124	Simazine D10					
DCPU 2	5.49	205.01	127		0.08	0.24	0.00	n.d.	5
DCPU 1	5.49	205.03	162						
Propoxur 1	5.74	210.1	168.1	Propoxur D3	0.16	0.48	0.00	n.d.	10
Propoxur 2	5.74	210.1	111	Propoxur D3					
Metribuzin 1	5.75	215.1	187	Metribuzin D3	0.09	0.28	7.33	3.26	6
Metribuzin 2	5.75	215.1	84	Metribuzin D3					
Atrazine 1	5.88	216.1	174	Atrazine D5	0.04	0.13	0.00	n.d.	3

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
Atrazine 2	5.88	216.1	96	Atrazine D5					
DCPMU 1	5.68	219.01	127		0.05	0.14	2.16	0.96	3
DCPMU 2	5.68	219.02	162						
Dichlorvos 1	5.53	221	109		0.06	0.17	0.03	0.01	4
Dichlorvos 2	5.53	223	109						
Carbofuran 1	5.88	222.1	165.2	Carbofuran D3	0.01	0.04	0.00	n.d.	1
Carbofuran 2	5.88	222.1	123	Carbofuran D3					
Methiocarb 1	6.4	226.1	169.2	Methiocarb D3	0.11	0.33	0.12	0.06	7
Methiocarb 2	6.4	226.1	121	Methiocarb D3					
Ametryn 1	6.07	228.2	186		0.20	0.61	0.12	0.05	16
Ametryn 2	6.07	228.2	116						
Tebuthiuron 1	5.7	229.2	172		0.06	0.19	0.00	n.d.	4
Tebuthiuron 2	5.7	229.2	116						
Propazine 1	6.15	230	146	Propazine D6	0.03	0.08	0.07	0.03	2
Propazine 2	6.15	230	188	Propazine D6					
Terbuthylazine 1	6.24	230.1	174	Terbuthylazine D5	0.14	0.41	0.17	0.07	10
Terbuthylazine 2	6.24	230.1	104	Terbuthylazine D5					
Asulam 1	3.83	231	156		0.04	0.12	0.00	n.d.	3
Asulam 2	3.83	231	108						
Naproxen 1	6.4	231.2	185.1		0.10	0.30	0.05	0.02	6
Naproxen 2	6.4	231.2	170.1						
Diuron 1	5.88	233.05	72	Diuron D6	0.12	0.36	0.30	0.13	8
Diuron 2	5.88	233.05	46	Diuron D6					
Fluometuron 1	5.59	233.1	72		0.07	0.22	0.10	0.04	5
Fluometuron 2	5.59	233.1	46						
Carbamazepine 1	6.11	237.2	194	Carbamazepine D10	0.17	0.51	0.86	0.38	8
Carbamazepine 2	6.11	237.2	193	Carbamazepine D10					
Prometryn 1	6.31	242.2	200.1	Prometryn D6	0.13	0.38	0.26	0.12	9
Prometryn 2	6.31	242.2	158	Prometryn D6					
Terbutryn 1	6.39	242.2	91.2		1.69	5.08	9.26	4.11	68
Terbutryn 2	6.39	242.2	71.1						
Picloram 1	3.8	243	197		0.11	0.33	0.00	n.d.	8
Picloram 2	3.8	243	143						
Hexazinone 1	6.1	253.2	171	Hexazinone D6	0.25	0.74	0.00	n.d.	18
Hexazinone 2	6.1	253.2	71	Hexazinone D6					
Fluroxypyr 1	5.35	255	209		0.07	0.22	0.04	0.02	5
Fluroxypyr 2	5.35	255	181						
Imidacloprid 1	5.26	256.1	209.1	Imidacloprid D4	0.04	0.13	0.14	0.06	3
Imidacloprid 2	5.26	256.1	175	Imidacloprid D4					
Bromacil 1	5.55	261.2	205	Bromacil D3	0.09	0.28	0.00	n.d.	5
Bromacil 2	5.55	263.2	207	Bromacil D3					
Tramadol 1	4.6	264.2	58	Tramadol D6	0.08	0.25	0.62	0.27	5
Tramadol 2	4.6	264.2	42	Tramadol D6					
Atenolol 1	2.82	267.2	190	Atenolol D7	0.03	0.10	0.05	0.02	2
Atenolol 2	2.82	267.2	145	Atenolol D7					
DesmethylDiazepam 1	6.58	271.2	140.1		0.06	0.17	0.00	n.d.	4
DesmethylDiazepam 2	6.58	271.2	165.1						

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
Imazapic 1	5.34	276.1	231.1		0.09	0.27	0.00	n.d.	5
Imazapic 2	5.34	276.1	163						
Venlafaxine 1	5.05	278.2	58	Venlafaxine D6	0.04	0.12	0.08	0.03	3
Venlafaxine 2	5.05	278.2	121	Venlafaxine D6					
Metalaxyl 1	6.42	280.2	220.1		0.05	0.16	0.02	0.01	3
Metalaxyl 2	6.42	280.2	192.1						
Pendimethalin 1	7.51	282.1	212.1		0.08	0.24	0.10	0.04	7
Pendimethalin 2	7.51	282.1	194.1						
Metolachlor 1	6.87	284.2	252	Metolachlor D6	0.26	0.79	0.02	0.01	18
Metolachlor 2	6.87	284.2	176	Metolachlor D6					
Imazethapyr 1	5.72	290.1	177.1		0.04	0.12	0.01	n.d.	3
Imazethapyr 2	5.72	290.1	106						
Codeine 1	4.02	300.2	215.1	Codeine D3	0.08	0.25	0.10	0.05	6
Codeine 2	4.02	300.2	165.1	Codeine D3					
Temazepam 1	6.69	301.2	255.1	Temazepam D5	0.09	0.27	0.15	0.07	6
Temazepam 2	6.69	301.2	283.1	Temazepam D5					
Fenamiphos 1	6.7	304.15	217.1		0.07	0.21	0.00	n.d.	4
Fenamiphos 2	6.7	304.15	202.1						
Diazinon 1	6.86	305.3	169.1	Diazinon D10	0.02	0.07	0.07	0.03	2
Diazinon 2	6.86	305.3	153	Diazinon D10					
Tebuconazole 1	6.69	308.15	70	Tebuconazole D6	0.05	0.15	0.02	0.01	3
Tebuconazole 2	6.69	310.15	70	Tebuconazole D6					
Fluoxetine 1	5.55	310.1	44	Fluoxetine D6	0.27	0.82	0.29	0.13	25
Fluoxetine 2	5.55	310.1	148	Fluoxetine D6					
Desmethyl Citalopram 1	5.32	311.3	109		0.04	0.13	0.00	n.d.	4
Desmethyl Citalopram 2	5.32	311.3	262.2						
Citalopram 1	5.34	325.3	109	Citalopram D6	0.10	0.31	0.88	0.39	5
Citalopram 2	5.34	325.3	262.2	Citalopram D6					
Fluazifop 1	6.39	328.2	282.2	Fluazifop D4+	0.06	0.18	0.04	0.02	4
Fluazifop 2	6.39	328.2	254.1	Fluazifop D4+					
Malathion 1	6.69	331.1	127		0.08	0.25	0.00	n.d.	9
Malathion 2	6.69	331.1	99						
Propiconazole 1	7	342	159	Propiconazole D5	0.04	0.12	0.05	0.02	3
Propiconazole 2	7	344	161	Propiconazole D5					
Prothioconazole 1	0.1	344	154				0.00	n.d.	
Prothioconazole 2	0.1	344	290						
Chlorpyriphos 1	7.37	350.1	198		0.08	0.24	0.02	0.01	7
Chlorpyriphos 2	7.37	350.1	97						
Haloxyfop + 1	6.72	362.2	316.2	Haloxyfop D4+	0.06	0.19	0.02	0.01	4
Haloxyfop + 2	6.72	362.2	288	Haloxyfop D4+					
Metsulfuron-Methyl 1	6.31	382.1	167	Metsulfuron-methyl D3	0.05	0.15	1.08	0.48	2
Metsulfuron-Methyl 2	6.31	382.1	199	Metsulfuron-methyl D3					
Tadalafil 1	6.61	390.2	268.1		0.10	0.29	0.00	n.d.	6
Tadalafil 2	6.61	390.2	204						
Verapamil 1	5.71	455.2	165.1		0.05	0.16	0.05	0.02	4
Verapamil 2	5.71	455.2	303.2						
Sildenafil 1	5.7	475.2	58		0.08	0.25	0.00	n.d.	7

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
Sildenafil 2	5.7	475.2	283.1						
Atorvastatin 1	6.8	559.5	440.3	Atorvastatin D5	0.26	0.77	0.40	0.18	19
Atorvastatin 2	6.8	559.5	250.2	Atorvastatin D5					
Iopromide 1	4.15	792	573.1	Iopromide D3	0.17	0.50	0.00	n.d.	11
Iopromide 2	4.15	792	559.1	Iopromide D3					
Haloxyfop-Me 1	7.04	376	316						
Haloxyfop-Me 2	7.04	376	288						
Haloxyfop-Me 4	7.04	376	272						

Table S-6. MS and quality assurance information for PPCPs and pesticides analysed in ESI negative mode.

Analyte ID negative	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)	Mass labelled	ILOD (ng/mL)	ILOQ (ng/mL)	LOR (ng/mL)	MilliQ blank (ng/mL)	Repeatability (%cv)
Salicylic acid 1	4.43	137	93		0.16	0.49	59.48	26.44	7
Salicylic acid 2	4.43	137	65						
Acesulfame 1	1.13	162	82	Acesulfame D4	0.13	0.40	214.20	95.20	6
Acesulfame 2	1.13	162	78	Acesulfame D4					
MCPA 1	6	199	141	MCPA D6	0.18	0.53	0.00	0.00	6
MCPA 2	6	201	143	MCPA D6					
Ibuprofen 1	6.56	205.1	161	Ibuprofen D3	0.15	0.45	0.12	0.05	9
Ibuprofen 2	6.56	205.1	159	Ibuprofen D3					
Mecoprop 1	6.21	213	141		0.11	0.34	0.00	0.00	5
Mecoprop 2	6.21	215	143						
2,4-D 1	5.94	219	161	2,4-D 13C6	0.20	0.61	0.08	0.03	9
2,4-D 2	5.94	221	163	2,4-D 13C6					
Dicamba 1	5.15	219	175		0.36	1.08	5.45	2.42	18
Dicamba 2	5.15	221	177						
2,4-DB 1	6.51	247	161				0.00	0.00	
2,4-DB 2	6.51	249	163						
2,4,5-T 1	6.3	252.9	194.9		0.31	0.93	0.00	0.00	13
2,4,5-T 2	6.3	254.9	196.9						
Triclopyr 1	6.31	254	196		0.37	1.10	0.00	0.00	23
Triclopyr 2	6.31	256	198						
Triclopyr 3	6.31	255.9	200						
Bromoxynil 1	5.9	273.8	78.9		0.21	0.63	0.01	0.01	11
Bromoxynil 2	5.9	275.8	78.9						
Triclosan 1	6.95	287	35		0.10	0.29	0.92	0.41	7
Triclosan 2	6.95	289	35						
Hydrochlorothiazide 1	2.96	296	269	Hydrochlorothiazide 13CD2	0.07	0.22	0.87	0.39	4
Hydrochlorothiazide 2	2.96	296	205	Hydrochlorothiazide 13CD2					
Furosemide 1	5.78	329	285		0.40	1.21	3.34	1.48	19
Furosemide 2	5.78	329	205						
Diketonitrile- 1	5.65	358.2	79		0.08	0.25	0.02	0.01	4
Diketonitrile- 2	5.65	358.2	64						
Haloxyfop- 1	6.72	360	288	Haloxyfop D4-	0.22	0.67	0.05	0.02	9
Haloxyfop- 2	6.72	362	290	Haloxyfop D4-					

Table S-7. MS information for PPCP and pesticide labelled standards.

	POSITIVE		
Analyte ID	**Retention time (Min)**	**Q1 Mass (Da)**	**Q3 Mass (Da)**
Paracetamol D4 1	1.54	156.1	114.1
Paracetamol D4 2	1.54	156.1	69.1
Methomyl D3 1	4.38	166	88
Methomyl D3 2	4.38	166	106
Nicotine D4 1	0.87	167.1	136
Nicotine D4 2	0.87	167.1	121
Atrazine Desisopropyl D5 1	4.04	179.1	137.1
Atrazine Desisopropyl D5 2	4.04	179.1	101.2
Cotinine D3 1	3.35	180.1	80
Cotinine D3 2	3.35	180.1	101
Gabapentin D10 1	3.01	182.1	164
Gabapentin D10 2	3.01	182.1	147
Paraxanthine D3 1	3.98	184	127
Paraxanthine D3 3	3.98	184	72
5HIAA D2 1	0.1	194.1	148
5HIAA D2 2	0.1	194.1	120
Atrazine desethyl D6 1	4.78	194.2	147.1
Atrazine desethyl D6 2	4.78	194.2	111.1
Hydroxycotinine D3 1	1.37	196.1	134.1
Hydroxycotinine D3 2	1.37	196.1	80
Caffeine 13C3 1	4.75	198.3	140.1
Caffeine 13C3 2	4.75	198.3	112.1
DEET D7 1	6.24	199.2	126.1
DEET D7 2	6.24	199.2	98.2
Tebuthylazine desethyl D9 1	5.52	211.1	147
Tebuthylazine desethyl D9 2	5.52	211.1	105
Simazine D10 1	5.52	212	137
Simazine D10 2	5.52	212	134
Propoxur D3 1	5.73	213.1	171.1
Propoxur D3 2	5.73	213.1	111
Metribuzin D3 1	5.74	218	190
Metribuzin D3 2	5.74	218	84
Atrazine D5 1	5.87	221.1	179
Atrazine D5 2	5.87	221.1	101
Carbofuran D3 1	5.88	225	165
Carbofuran D3 2	5.88	225	123
Methiocarb D3 1	6.4	229.1	169
Methiocarb D3 2	6.4	229.1	121
Terbuthylazine D5 1	6.23	235.1	179
Terbuthylazine D5 2	6.23	235.1	101
Propazine D6 1	6.14	236	147
Propazine D6 2	6.14	236	194
Diuron D6 1	5.86	240.9	78.2

	POSITIVE		
Analyte ID	**Retention time (Min)**	**Q1 Mass (Da)**	**Q3 Mass (Da)**
Diuron D6 2	5.86	240.9	52.1
Carbamazepine D10 1	6.08	247.2	204.1
Carbamazepine D10 2	6.08	247.2	202.1
Prometryn D6 1	6.3	248.2	159
Prometryn D6 2	6.3	248.2	206
Hexazinone D6 1	6.08	259.3	177.2
Hexazinone D6 2	6.08	259.3	77.2
Imidacloprid D4 1	5.25	260.2	179.3
Imidacloprid D4 2	5.25	260.2	213.2
Bromacil D3 1	5.55	264.1	208
Bromacil D3 2	5.55	266.1	210
Tramadol D6 1	4.69	268.2	58
Tramadol D6 2	4.69	268.2	42
Atenolol D7 1	2.76	274.1	145.1
Atenolol D7 2	2.76	274.1	190.1
Venlafaxine D6 1	5.05	284.2	64
Venlafaxine D6 2	5.05	284.2	121
Metolachlor D6 1	6.86	290.2	258.2
Metolachlor D6 2	6.86	290.2	182.2
D4 Acetyl Sulfamethoxazole 1	0.1	300.1	138.1
D4 Acetyl Sulfamethoxazole 2	0.1	300.1	69.1
Codeine D3 1	4.01	303.3	152
Codeine D3 2	4.01	303.3	115
Temazepam D5 1	6.69	306.2	260.1
Temazepam D5 2	6.69	306.2	288.1
Tebuconazole D6 1	6.68	314.2	72
Tebuconazole D6 2	6.68	314.2	125
Diazinon D10 1	6.84	315.1	170
Diazinon D10 2	6.84	315.1	154
Fluoxetine D6 1	5.54	316.2	44
Fluoxetine D6 2	5.54	316.2	154.2
Norfloxacin D5 1	4.31	325.1	231.1
Norfloxacin D5 2	4.31	325.1	281.2
Citalopram D6 1	5.34	331.1	109
Citalopram D6 2	5.34	331.1	262.1
Fluazifop D4+ 1	6.38	332.1	285.2
Fluazifop D4+ 2	6.38	332.1	257.1
Propiconazole D5 1	6.97	347	159
Propiconazole D5 2	6.97	349	161
Propiconazole D5 3	6.97	347	123
Haloxyfop D4+ 1	6.72	366	319.2
Haloxyfop D4+ 2	6.72	366	291.2
Metsulfuron-methyl D3 1	6.3	385.1	170
Metsulfuron-methyl D3 2	6.3	385.1	144
Atorvastatin D5 1	6.8	564.3	445.3
Atorvastatin D5 2	6.8	564.3	255.1

S-28

POSITIVE

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)
Iopromide D3 1	4.15	794.8	575.9
Iopromide D3 2	4.15	794.8	561.9

NEGATIVE

Analyte ID	Retention time (Min)	Q1 Mass (Da)	Q3 Mass (Da)
Acesulfame D4 1	1.11	166	86
Acesulfame D4 2	1.11	166	78
DCPA int std 1	0.1	205	161
DCPA int std 2	0.1	203	159
MCPA D6 1	5.98	205.1	147.1
MCPA D6 2	5.98	207.1	149.1
2,4-D 13C6 1	5.94	225	167
2,4-D 13C6 2	5.94	227	169
Hydrochlorothiazide 13CD2 1	2.94	298.9	269.9
Hydrochlorothiazide 13CD2 2	2.94	298.9	205.9
Haloxyfop D4- 1	6.71	364	292
Haloxyfop D4- 2	6.71	366	294
Ibuprofen D3 1	6.55	208.1	164
Ibuprofen D3 2	6.55	208.1	161

Table S-8. Daily PFAS discharge from WWTPs in the upper stretch of the Brisbane River estuary between Aug. 6 (Day 1) and Aug. 12 (Day 7) 2017.

	Goodna WWTP effluent discharge (mg/day)							Bundamba WWTP effluent discharge (mg/day)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
PFBA	29.83	35.28	28.68	28.33	27.19	22.63	25.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFPeA	141.99	132.00	129.39	116.93	129.62	113.34	114.64	0.14	0.12	0.14	0.10	0.11	0.15	0.11
PFHxA	248.44	238.81	223.21	216.17	219.74	209.31	212.51	0.42	0.42	0.37	0.41	0.37	0.39	0.39
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	353.56	349.67	314.84	304.97	324.33	316.77	332.38	0.64	0.71	0.72	0.66	0.73	0.77	0.66
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	30.19	35.40	n.d.	27.47	27.81	27.63	31.70	n.d.	n.d.	n.d.	0.68	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFPeS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	21.24	19.72	17.82	31.45	20.42	17.96	20.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDODS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-Et FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-Me FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-Me FOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
N-Et FOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10:2 FTS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8:2 FTS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6:2 FTS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.10	0.01	<LOQ	<LOQ	0.12	<LOQ
4:2 FTS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-Me FOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-Et FOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFECHS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6:2 PAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	<LOQ	<LOQ
8:2 PAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.73	2.00	4.35	12.14
SAmPAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.87	2.65	6.08	19.23
6:2 DiPAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8:2 DiPAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6:2 8:2 DiPAP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S-9. Daily PPCPs and pesticides discharge from WWTPs in the upper stretch of the Brisbane River estuary between Aug. 6 (Day 1) and Aug. 12 (Day 7) 2017.

	Goodna WWTP effluent discharge (mg/day)							Bundamba WWTP effluent discharge (mg/day)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Salicylic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acesulfame	4052.14	3048.63	2411.18	2401.22	3401.18	3459.25	4984.43	756.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MCPA	918.75	1098.16	509.58	619.93	508.73	823.31	796.19	7.22	7.61	10.72	5.28	3.70	2.33	2.79
Ibuprofen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mecoprop	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.22	0.23	0.38	0.34	0.38	<LOQ	<LOQ
2,4-D	80.13	85.68	70.98	77.96	73.78	89.37	87.38	12.40	0.70	0.54	0.35	0.46	0.43	0.43
Dicamba	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-DB	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4,5-T	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Triclopyr	163.02	120.42	98.16	181.95	124.91	76.92	83.67	0.08	<LOQ	0.08	<LOQ	0.10	0.16	0.20
Bromoxynil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	<LOQ	0.05	0.06	n.d.	n.d.	n.d.
Triclosan	86.95	215.15	152.29	138.22	102.71	60.74	241.74	0.06	0.02	0.04	0.03	0.02	0.03	0.05
Hydrochlorothia-zide	5701.91	5336.66	5176.19	4906.97	4993.58	4575.21	4839.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furosemide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diketonitrile	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Haloxyfop -	42.73	46.47	36.15	44.23	41.82	50.10	41.30	0.14	0.12	0.11	0.09	0.09	0.10	0.08
Paracetamol	2546.62	381.83	10371.57	186.84	1120.17	937.78	226.25	6.12	17.06	n.d.	37.71	26.35	n.d.	n.d.
3,4 DiCl Aniline	16.96	98.31	85.08	66.72	32.96	26.19	54.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methomyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nicotine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gabapentin	$1.25 \cdot 10^6$	$7.31 \cdot 10^5$	$1.28 \cdot 10^6$	$1.20 \cdot 10^6$	$1.15 \cdot 10^6$	$4.64 \cdot 10^4$	$7.29 \cdot 10^5$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Desisopropyl Atra-zine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cotinine	77.45	82.01	78.29	73.62	79.81	70.73	84.46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Paraxanthine	455.19	374.14	294.56	296.42	294.69	285.62	401.11	0.23	0.65	0.18	2.19	0.29	0.27	n.d.
Simazine hydroxy	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Desethyl Atrazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clopyralid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5HIAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEET	579.20	418.09	435.00	333.42	318.23	311.49	718.61	0.46	0.55	0.53	0.51	0.67	0.83	0.62
Hydroxycotinine	425.15	526.31	427.06	431.36	406.95	407.00	567.34	n.d.	5.60	0.94	3.38	0.90	n.d.	0.71
Caffeine	233.76	259.07	198.22	203.65	285.77	219.65	234.68	n.d.	n.d.	n.d.	0.24	n.d.	n.d.	n.d.
Ametryn hydroxy	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pyrimethanil	8.06	9.50	6.84	13.36	8.14	n.d.	7.42	n.d.	0.00	0.00	n.d.	0.00	0.01	0.00
Terbuthylazine de-sethyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	0.04	0.03	0.07	0.01	0.01
Simazine	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	0.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DCPU	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propoxur	145.24	107.83	95.32	68.51	51.85	41.43	42.82	0.05	0.13	0.15	0.14	0.17	0.15	0.12
Metribuzin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Atrazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DCPMU	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dichlorvos	n.d.	n.d.	n.d.	n.d.	n.d.	8.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbofuran	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methiocarb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

	Goodna WWTP effluent discharge (mg/day)							Bundamba WWTP effluent discharge (mg/day)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Ametryn	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tebuthiuron	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Propazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Terbuthylazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
Asulam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01	0.00	0.01	0.01	0.01	0.01
Naproxen	92.56	113.32	98.49	106.11	77.75	97.93	96.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diuron	552.81	524.47	750.52	604.29	621.74	546.69	734.52	0.67	0.74	0.87	0.69	0.95	0.95	0.60
Fluometuron	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	4596.63	4338.89	4554.81	3913.41	3032.87	3883.69	4535.88	6.38	n.d.	n.d.	0.09	0.46	0.11	0.04
Prometryn	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Terbutryn	6.15	10.90	7.29	6.16	3.55	2.93	13.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Picloram	203.09	248.54	193.77	258.99	175.96	124.17	133.63	0.08	0.56	0.59	0.56	0.66	0.72	0.54
Hexazinone	2748.58	1174.71	471.93	296.88	171.99	106.72	1414.82	0.05	<LOQ	0.05	0.05	0.05	0.05	0.06
Fluroxypyr	62.93	86.94	79.55	102.99	92.55	105.52	73.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Imidacloprid	1673.71	1292.14	982.37	838.30	759.74	938.65	1179.54	1.46	1.37	1.45	1.48	1.56	1.52	1.29
Bromacil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tramadol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Atenolol	356.21	334.39	365.85	299.78	294.38	279.58	361.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DesmethylDiaze-pam	73.73	105.85	95.25	89.47	78.47	73.38	93.86	0.27	0.07	0.07	0.10	0.11	0.06	0.10
Imazapic	155.75	477.70	410.95	272.97	231.46	111.32	585.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Venlafaxiine	6019.19	5975.26	5640.89	5800.64	14222.64	20613.01	5901.24	1.82	1.32	1.80	1.45	2.65	2.10	0.20
Metalaxyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pendimethalin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.62	3.29	3.08	6.02	5.83
Metolachlor	<LOQ	46.98	42.28	<LOQ	<LOQ	<LOQ	<LOQ	0.16	0.21	0.25	0.26	0.24	0.14	0.13
Imazethapyr	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Codeine	1262.53	1348.41	1402.97	1466.35	1501.99	1354.81	1596.40	0.03	0.03	0.04	0.29	0.37	0.12	0.05
Temazepam	1021.16	659.25	641.31	780.16	912.62	795.40	947.98	4.34	2.83	2.98	2.85	3.30	2.55	2.25
Fenamiphos	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diazinon	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tebuconazole	148.14	118.75	127.83	166.99	177.53	218.41	244.77	0.07	0.07	0.08	0.07	0.08	0.08	0.07
Fluoxetine	277.04	217.45	232.82	214.76	241.72	245.84	236.30	2.36	0.40	0.44	0.45	0.43	0.38	0.33
Desmethyl Cital-opram	921.16	1952.79	2010.02	1346.77	1158.65	725.59	1970.48	0.76	1.87	2.76	3.36	2.00	2.42	3.34
Citalopram	2384.29	2289.29	1508.76	2331.65	2581.95	1918.35	1632.41	0.13	0.10	0.16	0.08	0.20	0.19	0.03
Fluazifop	<LOQ	73.80	65.22	<LOQ	<LOQ	n.d.	147.94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Malathion	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propiconazole	192.02	149.34	130.19	194.33	207.09	254.40	183.23	0.13	0.15	0.14	0.13	0.16	0.15	0.13
Prothioconazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorpyriphos	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Haloxyfop +	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10	0.09	0.08	0.06	0.07	0.06	0.06
Metsulfuron-Me-thyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.17	n.d.	n.d.	n.d.	n.d.	n.d.
Tadalafil	n.d.	n.d.	7.75	6.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Verapamil	199.60	331.01	305.96	313.61	230.88	125.42	590.22	0.00	n.d.	0.00	0.00	0.00	0.00	0.00
Sildenafil	142.21	408.35	316.85	230.69	152.19	77.65	364.28	<LOQ	n.d.	<LOQ	n.d.	<LOQ	n.d.	n.d.
Atorvastatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Iopromide	$1.57 \cdot 10^4$	$7.31 \cdot 10^4$	$8.21 \cdot 10^4$	$5.84 \cdot 10^4$	$4.12 \cdot 10^4$	$4.84 \cdot 10^4$	$3.78 \cdot 10^4$	146.75	272.26	341.15	264.32	242.00	124.90	112.51

Table S-10. PFAS concentrations in surface water samples.

Concentration (ng/L)	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxDA	PFODA	PFBS	PFPeS	PFHxS
29/03/2017	10.89	11.69	17.07	n.d.	11.91	1.77	2.76	0.39	<LOQ	n.d.	n.d.	n.d.	n.d.	6.38	3.48	23.55
31/03/2017	54.06	4.25	5.27	n.d.	3.64	n.d.	0.61	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	1.83	1.05	9.01
01/04/2017	8.28	7.00	8.16	n.d.	5.15	0.96	0.95	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	2.48	1.52	10.88
02/04/2017	3.58	n.d.	1.33	n.d.	0.75	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.55	n.d.	2.53
03/04/2017	4.16	3.10	3.70	n.d.	2.45	n.d.	0.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.17	0.68	5.47
04/04/2017	4.45	1.76	2.63	n.d.	1.81	n.d.	0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.97	0.47	4.26
05/04/2017	1.71	1.57	2.38	n.d.	1.49	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.87	0.42	4.01
06/04/2017	4.16	1.57	2.28	n.d.	1.31	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.81	0.41	3.78
07/04/2017	3.55	1.46	2.28	n.d.	1.30	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	0.99	0.42	3.80
09/04/2017	2.47	1.78	2.56	n.d.	1.52	n.d.	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.03	0.46	4.26
10/04/2017	2.41	1.60	2.69	n.d.	1.57	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.14	0.52	4.28
12/04/2017	3.65	1.86	2.74	n.d.	1.60	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.22	0.50	4.06
14/04/2017	4.78	1.90	3.05	n.d.	1.87	n.d.	0.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.28	0.52	4.38
15/04/2017	4.64	2.08	3.38	n.d.	2.17	n.d.	0.29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.56	0.61	5.10
17/04/2017	3.03	2.27	3.34	n.d.	2.13	n.d.	0.37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.59	0.58	4.66
19/04/2017	3.94	2.24	3.63	n.d.	2.48	n.d.	0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.33	0.61	4.62
26/04/2017	4.65	2.63	4.09	n.d.	2.68	n.d.	0.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.54	0.70	5.34
03/05/2017	3.18	2.73	4.27	n.d.	3.07	n.d.	0.65	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	1.75	0.66	5.33
11/05/2017	5.04	2.64	4.02	n.d.	2.89	n.d.	0.42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.43	0.62	5.05
17/05/2017	4.37	2.99	4.43	n.d.	3.26	n.d.	0.47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.63	0.67	5.04
24/05/2017	5.59	3.35	5.40	n.d.	4.64	1.04	0.55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.85	0.81	5.66
31/05/2017	5.99	3.94	5.69	n.d.	4.68	n.d.	0.68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.09	0.88	6.53
07/06/2017	4.39	3.62	5.76	n.d.	4.76	n.d.	0.65	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	1.77	0.80	5.77
21/06/2017	7.10	4.48	6.96	n.d.	10.27	0.81	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.07	0.93	9.25
29/06/2017	6.77	4.33	6.86	n.d.	6.54	0.72	0.98	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	2.17	0.87	6.85
05/07/2017	5.07	4.57	7.45	n.d.	6.93	0.92	1.18	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	2.21	1.02	6.96
19/07/2017	6.25	5.06	8.43	n.d.	7.61	0.89	0.93	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	2.54	1.16	8.14
27/07/2017	7.54	5.12	8.48	n.d.	7.99	0.82	1.01	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	2.64	1.26	8.61
02/08/2017	6.82	4.82	9.19	n.d.	7.40	0.83	1.09	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	2.93	1.41	9.16
30/08/2017	9.41	6.28	11.09	n.d.	11.04	1.18	1.74	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	3.91	1.49	9.70
17/09/2017	7.54	6.02	10.52	n.d.	10.56	0.97	1.13	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	3.02	1.28	8.42
27/09/2017	11.80	6.10	12.28	n.d.	13.18	1.09	1.21	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	3.14	1.56	9.53
04/10/2017	3.46	5.01	10.42	n.d.	11.05	0.93	1.20	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	2.79	1.31	8.59
18/10/2017	7.97	7.97	13.52	n.d.	12.21	1.29	1.77	<LOQ	<LOQ	n.d.	< LOQ	n.d.	n.d.	3.69	1.87	11.48
01/11/2017	9.43	7.88	13.69	n.d.	13.01	1.41	1.43	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	4.68	2.09	13.72
16/11/2017	7.57	6.47	11.71	9.64	12.12	1.80	1.72	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	4.53	1.81	11.56
12/12/2017	7.23	7.04	13.27	n.d.	9.58	1.14	1.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.45	3.13	19.85

Concentration (ng/L)	PFHpS	PFOS	PFNS	PFDS	PFDoDS	FOSA	FOSAA	NEtFOSAA	NMeFOSAA	NMeFOSA	NEtFOSA	10:2FTS	8:2FTS
29/03/2017	1.18	34.43	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/03/2017	0.54	12.65	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
01/04/2017	0.48	9.80	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/04/2017	< LOQ	3.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
03/04/2017	0.24	4.42	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
04/04/2017	<LOQ	5.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
05/04/2017	<LOQ	4.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
06/04/2017	<LOQ	4.12	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
07/04/2017	<LOQ	4.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
09/04/2017	<LOQ	4.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10/04/2017	<LOQ	4.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/04/2017	<LOQ	4.77	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14/04/2017	<LOQ	5.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15/04/2017	0.20	5.99	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/04/2017	0.20	5.78	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19/04/2017	0.21	5.24	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26/04/2017	0.21	6.55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
03/05/2017	0.20	7.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11/05/2017	0.18	5.85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/05/2017	0.18	6.23	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.
24/05/2017	0.21	7.14	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/05/2017	0.26	7.64	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
07/06/2017	0.26	6.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21/06/2017	1.40	21.08	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29/06/2017	0.25	7.55	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.
05/07/2017	0.28	9.35	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	0.68	n.d.	n.d.	n.d.
19/07/2017	0.34	7.99	n.d.	n.d.	n.d.	< LOQ	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
27/07/2017	0.34	8.15	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/08/2017	0.35	8.96	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30/08/2017	0.38	9.26	n.d.	n.d.	< LOQ	0.63	n.d.	0.53	n.d.	n.d.	n.d.	n.d.	n.d.
17/09/2017	0.34	7.52	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27/09/2017	0.38	8.31	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
04/10/2017	0.33	7.25	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.
18/10/2017	0.47	10.35	n.d.	n.d.	n.d.	0.45	< LOQ	< LOQ	0.32	n.d.	n.d.	n.d.	n.d.
01/11/2017	0.55	14.61	n.d.	n.d.	n.d.	0.52	n.d.	< LOQ	0.45	n.d.	n.d.	n.d.	n.d.
16/11/2017	0.47	14.03	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/12/2017	0.85	26.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Concentration (ng/L)	6:2FTS	4:2FTS	NMeFOSE	NetFOSE	PFECHS	6:2PAP	8:2PAP	SamPAP	6:2DiPAP	8:2DiPAP	6:28:2DiPAP
29/03/2017	0.47	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/03/2017	0.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	689.88	n.d.	n.d.
01/04/2017	0.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
03/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
04/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
05/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
06/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
07/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
09/04/2017	<LOQ	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	38.48	n.d.	n.d.
15/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33.43	n.d.	n.d.
17/04/2017	<LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	63.28	n.d.	n.d.
19/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26/04/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
03/05/2017	<LOQ	n.d.	< LOQ	0.80	n.d.	n.d.	n.d.	n.d.	79.83	n.d.	n.d.
11/05/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/05/2017	<LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24/05/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/05/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	401.53	n.d.	n.d.
07/06/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21/06/2017	0.36	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29/06/2017	<LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	79.57	n.d.	n.d.
05/07/2017	<LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19/07/2017	<LOQ	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27/07/2017	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/08/2017	<LOQ	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30/08/2017	<LOQ	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/09/2017	0.54	n.d.	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27/09/2017	0.33	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
04/10/2017	<LOQ	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18/10/2017	0.30	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
01/11/2017	<LOQ	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16/11/2017	<LOQ	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/12/2017	0.35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S-11. PPCP and pesticide concentrations in surface water samples.

Concentration (ng/L)	Salicylic acid	Acesulfame	MCPA	Ibuprofen	Mecoprop	2,4-D	Dicamba	2,4-DB	2,4,5-T	Triclopyr	Bromoxynil	Triclosan
29/03/2017	n.d.	321.52	31.30	22.55	n.d.	36.87	97.79	n.d.	n.d.	1.99	n.d.	n.d.
31/03/2017	n.d.	n.d.	26.51	n.d.	n.d.	23.45	215.23	n.d.	n.d.	<LOQ	n.d.	n.d.
01/04/2017	n.d.	n.d.	20.88	n.d.	n.d.	17.30	161.20	n.d.	n.d.	n.d.	n.d.	n.d.
02/04/2017	n.d.	n.d.	11.55	n.d.	n.d.	32.98	160.50	n.d.	n.d.	<LOQ	n.d.	n.d.
03/04/2017	n.d.	n.d.	25.72	n.d.	n.d.	31.84	145.31	n.d.	n.d.	<LOQ	n.d.	n.d.
04/04/2017	n.d.	n.d.	11.83	n.d.	n.d.	35.32	124.11	n.d.	n.d.	<LOQ	n.d.	2.26
05/04/2017	n.d.	n.d.	10.50	n.d.	n.d.	36.89	133.78	n.d.	n.d.	1.60	n.d.	1.79
06/04/2017	n.d.	n.d.	10.49	n.d.	n.d.	36.58	166.72	n.d.	n.d.	1.73	n.d.	1.68
07/04/2017	n.d.	n.d.	11.65	n.d.	n.d.	32.16	168.99	n.d.	n.d.	1.52	n.d.	2.35
09/04/2017	n.d.	n.d.	9.28	n.d.	n.d.	32.80	136.92	n.d.	n.d.	1.78	n.d.	n.d.
10/04/2017	n.d.	n.d.	8.90	n.d.	n.d.	33.09	148.45	n.d.	n.d.	1.99	n.d.	1.25
12/04/2017	n.d.	n.d.	8.09	n.d.	n.d.	31.98	132.57	17.00	n.d.	<LOQ	n.d.	n.d.
14/04/2017	n.d.	n.d.	8.93	n.d.	n.d.	30.13	80.76	n.d.	n.d.	1.71	n.d.	n.d.
15/04/2017	n.d.	n.d.	9.26	n.d.	n.d.	28.82	133.82	n.d.	n.d.	1.55	n.d.	n.d.
17/04/2017	n.d.	n.d.	9.81	n.d.	n.d.	25.81	110.69	n.d.	n.d.	<LOQ	n.d.	n.d.
19/04/2017	n.d.	n.d.	8.90	n.d.	n.d.	24.39	118.16	n.d.	n.d.	<LOQ	n.d.	n.d.
26/04/2017	n.d.	n.d.	8.79	n.d.	n.d.	21.76	97.25	n.d.	n.d.	<LOQ	n.d.	n.d.
03/05/2017	n.d.	401.21	9.11	n.d.	n.d.	17.41	78.20	n.d.	n.d.	1.53	n.d.	1.53
11/05/2017	n.d.	n.d.	8.59	n.d.	n.d.	14.32	88.99	n.d.	n.d.	1.88	n.d.	n.d.
17/05/2017	n.d.	n.d.	8.98	n.d.	n.d.	12.22	70.50	n.d.	n.d.	1.73	n.d.	n.d.
24/05/2017	n.d.	n.d.	10.09	n.d.	n.d.	11.82	79.93	n.d.	n.d.	1.92	n.d.	n.d.
31/05/2017	n.d.	n.d.	12.43	n.d.	n.d.	21.62	55.64	n.d.	n.d.	2.03	n.d.	n.d.
07/06/2017	n.d.	n.d.	12.51	n.d.	n.d.	19.53	77.38	n.d.	n.d.	4.22	n.d.	n.d.
21/06/2017	n.d.	n.d.	15.97	n.d.	n.d.	39.68	91.75	n.d.	n.d.	4.75	n.d.	n.d.
29/06/2017	n.d.	n.d.	16.15	n.d.	n.d.	39.13	77.83	n.d.	n.d.	4.33	n.d.	n.d.
05/07/2017	n.d.	n.d.	16.41	n.d.	n.d.	36.49	87.53	n.d.	n.d.	4.47	n.d.	n.d.
19/07/2017	n.d.	434.34	29.52	n.d.	n.d.	41.98	103.60	n.d.	n.d.	4.48	n.d.	n.d.
27/07/2017	n.d.	n.d.	21.74	n.d.	n.d.	34.09	68.49	n.d.	n.d.	3.62	n.d.	n.d.
02/08/2017	n.d.	n.d.	27.13	n.d.	n.d.	40.58	57.68	n.d.	n.d.	2.84	n.d.	n.d.
30/08/2017	n.d.	314.31	23.40	n.d.	n.d.	28.39	80.56	n.d.	n.d.	4.07	n.d.	n.d.
17/09/2017	n.d.	883.25	62.51	n.d.	n.d.	207.40	127.24	n.d.	n.d.	5.70	n.d.	n.d.
27/09/2017	n.d.	404.67	57.63	n.d.	n.d.	254.33	114.07	n.d.	n.d.	4.57	n.d.	n.d.
04/10/2017	n.d.	n.d.	6.81	n.d.	n.d.	23.16	58.58	n.d.	n.d.	3.29	n.d.	n.d.
18/10/2017	n.d.	n.d.	30.01	n.d.	<LOQ	32.55	117.86	n.d.	n.d.	3.44	n.d.	n.d.
01/11/2017	n.d.	700.45	27.62	n.d.	n.d.	27.30	50.38	n.d.	n.d.	<LOQ	n.d.	n.d.
16/11/2017	198.99	n.d.	26.14	n.d.	n.d.	17.75	73.34	n.d.	n.d.	2.84	n.d.	n.d.
12/12/2017	n.d.	n.d.	39.47	n.d.	n.d.	38.94	81.42	n.d.	n.d.	2.28	n.d.	n.d.

Concentration (ng/L)	Hydrochlorothiazide	Furosemide	Diketonitrile	Haloxyfop -	Paracetamol	3,4 DiCl Aniline	Methomyl	Nicotine	Gabapentin	Desisopropyl Atrazine	Cotinine
29/03/2017	10.22	n.d.	0.55	3.69	n.d.	n.d.	0.39	n.d.	46.14	8.16	6.97
31/03/2017	n.d.	n.d.	n.d.	n.d.	84.32	n.d.	0.75	n.d.	n.d.	1.50	1.73
01/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30	n.d.	n.d.	2.10	0.97
02/04/2017	n.d.	n.d.	n.d.	3.80	n.d.	n.d.	180.33	n.d.	n.d.	0.95	0.84
03/04/2017	2.23	n.d.	n.d.	2.58	n.d.	n.d.	46.17	n.d.	18.46	1.72	1.25
04/04/2017	2.17	n.d.	n.d.	3.32	n.d.	n.d.	29.46	n.d.	14.04	1.70	1.17
05/04/2017	2.69	n.d.	n.d.	4.20	n.d.	n.d.	27.23	n.d.	10.24	1.31	1.53
06/04/2017	3.39	n.d.	n.d.	4.43	n.d.	n.d.	26.15	n.d.	11.07	1.31	1.33
07/04/2017	3.23	n.d.	n.d.	4.25	n.d.	n.d.	18.99	n.d.	9.06	1.66	0.98
09/04/2017	4.99	n.d.	n.d.	4.69	n.d.	n.d.	14.30	n.d.	8.96	1.28	1.38
10/04/2017	5.87	n.d.	<LOQ	5.33	n.d.	n.d.	14.67	n.d.	9.96	1.38	1.26
12/04/2017	5.45	n.d.	n.d.	4.48	n.d.	n.d.	11.62	n.d.	7.82	1.38	1.18
14/04/2017	6.95	n.d.	<LOQ	5.42	n.d.	n.d.	12.98	n.d.	11.26	1.39	1.44
15/04/2017	6.19	n.d.	<LOQ	4.69	n.d.	n.d.	9.66	n.d.	11.36	1.70	1.40
17/04/2017	6.59	n.d.	<LOQ	4.49	n.d.	n.d.	9.20	n.d.	11.93	1.39	1.49
19/04/2017	8.68	n.d.	<LOQ	4.10	n.d.	n.d.	9.81	n.d.	17.31	1.38	1.53
26/04/2017	11.91	n.d.	<LOQ	3.88	n.d.	n.d.	9.42	n.d.	17.74	1.53	1.77
03/05/2017	12.15	n.d.	<LOQ	3.73	n.d.	n.d.	8.13	n.d.	19.73	1.73	1.94
11/05/2017	16.62	n.d.	0.38	4.67	n.d.	n.d.	7.83	n.d.	25.72	1.61	2.42
17/05/2017	14.96	n.d.	0.38	4.83	n.d.	n.d.	6.78	n.d.	27.41	1.60	2.57
24/05/2017	16.56	n.d.	0.48	5.50	24.11	n.d.	7.30	n.d.	33.53	1.80	3.29
31/05/2017	16.37	n.d.	<LOQ	4.53	5.23	n.d.	4.64	n.d.	32.68	1.60	3.06
07/06/2017	19.17	n.d.	0.35	4.32	n.d.	n.d.	5.95	n.d.	36.58	1.85	3.66
21/06/2017	19.22	n.d.	0.39	5.95	7.00	n.d.	4.54	n.d.	38.23	1.64	4.66
29/06/2017	16.23	n.d.	0.36	4.92	n.d.	n.d.	3.36	n.d.	37.58	1.92	5.14
05/07/2017	20.84	n.d.	0.52	4.62	n.d.	n.d.	3.23	n.d.	42.15	1.77	5.44
19/07/2017	26.13	n.d.	0.57	5.26	11.99	n.d.	2.92	n.d.	52.62	2.23	6.36
27/07/2017	18.69	n.d.	0.42	5.62	n.d.	n.d.	2.00	n.d.	47.74	1.88	5.75
02/08/2017	24.44	n.d.	0.35	4.83	n.d.	n.d.	1.97	n.d.	44.17	2.12	6.42
30/08/2017	14.92	n.d.	0.67	4.27	n.d.	n.d.	1.04	n.d.	46.21	2.26	8.83
17/09/2017	19.20	n.d.	1.42	16.29	n.d.	n.d.	1.84	n.d.	54.87	2.61	8.91
27/09/2017	13.24	n.d.	1.99	17.87	9.09	n.d.	0.90	n.d.	38.52	2.13	11.96
04/10/2017	5.63	n.d.	1.92	15.99	n.d.	n.d.	0.61	n.d.	40.96	1.57	5.97
18/10/2017	13.24	n.d.	1.17	11.02	n.d.	n.d.	0.65	n.d.	57.73	3.64	9.15
01/11/2017	7.97	n.d.	<LOQ	4.13	n.d.	n.d.	2.03	n.d.	32.89	2.98	7.69
16/11/2017	7.98	n.d.	0.66	3.43	n.d.	0.82	3.28	n.d.	33.06	3.04	7.01
12/12/2017	7.20	n.d.	0.68	3.78	n.d.	n.d.	1.48	n.d.	46.28	3.91	6.30

Concentration (ng/L)	Paraxanthine	Simazine hydroxy	Desethyl Atrazine	Clopyralid	5HIAA	DEET	Hydroxycotinine	Caffeine	Ametrynhydroxy	Pyrimethanil	Terbuthylazine des ethyl
29/03/2017	n.d.	8.59	3.03	n.d.	n.d.	12.72	4.51	12.22	5.77	n.d.	0.61
31/03/2017	3.03	<LOQ	n.d.	n.d.	n.d.	8.40	3.60	9.28	<LOQ	n.d.	n.d.
01/04/2017	3.18	1.44	0.27	n.d.	n.d.	5.33	n.d.	4.78	1.42	n.d.	n.d.
02/04/2017	n.d.	n.d.	0.89	n.d.	n.d.	5.33	n.d.	3.72	2.66	n.d.	n.d.
03/04/2017	n.d.	<LOQ	0.71	n.d.	n.d.	7.35	1.35	7.12	2.87	n.d.	n.d.
04/04/2017	2.07	<LOQ	1.00	n.d.	n.d.	7.76	1.39	9.40	3.06	n.d.	n.d.
05/04/2017	n.d.	<LOQ	1.06	n.d.	n.d.	8.31	1.30	7.34	3.46	n.d.	n.d.
06/04/2017	n.d.	<LOQ	1.09	n.d.	n.d.	8.90	1.54	10.87	3.32	n.d.	n.d.
07/04/2017	n.d.	<LOQ	0.98	n.d.	n.d.	7.08	n.d.	2.86	3.24	n.d.	n.d.
09/04/2017	n.d.	<LOQ	1.21	n.d.	n.d.	11.16	1.43	9.70	3.49	n.d.	n.d.
10/04/2017	n.d.	<LOQ	1.23	n.d.	n.d.	9.22	1.49	6.32	3.36	n.d.	n.d.
12/04/2017	n.d.	<LOQ	1.30	n.d.	n.d.	12.40	1.33	5.26	3.38	n.d.	n.d.
14/04/2017	n.d.	<LOQ	1.29	n.d.	n.d.	8.33	1.79	19.74	3.01	n.d.	n.d.
15/04/2017	n.d.	<LOQ	1.46	n.d.	n.d.	12.70	1.56	6.70	3.09	n.d.	n.d.
17/04/2017	n.d.	1.24	1.20	n.d.	n.d.	17.41	1.51	7.26	3.68	n.d.	n.d.
19/04/2017	n.d.	<LOQ	1.24	n.d.	n.d.	15.11	1.71	19.74	3.04	n.d.	n.d.
26/04/2017	n.d.	<LOQ	1.45	n.d.	n.d.	13.32	2.08	7.32	3.30	n.d.	n.d.
03/05/2017	n.d.	<LOQ	1.45	n.d.	n.d.	37.66	2.25	6.64	3.63	n.d.	n.d.
11/05/2017	n.d.	1.34	1.43	n.d.	n.d.	14.87	2.88	6.52	4.26	n.d.	n.d.
17/05/2017	4.47	1.22	1.60	n.d.	n.d.	28.61	2.84	22.62	3.85	n.d.	n.d.
24/05/2017	n.d.	1.38	1.68	n.d.	n.d.	46.77	3.56	9.26	4.46	n.d.	n.d.
31/05/2017	4.29	1.24	1.63	n.d.	n.d.	38.52	3.11	7.69	3.70	n.d.	n.d.
07/06/2017	n.d.	1.46	1.66	n.d.	n.d.	36.81	4.04	10.82	4.17	n.d.	n.d.
21/06/2017	n.d.	1.52	1.34	n.d.	n.d.	31.34	4.30	16.01	4.20	n.d.	n.d.
29/06/2017	n.d.	1.86	1.39	n.d.	n.d.	37.65	4.50	23.51	4.83	n.d.	n.d.
05/07/2017	3.09	1.80	1.33	n.d.	n.d.	40.37	4.92	15.64	5.03	n.d.	n.d.
19/07/2017	n.d.	1.87	1.68	n.d.	n.d.	44.03	5.94	17.73	4.57	n.d.	n.d.
27/07/2017	n.d.	1.46	1.48	n.d.	n.d.	46.47	4.78	11.06	3.54	n.d.	0.31
02/08/2017	n.d.	<LOQ	1.80	n.d.	n.d.	64.02	5.61	13.46	2.77	n.d.	0.34
30/08/2017	n.d.	2.58	1.84	n.d.	n.d.	n.d.	5.78	8.58	5.12	n.d.	0.52
17/09/2017	4.20	2.85	2.25	n.d.	n.d.	196.17	6.75	19.36	6.41	n.d.	0.69
27/09/2017	n.d.	2.52	2.24	n.d.	n.d.	136.40	5.88	102.31	6.96	n.d.	0.65
04/10/2017	n.d.	2.60	1.44	n.d.	n.d.	56.01	3.65	8.47	7.16	n.d.	0.47
18/10/2017	n.d.	2.38	2.34	1.90	n.d.	83.31	5.12	15.42	5.08	n.d.	0.58
01/11/2017	n.d.	<LOQ	1.88	n.d.	n.d.	195.36	4.44	18.97	2.02	n.d.	0.44
16/11/2017	n.d.	2.90	2.02	n.d.	n.d.	467.51	4.79	22.22	4.55	n.d.	n.d.
12/12/2017	n.d.	3.04	1.99	n.d.	n.d.	420.86	4.31	15.15	4.63	n.d.	0.51

Concentration (ng/L)	Simazine	DCPU	Propoxur	Metribuzin	Atrazine	DCPMU	Dichlorvos	Carbofuran	Methiocarb	Ametryn	Tebuthiuron	Propazine
29/03/2017	51.36	n.d.	n.d.	n.d.	12.91	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
31/03/2017	19.05	n.d.	n.d.	n.d.	3.65	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
01/04/2017	12.76	n.d.	n.d.	n.d.	1.26	n.d.	n.d.	n.d.	n.d.	n.d.	0.55	n.d.
02/04/2017	0.81	n.d.	n.d.	n.d.	4.56	n.d.	n.d.	n.d.	n.d.	n.d.	0.76	n.d.
03/04/2017	7.86	n.d.	n.d.	n.d.	2.85	n.d.	n.d.	n.d.	n.d.	n.d.	0.36	n.d.
04/04/2017	6.08	n.d.	n.d.	n.d.	4.11	n.d.	n.d.	n.d.	n.d.	n.d.	0.77	n.d.
05/04/2017	1.91	n.d.	n.d.	n.d.	4.15	n.d.	n.d.	n.d.	n.d.	n.d.	1.17	n.d.
06/04/2017	1.54	n.d.	n.d.	n.d.	4.58	n.d.	n.d.	n.d.	n.d.	n.d.	1.30	n.d.
07/04/2017	2.90	n.d.	n.d.	n.d.	4.21	n.d.	n.d.	n.d.	n.d.	n.d.	1.53	n.d.
09/04/2017	1.69	n.d.	n.d.	n.d.	5.03	n.d.	n.d.	n.d.	n.d.	n.d.	1.69	n.d.
10/04/2017	1.82	n.d.	n.d.	n.d.	5.59	n.d.	n.d.	n.d.	n.d.	n.d.	1.76	n.d.
12/04/2017	1.41	n.d.	n.d.	n.d.	6.68	n.d.	n.d.	n.d.	n.d.	n.d.	2.06	n.d.
14/04/2017	2.27	n.d.	n.d.	n.d.	6.22	n.d.	n.d.	n.d.	n.d.	n.d.	1.87	n.d.
15/04/2017	2.50	n.d.	n.d.	n.d.	7.11	n.d.	n.d.	n.d.	n.d.	n.d.	1.81	n.d.
17/04/2017	2.08	n.d.	n.d.	n.d.	6.83	n.d.	n.d.	n.d.	n.d.	n.d.	1.83	n.d.
19/04/2017	2.92	n.d.	n.d.	n.d.	7.12	n.d.	n.d.	n.d.	n.d.	n.d.	1.86	n.d.
26/04/2017	2.61	n.d.	n.d.	n.d.	8.56	n.d.	n.d.	n.d.	n.d.	n.d.	1.82	n.d.
03/05/2017	3.16	n.d.	n.d.	n.d.	9.19	n.d.	n.d.	n.d.	n.d.	n.d.	1.81	n.d.
11/05/2017	2.56	n.d.	n.d.	n.d.	10.37	n.d.	n.d.	n.d.	n.d.	n.d.	1.87	n.d.
17/05/2017	3.08	n.d.	n.d.	n.d.	12.74	n.d.	n.d.	n.d.	n.d.	n.d.	1.64	n.d.
24/05/2017	6.62	n.d.	n.d.	n.d.	13.88	n.d.	n.d.	n.d.	n.d.	n.d.	1.60	n.d.
31/05/2017	5.17	n.d.	n.d.	n.d.	11.82	n.d.	n.d.	n.d.	n.d.	n.d.	1.37	n.d.
07/06/2017	6.47	n.d.	n.d.	n.d.	13.13	n.d.	n.d.	n.d.	n.d.	n.d.	1.44	n.d.
21/06/2017	7.32	n.d.	n.d.	n.d.	12.11	n.d.	n.d.	n.d.	n.d.	n.d.	1.22	n.d.
29/06/2017	9.22	n.d.	n.d.	n.d.	11.96	n.d.	n.d.	n.d.	n.d.	n.d.	1.28	n.d.
05/07/2017	9.16	n.d.	n.d.	n.d.	13.09	n.d.	n.d.	n.d.	n.d.	n.d.	1.34	n.d.
19/07/2017	10.28	n.d.	n.d.	n.d.	19.55	n.d.	n.d.	n.d.	n.d.	n.d.	1.18	n.d.
27/07/2017	9.78	n.d.	n.d.	n.d.	19.87	n.d.	n.d.	n.d.	n.d.	n.d.	0.83	n.d.
02/08/2017	10.56	n.d.	n.d.	n.d.	19.57	n.d.	n.d.	n.d.	n.d.	n.d.	0.66	n.d.
30/08/2017	10.49	n.d.	n.d.	n.d.	17.50	n.d.	n.d.	n.d.	n.d.	n.d.	0.87	n.d.
17/09/2017	15.04	n.d.	n.d.	n.d.	42.55	n.d.	n.d.	n.d.	n.d.	n.d.	1.01	n.d.
27/09/2017	11.42	n.d.	n.d.	n.d.	45.40	n.d.	n.d.	n.d.	n.d.	n.d.	0.88	n.d.
04/10/2017	9.04	n.d.	n.d.	n.d.	34.51	n.d.	n.d.	n.d.	n.d.	n.d.	0.73	n.d.
18/10/2017	17.78	n.d.	n.d.	n.d.	27.25	n.d.	n.d.	n.d.	n.d.	n.d.	0.84	n.d.
01/11/2017	11.51	n.d.	n.d.	n.d.	12.14	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
16/11/2017	10.51	n.d.	n.d.	n.d.	12.19	n.d.	n.d.	n.d.	n.d.	n.d.	0.62	n.d.
12/12/2017	26.67	n.d.	n.d.	n.d.	11.38	n.d.	n.d.	n.d.	n.d.	n.d.	0.48	n.d.

Concentration (ng/L)	Terbuthylazine	Asulam	Naproxen	Diuron	Fluometuron	Carbamazepine	Prometryn	Terbutryn	Picloram	Hexazinone	Fluroxypyr
29/03/2017	5.71	n.d.	n.d.	7.62	0.94	70.02	0.95	n.d.	n.d.	3.63	3.77
31/03/2017	n.d.	n.d.	n.d.	5.99	n.d.	1.67	n.d.	n.d.	n.d.	n.d.	5.53
01/04/2017	n.d.	n.d.	n.d.	4.31	n.d.	2.01	1.75	n.d.	n.d.	n.d.	5.26
02/04/2017	n.d.	n.d.	n.d.	0.84	n.d.	n.d.	2.10	n.d.	n.d.	<LOQ	n.d.
03/04/2017	n.d.	n.d.	n.d.	1.80	n.d.	2.48	1.79	n.d.	n.d.	n.d.	n.d.
04/04/2017	n.d.	n.d.	n.d.	1.28	n.d.	2.91	2.40	n.d.	n.d.	n.d.	n.d.
05/04/2017	n.d.	n.d.	n.d.	1.64	n.d.	3.15	2.51	n.d.	n.d.	<LOQ	n.d.
06/04/2017	n.d.	n.d.	n.d.	1.27	n.d.	3.90	2.41	n.d.	n.d.	<LOQ	n.d.
07/04/2017	n.d.	n.d.	n.d.	0.91	n.d.	3.11	2.07	n.d.	n.d.	<LOQ	n.d.
09/04/2017	n.d.	n.d.	n.d.	1.24	n.d.	6.62	1.93	n.d.	n.d.	<LOQ	n.d.
10/04/2017	n.d.	n.d.	n.d.	1.52	<LOQ	7.02	1.98	n.d.	n.d.	<LOQ	n.d.
12/04/2017	n.d.	n.d.	n.d.	1.37	<LOQ	8.92	1.76	n.d.	n.d.	<LOQ	n.d.
14/04/2017	n.d.	n.d.	n.d.	1.61	<LOQ	7.92	1.74	n.d.	n.d.	<LOQ	n.d.
15/04/2017	n.d.	n.d.	n.d.	1.76	<LOQ	9.70	1.65	n.d.	n.d.	<LOQ	n.d.
17/04/2017	n.d.	n.d.	n.d.	2.31	<LOQ	8.36	1.59	n.d.	n.d.	<LOQ	n.d.
19/04/2017	n.d.	n.d.	n.d.	2.15	<LOQ	12.95	1.54	n.d.	n.d.	<LOQ	n.d.
26/04/2017	n.d.	n.d.	n.d.	2.73	<LOQ	15.92	1.67	n.d.	n.d.	1.08	n.d.
03/05/2017	n.d.	n.d.	n.d.	3.41	0.30	21.73	1.45	n.d.	n.d.	1.04	n.d.
11/05/2017	n.d.	n.d.	n.d.	4.08	0.32	20.62	1.49	n.d.	n.d.	1.30	n.d.
17/05/2017	n.d.	n.d.	n.d.	5.26	0.35	41.29	1.41	n.d.	n.d.	1.12	n.d.
24/05/2017	n.d.	n.d.	n.d.	6.94	<LOQ	46.37	1.43	n.d.	n.d.	1.22	n.d.
31/05/2017	n.d.	n.d.	n.d.	6.06	0.31	39.21	1.23	n.d.	n.d.	0.99	n.d.
07/06/2017	n.d.	n.d.	n.d.	8.17	0.33	43.82	1.32	n.d.	n.d.	1.19	n.d.
21/06/2017	3.04	n.d.	n.d.	9.96	0.32	45.83	1.18	n.d.	n.d.	1.10	n.d.
29/06/2017	n.d.	n.d.	n.d.	9.75	0.30	66.16	1.17	n.d.	n.d.	1.20	n.d.
05/07/2017	7.72	n.d.	n.d.	10.88	0.37	50.85	1.24	n.d.	n.d.	1.23	n.d.
19/07/2017	12.71	n.d.	n.d.	11.46	0.34	57.92	1.06	n.d.	n.d.	1.70	n.d.
27/07/2017	39.90	n.d.	n.d.	11.89	<LOQ	56.95	1.11	n.d.	n.d.	1.84	n.d.
02/08/2017	38.89	n.d.	n.d.	11.03	<LOQ	85.65	1.36	n.d.	n.d.	2.60	n.d.
30/08/2017	69.14	n.d.	n.d.	11.79	<LOQ	84.95	0.82	n.d.	n.d.	2.59	n.d.
17/09/2017	98.14	n.d.	n.d.	26.30	0.69	71.39	1.18	n.d.	n.d.	3.46	4.56
27/09/2017	83.06	n.d.	n.d.	22.09	1.00	89.07	1.15	n.d.	n.d.	2.93	12.31
04/10/2017	76.57	n.d.	n.d.	17.53	0.88	69.41	1.06	n.d.	n.d.	2.15	9.38
18/10/2017	59.04	n.d.	n.d.	20.27	0.51	78.21	0.67	n.d.	n.d.	3.85	6.23
01/11/2017	22.87	n.d.	n.d.	9.59	n.d.	94.63	n.d.	n.d.	n.d.	4.28	1.86
16/11/2017	14.55	n.d.	n.d.	10.94	<LOQ	73.66	n.d.	n.d.	n.d.	4.97	n.d.
12/12/2017	8.33	n.d.	n.d.	10.74	n.d.	63.29	<LOQ	n.d.	n.d.	4.35	n.d.

Concentration (ng/L)	Imidacloprid	Bromacil	Tramadol	Atenolol	Desmethyl-Diazepam	Imazapic	Venlafaxine	Metalaxyl	Pendimethalin	Metolachlor	Imazethapyr
29/03/2017	15.86	n.d.	28.64	2.20	0.91	n.d.	11.23	<LOQ	n.d.	44.88	0.17
31/03/2017	9.38	n.d.	0.98	0.98	n.d.	0.73	0.45	n.d.	n.d.	8.95	n.d.
01/04/2017	13.02	n.d.	1.13	n.d.	n.d.	n.d.	0.16	4.32	n.d.	16.07	n.d.
02/04/2017	21.47	n.d.	0.83	0.35	n.d.	n.d.	0.53	0.23	n.d.	141.62	0.47
03/04/2017	15.18	n.d.	2.30	0.38	<LOQ	n.d.	0.83	0.57	n.d.	75.22	0.31
04/04/2017	16.93	n.d.	3.09	0.54	n.d.	n.d.	1.62	0.76	n.d.	259.49	0.41
05/04/2017	18.13	n.d.	2.92	0.61	n.d.	n.d.	1.86	0.84	n.d.	115.44	0.56
06/04/2017	17.76	n.d.	3.75	1.06	<LOQ	n.d.	2.49	0.82	n.d.	96.07	0.65
07/04/2017	15.26	n.d.	3.84	0.70	<LOQ	n.d.	2.49	0.74	n.d.	84.97	0.60
09/04/2017	16.18	n.d.	5.66	1.05	<LOQ	n.d.	3.60	0.60	n.d.	234.17	0.49
10/04/2017	17.59	n.d.	6.42	1.23	<LOQ	n.d.	4.79	0.54	n.d.	259.37	0.60
12/04/2017	15.88	n.d.	6.74	1.32	<LOQ	n.d.	4.89	0.57	n.d.	85.86	0.50
14/04/2017	17.34	n.d.	9.27	3.89	0.24	n.d.	6.29	0.57	n.d.	78.27	0.43
15/04/2017	16.18	n.d.	8.38	1.50	<LOQ	n.d.	6.48	0.52	n.d.	77.83	0.40
17/04/2017	14.84	n.d.	9.40	1.82	0.25	n.d.	6.09	0.57	n.d.	75.71	0.44
19/04/2017	16.37	n.d.	11.41	1.79	0.29	n.d.	9.49	0.50	n.d.	75.23	0.52
26/04/2017	16.79	n.d.	16.56	2.33	0.32	n.d.	11.75	0.60	n.d.	84.98	0.57
03/05/2017	16.02	n.d.	18.88	13.11	0.55	n.d.	11.08	0.56	n.d.	75.27	0.49
11/05/2017	16.32	n.d.	23.87	10.46	0.72	n.d.	15.91	0.64	n.d.	73.04	0.64
17/05/2017	16.49	n.d.	26.40	8.14	0.72	n.d.	16.83	0.57	n.d.	102.49	0.48
24/05/2017	17.17	n.d.	34.61	25.11	0.86	n.d.	21.54	0.59	n.d.	103.57	0.52
31/05/2017	15.61	n.d.	32.02	13.46	0.89	n.d.	19.90	0.54	n.d.	54.48	0.42
07/06/2017	17.31	n.d.	42.26	12.95	1.01	n.d.	25.67	0.55	n.d.	55.71	0.50
21/06/2017	17.15	n.d.	46.03	14.22	1.06	n.d.	32.37	0.55	n.d.	87.58	0.47
29/06/2017	16.19	n.d.	50.75	15.11	1.39	n.d.	33.70	0.52	n.d.	92.02	0.41
05/07/2017	16.77	n.d.	63.52	14.42	1.59	n.d.	37.63	0.61	n.d.	79.16	0.47
19/07/2017	22.99	n.d.	68.15	13.79	1.52	n.d.	41.33	0.57	n.d.	53.81	0.77
27/07/2017	19.23	n.d.	61.13	10.95	1.22	0.68	41.10	0.44	n.d.	82.14	0.79
02/08/2017	21.02	n.d.	74.46	12.90	1.05	n.d.	43.57	0.32	n.d.	94.82	0.47
30/08/2017	17.82	n.d.	49.96	7.47	1.62	n.d.	28.47	0.45	n.d.	47.97	0.59
17/09/2017	133.18	n.d.	63.98	12.30	1.90	2.34	42.35	0.76	n.d.	96.50	0.69
27/09/2017	261.85	n.d.	48.74	10.39	1.97	3.17	41.09	0.92	n.d.	143.16	0.28
04/10/2017	157.40	n.d.	42.69	8.00	2.20	3.19	38.79	0.78	n.d.	125.81	0.37
18/10/2017	178.19	n.d.	55.84	8.93	1.82	2.54	40.11	0.58	n.d.	53.90	0.40
01/11/2017	67.05	n.d.	38.45	10.24	0.73	n.d.	27.44	n.d.	n.d.	43.59	n.d.
16/11/2017	48.77	n.d.	41.84	7.70	1.46	n.d.	29.76	0.35	n.d.	38.08	0.16
12/12/2017	21.82	n.d.	31.84	3.71	1.18	n.d.	23.31	0.27	n.d.	37.68	0.31

Concentration (ng/L)	Codeine	Temazepam	Fenamiphos	Diazinon	Tebuconazole	Fluoxetine	Desmethyl Citalopram	Citalopram	Fluazifop	Malathion	Propiconazole
29/03/2017	n.d.	23.24	n.d.	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/03/2017	0.79	0.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
01/04/2017	n.d.	0.91	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/04/2017	n.d.	n.d.	n.d.	0.14	n.d.	n.d.	<LOQ	n.d.	8.95	n.d.	n.d.
03/04/2017	n.d.	1.10	n.d.	0.30	n.d.	n.d.	n.d.	n.d.	2.43	n.d.	n.d.
04/04/2017	n.d.	1.12	n.d.	0.40	n.d.	n.d.	n.d.	n.d.	1.55	n.d.	n.d.
05/04/2017	0.54	1.02	n.d.	0.61	n.d.	n.d.	n.d.	n.d.	1.25	n.d.	n.d.
06/04/2017	0.67	1.17	n.d.	0.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
07/04/2017	0.43	1.32	n.d.	0.72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
09/04/2017	0.57	2.09	n.d.	0.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10/04/2017	n.d.	2.41	n.d.	1.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/04/2017	0.58	2.74	n.d.	1.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14/04/2017	1.38	3.22	n.d.	0.95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15/04/2017	0.93	3.54	n.d.	0.93	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/04/2017	0.67	4.10	n.d.	0.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19/04/2017	0.71	4.44	n.d.	0.83	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26/04/2017	0.62	6.21	n.d.	0.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
03/05/2017	0.77	7.81	n.d.	0.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11/05/2017	0.80	8.57	n.d.	0.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/05/2017	0.80	10.37	n.d.	0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24/05/2017	1.17	12.16	n.d.	1.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31/05/2017	1.14	12.33	n.d.	0.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
07/06/2017	1.62	13.54	n.d.	0.75	n.d.	n.d.	n.d.	1.99	n.d.	n.d.	n.d.
21/06/2017	2.49	15.56	n.d.	0.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29/06/2017	3.01	18.14	n.d.	0.74	n.d.	n.d.	n.d.	2.40	n.d.	n.d.	n.d.
05/07/2017	3.90	19.89	n.d.	0.78	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19/07/2017	4.86	22.16	n.d.	0.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27/07/2017	4.23	23.19	n.d.	0.79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
02/08/2017	5.34	23.18	n.d.	0.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30/08/2017	n.d.	24.34	n.d.	0.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17/09/2017	1.33	27.15	n.d.	0.71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27/09/2017	0.71	28.11	n.d.	0.69	n.d.	n.d.	n.d.	3.14	n.d.	n.d.	n.d.
04/10/2017	0.57	23.73	n.d.	0.60	n.d.	n.d.	n.d.	3.64	n.d.	n.d.	n.d.
18/10/2017	1.34	26.89	n.d.	0.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
01/11/2017	0.87	20.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16/11/2017	1.83	19.50	n.d.	0.29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12/12/2017	0.88	14.96	n.d.	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Concentration (ng/L)	Prothioconazole	Chlorpyriphos	Haloxyfop +	Metsulfuron-Methyl	Tadalafil	Verapamil	Sildenafil	Atorvastatin	Iopromide
29/03/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	432.30
31/03/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28.65
01/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.99
02/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.48
03/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.37
04/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	50.65
05/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	61.06
06/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	66.40
07/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	73.22
09/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	113.38
10/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	130.25
12/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	185.32
14/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	160.55
15/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	189.39
17/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	182.18
19/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	180.08
26/04/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	206.21
03/05/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	258.99
11/05/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	266.10
17/05/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	334.14
24/05/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	333.45
31/05/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	401.00
07/06/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	364.17
21/06/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	311.15
29/06/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	437.19
05/07/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	442.72
19/07/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	645.33
27/07/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	515.36
02/08/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	750.17
30/08/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	669.31
17/09/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	591.67
27/09/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	432.73
04/10/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.45	n.d.	289.05
18/10/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	570.55
01/11/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	450.16
16/11/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	364.64
12/12/2017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	357.21

Table S-12. Rate constant for chemical loss from the estuary segment and emission flux estimated by fitting the measured concentrations in river water to Eq. 2 (time invariant rate constant) for Type B compounds.

	k_r (1/day)			Haf-life (days)		Chemical input E (mg/day)			
	Median	95% CI	R^2	Median	95% CI	Modelled (Median)	Modelled (95% CI)	Estimated from WWTP	Esti/Mod
Carbamazepine	$1.0 \cdot 10^{-2}$	$7.4 \cdot 10^{-3}$ to $1.4 \cdot 10^{-2}$	0.94	69	51 to 97	4000	3300 to 4800	4100	1.03
Cotinine	$5.9 \cdot 10^{-3}$	$2.3 \cdot 10^{-3}$ to $1.0 \cdot 10^{-2}$	0.88	118	68 to 300	290	210 to 390	78	0.27
Desmethyl diazepam	$1.1 \cdot 10^{-2}$	$6.7 \cdot 10^{-3}$ to $1.7 \cdot 10^{-2}$	0.89	62	41 to 100	92	69 to 120	87	0.95
Diuron	$8.0 \cdot 10^{-3}$	$4.7 \cdot 10^{-3}$ to $1.2 \cdot 10^{-2}$	0.97	86	59 to 150	610	520 to 720	620	1.02
Gabapentin	$1.8 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$	0.88	40	27 to 58	3500	2600 to 4700	920000	260
Hydrochlorothiazide	$5.8 \cdot 10^{-2}$	$3.1 \cdot 10^{-2}$ to $1.3 \cdot 10^{-1}$	0.51	12	5.6 to 22	3800	2100 to 7800	5100	1.34
Hydroxycotinine	$1.5 \cdot 10^{-2}$	$9.8 \cdot 10^{-3}$ to $2.2 \cdot 10^{-2}$	0.87	46	31 to 71	360	260 to 490	460	1.27
Iopromide	$2.3 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$ to $3.9 \cdot 10^{-2}$	0.74	30	18 to 48	50000	34000 to 77000	51000	1.02
Temazepam	$1.4 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$ to $1.8 \cdot 10^{-2}$	0.94	51	40 to 67	1500	1200 to 1800	83	0.55
Tramadol	$1.7 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$	0.83	40	27 to 62	4100	3000 to 5600	-	-
Venlafaxine	$1.8 \cdot 10^{-2}$	$1.3 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$	0.89	38	27 to 52	3000	2300 to 3800	9200	3.1

Table S-13. Rate constant for chemical loss from the estuary segment and emission flux estimated by fitting the measured concentrations in river water to Eq. 5 (UV-radiation intensity dependant rate constant) for Type B compounds. Presented half-lives are based on the mean of the daily UV indices when water samples were taken.

	k_{rp} (1/day)			Haf-life (days)		Chemical input E (mg/day)			
	Median	95% CI	R^2	Median	95% CI	Modelled (Median)	Modelled (95% CI)	Estimated from WWTP	Esti/Mod
Carbamazepine	$4.6 \cdot 10^{-3}$	$3.2 \cdot 10^{-3}$ to $6.0 \cdot 10^{-3}$	0.94	150	110 to 210	3000	2600 to 3400	4100	1.37
Cotinine	$3.3 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$ to $5.0 \cdot 10^{-3}$	0.91	210	140 to 380	250	210 to 300	78	0.31
Desmethyl diazepam	$4.9 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$ to $1.1 \cdot 10^{-2}$	0.91	140	63 to 210	66	56 to 77	87	1.32
Diuron	$1.0 \cdot 10^{-2}$	$7.1 \cdot 10^{-3}$ to $1.4 \cdot 10^{-2}$	0.98	66	48 to 98	640	560 to 740	620	0.97
Gabapentin	$7.2 \cdot 10^{-3}$	$4.8 \cdot 10^{-3}$ to $1.0 \cdot 10^{-2}$	0.85	96	68 to 150	2100	1700 to 2700	920000	440
Hydrochlorothiazide	$3.9 \cdot 10^{-2}$	$2.8 \cdot 10^{-2}$ to $5.9 \cdot 10^{-2}$	0.85	18	12 to 25	2900	2200 to 4100	5100	1.76
Hydroxycotinine	$6.6 \cdot 10^{-3}$	$4.6 \cdot 10^{-3}$ to $8.9 \cdot 10^{-3}$	0.89	110	78 to 150	230	190 to 270	460	2.0
Iopromide	$1.1 \cdot 10^{-2}$	$8.0 \cdot 10^{-3}$ to $1.6 \cdot 10^{-2}$	0.80	61	44 to 87	32000	26000 to 41000	51000	1.59
Temazepam	$7.0 \cdot 10^{-3}$	$6.2 \cdot 10^{-3}$ to $7.8 \cdot 10^{-3}$	0.98	99	89 to 112	1100	1000 to 1200	83	0.75
Tramadol	$1.0 \cdot 10^{-2}$	$8.1 \cdot 10^{-3}$ to $1.2 \cdot 10^{-2}$	0.93	69	56 to 85	3200	2800 to 3600	-	-
Venlafaxine	$9.6 \cdot 10^{-3}$	$7.8 \cdot 10^{-3}$ to $1.2 \cdot 10^{-2}$	0.94	72	59 to 88	2100	1900 to 2400	9200	4.4

Table S-14. Rate constant for chemical loss from the estuary segment and emission flux estimated by fitting the measured concentrations in river water to Eq. 7 (temperature dependant rate constant) for Type B compounds. Presented half-lives are based on the mean of the daily water temperatures when water samples were taken.

	k_{rT} (1/day)			Haf-life (days)		Chemical input E (mg/day)			
	Median	95% CI	R^2	Median	95% CI	Modelled (Median)	Modelled (95% CI)	Estimated from WWTP	Esti/Mod
Carbamazepine	$1.1 \cdot 10^{-2}$	$7.5 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}$	0.95	64	47 to 92	3800	3100 to ???*	4100	1.08
Cotinine	$9.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-3}$ to $1.4 \cdot 10^{-2}$	0.92	77	51 to 140	250	190 to 330	78	0.31
Desmethyl diazepam	$1.3 \cdot 10^{-2}$	$8.7 \cdot 10^{-3}$ to $1.8 \cdot 10^{-2}$	0.92	54	39 to 80	75	53 to 100	87	1.16
Diuron	$7.9 \cdot 10^{-3}$	??? to ???*	0.97	88	??? to ???*	610	??? to ???*	620	1.02
Gabapentin	$1.8 \cdot 10^{-2}$	??? to $2.7 \cdot 10^{-2}$*	0.88	38	25 to ???*	3500	2600 to ???*	920000	260
Hydrochlorothiazide	$2.7 \cdot 10^{-1}$	$1.2 \cdot 10^{-1}$ to ???*	0.81	2.5	??? to 6*	9600	4700 to ???*	5100	0.53
Hydroxycotinine	$1.8 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$ to $2.6 \cdot 10^{-2}$	0.88	40	26 to 60	350	250 to 480	460	1.31
Iopromide	$3.3 \cdot 10^{-2}$	$2.0 \cdot 10^{-2}$ to $5.6 \cdot 10^{-2}$	0.79	21	12 to 34	52000	35000 to ???*	51000	0.99
Temazepam	$1.9 \cdot 10^{-2}$	$1.6 \cdot 10^{-2}$ to $2.1 \cdot 10^{-2}$	0.98	37	33 to 43	1300	1200 to 1500	83	0.64
Tramadol	$2.9 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$ to $4.0 \cdot 10^{-2}$	0.91	24	17 to 34	4000	3100 to ???*	-	-
Venlafaxine	$2.5 \cdot 10^{-2}$	$1.9 \cdot 10^{-2}$ to $3.3 \cdot 10^{-2}$	0.93	27	21 to 36	2900	2300 to 3500	9200	3.2

**: Denotes that the software was unable to calculate the lower and/or upper boundary.*

Table S-15. Rate constant for chemical loss from the estuary segment derived by fitting the measured concentrations in river water to Eq. 4 (C: time invariant rate constant), Eq. 6 (UV: UV-radiation dependant constant). Eq. 8 (Tª: Temperature dependant constant) for Type C compounds. Presented half-lives for the UV- and temperature models are based on the mean of the daily UV indices or water temperatures when water samples were taken.

	k_r (1/day)			Half-life (days)		
	Median	95% CI	R^2	Median	95% CI	**Approach**
2,4-D	$2.6 \cdot 10^{-2}$	$2.3 \cdot 10^{-2}$ to $2.9 \cdot 10^{-2}$	0.99	27	24 to 30	C
	$2.3 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$	0.99	30	28 to 33	UV
	$3.2 \cdot 10^{-2}$	$2.4 \cdot 10^{-2}$ to $4.2 \cdot 10^{-2}$	0.92	22	17 to 29	Tª
Diazinon	$2.7 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$ to $3.6 \cdot 10^{-3}$	0.61	260	190 to 400	C
	$2.5 \cdot 10^{-3}$	$1.7 \cdot 10^{-3}$ to $3.4 \cdot 10^{-3}$	0.65	280	200 to 410	UV
	$7.8 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$ to $1.6 \cdot 10^{-2}$	0.65	89	44 to 320	Tª
Dicamba	$1.5 \cdot 10^{-2}$	$9.0 \cdot 10^{-3}$ to $2.2 \cdot 10^{-2}$	0.72	45	31 to 77	C
	$1.5 \cdot 10^{-2}$	$9.4 \cdot 10^{-3}$ to $2.1 \cdot 10^{-2}$	0.75	46	32 to 74	UV
	$6.2 \cdot 10^{-2}$	$1.8 \cdot 10^{-2}$ to $1.8 \cdot 10^{-1}$	0.82	11	4 to 39	Tª
Methomyl	$1.8 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$ to $2.2 \cdot 10^{-2}$	0.92	39	31 to 49	C
	$1.9 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$ to $2.3 \cdot 10^{-2}$	0.93	37	31 to 45	UV
	$3.8 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$ to $6.3 \cdot 10^{-2}$	0.94	18	11 to 32	Tª
Prometryn	$3.8 \cdot 10^{-3}$	$2.9 \cdot 10^{-3}$ to $4.7 \cdot 10^{-3}$	0.79	180	150 to 240	C
	$3.8 \cdot 10^{-3}$	$2.9 \cdot 10^{-3}$ to $4.9 \cdot 10^{-3}$	0.77	180	140 to 240	UV
	$2.2 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$ to $3.8 \cdot 10^{-2}$	0.89	32	18 to 59	Tª
Tebuthiuron	$5.1 \cdot 10^{-3}$	$4.2 \cdot 10^{-3}$ to $6.0 \cdot 10^{-3}$	0.89	140	110 to 160	C
	$4.8 \cdot 10^{-3}$	$3.7 \cdot 10^{-3}$ to $6.1 \cdot 10^{-3}$	0.89	140	110 to 190	UV
	$5.0 \cdot 10^{-3}$	$4.2 \cdot 10^{-3}$ to $9.1 \cdot 10^{-3}$	0.89	140	76 to 160	Tª

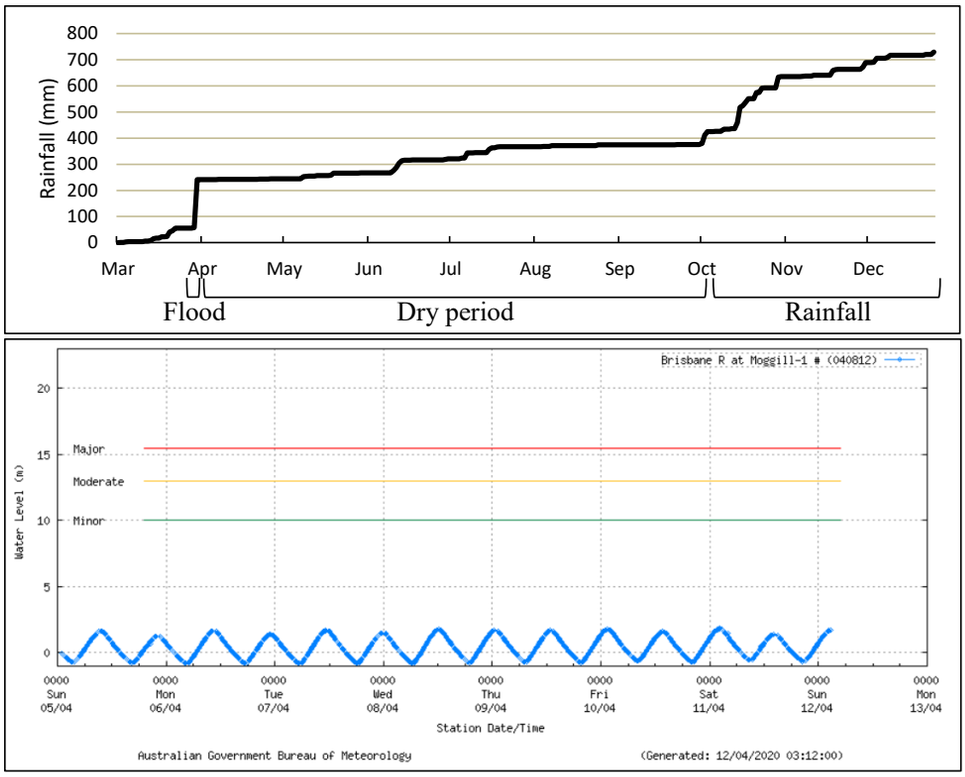

Figure S-1. Up: cumulative rainfall at Savage´s Crossing gauging station between March and December 2017 [1]. Down: example of tidal amplitude of the Brisbane River at Moggill between April 5 and April 12 2020.

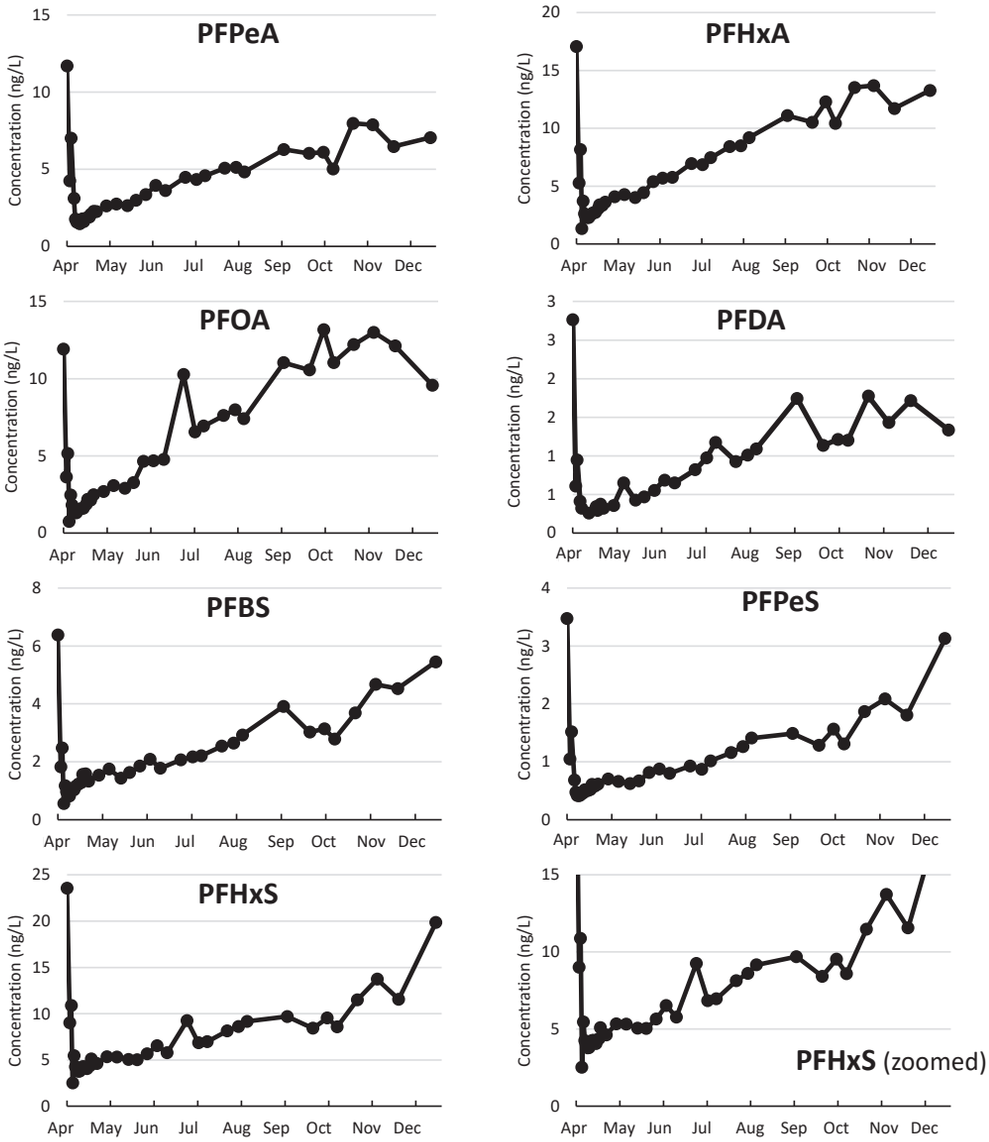

Figure S-2. Concentration of Type A chemicals during the whole sampling period. A zoom to the y-axis is provided for some substances to facilitate inspection of the trend. The x-axis labels mark the beginning of the month.

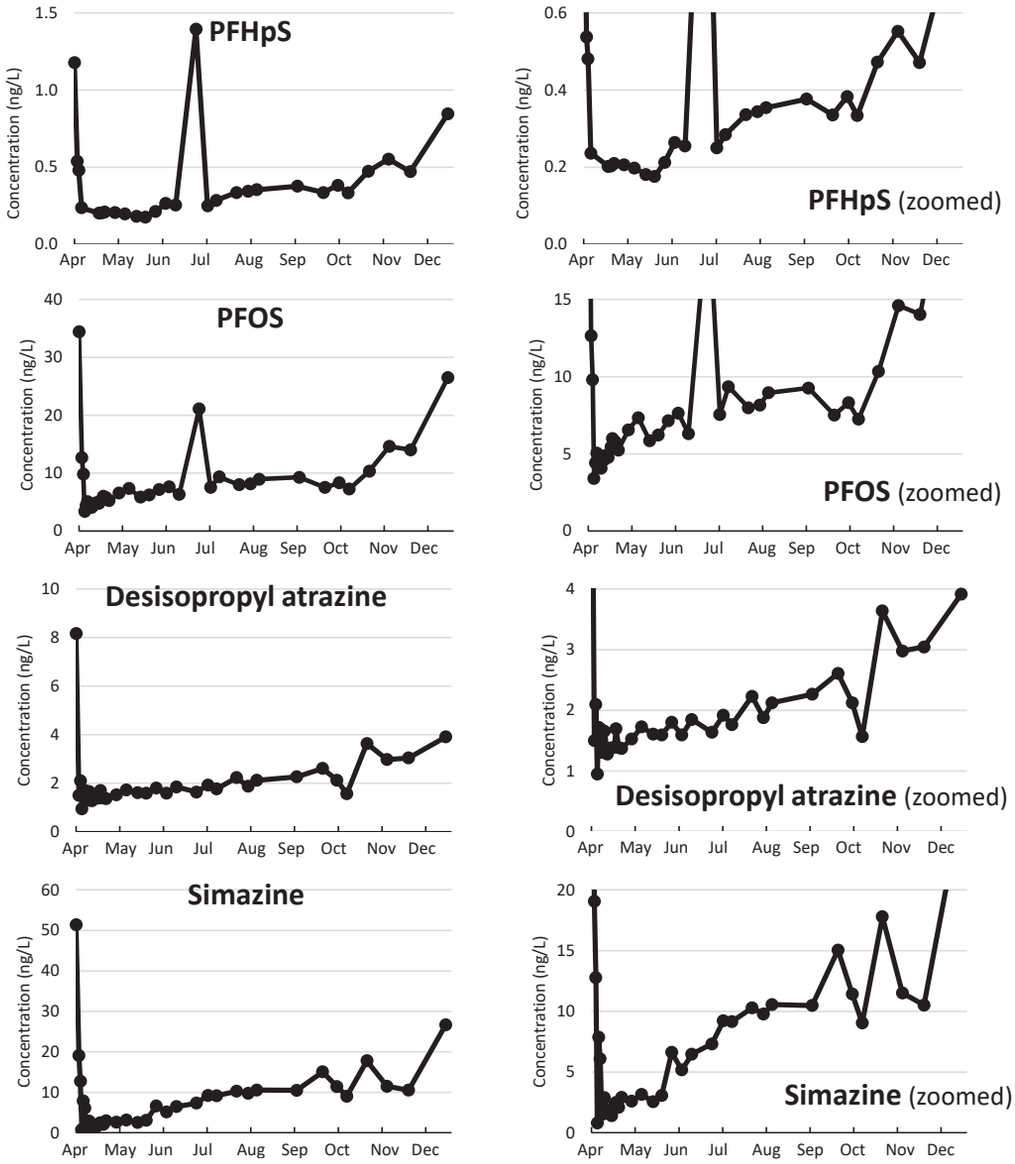

Figure S-2. (Continuation) Concentration of Type A chemicals during the whole sampling period. A zoom to the y-axis is provided for some substances to facilitate inspection of the trend. The x-axis labels mark the beginning of the month.

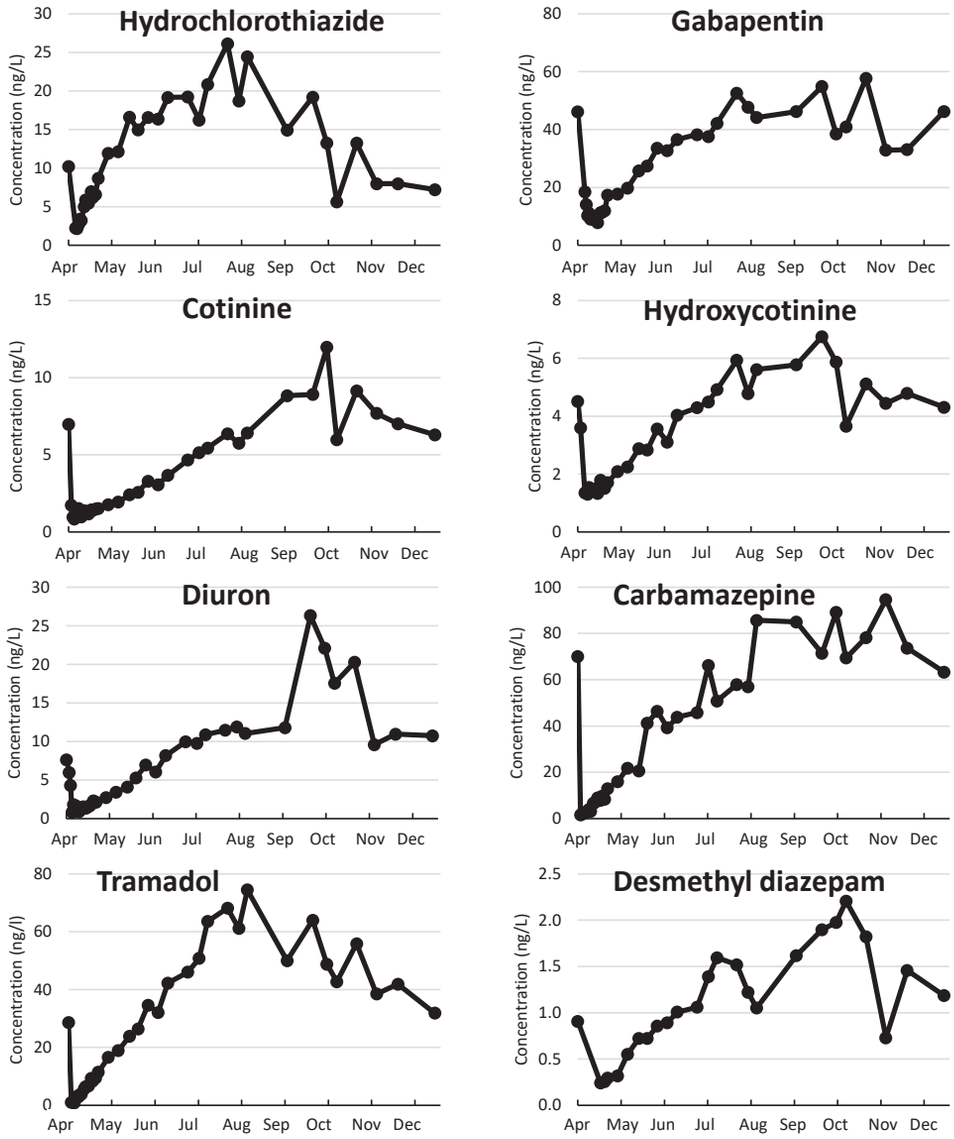

Figure S-3. Concentration of Type B chemicals during the whole sampling period. The x-axis labels mark the beginning of the month.

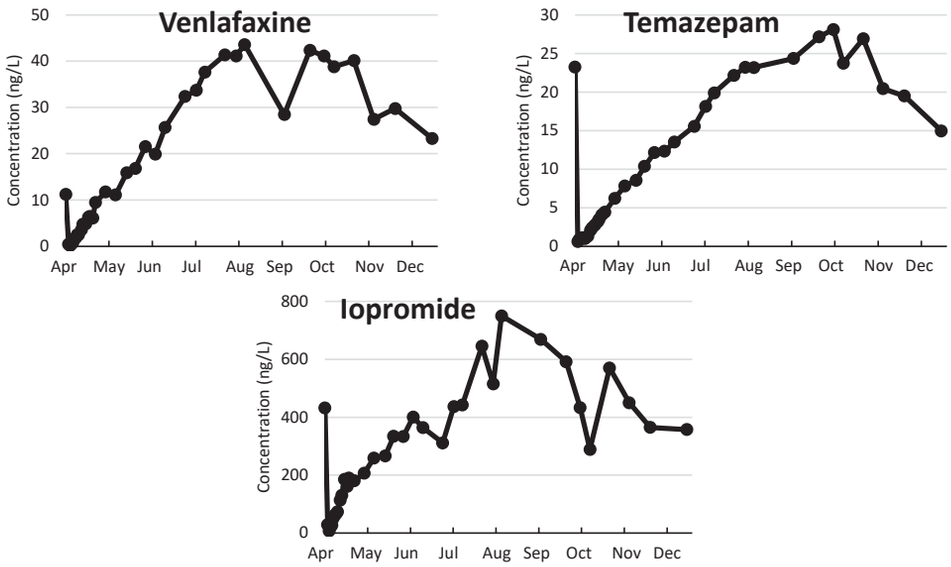

Figure S-3. (Continuation) Concentration of Type B chemicals during the whole sampling period. The x-axis labels mark the beginning of the month.

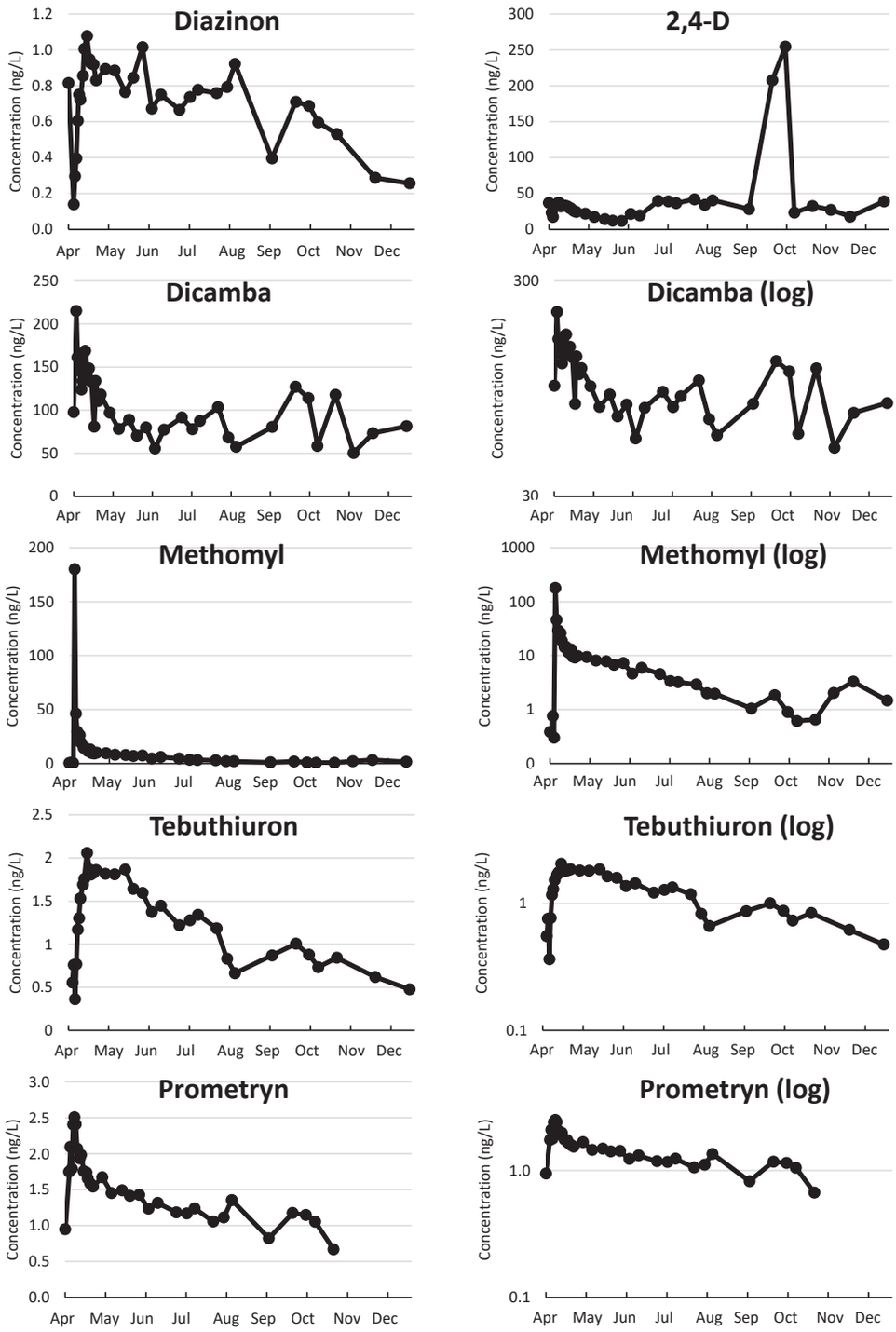

Figure S-4. Concentration of Type C chemicals during the whole sampling period. A semilogarithmic plot is provided for some substances to facilitate inspection of the elimination kinetics. The x-axis labels mark the beginning of the month.

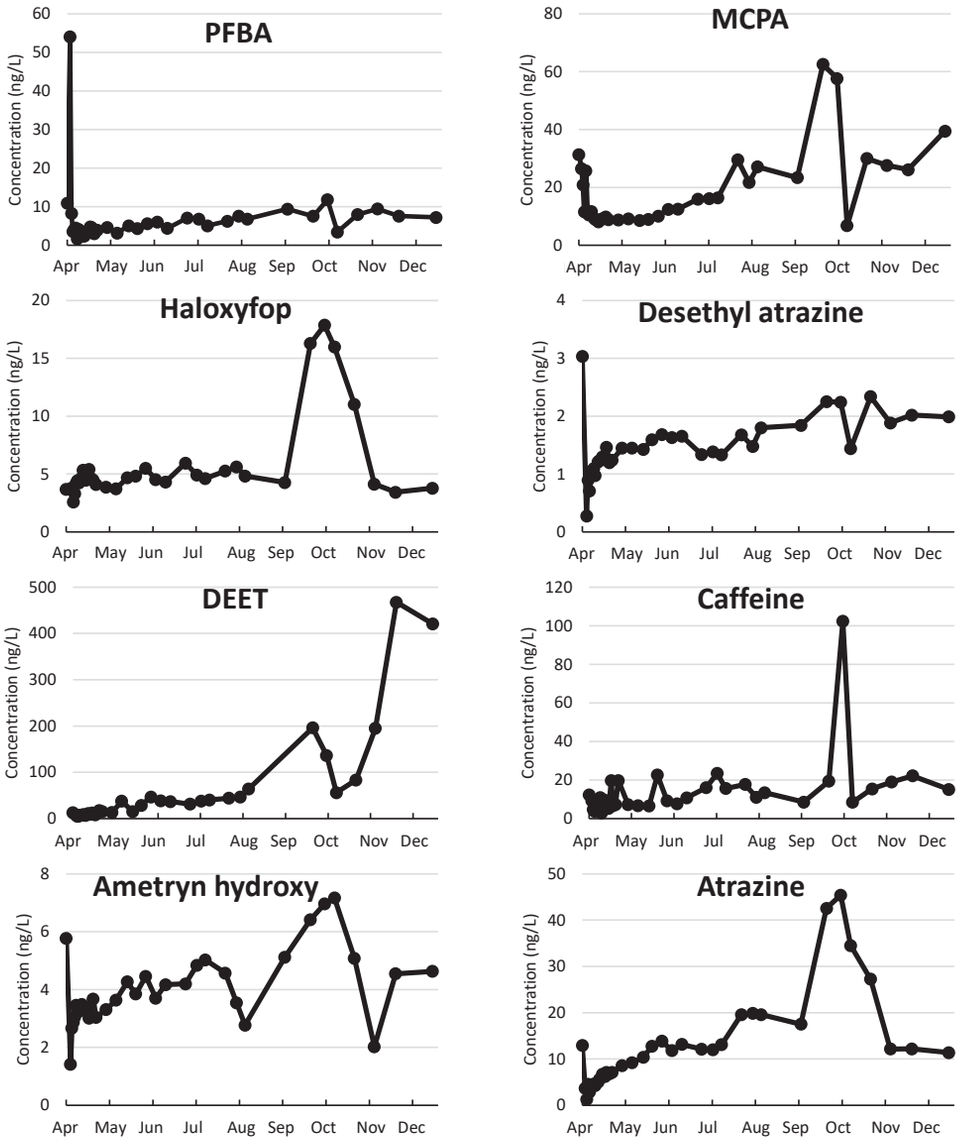

Figure S-5. Concentration trend during the whole sampling period for chemicals showing no consistent time treand. The x-axis labels mark the beginning of the month.

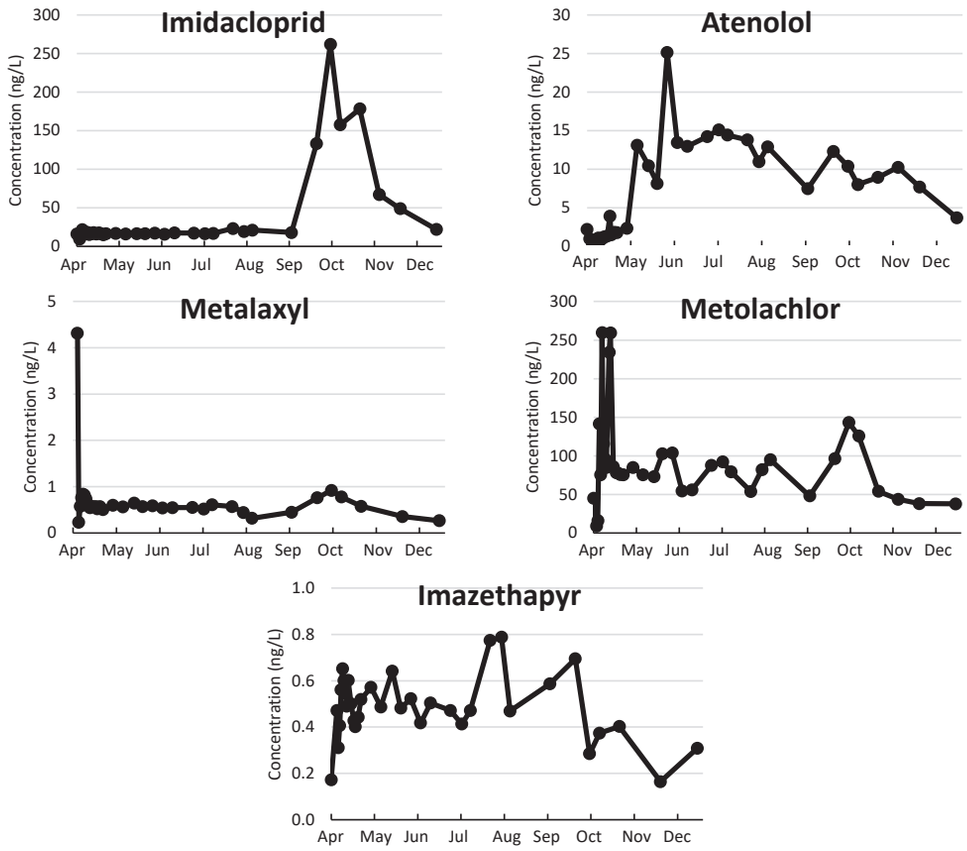

Figure S-5. (Continuation) Concentration trend during the whole sampling period for chemicals showing no consistent time treand. The x-axis labels mark the beginning of the month.

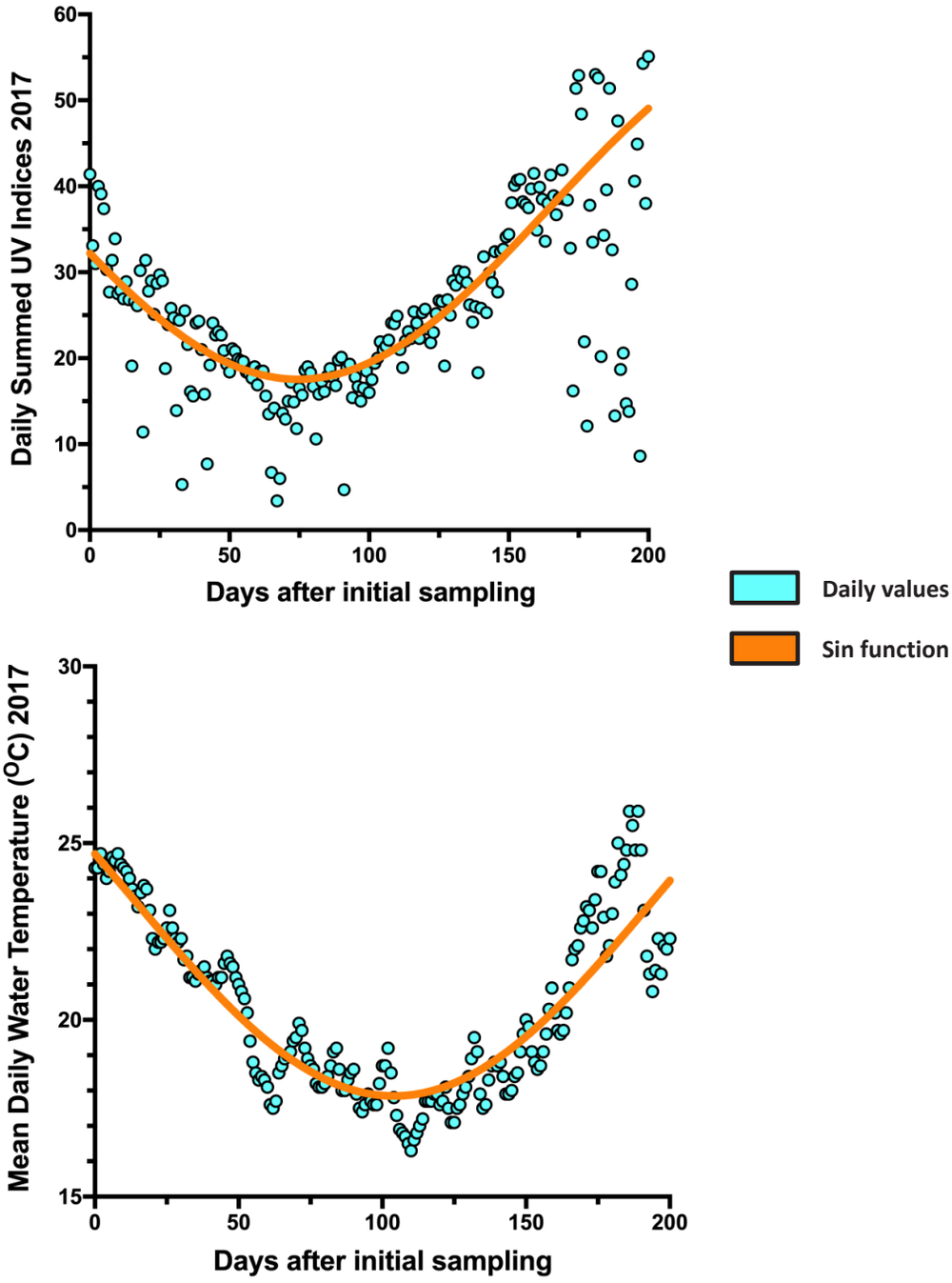

Figure S-6. Upper panel: Daily UV index at the Brisbane UV monitoring station, summarized from hourly UV index data [2] Lower panel: Daily average water temperature at the Savage´s Crossing station on the Brisbane River [1]. The time axis of both graphs extends from April 7 (day 0) to October 22 (day 200) 2017.

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Ampliación 2. Bioaccumulation of emerging contaminants in mussel (*Mytilus galloprovincialis*): Influence of microplastocs

In collaboration with Yolanda Picó and Julián Campo

A revised and improved version of this study has been already published:

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Abstract

Coastal environments are heavily influenced by human activities. Emerging contaminants (ECs) are one of the most important indicators of the anthropic influence on the environment, and they have recently shown to interact with microplastics (MPs). Mussels are suitable for in-lab bioacumulation studies, which provide insight about the occurrence and fate of contaminants in the organisms. In the present work, bioacummulation in *Mytilus galloprovincialis* of 20 contaminants, including pharmaceuticals and personal care products (PPCPs), pesticides, and perfluoroalkyl substances (PFASs) was assessed, along with the influence of microplastics during the process. Mussels were distributed in three groups: control (B), exposed to ECs (C) and exposed to ECs and polyethylene MPs (C+M). The study was carried out for 58 days separated in two stages (i) exposure during days 0-28, and (ii) depuration during days 29-58. Visceral mass and haemolymph of the mussels were extracted separately, using QuEChERS and solid phase extraction (SPE), respectively. Then, extracts are analysed via UHPLC-MS/MS. Results showed that 3 PPCPs, 4 pesticides and 3 PFASs accumulated in visceral mass with bioaccumulation factors (BCFs) ranging 6.7-120 L/kg/d. In addition, 2 PPCPs, 2 pesticides and PFPeA were detected in haemolymph showing BCFs ranging 0.9-3.3 L/kg/d. When comparing C and C+M, MPs worked as a vector for the accumulation of the PFASs: PFOA, PFOSs, PFDA and PFPeA; showing higher BCFs in the presence of MPs. Furthermore, the elimination of PFDA and PFOS was slower in the mussels exposed to MPs. On the other hand, the pesticides terbutylazine and chlorpyrifos showed lower BCFs and more rapid elimination in the mussels exposed to MPs.

1. Introduction

Recently, the ubiquitous distribution of emerging contaminants (ECs), mostly pharmaceuticals and personal care products (PPCPs), pesticides, perfluoroalkyl substances (PFASs) and microplastics (MPs) into the aquatic ecosystems has been identified as one of the prevalent topics in environmental risk assessment (Köck-Schulmeyer et al., 2021; Kroon et al., 2020; Pico et al., 2019). Many reports contributed information on the distribution of these ECs in abiotic environmental compartments as water and sediments (Barbieri et al., 2021; Calvo et al., 2021; Campo et al., 2016; Carmona et al., 2017; Sadutto et al., 2021b). However, several gaps remain on the accumulation and distribution of these compounds in biota because only very partial data are available, as ecotoxicity studies rarely include tissue concentrations and cover few analytes compared to the large number of contaminants that may be present even in a single sample (Çolakoglu et al., 2020; Di Poi et al., 2016; Miller et al., 2018; Pes et al., 2021; Sanchís et al., 2018).

The mixtures of contaminants rather than isolated contaminants resemble more the actual situation that can be found in the environment, as a couple of pesticides, several PPCPs and a few PFASs can coexist in the same sample with a certain level of MPs, which are one of the most important contaminants (Picó et al., 2020; Picó et al., 2021). This type of interaction is important, as several studies have established the capacity of MPs to adsorb some contaminants and increase their bioaccumulation in living organisms, increasing the occurrence of synergistic effects between several contaminants. Vieira et al. (2021) already summarized the physicochemical properties and compiled various adsorption models and their relationships to the dynamics of the environment to elucidate specific MPs-ECs relationships and interactions. Other studies explore which metabolic pathways may be affected by various contaminants including combinations of the parent compounds and their metabolites. Cui et al. (2019) demonstrated that in the livers and gills of zebrafish exposed to carbosulfan, carbofuran, and 3-hydroxycarbofuran, activities of catalase, superoxide dismutase, and glutathione-S-transferase changed in most cases, and the content of malondialdehyde increased, indicating that carbosulfan and its metabolites induced varying degrees of oxidative stress. The metabolites were more persistent and toxic to zebrafish and exhibit coincident synergistic effects in combination. Several studies also suggested that the common target for MPs and veterinary antibiotics in mussels is the innate immune response via phagocytosis (Han et al., 2021; Zhou et al.,

2021). The bioaccumulation potential of mixtures of contaminants is of great significance for the evaluation of the environmental ecological effects. Although there are a significant and growing number of studies on sorption of ECs in MPs, this topic remains controversial and with many gaps due to its complexity.

Molluscs are bioindicator organisms typically used to study the toxicity and bioaccumulation of environmental contaminants, due to their abundance and diversity in different aquatic environments, their physiological characteristics (e.g., sessility and filter-feeding) and their ecological and economic relevance (Woolnough et al., 2020). Several published works showed that bivalves did not regulate the levels of some metals in their body reflecting the metal contamination from surrounding area (Mejdoub et al., 2018; Yigit et al., 2018). In fact, mussels are well known as good bioindicators for metal monitoring. These organisms have been selected to investigate nanomaterials toxicity specially of those nanoparticles based on CdS quantum dots (Jimeno-Romero et al., 2019). This sensitive filter-feeding species have been also used extensively to study bioaccumulation of many ECs including PAHs (Arienzo et al., 2019; Yakan et al., 2017), UV-filters (Vidal-Liñán et al., 2018) and veterinary medicinal products (Brooks et al., 2019). Furthermore, intake of MPs by mussels has been reported by the literature (Renzi et al., 2018; Sparks et al., 2021). Elimination half-lives for teflubenzuron and emamectin benzoate, suggest that these chemicals accumulate in blue mussels (Brooks et al., 2019). Uptake and accumulation of waterborne 4-MBC, BP-4 and OC was very rapid (Vidal-Liñán et al., 2018). The kinetics of bioaccumulation of BP-4 and OC significantly fitted to an asymptotic model with high bioaccumulation factors (BCFs). Measured bioaccumulation of the hydrophilic chemical BP-4 was much higher than predicted by K_{ow}-based bioconcentration models, which would lead to a marked underestimation of actual risk. All these results pinpointed the interest to study bioaccumulation of mixtures of contaminants as a first step to elucidate environmental risk and effects.

The aim of this study is to assess the accumulation of a combination of 20 commonly used ECs belonging to different chemical classes including pesticides, PFASs, PPCPs and MPs in visceral tissue and haemolymph of mussels (*M. galloprovincialis*). Due to their ubiquity, high water filtration rates and potential for bioaccumulation of chemicals the marine mussels are the most common organisms used to monitor chemical pollution worldwide. The influence of the presence or not of MPs on the bioaccumulation behaviour of the other contaminants of the mixture is also evaluated. Furthermore, these

data are used to estimate the BCFs based on kinetic models for most of the contaminants. The results of this study shed light on the distribution of ECs and the concentration that these compounds can reach in tissues. These data are important from the point of view of exposure, the behaviour of different mixtures of contaminants and ecotoxicity.

2. Materials and methods

2.1. Reagents and materials

Detailed information about the reagents and materials employed in the present study can be found in **Text S-1** of the supplementary material.

2.2. Experimental design

The bioaccumulation study was carried out during October-December 2017 in the installations of the Scientific Park of the University of Valencia, Spain. With 200 L aquariums containing 160 L of sea water purified using sand filters and UV. The specific characteristic and conditions were salinity 35 ppt, temperature 18 ºC, pH 8.0 ± 0.2, water was continuously oxygenated (O_2 > 80%) and a 12 h day/night cycle. Mussels (*M. galloprovincialis*) were purchased from a local market and placed in the aquariums for acclimation for two weeks. Causalities were around 20% during the first 2-3 days, likely due to the conditions in the market. Then mussels were randomly distributed in three groups, with a total of 83 mussels per group: control group (B), the group exposed to the ECs mix (C) and the group exposed to ECs and MPs (C+M).

The study was carried out for 58 days separated in two stages. The exposure stage took place during days 0-28, when mussels were exposed to contaminants via water (10 µg/L and food (10 ng per specimen and day). Although 10 µg/L is a concentration higher than the typically found in aquatic Mediterranean environments (Campo et al., 2016; Köck-Schulmeyer et al., 2021; Sadutto et al., 2021a), some compounds have been detected at similar and higher concentrations in aquatic environments (Barbieri et al., 2021; Calvo et al., 2021) and WWTP effluents (Golovko et al., 2021; Sadutto et al., 2021a). Furthermore, it was still significantly lower than in other bioaccumulation studies (Cui et al., 2019; Le Bris and Pouliquen, 2004; Mezzelani et al., 2016). Depuration stage (mussels were not exposed to any contaminants) was from days 28-58.

2.2.1. Exposure stage

The contaminants were inoculated through both, water and food. It was decided that compounds with K_{ow} > 4 (**Table S-1**) were inoculated through food and the other were inoculated in the water. However, few considerations were taken into account: although K_{ow} reported for PFOA, PFOS and PFDA were > 4, these values were not from experimental studies, but simulated with the EPI Suite software, and since these compounds are known to be soluble and often detected in water (Campo et al., 2016; Lam et al., 2016), they were inoculated in the water. On the other hand, in-lab brief solubility tests performed for imazalil, chlorfenvinphos and etoricoxib (K_{ow} 3.82, 3.81 and 3.7, respectively) with the aquarium water, showed that chlofenvinphos was just partially soluble, and hence inoculated in the food.

The water of the aquariums of C and C+M was spiked with a mix of contaminants containing acetaminophen, atenolol, caffeine, diclofenac, etoricoxib, ibuprofen, metformin, naproxen, salicylic acid, vildagliptin, bentazone, imazalil, terbuthylazine, PFBS, PFPeA. PFOA. PFOS. and PFDA in MeOH. Water was spiked to reach a concentration of 10 µg/L of contaminants. Every 2-5 days (**Table S-2 and S-3**), part of the water from the aquariums was replaced by clean water, which was spiked with the compounds mix at the initial concentrations. During that spiking process was also added enough amount of contaminants to compensate their degradation in the aquarium water, based on the reference half-lives reported for water (**Table S-3**). Water samples from group C aquarium were taken almost daily (**Table S-2**) (and always prior re-spiking) to verify the real concentration of the ECs in the water. Additionally, water samples were taken sporadically from C+M to ensure that they had similar concentrations. Every time that C and C+M aquariums were spiked with the compounds mix, the same volume of MeOH was added to aquarium B to eliminate any potential difference between groups due to MeOH exposure.

Mussels were checked every day for feeding and to remove any possible corpses. They were fed daily with a plankton solution. This solution was different depending on the groups: a) C plankton was spiked with 10 µL per mussel remaining in the aquarium of a mix containing the compounds chlorfenvinphos, chlorpyrifos and triclosan at 1000 ng/mL in MeOH (10 ng of contaminant per mussel), b) C+M was fed with plankton spiked at the same concentration and polyethylene MPs and c) plankton for B was spiked with the same

amount of MeOH than C and C+M. The quantities were daily adjusted depending on the number of specimens to ensure an administration of ≈16 mg of plankton, 10 ng of contaminants and/or 1 mg of MPs per specimen. MPs suspended in the water were removed manually with every water replacement.

2.2.2. Depuration stage

At the end of day 28 (after the mussel sampling), the aquariums were completely emptied, rinsed and filled with clean water. During this process, no contaminants or MPs were added to any of the three groups. Water was sampled sporadically to check if any contaminants remain in the system even through the frequent rinses (**Table S-2**). Mussels were fed daily and water was replaced as described above until the end of the experiment.

2.3. Sampling

Five mussels were randomly sampled from each group the days 0 (before spiking the water), 2, 4, 7, 14, 28, 29, 30, 32, 35, 42 and 58 of the experiment. The last day all the mussels remaining were sampled (19-20 mussels per group). Just after the sampling, mussels´ maximum length was measured.

For haemolymph extraction, the shells were filed using a steel file. Then the haemolymph of each mussel was extracted from the posterior adductor muscle using a 1 mL syringe with a 25G needle. The volume extracted was 0.4-1.0 mL per mussel. Two hundred microliters of haemolymph from the five individuals of each group (and day) were pooled and stored in 15 mL falcon tubes at -20 ºC until extraction.

Mussel shell was removed, and visceral mass was washed with Milli-Q water and weighted. The five mussels of each group were then pooled, homogenised using a blender and stored at -20 ºC in 50 mL falcon tubes until extraction.

2.4. Extraction, LC-MS/MS analysis and quality assurance

Water extraction was performed employing solid phase extraction (SPE) following the method by Carmona et al. (2017). For mussel visceral mass extraction, the method developed by Álvarez-Ruiz et al. (2021b) combining QuEChERS extraction and EMR-Lipid clean-up was employed and for haemolymph, SPE was used as described by

Álvarez-Ruiz et al. (2021a). Additional information can be found in **Text S-2** of the supplementary material.

Analysis was performed via LC-MS/MS as previously described by Álvarez-Ruiz et al. (2021a) using an Agilent 1260 UHPLC coupled to an Agilent 6410 Mass Spectrometer QQQ both from Agilent technologies. Further information about the analytical method, compounds transitions and quality assurance can be found in **Texts S-3** and **S-4,** and **Tables S-4** and **S-5**.

2.5. MPs examination

One specimen of each group was digested following the procedure by Dehaut et al. (2016) and also inspected for the search of MPs. Briefly, visceral mass of each mussel was placed in glassware jars with KOH 10% solution and incubated for 24 h at 60 ºC. The resulting mixture was passed through glass filters. Each jar was rinsed three times with MilliQ water to drag the possible particles from the walls. Filters were then dried for 24 hours at room temperature and then visually inspected using the stereomicroscope. Additionally, test samples (TS) of *M. galloprovincialis* from a local market were spiked with the same MPs as the employed during the bioaccumulation tests; and then digested as described above to serve as a reference for MP identification.

2.6. Modelling

According to Landrum et al. (1992) the models to describe toxicant kinetics can be divided generally into two classes: compartment-based models and models based on organism physiology. In this study, we used a compartment-based model, which describes toxicant movement between compartments. A compartment represents the amount of a compound that behaves as though it exists in a homogeneously well-mixed container and moves across the compartment boundary with a single uptake or elimination rate coefficient. In our case, the compartment model is defined, and its mathematical equivalency demonstrated for a simple two-compartment model containing water and mussel compartments. The water represents the source and the organism, the contaminant sink. It is assumed that the contaminant is well mixed and homogeneous within each compartment. For this specific comparison, we also assume that no compound biotransformation occurs. The models use the underlying assumption that the rate constants and clearances remain constant over time. If the organism undergoes

physiological change, this assumption can be violated (Landrum et al., 1992). These models also assume that the transfer between compartments is first order, and the flux across the boundary depends on the concentration in the respective compartment (Li et al., 2020). Consequently, the net flux is the sum of the uptake and loss fluxes across the compartment boundaries.

Thus, the two-compartment model for accumulation (**Eq. 1**) from water is:

$$\frac{\mathrm{d}C_a}{\mathrm{d}t} = (k_u \cdot C_w) - (k_d \cdot C_a) \quad (1)$$

Where k_u is the uptake rate coefficient (L/kg/d), k_d is the depuration rate coefficient (1/d), *Ca* is the concentration accumulated in mussels (mg/kg), C_w is the concentration in water (mg/L) and t = time (h).

If C_w is held constant, as ideally occurs in flow through experiments and is often assumed for field exposures, **Eq. 1** can be exactly integrated to yield **Eq. 2**:

$$C_a(t) = \frac{C_w k_u}{k_{de}}(1 - e^{-k_{de}t}) \quad (2)$$

Where *Ca(t)* is the concentration accumulated in mussels (mg/kg) at time t, k_{de} is the depuration rate coefficient during the exposure stage (1/d) (loss of the toxicant that occurs while the organism is still exposed to the toxicant) and t is the time (days). k_u and k_{de} were estimated by least square fits of the accumulation data to equation (**Eq. 1**) model.

The BCF is usually calculated as the ratio of the uptake rate coefficient to the depuration rate coefficient*: BCF* = k_u/k_{de}, with units L/kg. **Eq. 2** can be rearranged (**Eq. 3**) to obtain directly the confidence intervals of BCF:

$$C_a(t) = C_W BCF(1 - e^{-k_{de}t}) \quad (3)$$

For the estimation of k_{dd} during the depuration stage, since mussels were not exposed to contaminants, K_u and C_w were neglected in **Eq. 1** and later integrated yielding to **Eq. 4**:

$$C_a(t) = (e^{-k_{dd}t}) \quad (4)$$

Where k_{dd} is the the depuration rate coefficient during the depuration stage (1/d).

The fit of the curves was performed using the Solver tool from Microsoft® Office Excel Professional Plus 2016, fitting k_{de} and k_u with **Eq.2** to the lowest Root-Mean-Square Error (RMSE). And fitting k_{dd} with **Eq.4** to the lowest RMSE for the depuration stage.

3. Results and discussion

3.1. Mussels characteristics and water concentration

Information relative to length, weight and casualties of the mussels during the experiment can be found in **Text S-5**.

Water concentration was quite stable for bentazone, caffeine, diclofenac, etoricoxib, naproxen, vildagliptin, PFBS, PFPeA and terbuthylazine. While it was variable for the others, generally showing a decrease in the concentration along time (**Table S-6)**. Further discussion about the contaminants in water can be found in **Text S-6**.

ECs concentrations in water are important to assess their bioaccumulation. Mean concentrations of water in aquarium C (**Fig. 1**) were used as C_w for modelling with **Eq. 2**. RSDs were <20% when compared with water concentrations of C+M in a given day. However, all the compounds (except terbuthylazine and chlorfenvinphos) reached concentrations close to steady state (in both visceral mass and haemolymph) in ≤48 hours (**Tables S-7 and S-8**). This, together with the variation of ECs concentration in water throughout the experiment, introduced uncertainty in the modelling during the exposure stage, so the results should be interpreted with caution. On the other hand, depuration stage was not affected by this uncertainty and water analysis served to verify that most ECs were removed from the aquariums after day 28 (**Table S-6**) where only scarce traces of few compounds such as PFASs or pesticides (probably due to glass adherence)

remained. These results provided valuable insight for future bioaccumulation studies, especially the related to their complexity and probable variability. It is highly recommended for future bioaccumulation studies to monitor the concentration of the water and specimens continuously (e.g. hourly) in the first 24-48 hours to observe possible variations in this critical stage where the major part of the bioaccumulation may occur.

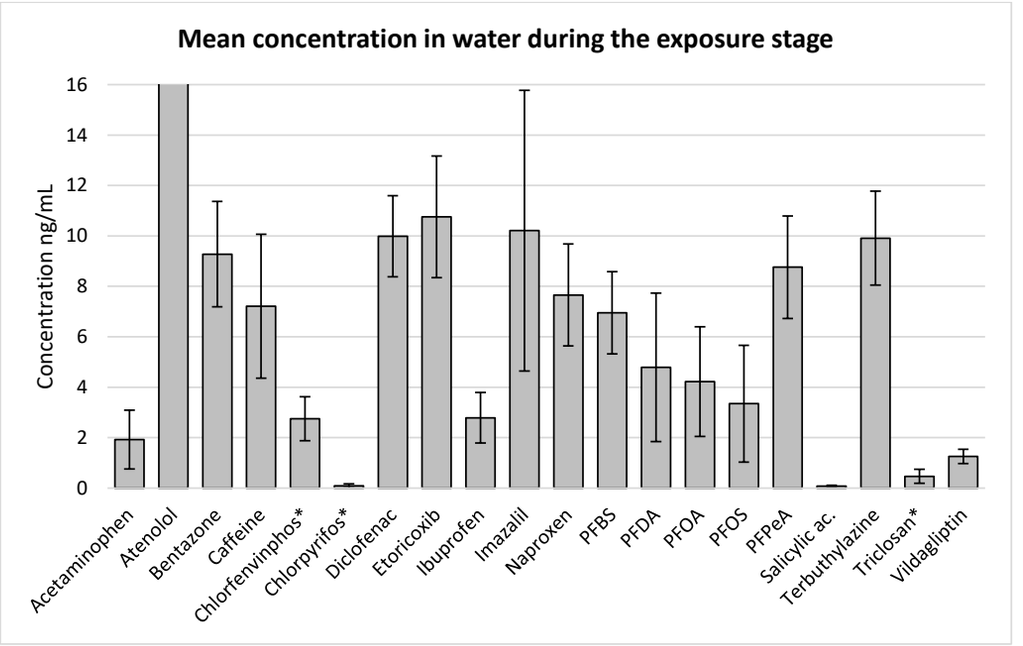

Figure 1: *Mean concentration in water of the compounds spiked*
**These compounds were introduced through the food*

3.2. Modelling considerations

The criteria followed for compound modelling during the exposure stage was that at least 4 (80% no taking in account day 0) samples provided concentrations above quantification limits (>LOQs). Similar as for the depuration stage where at least 5 (83%) samples with concentrations >LOQs were required. Following this criteria chlorfenvinphos, chlorpyrifos, diclofenac, etoricoxib, imazalil, PFDA, PFOA, PFOS, terbuthylazine and triclosan were modelled for visceral mass, while caffeine, etoricoxib, imazalil, PFPeA and terbuthylazine were modelled for haemolymph (**Table 1**).

Table 1: Results for the mussels exposed to ECs (C) and the mussels exposed to ECs and microplastics (C+M). Uptake (k_u) and depuration (k_{de}) rate coefficients for the exposure stage estimated with **Eq.2**. Depuration rate coefficient (k_{dd}) and half-life of the compounds for the depuration stage calculated with Eq.4. RMSE and BCF of the compounds modelled.

		Exposure Stage				Depuration Stage		
Visceral mass		K_{de} (1/d)	K_u (L/kg/d)	BCF (L/kg)	RMSE	K_{dd} (1/d)	Half-life (days)	RMSE
Chlorfenvinphos	C	8.6×10^{-2}	8.1	94	49	n.e.	-	-
	C+M	0.22	22	98	34	5.2×10^{-2}	14	8.5
Chlorpyrifos	C	6.5×10^{-2}	990	15000	300	2.6×10^{-2}	26	51
	C+M	1.02	3900	3800	190	0.30	2.3	58
Diclofenac	C	1.4	19	13	14	n.d.	-	-
	C+M	1.6	16	9.8	25	n.d.	-	-
Etoricoxib	C	2*	13	6.4	17	<LOQ	-	-
	C+M	0.92	6.2	6.7	23	<LOQ	-	-
Imazalil	C	2*	105	53	40	4.7×10^{-2}	15	19
	C+M	0.70	55	78	95	7.7×10^{-2}	9	34
PFDA	C	1.2	110	92	200	0.11	6.4	25
	C+M	0.74	91	120	110	5.0×10^{-2}	14	23
PFOA	C	1.6	5.1	3.3	3.6	n.d.	-	-
	C+M	1.1	4.9	4.3	8.6	n.d.	-	-
PFOS	C	2*	96	48	76	0.12	5.7	14
	C+M	2*	160	81	80	7.5×10^{-2}	9.3	5.6
Terbuthylazine	C	0.32	4.0	13	25	2.1×10^{-2}	32	2.9
	C+M	0.30	3.6	12	5.7	6.8×10^{-2}	10	1.7
Triclosan	C	n.e.	n.e	-	-	n.d.	-	-
	C+M	2*	240	120	43	<LOQ	-	-
Haemolymph								
Caffeine	C	<LOQ	<LOQ	-	-	n.d.	-	-
	C+M	2*	2.5	1.2	4.6	n.d.	-	-
Etoricoxib	C	2*	1.8	0.90	4.9	n.d.	-	-
	C+M	n.e.	n.e.	-	-	n.d.	-	-
Imazalil	C	1.04	1.3	1.2	3.4	<LOQ	-	-
	C+M	2*	2.8	1.4	3.5	<LOQ	-	-
PFPeA	C	0.97	2.5	2.5	12	n.d.	-	-
	C+M	2*	6.6	3.3	19	n.d.	-	-
Terbuthylazine	C	0.23	0.43	1.9	4.8	n.d.	-	-
	C+M	0.74	1.02	1.4	2.1	n.d.	-	-

**: the fit hits the $k_{de} \leq 2$ constrain.*
n.d.: not detected in any sample.
<LOQ: no samples detected above LOQ.
n.e.: not enough samples detected above the LOQ to fulfil the criteria.

Naproxen presented unusual concentrations in samples C day 0 (717 ng/g) and C+M day 4 (2380 ng/g), which are around 20 and 70 times higher than other concentrations. Although naproxen was not detected in the procedural blanks, this results are likely due to a contamination issue (at day 0 all the mussels were under the same conditions, hence differences between groups are not applicable and mussels from the 3 groups should have provided the same concentration), therefore naproxen was not modelled. Salicylic ac. presented inconsistent concentrations (**Tables S-7 and S-8**), likely due to interfering compounds naturally present in the mussel (Álvarez-Ruiz et al., 2021b), and hence the results were considered not representative and not modelled. Although chlorfenvinphos and chlorpyrifos were added to the food, C_w was set as the mean concentration for modelling purposes during exposure stage; so their results should be interpreted with caution. Finally, acetaminophen, atenolol, bentazone, ibuprofen, PFBS and vildagliptin were not detected in any mussel sample, suggesting that these ECs do not bioaccumulate.

As can be seen in **Fig. 2**, concentration on mussels was closed to steady state in the first 48 h for some compounds. This made that solver was not able to interpret the exposure data as an exponential regression function. Therefore, k_{de} was constrained to ≤ 2 (**Table 2**), since a $k_{de} > 2$ would be very unlikely for the compounds analysed (**Table S-3**). This also enabled comparison between groups and compounds.

Table 2: Ratio between the BCFs and half-lives calculated for C and C+M groups. Values close to 1 indicate similar values, >1 values indicates higher BCF or half-life in the C+M group and <1 values indicates higher BCF or half-life in the C group.

	BCF (C+M/C)	Half-life (C+M/C)
Visceral mass		
Chlorfenvinphos	1.04	-
Chlorpyrifos	0.25	0.09
Diclofenac	0.75	-
Etoricoxib	1.05	-
Imazalil	1.47	0.60
PFDA	1.30	2.19
PFOA	1.30	-
PFOS	1.69	1.63
Terbuthylazine	0.92	0.31
Triclosan	-	-
Haemolymph		
Caffeine	-	-
Etoricoxib	-	-
Imazalil	1.17	-
PFPeA	1.32	-
Terbuthylazine	0.74	-

3.3. Bioaccumulation and depuration

In the literature, few bioaccumulation tests have been conducted with dioxins and dioxin-like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), toxic metals (Pb, Cd, and Hg) and UV filters (Çolakoglu et al., 2020; Vidal-Liñán et al., 2018; Yakan et al., 2017). However, in contrast to the increasingly presence of ECs in sea and sea live, limited data exist about the occurrence of PPCPs, pesticides, PFASs or microplastics in mussels and even less in *M. galloprovincialis.* Experimental information on their bioaccumulation is even scarcer (Mezzelani et al., 2016) and deserves further research.

In the present study, high ECs levels were found in exposed mussels regardless of the via of inoculation. In most cases, concentrations > 120 ng/g wet weight (w.w.) (PFOS, terbuthylazine, diclofenac, chlorfenvinphos) were measured from the first sampling day reaching values > 400 ng/g w.w. for PFDA, imazalil and chlorpyrifos. When mussels were fed with contaminants through water, kinetic curves obtained followed the same trends: rapid increase and stabilization, not being this behaviour so obvious when contaminants were inoculated through food. For the former, kinetics of bioaccumulation fitted to the asymptotic model described by Eq. 1 and 2.

Mezzelani et al. (2016) studied the ecotoxicity of pharmaceuticals by comparing the bioaccumulation and the responsiveness of *M. galloprovincialis* toward five different non-steroidal anti-inflammatory drugs (NSAIDs): acetaminophen, diclofenac, ibuprofen, ketoprofen and nimesulide. Mussels were exposed to 25 µg/L of studied NSAIDs. According to their results, mussels showed a significant bioaccumulation of diclofenac, ibuprofen, nimesulide, from below detection limits (LODs) up to 14.9 ± 7.89 ng/g, 1.63 ± 1.00 ng/g, and 30.22 ± 13.50 ng/g dry weight d.w., respectively. Tissue concentrations of diclofenac tended to be lower than in our study. Their concentrations of ibuprofen detected in mussel were quite low, which may explain why it was not detected in the present study with a concentration in water significantly lower (2.8 ± 1 µg/L). No variations were observed after 14 days for acetaminophen (also not observed to bioaccumulate in the present study) and ketaprofen, which they confirmed to not bioaccumulate by the lack of detectable accumulation on wild mussels.

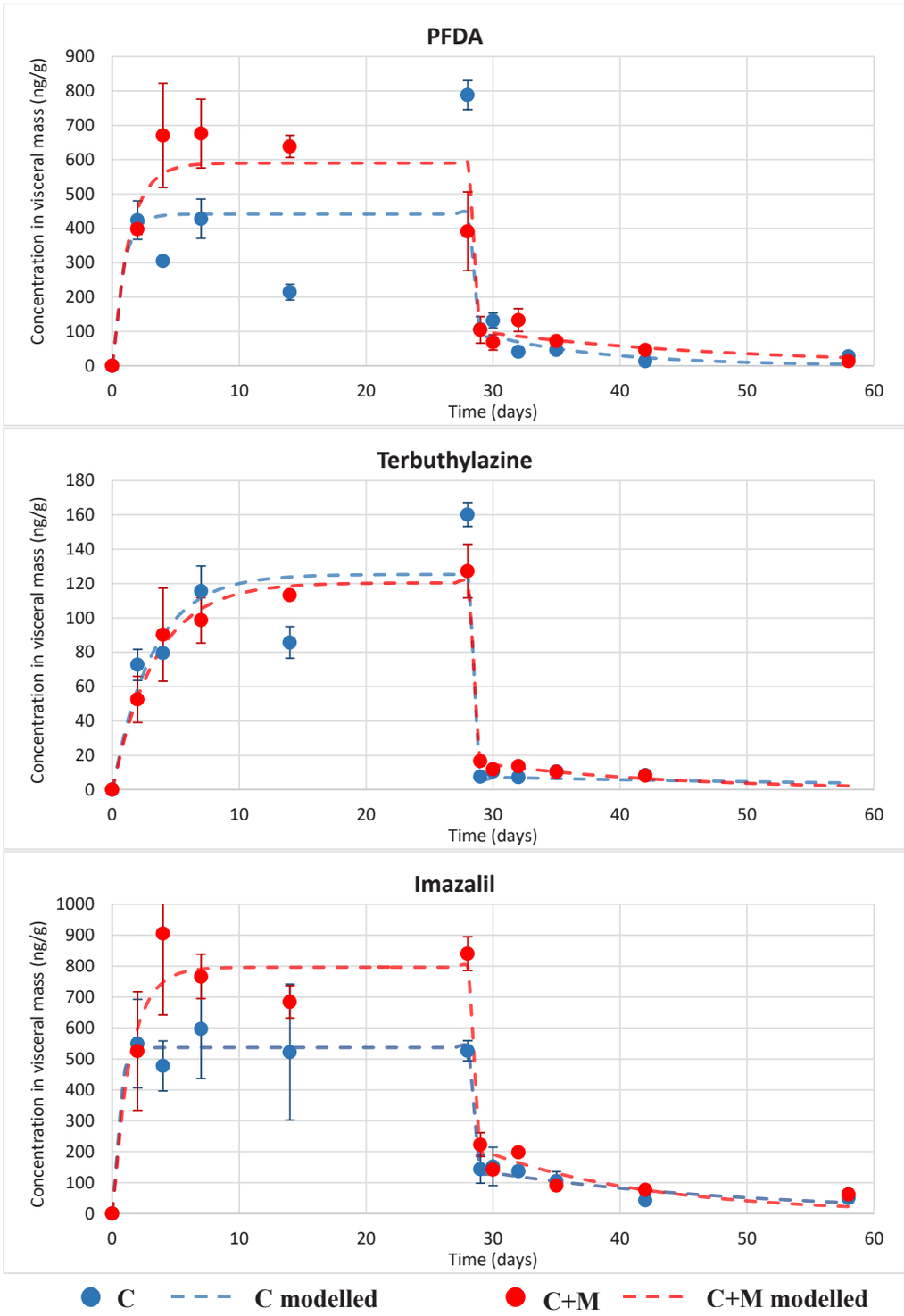

Figure 2: *Concentration measured (dots) and modelled (no-continuous line) for PFDA, terbuthylazine and imazalil, for both exposure stage (days 0-28) and depuration stage (days 29-58). Bars represent the ±SD of the measured concentrations.*

Mezzelani et al. (2016) studied the ecotoxicity of pharmaceuticals by comparing the bioaccumulation and the responsiveness of *M. galloprovincialis* toward five different non-steroidal anti-inflammatory drugs (NSAIDs): acetaminophen, diclofenac, ibuprofen, ketoprofen and nimesulide. Mussels were exposed to 25 µg/L of studied NSAIDs. According to their results, mussels showed a significant bioaccumulation of diclofenac, ibuprofen, nimesulide, from below detection limits (LODs) up to 14.9 ± 7.89 ng/g, 1.63 ± 1.00 ng/g, and 30.22 ± 13.50 ng/g dry weight d.w., respectively. Tissue concentrations of diclofenac tended to be lower than in our study. Their concentrations of ibuprofen detected in mussel were quite low, which may explain why it was not detected in the present study with a concentration in water significantly lower (2.8 ± 1 µg/L). No variations were observed after 14 days for acetaminophen (also not observed to bioaccumulate in the present study) and ketaprofen, which they confirmed to not bioaccumulate by the lack of detectable accumulation on wild mussels.

In a similar research, Vidal-Liñán et al. (2018) studied bioaccumulation kinetics of organic UV filters in *M. galloprovincialis*. Mussels were exposed for 30 days to experimental solutions containing 1 µg/L of UV filters followed by a 20 days depuration period. These authors also observed that the uptake of some compounds was very rapid, reaching the first day tissular concentrations of 263-418 ng/g d.w., for three of the five UV filters analysed. This is in accordance with the concentrations detected after 48 h in the present study and suggests that a significant part of the bioaccumulation of the studied contaminants occurs in the first 24-48 h.

In relation to kinetic constants, different values were modelled in the present study (**Table 1**), with k_u in visceral mass ranging from 3.6 L/kg/day (terbuthylazine) to 240 L/kg/day for PFDA, with the exception of chlorpyrifos (990 and 3900 L/kg/day for C and +M respectively) and k_{de} from 6.5 $x10^{-2}$ 1/day (chlorpyrifos) to 1.6 1/day (diclofenac and PFOA). Theses coefficients yielded BCFs ranging 0.90 to 120 L/kg, except, one more time, for chlorpyrifos (15000 and 3800 L/kg for C and C+M, respectively). Vidal-Liñan et al. calculated values for k_u of 169.8 and 281.7 L/kg/day, and k_{de} of 0.19 and 0.13 1/day for benzophenone-4 and octocrylene, respectively. These authors also presented high BCF values of 905 and 2210 L/kg, respectively.

Haemolymph results showed similar behavior for etoricoxib, imazalil and terbuthylazine as they showed in visceral mass, but with lower concentrations and BFCs (**Table 1**). At

the beginning of the exposure stage concentration in haemolymph was similar (or lower) to concentration in water for compounds such as imazalil, etoricoxib or caffeine. However, when the concentration of these ECs decreased in water, it remained stable or increased in the haemolymph. (**Table S-8**), suggesting bioaccumulation and being PFPeA (up to 56.4 ng/mL) and terbuthylazine (up to 22.2 ng/mL) the compounds with higher concentrations. Caffeine and PFPeA were exclusively detected in haemolymph, suggesting a probable specific accumulation. Unfortunately, previous works regarding bioaccumulation in haemolymph or these specific compounds were not found to compare.

Depuration rates during depuration (k_{dd}) stage were generally lower than during exposure stage (k_{de}) in our study (yielding elimination half-lives of 2.3 to 32 days for chlorfenvinphos, chlorpyrifos, imazalil, terbuthylazine, PFDA and PFOS). Concentrations at the end of the depuration phase were higher than predicted, similarly to the results reported by Vidal-Liñán et al. (2018) who explained that it may be due to accumulation in a second compartment, termed peripheral compartment, which functions as a storage compartment with virtually no elimination pathway. This pattern of accumulation was found in bivalves for chemicals that are preferentially stored in certain organs such as the digestive gland or the fat tissues. Le Bris and Pouliquen (2004) studied kinetics of the veterinary antibiotics oxolinic acid and oxytetracycline in different compartments (foot, muscles, mantle, viscera, gills and shell) of blue mussels (*Mytilus edulis*) showing that uptake was fast in the soft parts of the mussels, especially in viscera (for oxolonic acid) and gills (for oxytetracyclinen), which BCFs were higher than when estimated for the whole body.

Xie et al. (2019) studied bioaccumulation of PPCPs in biota samples from the Pearl River Delta using a steady state model where the BCFs were calculated as the ratio of their concentration in the organism and in water. The average BCFs of the PPCPs were 6272 L/kg in mussel, 10583 L/kg in oyster, 10313 L/kg in scallop, 174 L/kg in conch, 7189 L/kg in yellow grouper, 685 L/kg in topmouth culter, and 379 L/kg in orbfish. Similarly, the bioaccumulations of three antibiotics was assessed in molluscs from Hailing Island, South China (Chen et al., 2015) also using steady state models; estimated BCFs ranging 2-6488 L/kg. Most of these BCFs are two orders of magnitude higher than those calculated in our study (3.2-94.2 L/kg) highlighting the overestimation produced when using steady state models to calculate BCFs in relation to kinetic ones. This overestimation has influence the currently used classification that considered a compound

as bioaccumulative if BCF > 5000 L/kg and potentially bioaccumulative if BCF ranged 2000-5000 L/kg (Chen et al., 2015). Our results contradict these assumptions, since chlorfenvinphos, etoricoxib, imazalil, PFDA, PFOS and terbuthylazine were detected in the depuration stage (and therefore were bioaccumulated) with BCFs several orders of magnitude lower. This is also supported by Vidal-Liñán et al. (2018) who observed bioaccumulation with BCFs ranging 1000-2000 L/kg. However, the rapid increase of the concentration in mussel in the present study could also provide underestimated BCFs. As stated by Vidal-Liñán et al. (2018) much care must be taken when environmental risk is quantified on the basis of modelled rather than experimentally recorded parameters. Bioaccumulation of proteinophilic chemicals such as PFASs cannot be predicted on the basis of their partition between octanol or any other lipophilic surrogate and water (Kelly et al., 2009). This is in accordance with our results since differences in BCFs between the different contaminants (**Table 1**) did not correlate with their different K_{ow} (**Table S-3**). Unfortunately, bioaccumulation studies for the pesticides, PFASs and most of the PPCPs assessed in this work were not found. The particularities of each compound and complexity of metabolic pathways, require further and extensive research about bioaccumulation and elimination of ECs in mussels, and more concretely in *M. galloprovincialis*, which is also crucial for the human risk assessment of this widely consumed mollusc.

3.4. MPs identification

Previous works recommended the chemical confirmation by techniques such as FTIR (Picó et al., 2021; Sparks et al., 2021) or Raman analysis (Dehaut et al., 2016) for MP's identification. However, the visual inspection employing a stereomicroscope is also widely extended (Dehaut et al., 2016; Picó et al., 2021; Renzi et al., 2018; Sparks et al., 2021). In this study, TS with spiked MPs were used as reference for visual inspection. TS showed that added MPs had a bright white coloured globular structure and most of them were not stained during the digestion process (**Fig. 3**). The fact that the MPs were not stained during the digestion was important for their identification since the filters and residues remaining after the digestion had a slight ochre coloration. However, the colour of the added MPs still made difficult their identification, especially when their size was below 50 μm, which is relevant since the smaller the MP the more potential to influence in the bioaccumulation of organic contaminants (Wang et al., 2020a). This did not allow thorough identification and count of the MPs. Hence, the objects that matched with the

TS were identified as "suspected MPs", which were present in all the C+M from the second day of the experiment. On the other hand, suspected MPs were not observed in B and C samples. The addition of MPs with a characteristic colouration different from the sample (e.g. red for mussels), is highly recommended for future studies in order to make a visual identification of the MPs added.

Filaments were also observed in several samples independently of the group (**Fig. 3**). When found, they were between 1 to 4 filaments per mussel and their length ranged 300-2000 μm roughly. The presence of filaments was previously reported in mussels for human consumption (Renzi et al., 2018; Sparks et al., 2021), and, most likely, they would have entered the mussels while they were in the sea (before they were harvested), during the depuration and transport or even in the same aquariums.

3.5. Influence of MPs in bioaccumulation and depuration

Several compounds showed significant differences in the estimated BCF and elimination half-lives for C and C+M groups (**Table 2**). PFASs showed higher BCFs and half-lives when mussels were exposed to MPs (**Fig. 4**). Contrarily, chlorpyrifos, diclofenac and terbuthylazine showed lower BCFs. Chlorfenvinphos and etoricoxib showed no significant differences; and triclosan and caffeine had data just from one of the groups. Imazalil showed a characteristic behaviour with higher BCF for C+M but at the same time higher elimination half-life for C.

All the PFASs modelled presented higher BCFs in the presence of MPs, PFASs have showed to adhere to plastic materials (Llorca et al., 2018), and Islam et al. (Islam et al., 2021) showed that polyethylene MPs act as a vector for PFOS accumulation in *Scrobivularia plana* clams. This is in accordance with the results obtained in the present study, where PFDA, PFOA, PFOS and PFPeA showed 30-69% more bioaccumulation in the presence of MPs. Regarding the elimination half-lives, Abidli et al. (2021) showed that the exposure to polyethylene MPs significantly reduced the filtration rate of *M. galloprovincialis* mussels, which could reduce their capability for depurating contaminants. However, this would not explain why several compounds (e.g. terbuthylazine) showed higher elimination half-lives in C group (**Table 2**). Since polyethylene MPs were found in mussels during the depuration stage (**Fig. 3**), it is possible that mussels continued being exposed to (and somehow accumulating) the

PFASs adhered to those MPs, yielding lower depuration rate estimations. Previous studies about this phenomenon were not found and further research is needed to elucidate it.

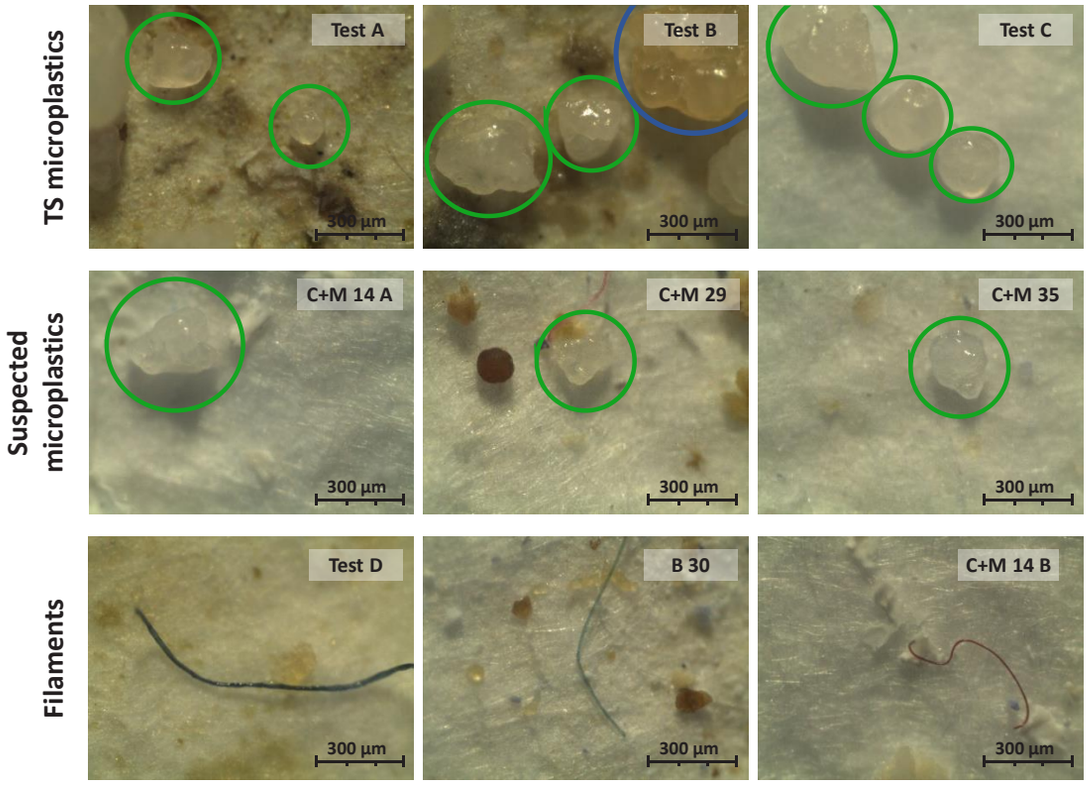

***Figure 3**: Example pictures of the different objects found in mussels after been digested. Green: suspected microplastics. Blue: stained suspected microplastic.*

The study performed with crustaceans (*Porcellio scaber*) by Dolar et al. (2021) showed that the presence of MPs reduced the bioavailability of chlorpyriphos. This may explain the significant higher BCF in absence of MPs for chlorpyrifos (15000 L/kg in C versus 3800 L/kg in C+M). However, the BCF estimation for C is strongly influenced by the samples from day 28, furthermore it presented the highest uncertainty, among all the contaminants analysed, for both exposure (RMSE 300 and 190 for C and C+M, respectively) and depuration stages (RMSE 51 and 58 for C and C+M, respectively). Hence, chlorpyrifos results should be taken with caution.

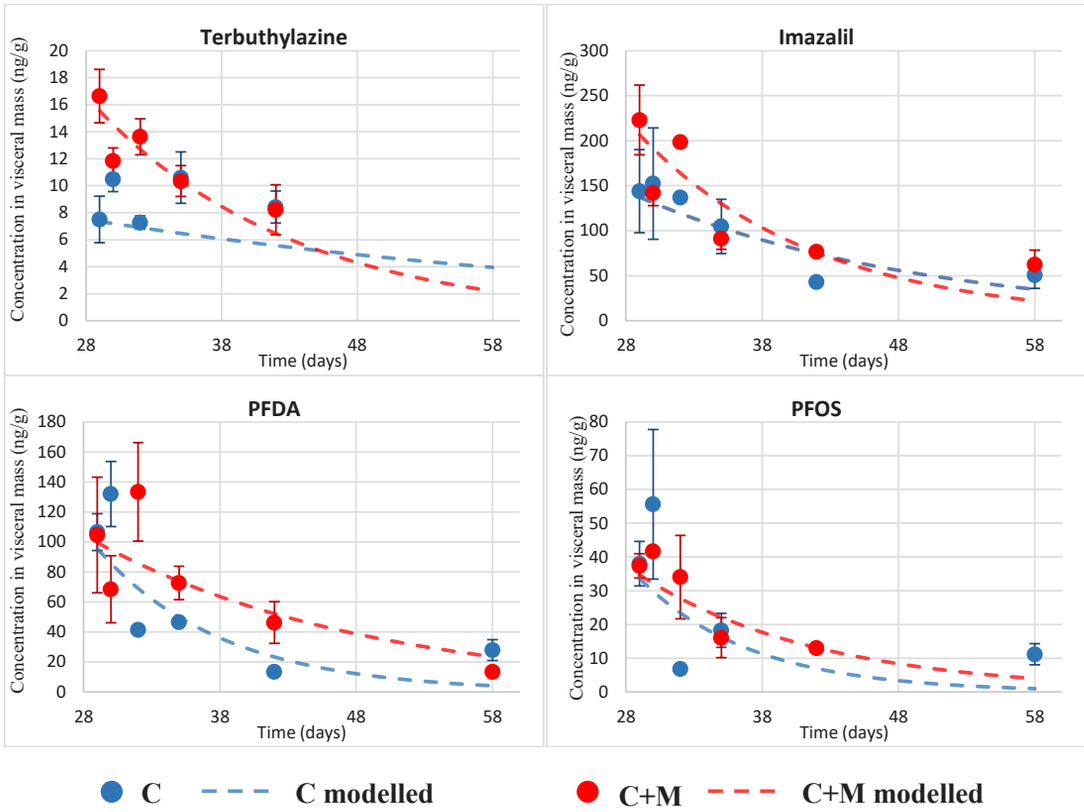

Figure 4: *Concentration measured (dots) and modelled (no-continuous line) for terbuthylazine, imazalil, PFDA and PFOS, for depuration stage (days 29-58). Bars represent the ±SD of the measured concentrations.*

Specific information about alterations of bioaccumulation or depuration due to MPs was not found for the pesticides imazalil, chlorfenvinphos and terbuthylazine and the pharmaceuticals etoricoxib and diclofenac. However, terbuthylazine and other pesticides were adsorbed by MPs (Wang et al., 2020a) and previous studies suggest that (as for chlorpyrifos) this may result in a reduced bioavailability of the pesticides (Bhagat et al., 2021; Dolar et al., 2021), which would explain terbuthylazine results (**Table 2**). Although diclofenac is a pharmaceutical, it has also shown to have a high sorption coefficient for polyethylene MPs (Elizalde-Velázquez et al., 2020; Vieira et al., 2021), and possible reduced bioavailability would be in accordance with its lower bioaccumulation in the presence of MPs. Chlorfenvinphos and etoricoxib showed no significant differences between the BCFs of both groups. They may not be adsorbed by polyethylene MPs or rather the conditions (pH, shape and size of MPs, etc.) were not appropriate to favour sorption. Imazalil showed a particular behaviour (**Fig. 2 and 4**), with higher BCF in C+M suggesting that MPs increase its bioaccumulation, but, at the same time, presented a lower

elimination half-life in C+M (9 days) compared to C (15 days). This behaviour is unusual when compared with the other compounds where a higher BCF in C+M also implied a higher half-life in that group (such as PFOS), and vice versa (such as chlorpyrifos). Further research is needed to explain its behaviour.

Most of triclosan concentrations in C were below the LOQs or the LODs (**Table S-7**). The higher concentrations found in C+M may suggest a higher bioaccumulation in the presence of MPs. This would be in accordance with the study of Sheng et al. (2021), which showed that MPs increased bioaccumulation of triclosan in zebrafish´ brain and liver as with the study of Webb et al. (2020) who also observed higher concentrations of triclosan in mussels in presence of MPs . A similar behaviour may explain the results of caffeine in haemolymph that has also shown to be adsorbed by MPs (Santana-Viera et al., 2021) or chlorfenvinphos during the depuration stage (**Tables S-7 and S-8**), which also present concentrations below LOD and LOQ for group C. However, the results are insufficient to reach a definitive conclusion.

Many works agree that sorption of contaminants by MPs depends on many factors such as the type of plastic, their shape, size, pH, temperature, etc. and that further research is needed to provide a full picture of the sorption variables and mechanisms (Bhagat et al., 2021; Wang et al., 2020a; Wang et al., 2020b). Since sorption mechanisms are closely related with the capability of MPs for influencing the accumulation or bioavailability of a given compound, their further research is crucial to assess their combined risk for fauna, flora and humans.

4. Conclusions and further research

A total of 3 PPCPs, 4 pesticides and 3 PFASs showed to bioaccumulate in visceral mass, with 4 pesticides and 2 PFASs consistently present during the depuration stage. On the other hand, caffeine and PFPeA were exclusively detected in haemolymph (along to 1 PPCP and 2 pesticides more), suggesting probable tissue specific accumulation for these compounds.

Results suggest that MPs favour the bioaccumulation of PFASs and also difficult their elimination from the organism. On the other hand, several pesticides showed to have a

lower bioaccumulation and faster depuration in the presence of MPs, probably due to low bioavailability since they were adsorbed in the MPs. However, the current knowledge about the bioaccumulation and bioavailability of contaminants in the presence of MPs is scarce. The assessment of MPs influence in the bioaccumulation of organic contaminants requires deep knowledge about the sorption mechanisms of the contaminants to the different MPs in given environmental conditions.

In order to correctly assess the environmental and human risk related to the bioaccumulation and depuration of ECs and influence of MPs, and since bioaccumulation prediction using K_{ow} is not completely reliable, further research is needed for: a) providing insigth about the metabolism of pesticides, PPCPs, PFASs and ECs in general in mussels, and b) stablishing MPs' ECs sorption and bioavailability mechanisms under different conditions, especially to fill the lack of knowledge about PPCPs and pesticides.

5. Acknowledgements

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Supplementary Information

Bioaccumulation of emerging contaminants in mussel (*Mytilus galloprovincialis)*: Influence of microplastics

In collaboration with Yolanda Picó and Julián Campo

Table of contents

S-1

Text S-1. Reagents and materials

LC grade methanol (MeOH) ≥ 99.8% purity, acetonitrile (ACN) ≥ 99.9% purity and trisodium citrate dehydrate were from VWR Chemicals® (Radnor, Pennsylvania). Magnesium sulphate ($MgSO_4$), disodium hydrogen citrate sesquihydrate and ammonium formate were from Alfa Aesar (Karlsruhe, Germany). Formic acid (CH_2O_2) was provided by ACROS ORGANICS (Geel, Belgium). Potassium hydroxide (KOH) was from Scharlau (Barcelona, Spain).

EMR-Lipid dispersive solid phase extraction (dSPE) clean-up was from Agilent Technologies (Santa Clara, CA, USA). Strata™-X 33µm Polymeric reversed phase, 200 mg/6 mL cartridges and Phree™ Phospholipid Removal Solutions 1 mL tubes were from Phenomenex® (Torrance, CA, USA). The 1 mL polypropylene syringes BD Plastipak™ and the needles 25G x 5/8" 0.5x16 mm BD Microlance™ were from BD (Madrid, Spain). The VISIPREP™ manifold was distributed by Supelco. Nylon 0.22 µm filters were purchased from Membrane Solutions (Plano, TX, USA) and polypropylene/polyethylene syringes manufactured by BRAUN and distributed by Scharlab S.L., (Barcelona, Spain). Evaporation was performed using a SBHCONC/1 concentrator combined with a SBH130D/3 heating plate, both provided by Stuart® (Stafford, United Kingdom). High purity water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). Glass fiber filters 0.45 µm were from Advantec MFS (Dublin, CA, USA). The 15 and 50 mL polypropylene centrifuge falcon tubes were from VWR International Eurolab (Barcelona, Spain). The 2 mL amber glass vials with stoppers 99 mm + Septum Sil/PTFE used to inject the samples were from Análisis Vínicos S.L. (Tomelloso, Spain), and the 250 µL polypropylene inserts were from Agilent Technologies. Oxidized polyethylene microplastics were purchased from Gran Velada (Zaragoza, Spain) and the stereomicroscope was LEICA S8AP0 connected to a LEICA EC3 camera, from Leica microsystems (Switzerland). Phytoplankton (*Dunaliella salina*) was purchased from Monzon Biotech Ltd. (Huesca, Spain).

The analytical standards of pharmaceuticals (acetaminophen, atenolol, caffeine, diclofenac, etoricoxib, ibuprofen, metformin, naproxen, salicylic acid, triclosan, vildagliptin), pesticides (bentazone, chlorfenvinphos, chlorpyrifos, imazalil, terbuthylazine) and PFASs [perfluoropentanoic acid (PFPeA) and perfluorobutanesulfonate (PFBS)] were from Sigma-Aldrich (Steinheim, Germany).

While perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA) and perfluorooctanesulfonate (PFOS) were from Wellington (Ontario, Canada).

The surrogate (internal) standards acetaminophen-d3, atenolol-d7 and ibuprofen-d3 were from Sigma Aldrich. Caffeine-d9, chlorfenvinphos-d10 (diethyl D5), chlorpyrifos-d10 (diethyl D10) and vildagliptin-d3 were from LGC Standards. Diclofenac-d4 and triclosan-d3 were purchased in Toronto Chemicals Research (Toronto Canada), and PFOA-d4 (MPFOA), PFOS-d4 (MPFOS) and PFDA-d4 (MPFDA) were from Wellington.

Text S-2. Extraction procedures

Water was extracted by solid phase extraction (SPE) following the method by Carmona et al. (2017). Briefly, 250 mL of water were passed dropwise through Strata-X cartridges previously conditioned with 6 mL of MeOH and 6 mL of MilliQ water. Then, cartridges were dried using vacuum for 15 minutes and analytes eluted with 6 mL of MeOH at gravity flow. The extracts were blowed down to dryness under a gentle stream of nitrogen. The residue was then reconstituted with 5 mL 70:30 water-MeOH (v/v), sonicated for 30 s, vortexed and stored in vials with inserts at -20 ºC until analysis.

For mussel visceral mass extraction, the method developed by Álvarez-Ruiz et al. (2021b) combining QuEChERS extraction and EMR-Lipid clean-up was employed. One g of mussel was placed in 50 mL Falcon tubes, spiked with 200 µl of internal standards mix at a concentration of 1 mg/L. Then, 7.5 mL of MilliQ water and 10 mL of ACN were added and vortex 3 min. Next, 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of disodium hydrogen citrate sesquihydrate and 1 g of trisodium citrate dehydrate were added and the tube was vigorously shaken for 3 min followed by centrifugation during at 3500 rpm (2465 rcf.). In a 15 mL Falcon tube, the EMR-Lipid mixture was activated with 5 mL of MilliQ water and vortex for 30 s. Then, 5 mL of the QuEChERS supernatant were added, the tube was vortex for 30 s and centrifuged for 5 min at 3500 rpm, and 5 mL of the resulting supernatant were added to another 15 mL Falcon tube containing 1600 mg of $MgSO_4$ and 400 mg of NaCl. The tube was shaken vigorously for 30 s and centrifuged for 5 min at 3500 rpm. The extract was filtered using Nylon 0.22 µm filters and stored in vials with inserts at -20 ºC until analysis.

Haemolymph was extracted using SPE as described by Álvarez-Ruiz et al. (2021a). Briefly, 100 µL of haemolymph were loaded in Phree cartridges followed by 600 µL of a solution of MeOH 1% formic acid containing IS mix at concentration of 23.33 ng/mL. After precipitation during 2 minutes, vacuum was applied to let the sample pass through the cartridges drop wise. The extracts were stored in vials with inserts at -20 ºC until analysis.

Text S-3. LC-MS/MS analysis

Analysis was performed via LC-MS/MS as previously described by Álvarez-Ruiz et al. (2021a) using an Agilent 1260 UHPLC from Agilent technologies coupled to an Agilent 6410 Mass Spectrometer QQQ also from Agilent technologies, with electrospray ionization (ESI) in both negative and positive ionization modes (nebulizer gas 15 psi, gas flow 11 L/min. ion-spray voltage 4 kV and temperature 300ºC) operated in multiple reaction monitoring mode (MRM). The column used for pharmaceuticals and PFASs analysis was a Kinetex® XB-C_{18} Column (1.7 µm 100 Å, 50 x 2.1 mm) and the column used for pesticides and etoricoxib analysis was Luna® C_{18}(2) Column (3 µm 100 Å, 150 x 2 mm), both from Phenomenex. When operated in negative ionization mode the mobile phases employed were (A) H_2O 2.5 mM NH_4F and (B) MeOH 2.5 mM NH_4F. For the positive ionization mode, the mobile phases employed were (A) H_2O 0.1% formic acid and (B) MeOH 0.1% formic acid. The linear gradient for separation was as follows: 0 min (70% A), 12 min (5% A), 22 min (5% A), 23 min (70% A) and 30 min (70% A). The injection volume was 5 µl and column temperature was held at 45 ºC. MS information of the compounds analysed is available in **Tables S-4** and **S-5**.

Text S-4. Quality assurance

A Blank (MilliQ water for water, and hemolymph samples and an empty falcon tube for visceral mass samples) was included in every batch of maximum 10 samples, and if detected in the blanks, the concentration was subtracted from the final concentration of the samples. Water samples had one replicate while mussel and haemolymph samples were extracted in triplicate. A set of 8 points (5, 10, 25, 50, 75, 100, 200, 500 ng/mL) calibration standards (in 7:3 water:MeOH for water, ACN for visceral mass and 1:7 water:MeOH for haemolymph) was injected at the beginning and the end of each

analytical sequence. For the calibration curve a regression coefficient of $R^2 > 0.99$ was accepted. Furthermore, the 100 ng/mL calibration point was injected every 15 samples to check instrumental variation. Limits of quantification (LOQs) for water were determined as nine times the standard deviation of the signal from repeated injection 6x of the lowest level standard (5 ng/mL). LOQs for haemolymph and visceral mass were taken from the references (Álvarez-Ruiz et al., 2021a; Álvarez-Ruiz et al., 2021b). Quantification was performed by interpolating the area of the peaks into the calibration curve generated by the area of the peaks of the 8 points set.

Text S-5. Mussels' characteristics

The maximum length of the mussels ranged from 3.0 to 7.5 cm with an average of 6.1 cm. The weight of the visceral mass ranged from 1.4 to 8.8 g with an average of 3.7 g. Significant changes in the weight and length of the specimens along the study were not observed. As well as significant differences were not observed between B, C and C+M, which mussels showed average lengths of 5.93±0.77 cm, 6.17±0.64 cm and 6.29±0.64 cm, and average weights of 4.0±1.82 g, 3.80±1.65 g and 3.34±1.27 g, respectively. Since changes in the weight and/or length in specimens of the same group along the experiment were not observed, any differences between groups were more likely due to the randomness of the distribution at the beginning of the study.

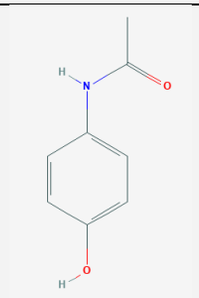
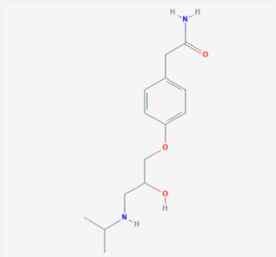
During the experiment, few casualties were registered. They did not show significant difference between groups, with a total of eight deceases for group C and nine deceases for groups B and C+M. The casualties were dispersed along the whole experimental period not showing any pattern or distinction between exposure and depuration periods.

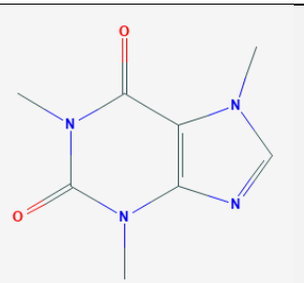
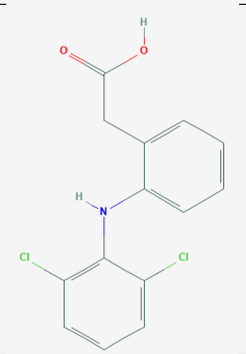
Text S-6. Concentration of contaminants in water

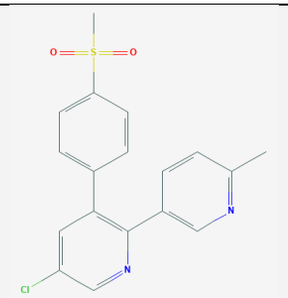
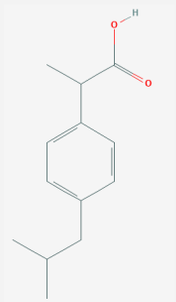
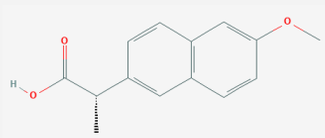
Although reference half-lives (**Table S-3**) were used to estimate degradation in the aquarium, the half-life of a given compound depends on the given conditions (temperature, UV-radiation, pH etc.), and variations in half-lives of 1 or 2 magnitude orders have been observed previously (Seller et al., 2020). The conditions in the aquariums (temperature, salt, microorganism, mussel excretions, etc.) likely implied important variations in the persistence of the compounds, respect to the references. Hence,

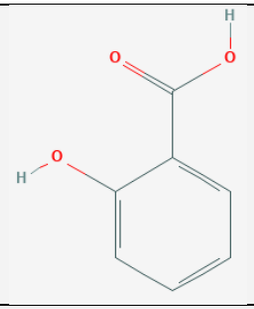
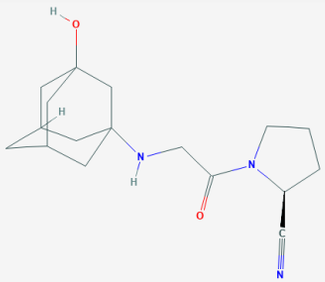
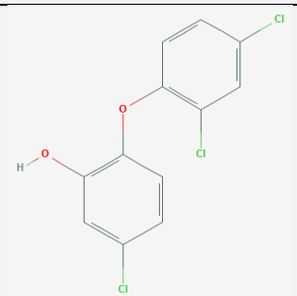
imazalil, acetaminophen, atenolol and ibuprofen results suggest that their persistence in the aquariums was significantly lower than in the references. Atenolol was spiked day 0 at 40 ng/mL in water due to a procedural error. It was decided to proceed with this concentration (40 ng/mL) applying the same methodology as for the rest of compounds but escalated 4 times. Since internal standard for imazalil was not available, the high concentrations detected could be due to interferences such as salt, or mussel excretions. Salicylic acid reported half-lives are very variable 1-142 days (PubChem) and its concentration may be the consequence of rapid degradation (under these specific conditions) in combination with the excretion of 3,4-dihydroxybenzaldehyde by mussels, which has been suggested to interfere with the signal of salicylic acid when analysed by LC-MS/MS (Álvarez-Ruiz et al., 2021b), and would explain the low constant levels detected in water (**Table S-6**). Since PFASs are known to adhere to glass (ISO-25101:, 2009; Lath et al., 2019) and plastic (Llorca et al., 2018) materials, their decrease maybe explained by the so called "diluting effect" (Wang et al., 2020). Chlorfenvinphos, chlorpyrifos and triclosan initially spiked to the plankton were detected in water, which likely explains their variable concentrations. Previous bioaccumulation studies showed that maintaining contaminants at a constant concentration is a very difficult task and is usual to experience variations (especially concentration decrease) along the experiment (Liu et al., 2016; Vidal-Liñán et al., 2018).

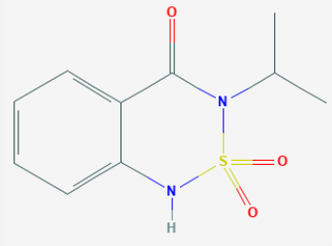
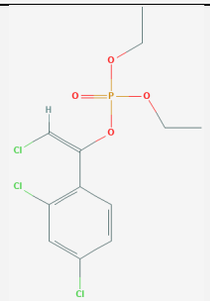
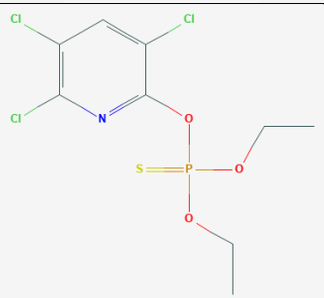
Table S-1: Information about the compounds analysed.

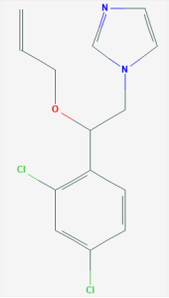
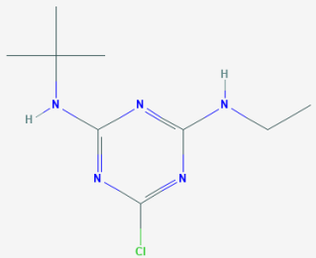
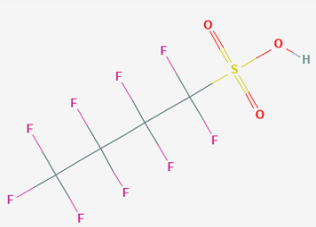
Common name(s)	Structure	Characteristics	Physico-chemical properties	Ref.
Acetaminophen Paracetamol		Family: pharmaceutical IUPAC name: N-(4-Hydroxyphenyl)acetamide Molecular formula: $C_8H_9NO_2$ Molecular weight: 151.16 g/mol	pKa: 9.38 pKa2: - Log Kow: 0.46 Solubility in solvents: ACN 32.83 g/kg[a]; MeOH; acetone Half-life in water: 15 days[f]	[a](Granberg and Rasmuson, 1999)
Atenolol		Family: pharmaceutical IUPAC name: 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy] phenyl]acetamide Molecular formula: $C_{14}H_{22}N_2O_3$ Molecular weight: 266.34 g/mol	pKa: 9.6 pKa2: - Log Kow: 0.16 Solubility in solvents: MeOH 5 g/L[a] Half-life in water: 38 days[f]	[a](Cayman, 2020)

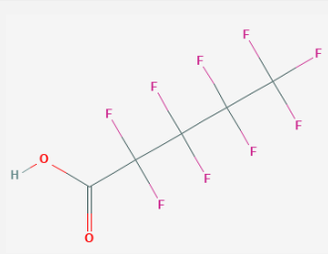
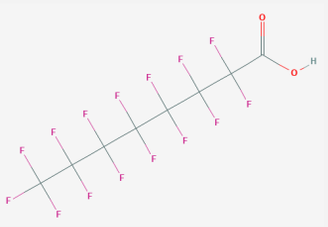
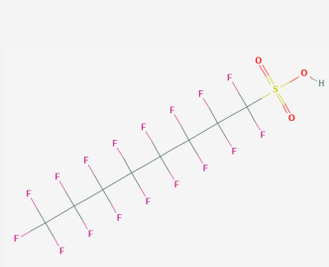
Caffeine		Family: pharmaceutical IUPAC name: 1,3,7-trimethylpurine-2,6-dione Molecular formula: $C_8H_{10}N_4O_2$ Molecular weight: 194.19 g/mol	pKa: 14 pKa2: 10.4 Log Kow: -0.07 Solubility in solvents: acetone 20 g/L[a]; ethanol 20 g/L[a] Half-life in water: 15 days[f]	[a](Ramalakshmi and Raghavan, 1999)
Diclofenac		Family: pharmaceutical IUPAC name: 2-[2-(2,6-dichloroanilino)phenyl] acetic acid Molecular formula: $C_{14}H_{11}Cl_2NO_2$ Molecular weight: 296.1 g/mol	pKa: 4.15 pKa2: - Log Kow: 1.9[a] Solubility in solvents: ACN <1g/L; MeOH; acetone Half-life in water: 8 days[b]	[a](Scheytt et al., 2005) [b](Tixier et al., 2003)

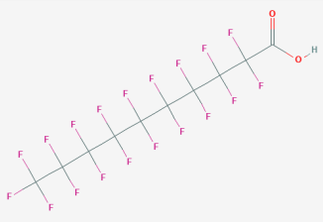
Etoricoxib		Family: pharmaceutical IUPAC name: 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine Molecular formula: $C_{18}H_{15}ClN_2O_2S$ Molecular weight: 358.8 g/mol	pKa: 19.69[d] pKa2: 4.96[d] Log Kow: 3.7[d] Solubility H_2O: 3.28 Solubility solvents: - Half-life in water: 180 days[f]	
Ibuprofen		Family: pharmaceutical IUPAC name: 2-[4-(2-methylpropyl)phenyl] propanoic acid Molecular formula: $C_{13}H_{18}O_2$ Molecular weight: 206.28 g/mol	pKa: 5.2 pKa2: 4.91 Log Kow: 2.48[a] Solubility solvents: ACN 425 g/L[a] Half-life in water: 32 days[b]	[a](Scheytt et al., 2005) [b](Filippa and Gasull, 2013) [c](Tixier et al., 2003)
Naproxen		Family: pharmaceutical IUPAC name: (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid Molecular formula: $C_{14}H_{14}O_3$ Molecular weight: 230.26 g/mol	pKa: 4.15 pKa2: - Log Kow: 3.18 Solubility solvents: ACN 57 g/L[a]; MeOH 60 g/L[a]; acetone 193 g/L[a] Half-life in water: 14 days[b]	[a](Filippa and Gasull, 2014) [b](Tixier et al., 2003)

Salicylic acid		Family: pharmaceutical IUPAC name: 2-hydroxybenzoic acid Molecular formula: $C_7H_6O_3$ Molecular weight: 138.12 g/mol	pKa: 2.98 pKa2: 13.6 Log Kow: 2.26 Solubility solvents: MeOH 384.6g/kg[a]; acetone 333 g/L[a] Half-life in water: 30 days	[a](ECHA)
Vildagliptin		Family: pharmaceutical IUPAC name: (2S)-1-[2-[(3-hydroxy-1-adamantyl)amino]acetyl] pyrrolidine-2-carbonitrile Molecular formula: $C_{17}H_{25}N_3O_2$ Molecular weight: 303.4 g/mol	pKa: 1.47[a] pKa2: 9.03[a] Log Kow: 1.12[a] Solubility solvents: soluble in polar organic solvents[b] Half-life in water: 65 days[f]	[a](MIC) [b](Cayman, 2013)
Triclosan		Family: pharmaceutical IUPAC name: 5-chloro-2- (2,4-dichlorophenoxy) phenol Molecular formula: $C_{12}H_7Cl_3O_2$ Molecular weight: 289.5 g/mol	pKa: 7.9 pKa2: - Log Kow: 4.76 Solubility solvents: acetone >1 kg/kg[a]. More soluble in acetone than in ACN, but more soluble in ACN than MeOH[b] Half-life in water: 8 days	[a](EC, 2010) [b](Delgado et al., 2012)

Bentazone		Family: pesticide IUPAC name: 2,2-dioxo-3-propan-2-yl-1H-2λ6,1,3-benzothiadiazin-4-one Molecular formula: $C_{10}H_{12}N_2O_3S$ Molecular weight: 240.28 g/mol	pKa: 3.3 pKa2: 2.92 Log Kow: 2.34 Solubility solvents: MeOH >300 g/L[a]; acetone >300 g/L[a] Half-life in water: 80 days[e]	[a](He)
Chlorfenvinfos		Family: pesticide IUPAC name: [(E)-2-chloro-1-(2,4-dichlorophenyl) ethenyl] diethyl phosphate Molecular formula: $C_{12}H_{14}Cl_3O_4P$ Molecular weight: 359.6 g/mol	pKa: - pKa2: - Log Kow: 3.81 Solubility solvents: miscible in organic solvents[e] Half-life in water: 7 days[e]	
Chlorpyrifos		IUPAC name: diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ5-phosphane Molecular formula: $C_9H_{11}Cl_3NO_3PS$ Molecular weight: 350.6 g/mol	pKa: - pKa2: - Log Kow: 4.96 Solubility solvents: MeOH 450 g/L; acetone 6500 g/L Half-life in water: 5 days[e]	

Imazalil Enilconazole		Family: pesticide IUPAC name: 1-[2-(2,4-dichlorophenyl)-2-prop-2-enoxyethyl]imidazole Molecular formula: $C_{14}H_{14}Cl_2N_2O$ Molecular weight: 297.2 g/mol	pKa: 6.53 pKa2: - Log Kow: 3.82 Solubility solvents: MeOH 500 g/L[e] Half-life in water: 7.8 days[e]	
Terbuthylazine		Family: pesticide IUPAC name: 2-N-tert-butyl-6-chloro-4-N-ethyl-1,3,5-triazine-2,4-diamine Molecular formula: $C_9H_{16}ClN_5$ Molecular weight: 229.71 g/mol	pKa: 2 pKa2: - Log Kow: 3.4 Solubility solvents: acetone 41.7 g/L Half-life in water: 6 days[e]	
PFBS Perfluorobutanesulfonic acid		Family: PFAS IUPAC name: 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonic acid Molecular formula: $C_4HF_9O_3S$ Molecular weight: 300.1 g/mol	pKa: 3.31 pKa2: - Log Kow: 1.82[f] Solubility solvents: - Half-life in water: 180 days[f]	

PFPeA Perfluoropentanoic acid Perfluorovaleric acid		Family: PFAS IUPAC name: 2,2,3,3,4,4,5,5,5-nonafluoropentanoic acid Molecular formula: $C_5HF_9O_2$ Molecular weight: 264.05 g/mol	pKa: 0.40[a] pKa2: - Log Kow: 3.262[f] Solubility solvents: - Half-life in water: 60 days[f]	[a](ChemicalBook, 2017)
PFOA Perfluorooctanoic acid		Family: PFAS IUPAC name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid Molecular formula: $C_8HF_{15}O_2$ Molecular weight: 414.07 g/mol	pKa: 1.3 pKa2: 2.8 Log Kow: 4.81[f] Solubility solvents: - Half-life in water: 180 days[f]	
PFOS Perfluorooctanesulfonic acid		Family: PFAS IUPAC name: 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid Molecular formula: $C_8HF_{17}O_3S$ Molecular weight: 500.13 g/mol	pKa: <1 pKa2: - Log Kow: 4.49[f] Solubility solvents: ACN 15 g/L[a] Half-life in water: 180 days[f]	[a](Meng et al., 2017)

PFDA Perfluorodecanoic acid		Family: PFAS IUPAC name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluorodecanoic acid Molecular formula: $C_{10}HF_{19}O_2$ Molecular weight: 514.08 g/mol	pKa: - pKa2: - Log Kow: 6.15[f] Solubility solvents: - Half-life in water:180 days[f]	

The data and chemical structures were obtained from PubChem (PubChem, 2021a), except when it is expressly indicated with the following marks:
***a, b, c**: data from the first referenced source for that compound.*
***d**: data estimated by DRUGBANK (DRUGBANK).*
***e**: data from (UHPPDB)University of Hertfordshire, PPDB: Pesticide Properties DataBase.*
***f**:data estimated using Estimation Program Interface (EPI) Suite™.*

Table S-2: Water replacement and sampling during the exposure stage.

	Water replacement (L)	Water sampling (mL)	Mussel sampling	Nº of casualties
Day 0	30	-	Yes	-
Day 1	-	400	-	-
Day 2	-	400	Yes	-
Day 3	30	400	-	1
Day 4	-	400	Yes	1
Day 5	-	400	-	2
Day 6	30	-	-	1
Day 7	-	400	Yes	-
Day 8	-	400	-	-
Day 9	-	400	-	-
Day 10	30	400	-	-
Day 11	-	400	-	-
Day 12	-	400	-	-
Day 13	30	400	-	-
Day 14	-	400	Yes	1
Day 15	-	400	-	-
Day 16	-	400	-	-
Day 17	-	400	-	1
Day 18	30	400	-	-
Day 19	-	400	-	-
Day 20	30	400	-	-
Day 21	-	400	-	-
Day 22	-	400	-	2
Day 23	-	400	-	-
Day 24	30	400	-	-
Day 25	-	400	-	1
Day 26	-	-	-	-
Day 27	30	400	-	-
Day 28	160	400	Yes	3

Table S-3: Water replacement and sampling during the depuration stage.

	Water replacement (L)	Water sampling (mL)	Mussel sampling	Nº of casualties
Day 29	-	400	Yes	-
Day 30	-	-	Yes	2
Day 31	30	-	-	-
Day 32	-	-	Yes	-
Day 33	-	-	-	-
Day 34	30	-	-	-
Day 35	-	-	Yes	1
Day 36	-	-	-	1
Day 37	-	-	-	-
Day 38	30	-	-	-
Day 39	-	-	-	-
Day 40	-	-	-	2
Day 41	30	-	-	-
Day 42	-	-	Yes	-
Day 43	-	-	-	-
Day 44	-	-	-	-
Day 45	30	-	-	1
Day 46	-	-	-	-
Day 47	-	-	-	3
Day 48	30	400	-	-
Day 49	-	-	-	-
Day 50	-	-	-	-
Day 51	-	-	-	-
Day 52	30	-	-	-
Day 53	-	-	-	-
Day 54	-	-	-	-
Day 55	30	-	-	1
Day 56	-	-	-	-
Day 57	-	-	-	-
Day 58	-	400	Yes	2

Table S-4: External standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)	Mass labelled	Visceral mass (ng/g)		Haemolymph (ng/mL)		Water ng/L	
					MLOQ	Blank	MLOQ	Blank	ILOQ	Blank
POSITIVE COMPOUNDS										
Etoricoxib 1	359	280	14		11	-	10.8	-	$4.54x10^{-2}$	-
Etoricoxib 2	359	279	14							
Chlorfenvinphos 1	359	155	18.4	Chlorfenvinphos-d10	19	8.3	8.8	-	$5.39x10^{-2}$	-
Chlorfenvinphos 2	359	127	18.4	Chlorfenvinphos-d10						
Chlorpyrifos 1	350	198	20.1	Chlorpyrifos-d10	0.78	-	2.8	4.6	$2.06x10^{-2}$	-
Chlorpyrifos 2	350	97	20.1	Chlorpyrifos-d10						
Vildagliptin 1	304	154	0.7	Vildagliptin-d3	4.8	-	5.1	-	$8.13x10^{-2}$	-
Vildagliptin 2	304	91	0.7	Vildagliptin-d3						
Imazalil 1	297	201	14.3		4.4	-	8.2	-	$2.78x10^{-2}$	-
Imazalil 2	297	159	14.3							
Atenolol 1	267	91	1.0	Atenolol-d7	63	-	10.9	-	$6.23x10^{-2}$	-
Atenolol 2	267	77	1.0	Atenolol-d7						
Terbuthylazine 1	230	174	17.6		6.1	-	9.2	-	$3.74x10^{-2}$	-
Terbuthylazine 2	230	96	17.6							
Caffeine 1	195	138	1.6	Caffeine-d9	13	-	8.8	-	$3.80x10^{-2}$	-
Caffeine 2	195	110	1.6	Caffeine-d9						
Acetaminophen 1	152	110	0.8	Acetominophen-d3	9.7	-	12.3	-	$5.32x10^{-2}$	-
Acetaminophen 2	152	92	0.8	Acetominophen-d3						
Bentazone 1	241	107	17.7		46	-	57.3	-	$3.88x10^{-2}$	-
Bentazone 2	241	199	17.7							
NEGATIVE COMPOUNDS										
Salicylic acid 1	137	93	1			-	8.46	-	$9.01x10^{-2}$	-
PFDA 1	513	469	14	MPFDA	9.9	2.9	19.3	-	$3.30x10^{-2}$	-

PFDA 2	513	269	14	MPFDA						
PFOS 1	499	99	13.5	MPFOS	8.5	-	0.89	-	$1.45x10^{-1}$	-
PFOS 2	499	80	13.5	MPFOS						
PFOA 1	413	369	12.9	MPFOA	3.3	5.6	10.9	-	$5.42x10^{-2}$	-
PFOA 2	413	169	12.9	MPFOA						
PFBS 1	299	99	7.5		17	-	19.3	-	$1.09x10^{-1}$	-
PFBS 2	299	80	7.5							
Diclofenac sodium 1	294	250	11.9	Diclofenac-d4	29	-	16.7	-	$9.34x10^{-2}$	-
Diclofenac sodium 2	294	178	11.9	Diclofenac-d4						
Triclosan 1	289	35	14.8	Triclosan-d3	37	-	66	-	$9.91x10^{-2}$	-
Triclosan 2	287	35	14.8	Triclosan-d3						
PFPeA 1	263	219	5.8		13	-	12.9	-	$1.64x10^{-1}$	-
Naproxen 1	229	185	10		11	-	8.2	-	$7.05x10^{-2}$	-
Naproxen 2	229	170	10							
Ibuprofen 1	205	159	12.8	Ibuprofen-d3	68	-	10.5	-	$6.35x10^{-2}$	-

Table S-5: Isotopically labelled standards LC-MS/MS information.

Analyte ID	Q1 Mass (Da)	Q3 Mass (Da)	Retention time (min)
POSITIVE COMPOUNDS			
Chlorfenvinphos-d10 1	369	101	18.4
Chlorfenvinphos-d10 2	369	170	18.4
Chlorpyrifos-d10 1	360	199	20.1
Chlorpyrifos-d10 2	360	99	20.1
Vildagliptin-d3 1	307	157	0.7
Vildagliptin-d3 2	307	93	0.7
Atenolol-d7 1	274	145	0.7
Atenolol-d7 2	274	79	0.7
Caffeine-d9 1	204	144	1.3
Caffeine-d9 2	204	116	1.3
Acetominophen-d3 1	155	111	0.9
Acetominophen-d3 2	155	65	0.9
NEGATIVE COMPOUNDS			
MPFDA 1	515	270	14
MPFDA 2	515	470	14
MPFOS 1	503	99	13.5
MPFOS 2	503	80	13.5
MPFOA 1	417	372	12.9
MPFOA 2	417	169	12.9
Diclofenac-d4 1	298	254	11.9
Triclosan-d3 1	290	35	14.8
Ibuprofen-d3 1	208	164	12.8

Table S-6: Concentration (µg/L) found in aquarium water of the compounds spiked in food and water during the exposure phase (EP): from day 1 to 28, and the depuration phase: from day 29 to 58.

	Acetaminophen	Atenolol	Bentazone	Caffeine	Chlorfenvinphos*	Chlorpyrifos*	Diclofenac	Etoricoxib	Ibuprofen
Day 1	4.5	37.5	13.8	8.1	1.8	$4.5x10^{-2}$	7.3	15.0	4.5
Day 2	3.9	56.2	13.0	9.3	1.5	$6.2x10^{-2}$	11.3	13.8	5.5
Day 3	3.9	35.6	12.8	9.8	1.6	$6.7x10^{-2}$	13.2	13.2	2.3
Day 4	3.5	41.5	11.5	8.3	2.3	$6.6x10^{-2}$	13.1	13.1	5.0
Day 5	2.5	31.1	9.0	7.9	2.9	$6.4x10^{-2}$	10.9	9.3	2.2
Day 7	3.5	37.2	10.4	10.0	2.8	$5.8x10^{-2}$	10.1	11.2	1.4
Day 8	3.6	29.1	10.3	10.8	2.6	$6.0x10^{-2}$	10.3	10.6	2.4
Day 9	1.8	33.1	10.4	10.5	2.7	$6.6x10^{-2}$	10.2	12.3	1.9
Day 10	1.6	24.0	10.0	8.3	2.4	$7.7x10^{-2}$	11.4	11.7	1.7
Day 11	1.5	27.8	6.4	3.1	3.0	$4.5x10^{-2}$	8.0	7.3	2.4
Day 12	2.2	40.5	12.2	4.8	3.9	$8.2x10^{-2}$	12.4	17.7	3.2
Day 13	1.3	39.2	9.7	3.8	4.5	$7.7x10^{-2}$	10.8	11.4	2.7
Day 14	1.9	36.2	10.3	5.5	3.7	0.49	9.6	11.7	2.8
Day 15	1.4	21.4	7.8	4.6	3.5	$8.1x10^{-2}$	9.1	9.1	2.5
Day 16	1.3	21.9	8.7	3.4	3.4	$5.6x10^{-2}$	9.5	10.1	2.6
Day 17	0.78	25.8	6.9	3.5	3.0	$6.6x10^{-2}$	8.3	7.6	2.0
Day 18	0.81	26.9	8.0	3.4	4.0	$5.9x10^{-2}$	9.9	10.1	2.3
Day 19	0.93	26.6	8.2	4.1	3.4	$6.6x10^{-2}$	8.6	9.6	2.7
Day 20	1.1	20.0	7.6	3.7	4.1	0.10	9.4	10.2	2.6
Day 21	1.7	20.1	7.9	11.7	1.8	$7.0x10^{-2}$	10.0	10.3	4.0
Day 22	1.2	15.9	8.0	10.5	2.0	$5.4x10^{-2}$	12.2	9.1	2.4
Day 23	1.0	16.6	8.3	9.2	2.1	$6.8x10^{-2}$	9.1	9.5	2.9
Day 24	0.67	17.1	8.2	9.9	2.0	$5.6x10^{-2}$	10.0	10.1	1.6
Day 25	1.5	11.8	7.2	6.3	3.2	$8.8x10^{-2}$	8.1	9.0	3.5
Day 27	0.67	10.6	5.8	7.2	1.5	$4.5x10^{-2}$	7.2	6.7	2.3
Day 28	1.0	10.6	8.9	9.6	1.8	$6.5x10^{-2}$	9.9	9.8	2.8
Day 29	n.d.	<LOQ	0.162	0.19	0.20	$7.5x10^{-2}$	0.5	0.22	0.41
Day 48	n.d.	n.d.	$9.0x10^{-2}$	$4.3x10^{-2}$	$9.9x10^{-2}$	$7.9x10^{-2}$	0.4	0.18	n.d.
Day 58	n.d.	n.d.	n.d.	<LOQ	$6.2x10^{-2}$	$5.6x10^{-2}$	0.3	0.12	n.d.
EP mean	1.9	27.5	9.3	7.2	2.7	$8.2x10^{-2}$	10.0	10.8	2.8
EP STD	1.2	11.1	2.1	2.9	0.86	$8.5x10^{-2}$	1.6	2.4	1.0
EP RSD	61	40	23	40	32	103	16	22	36

**: these compounds were added to the food instead of spiked to the water.*

	Imazalil	Naproxen	PFBS	PFDA	PFOA	PFOS	PFPeA	Salicylic ac.	Terbuthylazine	Triclosan*	Vildagliptin
Day 1	27.06	9.3	9.6	9.3	9.3	10.0	9.4	<LOQ	8.8	n.d.	1.4
Day 2	19.52	7.8	9.0	5.0	5.5	3.6	9.7	0.18	7.4	0.39	1.5
Day 3	21.09	3.0	3.6	9.5	2.6	6.4	3.8	0.12	8.8	n.d.	1.6
Day 4	17.89	8.2	9.2	8.5	7.2	5.4	10.2	0.11	9.1	0.46	0.95
Day 5	11.00	5.9	6.8	12.3	5.6	9.4	7.4	<LOQ	6.8	0.38	0.76
Day 7	8.92	3.0	3.4	7.2	2.0	4.4	3.6	<LOQ	9.6	0.40	1.0
Day 8	10.56	8.0	8.0	3.7	3.9	3.0	8.9	<LOQ	9.5	0.42	1.1
Day 9	11.91	7.6	7.5	4.8	2.9	3.2	9.3	<LOQ	8.9	0.86	1.6
Day 10	10.92	7.9	7.7	3.6	2.3	2.5	9.6	$9.9x10^{-2}$	8.7	0.97	0.87
Day 11	9.40	5.4	5.6	5.6	6.0	3.6	6.4	<LOQ	8.0	0.39	1.1
Day 12	11.27	11.0	8.5	3.0	5.3	2.0	10.6	<LOQ	14.0	0.64	1.8
Day 13	8.06	9.2	8.1	4.1	5.7	2.5	9.9	<LOQ	11.3	0.71	1.5
Day 14	13.26	6.2	5.9	5.2	4.5	2.5	6.9	<LOQ	12.8	0.61	1.2
Day 15	8.55	6.9	6.7	5.4	5.8	3.7	8.5	<LOQ	10.4	0.67	1.1
Day 16	6.86	7.7	7.4	3.8	5.2	2.2	9.8	<LOQ	10.5	0.36	1.5
Day 17	5.78	6.9	6.3	3.4	4.8	2.3	8.4	<LOQ	8.5	0.39	1.3
Day 18	5.60	8.7	8.0	4.0	5.1	2.6	10.5	<LOQ	11.0	0.36	1.4
Day 19	8.39	7.9	7.8	2.6	5.4	1.7	10.0	<LOQ	11.7	n.d.	1.7
Day 20	7.49	8.2	8.0	4.5	5.3	2.5	10.3	<LOQ	13.4	0.36	1.4
Day 21	8.91	9.8	7.6	1.8	2.6	1.7	10.6	$9.6x10^{-2}$	10.4	0.93	1.4
Day 22	5.58	6.8	5.1	3.5	1.4	2.4	7.0	<LOQ	9.9	0.65	1.3
Day 23	4.99	10.1	7.2	1.1	1.6	1.0	10.5	0.11	9.6	0.75	1.1
Day 24	4.87	5.5	4.2	1.1	0.74	1.0	6.4	<LOQ	8.5	0.51	1.3
Day 25	6.69	9.3	7.6	9.0	6.5	5.1	11.1	<LOQ	12.6	n.d.	0.91
Day 27	4.38	8.0	5.3	1.0	1.4	1.1	8.6	<LOQ	7.7	0.42	0.78
Day 28	6.45	10.8	6.6	1.2	1.2	1.1	10.5	$9.0x10^{-2}$	9.9	n.d.	1.1
Day 29	n.d.	0.19	0.39	0.12	0.12	0.25	0.27	<LOQ	0.37	n.d.	n.d.
Day 48	n.d.	$9.7x10^{-2}$	0.30	<LOQ	<LOQ	0.23	0.16	<LOQ	0.31	n.d.	n.d.
Day 58	n.d.	<LOQ	0.29	<LOQ	<LOQ	0.20	<LOQ	<LOQ	0.23	n.d.	n.d.
EP mean	10.2	7.7	7.0	4.8	4.2	3.3	8.8	0.12	9.9	0.46	1.3
EP STD	5.6	2.0	1.6	2.9	2.2	2.3	2.0	$3.0x10^{-2}$	1.9	0.28	0.28
EP RSD	54	26	23	61	51	69	23	26	19	59	23

**: these compounds were added to the food instead of spiked to the water.*

Table S-7: Concentration (ng/g w.w.) found in mussel visceral mass of the compounds spiked in food and aquarium water. For the groups: control (B), exposed to contaminants (C) and exposed to contaminants and microplastics (C+M)

C	Acetaminophen	Atenolol	Bentazone	Caffeine	Chlorfenvinphos
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	54.1
Day 4	n.d.	n.d.	n.d.	n.d.	93.6
Day 7	n.d.	n.d.	n.d.	n.d.	171
Day 14	n.d.	n.d.	n.d.	n.d.	95.2
Day 28	n.d.	n.d.	n.d.	n.d.	266
Day 29	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 30	n.d.	n.d.	n.d.	n.d.	37.1
Day 32	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 35	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 42	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 58	n.d.	n.d.	n.d.	n.d.	<LOQ
C+M					
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	63.1
Day 4	n.d.	n.d.	n.d.	n.d.	148
Day 7	n.d.	n.d.	n.d.	n.d.	265
Day 14	n.d.	n.d.	n.d.	n.d.	216
Day 28	n.d.	n.d.	n.d.	n.d.	279
Day 29	n.d.	n.d.	n.d.	n.d.	51.5
Day 30	n.d.	n.d.	n.d.	n.d.	38.7
Day 32	n.d.	n.d.	n.d.	n.d.	34.1
Day 35	n.d.	n.d.	n.d.	n.d.	47.0
Day 42	n.d.	n.d.	n.d.	n.d.	32.7
Day 58	n.d.	n.d.	n.d.	n.d.	<LOQ
B					
Day 0	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 7	n.d.	n.d.	n.d.	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 28	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 29	n.d.	n.d.	n.d.	n.d.	<LOQ
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	<LOQ

n.d.: not detected

C	Chlorpyrifos	Diclofenac	Etoricoxib	Ibuprofen	Imazalil	Naproxen
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	717
Day 2	201	126	96.0	n.d.	550	39.9
Day 4	302	136	73.7	n.d.	478	17.8
Day 7	594	121	67.1	n.d.	597	27.2
Day 14	104	160	53.9	n.d.	522	27.6
Day 28	1210	120	50.5	n.d.	527	18.9
Day 29	44.7	n.d.	<LOQ	n.d.	144	n.d.
Day 30	35.8	n.d.	<LOQ	n.d.	153	n.d.
Day 32	163	n.d.	n.d.	n.d.	137	n.d.
Day 35	26.3	n.d.	n.d.	n.d.	105	n.d.
Day 42	10.0	n.d.	n.d.	n.d.	43.0	n.d.
Day 58	8.4	n.d.	n.d.	n.d.	50.8	n.d.
C+M						
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	249	91.3	46.7	n.d.	526	<LOQ
Day 4	377	139	116	n.d.	906	2380
Day 7	637	110	61.4	n.d.	767	14.0
Day 14	101	78	58.0	n.d.	685	24.6
Day 28	158	69.6	63.9	n.d.	841	21.5
Day 29	357	n.d.	n.d.	n.d.	223	n.d.
Day 30	106	n.d.	<LOQ	n.d.	142	n.d.
Day 32	149	n.d.	<LOQ	n.d.	198	n.d.
Day 35	11.3	n.d.	n.d.	n.d.	91.4	n.d.
Day 42	12.1	n.d.	n.d.	n.d.	76.8	n.d.
Day 58	16.1	n.d.	n.d.	n.d.	62.4	n.d.
B						
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

S-23

C	PFBS	PFDA	PFOA	PFOS	PFPeA	Salicylic ac.	Terbuthylazine	Triclosan	Vildagliptin
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	974	n.d.	n.d.	n.d.
Day 2	n.d.	424	15.1	190	n.d.	2030	72.7	54.3	n.d.
Day 4	n.d.	305	10.2	95	n.d.	1560	79.6	<LOQ	n.d.
Day 7	n.d.	428	15.9	141	n.d.	709	116	n.d.	n.d.
Day 14	n.d.	215	8.7	84	n.d.	2390	85.7	65.5	n.d.
Day 28	n.d.	788	18.2	291	n.d.	6920	160	<LOQ	n.d.
Day 29	n.d.	107	n.d.	38.0	n.d.	2780	7.5	n.d.	n.d.
Day 30	n.d.	132	n.d.	55.6	n.d.	2980	10.5	n.d.	n.d.
Day 32	n.d.	42	n.d.	6.8	n.d.	4870	7.3	n.d.	n.d.
Day 35	n.d.	47	n.d.	18.3	n.d.	2230	10.6	n.d.	n.d.
Day 42	n.d.	13	n.d.	n.d.	n.d.	2040	8.4	n.d.	n.d.
Day 58	n.d.	28	n.d.	11.2	n.d.	3380	<LOQ	n.d.	n.d.
C+M									
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	1250	n.d.	n.d.	n.d.
Day 2	n.d.	398	31.5	292	n.d.	613	52.5	131	n.d.
Day 4	n.d.	671	16.9	298	n.d.	1420	90.2	<LOQ	n.d.
Day 7	n.d.	676	13.1	340	n.d.	2160	98.6	50.1	n.d.
Day 14	n.d.	638	21.8	311	n.d.	4150	113	52.2	n.d.
Day 28	n.d.	392	8.2	116	n.d.	2860	127	46.5	n.d.
Day 29	n.d.	105	n.d.	37.3	n.d.	2750	16.6	<LOQ	n.d.
Day 30	n.d.	68.5	n.d.	41.7	n.d.	3980	11.8	<LOQ	n.d.
Day 32	n.d.	133	n.d.	34.0	n.d.	3960	13.6	n.d.	n.d.
Day 35	n.d.	72.7	n.d.	11.9	n.d.	2510	10.3	n.d.	n.d.
Day 42	n.d.	46.2	n.d.	13.0	n.d.	1670	8.2	<LOQ	n.d.
Day 58	n.d.	13.5	n.d.	<LOQ	n.d.	3500	<LOQ	<LOQ	n.d.
B									
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	164	n.d.	n.d.	n.d.
Day 2	n.d.	<LOQ	n.d.	n.d.	n.d.	315	n.d.	n.d.	n.d.
Day 4	n.d.	<LOQ	n.d.	n.d.	n.d.	51.8	n.d.	n.d.	n.d.
Day 7	n.d.	<LOQ	n.d.	<LOQ	n.d.	136	n.d.	n.d.	n.d.
Day 14	n.d.	10.8	n.d.	<LOQ	n.d.	202	n.d.	n.d.	n.d.
Day 28	n.d.	<LOQ	n.d.	<LOQ	n.d.	247	n.d.	n.d.	n.d.
Day 29	n.d.	<LOQ	n.d.	n.d.	n.d.	176	n.d.	n.d.	n.d.
Day 30	n.d.	10.7	n.d.	<LOQ	n.d.	227	n.d.	n.d.	n.d.
Day 32	n.d.	12.9	n.d.	<LOQ	n.d.	340	n.d.	n.d.	n.d.
Day 35	n.d.	10.2	n.d.	n.d.	n.d.	200	n.d.	n.d.	n.d.
Day 42	n.d.	10.5	n.d.	<LOQ	n.d.	238	n.d.	n.d.	n.d.
Day 58	n.d.	<LOQ	n.d.	n.d.	n.d.	211	n.d.	n.d.	n.d.

n.d.: not detected

Table S-8: Concentration (ng/mL) found in mussel haemolymph of the compounds spiked in food and aquarium water. For the groups: control (B), exposed to contaminants (C) and exposed to contaminants and microplastics (C+M)

C	**Acetaminophen**	**Atenolol**	**Bentazone**	**Caffeine**	**Chlorfenvinfos**
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	<LOQ	n.d.
Day 4	n.d.	n.d.	n.d.	<LOQ	<LOQ
Day 7	n.d.	n.d.	n.d.	<LOQ	<LOQ
Day 14	n.d.	n.d.	n.d.	<LOQ	<LOQ
Day 28	n.d.	n.d.	n.d.	<LOQ	<LOQ
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.
C+M					
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	11.3	n.d.
Day 4	n.d.	n.d.	n.d.	<LOQ	<LOQ
Day 7	n.d.	n.d.	n.d.	9.2	<LOQ
Day 14	n.d.	n.d.	n.d.	13.0	<LOQ
Day 28	n.d.	n.d.	n.d.	11.1	10.1
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.
B					
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	n.d.	n.d.	n.d.	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

S-25

C	Clorpyriphos	Diclofenac	Etoricoxib	Ibuprofen	Imazalil	Naproxen
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	12.2	n.d.	11.5	<LOQ
Day 4	n.d.	n.d.	<LOQ	n.d.	9.9	n.d.
Day 7	n.d.	n.d.	13.5	n.d.	18.5	11.5
Day 14	n.d.	n.d.	11.3	n.d.	12.7	n.d.
Day 28	n.d.	n.d.	11.4	n.d.	8.6	14.4
Day 29	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C+M						
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	11.7	n.d.	15.8	<LOQ
Day 4	n.d.	n.d.	<LOQ	n.d.	12.2	<LOQ
Day 7	n.d.	n.d.	11.7	n.d.	12.7	<LOQ
Day 14	n.d.	n.d.	<LOQ	n.d.	11.0	n.d.
Day 28	n.d.	n.d.	14.0	n.d.	20.7	11.6
Day 29	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B						
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

S-26

C	PFBS	PFDA	PFOA	PFOS	PFPeA	Salicylic ac.	Terbuthylazine	Triclosan	Vildagliptin
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	<LOQ	n.d.	16.2	n.d.	<LOQ	n.d.	n.d.
Day 4	n.d.	n.d.	<LOQ	n.d.	31.8	n.d.	11.5	n.d.	n.d.
Day 7	n.d.	n.d.	n.d.	n.d.	24.9	n.d.	22.2	n.d.	n.d.
Day 14	n.d.	n.d.	<LOQ	n.d.	<LOQ	n.d.	13.9	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	34.9	n.d.	18.7	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C+M									
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	41.5	n.d.	10.7	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	27.3	n.d.	13.6	n.d.	n.d.
Day 7	n.d.	n.d.	<LOQ	n.d.	<LOQ	n.d.	9.7	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	18.28	n.d.	14.9	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	56.4	n.d.	15.90	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B									
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

S-27

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Conclusiones generales

04

1. Las muestras de biota tienen una gran complejidad debido a la presencia de compuestos interferentes, como proteínas, lípidos y pigmentos, entre otros. Siendo la eliminación de lípidos uno de los mayores retos. Además, algunos compuestos objetivo pueden estar presentes de forma natural en las muestras de biota.

2. La utilización de extracción QuEChERS, en combinación con un sistema de purificación (clean-up) apropiado, permite extraer de forma satisfactoria un amplio abanico de compuestos orgánicos (PPCPs, plaguicidas, PFAS y drogas de abuso) presentes en matrices bióticas sólidas complejas, tales como mejillón (masa visceral) o anguila (músculo e hígado).

3. La purificación mediante extracción en fase sólida dispersiva elimina una gran cantidad de interferentes, especialmente en aquellos casos que se emplean sorbentes específicos para la eliminación de lípidos, proteínas, fosfolípidos u otros interferentes en concreto.

4. La SPE es capaz de extraer fármacos presentes en plantas de forma satisfactoria. Asimismo, es capaz de extraer un amplio abanico de compuestos (PPCPs, plaguicidas, PFAS y drogas de abuso) presentes en hemolinfa de mejillón. Además, se observó una mejora significativa de la señal cromatográfica cuando se empleó metanol, en lugar de acetonitrilo, para la extracción de hemolinfa.

5. Las metodologías no dirigidas (o non-target) son capaces de identificar un amplio número de compuestos incluyendo metabolitos. Lo que permite evaluar la metabolización y distribución de los compuestos orgánicos en biota.

6. A la hora de emplear técnicas non-target, SWATH proporciona mayor cantidad de información y detecta una cantidad significativamente mayor de compuestos que IDA. No obstante, aunque IDA proporciona menor cantidad de información, esta tiene una mayor calidad, lo que hace que el proceso de identificación de compuestos sea más rápido y sencillo.

7. El desarrollo de métodos multiresiduo capaces de extraer una amplia variedad de compuestos en un solo proceso, es todavía escaso. El desarrollo de estas metodologías ayudaría a ahorrar tiempo y recursos, además de dar lugar a estudios más eficientes y respetuosos con el medio ambiente.

8. Las EDARs siguen siendo una de las principales fuentes de vertido de contaminantes de origen antropogénico al medio acuático. Incluyendo el vertido de PPCPs, PFASs, plaguicidas y microplásticos.

9. Los resultados de la presente tesis indican que las lluvias y las subsiguientes aguas de escorrentía provenientes de campos de cultivo, podrían ser el origen de episodios puntuales y agudos de contaminación en ecosistemas acuáticos.

10. La presencia de PPCPs, plaguicidas y PFASs es ubicua en los ambientes acuáticos, como se ha mostrado al ser detectados en diferentes partes del mundo (España, Arabia Saudita y Australia), incluyendo agua, sedimentos y biota. Estos compuestos también están presentes en el suelo y las plantas que se encuentran en los alrededores de ambientes acuáticos contaminados.

11. La utilización de modelos de balance de masas, junto con el uso de compuestos de referencia persistentes, como los PFASs. Permite estimar las vidas medias de compuestos orgánicos en ambientes naturales no controlados (como un estuario) y, por lo tanto, evaluar su destino ambiental.

12. Varios compuestos pueden ser bioacumulados en mejillón. Un total de 3 PPCPs, 4 plaguicidas y 3 PFASs mostraron signos de bioacumulación en masa visceral. Estando 6 de ellos (4 plaguicidas y 2 PFASs) presentes en la masa visceral durante toda la fase de depuración. Por otro lado, la cafeína y el PFPeA se detectaron solo en hemolinfa (junto con otro PPCP y otros 2 plaguicidas), lo que podría indicar una bioacumulación específica en este tejido para dichos compuestos.

13. La presencia de microplásticos podría favorecer la bioacumulación de PFASs, así como ralentizar su eliminación del organismo. Por contrario, la presencia de microplásticos podría reducir la bioacumulación de plaguicidas, así como acelerar su eliminación del organismo.

14. La información disponible sobre la bioacumulación y eliminación de compuestos orgánicos en biota, así como la posible interacción de estos compuestos con los microplásticos, es muy escasa. Por lo que es de especial interés seguir investigando sobre estas cuestiones.

5.0

General conclusions

1. Biota samples entail a great complexity due to interfering compounds such as proteins, lipids or pigments, among others, being lipid removal one of the main challenges. Furthermore, some target compounds might be naturally present in the biota samples.

2. The use of QuEChERS extraction combined with a proper clean-up can satisfactorily extract a wide range of organic compounds including PPCPs, pesticides, PFASs and illicit drugs, from complex solid biota matrices, such as, mussel (visceral mass) and eel (muscle and liver).

3. The dispersive solid-phase extraction clean-up eliminates a high number of interferers. Especially when some alternative methodologies specifically designed for the removal of lipid, proteins, phospholipids and other common interferers are employed.

4. SPE is a method able to extract satisfactorily pharmaceuticals from plants and a wide range of compounds (including PPCPs, pesticides and their metabolites, PFASs and illicit drugs) from mussel haemolymph. An analyte's signal improvement is observed if methanol is employed as solvent in haemolymph extraction, rather than acetonitrile.

5. Non-target approaches identify a wide number of compounds including metabolites, also enabling the assessment of the metabolisation and distribution of organic compounds in biota.

6. In non-target acquisition, SWATH provides more information and detects more analytes than IDA. On the other hand, although IDA provides less information, this has a higher quality, making the compound identification faster and easier.

7. The development of multi-residue methods, which can extract a wide range of compounds at the same time is still scarce. Developing these methods would save time and resources, hence providing more efficient and greener studies.

8. WWTPs are still one of the main sources of anthropogenic contaminants to the aquatic environments, including the discharge of PPCPs, PFASs, pesticides and microplastics.

9. Results indicate that rainfall events and subsequent discharge of runoff water from crops, may generate important punctual pollution episodes of pesticides in the aquatic environments.

10. Occurrence of PPCPs, pesticides, and PFASs is ubiquitous in aquatic

environments around the world (Spain, Saudi Arabia and Australia) including water, sediments and biota. These compounds are also present in the vegetation and soil surrounding polluted aquatic environments.

11. The use of mass balance models, along with the use of persistent compounds, such as PFASs, as benchmarking compounds, can estimate the half-lives of organic compounds in a natural non-controlled environment, such as a river estuary, and therefore to assess their fate.

12. Some organic compounds are susceptible to be accumulated in mussel. A total of 3 PPCPs, 4 pesticides and 3 PFASs showed to bioaccumulate in visceral mass, with 4 pesticides and 2 PFASs consistently present during the depuration stage. On the other hand, caffeine and PFPeA were exclusively detected in haemolymph (along to 1 PPCP and 2 pesticides more), suggesting probable tissue specific accumulation for these compounds.

13. The presence of microplastics may favour the bioaccumulation of PFASs and, at the same time, difficult their elimination from the organism. On the other hand, the presence of microplastics may reduce the bioaccumulation of pesticides and favour their elimination from the organism.

14. The information related to the bioaccumulation and elimination of emerging contaminants in biota and the information about how microplastics can affect to these processes, are very scarce. Then, there is a prominent need of further research.

ANEXOS

En esta sección se describen las abreviaturas más comunes de la presente tesis doctoral. Igualmente, todas las abreviaturas están definidas en los diferentes artículos

ACN	Acetonitrile – Acetonitrilo
AQ	Acid QuEChERS – QuEChERS ácido
BCF	Bioconcentration factor – Factor de bioconcentración
CE	Collision energy – Energía de colisión
d.w.	Dry weight – Peso seco
dSPE	Dispersive solid phase extraction – Extracción en fase sólida dispersiva
E%	Efficiency – Eficiencia
EMR	Enhanced matrix removal – Eliminación de matriz mejorada
EP	Emerging pollutant – Contaminante emergente
ESI	Elecntrospray ionization – Ionización por electrospray
FSWATH	Sequential window acquisition of all theoretical fragments with fixed windows – Adquisición de ventana secuencial de todos los fragmentos teóricos con ventanas fijas
FTS	Fluorotelomer sulfonates – Sulfonatos fluorotelomércos
GC	Gas chromatography – Cromatografía de gases
GCB	Graphitized carbon black – Carbono negro grafitizado
HPLC	High performance liquid chromatography – Cromatografia líquida de alta eficacia
HRMS	High resolution mass spectrometry – Espectrometría de masas de alta resolución
IDA	Information dependant acquisition – Adquisición dependiente de informacion
IIA	Information independent acquisition – Adquisición independiente de informacion
Inter-R	Reproducibility – Reproducibilidad
Intra-R	Repeatability – Repetitividad
IS	Internal standard – Patrón interno
LC	Liquid chromatography – Cromatografía líquida

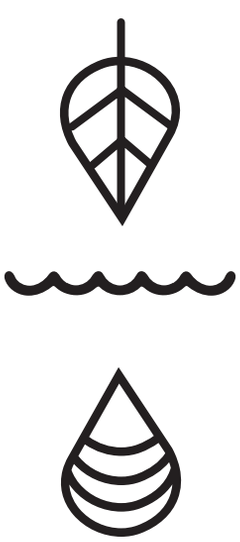
LOD	Limit of detection – Límite de detección
LOQ	Limit of quantification – Límite de cuantificación
LOR	Limit of report – Límite de reporte
ME	Matrix effect – Efecto matriz
MeOH	Methanol – Metanol
MP	Microplastic – Microplástico
MRM	Multiple reaction monitoring – Monitorización de reacciones seleccionadas múltiples
MS	Mass Spectrometry – Specntrometría de masas
MS/MS	Tandem mass spectrometry – espectrometría de masas en tándem
n.d.	Not detected – No detectado
NSAID	Non-steroidal anti-inflammatory drug – Antiinflamatorio no esteroideo
PAH	Polycyclic aromatic hydrocarbon – Hidrocarburo aromático policíclico
PAP	Perfluoroalkyl phosphate – Fosfato perfluoroalquilado
PCP	Personal care product – producto del cuidado personal
POP	Persistent organic compound – Compuesto orgánico persistente
PFAS	Perfluoroalkyl substances – Sustancias perfluoroalkiladas
PFCA	Perfluorocarbolxylic acid – Ácido perfluorocarboxílico
PFSA	Perfluorosulfonate – Perfluorosulfonato
PPCPs	Pharmaceuticals and personal care products – Fármacos y productos del cuidado personal
QqQ	Triple quadrupole – Triple cuadrupolo
QToF	Quadrupole time of light – Cuadrupolo tiempo de vuelo
QuEChERS	Quick, easy, cheap, effective, rugged and safe – Rápido, fácil, barato, efectivo, robusto y seguro
R^2	Coefficient of determination – Coeficiente de determinación
R%	Absolute recoveries – Recuperaciones absolutas
RR%	Relative recoveries – Recuperaciones relativas
RSD	Relative standard deviation – Desviación estándar relativa
RT	Retention time – Tiempo de retención

SD	Standard deviation – Desviación estándar
SE	Solvent extraction – Extracción por solvente
SPE	Soild phase extraction – Extracción en fase sólida
SQ	Standard QuEChERS – QuEChERS estándar
SWATH	Sequential window acquisition of all theoretical fragments – Adquisición de ventana secuencial de todos los fragmentos teóricos
TD	Tentative detected – Detección tentativa
UAE	Ultrasound assisted extraction – extracción asistida por ultrasonidos
UHPLC	Ultra-high performance liquid chromatography – Cromatografía de líquidos de ultra alta resolución
UV index	Ultraviolet index – Índice ultravioleta
VSWATH	Sequential window acquisition of all theoretical fragments with variable windows – Adquisición de ventana secuencial de todos los fragmentos teóricos con ventanas variables
w.w.	Wet weigth – Peso húmedo
WWTP	Wastewater treatment plant – Estación depuradora de aguas residuales

Compounds - Compuestos

CBX IBU	Carboxyibuprofen – Carboxiibuprofeno
FOSA	Perfluorooctanesulfonamide – Perflorooctanosulfonamida
FOSAA	Perfluorooctanesulfonamide acetic acid – Ácido acético perflorooctanosulfonamida
IBU	Ibuprofen – Ibuprofeno
OH IBU	Hydroxyibuprofen – Hidroxi-ibuprofeno
PFBA	Perfluorobutanoic acid – Ácido perfluorobutanóico
PFBS	Perfluorobutanesulphonate – Sulfonato de perfluorobutano
PFDA	Perfluorodecanoic acid – Ácido perfluorodecanóico
PFDoDA	Perfluorododecanoic acid – Ácido perfluorododecanóico
PFDoDS	Perfluordodecanesulphonate – Sulfonato de perfluorododecano

PFDS	Perfluorodecanesulfonic acid – Sulfonato de perfluorodecano
PFHpA	Perfluoroheptanoic acid – Ácido perfluoroheptanóico
PFHpS	Perfluoroheptanesulphonate – Sulfonato de perfluoroheptano
PFHxA	Perfluorohexanoic acid – Ácido perfluorohexanóico
PFHxDA	Perfluorohexadecanoic acid – Ácido perfluorohexadecanóico
PFHxS	Perfluorohexanesulsulphonate – Sulfonato de perfluorohexano
PFNA	Perfluorononanoic acid – Ácido perfluorononanóico
PFNS	Perfluorononanesulfonate – Sulfonato de perfluorononano
PFPeA	Perfluoropentanoic acid – Ácido perfluoropentanóico
PFPeS	Perfluoropentanesulsulphonate – Sulfonato de perfluoropentano
PFOA	Perfluorooctanoic acid – Ácido perfluorooctanóico
PFODA	Perfluorooctadecanoic acid – Ácido perfluorooctadecanóico
PFOS	Perfluorooctanesulsulphonate – Sulfonato de perfluorooctano
PFTeDA	Perfluorotetradecanoic acid – Ácido perfluorotetradecanóico
PFTrDA	Perfluorotridecanoic acid – Ácido perfluorotridecanóico
PFUnDA	Perfluoroundecanoic acid – Ácido perfluoroundecanóico

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