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Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants

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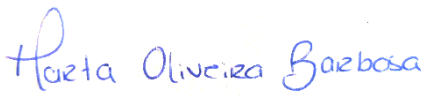
Other extra acknowledgments are included in the final of the individual chapters and in the respective PDF version of each research article, Appendix A – E.

Statement of Originality

I certify that this work does not contain any material that has been used nor will be for the award of any other degree or diploma in my name or anyone, in any university or institution. In addition, I certify that, to the best of my knowledge, this work does not contain any material previously published or written by another person, except where due reference has been made in the text.



Marta Oliveira Barbosa

Statement

In order to fulfil the Rules of Ethics of the Doctoral Program of Chemical and Biological Engineering (PDEQB), we hereby declare that all the contents of the thesis presented by Marta Sofia Oliveira Barbosa, entitled “Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants”, are exclusively from the author with the collaborations mentioned in the thesis. The full agreement of the co-authors on the inclusion of the manuscripts in the present thesis can be found in Appendix F.

“*Partimos a pedra*
Abrimos caminho
Rasgamos montanhas
Mas nunca sozinho

Corremos na selva
No sol do deserto
No escuro da sombra
Ficando mais perto

Quem no fundo quer
Vai atrás
Quem de longe vê
Parte pedra
Vai atrás.”

Tiago Bettencourt,
“Partimos a Pedra”

Aos meus.

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Abstract

The presence of organic micropollutants in the environment is a worldwide concern. These pollutants, usually found at residual concentrations (ng L^{-1} to µg L^{-1}), are not completely removed by the conventional wastewater treatment technologies and are continuously discharged into receiving water bodies that might be sources for drinking water (DW) supply. The occurrence of these compounds in such water matrices may cause adverse ecological and human health effects and, to tackle this problem, the monitoring of some priority substances (PSs, Directive 2013/39) and contaminants of emerging concern (CECs, Watch Lists of Decisions 2015/495, 2018/840 and 2020/1161) has been recommended in the European Union (EU). Thus, the fate and behavior of these PSs and CECs in water compartments require further investigation, and the development of robust and reliable multi-residue analytical methods that enable the determination of these pollutants is crucial in this context. Bearing this in mind, the main objective of this PhD project is the development of an eco-friendly and low-cost analytical tool to simultaneously determine a wide variety of PSs and CECs defined in recently launched EU legislation.

The first stage of this work was dedicated to the optimization and validation of analytical methodologies based on solid-phase extraction (SPE) followed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), for characterization of DW and surface water (SW). A green analytical method for determination of twenty-one organic micropollutants was successfully applied to tap, fountain, and well waters collected in different locations. The occurrence of thirteen compounds was demonstrated and the hazard quotients estimated for the quantified micropollutants suggested no adverse effects to humans.

This analytical method was also applied to characterize water samples before and after bench-scale photolysis and ozonation experiments, with only eight of the twenty-one spiked compounds being completely removed by these treatment options.

A spatiotemporal campaign in four stressed Portuguese rivers (Ave, Leça, Antuã and Cértima) was then performed to assess the variations and the distribution of a larger group of analytes (thirty-nine) that included new CECs integrated in the Watch List or identified as frequent in other countries. A widespread occurrence of micropollutants was observed with twenty-six compounds being detected, thirteen of which present in all rivers. Leça River was then considered as a case study for assessment of fluorescence excitation-emission matrices and the results matched the spatial distribution tendency of pollutants along the river, highlighting the industrial areas and urban wastewater treatment plants (WWTPs) as input sources of these substances.

The studies referred above allowed to establish reference methods for analysis of the organic micropollutants and to select eight target multi-class compounds for succeeding research works included in this thesis, based on their frequency of detection and higher concentrations in DW and SW samples. Five pesticides (acetamiprid, atrazine, isoproturon, metaflumizone and methiocarb), two pharmaceuticals (carbamazepine and diclofenac), and one industrial compound (perfluorooctanesulfonic acid - PFOS) were selected. In these following studies, the investigation was focused on tailoring the texture and surface chemistry of carbon materials aiming at the development of a multi-layer carbon-based SPE cartridge with high selectivity/specificity for the target compounds pre-concentrated by SPE and determined by UHPLC-MS/MS in water monitoring campaigns.

Pristine and functionalized multi-walled carbon nanotubes (MWCNTs) were the first materials applied as sorbent in SPE for extraction of the target micropollutants. The reusable SPE cartridge developed with pristine MWCNTs gave recoveries higher than

60% for five out of eight target compounds, using a low amount of material (50 mg) and a solvent considered more eco-friendly (ethanol) than the conventional ones. Moreover, the controlled HNO_3 hydrothermal oxidation methodology applied for functionalization of MWCNTs allowed to establish some important correlations between both the synthesis conditions and the oxygen-containing surface functionalities introduced on the material surface; as well as between the type and amount of those functionalities and the recoveries obtained for the target compounds. The introduction of oxygenated groups on the surface material affected the recovery values in different ways. The recovery was improved for methiocarb and PFOS, and dropped for acetamiprid, diclofenac and carbamazepine. In the specific case of metaflumizone, pristine and functionalized MWCNTs were both ineffective options.

Pristine and oxidized carbon xerogels (CXs) were then synthesized and investigated for extraction of the same target analytes. In this case, the introduction of oxygenated surface groups in CXs resulted in a decline of the recovery for the vast majority of the micropollutants. Interestingly, the recoveries were quite high for metaflumizone (69 ± 5%), which was not recovered when MWCNTs were tested. Thus, a SPE carbon-based cartridge with both materials (CXs and MWCNTs) in multi-layer configuration was developed. This novel cartridge was reusable and capable of extracting all the multi-class target compounds in a single procedure. Hence, an analytical methodology based on SPE-UHPLC-MS/MS was validated with the multi-layer carbon cartridges and used to monitor the target analytes in water samples collected before (SW) and after three DW treatment plants. The potential of the developed method for monitoring SW and DW was confirmed and six out of eight compounds were quantified.

Summarizing, the multi-layer carbon-based cartridge fabricated in the framework of this PhD thesis provides an analytical tool for monitoring PSs and CECs in SW and

DW, presenting advantages when compared with the marketed cartridges (i.e. being cheaper, reusable, and more eco-friendly). Moreover, this work contributes to the knowledge on the occurrence and fate of organic micropollutants in the aquatic compartments, emphasizing that actions are needed to preserve a good environmental status of stressed European waterbodies.

Resumo

A presença de micropoluentes orgânicos no ambiente tem sido alvo de preocupação a nível mundial. Estes poluentes, geralmente detetados em concentrações vestigiais (ng L^{-1} a µg L^{-1}), não são completamente removidos nos processos convencionais de tratamento de águas residuais, sendo continuamente descarregados em cursos de água que por sua vez são utilizados como fontes de abastecimento de água potável. A ocorrência de micropoluentes orgânicos neste tipo de matrizes aquáticas pode resultar em consequências adversas para o ambiente e para a saúde humana. De forma a mitigar este problema, a monitorização de substâncias prioritárias (PSs - *priority substances*, Diretiva 2013/39) e de contaminantes de preocupação emergente (CECs - *contaminants of emerging concern*, da lista de vigilância das Decisões 2015/495, 2018/840 e 2020/1161) tem sido recomendada na União Europeia (UE). O destino e os efeitos destes poluentes nos compartimentos aquáticos requerem estudos adicionais e o desenvolvimento de métodos analíticos robustos e fiáveis, que permitam detetar uma vasta gama de micropoluentes neste tipo de matrizes. O principal objetivo do presente projeto de doutoramento consiste no desenvolvimento de uma ferramenta analítica para a determinação simultânea de uma grande variedade de PSs e CECs, estabelecidos no quadro legal da União Europeia.

A primeira fase deste trabalho foi dedicada à otimização e validação de metodologias analíticas baseadas na extração em fase sólida (SPE - *solid-phase extraction*) seguida de cromatografia líquida de ultra-alta eficiência associada, em tandem, à espectrometria de massa (UHPLC-MS/MS - *ultra-high performance liquid chromatography coupled to tandem mass spectrometry*), para aplicação em água potável e de superfície. Foi assim desenvolvido um método analítico que permitiu

demonstrar a ocorrência de treze dos vinte e um micropoluentes orgânicos em estudo, em amostras de água da rede de abastecimento, fontes e poços, provenientes de diferentes locais. Foram estimados os quocientes de risco para os micropoluentes encontrados, não tendo sido revelados efeitos adversos para os seres humanos. A metodologia desenvolvida foi também aplicada em amostras de água da rede, contaminadas com os vinte e um micropoluentes, para avaliar a eficácia de diferentes processos de tratamento, à escala laboratorial, como a fotólise e a ozonização. Os resultados obtidos mostraram que apenas oito compostos foram totalmente removidos por estes processos.

No que diz respeito à análise de águas superficiais, realizou-se uma campanha de monitorização em quatro rios portugueses (Ave, Leça, Antuã e Cértima), com o objetivo de avaliar as variações e a distribuição espacial e temporal de um maior grupo de micropoluentes (trinta e nove). Este grupo incluiu novos CECs e outros compostos identificados como frequentes noutros países. Observou-se uma ocorrência generalizada dos micropoluentes, no espaço e no tempo, com a deteção de vinte e seis compostos, dos quais treze foram determinados em todos os rios. O rio Leça foi utilizado como caso de estudo para a avaliação de matrizes de excitação-emissão de fluorescência. Os resultados obtidos corresponderam à tendência de distribuição dos poluentes ao longo do curso do rio, destacando-se as áreas mais industrializadas e as estações de tratamento de águas residuais urbanas, como fontes de entrada de micropoluentes nos cursos de água superficial.

Os estudos referidos anteriormente permitiram estabelecer os métodos analíticos de referência e selecionar os oito compostos modelo, com base na frequência de deteção e nas concentrações mais elevadas encontradas em amostras de água potável e de superfície, recolhidas em Portugal. Deste modo, para a continuação deste projecto foram selecionados como compostos modelo: cinco pesticidas

(acetamiprida, atrazina, isoproturão, metaflumizona e metiocarbe), dois compostos farmacêuticos (carbamazepina e diclofenac) e um composto industrial (ácido perfluorooctanossulfónico). Os trabalhos desenvolvidos posteriormente centraram-se no estudo da química de superfície e das propriedades texturais de materiais de carbono para o desenvolvimento de um cartucho multicamada com elevada seletividade/especificidade para a pré-concentração por SPE dos compostos-alvo, e posterior análise por UHPLC-MS/MS, em campanhas de monitorização de cursos de água.

Os primeiros materiais estudados como adsorventes para a extração dos oito micropoluentes selecionados foram as amostras originais de nanotubos de carbono de parede múltipla (MWCNTs - *multi-walled carbon nanotubes*) antes e depois da respetiva funcionalização. O cartucho para SPE desenvolvido com os MWCNTs originais é reutilizável e permitiu obter recuperações superiores a 60% para cinco dos oito compostos em estudo, utilizando uma baixa quantidade de material (50 mg) e um solvente mais ecológico (etanol) quando comparado com alternativas mais convencionais. A oxidação hidrotérmica controlada com HNO_3 e aplicada aos MWCNTs permitiu estabelecer algumas correlações importantes entre as condições de síntese e os grupos funcionais oxigenados introduzidos no material; e entre o tipo e quantidade desses grupos funcionais e as recuperações obtidas para os compostos-alvo.

A introdução de grupos oxigenados na superfície do material afetou a recuperação dos compostos de diferentes formas, aumentando as recuperações de metiocarbe e ácido perfluorooctanossulfónico e diminuindo as de acetamiprida, diclofenac e carbamazepina. No caso específico da metaflumizona, os MWCNTs originais e funcionalizados foram ineficientes como adsorventes para SPE.

Foram também sintetizados e testados xerogéis de carbono (CXs - *carbon xerogels*) para a extração dos oito compostos-alvo já referidos. Neste caso, a introdução de grupos oxigenados na superfície do material reduziu os valores de recuperação obtidos para a maioria dos micropoluentes estudados. Curiosamente, as recuperações foram bastante elevadas para a metaflumizona (69 ± 5%), um composto que não foi possível recuperar quando foram utilizados MWCNTs em vez de CXs. Por este motivo foi assim desenvolvido um cartucho utilizando CXs e MWCNTs, posicionados em multicamada. Este cartucho inovador é reutilizável e capaz de extrair, em simultâneo, os vários micropoluentes orgânicos em estudo. Com este cartucho foi validada uma metodologia analítica baseada em SPE-UHPLC-MS/MS, que foi aplicada na monitorização de amostras de água recolhidas a montante e a jusante de três estações de tratamento de água para consumo. A aplicabilidade deste método foi confirmada com sucesso, quantificando seis dos oito compostos em estudo nas amostras de água analisadas.

Em suma, o cartucho multicamada à base de materiais de carbono, produzido no âmbito da presente tese de doutoramento, fornece uma ferramenta analítica para a monitorização de PSs e CECs em cursos de água, apresentando como vantagem ser reutilizável, mais económico e ecológico, quando comparado com os cartuchos comerciais. Este trabalho foi fundamental para a aquisição de novos conhecimentos relacionados com a ocorrência e proliferação de micropoluentes orgânicos nos recursos hídricos estudados, alertanto para a necessidade de serem implementadas medidas de preservação do bom estado ambiental destes recursos.

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List of Abbreviations

ADD	Average daily dose
ADI	Acceptable daily intake
AF	Assessment factor
AOP	Advanced oxidation processes
AOT	Advanced oxidation technologies
BET	Brunauer-Emmett-Teller
BHT	2,6-di-tert-butyl-4-methylphenol
CEC	Contaminant of emerging concern
CX	Carbon xerogel
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DW	Drinking water
DWTP	Drinking water treatment plant
E1	Estrone
E2	Beta-estradiol
EDI	Estimated daily intake
EDTA	Ethylenediamine tetraacetic acid
EE2	17-alpha-ethinylestradiol
EEM	Excitation-emission matrix
EHMC	2-ethylhexyl-4-methoxycinnamate
EQS	Environmental quality standard
EU	European Union

GAC	Green analytical chemistry
GW	Groundwater
HLB	Hydrophilic-Lipophilic-Balanced
HPLC	High performance liquid chromatography
HQ	Hazard quotient
HRT	Hydraulic retention time
MAC	Maximum allowable concentration
MAX	Mixed-mode Anion-eXchange
MCX	Mixed-mode Cation eXchange
MDL	Method detection limit
ME	Matrix effect
MQL	Method quantification limit
MWCNT	Multi-walled carbon nanotube
NSAID	Non-steroidal anti-inflammatory drug
OMP	Organic micropollutant
PFOS	Perfluorooctanesulfonic acid
pH_{PZC}	pH at point of zero charge
PS	Priority substance
QC	Quality control
RSD	Relative standard deviation
S/N	Signal-to-noise ratio
S_{BET}	Specific surface area
S_{meso}	Non-microporous specific surface area
SP	Sampling point
SPE	Solid phase extraction

SRM	Selected reaction monitoring
SW	Surface water
TGA	Thermogravimetric analysis
TOC	Total organic carbon
TPD	Temperature programmed desorption
UHPLC – MS/MS	Ultra-high performance liquid chromatography with tandem mass spectrometry
UV	Ultraviolet
V_{micro}	Micropore volume
V_{total}	Total pore volume
WL	Watch list
WWTP	Wastewater treatment plant

Chapter 1

General Introduction

1.1. Overview of the problematic

Nowadays, one of the major problems that humanity faces concerns about the quantity and/or quality of water. Less than 1% of the Earth's water is available for human consumption and, according to the more recent report by WHO (World Health Organization) and UNICEF (United Nations International Children's Emergency Fund), around 10% of the world's population had no access to basic drinking water (DW) services in 2017: 206 million people used limited services (i.e., water from an improved source for which collection time exceeds 30 min for a round trip, including queuing); 435 million used unimproved sources (i.e., water from an unprotected dug well or unprotected spring); and 144 million used surface water (SW) (i.e., water directly from a river, dam, lake, pond, stream, canal or irrigation canal) [1]. The improvement of the quality of life and the sustainable development of the world depend on measures to meet the environmental protection and the correction of water problems.

Throughout the past three decades, the research on the impact of water pollution has been mostly centered on conventional pollutants, namely heavy metals and persistent organic pollutants, and this subject was extensively reviewed [2-5]. The reduction of emissions in the developed countries, through the launch of policy guidelines to penalize illegal usages and/or discharges, took this type of substances from the priority context [6]. However, in recent years, an increasing attention was raised about the fate and effects of a large group of organic micropollutants on the water compartments, some of them already outlined in the EU legislation. These pollutants are found at trace or ultra-trace concentrations (ng L^{-1} to µg L^{-1}) and include pharmaceuticals compounds, pesticides, personal care products, UV-filters, industrial compounds, steroid hormones, drugs of abuse, among others [7, 8]. Some of these

pollutants are considered “pseudo-persistent” since their transformation/removal rates are overcome by their continuous introduction into the environment. Additionally, their recalcitrant character together with their polarity favors their dispersion and interchange between the aquatic environments.

Micropollutants are released into the environment through different sources (e.g., industry, agriculture/livestock, houses/hospitals) [8, 9]. Nonetheless, it is consensual that the most significant entry route for micropollutants is the release from the conventional wastewater treatment plants (WWTPs). Several studies have shown that most of them are not completely removed by conventional treatments, being discharged into receiving water bodies (rivers, lakes and seas), which may be used as sources for DW supply [10-13].

A great concern about the occurrence of micropollutants in the aquatic resources and the subsequent effects on humans and biota have been highlighted in the last decade. It is difficult to predict which environmental and public health implications may arise from the occurrence of organic micropollutants in freshwater ecosystems, since the concentrations usually found in the environment are lower than those able to cause direct negative effects [14]. The main problematic related to the frequent occurrence of recalcitrant compounds is the long-term exposition that can lead to serious chronic effects, as reported by several studies [15-17]. Their constant but imperceptible effects can gradually accumulate, finally leading to irreversible changes on both wildlife and human beings [18].

In this context, it is important to set up fast, sensitive and reliable analytical methods enabling the determination of a wide range of these pollutants in aquatic compartments, at residual levels usually found. Several analytical techniques have been improved to achieve a high sensitivity and reproducibility for the detection of organic micropollutants in the environment. Considering the wide resources and time

consumption involved in this task, the novel developed analytical methods should meet the multi-class purpose, being able to determine trace levels of a large number of compounds (chemically heterogeneous) and simultaneously reduce the most time-consuming cleanup and extraction step [8, 19].

The sample preparation to clean up the interferences and concentrate the target compounds remains as one of the essential steps of the analytical procedure during environmental analysis [20, 21]. Solid-phase extraction (SPE) is the most employed sample preparation method for the pre-concentration of analytes and removal of interferences in aqueous samples. It is well known that the selection of the most appropriate sorbent is the crucial step when using SPE. In regard to appropriate sorbents, several materials have been studied, namely synthetic resins and its derivatives, carbon materials and biological substrates [22]. Innovation in materials science may provide new tools for analytical sample preparation. In recent years, carbon-based materials have been investigated as sorbents in sample preparation [22-25]. The characteristic structures of this type of materials allow them to interact with organic molecules via non-covalent forces. These interactions and their hollow or layered nanosized structures make them potential candidates for use as adsorbents in SPE [23]. Filling the bridge between the materials science and analytical chemistry by interdisciplinary studies in the area of carbon materials may be a step forward to the development of a more efficient and eco-friendly analytical tool for SPE procedures.

1.2. EU priority substances and contaminants of emerging concern

The increasing demand for water protection and treatment by environmental organizations and population in general was one of the major reasons why the European Commission (EC) set water protection as one of its top work priorities.

Priority substances (PSs) are “individual pollutants or groups of pollutants presenting a significant risk to or via the aquatic environment, including such risks to waters used for the abstraction of drinking water”, according to Article 16 of the Water Framework Directive (WFD) 2000/60/EC [26]. Furthermore, some pollutants not regulated yet were now recognized as emerging compounds. The word “emerging” means that these substances are still unregulated or in the process of regulation and have been recently found in the environment or potentially cause negative effects on aquatic life at environmental concentrations [27]. These types of pollutants are so-called contaminants of emerging concern (CECs) due to the unidentified risk to the environment and to the human health, related to their presence, frequency of occurrence, or source [28]. Many pesticides, industrial compounds, pharmaceuticals, steroids and hormones, disinfection by-products, among others, belong to the definition of CECs.

Although there are no legal discharge limits for micropollutants, a few regulations have been published. The Directive 2013/39/EU includes 45 PSs/groups of PSs (organic micropollutants) and also certain other pollutants with defined environmental quality standards to be considered [29]. In 2015, the EC published the Decision 2015/495/EU on the establishment of the 1st watch list (WL) of substances for Union-wide monitoring in SW bodies [30]. The 1st WL included 10 substances or groups of substances, namely: 1 synthetic and 2 natural hormones, 1 pain killer, 5 neonicotinoid insecticides, 3 macrolide antibiotics, 1 sunscreen agent, 2 herbicides, 1 insecticide and 1 industrial product. An extensive review on the occurrence of CECs from the 1st WL is presented in *Chapter 2*, *Part A*. More recently, in 2018, a 2nd WL (Decision 2018/840/EU) was adopted, removing 5 substances from the previous WL (due to sufficiently high-quality monitoring data already available) and recommending 3 new substances to be monitored [31]. This 2nd WL also gives the relevant Predicted No Effect

Concentrations (PNECs) and identifies possible analytical methods of analysis for the substances included. More recently, a 3rd WL listed in the Decision 2020/1161/EU was approved by the EC [32]. This latest version maintains 3 substances present in the 2nd WL (i.e., the insecticide metaflumizone and the antibiotics amoxicillin and ciprofloxacin), and adds several other substances: 2 antibiotics, which are often prescribed together to overcome antimicrobial resistance; 10 azole substances, used as either pharmaceuticals or pesticides; an anti-depressant pharmaceutical and its metabolite; and 2 pesticides used as fungicides.

The biggest focus of the WL is to better evaluate risks from chemicals found in SW bodies, based on the mechanism introduced by the Directive 2013/39/EU. Member states have to monitor this type of pollutants at least once per year for up to 4 years. The monitoring of those substances should generate high-quality data on their concentrations in the aquatic environment, improving the available data.

1.3. Analytical methods for determination of micropollutants in surface and drinking water

Highly sensitive and reproducible methods are required to determine the trace levels of micropollutants in environmental compartments, and several techniques have been improved to reach this goal. Considering the wide resources and time consumption involved in this task, the novel developed analytical methods should meet the multi-residue or multi-class purposes, being able to determine trace levels of a large number of different compounds and simultaneously reduce the cleanup and extraction steps [33, 34]. The setup of a multi-residue method implies a thorough overview of the diverse steps involved in the analytical process. Two main issues can be distinguished during the

development of a method for analysis of environmental samples: (i) sample preparation; and (ii) analytical separation and detection.

Sample preparation, is often highly time-consuming, comprising limited automated processes, which can jeopardize the reproducibility [35]. The main goal of sample preparation is to obtain a sample extract enriched in target analytes and free of other components present in the matrix, as far as possible. Basically, it encompasses the following steps: (i) extraction of low amounts of the target analytes from the sample matrix; (ii) concentration of those analytes; and (iii) removal of other substances which may be co-extracted and simultaneously concentrated, consequently hampering the efficiency of the analytical method [36, 37]. Entire effort in the development of this analytical procedure may result in a considerable increase in the yield and quality of the results obtained [35].

As referred above, SPE is the most popular sample preparation technique for environmental samples. The principle of this technique involves partitioning between a liquid phase containing the analytes and a solid sorbent phase. The SPE procedure consists of five main steps: conditioning, sample loading, washing, dryness and elution (Fig. 1.1.).

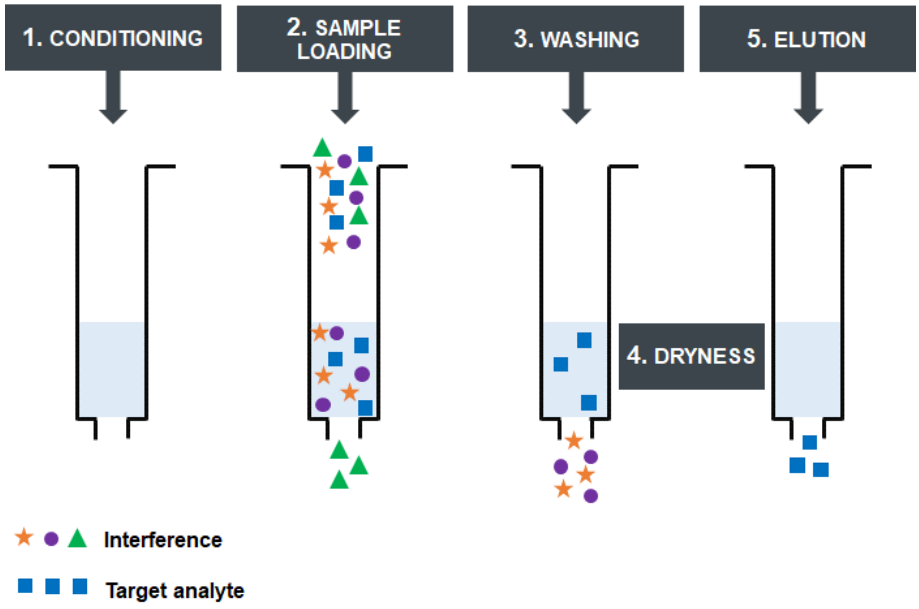

Fig. 1.1. Schematic representation of an ideal SPE procedure, with pre-concentration of the target analyte and an efficient removal of interferences.

The objectives of these stages are the extraction, concentration of the target analytes, elimination of interferences, removal of the residual water and, finally, desorption of the analytes. Owing to its high versatility, SPE is used for several purposes, such as purification, trace enrichment, desalting, derivatization of analytes in the cartridge and fractionation of the sample extract in different groups of compounds. To achieve optimal SPE extraction conditions, the selection of the sorbent is an important factor because it will affect the selectivity, affinity and capacity [38]. The choice depends strongly on the nature of the analytes and their physical and chemical properties, which define the interactions with the selected sorbent. Nevertheless, results also depend on the sample matrix and its interactions with both sorbent and analytes [38]. It is still important to refer that the extraction efficiency is affected, not only, by the type of sorbent, but also by the solvent used, sample pH and sample volume loaded. These parameters have to be carefully optimized to obtain good results [39].

After the optimization of the procedure of sample preparation, the analytical separation and detection of organic compounds can be accomplished by liquid chromatography (LC) (e.g., reversed-phase LC, hydrophilic interaction LC, mixed-mode LC), supercritical fluid chromatography, ion chromatography, capillary electrophoresis and gas chromatography (GC), according to the volatility, polarity and thermal stability of the analytes [40]. Whilst volatile or semi-volatile compounds may be analyzed by GC, more polar or thermolabile non-polar compounds are analyzed by LC, with no need of prior derivatization [37]. Fast and high-resolution LC systems are currently available, with high resolution and separation efficiency, as ultra-high performance liquid chromatography (UHPLC), enabling to work at pressures up to 1300 bar using sub-2-μm particle packed columns [41].

The detection of a wide range of organic contaminants at trace or ultra-trace concentrations, including organic micropollutants in environmental matrices, is a challenging task. Hyphenated chromatography-mass spectrometry (MS) techniques, such as LC-MS, are presently the methods of election for the analysis of organic micropollutants in water samples due to the notable improvement of the method detection limits achieved by these techniques [41, 42].

1.4. Carbon-based materials for solid-phase extraction

Trends in sample preparation are centred on offering highly selective, reproducible, fast and robust methods, with eco-friendly and cheap procedures, though the latter is not so easily accomplished. In order to achieve this, the introduction of new sorbent materials is possibly one of the most important research lines in this field. The application of nanomaterials has allowed the development of a huge number of research papers in which nanomaterials, with different properties (i.e., different

physicochemical characteristics, morphologies, and compositions), have been studied as extraction sorbents [25, 43, 44]. The use of new materials in SPE cartridges has been explored to achieve more selective materials with higher adsorption capacity and enrichment factors, and to expand the availability of cheaper, more easily synthesized sorbents [43, 45].

Regarding the diverse types of nanomaterials available nowadays, carbon-based nanostructured materials have been explored and many review articles [43, 44, 46-52] addressed recently the importance of this type of materials to develop new analytical sample preparation procedures, namely SPE [53], dispersive SPE [54], solid-phase microextraction [55], and stir-bar sorptive extraction [56]. Carbon materials are available in several allotropic forms, such as fullerenes, carbon nanotubes (single-, double- and multi-walled), carbon nanofibers, nanodiamonds, graphene and its derivatives (such as graphene oxide), carbon xerogels, aerogels, and cryogels, among others. The large adsorption surface-to-volume ratios, high affinity, easy modification with functional groups and easy covalent or non-covalent functionalization, render carbon materials as a great option for their application as sorbents in SPE cartridges for extraction of a wide range of analytes from different matrices [23, 27].

The application of carbon-based materials as sorbent solutions in SPE for extraction of organic micropollutants from water samples is discussed in *Part B* of *Chapter 2*.

1.5. Scope and objectives

Several micropollutants (ng L^{-1} to µg L^{-1}) end up in environmental compartments, such as surface, ground and drinking water. SPE is the most used pre-concentration technique for determination of organic micropollutants in water samples. The main motivation of this work is based on the lack of an eco-friendly and low-cost analytical tool to simultaneously determine a wide variety of organic PSs and CECs defined in recently launched EU legislation.

This PhD project is focused on tailoring the texture and surface chemistry of carbon materials to develop a multi-layer carbon-based SPE cartridge with high selectivity/specificity for adsorption/desorption of some target organic PSs and CECs, in order to pre-concentrate by SPE and determine by UHPLC-MS/MS these micropollutants in SW and DW, when monitoring EU water matrices.

Thus, the specific goals of this work can be listed as:

(i) To develop and validate analytical methodologies by using UHPLC-MS/MS for SW and DW matrices;

(ii) To obtain different carbon materials with controllable texture and surface chemistry for adsorption/desorption of PSs and CECs;

(iii) To develop and validate the concept of a SPE carbon-based cartridge layer-by-layer assembled with different carbon materials as sorbents (i.e., presenting different physical-chemical characteristics) to extract a wide range of multi-class PSs and CECs;

(iv) To monitor PSs and CECs in SW and DW, by using a simple, efficient, and timeless procedure.

1.6. Thesis outline

The present thesis is organized in 7 chapters.

Chapters 2 (Part A), 3, 4 and 5 correspond to the work already published and *Chapter* 6 to one submitted for publication. The information presented in these chapters is similar to that already published, with the exception of some formatting changes, in accordance with the guidelines of the Doctoral Program of Chemical and Biological Engineering (PDEQB). The original version of these works and the respective supplementary information are presented in Appendices A – E.

In this introductory chapter, *Chapter 1*, a brief description is given to present the problematic of EU-relevant organic micropollutants in water compartments and the potential of carbon-based materials as sorbents in SPE to extract and pre-concentrate this type of analytes before the UHPLC-MS/MS analysis. The scope and the major purpose of this project are also presented.

Chapter 2 comprises the state-of-the-art and is divided in two different parts, namely *Part A* and *Part B*. In *Part A,* a critical review on the occurrence and removal of organic micropollutants is presented, focused on the CECs of the 1st WL (Decision 2015/495/EU). *Part B* provides a literature overview on reports dealing with the application of carbon-based materials in SPE cartridges. This overview is based on studies developed for determination of EU-relevant organic micropollutants in real water samples.

Chapter 3 describes the entire optimization and validation of a green analytical methodology (i.e., employing ethanol as solvent) for multi-class determination of 21 organic micropollutants (PSs and CECs) in DW samples. The offline SPE-UHPLC-MS/MS method was successfully applied to samples from diverse sources (tap, fountain, and well waters) from different locations in the north of Portugal, as well as

before and after bench-scale treatment experiments of tap water samples spiked with organic micropollutants at ng L^{-1} levels.

In *Chapter 4*, a monitoring campaign of 39 EU-relevant organic micropollutants in 4 Portuguese rivers (Ave, Leça, Antuã and Cértima) was performed. Contamination levels of the target substances in these rivers were investigated during the dry and wet seasons. SW samples were collected at different points on each river and analyzed by an offline SPE-UHPLC-MS/MS method that was previously validated.

Chapter 5 explores the pristine and functionalized multi-walled carbon nanotubes (MWCNTs) as SPE sorbents for the simultaneous extraction of 8 EU multi-class organic micropollutants in SW before UHPLC-MS/MS analysis. Careful optimization of the parameters that influenced the extraction efficiency was performed. Moreover, a systematic study of these extraction efficiencies upon application of a controlled HNO_3 hydrothermal oxidation methodology to pristine MWCNTs is reported for the first time.

In *Chapter 6*, carbon xerogels (CXs) are prepared, characterized and validated as potential sorbents for SPE. CXs and MWCNTs are loaded in empty SPE cartridges, varying the multi-layer configurations (order/type of carbon layer). The most efficient multi-layer carbon-based SPE cartridges are used to concentrate SW and DW samples prior to the UHPLC-MS/MS. A monitoring campaign is also performed to identify and quantify the target PSs and CECs before and after drinking water treatment.

Finally, *Chapter 7* is dedicated to the general conclusion and suggestions for future work.

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Chapter 2

State-of-the-Art

Part A.

Occurrence and removal of organic micropollutants:
An overview of the watch list of EU Decision 2015/49

Part B.

Analysis of organic micropollutants:
An overview of carbon-based materials for solid-phase extraction cartridges

Part A

Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/49

Abstract

Although there are no legal discharge limits for micropollutants into the environment, some regulations have been published in the last few years. Recently, a watch list of substances for European Union-wide monitoring was reported in the Decision 2015/495/EU of 20 March 2015. Besides the substances previously recommended to be included by the Directive 39/2013/EU, namely two pharmaceuticals (diclofenac and the synthetic hormone 17-alpha-ethinylestradiol (EE2)) and a natural hormone (17-beta-estradiol (E2)), the first watch list of 10 substances/groups of substances also refers three macrolide antibiotics (azithromycin, clarithromycin and erythromycin), other natural hormone (estrone (E1)), some pesticides (methiocarb, oxadiazon, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid and triallate), a UV filter (2-ethylhexyl-4-methoxycinnamate) and an antioxidant (2,6-di-tert-butyl-4-methylphenol) commonly used as food additive. Since little is known about the removal of most of the substances included in the Decision 2015/495/EU, particularly regarding realistic concentrations in aqueous environmental samples, this review aims to: (i) overview the European policy in the water field; (ii) briefly describe the most commonly used conventional and advanced treatment processes to remove micropollutants; (iii) summarize the relevant data published in the last decade, regarding occurrence and removal in aqueous matrices of the 10 substances/groups of substances that were recently included in the first watch list for European Union monitoring (Decision 2015/495/EU); and (iv) highlight the lack of reports concerning

some substances of the watch list, the study of un-spiked aquatic matrices and the assessment of transformation by-products.

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2.A-1. Introduction

Water is a valuable resource, crucial to all living organisms and for multiple human activities, such as domestic uses, agriculture and industry. However, several contaminants of emerging concern (CECs) end up in vital aquatic compartments, such as surface water, groundwater and even drinking water, at concentrations between few ng L^{-1} and several µg L^{-1} [1], with negative impact on water quality. The occurrence of CECs in the environment is reported in thousands of publications during the last decades and reviewed by many authors [2-10], demonstrating an increasing concern about them. For instance, a series of periodic review articles focused on occurrence, fate, transport and treatment of CECs were published annually since 2007 [11] until 2011 [7], and then works on occurrence, fate and transport of CECs were reviewed separated from treatment since 2012 [10] until 2015 [6], due to the significant increase in the number of publications dealing with this particular topic.

CECs can be natural or anthropogenic substances such as pesticides, industrial compounds, pharmaceuticals, personal care products, steroid hormones, drugs of abuse and others [12]. Sources of CECs include: (i) industrial wastewaters; (ii) runoff from agriculture, livestock and aquaculture; (iii) landfill leachates; and (iv) domestic and hospital effluents, from which micropollutants might follow many pathways [13], as represented in Fig. 2.A-1.

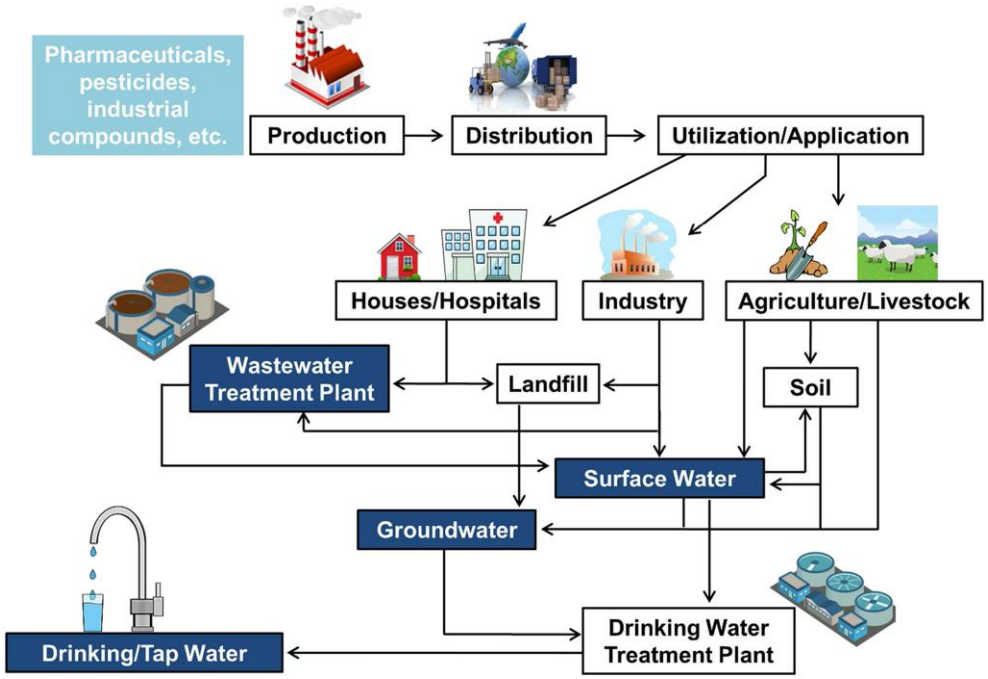


Fig. 2.A-1. Representative sources and routes of micropollutants in the environment.

The management of industrial effluents resulting from the production of pharmaceuticals, personal care products, pesticides and other compounds, has been properly done in several countries where regulations are already implemented, but more strict regulations are still needed in other regions of the world. The runoff from the agriculture and livestock areas is another important source of micropollutants, particularly in the case of pesticides used to improve productivity, as well as steroid hormones and antibiotics used for livestock [14, 15]. In addition, many contaminants and their intermediates can reach the fields when they are irrigated with treated wastewater and, as consequence, the receiving waters can also contain these substances [16]. Other source of CECs is the leakage from landfills and sewage treatment facilities, industrial waste systems and septic tanks [17]. The release of effluents from municipal wastewater treatment plants (WWTPs) is other important

route for the appearance of micropollutants in the aquatic environment [18], the wastewaters treated in these plants mainly resulting from domestic and/or industrial activities, as well as from hospitals.

In fact, most of the conventional WWTPs are not designed to completely eliminate organic compounds at low concentration, making the treatment processes vulnerable to such problem of pollution [18]. In this context, the non-degradable or partially removed compounds in WWTPs are likely to be detected in surface waters. In the cases of sewage sludge and soils, micropollutants can desorb and runoff to surface waters or undergo direct leaching to groundwater aquifers with consequent contamination of drinking water [19].

Agricultural reuse of sewage sludge in particular as fertilizer, is a common practice to improve the soil structure and provide nutrients but can represent a source of environmental contamination [20]. Moreover, sewage sludge solids sourced by wastewaters can be considered a sink of hazardous substances (e.g., such as pathogens, heavy metals and organic pollutants) that will accumulate in soils [20]. Due to the increasing concern about human health impacts, land application gained interest to convert sludge into a safer material through the treatment by anaerobic digestion, composting or other biological processes [21]. While composting is a controlled bio-oxidative process that converts sludge into stable and humic like materials, anaerobic digestion occurs in the absence of oxygen and has two main end products, a methane-rich biogas used as renewable energy source and the digested used as a fertilizer [21]. Removal of toxic organic contaminants by these processes was reported; however, their complete mineralization is difficult due to the adsorption mechanism and the formation of intermediates [21].

The fate and distribution of CECs will depend on the D_{OW}, which is a pH-dependent n-octanol–water distribution ratio that simultaneously considers hydrophobicity and

ionogenicity [22, 23]. Although most regulators use octanol-water partitioning coefficient (K_{OW}) to evaluate the hydrophobic partitioning, the environmental fate and transport should be based in the parameter D_{OW}, which is more accurate for organic ionizable compounds.

The contamination of environmental compartments, such as surface water, groundwater and soils, which are continuously interrelated, may cause cumulative negative effects along multigenerational exposure in aquatic organisms and/or affecting the human's health by drinking water contamination [24]. A great concern about the occurrence of micropollutants in the aquatic resources and the subsequent effects on humans and biota has been highlighted in the last few years. However, it is difficult to predict which environmental and public health implications may arise from the occurrence of CECs in freshwater ecosystems, since the individual concentrations usually found in the environment are lower than those able to cause direct negative effects [25]. For instance, concerning pharmaceuticals, toxicology studies have shown that they might have direct toxicity towards certain aquatic organisms [26]. The main issues related to the frequent occurrence of recalcitrant compounds are their simultaneous presence as complex mixtures and the long-term exposition that can lead to serious chronic effects, as reported by several studies [27, 28]. Their constant but imperceptible effects can gradually accumulate, finally leading to irreversible changes on both wildlife and human beings [29, 30].

Natural attenuation is a low-cost and simple process comprising physical, chemical and/or biological mechanisms to reduce contaminants concentration [31, 32]. Volatilization, dispersion, dilution, sorption, photolysis, biodegradation/transformation are the main natural attenuation processes [31, 32]. While volatilization has a minor impact, dispersion and dilution can lead to a significant decrease on the concentration of contaminants [33]. The dilution can decrease their concentration to levels where no

significant effects are verified for aquatic organisms. Sorption to sediments and suspended solids also reduce the concentration of CECs, but accumulation is enhanced. Indirect or direct photolysis can lead to removal of contaminants but is highly dependent on the presence of suspended matter and solar radiation. CECs can also be degraded by biodegradation/transformation, by bacterial enzymes [32].

The upgrading of the treatment processes for effluents generated by conventional WWTPs might minimize the discharge of micropollutants into the receiving waters and can even improve the overall quality status of effluents for possible reuse [34, 35]. The design improvement of WWTPs to include advanced treatment technologies, aiming to transform CECs into less harmful compounds or even to mineralize them, is one of the promising strategies to achieve this aim, as recently implemented in Switzerland. Advanced water treatment processes include adsorption (e.g., granular activated carbon), membrane and advanced chemical/oxidation technologies [36]. Other option is the implementation of natural systems to depurate water, such as riverbank filtration, aquifer recharge and recovery and constructed wetlands, which are reviewed in the literature [5, 37, 38] and will not be discussed in this work.

2.A-1.1. European policy

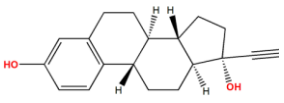
Although there are no legal discharge limits for micropollutants, some regulations have been published. The Directive 2000/60/EC was the first mark in the European water policy, which set up a strategy to define high risk substances to be prioritized [39]. A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by the Directive 2008/105/EC [40]. Two years ago, the European Union Directive 2013/39/EU recommended attention to the monitorization and treatment options for a group of 45 PSs [41], meeting the protection of the aquatic compartments and the human health. In that

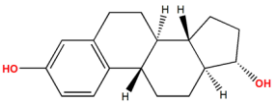
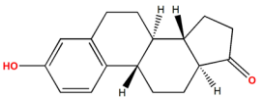
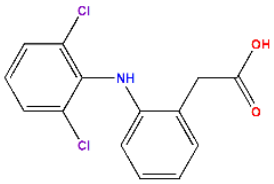
Directive, two pharmaceuticals (the non-steroid anti-inflammatory diclofenac and the synthetic hormone 17-alpha-ethinylestradiol EE2) and a natural hormone (17-beta-estradiol E2) were recommended to be included in a first watch list of 10 substances/groups of substances for European Union monitoring, to be launched within two years. In the first quarter of 2015, the Watch List of substances for European Union-wide monitoring (as set out in Article 8b of Directive 2008/ 105/EC) was amended in the Decision 2015/495/EU of 20 March 2015. Besides the abovementioned substances (diclofenac, EE2 and E2), three macrolide antibiotics (azithromycin, clarithromycin and erythromycin) were included, together with other natural hormone (estrone E1), some pesticides, a UV filter and an antioxidant commonly used as food additive, listed in Table 2.A-1. The frequent occurrence of CECs in the environment and the inefficiency of conventional WWTPs to remove such compounds, promoted the amendment of the framework to cover a larger set of hazardous compounds, as well as further recommendations for wastewater treatment steps or even new treatment scenarios. These actions should be implemented by the European Commission and regulated by the European country authorities.

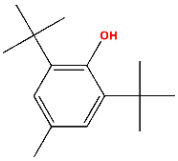
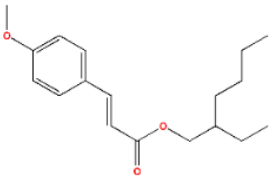
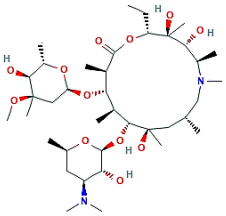
This review aims to summarize some relevant data of occurrence and removal in aqueous matrices of the 10 substances/groups of substances (i.e., a total of 17 organic compounds) enlisted in the first watch list for European Union monitoring, defined in the Decision 2015/495/EU. Studies on the occurrence of the referred substances (3 estrogens, diclofenac, 2,6-di-tert-butyl-4- methylphenol, 2-ethylhexyl-4-methoxycinnamate, 3 macrolide antibiotics, methiocarb, 5 neonicotinoids, oxadiazon and triallate) are shown in Table 2.A-1, for different aquatic compartments, namely wastewater, surface water and groundwater. Reports dealing with the removal of these 17 substances, only in real matrices, are overviewed below. The search comprised publications since 2005 (last decade) in Scopus database, using as

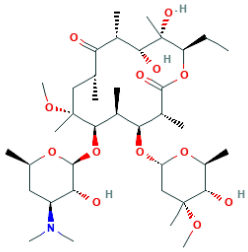
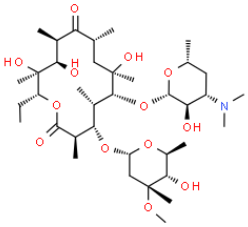
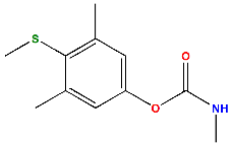
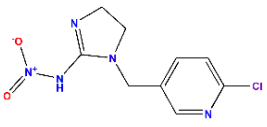
keywords each substance and the treatments herein reported. Most of the works refer to un- spiked aqueous environmental samples treated at lab, pilot or full scale, some describing the removal of these substances on spiked environmental matrices, and some including the comparison between the real matrix and ultrapure/deionized water. The first step of sample preparation is usually the filtration of the samples, and the works on occurrence take into account this step in the sample preparation protocol of the analytical method. Before such literature overview, the next sections (2.A-1.2, 2.A-1.3 and 2.A-1.4) present a brief description of the most commonly used conventional and advanced treatment processes.

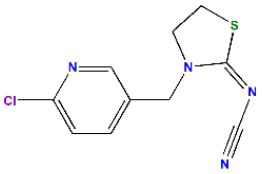
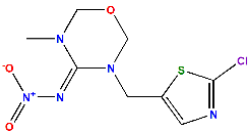
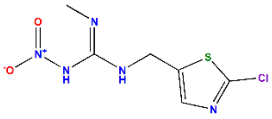
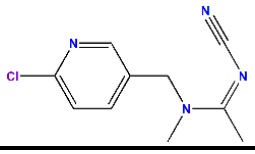
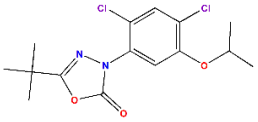
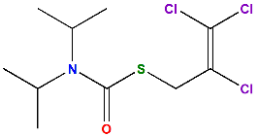
Table 2.A-1. List of 10 substances/groups of substances (total of 17 organic compounds) included in the watch list of EU Commission Decision 495/2015, and examples of their occurrence in different aquatic compartments, namely effluents of wastewater (WW), surface water (SW), and groundwater (GW). *n.a. refers to not available data.

Name of substance/group of substances	CAS number	(Substance) Structure	Concentration (ng L^{-1}) Matrix Locations (number of samples)	Ref.
17-Alpha-ethinylestradiol (EE2)	57-63-6		<1 – 8 WW Korea (n=120), Germany (n.a.), South Africa (n=12)	[42-44]
			0.2 – 1.9 SW China (n=3), Korea (n=120), Germany (n.a.), France (n=73)	[43, 45, 46]
			0.5 – 230 GW France (n=73), USA (n.a.)	[45-47]

17-Beta-estradiol (E2)	50-28-2		<1 – 88 WW China (n=3), Korea (n=120), Sweden (n=3), UK (n.a.), Germany (n.a.)	[42, 43, 48, 49]
			0.2 – 10.1 SW China (n=3), Korea (n=120), Germany (n.a.), Japan (n=517), France (n=71)	[43, 45, 46]
			0.3 – 147 GW France (n=73), USA (n.a.)	[45-47]
Estrone (E1)	53-16-7		<1 – 220 WW China (n=3), Korea (n=120), Sweden (n=3), UK (n.a.), Germany (n.a.)	[42, 43, 48, 49]
			0.5 – 69.1 SW China (n=3), Korea (n=120), Germany (n.a.), France (n=71)	[43, 45, 46]
			0.7 – 79 GW France (n=73), USA (n.a.)	[45-47]
Diclofenac	15307-86-5		14.9 – 4425 WW Spain (n.a.), Italy (n=3), USA (n.a.), Portugal (n=4)	[50-53]

			0.8 – 1043 SW Spain (n.a.), Vietnam (n.a), Costa Rica (n=86), Greece (n=30) 1.17 – 380 GW Spain (n=30), France (n=70)	[3, 54, 55]
2,6-di-tert-butyl-4-methylphenol (BHT)	128-37-0		49 - 620 SW USA (n=19), Sweden (n.a.)	[3, 45, 56]
2-ethylhexyl-4-methoxycinnamate (EHMC)	5466-77-3		4.7 – 505 WW China (n=17), Norway (n=5) 12 – 1040 SW Japan (n=23) 770 GW Spain (n=7)	[57, 58]
Macrolide antibiotics	83905-01-5	Azithromycin 	0.4 – 1220 WW Italy (n=3), Slovakia (n=3), USA (n.a.), Portugal (n=4) 0.6 – 90.8 SW Vietnam (n=2), China (n=24) 0.6 – 1620 GW Spain (n.a), China (n=69)	[59-61] [62]

	81103-11-9	Clarithromycin 	54 – 1890 WW Spain (n.a.), Italy (n=3), Slovakia (n=3), USA (n.a) 0.01 – 778 SW Vietnam (n=2), Spain (n=18), China (n=24) 0.2 – 20.5 GW Spain (n.a.), China (n=15)	[63]
	114-07-8	Erythromycin 	16 – 147.9 WW Spain (n.a.), Slovakia (n=3), USA (n.a.), China (n=3) 0.28 – 2246 SW Vietnam (n=2), Spain (n=18) 4.8 – 154.3 GW Spain (n=121), China (n=54)	[51, 53, 64, 65] [66, 67]
Methiocarb	2032-65-7		4.73 – 14.92 WW Spain (n=55)	
Neonicotinoids	105827-78-9 138261-41-3	Imidacloprid 	2 – 34.44 WW Spain (n=55) 1.1 – 105 SW Spain (n=24), USA (n=35), Greece (n=89), Portugal (n.a.), Australia (n=13)	[56, 67, 68]

	111988-49-9	Thiacloprid 	20 – 400 SW Australia (n=13)	[50-52, 64]
	153719-23-4	Thiamethoxam 	40 – 1580 SW Brasil (n.a.), Vietnam (n=11), Australia (n=13)	
	210880-92-5	Clothianidin 	20 – 420 SW Australia (n=13)	[66, 67, 69]
	135410-20-7 160430-64-8	Acetamiprid 	20 – 380 SW Australia (n=13)	
Oxadiazon	19666-30-9		4 – 1440 SW Canada (n=8)	[56, 67]
Triallate	2303-17-5		n.a.	n.a.

2.A-1.2. Treatment by conventional processes

The efficiency of a conventional WWTP varies depending on the characteristics of the pollutant and on the treatment process employed. The main mechanisms for removal

of micropollutants occurring during the secondary treatment at WWTPs are biological and/or chemical transformation and sorption [80, 81]. The most common employed processes are conventional activated sludge (CAS) and membrane biological reactors (MBRs).

The efficiency of a CAS system depends on the physicochemical characteristics of the substances and on the nature of the microbial community. The most important operational factors affecting the efficiency are the temperature, the hydraulic retention time (HRT) and the sludge retention time (SRT) [37, 82], a higher HRT favoring the removal of more refractory compounds and a higher SRT allowing a higher diversity of microorganisms [37]. The usual SRT in the CAS systems is 7 – 20 days and the biomass concentration 3 – 5 kg m^{-3}, with an HRT typically ranging from 2 to 24 h [80]. MBRs emerged as an alternative to CAS, integrating aerobic biodegradation and membrane separation, modestly more efficient than CAS in the extent of removal of several CECs [82]. MBR treatment differs mainly in the SRT that is normally longer (15 – 80 days) and the commonly higher biomass concentration (8 – 10 kg m^{-3}), HRT being often between 7 and 15 h [80]. Other important difference is the final stage using ultrafiltration (UF) or microfiltration (MF) membranes to separate the liquid from sludge. Therefore, MBR overcome the constraints of CAS treatment related to the sludge retention and settling characteristics, by applying these membranes to retain the biomass [37], decreasing the chemical oxygen demand while enhancing the removal of suspended solids and pathogens. Unlike the reports related to CAS, studies focusing on the performance of MBR processes to remove CECs are limited and difficult to compare due to the different operation conditions and target pollutants [83]. Verlicchi *et al.* [80] reviewed extensively the occurrence and removal of pharmaceutical compounds in municipal wastewater, comparing the effectiveness of the secondary treatment by CAS and MBR, with much more studies employing CAS

and using generally 24 h composite water samples, avoiding diurnal variability and favoring the inter-studies comparison. Pharmaceuticals and hormones that are now included in the watch list of Decision 495/2015 were referred in that review, where it was concluded that average removals found in the literature were superior employing MBR than CAS, namely between 26 and 44% for CAS and higher than 60% for MBR, except for azithromycin [80].

2.A-1.3. Formation of intermediates

Overall, most studies on both CAS and MBR have been focusing on the parent compounds and little attention has been given to the produced intermediates. It is noteworthy that biological or chemical reactions occurring in the secondary clarifiers might lead to the accumulation of metabolites/by-products [82]. There are also some compounds (e.g., pharmaceuticals, hormones, drugs of abuse that are excreted by humans and/or animals) that can be found at higher concentrations in the WWTPs effluents than in the respective influents, due to their excretion as conjugates that are broken in the WWTPs. These conjugates are generally metabolized during biological treatment and the parent compound is released, often increasing the concentrations of the parent compounds at the outlet of the WWTPs. For example, E1 can be detected in the secondary effluent of a WWTP at a higher concentration than that found in the raw influent, due to the oxidation of E2 that enters into the WWTP. This fact explains the occasional negative removal efficiencies, sometimes at high extents, with the greatest contribution of the biological transformation [80]. There are other causes for negative removals occurring during the WWTP treatment. In most cases, the sampling protocol does not consider the HRT and/or SRT and as consequence effluent does not correspond to the same plug of influent [72]. Sometimes the compounds can be released from particulate matter during treatment (e.g. macrolide

antibiotics released from feces particles) [83]. There are already some reports investigating the occurrence and removal of metabolites and/or intermediates; however, it is crucial to develop more studies on this matter, comprising the parent compounds, the possible by-products and the known metabolites in a broader and more comprehensive approach.

2.A-1.4. Separation by membrane technologies

Membrane filtration is mostly used for the removal of microorganisms and salts from water/wastewater. The most common membrane technologies include relatively low-pressure systems, such as MF and UF operating at pressures up to 5 and 10 bar, respectively, or high-pressure systems, namely nanofiltration (NF) operating at nearly 50 bar or reverse osmosis (RO) up to 70 bar (or 150 bar for high pressure RO systems) [82, 84, 85]. Among these types, the high pressure systems are more suitable for rejection of organic micropollutants, considering the size exclusion mechanism, but larger pores can be employed if electrostatic repulsion or adsorption are the main mechanisms involved in the process [82]. The parameters affecting the efficiency of the process include the molecular weight cut-off (MWCO), some membrane properties (e.g., hydrophobicity, surface roughness and charge) and physicochemical characteristics of the compounds to be rejected (e.g., molecular weight, p*K*a, octanol-water partitioning coefficient (K_{OW}) and polarity), among others [82]. Regarding the high pressure systems, the main characteristic of NF is the ion selectivity, where monovalent ions can pass through the membrane and multivalent anions are retained [85]. The rejection rates are high for organic compounds with molecular weights above 100 – 200 g mol^{-1} [86]. This process is typically applied for dye/color removal, but recent studies focused on the removal of emerging micropollutants from drinking water and wastewaters [87]. In RO the organic and

inorganic molecules are separated from the feed solution by their molecular weight (normally, less than 200 g mol^{-1}), size, charge and inability to permeate the active surface of the RO membrane [88]. The applications range from the production of ultrapure water, to the desalination of seawater for drinking water production and the treatment of industrial wastewaters [85]. More recently, RO was also applied for the removal of micropollutants, the process depending on complex interactions (e.g., steric, electrostatic/repulsion and hydrophobic) between the contaminants, the solution and the membrane [89]. Among the membrane processes, RO was considered as the ultimate treatment step yielding highest pollutant rejection efficiencies [90].

Forward osmosis (FO) and membrane distillation (MD) are some alternatives to the membrane processes exclusively based on hydraulic pressure. FO is an osmotically driven membrane process that consists on the osmotic pressure difference between the draw solution and the feed solution. Recently, FO has been more intensively investigated for water/wastewater treatment, as a single treatment or coupled to other membrane processes [84, 91]. MD (mainly developed for desalination) is based on a vapor pressure gradient across a porous hydrophobic membrane and can operate under different possible configurations (e.g., direct contact, vacuum, air gap and sweep gas MD) [92-94]. MD has also been studied to reject organic compounds in water treatment [95] since a complete rejection of inorganic ions and non-volatile substances is theoretically expected.

One of the major disadvantages in this type of processes is the production a concentrate containing all the retained compounds [96, 97]. The disposal of the concentrate can be performed by sewer disposal, evaporation ponds and deep well injection [98], but direct discharge to water bodies (oceans, surface and groundwater) is common and constitute potentially serious threat to ecosystems [99, 100]. Thus,

careful environmental practices are recommended to handle such a concentrated waste before discharge into the aquatic environment [101]. Different approaches for the treatment of membrane concentrates have been investigated, mainly using AOPs, but also coagulation/flocculation and adsorption with activated carbon were reported [96, 97, 102]. However, most of these emerging technologies have been developed at laboratory or pilot plant scale [99]. Good results have been achieved by AOPs for the removal of organic pollutants and persistent compounds, but the cost of these processes can limit their wide implementation at full scale [99, 101].

2.A-1.5. Degradation by advanced oxidation processes (AOPs)

Advanced oxidation processes (AOPs) are conceptually based on the production of highly reactive oxidizing species, such as hydroxyl radicals ($HO^{\bullet}$). AOPs are able to degrade unselectively organic pollutants [103] and can be used as pre- or post-treatment of a biological process. As pre-treatment, the aim of a single or a sequence of complementary AOPs is to obtain a more biodegradable effluent able to be treated by a conventional biological process. AOPs can be used as post-treatment to remove micropollutants and their by-products, ideally yielding as final products CO_2, H_2O and inorganic ions, if the aim is the direct discharge in natural water courses. One shortcoming often found in the application of AOPs for wastewater treatment is the frequent presence of radical scavengers in the wastewater, limiting the attack of the radicals to the organic pollutants. Commonly employed AOPs to investigate the treatment of micropollutants in real matrices, include the Fenton and photo-Fenton processes, (catalytic) wet peroxide/air oxidation, (catalytic) ozonation, heterogenous photocatalysis, electrochemical oxidation or combination of them. For the catalytic processes, different catalysts have been identified as the most active depending on the reaction system, including metal oxides (based on Ti, Cu, Zn, Mn, Fe, Co and Bi,

among others), supported noble metals (e.g., Ru, Pt, Pd, Ir and Rh), or even metal-free carbon materials such as activated carbons, carbon xerogels, carbon nanotubes, carbon foams and fibers and graphite [12].

Briefly, the Fenton process, based on the Fenton reagent [104], employs H_2O_2 and a precursor of iron, generating $HO^{\bullet}$ at atmospheric pressure and room temperature. High efficiency, relatively cheap reagents, no need of energy to activate H_2O_2 and the consequent easy implementation and operation are the advantages of such treatment. Some disadvantages are the generation of a secondary waste (sludge) and the narrow range of optimal pH (2.5 e 3.0). The photo-assisted Fenton process can be more efficient than Fenton alone, mainly due to the faster regeneration of Fe^{2+} [105]. Other related options are electro-Fenton, where Fe^{2+} is produced from sacrificial cast iron anodes [106], or even photo-electro-Fenton [107].

The concept of catalytic wet peroxide oxidation is similar to that of the Fenton process, but in this case any catalyst can be used (not only iron species) and slightly higher temperatures (50–70 ºC) are typically employed (the operating pressure and temperature dramatically increasing in the case of wet air oxidation).

Regarding ozonation, this process involves the direct attack of ozone (quite selective for electron-rich organic molecules) mainly at low pH and/or indirect reactions through $HO^{\bullet}$ more prone at high pH [108, 109]. The main handicap of ozonation is the typical low efficiency to mineralize the organic pollutants, while natural organic matter (NOM) and carbonate ions can have a significant interference with the ozone decomposition rate [110]. For this reason, different heterogeneous catalysts are under investigation to improve the process [111-116].

Heterogeneous photocatalysis is other process that has been extensively investigated for water/wastewater treatment and is based on the use of wide band-gap semiconductors which generate electrons and holes (and subsequent chain reactions

including HO•) when irradiated with photons of energy higher than the semiconductor band-gap (i.e., $h_v \leq E_g$) [117, 118]. TiO_2 is the most widely used reference photocatalyst due to the outstanding activity, photochemical stability, good band gap energy, low cost and relatively low toxicity [119, 120]. The possible use of sunlight and the intrinsic anti-microbial ability of heterogeneous photocatalysis [121-124] are counterbalanced by its main shortcomings, such as the fast recombination of electron-hole pairs and the limited usage of solar light when bare TiO_2 is employed (i.e., only the UV fraction, near 3–5% of the overall spectrum) [125]. A recent approach is the hybridization of photocatalysis with membrane processes, with emphasis in the preparation of new filtration membranes with photocatalytic properties [126, 127].

Sonolysis, supercritical water oxidation, γ-ray irradiation, microwaves and pulsed electron beam are less commonly applied AOPs [12].

2.A-2. The Watch List: occurrence and removal

This section aims to overview the substances and group of substances of the watch list for European Union monitoring, defined in the Decision 2015/495/EU, regarding their occurrence in aqueous matrices as well as their removal by using the above-mentioned treatments. Scopus database was used and the keywords were the name of each substance and the following treatments: CAS, MBR (conventional processes); RO, MF, UF, NF, FO or MD (membrane technologies); and UV- and peroxide-based, Fenton-based, heterogeneous photocatalysis or ozonation-based processes (AOPs). The studies selected for this review were performed using realistic matrices. Considering the huge amount of literature available for estrogens (EE2, E2 and E1) and for diclofenac, only some examples of studies related to treatment processes for these particular substances (Tables 2.A-2 and 2.A-3) are included in this review.

2.A-2.1. EE2, E2 and E1

Steroid hormones include highly active biological compounds able to induce the therapeutic effect at very low doses. Within this group, estrogens are the most usually found in the aquatic environment, existing either as natural or synthetic substances and acting as endocrine-disrupting compounds (EDCs) [128, 129]. Estriol, E1 and E2 are natural estrogens mainly excreted from humans whereas EE2 is the most used oral contraceptive, also excreted by humans, causing injurious effects to the ecosystems such as feminization of male fishes, DNA and immunity alterations [3]. The effects of EDCs toward animals are well reported, for example, a 7-year experiment was developed [27] and it was concluded that the chronic exposure of fathead minnow to 5 – 6 ng L^{-1} of EE2 led to feminization of male fish and altered oogenesis in females. Some studies suggested that the effect of EDCs exposure on human health includes a decrease in male sperm count, an increase in testicular, prostate, ovarian and breast cancers and reproductive malfunctions [130]. The major concern is related to fetuses and newborn babies, because of their higher vulnerability [131]. Recently, Kabir *et al.* [132] reviewed extensively the mechanism of action and harmful effects of EDCs on human health; and Fuhrman *et al.* [133] highlighted the EDCs risk assessment, namely issues related to long-term and combined exposure, transgenerational and mixture effects. Due to the potential deleterious effects that can arise from their release into the environment, their occurrence is well described and reviewed by several authors [3, 134, 135]. Table 2.A-1 summarizes some studies on the occurrence of E1, E2 and EE2 (concentration, matrix and location), which are frequently found in water matrices, namely wastewater, surface and groundwater, at ng L^{-1} levels.

The removal of these hormones is reported in several studies (Fig. 2.A-2), varying depending on the processes (Table 2.A-2). Biological treatments coupled with membrane processes are reported as effective mean for elimination of these type of compounds [136]. As example, more than 90% of EE2 was removed in an advanced wastewater reclamation plant employing a biological treatment and MF [137]. Few studies were developed using other membrane technologies to remove E1, E2 and EE2 (Table 2.A-2), being highly removed by NF and/or RO [138, 139]. AOPs are promising to remove this type of pollutants, with ozonation having the highest efficiency (Table 2.A-2). Data regarding these compounds can be consulted in article reviews that have been published in the last few years and that already encompass a significant amount of information dealing with their removal from water [45, 140-144]. Concerning the studies on the removal of the substances of the watch list, it can be concluded that E1, E2 and EE2 were the most studied in the last decade, employing all the types of processes herein referred (Fig. 2.A-2).

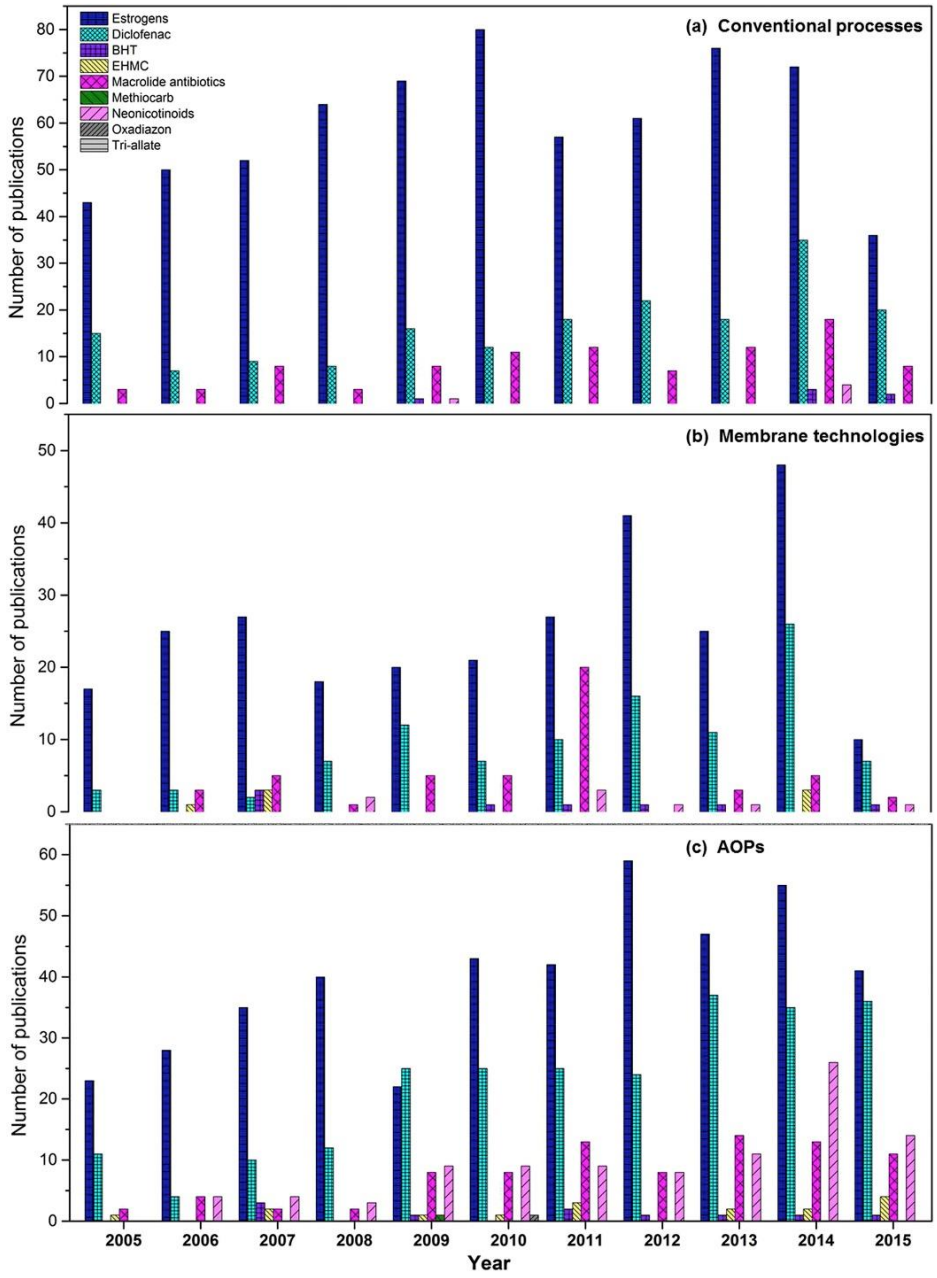


Fig. 2.A-2. Number of publications dealing with the removal of the 10 substances/groups of substances included in the first watch list for European Union monitoring (Decision 2015/495/EU). The search comprised publications since 2005 in Scopus database, using as keywords each substance and the treatments reported in the previous sections, namely **(a)** conventional processes (CAS or MBR); **(b)** membrane technologies (RO, MF, UF, NF, FO or MD); and **(c)** AOPs (UV-and peroxide based, Fenton based, heterogeneous photocatalysis or ozonation-based processes). In this particular search, any type of matrix (realistic and non-realistic) was considered.

Table 2.A-2. Some examples of studies dealing with the removal of E1, E2 and/or EE2. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
E1	ng L^{-1} level	3 pilot WWTPs, one employing CAS; Average flow rate: 107 dm^3 d^{-1}; SRT 3 d; HRT 7 h; 24 h composite samples; Burlington Skyway municipal WWTP; Ontario, Canada.	Removal efficiency of CAS was greater than 65% for E1.	[145]
E2 EE2 E1	14.5 ± 4.5 ng L^{-1} n.d. 3.2 ± 4.1 ng L^{-1}	Municipal WWTP with biological and chemical treatment; Average flow rate: 20,000 m^3 d^{-1}; Kristianstad; South Sweden.	Removals of 78% and > 47% were observed for E1 and E2, respectively.	[49]
E2	10 µg L^{-1} (spiked wastewater)	Lab-scale MBR and CAS; Industrial-municipal mixed wastewater before secondary treatment.	E2 was almost completely removed (99%) applying both treatments.	[146]
E1 E2 EE2	n.d./n.d.; up to 23.2/21.2 ng L^{-1}; up to 22.2/29.2 ng L^{-1}.	WWTPs: CAS or MBR coupled with UF or MF; 24 h composite samples composed by 4 h-aliquots collected by an automatic device; Granada, Spain.	The concentrations after the CAS and MBR treatments were respectively: up to 0.81 and 4.9 ng L^{-1} for E2 and up to 6.62 and 6.92 ng L^{-1} for EE2. MBR system was shown as good alternative to provide high-quality water for reuse. MBR with MF was more efficient for E2 removal.	[136]
EE2	140 ng L^{-1} (after primary clarifier).	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 m^3 d^{-1}; Gwinnett County, GA, USA.	After CAS and MF, the concentration of EE2 decreased by more than 90%. Ozonation oxidized the remaining compounds by more than 60%.	[137]

EE2 E1	8.73 ng L^{-1} 20.69 ng L^{-1}	Pilot-scale combination of MBR and NF or RO; MBR permeate flux: 10.5 L m^{-2} h (constant flux mode); 4-L samples of the influent and effluents of each MBR, NF and RO process.	Removal efficiencies higher than 70% (based on the detection limits) were verified for E1 and EE2 with each treatment process.	[138]
E1 E2 EE2	150 µg L^{-1} (spiked surface water)	Lab-scale UF prior to NF; NF experiments were conducted at 10 bar and 3.6 cm s^{-1} of cross-flow velocity; Surface water from Tagus river, Portugal.	High rejections (higher than 90%) were obtained for E1, E2 and EE2.	[147]
E2 EE2	0.2 µg L^{-1} (spiked wastewater)	MF and RO or MF prior to a pilot-scale UV/H_2O_2; LP-UV lamp; H_2O_2: 3 mg L^{-1}.	Removal of 99% was achieved in both cases.	[148]
E2 EE2 E1	1 mg L^{-1} (spiked surface water)	Multi-barrier approach; Lab-scale NF followed by LP-UV (λ_{max} = 245 nm) or indirect (H_2O_2-assisted) LP-UV; H_2O_2: 0, 20, 40, 60, 80 or 100 mg L^{-1}; Surface water.	Rejection of 71% verified by NF (for all the compounds). Direct photolysis led to high E1 removal, while a removal > 74% was obtained by indirect (H_2O_2) photolysis. The multi-barrier approach led to higher overall removals (80, 90 and 95% for E2, EE2 and E1, respectively).	[149]
E1 E2 EE2	1.65 – 3.59 µg L^{-1} (treated wastewater from the secondary clarifier)	Pilot plant O_3; O_3/UV; O_3/ H_2O_2 and O_3/UV/H_2O_2; O_3: 3.15 g h^{-1}; 5% of ozone in gas mixture.	A removal higher than 99.7% was observed for the 3 estrogens.	[150]
E1	3 µg L^{-1} – 5 mg L^{-1} (spiked wastewater)	Lab-scale O_3,UV,UV/H_2O_2,O_3/UV,O_3/ H_2O_2 and O_3 /UV/ H_2O_2; Annular reactor (750 mL); LP-UV lamp (λ_{max} = 253.7 nm); O_3: 0.33 – 1.31 mg L^{-1};	A complete removal after 30 min was achieved, employing all processes, except for UV (75 min). Ozonation achieved the higher removal rates of E1. Low TOC removal was observed for all the AOPs	[151]

		H_2O_2: 20, 40 and 60 mg L^{-1}; Municipal wastewater (London, OR, Canada).	tested, with the degradation rate decreasing with higher TOC values.	
E2 EE2 E1	0.035 mg g^{-1} dw 0.150 mg g^{-1} dw 0.125 mg g^{-1} dw	Lab-scale UV, H_2O_2 and UV/H_2O_2; Reactor with continuous recirculation (800 mL); 75 W LP Hg lamp (λ_{max} =253.7 nm); H_2O_2: 0.5 mol L^{-1}; pH 3; Spiked waste activated sludge.	E2, EE2 and E1 were removed respectively by 92%, 95% and 97%, after 2 min. UV/H_2O_2 was more efficient than UV or H_2O_2 alone. The sludge matrix influenced the degradation rate.	[152]
EE2	10 mg L^{-1} (spiked wastewater)	Catalytic ozonation; O_3: 20 mg L^{-1}; Catalysts: 5 g of commercial γ-Al_2O_3 or synthesized Co_3O_4/Al_2O_3; Ultrapure water and secondary effluents pre-treated to remove its carbonate/bicarbonate content by stripping; Municipal wastewater from a WWTP; Badajoz, Spain.	EE2 was removed in less than 10 min, regardless the matrix or the presence of catalyst. Comparing with single ozonation, catalytic ozonation enhanced the COD and TOC removals, especially in the presence of the Co_3O_4/Al_2O_3 catalyst.	[153]
EE2 E1	< 4.3 – 7.4 ng L^{-1} 1.6 – 2 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86 ─153 g Nm^{-3}; O_3 consumption: 0.6 and 0.9 g O_3 g DOC_0^{-1}; Wastewater; Austria.	The application of 0.6 g O_3 g DOC^{-1} increased the removal of these compounds (to values < LOD).	[154]
E2 EE2 E1	10 – 250 ng L^{-1} (spiked river water)	Ozonation; O_3: 3 - 4 mg L^{-1}; River water.	High removal (98 – 99%) after 10 min was achieved by ozonation process for all estrogens.	[155]

EE2 E2 E1	391.4. ± 59.3 ng L^{-1} 110.4 ± 55.4 ng L^{-1} 20.2 ± 3.3 ng L^{-1}	Lab-scale photolytic ozonation, ozonation and photocatalysis; O_3 flow rate: 150 Ncm^3 min^{-1}; O_3: 50 g Nm^{-3}; MP mercury vapor lamp (UV/Vis λ > 300 nm); TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.	Complete removal by photocatalytic ozonation was achieved for all estrogens, while EE2 was not completely removed using ozonation (77.2% only) and E1 was not completely removed using photocatalysis (61.8% only).	[156]
EE2	2.0 μM (spiked surface water)	Quartz photolysis tubes (1.4 cm i.d. × 20 cm) at a 45º angle were used in photodegradation experiments; Lake water from Lake Quinsigamond.	EE2 showed very high resistance to microbial degradation while rapid photodegradation under sunlight irradiation occurred (half-life of 23 h).	[157]

AOP, advanced oxidation process; CAS, conventional activated sludge; COD, chemical oxygen demand; DOC, dissolved organic carbon; dw, dry weight; GAC, granular activated carbon; HRT, hydraulic retention time; LP, low pressure; MBR, membrane biological reactor; MF, microfiltration; n.a., not available; n.d., not detected; NF, nanofiltration; RO, reverse osmosis; SRT, sludge retention time; TOC, total organic carbon; UF, ultrafiltration; WWTP, wastewater treatment plant.

2.A-2.2. Diclofenac

Regarding the non-steroidal anti-inflammatory drug (NSAID) diclofenac, it is considered harmful to several species at environmental concentrations, as indicated by Vieno *et al.* [158], who overviewed its occurrence, fate and transformation processes during treatment in WWTPs. Diclofenac is often detected in WWTP influents and effluents, surface waters and groundwater. Table 2.A-1 describes some studies on the occurrence in these aquatic compartments, with diclofenac found up to 4.4 µg L^{-1}. Information concerning the removal of diclofenac can be checked in article reviews that have been published in the last few years and that already include systematized data of its removal from water [159-163]. Diclofenac can be partially adsorbed on sludge and is usually poorly biodegradable, which means low removal rates during biological wastewater treatment (Table 2.A-3) [158, 164]. Membrane technologies to remove diclofenac have been used, but more research is needed (Table 2.A-3). Concerning AOPs, some studies dealing with heterogeneous photocatalysis and/or photo-Fenton are described in Table 2.A-3, with a moderate diclofenac removal, most using a pilot compound parabolic collector (CPC) plant and a high reaction time. Ozonation as single process, or combined with photolysis and/or photocatalysis, has been widely investigated showing a high performance for diclofenac removal. Overall, diclofenac is the second most studied substance of the watch list in the last 10 years, employing all the types of processes (Fig. 2.A-2).

Table 2.A-3. Some examples of studies dealing with removal of diclofenac. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial diclofenac concentration	Treatment and sampling conditions	Concluding remarks	Reference
Up to 12.7 ng L^{-1} (CAS) up to 38 ng L^{-1} (MBR)	WWTP with CAS or MBR coupled to UF or MF; 24 h composite samples composed by 4 h aliquots collected by an automatic device; Granada, Spain.	Mean removal rates were between 54 and 71% for MBR and approximately 79% for CAS.	[136]
30 mg L^{-1} (spiked surface water)	Photocatalysis and solar photolysis; Advanced lab-scale reactor immersion-well (UV-RS-1) made of Pyrex glass (cut-off <290 nm); Solution volume: 400 mL; MP Hg vapour lamp: TQ 150 Heraeus, Germany, 150 W; TiO_2 load: 0.1 g L^{-1}; pH 6.2.	Water quality showed high influence in the treatment efficiency. For river water, solar photolysis showed higher removal compared to TiO_2 photocatalysis, with 66% and 82% diclofenac removal for photocatalysis and direct sunlight, respectively.	[165]
0.05 µg L^{-1} (spiked surface water)	Kagithane Drinking Water Treatment Plant; Flow rate: 700,000 m^3 d^{-1}; Lab-scale NF; MF: cross-flow rate of 3 L min^{-1} and water flux of 137 L m^{-2} h^{-1}; Raw water sources: Terkos Lake and Alibeyköy Dam.	Diclofenac overall rejection was approximately 61%.	[166]
220 ng L^{-1} (after primary clarifier)	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 m^3 d^{-1}; Gwinnett County, GA, USA.	Diclofenac was removed by CAS, between 51 and 80%, achieving the LOQ.	[137]
WWTP1: 507 ng L^{-1}; WWTP2: 1450 ng L^{-1}.	WWTP1: parallel CAS and MBR serving 28,000 inhabitants and treating 5,544 m^3 d^{-1} by CAS and 7,237 m^3 d^{-1} by MBR;	Removal was not observed in both treatments of WWTP1 employing parallel CAS and MBR. Almost no removal occurred in WWTP2, with 1391 ng L^{-1} in the effluent of CAS.	[167]

	WWTP2: CAS serving 100,000 inhabitants and treating 20,846 m^3 d^{-1}; 24 h composite samples; Lede, Belgium.		
Up to 2400 ng L^{-1}	15 WWTPs designed for 6,850 to 756,000 population equivalents; Flow rates: 349 – 140,000 m^3 d^{-1}; Discharge points: main Portuguese rivers and Atlantic Ocean; 24 h composite influent and effluent samples; Portugal.	Concentration of diclofenac was quantified up to 670 ng L^{-1} in the effluent. The mean removal of this substance was 45.6%.	[53]
Up to 0.49 µg L^{-1}	4 WWTPs impacted by effluents from mid-size hospitals, corresponding to a WWTP inflow between 1 and 30%; Flow rates: 1300 – 103,000 m^3 d^{-1}; WWTP 1/3 - SBR and UV-tertiary treatment; WWTP 2 – CAS; WWTP 4 - oxidation ditch activated sludge process; 24 h composite samples; New York, USA.	WWTP 2 and 4, employing CAS or oxidation ditch activated sludge, were more efficient than the others for the removal of diclofenac.	[168]
Up to 6.34 ng L^{-1}	4 WWTPs; Chongqing, China; 2 WWTPs: anaerobic/anoxic/oxic (A/A/O) activated sludge process; 1 WWTP: CAST; 1 WWTP: OD.	Although diclofenac was quantified up to 4.7 ng L^{-1}, the removal was not assessed due to its detection in some cases, below than LOQ.	[169]
n.a.	MBR pilot plant in continuous operation (ca. 1% of diurnal hospital sewage); 2 h composite influent and effluent samples; Luxembourg.	Diclofenac was removed at an extent between 40 and 50%. UV was evaluated as post-treatment, improved degradation achieved by applying H_2O_2.	[170]
6.01 ng L^{-1}	4th largest WWTP in China, serving 1,540,000 equivalent inhabitants; 600,000 m^3 d^{-1}. CAST followed by chlorination; Grab samples collected according to the residence time in each treatment;	The removal obtained after secondary treatment was 41.8% for diclofenac. Chlorination led to a reduction of 8.6%.	[70]

	Southwest China.		
361 – 911 ng L^{-1}	Pilot-scale MF followed by RO; MF: flow rate of 2 m^3 h^{-1} and flux of 323 L m^2 h^{-1}; Residence time 3 min; RO: flow rate of 1 m^3 h^{-1} and permeate flux of 34 L m^2 h^{-1}; Residence time 50 min; Treated effluent; Girona, Spain.	High removal of diclofenac was observed. RO reduced the concentration of diclofenac in the MF permeate to levels below the limit of detection.	[171]
57 – 131 ng L^{-1}	Pilot-scale NF and RO; NF: Water flux between 12 and 62 L m^2 h^{-1}, depending on the type of membranes; RO: Water flux of 23.5 L m^2 h^{-1}; Treated effluent; Sydney, Australia.	RO was the most efficient treatment for the rejection of diclofenac, reaching concentrations lower than ng L^{-1}.	[172]
104.1 ng L^{-1}	Pilot-scale UF followed by a RO; UF permeate flux: 227 m^3 d^{-1}; RO permeate flux: 82 m^3 d^{-1}; Ansan, Gyeonggi-do, Korea.	Permeate UF: 69.7 ng L^{-1}; and permeate RO: n.d.; Concentration of diclofenac considerably decreased by UF. RO completely removed diclofenac.	[173]
750 ng L^{-1}	Pilot-scale NF; Flux: 1 – 2 L m^2 h^{-1}; 24 h composite sample; Giessen, Germany.	Diclofenac decreased by at least 65%.	[174]
605 ng L^{-1}	2-L reactors at 25 ^oC; UV/H_2O_2: 3 LP Hg lamps (λ_{max} = 254 nm); H_2O_2 consumed ranged from 0.04 to 0.72 mg H_2O_2 mg TOC^{-1}; Ozonation: 10 g O_3 Nm^{-3}; RO concentrates from a municipal WWTP.	UV/H_2O_2 exhibited higher performance than ozone in the removal of diclofenac, which had one of the lowest initial observed kinetic constants probably due to the matrix effects on the process.	[175]
935 ng L^{-1}	2-L reactors at 25 ^oC; UV/H_2O_2: 3 LP Hg lamps (λ_{max} = 254 nm); H_2O_2 consumed ranged from 0.01 to 0.90 mg H_2O_2 mg TOC^{-1};	Diclofenac was completely decomposed by UV, after the first minutes of treatment; it was also removed by UV/H_2O_2 and ozonation process.	[96]

	RO concentrates from a municipal WWTP in a coastal area of Catalonia, Spain.		
283 ng L^{-1}	Biological activated carbon (BAC) process to treat municipal wastewater RO concentrate; Lab scale during 320 days of operation; BAC, combined UV/ UV/H_2O_2–BAC and ozone–BAC.	54% of Diclofenac was removed by the BAC filter. The integration of the UV/ H_2O_2 or the ozonation processes was necessary to obtain a complete removal of diclofenac.	[100]
> 750 ng L^{-1}	Photocatalysis in a pilot-scale CPC plant under natural solar irradiation; TiO_2 load: 20 mg L^{-1}; Effluents of the biological treatment of El Ejido WWTP; Almería, Spain.	Complete diclofenac removal was achieved after 480 min.	[50]
671 – 4941 ng L^{-1}	Photo-Fenton in a pilot-scale CPC plant; Fe^{2+}: 5 mg L^{-1}; pH: 3 and 10; H_2O_2: 50 mg L^{-1}; Complexing agents (humic acid and ethylenediamine-N,N'-disuccinic acid); Effluents of the secondary treatment in a municipal WWTP; Almería, Spain.	Diclofenac was removed by 97% in the photo-Fenton process (pH 3), after 50 min. Photo-Fenton with humic acids at neutral pH resulted in a longer treatment time required to reach a similar degradation.	[176]
≈ 70 ng L^{-1}.	Bench-scale UV and UV/H_2O_2 (λ_{max} = 254 nm); H_2O_2: 7.8 mg L^{-1}; Volume and HRT: 35 L and 5 min, respectively; Capacity: 10 m^2 day^{-1}; Municipal WWTP; Japan.	A complete removal of diclofenac was observed for both processes.	[177]
10 µg L^{-1} (spiked surface water)	UV/H_2O_2 in a pilot plant with three parallel reactors with MP, LP or dielectric barrier discharge UV lamps. Pre-treated surface water (by coagulation, flocculation and sedimentation in a natural reservoir, micro-straining and dual layer rapid sand filtration)	The degradation of diclofenac was higher than 80%.	[178]

	from Meuse River (Netherlands), spiked with a mixture of 15 compounds.		
n.a.	Sulfate radical based homogeneous photo-Fenton involving peroxymonosulfate as an oxidant, ferrous iron (Fe(II)) as a catalyst and simulated solar irradiation as a light source; Biologically treated domestic wastewater effluents.	PMS/Fe(II)/UV–Vis advanced oxidation system using simulated solar irradiation has demonstrated better kinetic performances over TiO_2/UV–Vis system for clothianidin.	[179]
0.1 mg L^{-1} (spiked wastewater)	Heterogeneous photocatalysis and Photo-Fenton; Pilot-scale CPC solar plant at the Plataforma Solar de Almería (Spain); A: Photo-Fenton (pH 2; 5 mg L^{-1} of Fe^{2+}; 50 mg L^{-1} of H_2O_2; 5 mg L^{-1} of TiO_2); B: no pH adjustment; 50 mg L^{-1} of H_2O_2; 5 mg L^{-1} of Fe^{2+} (demineralized water); 5, 15 and 55 mg L^{-1} of Fe^{2+} (standard freshwater); 5 mg L^{-1} of Fe^{2+} (standard fresh water without $NaHCO_3$).	Solar TiO_2 photocatalysis showed complete diclofenac degradation. 20 − 50% of degradation in demineralised water was achieved in the dark (Fenton process) and photo-Fenton was the most effective treatment with a complete removal observed after 20 min. In standard fresh water, diclofenac was removed by Fenton process.	[180]
0.276 µg L^{-1}	Heterogeneous photocatalysis: Solardetox Acadus-2006 CPCs with 3.0 m^2 irradiated surface and 24 L of irradiated volume; TiO_2 load: 0.2 g L^{-1}; Effluent of a WWTP from the South East of Spain.	High diclofenac removal (≈ 88%) was observed after 3 h of treatment (bellow LOQ) applying solar TiO_2 photocatalysis.	[181]
10 mg L^{-1} (spiked wastewater)	Catalytic ozonation; O_3: 20 mg L^{-1}; Catalysts: 5 g of commercial γ-Al_2O_3 or synthesized Co_3O_4/Al_2O_3; Ultrapure water and secondary effluents pre-treated to partially remove its carbonate/bicarbonate content by stripping; Wastewater from a municipal WWTP; Badajoz, Spain.	Diclofenac removed in less than 10 min, regardless the matrix or the presence of catalyst. Comparing with single ozonation, catalytic ozonation enhanced the COD and TOC removals, in particular with a Co_3O_4/Al_2O_3 catalyst.	[153]

30 to 80 mg L^{-1} (spiked wastewater)	UVA, O_3, O_3/UVA; O_3/TiO_2; O_3/UVA/TiO_2; O_3: 5 – 30 g m^{-3}; HP mercury lamp; TiO_2 load: 0.5 and 2.5 g L^{-1}; Ultrapure water and urban wastewater from a municipal WWTP; Badajoz, Spain.	Complete removal of diclofenac by applying photocatalytic ozonation within 6 min (60 - 75% TOC reduction after 60 min, regardless the water matrix used. Photocatalytic ozonation showed the lowest ozone consumption compared to the other ozonation processes.	[182]
30 mg L^{-1} (spiked surface water)	Single ozonation and catalytic ozonation; O_3: 10 g m^{-3}; pH = 7; Catalysts: 1 g L^{-1} of lab-prepared Mn-Ce-O or a commercial (N-150) catalyst; Synthetic effluent and river water collected from Mondego River; Portugal.	The catalysts had no significant effect on diclofenac removal when compared with single ozonation. However, both catalysts increased the COD removal per mg of ozone applied.	[183]
n.a.	Bench-scale photolysis; 150 W MP Hg, which emits radiation between 200 and 450 nm; Municipal wastewater of secondary effluent of a biological WWTP; Portugal.	The degradation rate constants obtained for diclofenac in a filtered wastewater matrix were lower than in a pure water matrix.	[184]
2.5 mg L^{-1} (spiked wastewater)	Lab-scale TiO_2 photocatalysis; 125 W black light fluorescent lamp (300 - 420 nm); Catalyst load: 0.2–0.8 g L^{-1}; Urban WWTP effluent.	TiO_2 photocatalysis showed a high removal of diclofenac (≈98%).	[185]
100 µg L^{-1} (spiked wastewater)	Solar photo-Fenton in a pilot-scale solar CPC reactor; H_2O_2 dose = 0 – 50 mg L^{-1}; Fe = 5 mg L^{-1}; Municipal wastewater.	Diclofenac was completely removed (< LOQ) after 34 min.	[186]
464.8 ± 64.7 ng L^{-1}	Lab-scale photolytic ozonation, ozonation and photocatalysis; O_3: 50 g Nm^{-3}; O_3 flow rate: 150 Ncm^3 min^{-1}; MP mercury vapor lamp (UV/Vis λ > 300 nm);	For all processes in study, 100% removal was achieved.	[156]

	TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.		
13.5 – 52.0 µg L^{-1} (spiked wastewater)	Lab-scale ozonation; O_3: 5.5 – 8.5 mg L^{-1}; O_3 flow rate: 0.39 NL min^{-1}; Urban wastewater samples from the secondary clarifier of two WWTPs from West-Alcalá and Alcázar de San Juan; Spain.	High diclofenac removal (> 90%) was observed.	[187]
970 – 2300 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86 ─153 g Nm^{-3}; O_3 consumption: 0.6 and 0.9 g O_3 g DOC_0^{-1}; Municipal wastewater; Austria.	The application of 0.6 g O_3 g DOC^{-1} increased the removal of diclofenac (to values < LOQ).	[154]
5 – 20 mg L^{-1} (spiked wastewater)	UV-A/TiO_2 photocatalysis: 9 W lamp; Catalyst load: 50 – 1600 mg L^{-1}; H_2O_2 = 0.07 – 1.4 mM; Treated municipal effluent from Limassol; Cyprus.	UV-A/TiO_2 is an efficient method for the degradation and mineralization of diclofenac in treated municipal effluents.	[188]

BAC, Biological activated carbon; CAS, conventional activated sludge; CAST, cyclic activated sludge technology; COD, chemical oxygen demand; CPC, compound parabolic collector; GAC, granular activated carbon; HRT, hydraulic retention time; LOQ, limit of quantification; LP, low pressure; MP, medium pressure; MBR, membrane biological reactor; MF, microfiltration; n.a., not available; n.d., not detected; NF, nanofiltration; OD, oxidation ditch; RO, reverse osmosis; SBR, Sequential Batch Reactor; TOC, total organic carbon; UF, ultrafiltration, WWTP, wastewater treatment plant.

2.A-2.3. 2,6-di-tert-butyl-4-methylphenol

The anti-oxidant 2,6-di-tert-butyl-4-methylphenol (BHT) has been used as a common anti-oxidant to preserve and stabilize the freshness, nutritive value, flavor and color of food and animal feed products, since the 1950s [189, 190]. BHT can also improve the stability of pharmaceuticals and cosmetics and increase the durability of rubber and plastics. Approximately 40 countries allow the use of BHT as a direct or indirect food additive [190]. The use of BHT as a food additive does not appear to pose a public health risk. However, in the natural environment, BHT is degraded biologically to 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO), reported by generating peroxides in mice and rats and inducing cellular DNA damage [191]. The occurrence of the anti-oxidant BHT in the aquatic environment has been demonstrated (Table 2.A-1), with studies conducted in Sweden [57] and USA [58] reporting the presence of BHT in surface waters up to 620 ng L^{-1} and 49 ng L^{-1}, respectively. In other studies, BHT was detected in wastewaters (between 22 and 258 ng L^{-1}) [191], whereas higher values were quantified in surface waters (up to 1560 ng L^{-1}) and groundwater (up to 2156 ng L^{-1}) in Greece and Germany [190-192]. Additional data are needed to support assessments of human health risks associated with the exposure to this compound in the aquatic environment and to establish possible pathways of removal in aquatic systems. Considering the lack of studies on its removal (Fig. 2.A-2.), it is urgent to study its elimination from water matrices.

2.2.4. 2-ethylhexyl-4-methoxycinnamate

Organic UV filters are chemical filters used in many personal care products, alone or in formulations containing a physical filter like ZnO or TiO_2 nanoparticles [193]. Their occurrence in the environment has been described in several papers that have been

given a great attention to the aqueous matrices. These CECs reach the environment by two pathways, wash off from skin or through wastewater or swimming pool waters. Organic UV filters are likely to be present in sediments [194], where they might induce toxicological effects. Their known estrogenic effects on biota and humans was recently reviewed by Ramos *et al.* [195], who highlighted not only the recognized in vivo and in vitro estrogenic activity to fish and mammals, but also other non-estrogenic hormonal targets in such organisms. The UV filter 2-ethylhexyl-4-methoxycinnamate (EHMC), included in the watch list for Union-wide monitoring, is an EDC and was reported at concentrations levels of hundreds of μg kg^{-1} in diverse organisms including macroinvertebrates and fish [193]. Lake and rivers sediments are well characterized regarding this contaminant, which is usually present at μg kg^{-1} levels [60, 193, 194]. This compound was also detected up to 260 ng L^{-1} in tap water from Barcelona (Spain), one of the most frequently found of a group of five UV filters included in that study [63]. Little is known about the removal of EHMC in the aquatic environment (Table 2.A-4, Fig. 2.A-2), only three studies reporting its removal. The removal of EHMC varied (30 – 50%), depending on the respective treatment applied at the WWTP and season [61]. This UV filter was refractory to ozonation, without any degradation being observed after 15 min [196] or after 22 min, but could be removed by UV treatment [197].

Table 2.A-4. Studies dealing with removal of 2-ethylhexyl-4-methoxycinnamate (EHMC). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial EHMC concentration	Treatment and sampling conditions	Concluding remarks	Reference
n.a.	5 WWTPs equipped with different treatment levels: preliminary screening, primary sedimentation, secondary treatment; UV-disinfection, chlorination, sand filtration and RO; 24 h composite or grab samples, depending on the plant; Hong Kong.	Removal of EHMC varied depending on the season in the range of 30 –50%.	[61]
Up to 234 ng L^{-1}	Ozonation: nominal capacity of 3000 $m^3 h^{-1}$; 5 L glass reactor operating in semi-batch mode, at a temperature of 25 ^oC and pH 8.5; WWTP located in Madrid, which treats a mixture of domestic and industrial wastewater.	The UV filter EHMC was completely refractory to ozone.	[196]
23.6 ± 8.1 ng L^{-1}	UV, visible light, photocatalysis (visible light); O_3; 15 W LP mercury vapour lamp (λ_{max} = 254 nm); Xe 150 Xe-arc lamp with spectral emission in the visible region; Photocatalyst: ceria-doped TiO_2 at 0.5 g L^{-1}; O_3: 22 g Nm^{-3}; Mixture of domestic and industrial wastewater from the secondary clarifier of a 3000 $m^3 h^{-1}$ WWTP placed in Alcalá de Henares; Madrid, Spain.	EHMC was removed up to 50% after 15 min of UV-photolysis, mainly during the first 2 min. Visible light Xe-lamp driven photolysis led to an EHMC removal near 20% after 15 min. Removal was not enhanced, applying visible light Ce/TiO_2 photocatalysis. EHMC was not significantly removed by ozone.	[197]

n.a., not available; LP, low pressure; RO, reverse osmosis; WWTP, wastewater treatment plant.

2.A-2.5. Macrolide antibiotics

Among the different classes of pharmaceuticals present in the environment, particular importance has been given to antibiotics, which are the most often discussed pharmaceuticals due to their potential role in the development of resistant mechanisms by bacteria [198]. Macrolide antibiotics, such as clarithromycin, azithromycin and erythromycin are widely used in human and veterinary medicine, as well as in aquaculture, for the purpose of preventing or treating serious infections induced by pneumococci, staphylococci and streptococci [198, 199]. The conventional municipal WWTPs do not fully eliminate these drugs, which are found in WWTP effluents [199] and in other aquatic systems [56, 66, 67, 69]. These antibiotics have been extensively detected in wastewaters, surface and groundwater in several countries at ng L^{-1} levels, with some studies reporting antibiotics at several µg L^{-1} (Table 2.A-1). For instance, azithromycin, erythromycin and clarithromycin were found in effluents of a WWTP in Slovakia at ng L^{-1} levels [64]. Clarithromycin and erythromycin were reported in surface water in Spain and Vietnam [66, 69]. Lopez-Serna *et al.* [56] also reported the occurrence of the three macrolide antibiotics in groundwater (Spain) at range 1.6 – 1620 ng L^{-1}.

Elimination of this class of antibiotics in the environment has been reported in the last decade, for all the types of processes here discussed (Fig. 2.A-2). Biological treatments occurring at WWTPs are normally insufficient to remove such recalcitrant pharmaceuticals (Table 2.A-5). The combination of biological with advanced treatments can be fruitful, as example MBR and RO led to elimination rates above 99% [89] for the macrolides included in the watch list. Hence, advanced methods should be applied to deal with this environmental concern. Membrane technologies alone are not enough for the complete removal of such micropollutants (Table 2.A-5).

Studies reported in the literature employing AOPs for the removal of this type of antibiotics in environmental samples are focused only on photocatalysis [181, 198], revealing a lack of knowledge regarding the efficiency of other AOPs to remove this compounds in real scenarios. In fact, some studies with other AOPs were already published considering these compounds, but not using real matrices and, thus, they are out of the scope of the present review; for instance, UV/TiO_2 and ozonation were studied for the removal of clarithromycin and erythromycin, ozonation apparently being more effective for the parent compounds (complete degradation), while catalytic ozonation improved the mineralization of erythromycin [199, 200].

Table 2.A-5. Studies dealing with removal of macrolides (azithromycin, clarithromycin and erythromycin). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Erythromycin	2600 ng L^{-1} (after primary clarifier)	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 m^3 d^{-1}; 24 h composite samples; Gwinnett County, GA, USA.	Erythromycin was recalcitrant to the biological treatment, but it was removed at an extent of 74% by GAC adsorption. Ozonation oxidized the remaining compounds by more than 60%.	[137]
Azithromycin Clarithromycin Erythromycin	118 ng L^{-1}; 2020 ng L^{-1}; 49 ng L^{-1}.	MBR coupled to RO; Coastal WWTP Castell-Platja d'Aro, Spain.	The combination of MBR and RO led to removal rates above 99% for the target pollutants, with RO showing removal rates always higher than 99%. MBR removed 75 to 85% of the antibiotics, and the remaining non-degraded macrolides were removed by RO.	[89]
Azithromycin Clarithromycin Erythromycin	232.5-876.9 ng L^{-1}; > 0.1 µg L^{-1}; 4.11-42.01 ng L^{-1}.	WWTP1 serving with secondary treatment (anaerobic/anoxic/oxic (A/A/O) treatment + moving bed biofilm reactor (MBBR) + secondary clarifier) and tertiary treatment (rotary fiber disc filters (RFDFs)). WWTP2 with secondary treatment (C-Orbal OD process + secondary clarifier) and tertiary treatment (UV disinfection and RFDFs); 24 h composite wastewater samples at different sampling points; Wuxi City, Jiangsu Province, China.	Removal efficiencies were generally higher in the WWTP1 employing the A/A/O-MBBR process than those obtained by the conventional WWTP2 adopting the C-Orbal OD process, except for clarithromycin. The type of biodegradation process was the predominant factor in this study, the better performance being obtained with WWTP1.	[201]

Erythromycin	0.2 µg L^{-1}	1 WWTP serving 500,000 population equivalent, with an industrial inlet lower than 10% of the total load; with biological treatment, final clarification and tertiary treatment by phosphorus precipitation; 2 h composite influent and effluent samples, during 24 h Nancy, France.	No elimination was reported for erythromycin in the liquid phase. This antibiotic was also not adsorbed on the particulate matter or the sludge.	[202]
Azithromycin, Clarithromycin Erythromycin	406-611 ng L^{-1}; 785-941 ng L^{-1}; 164-210 ng L^{-1}.	1 WWTP equipped with MBR and UV treatment, serving 24,000 inhabitants; Membrane modules made of hollow-fibre membranes; Average flow rates: 8,800 m^3 d^{-1}; 24 h composite influent and effluent samples; Canada.	The degraded fraction of azithromycin was approximately 49% and that of erythromycin was negligible. Clarithromycin was not removed during MBR treatment, being even formed during treatment.	[83]
Azithromycin	up to 719 ng L^{-1}	15 WWTPs, designed for 6,850 to 756,000 population equivalents; Average flow rates: between 349 and 140,000 m^3 d^{-1}; Discharge points: Portuguese rivers and Atlantic Ocean; 24 h composite influent and effluent samples; Portugal.	The concentration of azithromycin in the effluent was up to 200 ng L^{-1}, with a mean removal of 94.6%.	[53]
Clarithromycin Erythromycin	up to 0.33 µg L^{-1}; up to 0.13 µg L^{-1}.	4 WWTPs impacted by effluents from mid-size hospitals (250 to 600 beds) corresponding to a WWTP inflow ranging between 1 and 30%; Average flow rates: between 1300 and 103,000 m^3 d^{-1};	WWTP 2 and 4, employing CAS or OD activated sludge process were more efficient than the others for the removal of clarithromycin and erythromycin.	[168]

		WWTP 1/3 - SBR and UV-tertiary treatment; WWTP 2 – CAS; WWTP 4 - OD activated sludge process; 24 h composite samples; New York, USA.		
Azithromycin Erythromycin	up to 661.9 ng L^{-1}; up to 338.2 ng L^{-1}.	2 municipal WWTPs with anaerobic/anoxic/oxic (A/A/O) activated sludge process, one of them employing a cyclic activated sludge technology (CAST) whereas the other having an OD; Chongqing, China.	WWTP using the OD biological treatment process had the higher efficiency to remove the macrolide antibiotics.	[169]
Clarithromycin Erythromycin	n.a.	MBR pilot plant in continuous operation ca. 1% of diurnal hospital sewage; 2 h composite influent and effluent samples; Luxembourg.	Erythromycin was almost totally removed by MBR, while clarithromycin was removed at extents between 40 and 50%. UV was evaluated as post-treatment, with improved degradation obtained by adding H_2O_2.	[170]
Azithromycin Erythromycin	330.27–376.5 ng L^{-1}; 238.6-275.4 ng L^{-1}.	4th largest WWTP in China, serving 1,540,000 equivalent inhabitants and treating 600,000 m^3 d^{-1}. CAST (anaerobic/anoxic/aerobic (A/A/A) treatment secondary clarifier) followed by chlorination; Grab samples collected according to the residence time in each treatment; Southwest China.	The removal obtained after secondary treatment was 75.6% for azithromycin and 42.8% for erythromycin. Chlorination led to a reduction of 8.0% for azithromycin. Erythromycin was not removed during chlorination.	[70]
Azithromycin Clarithromycin	160 – 279 ng L^{-1}; 1129 - 1570 ng L^{-1}.	Samples were collected in winter from four STP located in Kyoto and Shiga prefecture (Japan); STPs employed a wide variety of secondary treatment processes: CAS;	Removal efficiency of the macrolide antibiotics were higher using CAS (39 – 83%) and A/A (34 – 86%) processes than using A/A/A (41 – 53%) process.	[203]

		anaerobic/anoxic/aerobic (A/A/A) and anoxic/aerobic (A/A).		
Clarithromycin	up to 27.4 µg L^{-1}	MBR followed by NF and RO; Membrane surface of NF and RO modules: 2.5 m^2; Operation: cross flow membranes; NF/RO modules: maximum flux between 20 and 36 L $m^{-2}.h^{-1}$; Hospital wastewater, Germany.	Clarithromycin was completely removed by RO and NF treatments (< LOQ).	[204]
Erythromycin Clarithromycin	337 ± 19.2 ng L^{-1}; 377 ± 30.9 ng L^{-1}.	Pilot-scale UF and RO treatments in sequence; UF flux range of 25 – 47 L $m^{-2}.h^{-1}$; RO flux range of 22 – 31 L m^{-2} h^{-1}; Municipal WWTP; Tel-Aviv, Israel.	High removal rates were achieved after RO (99% for macrolides antibiotics).	[205]
Azithromycin Erythromycin	187 – 367 ng L^{-1}; 180 – 191 ng L^{-1}.	Pilot-scale MF followed by RO; MF: flow rate of 2 m^3 h^{-1} and flux of 323 L m^2 h^{-1}; Residence time 3 min; RO: flow rate of 1 m^3 h^{-1} and permeate flux of 34 L m^2 h^{-1}; Residence time 50 min; Municipal treated effluent; Girona, Spain.	High removals were observed for these pharmaceuticals compounds. Even though the pharmaceuticals were present in the MF permeate at levels higher than 100 ng L^{-1}, RO filtration reduced their loads to the low ng L^{-1} range or to below the method LOQ.	[171]
Clarithromycin	77 ng L^{-1}	2-L reactors at 25 ^oC; UV/H_2O_2: 3 LP Hg lamps (254 nm); H_2O_2 consumed ranged from 0.01 to 0.90 mg H_2O_2 mg TOC^{-1}; RO concentrates from a municipal WWTP in a coastal area of Catalonia, Spain.	Clarithromycin was completely removed by ozonation, but it was recalcitrant to UV (removal of 60%) and UV/H_2O_2 (removal of almost 80%).	[96]

Clarithromycin	46 ng L^{-1}	Biological activated carbon (BAC) process to treat municipal wastewater RO concentrate; Lab scale during 320 days of operation; BAC, combined UV/ UV/H_2O_2–BAC and ozone–BAC.	70% of clarithromycin was removed by the BAC filter. Pretreatment of RO brine with UV/ H_2O_2 or ozonation led to the removal of the pharmaceutical.	[100]
Clarithromycin Erythromycin	< 750 ng L^{-1}	Pilot-scale photocatalysis: CPC plant under natural solar irradiation; TiO_2 load: 20 mg L^{-1}; Municipal effluents collected downstream of the secondary biological treatment of El Ejido WWTP; Almería, Spain.	Using a low TiO_2 load (29.2 mm photoreactor), the treatment was not effective due to the slow reaction rate; 85% of the pollutants were degraded after 480 min. Increasing the light-path of the reactor, the performance was enhanced (90% of the pollutants removed after 300 min).	[50]
Clarithromycin Erythromycin	≈ 0.0275 µg L^{-1}; < 0.05 µg L^{-1}.	Pilot-scale photocatalysis: Solardetox Acadus-2006 CPCs; 3.0 m^2 irradiated surface; 24 L irradiated volume; TiO_2 load: 0.2 g L^{-1}; Wastewater.	Removal was high for all the compounds after 3 h of treatment (bellow LOQ).	[181]
Azithromycin	1653.84 ng L^{-1}	O_3/H_2O_2; O_3: 24 g O_3 Nm^{-3}; Gas flow: 0.36 Nm^3 h^{-1}; H_2O_2: 0.15 mL of a 30% (w/v) solution; Wastewater from the secondary clarifier from a sewage treatment plant of Alcalá de Henares; Madrid, Spain.	The removal for azithromycin was 89.6% after 5 min.	[206]
Clarithromycin	up to 0.1 µg L^{-1}	Bench-scale ozonation: at pH 8.5 (original) and at pH 7.0 (adjusted by adding H_2SO_4);	The elimination of clarithromycin was efficient: when the ratio of O_3/DOC was higher than 0.5. The LOQ was achieved and the removal was higher than 92%	[207]

		O_3 doses (g O_3/g DOC): 0.25, 0.5, 1.0, and 1.5; H_2O_2/O_3 molar ratio = 0, 0.25, and 0.5; 24 h composite samples of hospital wastewater effluents from a pilot MBR; Baden, Switzerland.	at both pH conditions. The average removal was 80% using a ratio of O_3/DOC of 0.25.	
Azithromycin Clarithromycin Erythromycin	n.d. 228 ng L^{-1}; 150 ng L^{-1}.	Ozonation of secondary effluent; O_3: 3 mg L^{-1}; Samples were collected from a municipal sewage treatment plant; Tokyo.	The removal efficiencies of all the target macrolides antibiotics were up to 80%.	[208]
Clarithromycin	363 – 469 ng L^{-1}	Lab-scale UV, UV/H_2O_2, solar irradiation, Fenton, solar photo-Fenton; UV-C irradiation (λ_{max} = 254 nm); H_2O_2: 25 mg L^{-1}; Fenton: 25 mg H_2O_2 L^{-1} and 5 mg Fe^{2+} L^{-1}; Photo-Fenton: 25 mg H_2O_2 L^{-1} and 5 mg Fe^{2+} L^{-1}; Municipal wastewater from Vidy WWTP; Lausanne, Switzerland.	From the five different treatments applied, only the UV-based processes were able to remove 80% of clarithromycin. After 30 min of treatment, the oxidation was significant, verified by COD and TOC removals. For the cases of solar light, Fenton and photo-Fenton processes, the degradation rates were lower.	[209]
Clarithromycin	469 ng L^{-1}	Solar Fenton treatment (natural solar driven oxidation) in a pilot-scale CPC plant; H_2O_2:50 mg L^{-1}; Fe^{3+}: 5 mg L^{-1}; Municipal wastewater from the El Ejido municipal WWTP; Almería, Spain.	Clarithromycin was completely degraded, applying photolytic and solar Fenton experiments, with a removal of 77% at the end of the treatment time (250 min), when present at low concentrations and at low Fenton reagent dosages.	[210]
Erythromycin	170 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86 ─153 g Nm^{-3};	The application of 0.6 g O_3 g DOC^{-1} increased the removal of erythromycin (to values < LOQ).	[154]

		O_3 consumption: 0.6 and 0.9 g O_3 g DOC_0^{-1}; Wastewater; Austria.		
Azithromycin Clarithromycin Erythromycin	139.9 ± 6.2 ng L^{-1} 116.4 ± 2.7 ng L^{-1} 27.0 ± 2.5 ng L^{-1}	Lab-scale photolytic ozonation, ozonation and photocatalysis; O_3: 50 g Nm^{-3}; O_3 flow rate: 150 Ncm^3 min^{-1}; MP mercury vapor lamp (UV/Vis λ > 300 nm); TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.	Completely removal by photocatalytic ozonation for all macrolide antibiotics, while by ozonation only erythromycin was totally eliminated. Photocatalysis was the less efficient process in study.	[156]
Erythromycin	0.7 - 0.9 µg L^{-1} (spiked wastewater)	Lab-scale ozonation; O_3: 5.5 – 8.5 mg L^{-1}; O_3 flow rate: 0.39 NL min^{-1}; Urban wastewater samples (spiked) from the secondary clarifier of two treatment plants from West-Alcalá and Alcázar de San Juan; Spain.	High removal of erythromycin (> 90%) was observed for both wastewaters studied.	[187]

BAC, Biological activated carbon; CAS, conventional activated sludge; CAST, cyclic activated sludge technology; CPC, compound parabolic collector; DOC, dissolved organic carbon; GAC, granular activated carbon; LOQ, limit of quantification; MBR, membrane biological reactor; MF, Microfiltration; n.a., not available; n.d., not detected; OD, oxidation ditch; RFDFs, rotary fiber disc filters; RO, Reverse osmosis; SBR, sequential batch reactor; UF, ultrafiltration; WWTP, wastewater treatment plant.

2.A-2.6. Methiocarb

Regarding pesticides, their use plays an important role in harvest quality and food protection, providing enormous benefits to increase production, as pests and diseases are usually responsible to damage up to one-third of crops [211]. As consequence of massive global consumption, pesticides and their degradation products spread through the environment and can contaminate water resources. Surface and groundwater located in intensive agricultural areas are more susceptible to pesticide contamination, which is a major concern if the water is used for human consumption [212]. The impact of these contaminants in the environment and to the wildlife is demonstrated by several injurious effects, such as the enhancement of the incidence of cancer, birth defects, genetic mutations, or other problems such as damage in the liver or in the central nervous system [213]. The occurrence of pesticides in aquatic compartments and their possible effects to public health are a topic of considerable environmental interest.

Methiocarb (also known as mercaptodimethur, mesurol, 3,5-dimethyl-4-(methylthio)phenyl methylcarbamate) is one of the most commonly used carbamate pesticides worldwide. This pesticide has been applied since 1960s for a variety of invertebrate pests and also as a bird repellent on fruit crops [214, 215]. The detected concentrations of methiocarb in wastewater and groundwater are generally low (Table 2.A-1); however, it poses a serious health threat to aquatic life and humans considering its high toxicity [214]. A negative removal of methiocarb was reported in a Spanish sewage treatment plant (Table 2.A-6), probably due to the limitations on the sampling procedure, where both HRT (24 – 72 h) and SRT (7.5 – 25 days) were not taken into consideration, consequently higher concentrations were found in the

effluents than in influents [72]. Recent studies related to the removal of this compound by advanced treatment options were not found in the literature.

Table 2.A-6. Studies dealing with removal of methiocarb. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial methiocarb concentration	Treatment and sampling conditions	Concluding remarks	Reference
3.77 – 5.74 ng L^{-1} (2010); 1.26 –105.31 ng L^{-1} (2011).	Sewage treatment plants monitored in the four River Basins of Ebro River; Spain.	The removal of methiocarb was negative. The higher concentrations in effluents than in influents were attributed to the sampling limitations: influent and effluent samples were collected at the same day, without considering the HRT (24 – 72 h) and SRT (7.5 – 25 days).	[72]

HRT, hydraulic retention time; SRT, sludge retention time

2.A-2.7. Neonicotinoids

In the last decade, the neonicotinoid group of insecticides has been one of the most broadly adopted conventional management tools to deal with insect pests of annual and perennial cropping systems. Benefits of the neonicotinoids include flexibility of application, a wide range of active ingredients and broad spectrum activity [216, 217]. This group includes imidacloprid, thiacloprid, thiamethoxam, clothianidin and acetamiprid, which are extremely toxic to all aquatic arthropods, except water fleas [76]. However, as a result of structural differences in the polypeptide subunit containing the neonicotinoid-binding region of the vertebrates' nicotinic acetylcholine receptors, neonicotinoids pose a relatively low risk to fish and mammals [76]. Neonicotinoids are systemic insecticides and are applied as seed dressings by sprays, owing to their solubility in water. Therefore, the main sources of this class of herbicides in the environment are the runoff from agriculture areas and leaching into groundwater, with the consequent subsurface discharge into wetlands and other surface waters [217]. As a result of their high water solubility and persistence in soil, neonicotinoids cause a threat for water contamination, mainly after storm events that produce runoff pulses [76]. Other sources of these compounds are soluble or insoluble fractions transported via snowmelt, decay of treated plants in water bodies, and deposition of treated seeds or soil into water bodies [217]. Recent studies from Spain, Portugal, USA, Australia and other countries (Table 2.A-1) have confirmed the occurrence of this group of pesticides in the aquatic ecosystems [72-78].

There is a lack of literature concerning the removal of this class of pesticides in the environment (Fig. 2.A-2). The majority of the reports refer to the performance of AOPs, dealing with their degradation at laboratory or pilot-scale conditions and mostly using spiked water or spiked simulated water [218]. Photolysis, photocatalysis and photo-

Fenton were applied to study the removal of these compounds from water, photocatalysis being the most applied (Table 2.A-7). Studies dealing with real waters and other treatment processes should be performed to bring a more realistic overview of the elimination of this group of pesticides. Some other studies with these substances were already published, but not using real matrices and, therefore, they are out of the scope of the present review; for instance the degradation of imidacloprid [219-223], thiamethoxam [219, 224, 225], clothianidin [219], and acetamiprid [226] were studied with photo-assisted and ozonation processes

Table 2.A-7. Studies dealing with removal of neonicotinoids (thiacloprid and acetamiprid). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Acetamiprid	< 0.05 μg L^{-1}	Pilot-scale photocatalysis: Solardetox Acadus-2006 CPCs 3.0 m^2 irradiated surface; 24 L of irradiated volume; TiO_2 load: 0.2 g L^{-1}; Water taken from the outlet of a WWTP from the South East of Spain.	High removal for all the emerging contaminants after 3 h of treatment (bellow LOQ).	[181]
Thiacloprid	0.05 – 0.38 mM (spiked spring water)	Photocatalysis: Lab-scale reactor operated in a circular 'closed-loop' mode; Six 18 W UV lamps (λ_{max} = 366 nm); ZnO load: 0.5 – 3.0 g L^{-1}; Thermal water collected from the spring of Kistelek, Hungary.	Very low degradation by direct photolysis. A removal of 86.6% was observed for thiacloprid, with a ZnO load of 2 g L^{-1} and pH 6.8. The efficiency of the thiacloprid removal in filtered and un-filtered thermal water was about two times lower than from the distilled water, indicating that the removal was due to the dissolved substances.	[227]
Thiacloprid	0.32 mM (spiked river water)	Lab-scale UV and UV/H_2O_2; 125 W HP mercury lamp (emission bands λ = 304, 314, 335, 366 nm) (λ_{max} =3 66 nm); H_2O_2 concentration: 0 to 162 mM; pH: 2.8 – 9; Spiked water from Begej river at Itebej, Serbia.	The removal rate of thiacloprid was influenced by the presence of HCO_3^-. Very low degradation rates were observed for single UV and H_2O_2. High removal of thiacloprid was achieved after 120 min of UV/H_2O_2. The removal rate for natural water was lower compared with distilled water (45 mM H_2O_2) at pH 8.2. However, the removal in natural water adjusted at pH 2.8 was higher than in distilled water due to the naturally occurring photosensitizers, i.e. dissolved organic matter.	[228]

Clothianidin	n.a.	Sulfate radical based homogeneous photo-Fenton involving peroxymonosulfate as an oxidant, ferrous iron (Fe(II)) as a catalyst and simulated solar irradiation as a light source; Biologically treated domestic wastewater effluents.	PMS/Fe(II)/UV–Vis advanced oxidation system using simulated solar irradiation has demonstrated better kinetic performances over TiO_2/UV–Vis system for clothianidin.	[179]
Acetamiprid	100 µg L^{-1} (spiked wastewater)	Pilot-scale photocatalysis: CPC; Wastewater of Almería, Spain.	The removal of acetamiprid was poor in the wastewater matrix.	[229]
Imidacloprid	60 mg L^{-1}	Pilot-scale CPC plant (60 L); Fe(III)-EDDS as complexing agent; Spiked tap water from the groundwater well of Plataforma Solar de Almería, Spain.	Photolysis of the complexing agent generated radical species able to act independently of carbonate scavengers that are present in natural waters.	[230]

CPC, compound parabolic collector; EDDS, ethylenediamine-N,N'-dissucinic acid; HP, high pressure; LOQ, limit of quantification; WWTP, wastewater treatment plant.

2.A-2.8. Oxadiazon

The oxadiazole herbicide oxadiazon [5-tert-butyl-3-(2,4- dichloro-5-propan-2-yloxyphenyl)-1,3,4-oxadiazol-2-one] has been habitually used to combat weeds in various agricultural crops such as rice, cotton, soybean, potato, peanut and onion. Oxadiazon is an organic contaminant causing a great environmental concern due to its relatively long half-life [231]. Previous studies on the leaching of oxadiazon in soils indicated that, the strong adsorption of the herbicide to soils reduces the displacement towards the sub-surface layers [232]. However, oxadiazon was found in surface water in Canada (Table 2.A-1) at ng L^{-1} levels [79]. In contrast, the removal of oxadiazon in aquatic matrices is still unknown.

2.A-2.9. Triallate

Triallate (S-2,3,3-trichloroallyl di-isopropyl thiocarbamate) is a carbamothioate herbicide widely used to control annual and perennial grasses in wheat, barley, legumes and a number of other crops [233, 234]. This pesticide is often used in mixture with other chemicals (chloridazon, isoproturon, metoxuron) and its use, in the last decades, has exceeded 500 tons per year in some European countries [233]. Triallate has a high hydrophobic partitioning [235], therefore it adsorbs to loam and clay soils and is not readily dissolved in water [236]. This information indicates that this herbicide is not likely to move through the soil, even though it has a long soil half-life (82 days). Nevertheless, if there is significant moisture and/or low levels of organic matter in the soil, triallate may become desorbed from soil particles [234]. Leaching and consequent groundwater contamination would be possible in such situations, but Environmental Protection Agency (EPA) suggests that triallate leaching does not cause a threat to the environment, since it is usually used where the water table is

relatively low [237]. A lack of knowledge exists about its occurrence and removal in the aquatic environment due to its chemical nature.

2.A-3. Conclusions

Despite the considerable amount of studies reported on the occurrence and removal of E1, E2, EE2, diclofenac and macrolide antibiotics (azithromycin, clarithromycin and erythromycin), a lack of knowledge exists concerning the pesticides (methiocarb, neonicotinoids, oxadiazon and triallate), the UV filter (EHMC) and the antioxidant (2,6-di-tert-butyl-4-methylphenol), which are included in the watch list of Decision 2015/495/EU for European Union monitoring. Thus, more investigation is needed regarding the occurrence and removal of neonicotinoids, EHMC and 2,6-di-tert-butyl-4-methylphenol and the performance of different treatments to remove the substances included in the watch list under realistic conditions. These compounds are usually present at residual concentrations, as mixtures in the different environmental compartments (e.g., municipal wastewater, surface water, groundwater, solid matrices) and comprehensive works considering it are scarce. As shown by different studies, the efficiency of the treatment processes can decrease considerably when realistic water matrices are used instead of simulated ones. For example, the presence of carbonates and bicarbonates can decrease the efficiency of AOPs, principally due to competition by $HO^{\bullet}$. Since multiple factors can affect the efficiency of the treatments, experiments should be performed as close as possible to the real conditions. Additionally, the formation of intermediates should be attempted in this type of studies, considering that the produced by-products might be more toxic and/or persistent than the parent compounds. Toxicological studies are needed to determine the deleterious effects on the ecosystems and human health of parent compounds

and by-products formed in real matrices. Considering the scale up of the treatment option, these processes can be expensive both in the implementation and maintenance, therefore it is of major importance to perform cost effectiveness analysis for each of them under a common base of comparison.

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Part B.

Analysis of organic micropollutants: An overview of carbon-based materials for solid-phase extraction cartridges

Abstract

Solid-phase extraction (SPE) is the most used sample preparation technique for pre-concentration and extraction of organic micropollutants in aqueous matrices. To achieve good SPE extraction conditions, the choice of the sorbent is a key step since it will affect important parameters (selectivity, affinity and capacity). The development of novel materials as sorbents for SPE has been extensively exploited to achieve more selective materials with higher adsorption capacity, and to expand the availability of cheaper, more easily synthesized sorbents. Carbon-based nanostructured materials are interesting materials for sample preparation, due to their unique properties, such as the high surface area and the possibility to be functionalized, which may increase their affinity toward target compounds. This part of *Chapter 2* aims to overview the application of carbon-based materials as sorbents in SPE cartridges for extraction and pre-concentration of EU organic micropollutants in realistic water matrices.

2.B-1 Introduction

Several analytical techniques have been improved to achieve high sensitivity and reproducibility for the detection of organic micropollutants in the environment. Considering the wide need of resources and great deal of time involved in this task, novel analytical methods should allow (i) the simultaneous determination of different chemical compounds in trace levels (i.e., meet the multi-class purpose), while (ii) shortening the time required for dealing with the cleanup of sample matrix and the extraction of analytes (i.e., the most time-consuming analytical steps) [1, 2]. Solid-phase extraction followed by ultra-high performance liquid chromatography-tandem mass spectrometry (SPE-UHPLC-MS/MS) is an advanced analytical method that has been routinely used for quantification of organic micropollutants in environmental samples. It is well known that the selection of the most appropriate sorbent is a crucial step when using SPE since the enrichment efficiency of the target analytes relies on the sorbent characteristics [3]. Several carbon materials have been studied for this purpose, including graphene, fullerene, carbon nanotubes (single and multi-walled) and carbon nanocones/disks [4-9]. Some of these materials have attracted increasing attention in this field and several reports dealing with their application in conventional SPE for extraction of PSs and CECs can be found in the literature. However, most of these studies are focused on one single target compound or on a specific class of compounds, generally pesticides [3, 10-16].

Part B of *Chapter 2* intends to: (i) provide a comprehensive snapshot of the application of carbon-based materials in SPE cartridges for extraction of EU-relevant PSs and CECs from real water samples; (ii) highlight the lack of studies in this field; and (iii) present and discuss the main difficulties and challenges on this research topic.

2.B-2. Literature survey on the application of carbon-based materials in SPE cartridges for extraction of EU micropollutants

Carbon nanostructured materials have unique physical and chemical properties which make them excellent candidates as sorbents. Moreover, they can be tailored to improve the sensitivity, enhance the selectivity and increase the analytical throughput. Table 2.B-1 summarizes the literature reporting the application of carbon materials in conventional SPE procedures. The search comprised publications in Scopus database, using as keywords the name of each substance defined in EU legislation (Directive 2013/39/EU, Decision 2015/495/EU, Decision 2018/840/EU and Decision 2020/1161/EU) and “carbon material” and “solid-phase extraction”. The publications selected for this report were only those considering realistic water matrices. A total of 33 publications were found using the defined search criteria. Figs. 2.B-1 a) and b) show the distribution of these publications in terms of the type of carbon material and class of EU organic micropollutants studied, respectively.

Table 2.B-1. Studies dealing with the application of carbon materials in conventional SPE: type of carbon-based material, target analyte, matrix, and recoveries (%) obtained. Pollutants included in these studies that are out of the scope of EU legislation are not discussed.

Carbon-based material	Target analyte	Matrix	Recovery (%)	Ref.
Multi-walled carbon nanotubes (MWCNT)	Atrazine and simazine	Tap and groundwater	85–95	[10]
	Atrazine and metabolites	Surface and underground water	86–110	[16]
	Atrazine and simazine	River water	87–97	[17]
	Atrazine and simazine	River, tap, reservoir and wastewater	83–104	[15]
	Atrazine	Tap, reservoir and stream water	81–108	[13]
	Chlorpyrifos	Mineral water, groundwater, and run-off water from an agricultural	70–100	[12]
	Chlorpyrifos	Well, tap and river water	94–98	[18]
	Atrazine	Tap and reservoir water	74–>99	[14]
	Methiocarb	Tap and surface water	92–96	[11]
	Alachlor	Tap and river water	82–85	[19]
	Thiamethoxam, acetamiprid and imidacloprid	Tap, ground and reservoir water	88–110	[3]
	[a] Diclofenac	Surface and tap water	95–106	[20]
	[a] PFOS	Tap and river water	88–90	[21]
	[a] PCP	River water	62–98	[22]

	PCP	Tap and river water	97–109	[23]
	PAHs	Tap, river and seawater	67–127	[24]
	PAHs	River, tap and wastewater	79–118	[25]
	Diclofenac	River water	79–94	[26]
	Atrazine and simazine	River, tap and wastewater	n.a.	[27]
	[b] 17-beta-estradiol	Lake water	93–97	[28]
	[a] Erythromycin, azithromycin and diclofenac	Surface and groundwater	93–112	[29]
Graphene-based materials: graphene, graphene oxide, graphene aerogel	[c] PCP	Environmental water	110–120	[30]
	[c] PCP	Pond and lake water	101–110	[31]
	[a] PAHs	Rain, river and tap water	85–110	[32]
	[c] PAHs	Pond and river water	89–114	[33]
	[d] PAHs	Well, tap river and wastewater	95–101	[34]
	[c] Chlorfenvinphos	Water	n.a.	[35]
	[c] Diclofenac	Wastewater	80–90	[9]
	[c] Ciprofloxacin	Tap and river water	72–108	[36]
	[e] Estrone, 17-beta-estradiol, estriol and 17-alpha-ethynylestradiol	Tap and river water	87–109	[37]
Carbon nanocones/ disks	PCP	Drinking, swimming pool, well and tank water	93–104	[8]

Fullerene C_{60}	Benzene	Drinking, river, rain, ground and wastewater	n.a.	[7]
Asphalt-derived porous activated carbon	PAHs	Tap water	88–98	[38]

[a] carbon material chemically modified; [b] molecularly imprinted poly(methacrylic acid) grafted on an iniferter-modified MWCNT by living-radical polymerization; [c] Graphene/ Graphene oxide with silica gel or supported on silica; [d] Graphene oxide/ polydimethylsiloxane-coated stainless-steel mesh; [e] Graphene arerogel; n.a. is not available; PFOS is perfluorooctanesulfonic acid; PAHs is polycyclic aromatic hydrocarbons; PCP is pentachlorophenol.

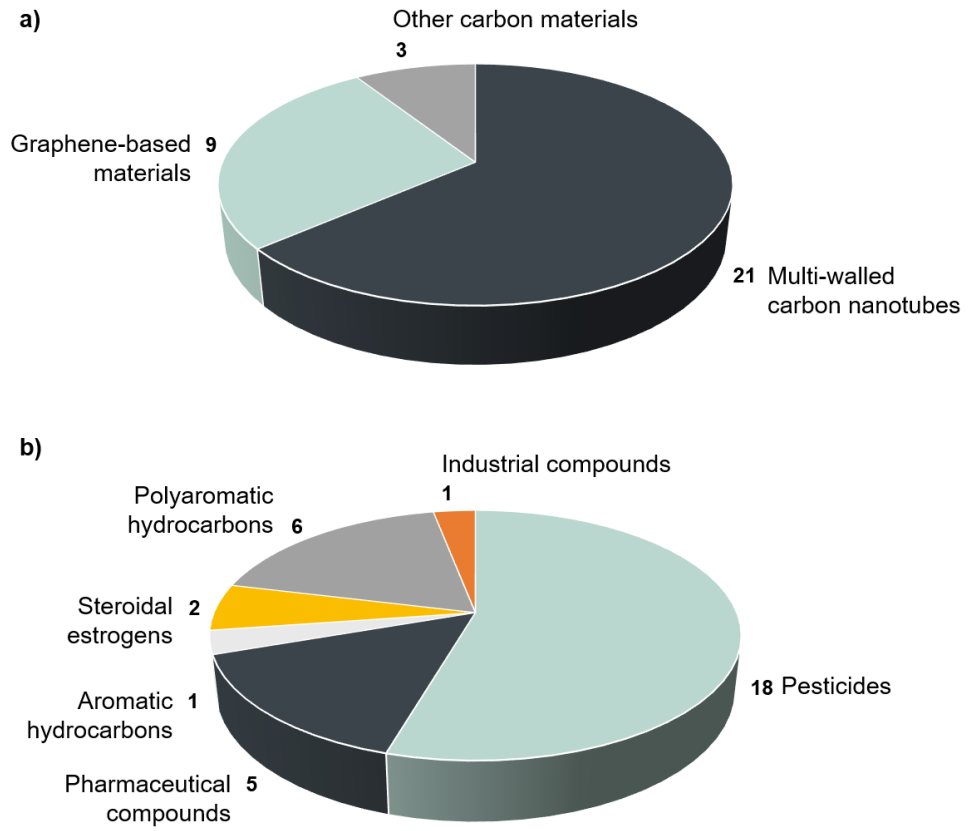

Fig. 2.B-1. Number of publications reported in the literature dealing with the application of carbon materials in SPE cartridges: **(a)** type of carbon material employed; and **(b)** class of EU organic micropollutant studied. The studies selected for this review were performed using realistic matrices.

Considering the application of carbon materials in conventional SPE for extraction of organic micropollutants listed in the abovementioned documents, it is possible to verify that carbon nanotubes, graphene and its derivates, fullerene, carbon nanocones/disks and activated carbon were already tested. MWCNTs are the most studied, followed by graphene-based materials (21 and 9 publications, respectively). Both materials showed excellent characteristics as sorbents, with recoveries higher than 62% in all studies herein reviewed (Table 2.B-1). Graphene may have some limitations when employed as sorbent in SPE, including the higher possibility to be

released from the SPE cartridge [39]. For this reason, the reports in Table 2.B-1 propose using silica as support of graphene. Fullerene, carbon nanocones/disks and activated carbon were reported only once, suggesting that these materials have attracted less attention for SPE in comparison with graphene or MWCNTs. However, the good recoveries obtained with these carbon materials make them interesting candidates and, therefore, should be more studied in the future.

Carbon materials such as carbon nanofibers, carbon aerogels/xerogels among others, have not yet been employed for the purpose here discussed, probably because they are less common and also due to the limited number of research groups preparing these materials [39]. Moreover, the application of chemically modified carbon materials is even scarcer in the literature, only four studies being reported so far (Table 2.B-1, publications marked with "[a]") [20-22, 29].

Concerning the target analytes, pesticides are the most studied micropollutants in water matrices, i.e., 18 of the 33 existing publications were focused on the extraction of this type of compounds (Fig. 2.B-1b). Furthermore, it is important to refer that only 1 of the 33 publications found has considered EU pollutants of different classes [29]. Indeed, more studies on the other classes of organic micropollutants targeted in EU legislation and/or considering multi-class compounds are missing in the literature.

2.B-3. Future perspectives

Carbon materials are good options to accomplish the urgent demand in analytical methodologies for determination of PSs and CECs at trace concentrations. However, it must be realized that the application of carbon-based materials is in its preliminary stage of research to prepare SPE sorbents. Some challenges are expected in the

development of carbon materials for sample preparation before the analysis of PSs and CECs, such as: (i) the application of carbon materials is still restricted to a small number of pollutants and many other important pollutants have not been tested with these materials; (ii) carbon materials must be prepared with tailored texture and surface chemistry and high selectivity/specificity for adsorption/desorption of the target organic micropollutants; and (iii) a better understanding of the interactive nature of adsorption will provide valuable information for the preparation of adequate carbon adsorbents for SPE cartridge, besides high purity and good stability. In fact, few studies have been conducted on the application of MWCNTs and graphene derivatives to assess the presence of organic micropollutants in diverse aquatic matrices, and none specifically for a wide set of the target PSs and CECs. Among these studies, an analytical methodology for extraction of multi-class compounds using SPE with different carbon materials in a single and unique cartridge is not reported yet. Further studies on a number of the aspects described above are required to advance the application of carbon-based materials in conventional SPE for the extraction of substances included in recently launched EU legislation.

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Chapter 3

Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal

Chapter 3

Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal

Abstract

Organic micropollutants present in drinking water (DW) may cause adverse effects for public health, and so reliable analytical methods are required to detect these pollutants at trace levels in DW. This work describes the first green analytical methodology for multi-class determination of 21 pollutants in DW: seven pesticides, an industrial compound, 12 pharmaceuticals, and a metabolite (some included in Directive 2013/39/EU or Decision 2015/495/EU). A solid-phase extraction procedure followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (offline SPE–UHPLC–MS/MS) method was optimized using eco-friendly solvents, achieving detection limits below 0.20 ng L^{-1}. The validated analytical method was successfully applied to DW samples from different sources (tap, fountain, and well waters) from different locations in the north of Portugal, as well as before and after bench-scale UV and ozonation experiments in spiked tap water samples. Thirteen compounds were detected, many of them not regulated yet, in the following order of frequency: diclofenac > norfluoxetine > atrazine > simazine > warfarin > metoprolol > alachlor > chlorfenvinphos > trimethoprim > clarithromycin ≈ carbamazepine ≈ PFOS > citalopram. Hazard quotients were also estimated for the quantified substances and suggested no adverse effects to humans.

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3.1. Introduction

Many micropollutants are not completely removed during conventional domestic wastewater treatment and are discharged into water bodies (such as rivers) that are then in turn used to supply drinking water treatment plants (DWTPs) providing tap water. Amoxicillin, naproxen, metoprolol, phenacetin, indomethacin, sulfamethoxazole and caffeine, are some of these refractory micropollutants, and despite their low concentrations in DW, they are of increasing public health concern [1,2]. Moreover, even if public health effects are not expected, chemical compounds may cause ecotoxicological adverse effects after long-term exposure, particularly when present as complex mixtures [3,4].

Some regulations on water pollution have been published in the last years. In the particular case of the European Union (EU), the requirements for a good chemical status of groundwater have been set out in Directive 2006/118/EC [5] and the values for a wholesome and clean water for human consumption in Directive 1998/83/EC [6]. Moreover, surface water protection was identified as one of the top work priorities at EU, due to the increasing demand for water protection and treatment by environmental organizations and population. Directive 2000/60/EC [7] was the first mark in the European water policy, which set up a strategy to define high risk substances to be prioritized. A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by Directive 2008/105/EC [8]. In 2013, Directive 39/2013/EU [9] recommended attention to the monitoring and the progress of innovative water/wastewater treatment technologies, identifying 45 PSs to meet the protection of the aquatic compartments and the human health. More recently, a set of substances for EU monitoring in surface water bodies was defined in the Watch List of Decision 2015/495/EU [10]. The

occurrence and removal of these substances has already been reviewed [11]. However, reports focused on the determination of organic micropollutants in DW, and in particular regarding contaminants of emerging concern (CECs), are still scarce and most countries do not have monitoring programs to routinely determine these micropollutants. In fact, the analytical challenge of measuring pollutants at low concentrations in environmental matrices, such as sludge and wastewater [12,13], has been a major research focus for scientists in the last decades, but much less attention has been given to DW [14]. In this context, it is crucial to develop sensitive and reproducible analytical methods that enable the determination of organic micropollutants belonging to different classes in DW.

The employment of an accurate and precise sample preparation as well as analytical techniques with high standards of sensitivity and reproducibility, such as ultra-high performance liquid chromatography (UHPLC), is required to assess the occurrence and respective removal of micropollutants after water treatment. Hyphenated chromatography-mass spectrometry techniques are presently the methods of choice for DW analysis (Table B-S3.1), with only few works dealing with both pharmaceuticals and pesticides [15,16], some with pesticides and/or their metabolites [17-19], and most referring only to pharmaceuticals and/or their metabolites [14,20-27]. Considering the resources and time consumed in these tasks, new analytical methods should incorporate multi-residue and environmentally friendly approaches, being able to determine trace levels of a wide range of chemically heterogeneous compounds and simultaneously reduce the cleanup and extraction steps using green solvents [28,29].

Green chemistry principles were introduced in the 90s, aiming to reduce the environmental impact of diverse chemical activities, including those used in research [30,31]. In this scenario, green analytical chemistry (GAC) plays an important role,

e.g., by reducing hazardous wastes, using reusable materials and/or employing “eco-friendly solvents” or “green solvents”. The last two terms have been applied to refer the solvents that have associated a lower environmental impact resulting from their production, use and disposal (life cycle assessment), and/or that allow minimizing health and safety impacts [32]. The main goals of GAC include the multi-analyte determination and the development of new (or modification of) analytical methodologies, through the replacement of toxic reagents by smaller amounts of safer reagents, preferentially obtained from renewable sources [29,32]. Several strategies have been used in LC-MS/MS, such as the reduction of the internal diameter and particle size (sub-2 μm) of chromatographic columns (to diminish eluent consumption), and the replacement of conventional mobile phases (consisting of acetonitrile and/or methanol) by environmental friendly alternatives like water, ethanol and carbon dioxide in the particular case of Supercritical Fluid Chromatography [30,33].

The aim of this work was the optimization and validation of an eco-friendly analytical method based on offline SPE-UHPLC-MS/MS, for the multi-class determination of organic micropollutants (12 pharmaceuticals, 1 metabolite, 7 pesticides and 1 industrial compound) in DW from northern Portugal. The targeted organic contaminants (Table B-S3.2) were selected based on their inclusion in EU regulations, some of the compounds being specified in Directive 2013/39/EU or in Watch List of Decision 2015/495/EU. The selected micropollutants were previously reported as toxic and frequently found in the aquatic environment [14,20,34]. The occurrence of the multi-class contaminants was investigated for the first time in DW samples from different sources (tap, fountain and well waters) and locations in northern Portugal, as well as the related hazard quotients (HQs) were determined. The HQs evaluation for these micropollutants could be a predictive way to assess the human health risk of

exposure to CECs, but only a few reports focused this approach for organic contaminants in DW [2,14,34-37]. The efficiency of two processes (UV and ozonation) typically employed for DW disinfection and/or degradation of organic pollutants in DWTPs was also verified using the analytical strategy proposed.

3.2. Experimental

3.2.1. Chemicals and materials

All reference standards (diclofenac sodium, tramadol hydrochloride, azithromycin dihydrate, clarithromycin, trimethoprim, warfarin, clopidogrel hydrogen sulfate, metoprolol tartrate, carbamazepine, citalopram hydrobromide, venlafaxine hydrochloride, fluoxetine hydrochloride, norfluoxetine oxalate, alachlor, atrazine, simazine, isoproturon, chlorfenvinphos, pentachlorophenol, clofibric acid and perfluorooctanesulfonic acid; > 98% purity) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of approximately 1000 mg L^{-1} were prepared in methanol, ethanol or acetonitrile, depending on the solubility of each analyte. Two working standard solutions containing all the target analytes at 200 µg L^{-1} and 20 µg L^{-1} were prepared by diluting each stock solution in ethanol. Surrogate standards (ketoprofen–d3, fluoxetine–d5 solution and atrazine–d5) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of 1000 mg L^{-1} of the isotopically labeled internal standards ketoprofen–d3 and atrazine–d5 were prepared in methanol, the same solvent of fluoxetine–d5 solution. An ethanolic working solution containing 1 mg L^{-1} of each isotopically labeled internal standard was prepared.

Methanol and acetonitrile (MS grade) were obtained from VWR International (Fontenay-sous-Bois, France). Ethanol (HPLC grade) and ethylenediaminetetraacetic acid (EDTA) (99%) were acquired from Fisher Scientific UK Limited (Leicestershire, UK). Sodium thiosulfate and L-ascorbic acid (99%) were purchased from Sigma-Aldrich (Steinhein, Germany). Ammonium acetate, ammonium hydroxide 25%, sulphuric acid and formic acid were obtained from Merck (Darmstadt, Germany). Ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ.cm, at 25 °C). HPLC grade solvents were filtered with 0.22 µm nylon membrane filters (Membrane Solutions, TX, USA). Oasis® HLB (Hydrophilic-Lipophilic-Balanced), Oasis® MCX (Mixed-mode Cation eXchange) and Oasis® MAX (Mixed-mode Anion-eXchange) cartridges (150 mg, 6 mL), obtained from Waters (Milford, MA, USA), were tested for SPE optimization. A pHenomenal® pH 1100L pH meter (VWR, Germany) was used for the pH adjustments.

3.2.2. Sample preparation

Tap waters were collected from the water supply network for use as matrix for the SPE optimization and validation of the method. The vacuum extraction and drying devices LiChrolut® used for SPE procedure were acquired from VWR (Merck Millipore, Billerica, MA, USA). In order to assess the best performance of SPE cartridges to extract the overall compounds, SPE optimization was performed by comparing Oasis® HLB, MCX and MAX cartridges. Oasis® MAX and MCX cartridges were conditioned sequentially with 4 mL of methanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. For HLB cartridges, the conditioning was performed at the same flow with 4 mL of methanol or ethanol and 4 mL of ultrapure water. The sample pH was optimized for HLB cartridges using methanol as conditioning solvent, by comparing the recoveries achieved with initial sample pH adjusted to 3, 7 and 9. For

MAX and MCX SPE procedures, samples were respectively alkalinized to pH 9 or acidified to pH 3, before loading. The pH adjustments were done with ammonium hydroxide or sulphuric acid. Sample loading was carried out with 250 mL of blank and spiked (35 ng L^{-1}) tap water samples at a constant flow rate of 10 mL min^{-1}, using the vacuum manifold unit connected to a vacuum pump. The washing step was performed with 4 mL of ultrapure water, 5% ammonium hydroxide aqueous solution, or 2% formic acid aqueous solution, for HLB, MAX and MCX, respectively. After washing, the cartridges were dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with: 4 mL methanol or ethanol for Oasis® HLB cartridges, 4 mL of methanol to extract the neutral compounds and weak bases in the case of Oasis® MAX and neutrals and weak acids in the case of Oasis® MCX. A second elution was performed for mixed-mode cartridges Oasis® MAX and MCX, respectively with a 2% formic acid methanolic solution (elution of acids) or 5% ammonium hydroxide methanolic solution (elution of basics). The LiChrolut® drying device was coupled to the vacuum extraction unit to evaporate the extracts to dryness with a gentle nitrogen stream. The dry residues were reconstituted in 300 µL of ethanol and the ethanolic extracts were filtered using 0.22 µm polytetrafluoroethylene syringe filters (Membrane Solutions, TX, USA). To assess the breakthrough volume, sample loading was tested with three volumes of non-spiked (blanks) and 35 ng L^{-1} spiked tap water samples, namely 250, 500 and 1000 mL, using the optimized SPE procedure. In order to improve the recovery rates, the chelating agent EDTA (100 mg L^{-1}) was tested as well as two dechlorination agents, ascorbic acid (10 mg L^{-1}) and sodium thiosulfate (30 mg L^{-1}). Analysis of reuse efficiency for the optimized SPE protocol was performed in three consecutive days.

3.2.3. UHPLC-MS/MS

A Kinetex™ 1.7 µm XB-C18 100 Å column (100 × 2.1 mm, i.d.) (Phenomenex, CA, USA) was used and different mobile phases were tested (acetonitrile, ethanol or methanol as organic phase and ammonium acetate, formic acid aqueous solutions or water as aqueous phase). The optimized mobile phase was ethanol/water (70/30, v/v), pH 7.0, performed at isocratic mode using a flow rate of 0.20 mL min^{-1}. Column oven and autosampler temperatures were set respectively at 35 and 4 ^{o}C and the volume of injection was 5 µL. An electrospray ionization source was used operating in both positive and negative ionization modes. The precursor ion and the two most abundant fragments were used for quantification by selected reaction monitoring (SRM) and identification (Table B-S3.3). The mass spectrometer parameters declustering potential, collision energy and collision cell exit potential of each analyte are described elsewhere [38]. The optimized conditions for MS parameters, using argon at 230 kPa as CID gas were: 2.5 dm^3 min^{-1} for nebulizing gas flow, 10 dm^3 min^{-1} for drying gas flow, 0.5 kV for capillary voltage, 450 ^{o}C for source temperature and 200 ^{o}C for desolvation temperature. Additional information is presented in the Text B-S3.1. Instrumentation (Appendice B).

3.2.4. Quality assurance/quality control

The offline SPE-UHPLC-MS/MS method validation was performed according to the international guidelines [39] and previous works [38, 40, 41], through the evaluation of the following parameters: selectivity, linearity and range, limits of detection and quantification, accuracy, precision and recovery. Chromatograms of non-spiked tap waters (blank extracts), standards extracted from the spiked tap waters at 35 ng L^{-1} and an ethanolic solution containing all the standards at a concentration

corresponding to the theoretical concentration after SPE, were compared to assess the selectivity. For recovery experiments, three quality control (QC) standard solutions were prepared, in triplicate in three consecutive days, by extracting tap water samples spiked with three different concentrations (3.5, 15 and 35 ng L^{-1}). The peak areas of the standards extracted from the spiked tap waters were compared with those of ethanolic solutions containing all the standards at the theoretical concentrations of recovered extracts, to assess the recovery of each SPE procedure. For target compounds detected in the blank matrix, the peak areas were subtracted from those obtained with the spiked matrix.

The internal standard calibration method was used to define the linearity and range for each target analyte. Triplicates of 250 mL tap water samples spiked with seven different standard concentrations (0.75, 1.5, 2.0, 4.0, 8.0, 20 and 40 ng L^{-1}) were prepared, the pH was adjusted to 3 and sodium thiosulfate solution was added to obtain a concentration of 30 mg L^{-1}. 10 µL of a working internal standards solution of 1 mg L^{-1} was added to each sample. These standard solutions were extracted by the optimized SPE procedure and reconstituted in 300 µL of ethanol to create the calibration curves, by injecting 5 µL in the UHPLC apparatus. Method detection (MDL) and quantification (MQL) limits were determined as described elsewhere [38,41] spiking water samples prior to the SPE procedure with ethanolic standard solution to achieve successively diluted samples. The minimum detectable amount of each compound giving a signal-to-noise (S/N) ratio of 3.3 and 10 gave MDL and MQL, respectively. The three triplicate QC solutions, described above, were also used to evaluate the accuracy of the method as well as the precision (intra- and inter-batch). The concentrations of the analytes in the SPE extracts calculated using the calibration curves were compared with the nominal concentration, in percentage, to determine

the accuracy. The relative standard deviation (RSD) of the intra-batch and inter-batch replicate analyses expressed the precision of the method [42, 43] In order to evaluate the possible carry-out effect, ethanol was injected after each set of triplicates. The stability of the compounds was assessed by calculating the RSD of the three QC extracts stored at 4 ºC in the autosampler, 24 and 48 h after reconstitution.

3.2.5. Matrix effect

The post-extraction addition method was used to assess the matrix effect [38, 41, 43]. The method was carried out on tap water samples, by comparison of three post-spiked extracts of blank samples and three extracts of non-spiked blank samples, using the optimized SPE procedure. The matrix effect (ME) was calculated as the ratio of the peak areas obtained for blank extracts spiked after SPE, subtracting those of the non-spiked blanks (A) and the peak areas of the standards solution with a similar concentration as the post-spiked extracts (B) through the following equation: ME (%) = A/B x 100 [41, 43]. The absence of matrix effect, the ionization enhancement and the ionization suppression are given respectively by values of 100%, > 100% or < 100%.

3.2.6. Application to drinking water samples and chemical treatment

Grab DW samples from different sources, namely tap water (n = 13), fountain water (n = 5) and well water (n = 5), were collected in the end of May 2015, from various locations of Portugal northwest region and analyzed by the proposed method. Samples were immediately stored at 4 °C until extraction, which was performed within 24 h. Before SPE, samples were acidified with sulphuric acid (pH 3) and sodium thiosulfate was added to each sample (30 mg L^{-1}) to reduce any residual chlorine that might be added as a disinfectant.

Tap water samples collected from the water supply network were spiked with the target analytes at 30 ng L^{-1}, to assess the applicability of the present UHPLC-MS/MS method to assess the removal of the target micropollutants by chemical processes. UV and ozonation experiments were performed as described elsewhere [44], and the removal of the target micropollutants was evaluated after 30 min, using a 1 L reactor loaded with 750 mL of the spiked samples, under magnetic stirring at 350 rpm.

3.2.7. Human health risk assessment

For those substances found in DW, a preliminary human health risk assessment was performed through the estimation of the HQ, according to previous works [35, 45]. HQ is given by the quotient of the estimated daily intake (EDI) and the acceptable daily intake (ADI):

$$HQ = \frac{EDI}{ADI} \qquad \text{(Eq. 3.1)}$$

where EDI values were calculated for the higher concentration of each substance quantified in tap, fountain or well water, as follows:

$$EDI = \frac{Concentration \times Ingestion\ rate}{Body\ weight} \qquad \text{(Eq. 3.2)}$$

by considering an average body weight of 70 kg for adults based on the average life expectancy at birth of the global population in 2013 of the World Health Organization, and a water intake of 2 L day^{-1} [35]. ADI for each pesticide was based on the Australian ADI list [46], whereas the values for pharmaceuticals were calculated from equation 3.3:

$$ADI = \frac{ADD}{AF} \qquad \text{(Eq. 3.3)}$$

where ADD is the average daily dose and AF is an assessment factor of 1000, which accounts for 10 from intra species variability, 10 for sensitivity in susceptible population groups and 10 for the differences between the ADD and the no observed effect concentration [35, 37].

3.3. Results and discussion

3.3.1. UHPLC-MS/MS optimization

Chromatographic separation was optimized using a sub-2 μm particle Kinetex™ column, allowing short and high resolution chromatographic runs. Since the present work deals with different groups of compounds with a vast range of physical-chemical characteristics (Table B-S3.1), the ideal mobile phase for certain target compounds might lead to low sensitivity for many other analytes. The mobile phase consisting of ethanol and ultrapure water, gave the best signal intensity and symmetric peaks as previously found for wastewater matrix [38]. The variation of organic/aqueous phase proportion and flow rate was optimized and a mixture of ethanol and ultrapure water (70/30, v/v), with a flow rate of 0.20 mL min^{-1} at isocratic mode was used. The column oven temperature was set at 35 °C, improving the resolution and peak shape of the analytes and reducing the analysis time to 15 min, as raising the temperature reduces the viscosity of the mobile phase.

3.3.2. MS/MS optimization

The tandem MS detection using a triple quadrupole enabled the simultaneous quantification of the 21 analytes at trace levels, as well as confirming their identity. The precursor ions of each compound were selected through the flow injection

analysis of each target analyte in full scan mode, under both positive and negative modes. From all the compounds studied in this work, 18 compounds and 2 internal standards had a higher intensity under positive mode of ionization, with the protonated molecular ion of each compound $[M+H]^+$ chosen as precursor ion, whereas 4 substances (3 compounds and 1 internal standard) were more intense in the negative ionization mode, using the deprotonated molecular ion of each compound $[M-H]^-$ as precursor ion. Most compounds presented two or more SRM and the most abundant product ion from each precursor ion (SRM1) was selected for quantification and the second most abundant (SRM2) was monitored for identity confirmation (Table B-S3.3), with a scan time of 100 ms per transition. In order to confirm the identity of the compounds, both the retention time (Table 3.1*)* and the ion ratio (SRM1/SRM2) of each analyte were used, according to European Commission Decision 2002/657/EC. Two pharmaceuticals and one pesticide (tramadol, fluoxetine and pentachlorophenol) had a poor fragmentation and only one SRM was monitored, a drawback overcome by the internal standard calibration using the respective surrogate standard.

Table 3.1. Retention time, range, linearity, method detection (MDL) and quantification (MQL) limits, accuracy, precision (intra- and inter-batch) and matrix effect for each target analyte.

Class and sub-class	Analyte	Retention time (min)	Range (ng L^{-1})	r^2	MDL[a] (ng L^{-1})	MQL[b] (ng L^{-1})	Accuracy (%)	Intra-batch precision RSD (%)	Inter-batch precision RSD (%)	Matrix effect (%)
Pharmaceuticals										
Anti-inflammatories	Diclofenac	1.27	0.75-40	0.9982	0.17	0.52	106.3 ± 10.5	1.67 - 8.48	10.1	22.2 ± 2.3
	Tramadol	5.65	0.75-40	0.9976	0.07	0.22	103.7 ± 9.3	2.28 - 3.55	12.9	117.1 ± 0.1
Antibiotics	Azithromycin	8.08	0.75-40	0.9969	0.20	0.61	93.4 ± 13.3	7.93 - 9.75	9.38	23.7 ± 8.4
	Clarithromycin	8.47	0.75-40	0.9957	0.11	0.32	104.1 ± 6.1	7.75 - 10.0	11.2	26.4 ± 11.5
	Trimethoprim	4.00	0.75-40	0.9993	0.07	0.21	97.1 ± 15.7	2.99 - 5.80	7.21	64.9 ± 13.3
Anticoagulant	Warfarin	1.28	0.75-40	0.9965	0.17	0.52	97.6 ± 15.1	7.67 - 15.2	10.6	193.4 ± 1.7
Antiplatelet agent	Clopidogrel	2.11	0.75-40	0.9982	0.01	0.04	112.1 ± 6.6	2.75 - 8.24	6.89	77.4 ± 10.3
Beta-blockers	Metoprolol	6.29	0.75-40	0.9984	0.05	0.15	109.3 ± 0.7	3.26 - 14.0	13.2	113.1 ± 5.6
Psychiatric drugs	Carbamazepine	1.32	0.75-40	0.9966	0.19	0.59	100.6 ± 3.5	9.76 – 15.0	8.38	30.4 ± 8.4
	Citalopram	6.06	0.75-40	0.9961	0.09	0.26	86.6 ± 6.4	5.09 - 11.4	14.5	113.2 ± 11.7
	Venlafaxine	6.84	0.75-40	0.9978	0.10	0.32	105.2 ± 5.4	1.11 - 4.60	14.5	108.9 ± 2.1
	Fluoxetine	8.86	0.75-40	0.9963	0.04	0.13	118.1 ± 0.3	0.77 - 3.96	5.19	95.2 ± 6.4
Metabolite	Norfluoxetine	8.93	0.75-40	0.9975	0.05	0.16	119.0 ± 0.2	3.04 - 6.79	6.99	95.2 ± 4.4

Pesticides										
Chloroacetanilide	Alachlor	1.65	0.75-40	0.9975	0.09	0.28	98.8 ± 0.3	6.39 - 14.9	8.97	99.2 ± 10.3
Triazine	Atrazine	1.33	0.75-40	0.9945	0.12	0.37	92.3 ± 2.8	2.60 - 6.47	7.86	52.5 ± 15.4
	Simazine	1.21	0.75-40	0.9983	0.15	0.46	84.9 ± 4.6	3.86 - 9.23	8.35	49.8 ± 2.4
Organophosphorus	Chlorfenvinphos	1.62	0.75-40	0.9971	0.18	0.54	98.6 ± 6.2	5.01 - 14.7	14.8	96.9 ± 2.0
Phenylurea	Isoproturon	1.34	0.75-40	0.9968	0.04	0.12	99.2± 3.4	2.00 - 4.10	5.02	34.4 ± 9.4
Organochlorine	Pentachlorophenol	1.55	0.75-40	0.9986	0.20	0.60	94.1 ± 6.8	7.75 - 13.2	8.65	57.5 ± 9.0
Herbicide	Clofibric acid	1.23	0.75-40	0.9995	0.14	0.42	92.7 ± 5.5	6.20 – 11.0	6.57	19.1 ± 6.5
Industrial compound	PFOS	1.07	0.75-40	0.9957	0.06	0.19	80.6 ± 6.2	5.30 - 13.5	4.51	48.7 ± 1.4

[a] MDL, method detection limit; [b] MQL, method quantification limit.

3.3.3. SPE optimization

A detailed optimization study was carried out on the most relevant parameters that affect recovery rates and matrix effects, namely the sample pH, the extraction solvents, the type of cartridges, the sample volume and the addition of chelating and dechlorination additives. Preliminary studies were performed to evaluate the performance of different sample pH, by extracting 250 mL of tap water samples through the versatile Oasis® HLB cartridges. The water samples were adjusted to different pH (3, 7 and 9) and extracted using a conventional solvent, i.e. methanol, as conditioning and eluting solvent. Acidic pH provided higher recoveries for acidic compounds, and in particular for pesticides and some pharmaceuticals (Fig. B-S3.1), whereas basic analytes were recovered better at higher pH but a lower influence of pH on the extraction efficiencies was found for these compounds. Thus, the best compromise was to adjust the sample pH to 3, in order to get the best recovery for as many analytes as possible.

Recoveries of Oasis® MCX cartridges useful for extraction of basic compounds and Oasis® MAX adequate for extraction of acidic compounds were then compared to Oasis® HLB cartridges. A recovery higher than 70% was achieved using Oasis® MCX for the antidepressants (citalopram, venlafaxine, fluoxetine) and for trimethoprim (Fig. 3.1). These results were expected, due to the high p*K*a of these compounds (near 9). Clofibric acid and diclofenac were highly recovered when extracted by Oasis® MAX cartridges (Fig. 3.1), owing to their acidic nature (p*K*a values of approximately 4). However, the versatile Oasis® HLB cartridges suitable for most compounds (acidic, basic and neutrals), provided higher recoveries for most analytes (Fig. 3.1), as observed in other works [15, 20]. Thus, Oasis® HLB was the adsorbent selected for the next recovery experiments, using sample pH adjusted to 3.

Different sample volumes were tested (250, 500 and 1000 mL) using Oasis® HLB cartridges and sample pH adjusted to 3 to determine the breakthrough volume, the volume that allows the maximum extraction efficiency and from which extraction efficiency declines [41]. The sample volume of 250 mL provided the highest recoveries for the majority of the compounds, except for fluoxetine and norfluoxetine, being selected as the optimized sample volume (data not shown). Although a higher volume would give a theoretical higher enrichment factor, the results showed that recovery rates for most compounds decreased using higher sample volumes, due to the aforementioned phenomenon of decrease of extraction efficiency above the so-called breakthrough volume, as previously described [47]. Although a clean matrix was studied in the present work, it is reported in other studies dealing with different matrices that even when using the same method, the recovery is not always better for matrices that are supposed to be cleaner [20, 48].

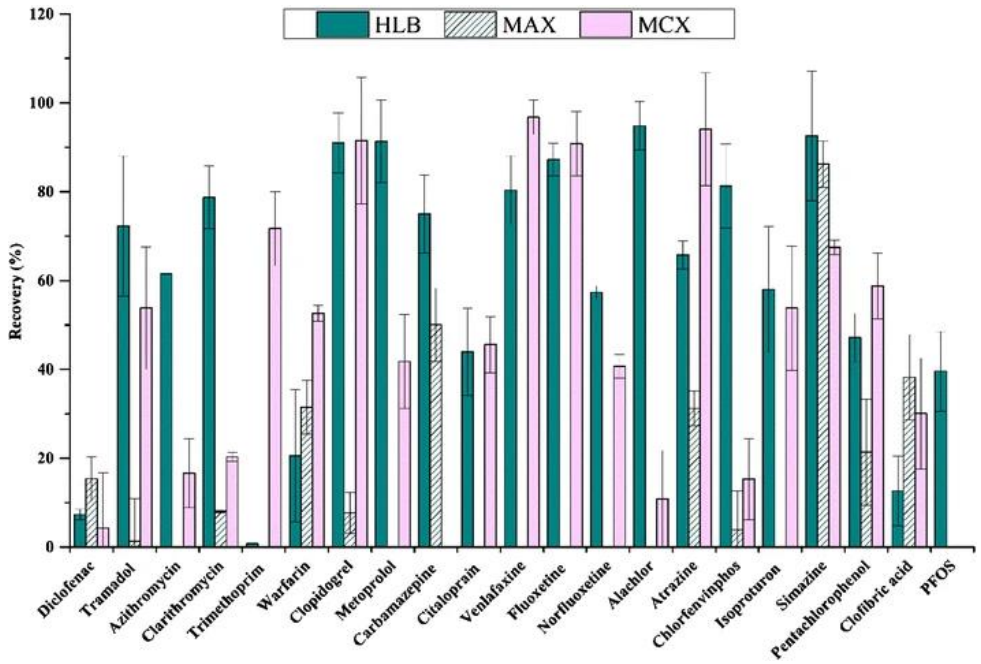

Fig. 3.1. Recoveries obtained for the target analytes with the following SPE conditions: HLB, MAX and MCX using methanol and extracting 250 mL of tap water samples, adjusted to pH 3 for HLB and MCX and pH 9 for MAX cartridges.

Afterwards, Oasis® HLB cartridges were employed to extract 250 mL of tap water samples at pH 3 (optimized for methanol), using ethanol as conditioning and elution solvent, due to the known toxicity of methanol, usually used for SPE. Ethanol (Fig. 3.2) gave recoveries slightly higher than methanol (Fig. 3.1) for the majority of compounds. Moreover, ethanol is considered a “green” solvent, i.e., minimizes the environmental impact resulting from the use of solvents, and follows the guidelines of GAC [28, 29]. In fact, several methods reported in the literature employ solvents such as methanol or acetonitrile, presenting high toxicity [14, 15, 20, 22, 23, 27]. Thus, ethanol was selected as solvent for the next experiments. This is the first SPE procedure proposed for extraction and cleanup of DW samples, employing ethanol as extracting and eluting solvent.

Subsequently, the chelating and dechlorination effects were studied. Whilst a solution of EDTA was added to the water samples to test the chelating effect, acid ascorbic or sodium thiosulfate were added to assess the dechlorination effect. Regarding to the addition of EDTA, it was possible to verify a slight improvement in the extraction efficiency of a few compounds (Fig. 3.2), compared with the results obtained for samples without additive, namely for chlorfenvinphos, clofibric acid, trimethoprim and diclofenac. This could be explained by the fact that these compounds might bind to residual metals present in the sample matrix, resulting in low extraction recoveries [20]. By adding EDTA, soluble metals bind to the chelating agent, increasing the extraction efficiency of some compounds that are available to be extracted and detected [20]. This phenomenon was previously observed in DW by several authors [14, 20, 23]. Concerning the dechlorination agents, the addition of sodium thiosulfate increased the overall extraction recoveries (Fig. 3.2), probably because it reduced the residual chlorine that had been added as a disinfectant in the DW supply [22]. The effects of filtering and/or aeration of the water samples and the simultaneous addition of EDTA and sodium thiosulfate were also studied, however the recovery efficiency was not improved. Therefore, sodium thiosulfate was used before SPE to enhance the recovery rates.

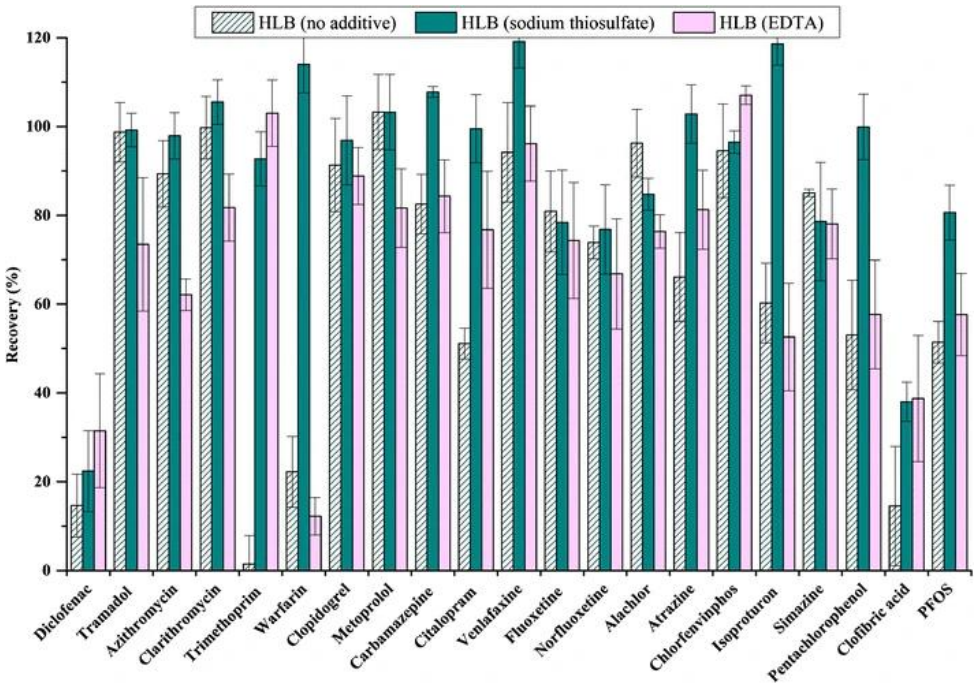

Fig. 3.2. Recoveries obtained for the target analytes with the following SPE conditions: HLB cartridges using ethanol, extracting 250 mL of tap water samples, adjusted to pH 3, without additives, with sodium thiosulfate, or EDTA as additives.

The main objective of the optimization of the sample preparation methodology was the development of a single SPE procedure, allowing the extraction of a large group of compounds with different physical-chemical characteristics. As result, and according to the higher recoveries obtained for most of the target compounds, the selected conditions were: Oasis® HLB cartridges, ethanol as conditioning and eluting solvent and 250 mL of water samples (pH 3) with sodium thiosulfate at 30 mg L^{-1} as dechlorination agent.

The recoveries obtained for reuse performance assessment of the cartridges showed that each reuse led to a loss of retention capacity of the cartridges, reflected by the decrease of the recovery of the compounds. The first reuse of the cartridges led to an average decrease of 14% on the recovery efficiency. The loss was higher for the

second reuse, with a decrease of approximately 50% in the recovery rates. Here, it was verified that although preconized by the supplier, reusing cartridges is not a good practice for analytical purposes that require a high reproducibility.

3.3.4. Matrix effect

The matrix effect was determined by the post-extraction addition method, to assess the influence of the matrix in the ionization process occurring in the ionization source of the mass spectrometer [38]. The percentage ratio between the post-spiked blank extracts and ethanolic standard solutions were between 19.1% and 193%. Although DW is considered a clean and simple matrix, a wide range of values was found for the matrix effect. Cotton *et al.* [49] also reported high matrix interferences for many compounds, only less than half of the analytes had matrix effect values within 80-120%. When LC-MS/MS methods are developed to determine various micropollutants in different matrices, e.g., DW, surface water and wastewater, matrix effects are usually calculated for only one of these matrices. Most compounds presented signal suppression, i.e. matrix effect < 100%, namely diclofenac, azithromycin, clarithromycin, trimethoprim, clopidogrel, carbamazepine, atrazine, simazine, isoproturon, pentachlorophenol, clofibric acid and PFOS (Table 3.1). Tramadol, metoprolol, citalopram and venlafaxine had a slight ionization enhancement (matrix effect > 100%) while the signal of warfarin was highly increased. Compounds with almost no matrix effect, under the conditions of the current work, were fluoxetine, norfluoxetine, alachlor and chlorfenvinphos.

3.3.5. Quality assurance/quality control

The trends of GAC were applied in the chromatographic optimization, namely the use of low volumes of non-toxic solvents [28, 29]. Enhanced productivity and reduced cost

are the main objectives for routine analysis, being possible using stationary phases with reduced column length and diameter [30, 33]. Also the new instruments operating at higher pressure allow using more viscous solvents as ethanol, which is less volatile than acetonitrile and has less toxicity and lower disposal costs than both acetonitrile and methanol, complying with the trends of GAC. The short run time and the low volume of a non-toxic organic phase as ethanol is a great achievement in the method development, in comparison to chromatographic methods for DW analysis using methanol [15, 20, 21] or acetonitrile [14, 22, 23, 27] as organic mobile phases, as well as methanol as solvent for conditioning and eluting the SPE cartridges [14, 15, 20, 22, 23]. In the present work, 21 compounds with diverse chemical nature (7 pesticides, 1 industrial compound, 12 pharmaceuticals and 1 metabolite) were determined in a single run (Fig. B-S3.2 (a, b)). In the limited literature for DW analysis, the number of compounds analyzed by LC-MS/MS varies up to ca. 80, most reports deal with pharmaceuticals [14, 20–23, 27], and a couple of them deal with both pharmaceuticals and pesticides [15, 16].

The offline SPE-UHPLC-MS/MS method was validated according to the international guidelines [39] and works published elsewhere [38, 41, 50], regarding recovery, accuracy, intra and inter-batch precision (Table 3.1). The recovery of the target analytes using the optimized SPE procedure was assessed, after pre-concentration of blank samples and 35 ng L^{-1} spiked samples. The recoveries evaluated for the DW matrix were reproducible and between 22.4% and 139% (Fig. 3.2). Peak areas of the target analytes found in the DW blank matrix were deducted for recovery rate evaluation. The dissimilar recoveries are owing to the wide chemistry nature of the target compounds and were taken into account, using the matrix match calibration curves and internal standards addition before SPE. For instance, Gros *et al.* [20] developed a multi-residue analytical method, with similar recoveries values for DW,

namely for cimetidine (24 ± 17%). In that work, recovery values for the same compounds were higher in other matrices such as surface and wastewaters. López-Serna *et al.* [48] also reported some low values of recovery (< 10%) for groundwater, and higher recoveries for matrices presumably more affected by interferents. Accuracy as well as intra and inter-batch precision were evaluated by analysis of the QC extracts. The accuracy ranged from 80.6% to 119% (Table 3.1), which is within the range of 80 –120%, according to the international criteria [39]. RSD of the triplicate measurements of the three QC was used to guarantee the precision of the method (Table 3.1), with intra-batch < 15.2% and inter-batch < 14.8%, meeting the international guidelines (RSD lower than 15% or 20% for the lower concentration QC) [39]. RSD of the triplicate analysis of the three QC samples after 24 and 48 h of reconstitution was lower than 5%. The calibration curves were generated using the internal calibration method through spiking samples with isotopically labeled internal standards, before SPE extraction. Three internal standards were used for three sets of compounds that were defined depending on the acid-basic nature (Table B-S3.3), as other published works dealing with multi-class determination [14, 20, 27], which use an internal standard for each set of compounds due to the high cost for routine environmental monitoring and difficulty to find suitable internal standards for each compound in a series of compounds with distinct properties. The coefficients of determination of the calibration curve extracts were higher than 0.99 in the range of 0.75–40 ng L^{-1} for all compounds (Table 3.1). The MDL and MQL were between 0.01–0.20 ng L^{-1} and 0.04–0.61 ng L^{-1}, respectively, allowing to detect the target contaminants at residual concentrations (few nanograms per liter levels).

3.3.6. Quantification of micropollutants in DW

The developed offline SPE-UHPLC-MS/MS method was applied to DW samples collected at the end of May 2015, from various locations of Portugal northwest region and from different sources (Table 3.2), namely tap water (n = 13) (Fig. B-S3.2 (c)), fountain water (n = 5) and well water (n = 5). Of the 21 investigated chemicals, 13 were detected in DW samples at ng L^{-1} levels, which is consistent with concentrations reported in other studies [14, 15, 20–23, 27, 51]. The most common chemicals observed were diclofenac, trimethoprim, warfarin, metoprolol, norfluoxetine, atrazine and simazine.

Regarding tap water, diclofenac, warfarin, norfluoxetine, atrazine and simazine were the compounds most frequently detected. The micropollutants found at highest concentrations were diclofenac and the pesticide chlorfenvinphos considered a PS, although well below 0.1 μg L^{-1} preconized for single pesticides in the Directive 1998/83/EC [6]. Concerning fountain water samples, diclofenac and atrazine were the most common micropollutants, being also found at the highest concentrations. The results obtained for well water samples showed that diclofenac was quantified in all the samples. Diclofenac, carbamazepine and the PS simazine were those found at the highest concentrations.

The comparison of the results obtained in this work with similar studies conducted by other authors (Table B-S3.4) is difficult, since the consumption of pharmaceutical compounds as well as the intensity of agricultural and industrial activities, varies among different regions. Carbamazepine, caffeine, ibuprofen and sulfamethoxazole were often reported in DW, being carbamazepine the most frequently found up to 40 ng L^{-1} [14, 15, 20–23, 27, 51]. Other compounds such as atenolol, clofibric acid, azithromycin, erythromycin, fluoxetine and diclofenac were also detected but at very

low levels [15, 20, 21, 23, 27]. It is important to emphasize the need of revision of the European policy regarding tap waters, considering that Directive 1998/83/EC is outdated in view of the studies reported in the last decade. The more recent Directive 2013/39/EU regulates surface waters, demanding more rigorous acceptable values than Directive 1998/83/EC [6] regulating water for human consumption. The same issue should be considered for groundwater regulated by Directive 2006/118/EC [5], considering that fountain and well waters used for human consumption can be sourced by this type of water.

3.3.7. Human health risk assessment

The maximum values of each micropollutant in DW were used to estimate the respective HQ. This prediction give insights about the human health risk assessment, by evaluating the probability of adverse effects: HQ values below 0.1 indicate no expected adverse effects; values between 0.1 and 1.0 suggest potential for adverse effects that should be considered, despite of the low risk; HQ values ranging from 1.0 to 10 indicate adverse effects or mild risk; a high risk is assumed only for HQ values above 10 [32]. The maximum measured concentrations observed for the targeted chemicals found in DW (Table 3.2) were used to calculate EDI, predicting the worst case scenario. Even so, the HQ for all micropollutants found in DW samples were between 4.56×10^{-6} and 4.49×10^{-3}, well below 0.1, so adverse effects are not likely to be expected at such concentrations. Risks assessment of simultaneous exposure to multiple contaminants was not considered, although some of these compounds are already recognized to trigger several additive, synergistic or antagonist effects [34, 36].

Table 3.2. Concentrations of micropollutants (ng L^{-1}) detected in tap, fountain and well water samples analyzed.

Class and sub-class	Analyte	Tap water (n=13)		Fountain water (n= 5)		Well water (n=5)	
Pharmaceuticals		**Concentration (ng L^{-1})**	**Frequency**	**Concentration (ng L^{-1})**	**Frequency**	**Concentration (ng L^{-1})**	**Frequency**
Anti-inflammatories	Diclofenac	<MQL–7.87	7/13	3.95–7.66	4/5	1.60–36.20	5/5
	Tramadol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotics	Azithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Clarithromycin	< MQL	1/13	n.d.	n.d.	1.14	1/5
	Trimethoprim	< MQL	1/13	< MQL	1/5	0.86	1/5
Anticoagulant	Warfarin	0.39–3.89	5/13	4.07	1/5	11.2	1/5
Antiplatelet agent	Clopidogrel	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beta-blockers	Metoprolol	< MQL	5/13	n.d.	n.d.	< MQL	1/5
Psychiatric drugs	Carbamazepine	3.34	1/13	n.d.	n.d.	58.8	1/5
	Citalopram	< MQL	1/13	n.d.	n.d.	n.d.	n.d.
	Venlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Fluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metabolite	Norfluoxetine	< MQL	13/13	< MQL	1/5	< MQL	1/5
Pesticides							
Chloroacetanilide	Alachlor	< MQL	4/13	n.d.	n.d.	3.07	1/5

Triazine	Atrazine	1.14–2.24	6/13	1.59–103	3/5	1.66	1/5
	Simazine	< MQL–1.45	4/13	< MQL–2.20	2/5	2.84–28.4	2/5
Organophosphorus	Chlorfenvinphos	2.46–6.50	2/13	0.49–3.89	2/5	n.d.	n.d.
Phenylurea	Isoproturon	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Organochlorine	Pentachlorophenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Herbicide	Clofibric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Industrial compound	PFOS	< MQL	1/13	n.d.	n.d.	11.7	1/5

MQL, method quantification limit; n.d., not detected; PFOS, Perfluorooctanesulfonic acid.

3.3.8. Removal of micropollutants in DW using UV radiation or ozonation

Tap water samples collected from the water supply network were post-spiked with the target micropollutants at ng L^{-1} level and submitted to UV radiation or ozonation to assess the removal of the target micropollutants using the eco-friendly analytical method (Fig. 3.3), since these processes are often applied in DWTPs.

Only 7 pharmaceuticals were completely removed by these water treatments: (i) tramadol, venlafaxine and azithromycin by both processes, azithromycin recently included in the first Watch List by the EU Decision 2015/495; (ii) clopidogrel, carbamazepine and isoproturon by ozonation; and (iii) the metabolite norfluoxetine by UV. Regarding the other micropollutants, the efficiency of the processes varied according to the substance. The results showed that, in general, UV radiation was more effective than ozonation for the removal of pesticides and for the industrial compound, whereas ozonation performed slightly better for pharmaceuticals. The feasibility of this UHPLC-MS/MS analytical method for monitoring chemical processes used to improve the quality of drinking water was shown.

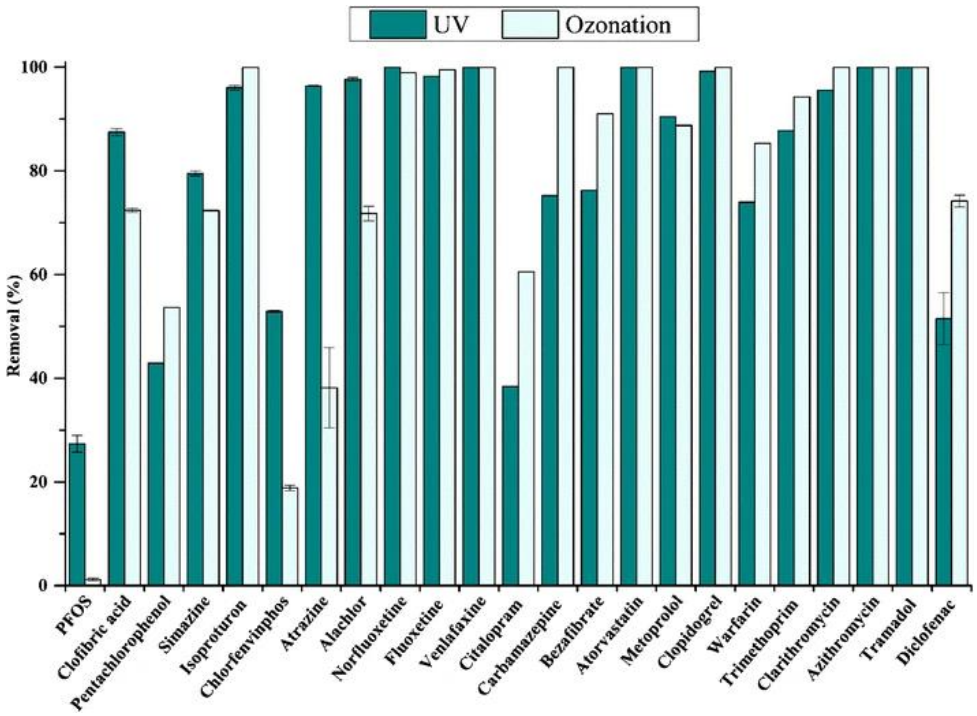

Fig. 3.3. Removal percentage of the micropollutants in spiked DW after the bench-scale UV or ozonation treatments.

3.4. Conclusions

The offline SPE-UHPLC-MS/MS that was developed and validated, to assess the occurrence and removal of 21 multi-class micropollutants in DW, has the great advantage of using an eco-friendly solvent (ethanol) for both SPE procedure and UHPLC analysis, according to the recent concerns about GAC applied to environmental analyses. Additional advantages presented by the method are: (i) low detection limits (below 1 ng L^{-1}); (ii) short run time; (iii) low volume of eluent employed for each analysis; (iv) the use of a single cartridge/SPE procedure to extract all the target analytes; (v) and the low volume of sample used. The potential of the offline SPE-UHPLC-MS/MS method for monitoring programs and evaluation of advanced

treatment options (UV and ozonation) was demonstrated in the selected case studies. For instance, analysis of tap, fountain and well water samples from different locations of Portugal northwest region, showed a widespread occurrence of micropollutants in such matrices, at ng L^{-1} levels. From the thirteen micropollutants detected in DW samples, the most common were diclofenac, trimethoprim, warfarin, norfluoxetine, atrazine and simazine; the feasibility of the method for monitoring DW treatment processes was also validated.

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Supplementary material

(Please see Appendix B)

Table B-S3.1. Hyphenated chromatography-mass spectrometry techniques for DW analysis.

Table B-S3.2. Target analytes, describing their class, sub-class, chemical structure, molecular weight (M*w*) and p*K*a.

Table B-S3.3. Optimized mass spectrometer parameters for SRM analysis of the target analytes.

Table B-S3.4. Comparison of occurrence data for the targeted pollutants in DW samples (ng L^{-1}), observed in the present study and reported in others.

Fig. B-S3.1. Recoveries obtained for the target analytes with the following SPE conditions: HLB cartridges using methanol, extracting 250 mL of tap water samples, adjusted to pH 3, 7 or 9.

Fig. B-S3.2. Total ion chromatograms of (a, b) QC sample spiked with the targeted chemicals at 15 ng L^{-1}; (c) tap water sample contaminated with atrazine, chlorfenvinphos and warfarin.

Chapter 4

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

Chapter 4

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

Abstract

The European Union (EU) has recommended the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2015/495) in surface waterbodies. The present study provides spatial distributions and temporal variations of a wide range of multi-class PSs and CECs in four stressed rivers in Portugal (Ave, Leça, Antuã, and Cértima). Thirteen micropollutants were found in all four rivers, including the priority pesticide isoproturon (up to 92 ng L^{-1}), various pharmaceuticals (up to 396 ng L^{-1}), and the UV-filter 2-ethyl-hexyl-4-methoxycinnamate (up to 562 ng L^{-1}) identified in Decision 2015/495. The industrial priority compound perfluorooctanesulfonic acid (PFOS) was found in three rivers (Antuã, Cértima, and Leça) below the method quantification limit, together with four pharmaceuticals not included in these EU guidelines. The already banned priority pesticide atrazine was detected in Ave, Antuã, and Leça (up to 41 ng L^{-1}) and simazine in Cértima and Leça (up to 26 ng L^{-1}). Acetamiprid and imidacloprid (included in Decision 2015/495) were only detected during the dry season in the Ave. Leça river was selected as a waterbody case study for assessment of fluorescence excitation-emission matrices (EEMs). These results matched the spatial distribution trend of micropollutants along the river, with stronger fluorescence response and higher concentrations being found downstream of industrial areas and urban wastewater treatment plants (WWTPs). Moreover, the fluorescence signature of surface water collected downstream of an urban WWTP aligned very well with that obtained for the

respective WWTP effluent. Thus, actions are needed to preserve a good environmental status of these stressed European waterbodies.

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4.1. Introduction

Environmental contamination of aquatic compartments by organic micropollutants is a subject of major concern in the last two decades [1, 2]. Surface waters are constantly exposed to such contaminants, which mainly originate from agricultural runoff and discharge of effluents from industrial and municipal wastewater treatment plants (WWTPs), with the latter considered the major source of some classes of micropollutants found in river waters [2, 3]. The occurrence of organic micropollutants in rivers at residual concentrations [4-12] can lead to adverse effects for aquatic wildlife and human health, limiting the use of water for recreation, irrigation, and consumption [13, 14].

To tackle these problems, current European Union (EU) recommendations suggest the regular monitoring of an extensive range of chemical and biological parameters in surface waters [15], including a list of 45 substances for priority action (priority substances, PSs) with environmental quality standards (EQS) set up for some compounds [16, 17] and a Watch List of contaminants of emerging concern (CECs) [18]. Moreover, Directive 2013/39/EU set the Maximum Allowable Concentration-EQS (MAC-EQS), corresponding to the concentration that should not be exceeded at any representative monitoring point for any given surface water body. In this context, the monitoring of PSs and CECs in surface waters is a useful tool not only to assess pollution sources, but also to ensure efficient management of water resources and the protection of aquatic flora and fauna [1].

The occurrence of particular PSs and CECs in Portuguese rivers has been reported in Ave river [19], Leça river [20], Douro river [21], Ria de Aveiro [22], and Guadiana river [23]. However, an integrated study comprising the spatial distributions and temporal variations of a wide range of PSs and CECs belonging to different classes

has not been concurrently conducted in multiple rivers. Therefore, the purpose of the present study was to perform two seasonal monitoring campaigns of 39 organic micropollutants in four stressed Portuguese rivers: i) Ave; ii) Leça; iii) Antuã; and, iv) Cértima. Contamination levels of the target compounds in these rivers were investigated during dry and wet seasons, and the water samples, collected at different points on each river, were analysed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), after sample preparation by solid-phase extraction (SPE).

Quantitative PS/CEC analyses require significant costs, which often limit the scope of the sampling plan for specific projects. For this reason, quick, inexpensive screening tools that provide insight into PS/CEC occurrence and concentration would not only allow optimization of sampling strategies, but also elicit new research questions on the occurrence and fate of PSs/CECs in surface water systems. Excitation-emission matrix (EEM) analyses are increasingly being used to describe the fluorescence properties of dissolved organic matter (DOM) for characterization [24], source-tracking [25], and fate/transformation [26] purposes. We posit that EEM analysis may serve as a useful screening tool for representative PSs/CECs given their chemical similarity with select molecules in the DOM matrix. This concept has been previously explored with respect to CEC occurrence [27] and transformation [28]. For example, Yang *et al.* (2013) [27] found significant correlations for caffeine, sulfamethoxazole, acetaminophen, and ciprofloxacin concentrations with the summed volume from regions I, II, and IV of the EEMs of water samples collected from the Pearl river (China). Nevertheless, the correlation of other PSs/CECs with specific EEM regional volumes needs to be explored to develop location-based screening tools for other watersheds, especially as the DOM matrix and PSs/CECs use vary by watershed. In this study, potential correlations between EEMs and PSs/CECs occurrence and

concentration were investigated in the Leça river. This report is the first to evaluate the spatiotemporal distribution of PSs/CECs and fluorescence EEMs, as well as the correlations between these water quality parameters, in a Portuguese river, and the results have important implications for other water systems around the world.

4.2. Materials and methods

4.2.1. Chemicals and materials

All reference standards (i.e., acetamiprid, alachlor, atenolol, atorvastatin, atrazine, azithromycin dihydrate, bezafibrate, carbamazepine, ceftiofur, chlorfenvinphos, citalopram hydrobromide, clarithromycin, clindamycin, clofibric acid, clopidogrel hydrogen sulfate, clothianidin, diclofenac sodium, diphenhydramine, 2-ethylhexyl-4-methoxycinnamate (EHMC), enrofloxacin, erythromycin, fluoxetine hydrochloride, hydrochlorothiazide, imidacloprid, isoproturon, ketoprofen, methiocarb, metoprolol tartrate, norfluoxetine oxalate, ofloxacin, perfluorooctanesulfonic acid (PFOS), propranolol, simazine, thiacloprid, thiamethoxam, tramadol hydrochloride, trimethoprim, venlafaxine hydrochloride, and warfarin; > 98% purity) and surrogate standards (i.e., acetamiprid-d3, azithromycin-d3, atrazine–d5, diclofenac-d4, fluoxetine–d5, ketoprofen–d3, methiocarb-d3, and ofloxacin-d3) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol (MS grade) and ethanol (HPLC grade) were acquired from VWR International (Fontenay-sous-Bois, France) and Fisher Scientific (Leicestershire, UK), respectively. Formic and sulfuric acid were obtained from Merck (Darmstadt, Germany), and ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ cm at 25 °C). Oasis® HLB (Hydrophilic-

Lipophilic Balanced) cartridges (150 mg, 6 mL), used for sample preparation, were purchased from Waters (Milford, MA, USA).

4.2.2. Sampling area

Two sampling campaigns were performed in the dry (September 2016) and wet seasons (February 2017). During the sampling period, the weather was characterized by a mean atmospheric temperature of 23 °C in September 2016 and 11 °C in February 2017. The average precipitation was 24.3 and 113.5 mm in September and February, respectively [29]. The selection of Ave, Leça, Antuã, and Cértima rivers was based on the following: recognized contamination due to adjacent land-use patterns (i.e., residential, agricultural, and industrial areas); the existence of tributaries and WWTPs that can have a negative impact on the quality of these water courses; and, the presence of drinking water treatment plants (DWTPs) that may be affected by surface water pollution. Sample collection was performed along the whole course of the four target rivers (8 sampling points (SPs) for Leça and Cértima rivers and 9 SPs for Ave and Antuã rivers, Fig. 4.1), comprising locations near the source and mouth, as well as strategic areas subject to impacts from urban, agricultural, or WWTP activities. The GPS coordinates of the SPs for each river are given in Table C-S4.1, Supplementary material.

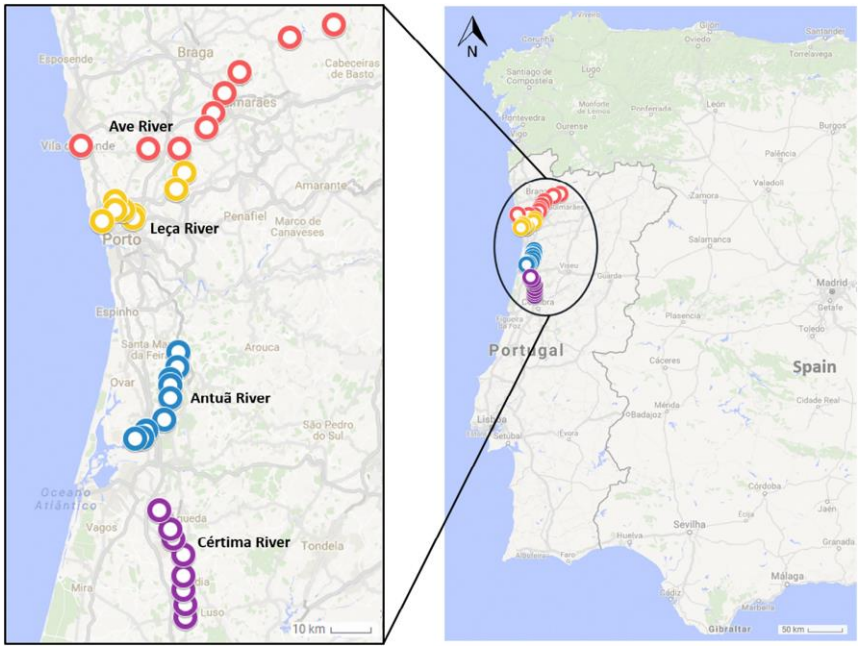

Fig. 4.1. Ave, Leça, Antuã, and Cértima rivers (Portugal) and the location of each sampling site.

4.2.2.1. Ave River

The Ave river, which is situated in the North of Portugal, has an extension of about 100 km and a drainage basin area of 1340 km^2. The headwaters are located in Cabreira Mountain (1260 m above mean sea level, a.m.s.l.), and the estuary is located in Vila do Conde, along the Atlantic coast. The most important tributaries are the Este and Vizela rivers at the right and left banks, respectively. The average flow in the Ave river was 2.96 $m^3\ s^{-1}$ in the dry season (September 2016) and 63.08 $m^3\ s^{-1}$ in the wet season (February 2017) [30]. The water resources are used for manufacturing and irrigation of rural activities. Most of the river basin area is used for agricultural and livestock activities [31]. Water quality problems observed in this area are associated with high industrial density, including the textile sector (largest industry), leather

tanning, rubber manufacture, and plastic production. Some industrial effluents are still illegally discharged into the water courses without treatment [19].

4.2.2.2. Antuã River

Antuã river (extension of 38 km) has its source at Romariz, Santa Maria da Feira (400 m a.m.s.l.) and drains into the Atlantic Ocean through the Ria de Aveiro. Antuã basin is one of the sub-basins of the Vouga river with a total area of approximately 149 km^2. The main tributaries are Pintor stream, Cercal stream, and Ínsua river on the left bank and Arrifana stream on the right bank. Antuã river is characterized by an average flow of 4 $m^3\ s^{-1}$, with the monthly average ranging between 0.6 $m^3\ s^{-1}$ in August and 10 $m^3\ s^{-1}$ in February [32]. Low water quality in this river stems from industrial and urban discharges, runoff from agricultural fields, and discharges from livestock farms. Agriculture is the principal activity within the borders of the Antuã river basin, which is situated near the city of Estarreja [33].

4.2.2.3. Cértima River

The 43 km long Cértima river is located in North-Central Portugal and serves as a sub-tributary of the Vouga river, which drains into the Atlantic Ocean through the Ria de Aveiro coastal lagoon. The river source is at Buçaco Mountain (380 m a.m.s.l.), and the basin drains an area of 538 km^2. In its lower section, the river valley opens widely to form Pateira de Fermentelos lake, a sensitive wetland classified as a Ramsar site (i.e., wetlands of international importance designated under the Ramsar Convention). The river narrows again at Requeixo, where the Cértima discharges into the Águeda river [34]. The Cértima river has a flow rate of 7.17 $m^3\ s^{-1}$ at SP8 in the wet season and 0.13 $m^3\ s^{-1}$ during the dry season [35]. The main tributaries are the Serra and Levira rivers and Ribeira do Pano [36]. Agriculture, domestic discharges,

and industrial activities are the major sources of chemical pollution in the Cértima river basin [13].

4.2.2.3. Leça River

On its flow towards the Atlantic Ocean, Leça river has an extension of 45 km and drains an area of 190 km^2 [20], with an average flow of 3.4 m^3 s^{-1} [37]. The source of Leça river is located at Monte Córdova, Santo Tirso (475 m a.m.s.l.) and its mouth is located at Leixões Harbor basin, an important international harbor having dock facilities for commercial, cruise, and fishing vessels and an oil terminal. The main tributaries are Ribeira do Arquitecto and Ribeira do Leandro, both on the right bank. The river receives effluents from several industries, some of which are untreated, and urban WWTPs [38]. Leça river was selected as a case study to evaluate the correlation of fluorescence EEMs to PS and CEC concentrations since it is located between highly urbanized and industrialized regions belonging to the Porto metropolitan area, which represents the largest Port in Northern Portugal. One sample collected after the secondary biological treatment stage of an urban WWTP was also analyzed for comparison with surface samples.

4.2.3. Sample collection and preparation

Surface water samples were collected in the middle of each river, using a bottle sampler. Subsequently, samples were transferred to 1 L amber glass bottles and stored at 4 °C until extraction, which was performed within 24 h. Leça river surface water and wastewater effluent samples were frozen at -20 °C, until analysis of the respective fluorescence EEMs. Several parameters, such as pH, conductivity, oxidation-reduction potential, temperature, salinity, dissolved oxygen, and total dissolved solids, were analyzed on site using a HI98194 Multiparameter Meter

(HANNA® instruments; Woonsocket, RI, USA). Before SPE, all samples were filtered through 1.2-µm glass-fiber filters (47 mm GF/C, WhatmanTM; Maidstone, United Kingdom) and the pH was adjusted to 3 using sulfuric acid.

4.2.4. SPE–UHPLC–MS/MS method

An offline SPE–UHPLC–MS/MS method was applied for quantification of the target organic micropollutants according to previous works [39, 40]. Briefly, Oasis® HLB cartridges were sequentially conditioned with 4 mL of ethanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. Sample loading of 500 mL of surface water samples was carried out at a constant flow rate of 10 mL min^{-1}, using a vacuum manifold unit. The washing step was performed with 4 mL of ultrapure water, and the cartridges were then dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of ethanol and the extracts were evaporated to dryness in a Centrivap Concentrator® device (LABCONCO® Corporation, Kansas City, MO, USA). The dried extracts were reconstituted in 250 µL of ethanol, and the resulting solutions were filtered through 0.22 µm polytetrafluoroethylene syringe filters (Membrane Solutions, Kent, WA, USA).

Surface water sample analysis was performed by UHPLC-MS/MS, using a Shimadzu Corporation apparatus (Tokyo, Japan), consisting of an UHPLC (Nexera) with two pumps (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a degasser (DGU-20A 5R), and a system controller (CBM-20A) with proper software (LC Solution Version 5.41SP1) coupled to a triple quadrupole mass spectrometer (Ultra Fast Mass Spectrometry series LCMS-8040). Analytical separation occurred along a Kinetex™ XB-C18 100 Å column (100 × 2.1 mm i.d.; 1.7 µm particle diameter) supplied by Phenomenex, Inc. (Torrance, CA, USA). The mobile phase consisted of (A) 0.1% formic acid aqueous solution and (B) methanol operated in gradient mode. Column

oven and autosampler temperatures were set at 35 °C and 4 °C, respectively, and the injection volume was 5 µL. Selected reaction monitoring (SRM) transitions between the precursor ion and the two most abundant fragment ions were evaluated to quantify and confirm the identity of each compound. SRM1 was used for quantification purposes and the ratio between SRM1 and SRM2 was used for qualitative confirmation, along with the analyte retention time. Detailed analytical parameters and method selectivity, linear range, and limits of detection and quantification are described in the supplementary material (Tables C-S4.2 and C-S4.3).

4.2.5. Fluorescence excitation-emission matrices (EEMs)

Fluorescence EEMs of Leça river and wastewater samples collected during the wet season were measured using a Horiba Aqualog fluorescence spectrophotometer (Horiba Scientific; Edison, NJ USA). For all samples, 3-mL aliquots were added to a 1-cm quartz cuvette for analysis. Excitation wavelengths were incrementally increased from 209 to 620 nm using 3-nm steps, and the emission spectrum was recorded at 244-822 nm with 2.33-nm steps. Fluorescence EEMs of environmental samples were blank-corrected using LC-MS grade water. Inner-filter effects were corrected using the controlled dilution approach [41, 42]. The 1st and 2nd order Rayleigh scattering lines were removed using the Horiba masking tool. A sealed Raman water fluorescence standard (Agilent Technologies; Santa Clara, CA USA) was used to convert all data to Raman Units [43]. Corrected EEMs were plotted in Matlab (Mathworks; Natick, MA, USA), and regional volumes were calculated according to Chen *et al.* [44]. The corrected EEMs were considered in terms of tyrosine (region I), tryptophan (region II), fulvic acid (region III), soluble microbial product (region IV), and humic acid (region V)-like fluorescence. Pearson correlations were conducted to assess relationships between CEC concentrations for the

compounds detected at all sampling sites (i.e., azithromycin, carbamazepine, and EHMC) and regional/total volumes from the EEM analysis. The correlations were considered statistically significant at a 95% confidence interval (p-value < 0.05). All statistical analyses were performed in R-studio 3.5.0.

4.3. Results and discussion

4.3.1. Physicochemical characterization

To assess the water quality and anthropogenic impacts in the four rivers, physicochemical parameters, namely pH, temperature, dissolved oxygen, conductivity, salinity, total dissolved solids, and turbidity, were measured at all sampling sites in both seasons (Table C-S4.4). The pH values ranged between 5.0 and 8.1 in the Ave, between 6.3 and 7.2 in the Leça, between 6.7 and 7.4 in the Antuã, and between 6.9 and 8.0 in the Cértima. This parameter affects the solubility of nutrients, and the values measured in all rivers (between 5 and 8) are optimal for plankton growth and nutrient availability [21]. The pH was generally higher during the wet season in comparison to the dry season. The pH was constant during each season along the Leça and Cértima rivers. In the Ave, pH increased from SP1 to SP9 (close to the mouth of the river), whereas pH increased slightly from SP1 to SP5 and then decreased from SP5 to SP9 in the Antuã. Dissolved oxygen concentrations varied slightly in each season but were generally higher during the wet season, except in the case of the Ave. In Ave and Leça, a gradient of conductivity, salinity, and total dissolved solids was detected from SP1 until the last SP, where the values were higher by at least one order of magnitude, since these SPs were located in the estuary. The same increasing trend was not observed between SP5 and SP9 of Antuã

river or for SP2 to SP3 and SP5 to SP9 of the Cértima river. These three physicochemical parameters (i.e., conductivity, salinity, and total dissolved solids) were typically higher during the dry season in all rivers.

4.3.2. Distribution and seasonal variation of target micropollutants in four Portuguese rivers

Thirteen micropollutants, namely azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, EHMC, fluoxetine, isoproturon, metoprolol, thiacloprid, tramadol, and venlafaxine, were found in all four rivers (Table C-S4.5). Many of these ubiquitously detected compounds are pharmaceuticals, and their occurrence is related to overall consumption and recalcitrance to wastewater treatment with domestic and hospital effluents being the main sources [45]. For pesticides, isoproturon was found below 5.0 ng L^{-1}, except in Leça river, where it was found at higher concentrations (9.42–92.5 ng L^{-1}), but still below the 1.0 µg L^{-1} MAC-EQS set in Directive 2013/39/EU and the maximum admissible concentration for pesticides in drinking water, established by Directive 98/83/EC as 0.1 µg L^{-1} for individual pesticides and 0.5 µg L^{-1} for their sum [46]. On the contrary, thiacloprid was quantified above the maximum value allowed for individual pesticides in drinking water at two SPs of Cértima river. Bezafibrate, enrofloxacin, PFOS, propranolol, and trimethoprim were also identified in the Antuã, Cértima, and Leça rivers. PFOS was always below the MAC-EQS set in Directive 2013/39/EU. Acetamiprid and imidacloprid were only detected in the Ave in the dry season, with acetamiprid below the method quantification limit (MQL) at three SPs and imidacloprid quantified at SP6 and below the MQL at SP9. In this river, the pharmaceuticals, ketoprofen (also in Cértima) and warfarin (also in Antuã), were quantified at almost all SPs in the dry

season. Atrazine was detected in the Ave, Antuã, and Leça rivers. This banned triazine pesticide was present at levels less than 1.58 ng L^{-1}, except at SP9 of Leça, where it reached 41 ng L^{-1}. Simazine, which is also a triazine pesticide, was detected in Cértima and Leça rivers, with the highest concentration (26 ng L^{-1}) measured at Leça SP9. Both triazine pesticides were always below their MAC-EQS (2.0 µg L^{-1} for atrazine and 4.0 µg L^{-1} for simazine). The sum of all pesticides in any case was below the maximum admissible concentration (0.5 µg L^{-1}) for pesticides in drinking water, defined in Directive 98/83/EC [46]. Atorvastatin was identified in the wet and dry seasons in the Antuã (up to 61 ng L^{-1}) and Leça rivers (up to 24 ng L^{-1}). The antidepressant drug citalopram was only quantified at 30 ng L^{-1} in the Antuã river. Overall, the concentrations of the micropollutants were generally lower in the wet season, which can be attributed to dilution effects associated with the higher flow rates in all rivers. For select compounds, the opposite trend was observed, namely higher concentrations were determined in the wet season. These findings may be attributed to seasonal differences in consumption (e.g., antibiotics), as reported in other works [47]. The lower temperatures and shorter daylight hours of the winter season may also impede biodegradation and phototransformation mechanisms resulting in less environmental transformation [48].

4.3.2.1. Ave River

Eighteen of the thirty nine target compounds were detected in the Ave. Figs. 4.2 and C-S4.1 show the spatial distribution and concentrations of micropollutants in dry and wet seasons. Some target compounds, namely acetamiprid, atrazine, clindamycin, diphenhydramine, imidacloprid, ketoprofen, metoprolol, and warfarin, were only quantified in the dry season, which may be related to the lower precipitation and flow rates observed. From the target micropollutants, those with frequency of occurrence

higher than 50% in the Ave were as follows: clindamycin, diphenhydramine, fluoxetine, ketoprofen, and warfarin in the dry season; the macrolide antibiotics (i.e., azithromycin and clarithromycin) and EHMC in the wet season; and isoproturon, thiacloprid, carbamazepine, and tramadol in both seasons. The highest concentrations determined in this river corresponded to the anti-inflammatory ketoprofen, which was found at all SPs during the dry season at concentrations between 50 and 217 ng L^{-1}. This anti-inflammatory compound was also determined at high concentrations in the Llobregat river (Spain) [49], due to its broad use in human medicine. EHMC was determined during the dry season at four SPs (SP1-4) at concentrations up to 168 ng L^{-1}. This UV-filter was also detected at almost all SPs during the wet season, with a maximum concentration of 132 ng L^{-1}. Similar EHMC concentrations were reported in other studies from Brazil (n.d. to 150 ng L^{-1}), Spain (mean: 24.2 ng L^{-1}), Japan, China, USA, and Arctic (up to 150 ng L^{-1}) [7, 50, 51]. The occurrence of EHMC in Hong Kong river water samples was reported at higher levels (4043 ng L^{-1}) [7]. Although UV-filters are widely used in personal care products to protect human skin from UV radiation, they are also applied in several materials, such as rubber, plastics, and paints, to prevent degradation [7]. The occurrence of EHMC in both seasons can be related to these applications. Downstream of SP6 in the Ave, a marked increase was observed for the concentration of many micropollutants (except for azithromycin, clarithromycin and EHMC), which can be explained by the presence of two urban WWTPs, which are considered sources, and other anthropogenic activities (e.g., agriculture and industry). Overall, the concentration of pollutants generally increased from the headwaters to the mouth of the Ave.

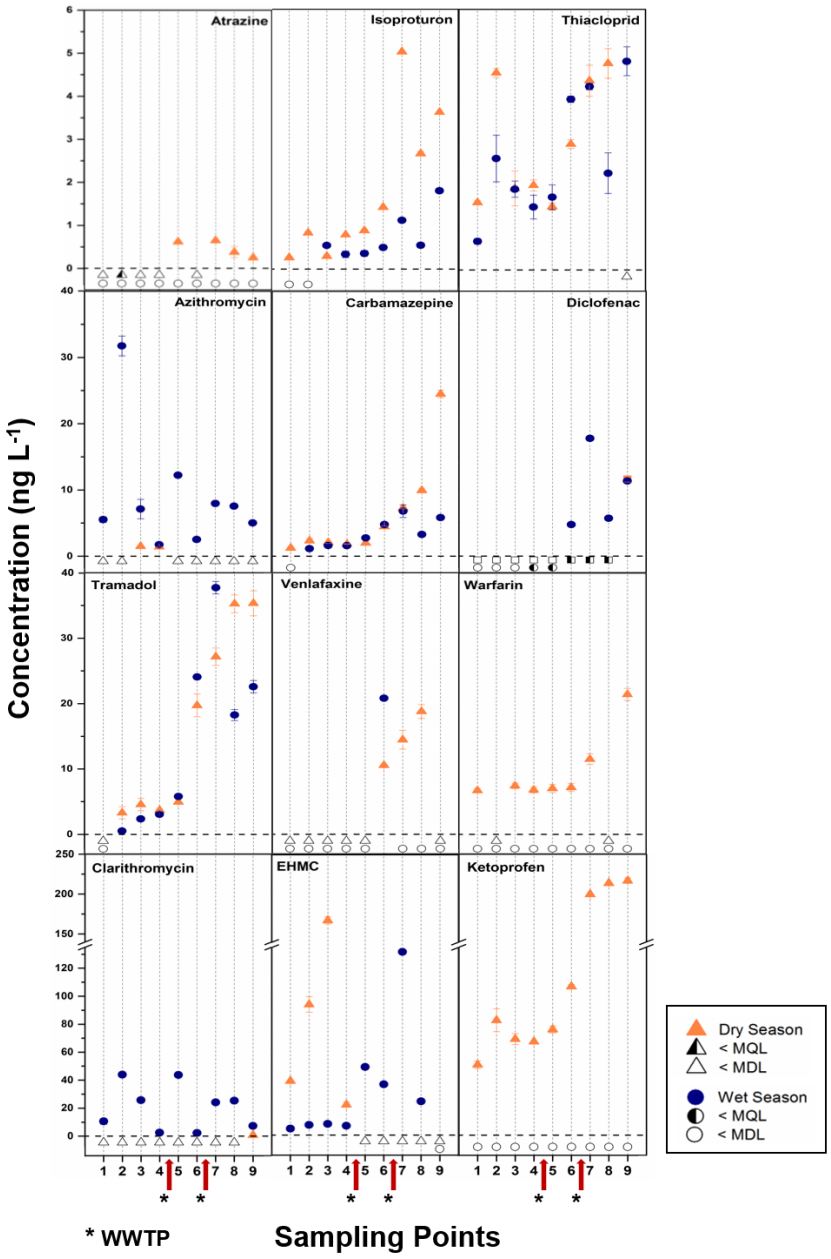

Fig. 4.2. Spatial distribution and concentrations of micropollutants in Ave river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Ave river, please see Fig. C-S4.1).

4.3.2.2. Antuã River

Twenty-two target organic micropollutants were found in the Antuã, including seventeen pharmaceuticals, three pesticides, a UV-filter, and an industrial compound (Figs. 4.3 and C-S4.2). Some of these analytes occur only in the dry season, namely the antibiotics enrofloxacin and trimethoprim, bezafibrate, metoprolol, and warfarin. The most frequently detected compounds (> 50%) in Antuã river samples during the two campaigns were the pesticides isoproturon and thiacloprid and the pharmaceuticals atorvastatin, azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, fluoxetine, tramadol, and venlafaxine. The highest concentration in this river was found for enrofloxacin in the dry season (343 ng L^{-1}), which may be due to the livestock production in the surrounding areas. In the wet season, venlafaxine registered the highest concentration at SP5 (199 ng L^{-1}). A significant increase in the concentrations of many contaminants was recorded downstream of Salgueiro WWTP (SP4). Several factors contribute to the variation of micropollutant concentrations along this river and between the different rivers investigated here. For instance, enrofloxacin and PFOS were determined in Antuã river and not detected in Ave river. Overall, flow rate, environmental factors (e.g., temperature, sunlight, nutrients), and fate/distribution mechanisms, such as adsorption to sediments or particulate matter, biodegradation, photodegradation, other abiotic processes, and uptake by biota, affect the concentrations of these compounds along the rivers of interest [52].

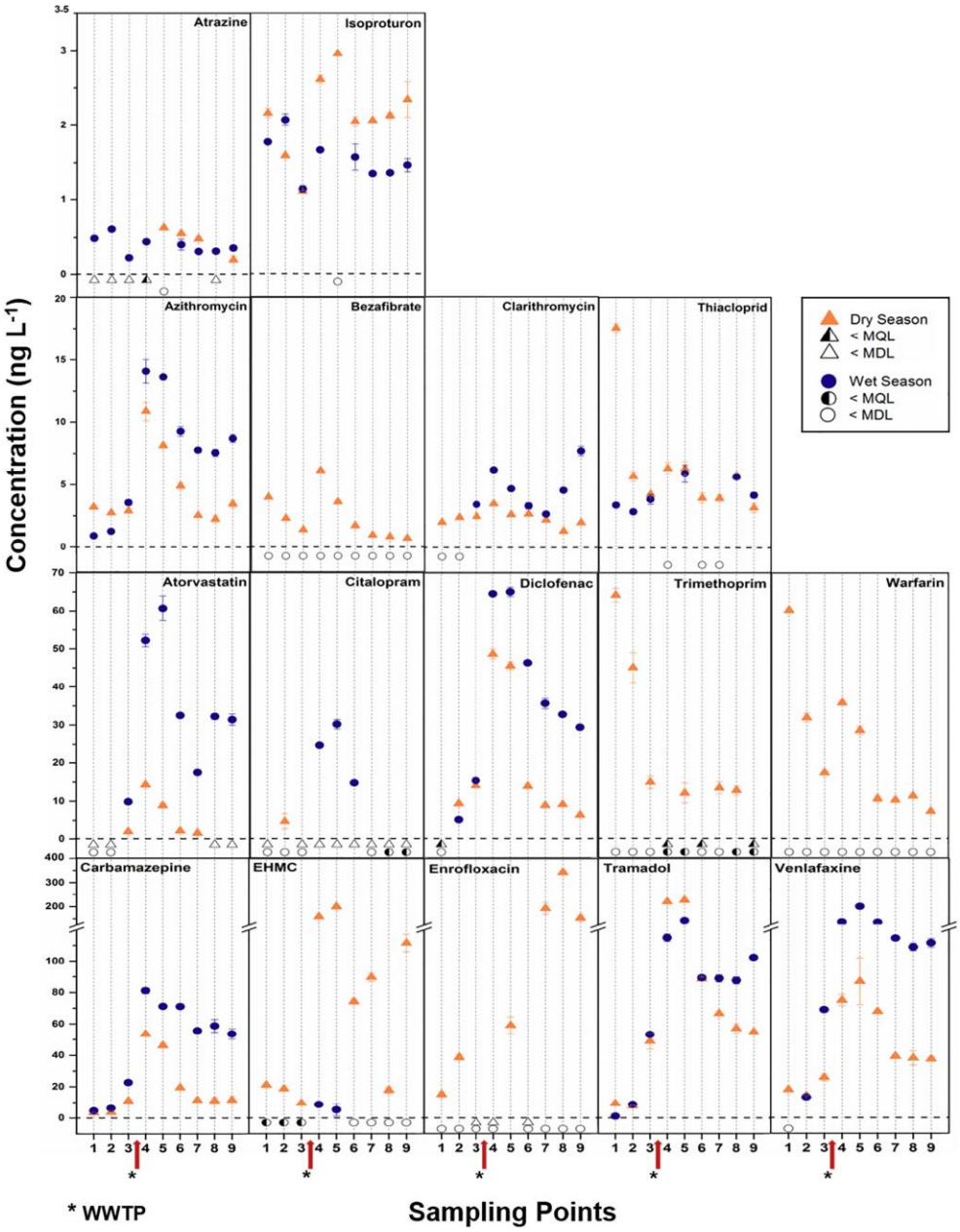

Fig. 4.3. Spatial distribution and concentrations of micropollutants in Antuã river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Antuã river, please see Fig. C-S4.2).

4.3.2.3. Cértima River

In Cértima river (Figs. 4.4 and C-S4.3), twenty compounds were determined at concentrations up to 755 ng L^{-1} during the wet and dry season monitoring campaigns. The high concentrations observed in this river can be related to its low flow rate (ca. 0.13 m^3 s^{-1}) during the dry season. In fact, some target compounds, namely the pharmaceuticals diphenhydramine, propranolol, metoprolol, trimethoprim, enrofloxacin, ketoprofen, and venlafaxine and the pesticide isoproturon, were quantified only in the samples collected during the dry season. The most frequently detected (> 50%) micropollutants in the Cértima varied by season: in the dry season, diphenhydramine, isoproturon, ketoprofen, the antibiotic enrofloxacin, and the neonicotinoid thiacloprid; in the wet season, azithromycin, bezafibrate, fluoxetine, and the pesticide simazine; and across both seasons, clindamycin, carbamazepine, diclofenac, EHMC, tramadol, and clarithromycin. The highest concentrations in this river were recorded for the neonicotinoid thiacloprid in the wet season (755 ng L^{-1}), a finding which may be due to agricultural leaching caused by precipitation events, and for the anti-inflammatory ketoprofen in the dry season (702 ng L^{-1}). With the exception of thiacloprid and PFOS, the concentrations of organic pollutants increased at SP2. The higher concentrations of most micropollutants in this area can be explained by the presence of Mealhada WWTP.

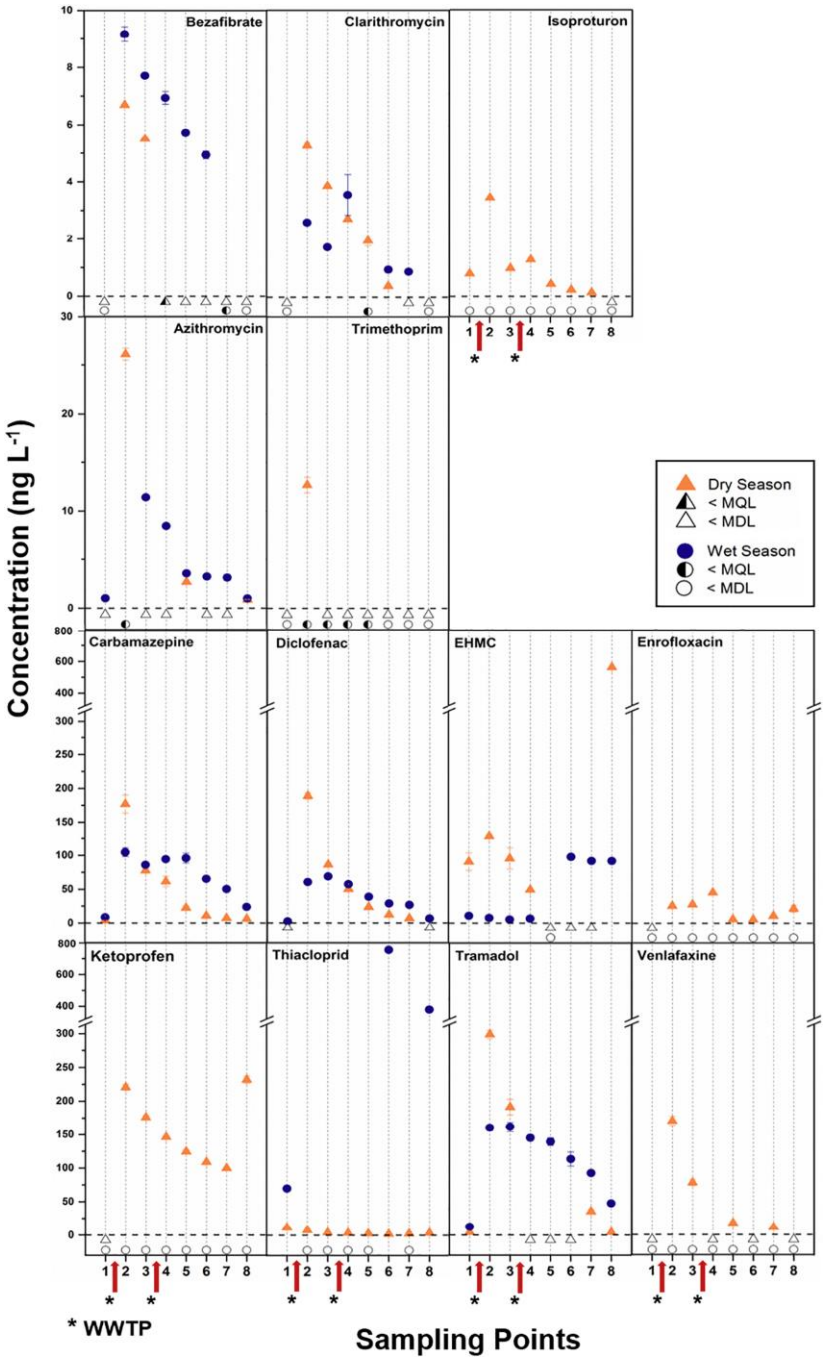

Fig. 4.4. Spatial distribution and concentrations of micropollutants in Cértima river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Cértima river please see Fig. C-S4.3).

4.3.2.4. Leça River

The levels of organic micropollutants determined in the eight SPs of Leça river are reported in Figs. 4.5 and C-S4.4. Twenty-one compounds were observed, including the antibiotics clindamycin, enrofloxacin, and trimethoprim, metoprolol, and the pesticides atrazine and simazine quantified only during the dry season, and azithromycin found only in the wet season. Twelve analytes, namely atorvastatin, diphenhydramine, fluoxetine, propranolol, bezafibrate, clarithromycin, carbamazepine, diclofenac, isoproturon, EHMC, tramadol, and venlafaxine, were detected in more than 50% of the river samples across both seasons. During the dry and wet seasons, tramadol was found at the highest concentration, with a maximum of 396 ng L^{-1} and 233 ng L^{-1}, respectively. This analgesic was frequently detected in all four rivers up to hundreds of ng L^{-1}, as also recently reported by Burns *et al.* (2018) [53] for two rivers in York (UK). EHMC, isoproturon, carbamazepine, diclofenac, and venlafaxine were also found at high concentrations during the two sampling campaigns and, together with tramadol, these micropollutants exhibited the highest detection frequencies and concentrations.

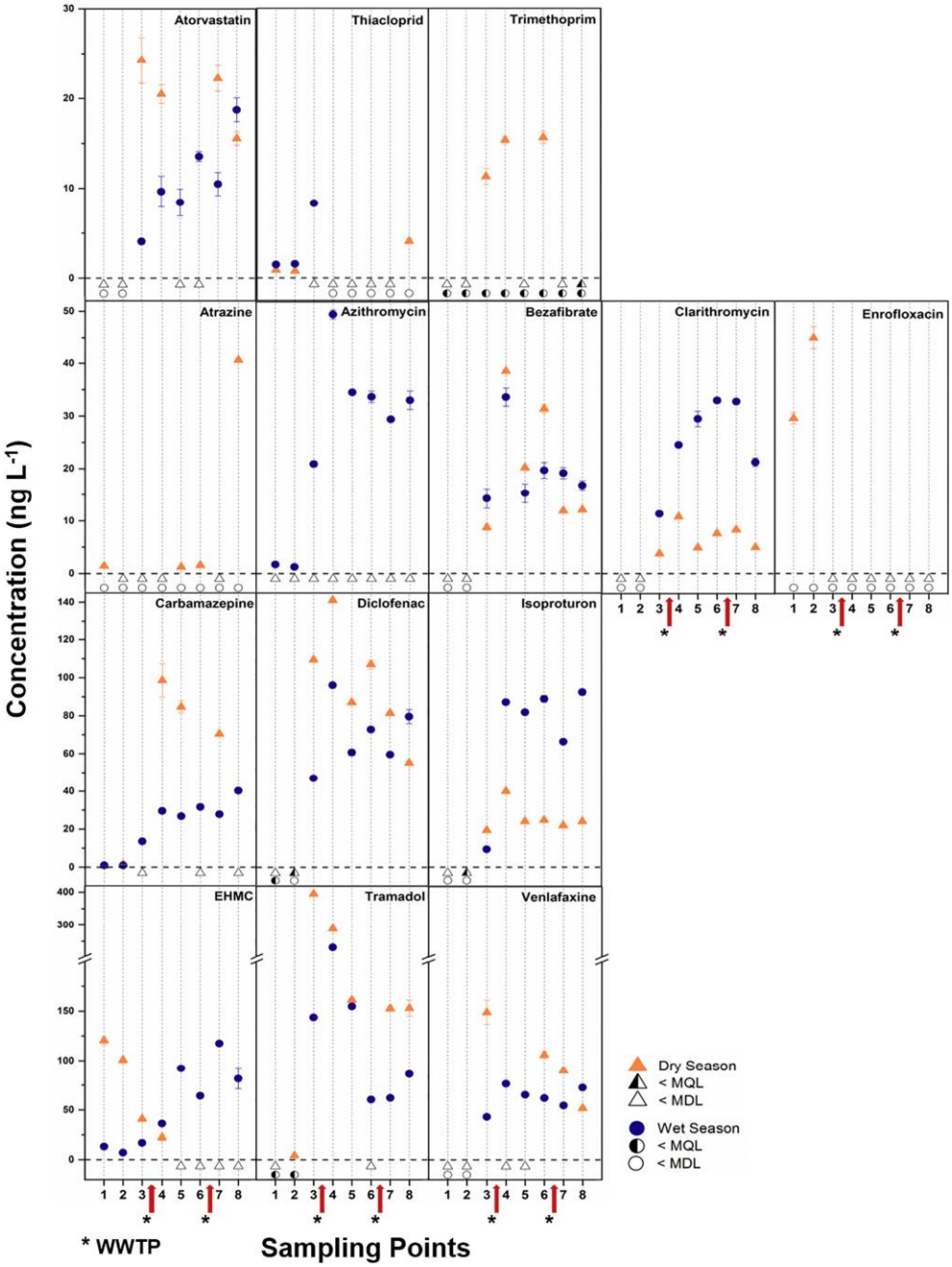

Fig. 4.5. Spatial distribution and concentrations of micropollutants in Leça river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Leça river, please see Fig. C-S4.4).

Parada and Ponte de Moreira WWTPs (SP4 and SP6, respectively) and the associated industrialized areas (SP4 and SP7) seemed to directly influence the levels of organic micropollutants in Leça river, increasing the concentration of most compounds. These data were well correlated with the fluorescence EEMs of the surface water and WWTP effluent samples (Fig. 4.6). The WWTP effluent sample exhibited a strong fluorescence response in all regions, and the fluorescence signature was similar to previous reports for wastewater [54, 55]. The fluorescence signatures of SP1 and SP2 were minimal. SP3 showed minor fluorescence in regions III (fulvic acid-like) and V (humic acid-like). The fluorescence response increased in all regions at SP4, downstream of the first WWTP. The SP5-8 samples showed similar fluorescence signatures in all regions, although the signal was slightly lower than at SP4. However, slight increases in the fluorescence response were observed at SP6, which is influenced by a WWTP, and SP7, which is surrounded by a highly industrialized area.

To better highlight the changes in fluorescence with sample location, the regional volumes of the environmental samples were normalized by the corresponding regional volumes from the WWTP sample. As indicated in Fig. 4.7, the relative presence of aromatic protein-, fulvic acid-, soluble microbial product-, and humic acid-like fluorescence increased between SP3 and SP4.

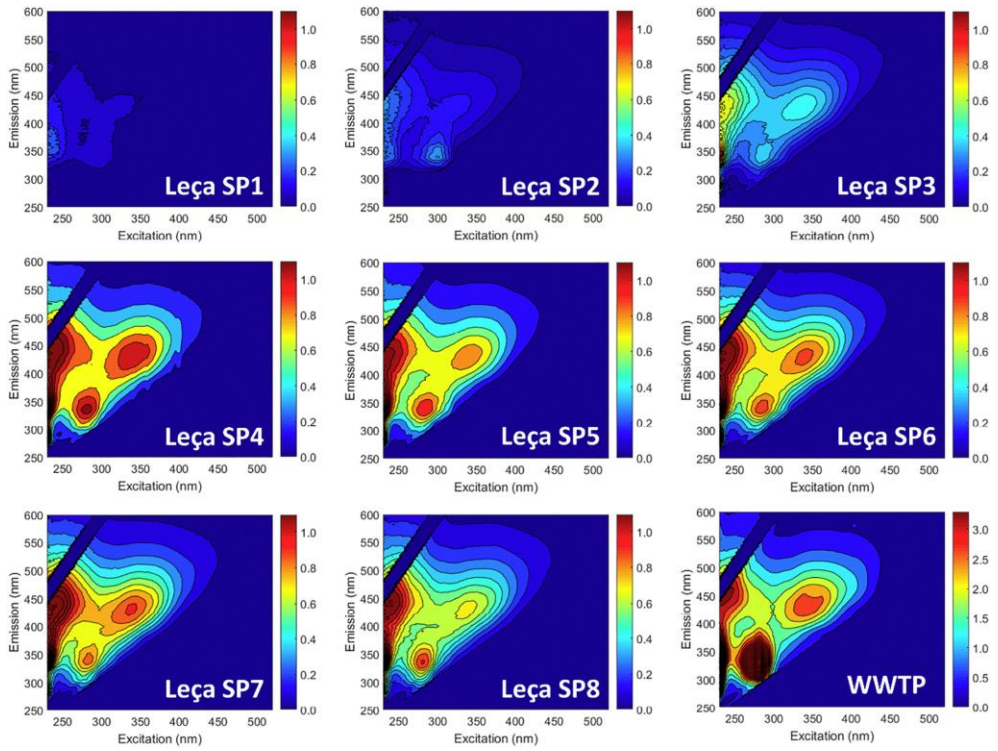

Fig. 4.6. Fluorescence EEMs for the eight Leça river samples and one WWTP sample.

Downstream of SP4, the fluorescence response associated with regions I and II decreased, presumably due to biotic/abiotic degradation mechanisms. The fluorescence in regions III, IV, and V remained fairly consistent from SP5 to SP8.

The similarity of CEC concentrations and regional EEM volumes along the Leça river is shown in Fig. 4.7. Azithromycin and carbamazepine concentrations were significantly, positively correlated with the EEM volumes for regions I-V; furthermore, significant correlations were observed for EHMC concentrations with fluorescence signatures from regions III, IV, V (Table C-S4.7 in the supplementary material). Significant correlations were also observed for all three CECs with the total fluorescence response, which was calculated as the sum of EEM volumes from

regions I-V. Based on these results, the fluorescence intensity in regions III, IV, and V may serve as useful screening tools to inform the presence of PSs/CECs in the Leça river. As DOM signatures and sources vary in other river systems, the EEM-based screening tool needs to be evaluated and refined for other watersheds.

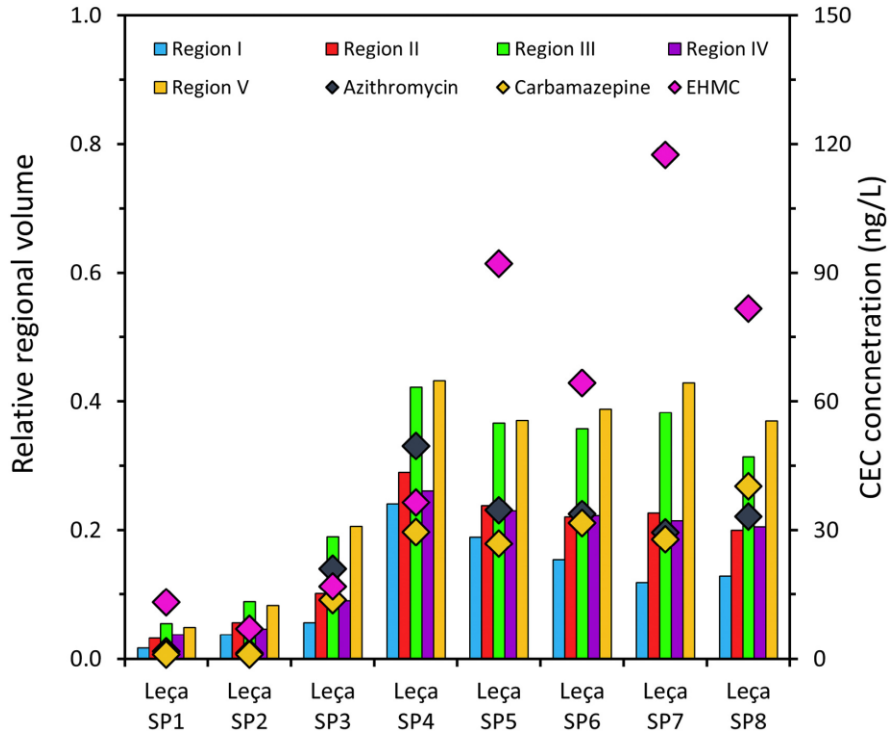

Fig. 4.7. The relative regional volume of environmental samples (Leça SP1–8) as a function of the WWTP sample for region I (tyrosine-like fluorescence), region II (tryptophan-like fluorescence), region III (fulvic acid-like fluorescence), region IV (soluble microbial product-like fluorescence), and region V (humic acid-like fluorescence). The average azithromycin, carbamazepine, and EHMC concentrations are plotted to highlight the correlation in EEM results and CECs concentrations.

While previous reports [25] have found correlations between the total and summed (e.g., region I, II, and IV) EEM volumes with particular CECs, the more specific correlations with particular regions identified in this study may better reflect CEC fate and transport along spatiotemporal gradients. In particular, strong correlations were

observed for azithromycin, carbamazepine, and EHMC concentrations with the region V fluorescence response, suggesting that these CECs exhibit similar fate and transport behavior as humic acid-like substances. Yang *et al.* (2017) [27] highlighted the use of parallel factor (PARAFAC) analysis to not only deconvolute fluorescence EEMs into a finite number of components, but also predict CEC degradation. This approach may provide further insight into the fate and transport of PSs/CECs in Portuguese rivers.

4.3.3. Occurrence of target micropollutants in surface waters

The comparison of many target micropollutants found in this study with results from other reports on seasonal surface water monitoring (Table C-S4.6) is complex since the production and usage of industrial products, the application of pesticides in agricultural activities, and the consumption of pharmaceutical compounds is different between locations. However, the macrolide antibiotic azithromycin, the anti-inflammatory chemicals diclofenac and ketoprofen, and the antidepressants fluoxetine and venlafaxine have been reported in surface water at levels similar to those found in the present work. For instance, azithromycin, fluoxetine, and venlafaxine were found in Lis river (Portugal) up to 30 ng L^{-1}, 20 ng L^{-1}, and 159 ng L^{-1}, respectively [52]. Diclofenac was quantified in surface water collected from China at a maximum of 170 ng L^{-1} [5]. Ketoprofen was detected in Spain up to 225 ng L^{-1} [56]. These findings indicate that a similar consumption pattern of these pharmaceutical compounds may occur in different regions of the world.

The pesticides atrazine and simazine were quantified in other studies at maximum levels well above those determined here. For example, in three different monitoring studies performed in Spain [57], Brazil [58], and Thailand [59], atrazine was found at maximum concentrations of 333, 320, and 800 ng L^{-1}, respectively. These

concentrations are more than one order of magnitude higher than those determined in the present study (e.g., 40.6 ng L^{-1}). Simazine was found in Australia at concentrations between 50 and 670 ng L^{-1} [60] and in Spain up to 207 ng L^{-1} [57]. The intense agricultural activity in those regions may explain the presence of triazine herbicides in surface water, even after these chemicals were phased out, since they can be illegally acquired and/or released from existing sediments/soils. In contrast, the other target pesticide, isoproturon, was determined at higher concentrations in the present study compared to values found in the literature [23, 61].

The UV-filter EHMC was determined in this work at a maximum value of 562 ng L^{-1} and a similar level was reported in Brazil (669 ng L^{-1}) [50]. This compound was also detected in surface water samples in Hong Kong at concentrations one order of magnitude higher, i.e., 4043 ng L^{-1} [7], than those determined in the present study. Importantly, this compound has also been shown to accumulate in aquatic and marine organisms [62], raising concerns about the high aqueous-phase concentrations detected here.

The concentrations of bezafibrate, carbamazepine, clarithromycin, and thiacloprid described in the literature are slightly higher than those reported in the target Portuguese rivers. The beta-blocker metoprolol was found at a maximum concentration of 25 ng L^{-1} in Ave river, while it was determined up to 448 ng L^{-1} in Beiyun river of Beijing, China [5, 63]. The maximum concentration of the antibiotic trimethoprim in the current study was 64 ng L^{-1} in Antuã river in the dry season, which was comparable to the maximum concentration (36 ng L^{-1}) found in Llobregat river, Spain [49] and lower than the maximum concentration (180 ng L^{-1}) reported in a monitoring study performed in the Los Angeles and San Gabriel rivers [64].

4.4. Conclusions

In this first simultaneous survey of specific PSs and CECs defined by EU documents in four stressed rivers (i.e., Ave, Leça, Antuã, and Cértima) in Portugal, 26 out of 39 target micropollutants were found at least in one of the selected rivers. Of the detected compounds, thirteen were consistently determined in all four rivers: azithromycin; carbamazepine; clarithromycin; clindamycin; diclofenac; diphenhyhydramine; EHMC; fluoxetine; isoproturon; metoprolol; thiacloprid; tramadol; and, venlafaxine. The highest concentrations were verified for ketoprofen in Ave river, tramadol in Leça river, enrofloxacin in Antuã river, and thiacloprid in Cértima river. These data highlight the different land-use patterns and contaminant sources found in the targeted rivers, with the occurrence and concentration distributions along particular rivers depending on location and seasonal variations. The increase in fluorescence response profiles for specific locations of the Leça river matched the distribution of micropollutants along this river. Although some of these compounds are already prioritized or defined in the Watch List, larger monitoring programs are needed for further prioritization and risk assessment of such contaminants. Given the significant correlations found for EEM regional volumes with CEC concentrations in the Leça river, preliminary EEM analysis may help to inform the design of future monitoring studies.

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Supplementary material

(Please see Appendix C)

Table C-S4.1. Sampling points of Ave, Leça, Antuã and Cértima Rivers and the respective GPS coordinates.

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Fig. C-S4.4. Spatial distribution and concentrations of micropollutants determined at concentrations below 30 ng L^{-1} in Leça river for dry and wet seasons.

Chapter 5

Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants

Chapter 5

Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants

Abstract

Pristine and functionalized multi-walled carbon nanotubes (MWCNTs) were investigated as adsorbent materials inside solid-phase extraction (SPE) cartridges for extraction and preconcentration of 8 EU-relevant organic micropollutants (with different p*K*a and polarity) before chromatographic analysis of surface water. The recoveries obtained were > 60% for 5/8 target pollutants (acetamiprid, atrazine, carbamazepine, diclofenac and isoproturon) using a low amount of this reusable adsorbent (50 mg) and an eco-friendly solvent (ethanol) for both conditioning and elution steps. The introduction of oxygenated surface groups in the carbon nanotubes by using a controlled HNO_3 hydrothermal oxidation method, considerably improved the recoveries obtained for PFOS (perfluorooctanesulfonic acid) and methiocarb, which was ascribed to the hydrogen bond adsorption mechanism, but decreased those observed for the pesticide acetamiprid and for two pharmaceuticals (carbamazepine and diclofenac), suggesting π-π dispersive interactions. Moreover, a good correlation was found between the recovery obtained for methiocarb and the amount of oxygenated surface groups on functionalized MWCNTs, which was mainly attributed to the increase of phenols and carbonyl and quinone groups. Thus, the HNO_3 hydrothermal oxidation method can be used to finely tune the surface chemistry (and texture) of MWCNTs according to the specific micropollutants to be extracted and quantified in real water samples.

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5.1. Introduction

In the last decades, a growing interest has been raised about the fate and effects of a large group of organic micropollutants (OMPs) on the aquatic environment. These pollutants found at trace concentrations (ng L^{-1} to µg L^{-1}) can be natural or anthropogenic substances, such as pharmaceutical compounds, pesticides, industrial compounds and steroid hormones [1]. Conventional wastewater treatment plants are not designed to completely remove many of these organic compounds at low concentrations, which are thus discharged into receiving water bodies, including groundwater and surface water (SW), reaching drinking water for human consumption [2]. Other sources of contamination include direct discharge and runoff, namely in the case of industrial compounds, pesticides applied in agriculture, and veterinary pharmaceuticals used for livestock and aquaculture [3, 4]. Most of these compounds are pseudo-persistent since their transformation/removal rates are overcome by their continuous release into the environment. Moreover, their recalcitrant character and polarity favours the dispersion and interchange between aquatic compartments [4, 5]. The presence of such OMPs in the aquatic environment is considered an important issue in terms of public health safety [6]. Therefore, the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2018/840 and Decision 2020/161) in SW bodies has been recommended within the European Union (EU). The comprehensive identification and quantification of PSs and CECs in freshwater samples is crucial to collect information on their sources, distribution and fate in the environment, to study the effects on ecosystems and human health, and to update the water policy in this field. To achieve this goal, it is important to set up fast, sensitive and reliable analytical methods

enabling the determination of a wide range of OMPs typically found at residual levels in aquatic compartments.

Despite the shortcomings of solid-phase extraction (SPE), such as the high volumes of organic solvents needed in comparison with miniaturized techniques, time consumption and high cost, this sample preparation technique is still the most employed for preconcentration of OMPs in water matrices due to the efficient removal of interferences, consequent reduction of matrix effects and high enrichment factors and recoveries often yielded [1, 7]. SPE is an essential preconcentration step prior to analysis by a sensitive and reproducible analytical technique such as ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The type of sorbent, its structure and interactions with the target analytes play an important role in SPE, reason why many carbon materials have been already reported as good candidates as filling materials for this purpose [8, 9].

Multi-walled carbon nanotubes (MWCNTs) are the most studied carbon materials for environmental applications of SPE. This fact can be attributed to: (i) the unique structure of MWCNTs that enables strong interactions with organic molecules through non-covalent forces (i.e., hydrophobic interactions, hydrogen bonding, π -π stacking, electrostatic forces and van der Waals forces); (ii) their large surface-to-volume ratio; (iii) good thermal and mechanical stability; and (iv) the possibility to control their affinity towards target compounds upon surface functionalization by chemical or physical methods [10]. MWCNTs have been investigated as SPE sorbents for sample preparation of water matrices and to pre-concentrate and extract OMPs such as pesticides (e.g. [11-16]), polycyclic aromatic hydrocarbons [17, 18], industrial compounds [19], macrolide antibiotics and nonsteroidal anti-inflammatory drugs [20, 21], with recoveries higher than 62%. However, the number of studies dealing with multi-class PSs and CECs with different physicochemical properties are still very

limited [20, 21]. Moreover, application of functionalized MWCNTs in SPE for extraction of EU-relevant OMPs is even scarcer in the literature. Only three studies were reported, namely for: (i) pentachlorophenol using MWCNTs oxidized with 8.0 mol L^{-1} of HNO_3 [22]; (ii) the industrial compound perfluorooctanesulfonic acid (PFOS) using amino-terminated alkyl-functionalized MWCNTs [19]; and (iii) thirteen pharmaceutical compounds, some of them defined in the EU Decisions (erythromycin, azithromycin and diclofenac), using MWCNTs treated with high concentrations of HNO_3 (4.0 mol L^{-1}), HCl (1.0 mol L^{-1}) and KOH (4.0 mol L^{-1}) [21], i.e. having pronounced environmental implications and costs.

In the present work, pristine and modified MWCNTs were investigated as SPE sorbents for the simultaneous extraction of 8 EU multi-class OMPs in SW before UHPLC-MS/MS analysis. The target compounds, namely 5 pesticides (acetamiprid, atrazine, isoproturon, metaflumizone and methiocarb), 2 pharmaceutical compounds (carbamazepine and diclofenac), and one industrial compound (PFOS) were strategically selected due to their high frequency of detection and/or their high levels of concentration in water matrices observed during the monitoring sampling campaigns performed by our research group in the last years [2, 6] and, in the specific case of metaflumizone due to its presence in the recently 3rd Watch List (Decision 2020/1161). A set of experiments was performed using pristine MWCNTs to study the parameters that influenced the extraction efficiency of the 8 OMPs spiked in SW samples, namely the sample pH and volume, the elution and extraction solvent and respective volumes, and the amount of MWCNTs packed in the cartridge. After optimizing these parameters, the cartridge packed with MWCNTs and the commercial cartridge Oasis HLB were compared, in terms of extraction efficiency, reusability, and costs. Then, we attempted to investigate a HNO_3 hydrothermal oxidation methodology reported by our group [23] to obtain a series of MWCNTs with meticulously introduced

surface oxygen functionalities. This methodology allows the fine control of the type and amount of surface groups introduced on carbon materials by adjusting the concentration of oxidizing agent employed in the treatment (HNO_3 concentration in the range 0.01 – 0.30 mol L^{-1}), as determined by different characterization techniques. This distinctive feature allowed establishing correlations between both the synthesis conditions and the oxygen-containing surface functionalities introduced on the MWCNTs; and the type and amount of those functionalities and the recoveries obtained for the 8 target OMPs; while employing much lower concentrations of oxidizing agent than those previously reported with similar hydrothermal methodologies [23-26]. Therefore, the novelty of this study relies on (i) the development of a systematic study, upon application of a controlled HNO_3 hydrothermal oxidation methodology to pristine MWCNTs; but also on (ii) the use of ethanol as elution solvent in the SPE procedure, when using MWCNT cartridges; and (iii) the study of metaflumizone for the first time in real water compartments.

5.2. Experimental section

5.2.1. Chemicals and materials

MWCNTs (NC3100, powder) with an average diameter of 9.5 nm, average length of 1.5 μm and > 95% purity were obtained from Nanocyl SA (Sambreville, Belgium). All reference standards (acetamiprid, atrazine, carbamazepine, diclofenac sodium, isoproturon, metaflumizone, methiocarb and PFOS; > 98% purity) and deuterated compounds used as internal standards (acetamiprid–d3, atrazine–d5, diclofenac-d4, fluoxetine–d5 and methiocarb-d3) were purchased from Sigma-Aldrich (Steinhein, Germany). The physicochemical properties of the target compounds can be found in

Table D-S5.1. Methanol and acetonitrile (MS grade), ethanol (HPLC grade), and hydrochloric acid were obtained from VWR International (Fontenay-sous-Bois, France). Individual stock solutions of 1000 mg L^{-1} of each reference and internal standard were prepared in methanol, ethanol or acetonitrile, depending on their solubility. Two ethanolic working solutions containing the 8 target compounds (2.5 mg L^{-1}) and the 5 internal standards (5.0 mg L^{-1}) were prepared by dilution of the individual stocks. Sulfuric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Sodium chloride was purchased from José Manuel Gomes dos Santos. Ultrapure water was supplied by a Milli-Q water system. Oasis HLB (Hydrophilic-Lipophilic-Balanced) cartridges (150 mg, 6 mL) were obtained from Waters (Milford, MA, USA), and the empty SPE cartridges (6 mL) with two frits (20 µm) (Bond Elut) were purchased from VWR International (Fontenay-sous-Bois, France). pH measurements were performed with a pHenomenal pH 1100L pH meter (VWR, Germany).

5.2.2. Surface functionalization of MWCNTs

Hydrothermal oxidation of the pristine MWCNTs was performed in a Teflon-lined stainless-steel autoclave (Mod. 4748, Parr Instruments, USA) with 125 mL of capacity, following the experimental procedure described elsewhere [27]. 75 mL of a HNO_3 solution (concentration in the range 0.01 – 0.30 mol L^{-1}) was transferred to a PTFE vessel and 0.2 g of the pristine MWCNTs was loaded. The PTFE vessel was placed into the stainless-steel autoclave, which was sealed and placed in an oven at 200 ^{o}C for 2 h. After this time, the autoclave was allowed to cool down until ambient temperature. The recovered material was washed several times with distilled water until a neutral pH of the rinsing water was attained, and then dried overnight at 120 ^{o}C. Additionally, a blank hydrothermal treatment with distilled water instead of the HNO_3 solution was performed. The resulting materials were labelled as MWCNT followed by

a subscript number corresponding to the concentration of HNO_3 employed in the hydrothermal treatment in mol L^{-1} (i.e., $MWCNT_0$, $MWCNT_{0.01}$, $MWCNT_{0.05}$, $MWCNT_{0.1}$, $MWCNT_{0.2}$, and $MWCNT_{0.3}$).

5.2.3. Characterization of MWCNTs

Temperature programmed desorption (TPD) was performed in a fully automated AMI-300 Catalyst Characterization Instrument (Altamira Instruments), equipped with a quadrupole mass spectrometer (Dymaxion, Ametek), as described elsewhere [27]. Briefly, TPD is a well-established advanced characterization technique assuming that all oxygen-containing surface groups are decomposed into CO_2 and CO upon heating under controlled operating conditions [28]. In this case, a low heating rate of 10 ^{o} C min^{-1}, and a high helium flow of 25 cm^3 min^{-1} were set to minimize secondary reactions during the experiments [26, 28]. The mass signals m/z = 28 and 44 were monitored during the thermal analysis, the corresponding TPD spectra being obtained. CO and CO_2 were calibrated at the end of each analysis with the respective gases. The concentrations of the different oxygen containing surface groups were then obtained by deconvolution analysis of the CO_2 and CO TPD spectra using a procedure established by our group [28, 29]. Accordingly, the peaks in the CO_2 TPD spectra were assigned to diverse functional groups, namely strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (CAn), and lactones (Lac). Similarly, the peaks in the CO TPD spectra were assigned to carboxylic anhydrides (Can), phenols (Ph), carbonyls and quinones (CQ), and basic surface groups (Bas), such as pyrones and chromenes. The width at half-height (W) of the peak was taken the same for Can and Lac in the CO_2 spectra, and the same W was considered for Ph and CQ in the CO spectra whenever peak shoulders were unclear. Thermogravimetric analysis (TGA) was performed in a Netzsch STA 490 PC/4/H Luxx thermal analyser, in

which the powder sample was heated from 50 to 900 ºC at 10 ºC min^{-1}, under an inert (N_2) gas flow. Regarding TPD and TGA analysis, selected experiments were performed in duplicate, the standard deviations (SD) never exceeding the values given in the caption of Fig. 5.3. Textural properties were determined from N_2 adsorption-desorption isotherms at -196 ºC, as described in our previous work [23], and included specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}), micropore volume (V_{micro}) and total pore volume (V_{total}). The pH at point of zero charge (pH_{PZC}) was obtained by pH drift tests [27].

5.2.4. MWCNTs SPE procedure

Commercial cartridge Oasis HLB were used for comparison purposes in this study (Text D-S1). After preparing the cartridges with 50 mg of each adsorbent (Fig. D-S5.1), the SPE protocol previously optimized (Text D-S2) for pristine MWCNTs (NC3100) was performed. Briefly, ethanol (4 mL) and ultrapure water (4 mL) were used to condition and equilibrate the cartridge at a flow rate of 1 mL min^{-1}. 500 mL of blank or spiked (200 ng L^{-1} of each target compound) SW sample previously acidified to pH 3 was loaded at 10 mL min^{-1}. 4 mL of ultrapure water was then added in the washing step, followed by 45 min of vacuum drying. For the elution step, 4 mL of ethanol was used and, after evaporation, the filtered reconstituted ethanolic extracts were analysed by UHPLC-MS/MS. All experiments were performed in triplicate and relative standard deviation (RSD) were estimated. For details on the SPE procedure, please see Supplementary Material (Text D-S5.1 and D-S5.2; Fig. D-S5.1).

5.2.5. Evaluation of the SPE recovery efficiency

The recovery efficiency (%) is the most important parameter supporting the selection of the optimal conditions for a given SPE procedure. Therefore, the performance of the

off-line SPE method was assessed considering the recovery efficiency for the 8 target analytes under study. The recovery was calculated as the ratio of the peak areas obtained for extracted spiked sample (A) and the peak areas of the post-spiked extracted sample (B), as described in Fig. D-S5.2 and Equation 5.1:

$$Recovery\ efficiency\ (\%) = 100\ \times\ (A/B) \qquad Eq.(5.1)$$

Since the matrix effect is considered the same in both A and B, and thus not accounted for, this approach allows evaluating exclusively the recovery promoted by the adsorbent material. Total Ion Current (TIC) chromatograms of the 8 target OMPs (200 ng L^{-1}) after SPE of a spiked sample and after post-spiking a blank extract using original MWCNT packed cartridges are showed in Fig. D-S5.3 a and b.

5.*2.6. UHPLC–MS/MS method*

A Shimadzu Corporation UHPLC-MS/MS (Tokyo, Japan) consisting of a Nexera UHPLC (two chromatographic pumps LC-30AD with a degasser DGU-20A 5R, an autosampler SIL-30AC, an oven CTO-20AC, and a system controller CBM-20A with a Shimadzu LC Solution Version 5.41SP1 software), and a Ultra Fast Mass Spectrometry series LCMS-8040 triple quadrupole mass spectrometer, was used for SW analysis. The chromatographic separation of the target compounds was performed by using a column Kinetex XB-C18 100 Å (100 × 2.1 mm i.d.; particle diameter of 1.7 µm) acquired to Phenomenex, Inc. (Torrance, CA, USA) operating under gradient mode of flow of the mobile phase water/ethanol (50/50, v/v). The column oven temperature was set at 35 °C. The autosampler temperature was set at 15 °C and the injection volume was 5 µL. The MS settings were: 2.5 $dm^3\ min^{-1}$ of nebulizing gas flow, 12.5 $dm^3\ min^{-1}$ of drying gas flow, capillary voltage of 0.5 kV, 400 °C and 250 °C for source and desolvation temperatures, argon at 230 kPa as CID gas. The quantification and

confirmation of the identity of each analyte was performed by Selected reaction monitoring (SRM). Along with the retention time of the analyte, the transition between the precursor ion and the most abundant fragment ion (SRM1) was used for quantification and the ratio between SRM1 and the transition between the precursor ion and the second most abundant fragment ion SRM2 was used for identity confirmation. All the analytical parameters used, namely SRM instrument parameters, retention time, linearity, and limits of detection and quantification, are detailed in the Supplementary Material (Tables D-S5.2 and D-S5.3).

5.2.7. Sample collection

SW samples (pH = 6.5 ± 0.1) were collected from Cavalum River (tributary of the Sousa River) located in Penafiel (40 km from Porto, Portugal). Samples were stored in amber glass bottles (1 L) at 4 °C until extraction, which was performed within 24 h. Before SPE, all samples were filtered through 1.2-µm glass-fiber filters (47 mm GF/C, Whatmam, Maidstone, United Kingdom) and the pH was adjusted using sulfuric acid or sodium hydroxide solutions, according to the SPE procedure (Section 5.2.4, Texts D-S1 and D-S2).

5.3. Results and discussion

5.3.1. Optimization of SPE procedure with pristine MWCNTs (NC3100) *cartridges*

In order to study the performance of pristine MWCNTs (NC3100) as SPE adsorbent for the simultaneous enrichment of the 8 target EU OMPs with different p*K*a and polarity range, the main experimental conditions affecting the extraction efficiency were

optimized, namely the sample pH and volume, the elution and extraction solvent and respective volumes, and the amount of MWCNTs packed in the cartridge.

Regarding the sample pH (3, 7 or 9), it determines the state of the target micropollutants in solution as ionic or molecular form, directly affecting the recovery efficiency of the process. When using ethanol as solvent (Fig. 5.1a), an acidic pH enabled higher recoveries of acetamiprid, atrazine, methiocarb and the industrial compound PFOS, while the recovery of the pharmaceutical compounds carbamazepine and diclofenac performed better at alkaline pH. Neutral pH led to lower recoveries, except for isoproturon. Methanol and acetonitrile were also tested as solvents (Fig. 5.1b), but ethanol (as conditioning and elution solvent) and an acidic sample pH allowed similar or slightly higher recoveries for most of the target compounds, in comparison with the other studied conditions. Moreover, ethanol is considered an eco-friendly (and greener) solvent [30], and thus selected for the next experiments. Different amounts of the adsorbent material packed in the SPE cartridge (between 25 and 150 mg) were then investigated (Fig. 5.1c), and the highest recoveries for the target compounds were obtained when using cartridges packed with 50 mg of MWCNTs (except in the case of the pesticide metaflumizone). Lower recoveries were obtained when using amounts below 50 mg of MWCNTs, which may be due to the limited adsorption capacity of this carbon material at these conditions. Lower recoveries were also obtained for amounts above 50 mg of MWCNTs, which may be explained by a lower desorption of OMPs from MWCNTs during the elution step. Bearing this in mind, 50 mg was considered the optimum amount of MWCNTs, and thus selected for the following experiments.

In what concerns the volume of the SW sample (Fig. 5.1d), the higher extraction efficiencies were obtained for the majority of the compounds when using a sample volume of 500 mL (except for atrazine and metaflumizone). The sample volume is expected to be directly proportional to the sample preparation enrichment factor (i.e.,

the ratio between the sample volume and the volume of reconstitution). However, the recoveries obtained for most compounds decreased when the sample volume increased from 500 to 1000 mL. This phenomenon may be ascribed to the SPE breakthrough volume, which is the highest sample volume that allows the maximum extraction efficiency, as observed in previous works [3, 6]. Using the optimum volume of SW sample for most OMPs (500 mL), different volumes of eluent (4 - 10 mL) were then tested (Fig. 5.1e). The recoveries obtained for the 8 OMPs under study slightly increased with the volume of ethanol used in the elution step, a lower volume of eluent being selected for the next experiments (i.e. lower costs) since the recoveries obtained were quite similar.

The reusability of the MWCNT cartridge is confirmed in Fig. 5.1g, similar recoveries being obtained in three consecutive cycles. Moreover, the recoveries achieved (> 60%) for 5 of these EU multi-class OMPs analyzed simultaneously (acetamiprid, atrazine, carbamazepine, diclofenac and isoproturon), using a low amount of adsorbent (50 mg of MWCNTs for 500 mL of SW samples at pH 3) and a conditioning and elution solvent considered “green” (ethanol - 4 mL), were comparable to those reported in the literature using more toxic solvents and a single compound or specific class of compounds (Table D-S5.4). Thus, the next step was to functionalize the MWCNTs in order to investigate the influence of the surface chemistry on the performance of this analytical tool.

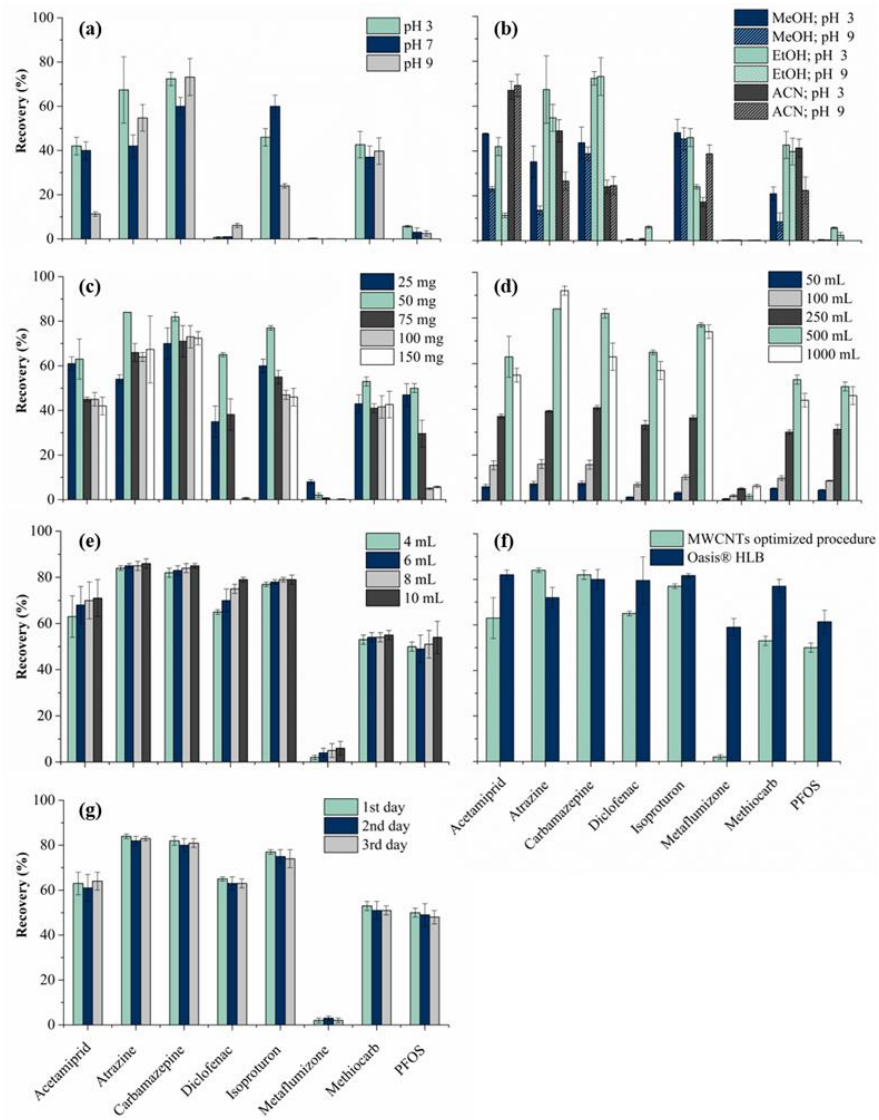

Fig. 5.1 Recoveries obtained for micropollutants (200 ng L^{-1} each), using: **(a)** different pH (3, 7 and 9) (fixed conditions: cartridges packed with 150 mg of MWCNTs, 500 mL of SW and 4 mL of ethanol as a solvent); **(b)** different solvents (4 mL of methanol, ethanol or acetonitrile) and pH (3 and 9) (fixed conditions: cartridges packed with 150 mg of MWCNTs, 500 mL of SW and 4 mL of solvent); **(c)** cartridges packed with different amounts of MWCNTs (25-150 mg) (fixed conditions: pH 3, 500 mL of SW and 4 mL of ethanol as a solvent); **(d)** different volumes (50 - 1000 mL) of SW (fixed conditions: cartridges packed with 50 mg of MWCNTs, pH 3 and 4 mL of ethanol as a solvent); **(e)** using different volumes (4 - 10 mL) of ethanol as elution solvent (fixed conditions: cartridges packed with 50 mg of MWCNTs, 500 mL of SW, pH 3); **(f)** MWCNT optimized cartridge (50 mg) and commercial cartridge Oasis HLB (experiments performed with 500 mL of SW samples (pH 3) and using ethanol as solvent (4 mL); and **(g)** recoveries obtained for micropollutants (200 ng L^{-1} each), extracting 500 mL of SW (pH 3) with 4 mL of ethanol as solvent, during consecutive reuse cycles performed with the same cartridge packed with MWCNTs (50 mg); n = 3 (RSD is represented as error bars).

5.3.1.1. Comparison of optimized SPE procedures for MWCNT and commercial cartridges

The comparison of enrichment performance of the MWCNT cartridge previously optimized and the commercial cartridge Oasis HLB was performed with SW samples. The optimized SPE methodology was applied and the recoveries of the 8 target micropollutants (spiked at 200 ng L^{-1} each) were obtained (Fig. 5.1f). A recovery higher than 60% was achieved for 3 pesticides (acetamiprid, atrazine and isoproturon) and the 2 pharmaceutical compounds (carbamazepine and diclofenac) when using both MWCNTs and commercial cartridges. However, the commercial cartridge Oasis HLB gave recovery values also higher than 60% for the industrial compound (PFOS) and the other 2 pesticides (metaflumizone and methiocarb). Except for metaflumizone, the overall recovery of the other 7 micropollutants was similar.

The textural properties of MWCNTs and Oasis HLB were investigated through N_2 adsorption-desorption isotherms, as described in Section 5.2.3. The results revealed that these materials have different porous characteristics (Table D-S5.5). Although the total pore volume (V_{total}) is similar for both adsorbents (1.264 and 1.284 $cm^3\ g^{-1}$ for MWCNTs and Oasis HLB, respectively), the specific surface area (S_{BET}) of Oasis HLB (756 $m^2\ g^{-1}$) is ca. 3.8-fold higher than that of MWCNTs (198 $m^2\ g^{-1}$). Consequently, the average pore diameter (d_{pore}) of MWCNTs (25.5 nm) is almost 4-fold higher than that of Oasis HLB (6.8 nm). Interestingly, the sorbent load in each cartridge Oasis HLB is 3 times higher than that of the cartridges packed with MWCNTs, which have a S_{BET} 3.8-fold lower. However, the SPE performances obtained for MWCNTs (Fig. 5.1f) cannot be explained by one unique parameter. Instead, the adsorption followed by elution/desorption of the organic micropollutants result from the interplay of many factors: the textural properties of the adsorbent material (Table D-S5.5); the functional

groups of the adsorbent and of the organic pollutants; the hydrophobic interactions between the target micropollutants (log K_{OW} of each target compound can be found in Table D-S5.1) and MWCNTs, and other adsorption mechanisms; the morphology of the adsorbent material; and the sample characteristics (for example, the dissolved organic matter present in the water matrix) [10].

In addition to the analytical performance of the SPE procedure, it is important to take into consideration the cost of the adsorbent material. Considering 2020 retail prices, each commercial cartridge Oasis HLB costs around 8 euros, while the whole cost associated to each cartridge packed with 50 mg of MWCNTs amounts to ca. 2 euros (including the empty polypropylene cartridge and two frits). This represents a possible cost reduction of 75%. Furthermore, these MWCNT cartridges are reusable, while commercial cartridges often are single-use disposable devices [6, 20].

5.3.2. Textural and surface chemistry characterization of MWCNTs

The type and overall amount of oxygen-containing surface groups were determined by TPD analysis, as described in Section 5.2.3. The CO_2 and CO TPD spectra of the hydrothermally treated MWCNTs (HNO_3 concentration in the range 0.01 – 0.30 mol L^{-1}) are shown in Figs. 5.2 a and b, respectively. For comparison, the TPD profiles determined for the pristine MWCNTs (original) and for MWCNTs after hydrothermal treatment with water (i.e., [HNO_3] = 0) are also included. The total amount of surface groups (released as CO_2 and CO) and the corresponding oxygen content (calculated from the total amounts of CO_2 and CO) are summarized in Table 5.1.

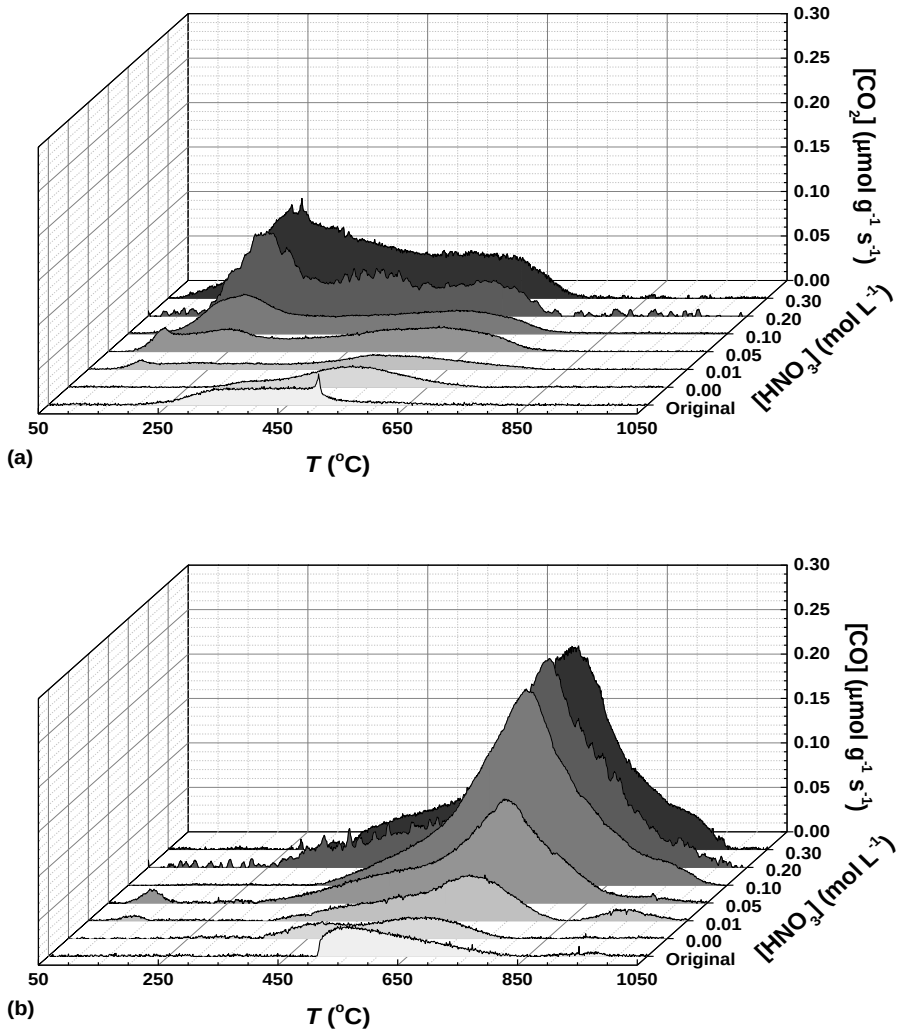

Fig. 5.2 TPD spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations: **(a)** CO_2 and **(b)** CO evolution with temperature.

The amounts of CO_2 and CO increase as the concentration of the oxidizing agent increases (up to 0.30 mol L^{-1}), confirming that MWCNTs are suitable to the inclusion of oxygenated functional groups through hydrothermal oxidation under mild conditions. The high level of oxidation in this type of carbon material can be associated to their structure, which provides a great number of defects where the oxidation

process can be started [27]. Nevertheless, only a slight increase in the amount of oxygenated surface groups is observed when the HNO_3 concentration is incremented from 0.20 to 0.30 mol L^{-1} (Fig. 5.3a and b, and Table 5.1). This phenomenon was already reported in a previous work on HNO_3 hydrothermal oxidation of carbon xerogels [23] and suggests that there is a maximum extent of surface functionalization achievable through this mild hydrothermal methodology. A prevalence of surface groups released as CO was found in contrast to those released as CO_2, the [CO]/[CO_2] ratio being higher than one for all the MWCNTs under study (Table 5.1). The amount of oxygen follows the same trend of the surface groups released as CO_2 and CO, as expected. Comparing the pristine MWCNTs and those hydrothermally treated with water (blank), both have similar (and low) amounts of oxygen surface groups, indicating that the hydrothermal treatment without addition of HNO_3 has no effect on the surface chemistry of MWCNTs.

Table 5.1 Properties of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations: amounts of CO_2 and CO released by TPD, [CO/CO_2] ratio, percentage of oxygen obtained from the analysis of the TPD spectra (assuming that all the surface oxygen is released as CO and/or CO_2), amount of volatiles (determined by TGA), amount of carboxylic acids (CA; corresponding to the sum of SA and LA, as determined by TPD), pH at the point of zero charge (pH_{PZC}), specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}) and total pore volume (V_{total}).

[HNO_3] (mol L^{-1})	Parameters									
	[CO_2] (µmol g^{-1})	[CO] (µmol g^{-1})	O (wt.%)	[CO]/[CO_2]	Volatiles (wt.%)	[CA] (µmol g^{-1})	pH_{PZC}	S_{BET} (m^2 g^{-1})	S_{meso} (m^2 g^{-1})	V_{total} (cm^3 g^{-1})
Original MWCNT	59	72	0.3	1.2	2.87	n.d.	6.9	198	198	1.264
0 (Blank)	59	72	0.3	1.2	2.92	5	6.9	188	188	1.581
0.01	71	150	0.5	2.1	3.41	20	6.7	202	202	1.148
0.05	156	327	1.0	2.1	3.75	54	6.1	229	204	1.115
0.10	178	590	1.5	3.3	4.41	93	5.5	250	237	1.566
0.20	304	656	2.0	2.2	4.80	152	5.2	261	255	1.669
0.30	392	671	2.3	1.7	5.12	225	5.0	262	262	2.193

n.d.: Not determined.

Correlations between the amount of oxygenated groups introduced on the surface of the MWCNTs (released as CO_2 and CO) and the concentration of HNO_3 employed in the hydrothermal treatment were obtained (Fig. 5.3a). Likewise, the contents of oxygen and volatiles (determined by TGA under inert atmosphere; Table 5.1) are also given as function of the HNO_3 concentration (Fig. 5.3b). As observed, the evolution of all the parameters under study can be described as function of the HNO_3 concentration by single exponential functions (r^2 in the range 0.972 – 0.999), which is in accordance with our previous results on hydrothermally treated MWCNTs [24], single-walled carbon nanotubes (SWCNTs) [25] and carbon xerogels [23-25]. These correlations are very useful to fine tune the surface chemistry of MWCNTs, as they allow a given amount of oxygenated surface groups to be obtained by setting the proper concentration of HNO_3 in the hydrothermal treatment.

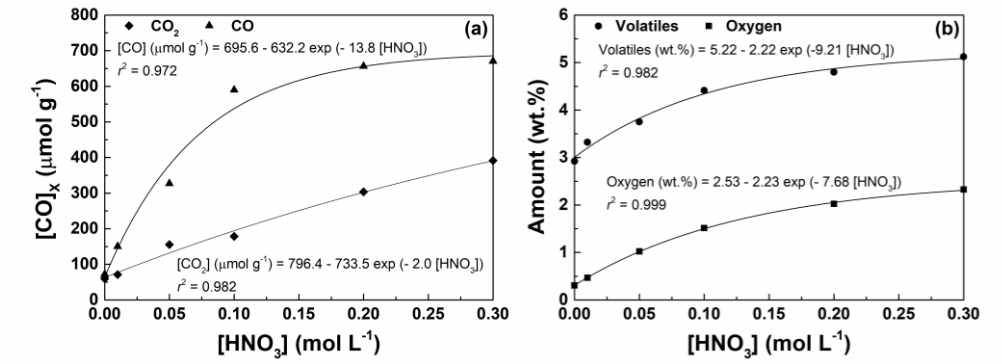

Fig. 5.3 (a) Amounts of CO_2 (*SD* ≤ 39 μmol g^{-1}) and CO (*SD* ≤ 48 μmol g^{-1}) released by TPD and **(b)** contents of oxygen (*SD* ≤ 0.19 wt.%) and volatiles (*SD* ≤ 0.49 wt.%) as function of the concentration of HNO_3 employed in the hydrothermal treatment of MWCNTs. Points represent experimental data, while lines represent non-linear fittings.

Deconvolution analysis of the CO_2 and CO TPD spectra was performed in order to identify and quantify the amounts of the different functionalities (Figs. 5.4 a and b, respectively, and Fig. D-S5.4, and corresponding results detailed in Tables D-S5.6 and

D-S5.7). As a representative example, Figs. 5.4 a and b show the deconvoluted CO_2 and CO spectra of $MWCTN_{0.3}$ (i.e., the material obtained after hydrothermal treatment with 0.30 mol L^{-1} HNO_3). As observed, the surface groups released as CO_2 were mainly assigned to strongly acidic carboxylic acids (SA, 172 µmol g^{-1}), followed by carboxylic anhydrides (CAn, 84 µmol g^{-1}) and lactones (Lac, 84 µmol g^{-1}), and less acidic carboxylic acids (LA, 53 µmol g^{-1}). Regarding the CO spectrum, the main contribution was assigned to carbonyls and quinones (CQ, 388 µmol g^{-1}), followed by phenols (Ph, 148 µmol g^{-1}) and carboxylic anhydrides (CAn, 84 µmol g^{-1}). Moreover, a minor contribution was found at high temperature, as revealed by the shoulder observed at around 900 ^{o}C (Fig. 5.4b), which can be attributed to basic surface groups (Bas, 64 µmol g^{-1}) [29]. Considering the results shown in Tables D-S5.6 and D-S5.7, the concentration of the oxygen functional groups generally increases with the concentration of HNO_3 employed in the hydrothermal treatment, as previously observed for the total amounts of CO_2 and CO.

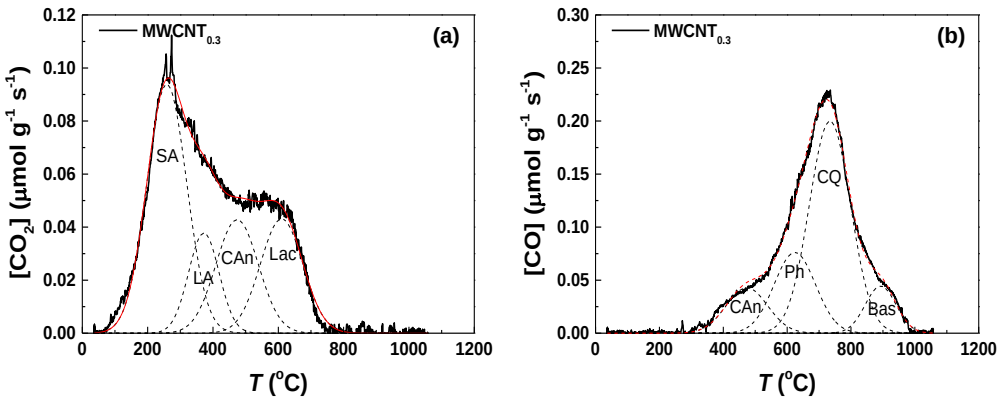

Fig. 5.4 Deconvolution results of **(a)** CO_2 and **(b)** CO TPD spectra of MWCNTs subjected to hydrothermal treatment with 0.30 mol L^{-1} HNO_3 ($MWCNT_{0.3}$). Dashed lines represent peaks assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (CAn), lactones (Lac), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes. Red lines represent cumulative peak fitting.

The effect of the hydrothermal treatment on the overall surface charge (assessed though pH_{PZC} measurements) of the resulting materials, was also studied (Table 5.1). As observed, materials with a more pronounced acidic character are gradually obtained as the HNO_3 concentration increases (i.e., pH_{PZC} is 6.9 for pristine MWCNTs and 5.0 for the MWCNTs treated with 0.30 mol L^{-1} HNO_3). This continuous decrease of the pH_{PZC} values can be ascribed to the increasingly significant amounts of carboxilic acids (CA) introduced on the carbon surface, as summarized in Table 5.1. A similar conclusion was achieved in a previous publication of our group, upon a thorough analysis of results obtained by Raman spectroscopy [31]. As shown in Fig. D-S5.5, the intensity ratio of the D band relative to the G mode (I_D/I_G) obtained by Raman spectroscopy plotted as a function of the total amount of functional groups determined by TPD revealed a linear function when characterizing single-walled carbon nanotubes treated with different HNO_3 concentrations. Thus, data obtained by TPD correlate well with data obtained by Raman spectroscopy or other techniques such as water adsorption/desorption [31]. The effect of the hydrothermal treatment on the textural properties of the MWCNTs was evaluated through N_2 adsorption-desorption isotherms. All the MWCNTs possess negligible microporosity (as revealed by the low adsorption obtained at low N_2 pressures); on the contrary, the prevalence of mesopores is revealed by the high adsorption observed at higher N_2 pressures (Fig. D-S5.6). The mesoporous nature of the MWCNTs is confirmed by the results given in Table 5.1. As observed, the surface area (both S_{BET} and S_{meso}) and pore volume (V_{pore}) increase as the concentration of HNO_3 employed in the hydrothermal treatment increases. The average diameter of the MWCNTs is 9.5 nm (technical description provided by the manufacturer) and the average internal diameter of the tubes is around 4 nm [31], i.e., almost 2-fold higher than the maximum diameter of micropores (2 nm). Therefore, as

expected, micropores were not found in the original sample (V_{micro} = 0) and the values were very low for the oxidized ones (i.e., within the error of the analysis).

Our results are in line with those previously reported in a study performed with SWCNTs, in which it was concluded that functionalization with HNO_3 causes the opening of the nanotube caps with no significant defects being additionally produced [32]. The progressive increase of S_{BET}, S_{meso} and V_{pore} can thus be ascribed to the opening of the nanotube caps, which enhances the accessibility to the inner part of the MWCNTs, rather than defect creation. As stated in a previous work, a gradual increase of both S_{BET} and the amount of oxygenated functional groups is observed when the hydrothermal oxidation treatment is performed with increasing HNO_3 concentrations [24]. This methodology leads to a slight increase of the S_{BET}, around 32% when comparing the pristine and the $MWCNT_{0.3}$ sample, i.e. from 198 $m^2\ g^{-1}$ in the pristine MWCNTs to 262 $m^2\ g^{-1}$ in the sample treated with 0.30 mol L^{-1} HNO_3. This increase of S_{BET} as consequence of the hydrothermal oxidation treatment with HNO_3 is similar to that obtained in a previous study of our group performed under similar conditions, i.e., 27% [33]. In that study, both pristine and oxidized (0.3 mol L^{-1} of HNO_3) MWCNTs were characterized by scanning electron microscopy (SEM) (Fig. D-S5.7), allowing to conclude that both samples consist of agglomerated carbon nanotubes, with no significant morphological changes being perceptible as a consequence of the HNO_3 hydrothermal oxidation [33]. When the total amounts of CO_2 and CO released by TPD are normalized by the S_{BET} (i.e., ($[CO_2]+[CO]$)/S_{BET}), and represented as a function of HNO_3 concentration (Fig. 5.5), two distinct stages in the exponential curve are observed: (i) in the first stage, the pronounced increase is associated with the functionalization of the accessible surface area of the original MWCNTs; while (ii) the final part of the curve corresponds to the functionalization of new surface area made accessible during the HNO_3 hydrothermal oxidation. The correlation obtained in Fig.

5.5 (with r^2 = 0.997) can be extended to other carbon materials obtained through the same hydrothermal functionalization methodology.

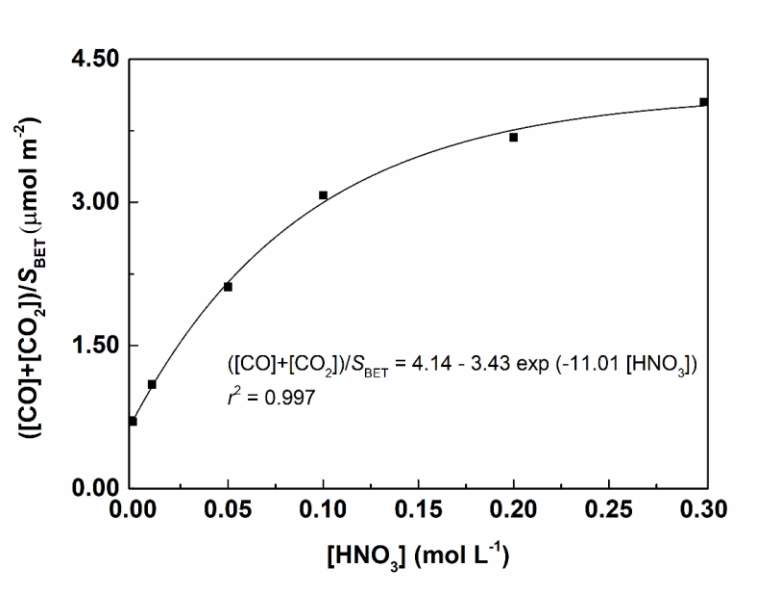

Fig. 5.5 ([CO_2] + [CO])/S_{BET} as a function of HNO_3 concentration.

5.3.4. Application of functionalized MWCNTs for extraction of EU multi-class OMPs

The applicability of the original and hydrothermally treated MWCNTs as sorbents for SPE of the 8 target OMPs was studied, as well as the influence of both textural and surface chemistry properties of these materials in the adsorption/desorption process. For that purpose, the recoveries of the target compounds were determined as described by Equation (5.1) (Section 5.2.5). The recoveries obtained revealed different behaviors for the OMPs under study (Fig. 5.6) due to their distinct classes and physicochemical properties. For instance, similar recoveries were achieved for the pesticides atrazine and isoproturon (around 80 and 70%, respectively) with all the samples of MWCNTs that were tested, indicating that the adsorption/desorption

process is not affected by the oxygenated surface groups introduced by the HNO_3 hydrothermal treatment. The SPE cartridges packed with the original MWCNTs (recoveries > 60%) performed better than the materials treated with HNO_3 for the pharmaceutical compounds diclofenac and carbamazepine and the neonicotinoid pesticide acetamiprid (recoveries < 60%). In the case of metaflumizone, the recovery obtained is ineffective with the original and treated MWCNTs packed in the SPE cartridges. On the other hand, performing SPE with $MWCNT_{0.30}$ leads to a significant improvement of the recoveries obtained for methiocarb and the industrial compound PFOS, when compared to the original MWCNTs.

Several mechanisms may simultaneously control the adsorption/desorption process of the organic pollutants on MWCNTs, including (i) π-π interactions, i.e., the interactions between bulk π systems present on the surface of MWCNTs and organic molecules with their benzene rings or C=C double bonds; (ii) hydrogen bonds with functional groups on the surface of the sorbent material; and (iii) electrostatic interactions due to of the charged carbon material surface [10, 34].

However, each mechanism could be affected differently by the environmental conditions, which makes the application of MWCNTs for SPE of different organic compounds from aqueous matrices a challenging research topic. The obtained results (Fig. 5.6) suggest that the HNO_3 hydrothermal treatment applied to MWCNTs affects the SPE efficiency of the target OMPs in two distinct ways. In the case of acetamiprid, diclofenac and carbamazepine, the dominant adsorption mechanism seems to be π-π dispersive interactions, which decrease with the increase of the oxygen-containing functional groups, most of them with electron-withdrawing properties. In contrast, for methiocarb and PFOS, the HNO_3 functionalization leads to higher recoveries, possibly due to the predominance of the hydrogen bond adsorption mechanism favored by the increase of the oxygen surface groups.

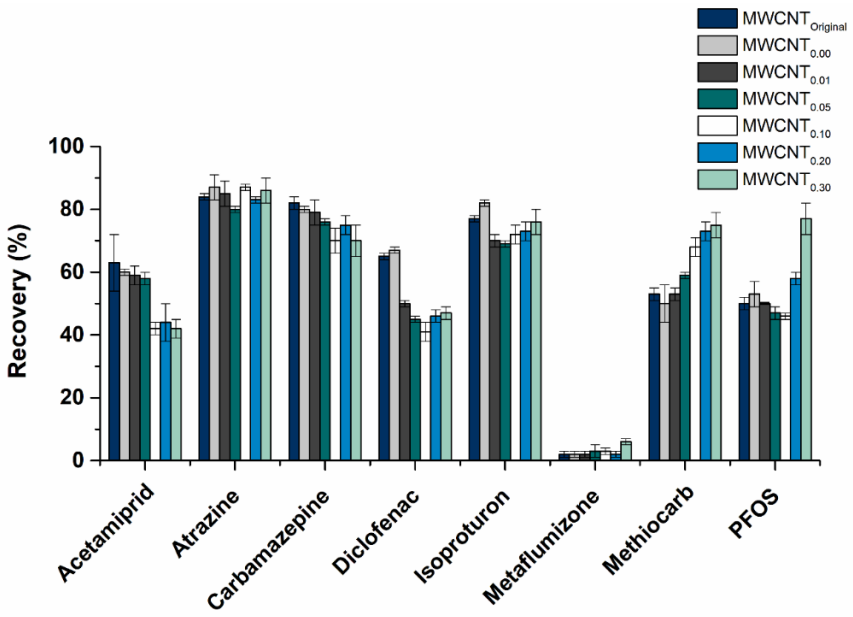

Fig. 5.6 Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with MWCNTs (50 mg) obtained after hydrothermal treatment with different HNO_3 concentrations (0-0.30 mol L^{-1}). Experiments performed with 500 mL of sample (SW; pH 3) and using ethanol as solvent (4 mL); n = 3 (RSD is represented as error bars).

In the case of the carbamate pesticide methiocarb, it is interesting to observe the continuous increase in the recovery values with the increase of the acid concentration. Thus, the recoveries obtained for methiocarb were plotted as a function of the total amount of functional groups introduced ($CO+CO_2$) divided by the respective S_{BET} (Fig. 5.7). A good linear correlation (r^2 = 0.995) was obtained, i.e., the total amount of CO and CO_2 divided by the S_{BET} has proved to be a good predictor of methiocarb recovery. For this carbamate pesticide, the HNO_3 functionalization of MWCNTs led to a continuous and significant increase in the SPE efficiency. In order to understand if this correlation was associated with any specific functional group previously determined by TPD, a similar analysis was made but with the amount of each surface group (SA, LA, CAn, Lac, Ph, CQ and Bas), instead of the total amount released as CO and CO_2.

Good correlations with Ph (r^2 = 0.916) and CQ groups (r^2 = 0.918) were also obtained (Figs. D-S8 a and b), suggesting that the presence of higher amounts of these functional groups on the MWCNT surface increases the affinity for methiocarb.

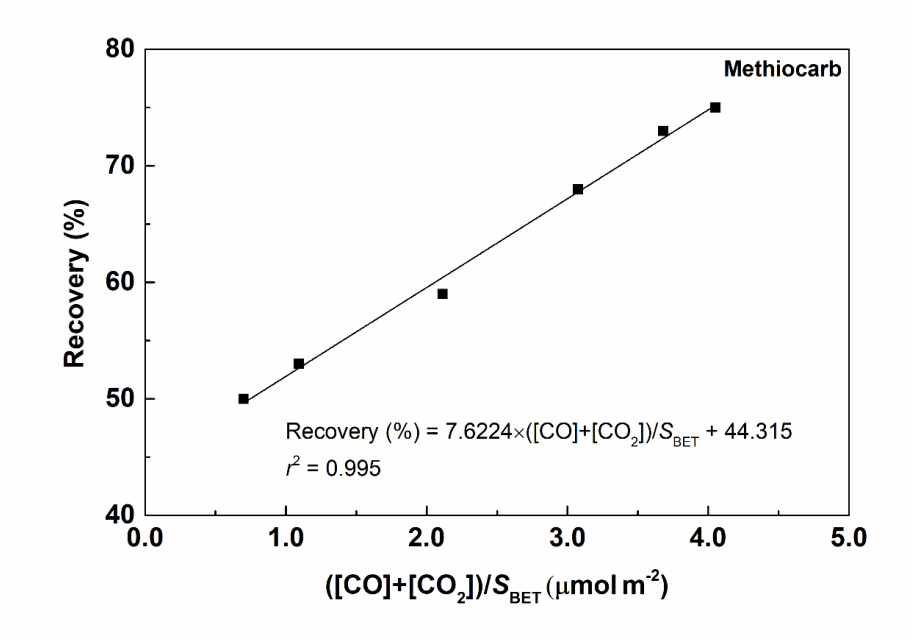

Fig. 5.7 Recovery obtained for methiocarb as a function of ($[CO_2]$ + [CO])/S_{BET}.

In the literature, there are several studies reporting the possible mechanisms of adsorption of some target compounds on MWCNTs. For example, in the case of atrazine, π–π dispersive and polar interactions were appointed as responsible for the adsorption on MWCNTs [35]. Regarding the industrial compound PFOS, a study conducted by Li *et al.* [36] concluded that hydrophobic interactions are the main mechanism of adsorption of PFOS. The pharmaceutical compound diclofenac was already studied, and diverse types of interactions were suggested, such as electrostatic and hydrophobic interactions and hydrogen bonding [34, 37]. In the case of carbamazepine, the π-π electron-donor-acceptor interactions, hydrogen bonding and hydrophobic interactions had a key role in the adsorption on MWCNTs [34, 37].

Therefore, the mechanism and the extraction performance result from the interplay of the characteristics of each pollutant and the properties of the sorbent material.

5.4. Conclusions

Pristine and modified MWCNTs were applied as adsorbent materials in conventional SPE for enrichment of 8 EU multi-class OMPs in SW samples and analysis by UHPLC-MS/MS. The optimized SPE procedure with pristine MWCNTs has the great advantage of using an eco-friendly solvent (ethanol) for both conditioning and elution steps. Additional advantages of this carbon-based cartridge are the small amount of adsorbent that is needed (50 mg), representing a ~75% cost reduction in comparison with the commercial cartridge (while obtaining similar recoveries), and the ability to be reused at least three times without substantial impact on the retention capacity of the adsorbent. The oxidation of the MWCNT surface (and thus the introduction of oxygenated functional groups) can affect the SPE recoveries in different ways. The dominant adsorption mechanism seems to be π-π dispersive interactions in the case of acetamiprid, diclofenac and carbamazepine (i.e. the recoveries were higher when using the original MWCNTs), whereas the hydrogen bond adsorption mechanism (favored by the increase of the oxygen surface groups) seems to be predominant in the case of methiocarb and PFOS. Moreover, a very good correlation between the recovery of methiocarb and the functionalities created on the MWCNTs was found, which was attributed to the phenol and carbonyl and quionone groups. The fine control of the surface chemistry and texture of MWCNTs, with the purpose of improving the selectivity and specificity of these materials, opens a window of opportunity for the development of more efficient and eco-friendly analytical tools for the analysis of EU-relevant OMPs, for instance by mixing MWCNTs with different textural and surface chemistry properties in the same SPE cartridge.

Acknowledgments

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Supplementary material

(Please see Appendix D)

Text D-S5.1. Reference SPE protocol.

Text D-S5.2. MWCNTs SPE optimization.

Table D-S5.1. Target compounds, class, structure, relative molecular mass (M_r), p*K*a and log K_{OW} values and solubility in water.

Table D-S5.2. Selected reaction monitoring (SRM) instrument parameters for tandem mass spectrometry analysis of target analytes.

Table D-S5.3. Retention time, range, linearity, instrument and method detection and quantification limits for each target analyte.

Table D-S5.4. Studies dealing with the application of MWCNTs in conventional SPE: target analyte and respective spiked level (ng L^{-1}), matrix, sample loading (mL), amount of MWCNTs packed in the cartridge (mg), conditioning and elution solvents, and recoveries (%) obtained. Pollutants included in these studies that are out of the scope of EU legislation are not discussed.

Table D-S5.5. Textural properties of MWCNTs (NC3100) and Oasis HLB: specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}), total pore volume (V_{total}) and average pore diameter (d_{pore}).

Table D-S5.6. Results obtained from the deconvolution of the CO_2 spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. T_M, W and A represent the temperature at the peak maximum, the width of the peak at half-height and the integrated peak area, respectively. Peaks were assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (Can) and lactones (Lac).

Table D-S5.7. Results obtained from the deconvolution of the CO spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. T_M, W and A represent the temperature at the peak maximum, the width of the peak at half-height and the integrated peak area, respectively. Peaks were assigned to carboxylic

anhydrides (Can), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes.

Fig. D-S5.1. Schematic representation (a) and photograph (b) of the lab-scale packing device designed to prepare MWCNT cartridges (6 at a time).

Fig. D-S5.2. Schematic representation of the experimental procedure carried out to evaluate the extraction efficiency (i.e. recovery in %) of each SPE method.

Fig. D-S5.3. Total Ion Current (TIC) chromatograms of the 8 target OMPs (200 ng L^{-1}) in: (a) a SPE extract of a spiked sample; and (b) a post-spiked blank extract, using cartridges packed with MWCNTs (50 mg).

Fig. D-S5.4. Deconvolution results of (a, c, e, g, i) CO_2 and (b, d, f, h, j) CO TPD spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. Dashed lines represent peaks assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (Can), lactones (Lac), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes. Red lines represent cumulative peak fitting.

Fig. D-S5.5. Intensity ratio of the D band relative to the G mode (I_D/I_G) obtained by Raman spectroscopy, as function of the total amount of evolved CO and CO_2 determined by TPD when functionalizing single-walled carbon nanotubes with different HNO_3 concentrations in the hydrothermal treatment. Lines designate the linear fit. Reprinted (adapted) with permission from The Journal of Physical Chemistry C, Vol. 115, G.E. Romanos, V. Likodimos, R.R.N. Marques, T.A. Steriotis, S.K. Papageorgiou, J.L. Faria, J.L. Figueiredo, A.M.T. Silva, P. Falaras, Controlling and Quantifying Oxygen Functionalities on Hydrothermally and Thermally Treated Single-Wall Carbon Nanotubes, 8534-8546. Copyright 2011, with permission of American Chemical Society.

Fig. D-S5.6. N_2 adsorption-desorption isotherms at -196 ^oC of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations.

Fig. D-S5.7. SEM micrographs of (a) pristine (MWp) and (b) functionalized (MWf) MWCNTs. Reprinted from The Journal of Membrane Science, Vol. 520, Sergio Morales-Torres, Carla M.P. Esteves, José L.Figueiredo, Adrián M.T. Silva, Thin-film composite forward osmosis membranes based on polysulfone supports blended with

nanostructured carbon materials, 326-336, Copyright 2016, with permission from Elsevier [License number: 4945340204301].

Fig. D-S5.8. Recovery obtained for methiocarb as a function of (a) (Ph)/S_{BET} and (b) (CQ)/S_{BET}.

Chapter 6

Carbon xerogels combined with carbon nanotubes as an innovative sorbent in solid-phase extraction cartridges to assess the occurrence of organic micropollutants in surface and drinking water

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Carbon xerogels combined with carbon nanotubes as an innovative sorbent in solid-phase extraction cartridges to assess the occurrence of organic micropollutants in surface and drinking water

Abstract

Carbon xerogels (CXs) were synthesized by polycondensation of resorcinol and formaldehyde, followed by thermal annealing. Solid-phase extraction (SPE) cartridges were prepared with CXs as filling material and tested for extraction and preconcentration of eight multi-class organic micropollutants present in water samples before chromatographic analysis. Five pesticides (acetamiprid, atrazine, isoproturon, metaflumizone, and methiocarb), two pharmaceuticals (carbamazepine and diclofenac), and one industrial compound (perfluorooctanesulfonic acid) were considered in this study. The recoveries obtained with the pristine CX material were relatively low for most of the compounds, except for metaflumizone (69 ± 5%). Moreover, the introduction of oxygenated surface groups in the carbon material led to a decrease in the recovery values. It was concluded that the adsorption/desorption process of the target micropollutants performed better on CXs with a less acidic surface, as is the case of pristine CXs. Thus, carbon-based cartridges were then prepared by adding pristine CXs and multi-walled carbon nanotubes (MWCNTs) in a multi-layer configuration. This cartridge was reusable and able to simultaneously extract the eight target micropollutants. The innovative cartridge was used to validate an analytical methodology based on SPE followed by ultra-high performance liquid chromatography-tandem mass spectrometry for monitoring these compounds (i) in surface water collected in rivers supplying three drinking water treatment plants and

(ii) in the resulting drinking water at the endpoint of the respective distribution systems. Their widespread occurrence up to hundreds of ng L^{-1} was verified in the following order of frequencies: carbamazepine > diclofenac > isoproturon > metaflumizone > atrazine ~ methiocarb. Therefore, the first study employing CXs and MWCNTs as sorbent material in multi-layer SPE cartridges is herein reported for determination of organic micropollutants in water samples.

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6.1. Introduction

Pesticides, industrial and pharmaceutical compounds, are widely used in diverse activities, and many are indispensable in daily life [1]. However, some of these compounds have been identified as priority substances (PSs, as defined in EU Directive 2013/39) [2] or contaminants of emerging concern (CECs, as defined in EU Decisions 2015/495, 2018/840, and 2020/1161) [3-5]. The recognized (PSs) or suspected (CECs) impact of these organic micropollutants (OMPs) in the environment and human health have been reported [6-8], in particular when occurring in surface (SW) and drinking water (DW). OMPs are released in effluents from conventional urban wastewater treatment plants (WWTPs), which are not designed to eliminate these compounds, and from several other activities (e.g., agriculture, livestock, and aquaculture), reaching water bodies and courses directly and/or through surface runoff [9, 10]. Therefore, the broad identification and quantification of PSs and CECs in aquatic compartments are essential to compile the data needed to evolve regulatory frameworks in the field of water policy (e.g., transport and fate in the environment and effects on human and ecological health).

Since OMPs are typically found at residual concentrations in the environment (ng L^{-1} to µg L^{-1}), it is important to employ an accurate and precise preconcentration step prior to the analysis by using a sensitive and reproducible analytical technique, such as ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Considering the wide resources and great deal of time involved in this task, novel analytical methods would ideally allow: (i) the simultaneous determination of several distinct chemical compounds at trace levels, thus meeting the multi-class purpose; and (ii) shortening the time required for cleanup of the sample matrix and the extraction of analytes, which are generally the most time-consuming

analytical steps [1, 11]. Solid-phase extraction (SPE) is the most popular sample preparation technique for environmental samples, recognized as a robust and straightforward tool for obtaining sample extracts enriched with target analytes and free of interfering species present in the matrix [12-14]. It is well known that the selection of the most appropriate sorbent is the key step in SPE, since the enrichment efficiency of the target analytes depends on the sorbent characteristics [15]. A wide range of sorbents is currently available. Nevertheless, chemically modified silica gel, polymeric sorbents and, more recently, carbon-based materials, are the most commonly used [16-18].

Carbon xerogels (CXs) can be obtained by sol-gel polycondensation of resorcinol with formaldehyde, followed by conventional drying and thermal annealing under inert atmosphere [19]. CXs present some advantages when compared with other carbon materials, such as the reproducibility of the synthesis procedure and the possibility to control their textural and surface chemistry properties [20]. Furthermore, these materials are characterized by having high porosity and surface area, controllable pore size, and also having the possibility of being shaped as desired [21, 22]. These attributes make CXs attractive materials for several applications, including fuel cells [23, 24], catalysis [25-27], and adsorption for either water treatment [28, 29] or analytical chemistry [30, 31]. Regarding water treatment by adsorption, some authors have been studying CXs for removal of specific PSs and CECs: (i) Moral-Rodriguez *et al.* [32] studied different CXs for the adsorption of diclofenac; (ii) Álvarez *et al.* [33] applied CXs as adsorbents for the removal of caffeine and diclofenac; and (iii) Carabineiro *et al.* [34] studied the adsorption capacity of CXs for the antibiotic ciprofloxacin. In the field of analytical chemistry, only two studies were found in the literature for the analysis of pollutants by using CXs in SPE: (i) one to determine the effectiveness of an oxidized CX material as sorbent in SPE to preconcentrate lead

from tap water samples [31]; and (ii) another to extract aryloxyphenoxypropionate herbicides from aquatic environmental samples by micro-SPE [30]. However, studies focused on the application of CXs as SPE adsorbent for extraction of other PSs [2] or CECs listed in the Watch List of the EU Decisions [3-5] were not found.

In the present work, pristine and hydrothermally modified CXs were synthetized, characterized, and applied as SPE adsorbents for the simultaneous extraction of 8 EU multi-class OMPs in water samples, namely 5 pesticides (acetamiprid, atrazine, isoproturon, metaflumizone, and methiocarb), 2 pharmaceuticals (carbamazepine and diclofenac) and 1 industrial compound (perfluorooctanesulfonic acid), followed by analysis with UHPLC-MS/MS. In fact, SPE-LC-MS/MS is the most common indicative analytical method suggested for these compounds in the abovementioned EU legislations. Moreover, SPE cartridges packed with multi-walled carbon nanotubes (MWCNTs) performed well for 7 out of these 8 target compounds in our previous study [35], but failed to recover metaflumizone, which is included in the very recent Watch List of EU Decision 2020/1161 [5]. Therefore, the main goal of the present study was to find a carbon material with a good performance for the recovery of metaflumizone, aiming at the development of a SPE carbon-based cartridge in multi-layer configuration performing well for all the 8 OMPs.

Additionally, an analytical method based on SPE-UHPLC–MS/MS was validated with the optimized multi-layer carbon cartridge and used to assess the occurrence of the 8 multi-class contaminants in SW samples strategically collected at different locations (i.e., nearby the admission point of 3 drinking water treatment plants - DWTPs - in northern Portugal) and in the DW yielded by those plants. To the best of our knowledge, this is the first time a (non-polymeric) multi-layer carbon cartridge was prepared for the extraction and preconcentration of OMPs. Moreover, the pesticide

metaflumizone listed in the recent Watch List of EU Decision 2020/1161 was quantified for the first time in environmental water compartments.

6.2. Experimental section

6.2.1. Chemicals and materials

Formaldehyde solution (37 wt.% in water, stabilized with 15 wt.% of methanol) and resorcinol (99 wt.%) were purchased from Sigma-Aldrich (Steinhein, Germany). Commercial MWCNTs Nanocyl 3100 (NC3100™, powder), with an average diameter of 9.5 nm, average length of 1.5 µm, and > 95% purity were purchased from Nanocyl SA (Sambreville, Belgium). All reference standards (acetamiprid, atrazine, carbamazepine, diclofenac sodium, isoproturon, metaflumizone, methiocarb and perfluorooctanesulfonic acid - PFOS; > 98% purity) and deuterated compounds used as internal standards (acetamiprid–d3, atrazine–d5, diclofenac-d4, and methiocarb-d3) were acquired from Sigma-Aldrich (Steinhein, Germany). The physicochemical characteristics of these analytes can be found in the Supplementary Material, Table E-S6.1. Hydrochloric acid, ethanol (HPLC grade), and methanol (MS grade) were purchased from VWR International (Fontenay-sous-Bois, France). Each stock solution was prepared in ethanol with 1000 mg L^{-1} of each reference or internal standard. The working solutions comprising all the target compounds or the internal standards (2.5 mg L^{-1} and 5.0 mg L^{-1}, respectively) were prepared by dilution of the individual stocks in ethanol. Sodium chloride was purchased from José Manuel Gomes dos Santos (Odivelas, Portugal). Sodium hydroxide, nitric acid, and sulfuric acid were obtained from Merck (Darmstadt, Germany). Empty SPE cartridges (6 mL) with two frits (20 µm) (Bond Elut™) were purchased from VWR International (Fontenay-sous-Bois,

France). Ultrapure water was provided by a Milli-Q water system, and the pH measurements were performed with a pHenomenal® pH 1100L pH meter (VWR, Germany).

6.2.2. Preparation and modification of carbon materials

Two types of carbon materials were used in this work, namely in-house prepared CXs and commercial MWCNTs. More details (i.e., textural and surface chemistry characterization) on the MWCNTs can be found in our previous publication [35]. The pristine CX material was prepared by polycondensation of resorcinol with formaldehyde using a molar ratio of 1:2, adapting the procedures described elsewhere [19, 21]. The sol-gel step was carried out at pH 6.0, sodium hydroxide solutions being added dropwise under continuous stirring until achieving the desired pH. This step is crucial since the accurate control of pH was determinant for the development of the mesoporous character of CXs [21].

The original CX material was modified by surface functionalization with HNO_3, following the hydrothermal procedure described elsewhere [36]. Specifically, the hydrothermal oxidation was conducted in a Teflon-lined stainless-steel autoclave (125 mL, Mod. 4748, Parr Instruments, USA). The HNO_3 solution (75 mL, at five different concentrations varying from 0.01 to 0.30 mol L^{-1}) was transferred to the PTFE vessel and 0.2 g of CXs was loaded. The PTFE vessel was placed inside the stainless-steel autoclave, which was sealed and kept in an oven at 200 ºC during 2 h. Subsequently, the autoclave was allowed to cool down until room temperature. The recovered CX sample was washed with distilled water until a neutral pH of the rinsing water was reached and then dried overnight at 120 ºC. Moreover, a blank hydrothermal treatment was performed with distilled water instead of the HNO_3 solution. The six resulting materials were denoted as CX followed by a subscript number corresponding to the

concentration of HNO_3 employed in mol L^{-1}, namely: CX_0, $CX_{0.01}$, $CX_{0.05}$, $CX_{0.10}$, $CX_{0.20,}$ and $CX_{0.30}$.

6.2.3. Characterization of carbon materials

The textural properties were determined from N_2 adsorption-desorption isotherms at -196 ºC, as described in our previous work [21] and included the following parameters: specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}) and total pore volume (V_{total}). Thermogravimetric analysis (TGA) was performed in a Netzsch STA 490 PC/4/H Luxx thermal analyser, in which the CX samples were heated from 50 to 900 ºC at 10 ºC min^{-1}, under an inert (N_2) gas flow. Temperature programmed desorption (TPD) was performed in a fully automated AMI-300 Catalyst Characterization Instrument (Altamira Instruments) with a quadrupole mass spectrometer (Dymaxion, Ametek), as described elsewhere [36]. The pH at point of zero charge (pH_{PZC}) was determined by pH drift tests [36].

6.2.4. SPE procedure

6.2.4.1. Preparation of the SPE cartridges

Handmade cartridges with single (i.e., containing a CX sample only) and multi-layer configuration (i.e., with both CX and MWCNT) were packed by using a device specially created for that purpose [35]. This process comprises several successive phases, namely: (i) a polyethylene frit (20 µm) was positioned on the bottom of an empty SPE cartridge (6 mL); (ii) a selected amount of carbon material was then introduced in the cartridge; (iii) the carbon sample was protected with another frit; and (iv) a slight compression was applied until a specific bed height was achieved. In the case of multi-

layer carbon cartridges, the steps (ii) and (iii) were repeated, to introduce the second layer of sorbent material.

6.2.4.2. Optimization of the SPE procedure

A design of experiments (DoE) was carried out for the optimization of SPE with CX-cartridges. A definitive screening design (DSD), which involved 14 randomized experiments with 5 factors (mass of sorbent material (mg), sample volume (mL), sample pH, type of solvent and solvent volume (mL)) and 3 levels (−1, 0 and 1), was applied in a first stage aiming the determination of the main parameters affecting the recovery efficiency of the 8 target OMPs. Afterwards, a Box-Benkhen Design (BBD) considering only the significant variables was employed (15 runs, random order) to find the best operating conditions with a minimum number of assays. The designated coded values for the variables, −1, 0 and 1, were used to represent low, middle, and high levels, respectively, and the coded and actual levels of the variables are listed in Table E-S6.2 for DSD and BBD. The DoE and data analysis were accomplished using Minitab® 19 (Minitab® Statistical Software, Pennsylvania, USA).

Regarding the SPE procedure itself, the conditioning solvents ethanol or methanol and ultrapure water were successively passed through the SPE cartridge at a flow rate of 1 mL min^{-1}. Immediately, sample loading was carried out with a determined volume of blank or spiked (200 ng L^{-1} of each target analyte) water sample at a constant flow rate of 10 mL min^{-1}, using a vacuum manifold unit connected to a vacuum pump. Then, the washing step was performed with 4 mL of ultrapure water. The cartridges were dried under vacuum for 45 min and the retained analytes were eluted with a specific volume of solvent (ethanol or methanol). The extracts obtained were evaporated to dryness in a Centrivap Concentrator® device (LABCONCO® Corporation, Kansas City, MO, USA). The dried extracts were reconstituted in 250 µL of solvent (ethanol or methanol),

and the resulting solutions were filtered through 0.22 μm polytetrafluoroethylene syringe filters (Membrane Solutions, Kent, WA, USA) and analysed by UHPLC-MS/MS. All experiments carried out during the SPE optimization study were performed in triplicate, the obtained relative standard deviations (RSD) being represented as error bars in the corresponding Figures.

6.*2.5. UHPLC–MS/MS method*

The water sample analysis was performed in an UHPLC-MS/MS apparatus (Shimadzu Corporation, Tokyo, Japan). This system consists of: (i) a Nexera UHPLC equipment with two LC-30AD chromatographic pumps and a DGU-20A 5R degasser; (ii) a CTO-20AC column oven; (iii) a SIL-30AC autosampler; (iv) a CBM-20A system controller coupled to a Shimadzu LC Solution Version 5.41SP1 software; and (v) a Ultra-Fast Mass Spectrometry series LCMS-8040 triple quadrupole mass spectrometer. A column Kinetex™ XB-C18 100 Å (100 × 2.1 mm i.d.; particle diameter of 1.7 μm) purchased from Phenomenex, Inc. (Torrance, CA, USA) and operating under gradient mode of flow of the mobile phase water/ethanol (50/50, v/v) was used for the chromatographic separation of the target OMPs. The temperature of the column oven was set at 35 °C, the autosampler temperature at 15 °C, and the injection volume was 5 μL. Regarding the MS settings, these were defined to: argon at 230 kPa as CID gas, 400 °C and 250 °C for source and desolvation temperatures, capillary voltage of 0.5 kV, 2.5 dm^3 min^{-1} and 12.5 dm^3 min^{-1} of nebulizing and drying gas flow, respectively. An electrospray ionization source was used operating in both positive and negative ionization modes. Selected reaction monitoring (SRM) was applied to quantify and confirm the identity of each target compound, by using SRM1 for quantification and the ratio between SRM1 and SRM2 for confirmation (Table E-S6.3 of the Supplementary

Material), along with the retention time of the analyte (Table E-S6.4 of the Supplementary Material).

6.2.6. Quality assurance and control of the analytical method

The validation of the SPE-UHPLC-MS/MS methodology was implemented in accordance with the international guidelines [37] and the previous works developed by our group [1, 38]. The evaluation of the selectivity, linearity and range, instrument and method limits of detection and quantification, accuracy, precision, recovery, and matrix effect, were performed. These results are detailed in the Supplementary Material (Tables E-S6.4 and E-S6.5).

The matrix effect was calculated as the ratio of: (*A*) the peak areas obtained for blank extracts spiked after SPE, subtracting those of the non-spiked blanks and (*B*) the peak areas of the standards solution with a similar concentration as the post-spiked extracts - Equation 1 [1].

$$\text{Matrix effect (\%)} = 100 \times (A/B) \qquad \text{Eq. (1)}$$

The recovery efficiency, i.e., the parameter that supports the selection of the optimum conditions for the SPE procedure, was calculated according to Equation 2.

$$\text{Recovery efficiency (\%)} = 100 \times (C/D) \qquad \text{Eq. (2)}$$

The recovery (%) was calculated as the ratio of: (*C*) the peak areas obtained for extracted spiked sample and (*D*) the peak areas of the post-spiked extracted sample. This approach for evaluating the recovery efficiency allows obtaining only the recovery provided by the adsorbent material, as the matrix effect is considered the same in *C* and *D* [35].

6.2.7. Water samples collection and preparation

SW samples specifically used in the optimization of the SPE method were collected from the Cavalum River located in Penafiel (40 km from Porto, Portugal), a tributary of the Sousa River. SW and DW samples used in the monitoring campaign were collected from 3 regions within the northwest of Portugal (served by 3 different DWTPs), in the period of August to October 2020. Specifically, SW was collected nearby the admission point of each DWTP, while DW was collected at an endpoint of the distribution system served by the corresponding plant. All the water samples were stored at 4 °C in amber glass bottles with 1 L of capacity until extraction (24 h). The samples were filtered through 1.2-µm glass-fiber filters (47 mm GF/C, Whatman™, Maidstone, United Kingdom) and, when needed, the pH was adjusted before the SPE step.

6.3. Results and discussion

6.3.1. Optimization of SPE procedure with pristine CX-cartridges

There are several factors that can affect the efficiency of SPE, namely the mass of sorbent material (mg), sample volume (mL), sample pH, type of solvent and solvent volume (mL). Therefore, a multivariate approach is recommended for this type of optimization involving a large number of parameters. However, some of the parameters mentioned above may not significantly affect the extraction efficiency, so its use in the analysis may be avoided. Thus, a screening study, preceding the optimization step, would be helpful to evaluate the significant factors involved in the analytical sample preparation method. With that goal in mind, a DSD was employed (please see Section 6.2.4.2 and Table E-S6.6), and the results were obtained for the 8 target compounds analysed using Pareto charts (Fig. E-S6.1), which are very useful as they allow ordering

the input factors by significance [39]. Regarding the results obtained, in general, the amount of CX packed in the cartridge (factor A), the volume of sample (factor B) and the sample pH (factor C) were the most determining factors for the recovery of the target compounds. In the specific case of acetamiprid (Fig. E-S6.1a), no factor was significant due to the low recoveries obtained. Overall, the results of this first screening revealed that 3 significant factors (A, B and C) should be considered in the optimization step and the 2 remaining factors should be fixed (i.e., factor D was selected as ethanol and factor E was set to 8 mL of solvent).

BBD was then used (please see Section 6.2.4.2 and Table E-S6.7) to determine the optimum operating conditions upon visual analysis of the corresponding response surfaces and contour plots (an example is shown in Fig. E-S6.2 for metaflumizone). To obtain the surfaces, 1 variable is always fixed. The results revealed that the regions of maximum responses for all target compounds correspond to a mass around 50 mg of CX sample (factor A), 1000 mL of water sample (factor B) and natural sample pH (factor C). These values were thus fixed and used in subsequent SPE tests.

6.3.2. Textural and surface chemistry characterization of CXs

Figs. 6.1a and b show the respective CO_2 and CO TPD spectra obtained with the hydrothermally treated CXs (HNO_3 concentration in the range 0.01 – 0.30 mol L^{-1}). The TPD profiles of the pristine CX material (original) and CXs after hydrothermal treatment with water (i.e., $[HNO_3]$ = 0 mol L^{-1}) are also shown for comparison purposes. The total amount of surface groups released as CO_2 and CO, and the corresponding oxygen content are given in Table 6.1.

Pristine CXs and the CXs treated with water ($[HNO_3]$ = 0) have low amounts of oxygen surface groups (ca. 2 wt.%), as shown in Table 6.1. The increase in the concentration

of HNO_3 (i.e., the oxidizing agent) leads to a rise in the amounts of CO_2 and CO released by TPD (Figs 6.1a and b, and Table 6.1) and, as a consequence, the oxygen contents also increase (Table 6.1). This trend proves that CXs are appropriate carbon materials for the incorporation of oxygenated functional groups through hydrothermal oxidation with HNO_3 under mild conditions. Moreover, the $[CO]/[CO_2]$ ratio is above 1 for all the CX samples (Table 6.1). Thus, the surface groups released as CO are dominant over those released as CO_2.

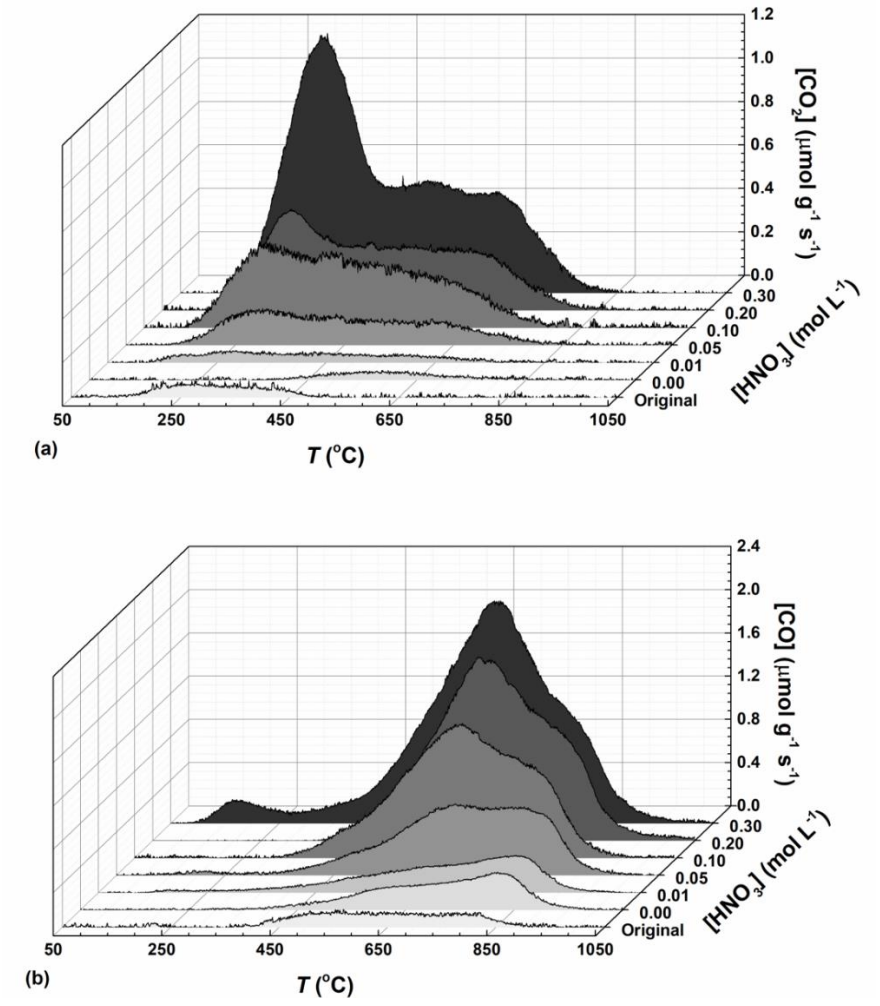

Fig. 6.1. TPD spectra of CXs subjected to hydrothermal treatment with different HNO_3 concentrations: **(a)** CO_2 and **(b)** CO evolution with temperature.

Table 6.1 Properties of the pristine CX material and CXs subjected to hydrothermal treatment with different HNO_3 concentrations: amounts of CO_2 and CO released by TPD, [CO/CO_2] ratio, percentage of oxygen obtained from the analysis of the TPD spectra (assuming that all the surface oxygen is released as CO and/or CO_2), amount of volatiles (determined by TGA), pH at the point of zero charge (pH_{PZC}), specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}) and total pore volume (V_{total}).

[HNO_3] (mol L^{-1})	**Parameters**								
	[CO_2] (µmol g^{-1})	**[CO] (µmol g^{-1})**	**O (wt.%)**	**[CO]/[CO_2]**	**Volatiles (wt.%)**	**pH_{PZC}**	**S_{BET} (m^2 g^{-1})**	**S_{meso} (m^2 g^{-1})**	**V_{total} (cm^3 g^{-1})**
(pristine CX)	174	626	1.6	--	2.32	7.4	699	256	1.267
0 (Blank)	112	1173	2.2	--	3.27	6.9	672	249	1.205
0.01	247	1326	2.9	5.4	4.80	6.6	637	246	1.225
0.05	770	2718	6.8	3.5	9.15	3.9	629	242	1.199
0.10	1752	4359	12.6	2.5	14.0	< 2	716	249	1.247
0.20	1965	5357	14.9	2.7	20.3	< 2	810	268	1.356
0.30	3920	7097	23.9	1.8	25.2	< 2	797	287	1.337

The amount of oxygenated groups released as CO_2 and CO and the contents of oxygen and volatiles (determined by TGA under inert atmosphere; Table 6.1) were correlated with the concentration of HNO_3 used in the hydrothermal treatment of CX. As observed, single exponential functions can correlate the evolution of all parameters under study as a function of the HNO_3 concentration (Figs. 6.2a and b). The correlations found are helpful to tune the amount of oxygenated surface groups introduced, i.e. by fixing the appropriate concentration of HNO_3 in the hydrothermal treatment. These results are in agreement with our previous studies on hydrothermally treated CXs [21], MWCNTs [40] and single-walled CNTs [41].

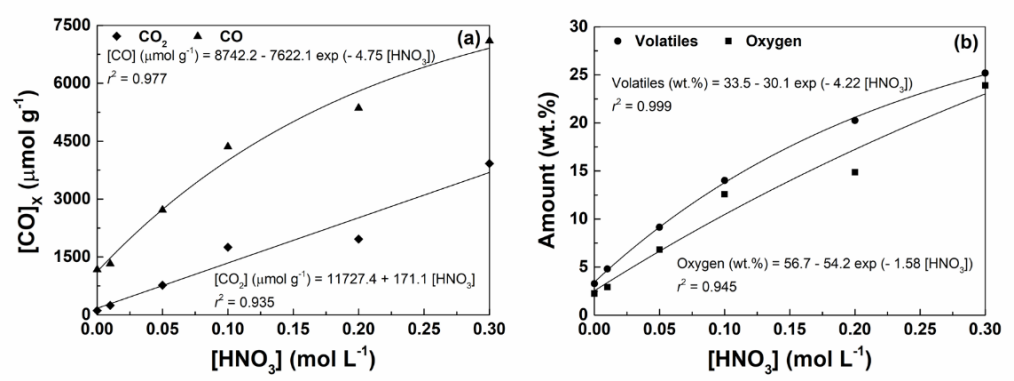

Fig. 6.2. (a) Amounts of CO_2 and CO released by TPD, and **(b)** contents of volatiles and oxygen as a function of the concentration of HNO_3 employed in the hydrothermal treatment of CXs. Points represent experimental data, while lines represent non-linear fittings.

When the total amounts of CO_2 and CO are normalized by the S_{BET} (i.e., $([CO_2]+[CO])/S_{BET}$), and represented as a function of HNO_3 concentration (Fig. 6.3), a good correlation is also obtained.

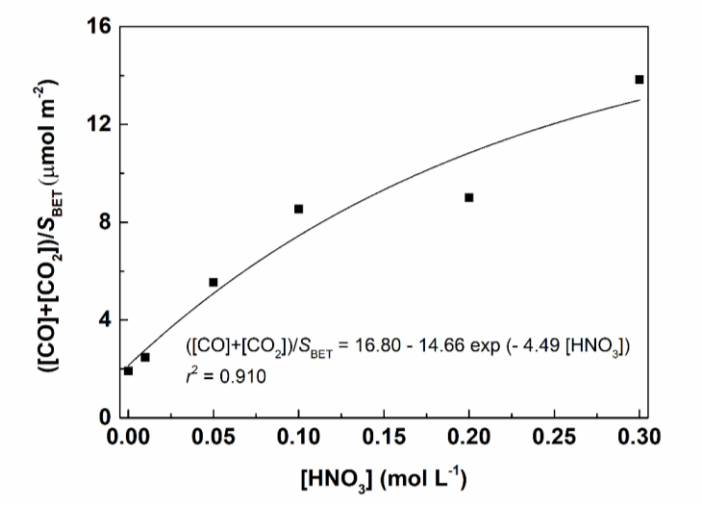

Fig. 6.3. ([CO_2] + [CO])/S_{BET} as a function of HNO_3 concentration.

The effect of the hydrothermal treatment on the overall surface charge of the resulting samples was studied by pH_{PZC} measurements (Table 6.1). The results revealed the markedly acidic nature of the CX samples treated with [HNO_3] ≥ 0.10 mol L^{-1}, as inferred from the values of pH_{PZC} < 2.

Regarding the textural properties of the CX materials, the effect of the hydrothermal treatment was evaluated through N_2 adsorption-desorption isotherms. In general, the surface area (both S_{BET} and S_{meso}) and pore volume (V_{total}) of CXs increase as the concentration of oxidizing agent used in the hydrothermal treatment increases (Table 6.1). The mesoporous character of all these materials can be confirmed by the high rise of adsorption of N_2 at high relative pressures (Fig. E-S6.3).

6.3.3. Application of pristine and functionalized CXs for extraction of EU multi-class OMPs

The pristine and hydrothermally treated CXs were tested as adsorbents for extraction of the 8 target EU-relevant OMPs. The effect of surface chemistry and textural

properties of the CX samples were evaluated in the adsorption/desorption process of these compounds by comparing the recovery efficiencies of the target compounds calculated by using Equation (2) (Section 2.6). The results showed that low recoveries are obtained with the original CX material for most compounds, except for metaflumizone (Fig. E-S6.4). Moreover, it was found that, in general, the HNO_3 hydrothermal treatment has a negative effect on the recovery of the target OMPs (Fig. E-S6.4). Other solvents (ethanolic solutions of 5% NH_4OH or 2% of CH_2O_2) and a second elution step were tested (Fig. E-S6.5) in an attempt to improve the recoveries obtained with the original CX material. However, no improvement was onserved, and the subsequent tests were thus performed with the former conditions (8 mL of an eco-friendly solvent, ethanol).

The PSs and CECs under study belong to different classes and possess different physicochemical properties (Table E-S6.1). Thus, the mechanisms controlling the adsorption/desorption of these compounds on CXs can be distinct. The main interactions comprise: (i) electrostatic interactions due to the charged carbon material surface; (ii) π-π interactions (between bulk π systems existing on the surface of the adsorbent material and the organic molecules with their benzene rings) or C=C double bonds; and (iii) hydrogen bonds with functional groups on the surface of the CX material [42, 43]. It is important to highlight that each of the interactions described above can be affected by the different constituents of environmental matrices. Therefore, performing a mechanistic study on the extraction of multi-class OMPs from real water matrices is a challenge. Nevertheless, the results obtained when employing the pristine and functionalized CXs as SPE sorbents suggest that a less acidic surface (i.e., with a lower amount of surface oxygen-containing groups) favors the adsorption/desorption process of the target OMPs (Fig. E-S6.4). This trend is clearly identified in the specific case of

the pesticide metaflumizone (Fig. E-S6.6) when the recoveries obtained for this CEC are plotted as a function of $([CO_2]+[CO])/S_{BET}$.

Comparing the results obtained in the present study with those obtained with MWCNTs in our previous work [35], it is possible to conclude that the MWCNTs are a more effective option as SPE sorbent for 7 out of the 8 target OMPs. Nevertheless, the CX-cartridges employed in the present study enable a better performance for the extraction of metaflumizone (recovery > 60%; against 2% obtained in the previous study with MWCNTs) [35]. These results prompted the study reported in the following Section, i.e. the application of a multi-layer carbon-based cartridge to extract the 8 target OMPs from water samples.

6.3.4. Multi-layer carbon-based cartridges for determination of EU multi-class OMPs: a proof of concept

In view of the results presented above, we decided to develop a multi-layer carbon-based SPE cartridge with high selectivity/specificity for adsorption/desorption of the 8 target OMPs in water matrices. No studies were found in the literature considering (non-polymeric) carbon materials in a multi-layer configuration inside SPE cartridges. The only three studies addressing multi-layer cartridges to extract OMPs (from water and olive oil) employs commercial materials developed by leading companies in this field: (i) graphitized carbon black (GCB, ENVI-Carb), polymeric weak anion exchanger (Oasis WAX), and polymeric weak cation exchanger (Oasis WCX) [44, 45]; and (ii) zirconia-coated silica and C18 [46]. A compromise between the SPE procedure optimized for CXs in the present work (relevant for metaflumizone) and the procedure optimized in our previous work for MWCNTs (that is not recommended for metaflumizone) was needed to obtain the best possible extraction efficiencies for all

the 8 target compounds. On this basis, the SPE conditions selected for the next tests were 1000 mL of water sample at neutral pH (7.0 ± 0.1).

6.3.4.1. Optimization of the multi-layer configuration

The selected materials (pristine CXs and MWCNTs) were loaded in empty SPE cartridges, varying the multi-layer configuration, namely the type of carbon material in each layer (bottom and top) and load of sorbents (25 and/or 50 mg). The configurations tested and the respective results are shown in Fig. 6.4. As observed, the order of the carbon layers (i.e., CXs or MWCNTs loaded on the bottom or top of the cartridge) has no significant impact on the recoveries obtained. On the other hand, the quantity of sorbent loaded has some influence on the recoveries of the pesticides atrazine and methiocarb, for which a higher amount of carbon material provided a better performance; and metaflumizone, for which the recovery increases around 20% with the lower quantity of sorbent. Similar results were obtained for the other target OMPs regardless of the sorbent load.

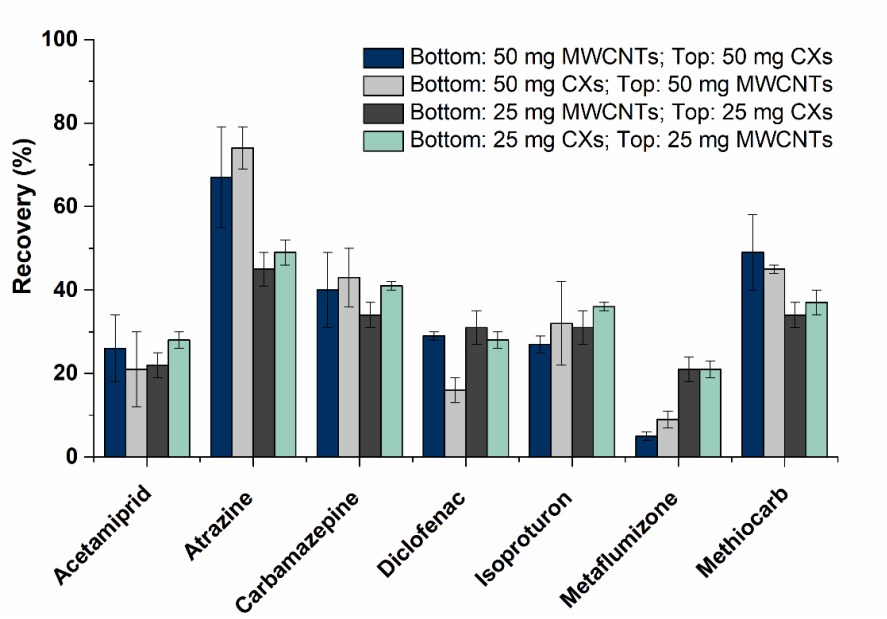

Fig. 6.4. Recovery obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges with different multi-layer configurations: type of carbon material (pristine CXs or MWCNTs) in each layer (bottom or top) and load of sorbents (25 and/or 50 mg). Experiments performed with 1000 mL of sample (SW; pH 7) and using ethanol as solvent (8 mL); *n* = 3 (RSD is represented as error bars).

The recoveries obtained with the best multi-layer carbon-based cartridges, particularly when considering metaflumizone (bottom: 25 mg of CXs; top: 25 mg of MWCNTs), were compared with those previously obtained with CXs and MWCNTs cartridges independently (Fig. E-S6.7). A non-cumulative effect is observed, since the recoveries obtained when the two carbon materials are packed together in the same cartridge are nearly half of those obtained when CXs (in the case of metaflumizone) and MWCNTs cartridges (for the other 7 OMPs) are used independently. Only the multi-layer carbon-based SPE cartridge is able to simultaneously extract the 8 target OMPs in a single procedure. Although the recoveries are low (> 20%), the procedure was proved to be very precise (RSD < 6%). Therefore, the multi-layer carbon cartridge's reusability was

studied, similar recoveries being obtained for the 8 OMPs during three successive cycles (Fig. 6.5). These reusability features are characteristic of carbon-based SPE cartridges [35], representing an advantage when compared to traditional single-use commercial cartridges [1, 47]. Moreover, this multi-layer cartridge is able to extract all the target analytes simultaneously, with a low load of sorbents and an eco-friendly solvent.

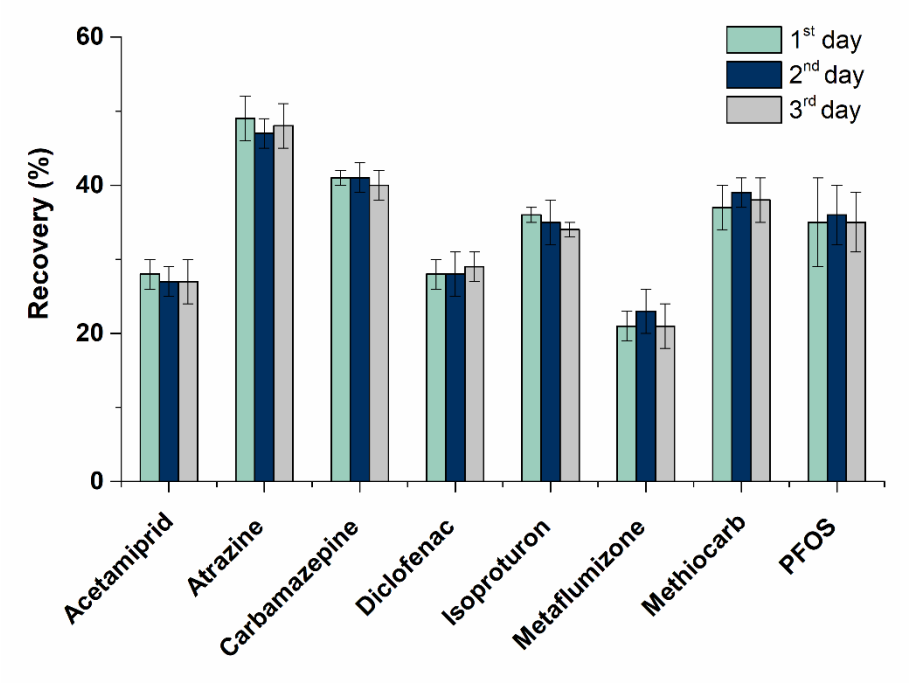

Fig. 6.5. Recoveries obtained for micropollutants (200 ng L^{-1} each), extracting 1000 mL of water (pH 7) with 8 mL of ethanol as solvent, during consecutive reuse cycles performed with the same multi-layer carbon-based cartridge (bottom: 25 mg of CX; top: 25 mg of MWCNT); n = 3 (RSD is represented as error bars).

6.3.4.2. Application in a monitoring campaign focusing on SW and DW

The developed multi-layer carbon-based SPE cartridge was used to concentrate and cleanup water samples prior to the analysis by UHPLC-MS/MS, in order to identify and quantify the target OMPs. A spatial monitoring program of SW collected from different

Portuguese rivers was performed, providing a mapping on the occurrence of PSs and CECs in the environment that persist and might contaminate DW. For this purpose, the SW and DW samples were collected before and after 3 DWTPs, i.e., nearby the admission point of each DWTP and at an endpoint of the distribution system served by that same plant, respectively. The results obtained are given in Table 6.2. As observed, 6 out of the 8 target compounds were detected in the collected water samples: atrazine, carbamazepine, diclofenac, isoproturon, metaflumizone, and methiocarb.

Regarding the target pesticides of this study, the neonicotinoid acetamiprid was not detected. The banned triazine pesticide, atrazine, was quantified in SW-3 and detected below the MQL in SW-1. Isoproturon was quantified in the 3 SW samples analysed (up to 339 ± 29 ng L^{-1}). Metaflumizone was quantified for the first time ever in both SW (SW-2 and SW-3) and DW (DW-2) samples. Methiocarb was found only once in SW-3 (138 ± 1 ng L^{-1}). Moreover, the industrial compound PFOS was not detected in this study. In fact, the target compounds listed as PSs (atrazine, isoproturon and PFOS) were always quantified below their maximum allowable concentration – environmental quality standards (MAC-EQS), as defined in Directive 2013/39/EU for surface water bodies. Additionally, the sum of all the pesticides was below the maximum admissible concentration (i.e., 0.5 µg L^{-1}) for this class of compounds in DW, as defined in Directive 98/83/EC.

The pharmaceutical compounds were detected at the highest concentrations and more frequently in both matrices (SW and DW), possibly due to their high prescription/usage nowadays. Carbamazepine, an anti-epileptic compound reported as recalcitrant in several monitoring studies [48, 50], was quantified up to 83.8 ± 6.6 ng L^{-1} in SW and 15.3 ± 2.4 ng L^{-1} in DW samples. The anti-inflammatory diclofenac was quantified at a high level in SW-1 (949 ± 24 ng L^{-1}). This broadly consumed pharmaceutical was withdrawn from the most recent Watch List (EU Decision 2020/1161). Nevertheless, it

is one of the compounds most frequently found and at highest concentrations in water bodies, being found even in DW samples [48, 53]. These results suggest that neither the natural mechanisms occurring along the river course (i.e., photodegradation, biodegradation, among others), nor the dilution factor are sufficient to completely eliminate these analytes that are released into the environment.

Summarizing, the results obtained allow concluding about the feasibility of employing this novel multi-layer carbon-based cartridge in a SPE-UHPLC–MS/MS method for monitoring PSs and CECs in SW and DW.

Table 6.2 Concentrations (ng L^{-1}) of target micropollutants found in SW and DW near to the DWTP 1, 2 and 3; [a] n.d. is not detected; [b] MQL is method quantification limit.

Analyte	Concentration (ng L^{-1})					
	DWTP-1		DWTP-2		DWTP-3	
	SW-1	DW-1	SW-2	DW-2	SW-3	DW-3
Acetamiprid	n.d. [a]	n.d.	n.d.	n.d.	n.d.	n.d.
Atrazine	< MQL [b]	n.d.	n.d.	n.d.	198 ± 2	n.d.
Carbamazepine	83.8 ± 6.6	15.3 ± 2.4	69.6 ± 16.3	12.6 ± 2.9	68.5 ± 5.8	n.d.
Diclofenac	949 ± 24	31.8 ± 5.3	116 ± 5	n.d.	264 ± 3	n.d.
Isoproturon	168 ± 5	n.d.	131 ± 19	n.d.	339 ± 29	n.d.
Metaflumizone	n.d.	n.d.	159 ± 33	15.5 ± 0.4	72.9 ± 3.9	n.d.
Methiocarb	n.d.	n.d.	n.d.	n.d.	138 ± 1	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

6.4. Conclusions

Pristine and HNO_3 hydrothermally treated CXs were synthesized and used as SPE sorbents for the extraction of 8 multi-class OMPs from water matrices. The introduction of oxygenated functional groups by oxidation of the CX surface negatively affected the recoveries obtained for the target analytes, indicating that the adsorption/desorption

process is more efficient on a less acidic surface. Overall, the recoveries obtained with pristine and functionalized CXs were low, except for metaflumizone (> 60%). Taking this into consideration, multi-layer carbon cartridges with pristine CXs and MWCNTs were tested as a proof of concept in the present work. The optimized cartridge configuration was able to extract the 8 target OMPs (with different p*K*a and polarity range) at once, using an eco-friendly solvent and low load of sorbents. Moreover, this cartridge can be reused at least three times without affecting the extraction efficiency. Therefore, an analytical methodology based on SPE-UHPLC–MS/MS was then validated using the innovative multi-layer carbon-based cartridge. The potential of this method for monitoring EU-relevant OMPs was demonstrated through a monitoring campaign focusing on SW and DW samples collected before and after DWTPs, which confirmed the occurrence of a wide range of OMPs (at ng L^{-1} levels). Among the OMPs quantified (6 in total), the most commonly found were carbamazepine and diclofenac. Metaflumizone, a CEC recently added to the Watch List in EU Decision 2020/1161, was quantified in water courses for the first time, highlighting the importance of including this new CEC in future monitoring programs.

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Supplementary material

(Please see Appendix E)

Table E-S6.1. Target compounds, class, structure, relative molecular mass (M_r), p*K*a, and log K_{OW} values and solubility in water.

Table E-S6.2. Selected variables and respective levels investigated in the definitive screening design (DSD) and Box-Benkhen design (BBD).

Table E-S6.3. Selected reaction monitoring (SRM) instrument parameters for tandem mass spectrometry analysis of target analytes.

Table E-S6.4. Retention time, range, linearity, instrument and method detection, and quantification limits for each target analyte.

Table E-S6.5. Recovery, accuracy, precision (intra- and inter-batch), and matrix effect for each target analyte.

Table E-S6.6. Design of trial runs (in coded form) for DSD and corresponding recovery values for the 8 target compounds. All the experiments were performed with pristine CX samples.

Table E-S6.7. Design of trial runs (in coded form) for BBD and corresponding recovery values for the 8 target compounds. All the experiments were performed with pristine CX samples.

Fig. E-S6.1. Pareto charts representation of the standardized effects originated by the main factors (A, B, C, D and E) for each target compound; response is recovery (%); α = 0.05. All the experiments were performed with pristine CX samples.

Fig. E-S6.2. Response surface and contour plots showing the effect of the mass of sorbent material (mg) (factor A), sample volume (mL) (factor B), and sample pH (factor C) on the recovery of metaflumizone: **(a)** Response surface and contour plots of recovery as a function of factors A and B (hold value – C = 7); **(b)** Response surface and contour plots of recovery as a function of factors A and C (hold value – B = 750 mL); **(c)** Response surface and contour plots of recovery as a function of factors B and C (hold value – A= 100 mg). All the experiments were performed with pristine CX samples.

Fig. E-S6.3. N_2 adsorption-desorption isotherms at -196 ^oC of CXs subjected to hydrothermal treatment with different HNO_3 concentrations: the subscript number in CX_0, $CX_{0.01}$, and $CX_{0.05}$ corresponds to the concentration of HNO_3 (mol L^{-1}).

Fig. E-S6.4. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with carbon xerogel (CX; 50 mg) obtained after hydrothermal treatment with different HNO_3 concentrations (0-0.30 mol L^{-1}). Experiments performed with 1000 mL of surface water (SW; pH 7) and using ethanol as solvent (8 mL); *n* = 3 (RSD is represented as error bars).

Fig. E-S6.5. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with CXs (50 mg) with different solvents (ethanol + 5% of NH_4OH, ethanol + 2% of CH_2O_2, and ethanol) and two elution steps (8 + 8 mL). Experiments performed with 1000 mL of SW (pH 7); n = 3 (RSD is represented as error bars).

Fig. E-S6.6. Recovery obtained for metaflumizone as a function of ($[CO_2]$ + $[CO]$)/S_{BET}.

Fig. E-S6.7. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with different carbon materials: multi-walled carbon nanotubes (MWCNTs), CXs and multi-layer (bottom: 25 mg of CXs; top: 25 mg of MWCNTs). Experiments performed with the optimized procedures for each type of sorbent; n = 3 (RSD is represented as error bars).

Chapter 7

Final Conclusions and Perspectives

7.1. Final conclusions

The outcome knowledge obtained during this PhD project has allowed to reach the proposed objectives that were based on groundbreaking and innovative features in two different areas of research, namely analytical chemistry and materials chemistry, and their combination in a single research topic of major international interest: environmental pollution and policy. Specifically, this project was focused on the development of analytical methodologies based on SPE-UHPLC-MS/MS for the simultaneous analysis of PSs and CECs in water matrices by using an innovative SPE cartridge layer-by-layer assembled with different carbon materials. The most relevant conclusions of the works described in this thesis are summarized below.

In order to build knowledge on the occurrence and removal of OMPs in DW, an eco-friendly multiresidue analytical method based on SPE-UHPLC-MS/MS was developed and validated for the analysis of 21 micropollutants (*Chapter 3*). The proposed method was successfully applied to tap, fountain, and well water samples from different locations of northwest Portugal, showing the occurrence of 13 out of 21 target OMPs. Additionally, the hazard quotients were estimated for the quantified micropollutants and suggested no adverse effects to humans.

In a subsequent work (*Chapter 4*), spatial and seasonal variations of a wide range of multi-class PSs and CECs were studied in Portuguese SW samples, and 26 out of 39 target micropollutants were found at least in one of the 4 stressed selected rivers. As expected, the results indicate that the occurrence and distribution of the OMPs along rivers are largely dependent on location and seasonal variations. This study was the first to assess the spatiotemporal distribution of EU-relevant micropollutants and the fluorescence EEMs, as well as the correlations between these water quality parameters. The fluorescence EEMs of SW matched the distribution of

micropollutants, indicating that preliminary EEM analysis may help to inform the design of future water courses monitoring campaigns.

The lack of a reusable, eco-friendly, and low-cost analytical tool to simultaneously determine multi-class organic PSs and CECs defined in recently launched EU regulation has driven the study of carbon materials as SPE sorbents for pre-concentration of these type of micropollutants. Thus, in the subsequent research work (*Chapter 5*), pristine and HNO_3 hydrothermal treated MWCNTs were studied for the extraction of 8 selected OMPs in SW samples before UHPLC-MS/MS analysis. Recoveries higher than 60% were achieved for 5 out of 8 target micropollutants. However, in the specific case of metaflumizone, the recovery obtained with this type of carbon material is negligible. The SPE procedure optimized with pristine MWCNTs presented great advantages when compared with the commercial cartridge, namely the cost reduction (~ 75%) and the capacity to be reused at least three times without substantial effect on the recovery values. The oxygen functional groups improved the recoveries of some micropollutants, proving that the fine tune of the surface chemistry and texture of MWCNTs unlocks an opportunity for the progress of more efficient and environmental friendly SPE procedures to analyse PSs and CECs in water.

Finally, pristine and oxidized CXs were produced and used as SPE sorbents in single and multi-layer cartridges (*Chapter 6*). In order to achieve satisfactory recoveries for the 8 target OMPs, a novel multi-layer carbon cartridge with pristine CXs and MWCNTs were tested as a proof of concept. The optimized multi-layer carbon cartridge presented several strengths: (i) the ability to simultaneously extract the 8 target OMPs (with different p*K*a and polarity range); (ii) the use of an eco-friendly solvent; (iii) the low amount of sorbents required; and (iv) the possibility to be reused at least three times. These cartridges were applied to a SPE-UHPLC-MS/MS methodology for monitoring the 8 PSs and CECs in SW and DW samples collected

before and after DWTPs and the results revealed that 6 compounds were quantified. Carbamazepine and diclofenac were the most frequent micropollutants and metaflumizone, a CEC recently listed in the 3rd WL, was quantified in aquatic compartments for the first time.

Although some of the compounds are already defined in the EU legislation, more monitoring programs are needed for further prioritization and risk assessment of such micropollutants. There is still a lot of missing information about many aspects of this environmental issue, particularly about the occurrence and fate in the aquatic compartments of this type of contaminants, and contamination prevention plans to guide the development of new and restrict legislation including them. Accurate and high quality data obtained in monitoring programmes is thus needed, which in turn have to be based upon well developed and validated analytical methodologies.

The results of this PhD thesis may contribute to this end by increasing the information available on the occurrence and fate of OMPs in water and providing a single analytical method for Union-wide monitoring of SW pollution affecting EU and world citizens.

7.2. Perspectives

The analytical methods developed and validated in this research work can be helpful in further studies concerning the water monitoring of the target OMPs. Particularly, the validated SPE-UHPLC-MS/MS method with the multi-layer carbon cartridge was established for future monitoring campaigns to provide a national mapping on the occurrence of PSs and CECs in the environment that persist and might even reach DW, assessing the performance of DWTPs.

For instance, a 1-year monitoring plan of some DWTPs can be now performed to quantify PSs and CECs at the different stages (influents, after each treatment step, and effluents), allowing to evaluate the performance of such DWTPs and the respective treatments applied (e.g., adsorption, coagulation, ozonation, UV, chlorination).

Studies on identification of degradation products are crucial for proper assessment of the environmental impact. Thus, efforts should be made to identify and include these by-products in the developed SPE-UHPLC-MS/MS method.

Since additional pollutants are continuously identified and there is a continuous updating of the WL for water monitoring, a number of other carbon materials (e.g., carbon nanofibers, graphene-derivates, nanodiamonds) with controllable texture and surface chemistry might be produced and studied for the development of new multi-layer cartridges with affinity/selectivity to extract the emerging compounds. Moreover, the so-called “cocktail effect” in aquatic environments is much more complex, i.e., single substances that are individually present at inoffensive concentrations in the case of mixtures may additively or synergistically pose a risk to ecosystems and human health. Moreover, the pollutants entering SW can interact with natural mineral salts and organic compounds, as well as with nutrients from WW, sewage, and

agricultural run-off. Therefore, further measures are needed to tackle the presence of “cocktail effect” substances in European water bodies. Bearing this in mind, an interesting approach for the multi-layer carbon-based cartridges would be to extend the study to extract other types of water contaminants and improve the overall recoveries.

In conclusion, the methodologies must be aligned, the strategies must be established, and several efforts must be pooled, in order to accomplish the mutual goal of aquatic environmental pollution control.

Appendices

Appendix A

Original version of Part A, Chapter 2:

Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/49

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Review

Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495

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ABSTRACT

Although there are no legal discharge limits for micropollutants into the environment, some regulations have been published in the last few years. Recently, a watch list of substances for European Union-wide monitoring was reported in the Decision 2015/495/EU of 20 March 2015. Besides the substances previously recommended to be included by the Directive 39/2013/EU, namely two pharmaceuticals (diclofenac and the synthetic hormone 17-alpha-ethinylestradiol (EE2)) and a natural hormone (17-beta-estradiol (E2)), the first watch list of 10 substances/groups of substances also refers three macrolide antibiotics (azithromycin, clarithromycin and erythromycin), other natural hormone (estrone (E1)), some pesticides (methiocarb, oxadiazon, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid and triallate), a UV filter (2-ethylhexyl-4-methoxycinnamate) and an antioxidant (2,6-di-tert-butyl-4-methylphenol) commonly used as food additive. Since little is known about the removal of most of the substances included in the Decision 2015/495/EU, particularly regarding realistic concentrations in aqueous environmental samples, this review aims to: (i) overview the European policy in the water field; (ii) briefly describe the most commonly used conventional and advanced treatment processes to remove micropollutants; (iii) summarize the relevant data published in the last decade, regarding occurrence and removal in aqueous matrices of the 10 substances/groups of substances that were recently included in the first watch list for European Union monitoring (Decision 2015/495/EU); and (iv) highlight the lack of reports concerning some substances of the watch list, the study of un-spiked aquatic matrices and the assessment of transformation by-products.

Contents

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1. Introduction

Water is a valuable resource, crucial to all living organisms and for multiple human activities, such as domestic uses, agriculture and industry. However, several contaminants of emerging concern (CECs) end up in vital aquatic compartments, such as surface water, groundwater and even drinking water, at concentrations between few ng L^{-1} and several μg L^{-1} (Matamoros and Bayona, 2006), with negative impact on water quality. The occurrence of CECs in the environment is reported in thousands of publications during the last decades and reviewed by many authors (Bell et al., 2011; Bu et al., 2013; da Silva et al., 2012; da Silva et al., 2013; Li et al., 2015; Li, 2014; Liu and Wong, 2013; Sima et al., 2014; Zhang et al., 2014a), demonstrating an increasing concern about them. For instance, a series of periodic review articles focused on occurrence, fate, transport and treatment of CECs were published annually since 2007 (Wells et al., 2007) until 2011 (Bell et al., 2011), and then works on occurrence, fate and transport of CECs were reviewed separated from treatment since 2012 (da Silva et al., 2012) until 2015 (Li et al., 2015), due to the significant increase in the number of publications dealing with this particular topic.

CECs can be natural or anthropogenic substances such as pesticides, industrial compounds, pharmaceuticals, personal care products, steroid hormones, drugs of abuse and others (Ribeiro et al., 2015). Sources of CECs include: (i) industrial wastewater; (ii) runoff from agriculture, livestock and aquaculture; (iii) landfill leachates; and (iv) domestic and hospital effluents, from which micropollutants might follow many pathways (Mompelat et al., 2009), as represented in Fig. 1.

The management of industrial effluents resulting from the production of pharmaceuticals, personal care products, pesticides and other compounds, has been properly done in several countries where regulations are already implemented, but more strict regulations are still needed in other regions of the world. The runoff from agriculture and livestock areas is another important source of micropollutants, particularly in the case of pesticides used to improve productivity, as well as steroid hormones and antibiotics used for livestock (Birkett and Lester, 2002; Song et al., 2007). In addition, many contaminants and their intermediates can reach the fields when they are irrigated with treated wastewater and, as consequence, the receiving waters can also contain these substances (Pedersen et al., 2003). Other source of CECs is the leakage from landfills and sewage treatment facilities, industrial waste systems and septic tanks (Matthiessen et al., 2006). The release of effluents from municipal wastewater treatment plants (WWTPs) is other important route for the appearance of micropollutants in the aquatic environment (Tijani et al., 2013), the wastewater treated in these plants mainly resulting from domestic and/or industrial activities, as well as from hospitals.

In fact, most of the conventional WWTPs are not designed to completely eliminate organic compounds at low concentrations, making the treatment processes vulnerable to such problem of pollution (Tijani et al., 2013). In this context, the non-degradable or partially removed compounds in WWTPs are likely to be detected in surface water. In the cases of sewage sludge and soils, micropollutants can desorb and runoff to surface water or undergo direct leaching to groundwater aquifers with consequent contamination of drinking water (Feng et al., 2013).

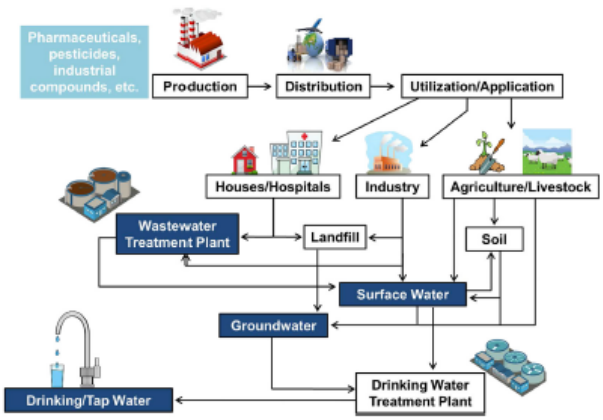


Fig. 1. Representative sources and routes of micropollutants in the environment.

Appendix A

Agricultural reuse of sewage sludge in particular as fertilizer, is a common practice to improve the soil structure and provide nutrients, but can represent a source of environmental contamination (Dichtl et al., 2007). Moreover, sewage sludge solids sourced by wastewater can be considered a sink of hazardous substances (e.g., such as pathogens, heavy metals and organic pollutants) that will accumulate in soils (Dichtl et al., 2007). Due to the increasing concern about human health impacts, land application gained interest to convert sludge into a safer material through the treatment by anaerobic digestion, composting or other biological processes (Zhang et al., 2014b). While composting is a controlled bio-oxidative process that converts sludge into stable and humic like materials, anaerobic digestion occurs in the absence of oxygen and has two main end products, a methane-rich biogas used as renewable energy source and the digested used as fertilizer (Zhang et al., 2014b). Removal of toxic organic contaminants by these processes was reported; however, their complete mineralization is difficult due to the adsorption mechanism and the formation of intermediates (Zhang et al., 2014b).

The fate and distribution of CECs will depend on the D_{ow}, which is a pH-dependent n-octanol–water distribution ratio that simultaneously considers hydrophobicity and ionogenicity (Wells, 2006, 2007). Although most regulators use octanol–water partitioning coefficient (K_{ow}) to evaluate the hydrophobic partitioning, the environmental fate and transport should be based in the parameter D_{ow}, which is more accurate for ionizable organic compounds.

The contamination of environmental compartments, such as surface water, groundwater and soils, which are continuously interrelated, may cause cumulative negative effects along multi-generational exposure in aquatic organisms and/or affect the human's health by drinking water contamination (Daughton, 2010). A great concern about the occurrence of micropollutants in the aquatic resources and the subsequent effects on humans and biota has been highlighted in the last few years. However, it is difficult to predict which environmental and public health implications may arise from the occurrence of CECs in freshwater ecosystems, since the individual concentrations usually found in the environment are lower than those able to cause direct negative effects (Quinn et al., 2009). For instance, concerning pharmaceuticals, toxicological studies have shown that they might have direct toxicity towards certain aquatic organisms (Crane et al., 2006). The main issues related to the frequent occurrence of recalcitrant compounds are their simultaneous presence as complex mixtures and the long term exposition that can lead to serious chronic effects, as reported by several studies (Kidd et al., 2007; Santos et al., 2010). Their constant but imperceptible effects can gradually accumulate, finally leading to irreversible changes on both wildlife and human beings (Daughton and Ternes, 1999; Jjemba, 2006).

Natural attenuation is a low-cost and simple process comprising physical, chemical and/or biological mechanisms to reduce contaminants concentrations (Khan et al., 2004; Kuppusamy et al., 2016). Volatilization, dispersion, dilution, sorption, photolysis, biodegradation/transformation are the main natural attenuation processes (Khan et al., 2004; Kuppusamy et al., 2016). While volatilization has a minor impact, dispersion and dilution can lead to a significant decrease on the concentrations of contaminants (Gurr and Reinhard, 2006). The dilution can decrease their concentration to levels for which no significant effects are verified for aquatic organisms. Sorption to sediments and suspended solids also reduce the concentration of CECs, but accumulation is enhanced. Indirect or direct photolysis can lead to removal of contaminants, but is highly dependent on the presence of suspended matter and solar radiation. CECs can also be degraded by biodegradation/transformation, by bacterial enzymes (Khan et al., 2004).

The upgrading of the treatment processes for effluents generated by conventional WWTPs might minimize the discharge of micropollutants into the receiving waters, and can even improve the overall quality status of effluents for possible reuse (Comninellis et al., 2008; De Luca et al., 2013). The design improvement of WWTPs to include advanced treatment technologies, aiming to transform CECs into less harmful compounds or even to mineralize them, is one of the promising strategies to achieve this aim, as recently implemented in Switzerland. Advanced water treatment processes include adsorption (e.g., granular activated carbon (GAC)), membrane and advanced chemical/oxidation technologies (Sudhakaran et al., 2013). Other option is the implementation of natural systems to depurate water, such as riverbank filtration (RBF), aquifer recharge and recovery (ARR) and constructed wetlands (CWs), which are reviewed in the literature (Li et al., 2014; Petrovic et al., 2009; Zhang et al., 2014a) and will not be discussed in this work.

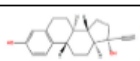
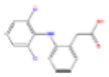
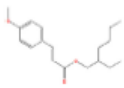
1.1. European policy

Although there are no legal discharge limits for micropollutants, some regulations have been published. The Directive 2000/60/EC was the first mark in the European water policy, which set up a strategy to define high risk substances to be prioritized (Directive, 2000). A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by the Directive 2008/105/EC (Directive, 2008). Two years ago, the European Union Directive 2013/39/EU recommended attention to the monitorization and treatment options for a group of 45 PSs (Directive, 2013), meeting the protection of the aquatic compartments and the human health. In that Directive, two pharmaceuticals (the non-steroid anti-inflammatory diclofenac and the synthetic hormone 17-alpha-ethinylestradiol – EE2) and a natural hormone (17-beta-estradiol – E2) were recommended to be included in a first watch list of 10 substances/groups of substances for European Union monitoring, to be launched within two years. In the first quarter of 2015, the watch list of substances for European Union-wide monitoring (as set out in Article 8b of Directive 2008/105/EC) was amended in the Decision 2015/495/EU of 20 March 2015. Besides the abovementioned substances (diclofenac, EE2 and E2), three macrolide antibiotics (azithromycin, clarithromycin and erythromycin) were included, together with other natural hormone (estrone – E1), some pesticides, a UV filter and an antioxidant commonly used as food additive, listed in Table 1. The frequent occurrence of CECs in the environment and the inefficiency of conventional WWTPs to remove such compounds, promoted the amendment of the framework to cover a larger set of hazardous compounds, as well as further recommendations for wastewater treatment steps or even new treatment scenarios. This actions should be implemented by the European Commission and regulated by the European country authorities.

This review aims to summarize some relevant data of occurrence and removal in aqueous matrices of the 10 substances/groups of substances (i.e., a total of 17 organic compounds) enlisted in the first watch list for European Union monitoring, defined in the Decision 2015/495/EU. Studies on the occurrence of the referred substances (3 estrogens, diclofenac, 2,6-di-tert-butyl-4-methylphenol, 2-ethylhexyl-4-methoxycinnamate, 3 macrolide antibiotics, methiocarb, 5 neonicotinoids, oxadiazon and triallate) are shown in Table 1, for different aquatic compartments, namely wastewater, surface water and groundwater. Reports dealing with the removal of these 17 substances, only in real matrices, are overviewed below. The search comprised publications since 2005 (last decade) in Scopus database, using as keywords each substance and the treatments herein reported. Most of the works refer to unspiked aqueous environmental samples treated at lab-, pilot- or

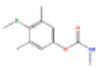
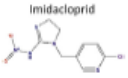
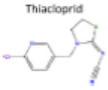
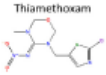
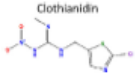
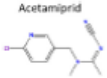
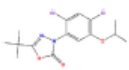
Appendix A

Table 1
List of 10 substances/groups of substances (total of 17 organic compounds) included in the watch list of EU Commission Decision 495/2015, and their occurrence in different aquatic compartments, namely effluents of wastewater (WW), surface water (SW), and groundwater (GW). *n.a. refers to not available data.

Name of substance/ group of substances	CAS number	(Substance) structure	Concentration (ng L^{-1}) Matrix Locations (number of samples)	Reference
17-Alpha-ethinylestradiol (EE2)	57-63-6		<1–8 WW Korea (n = 120), Germany (n.a.), South Africa (n = 12)	(Behera et al., 2011; Bolong et al., 2009; Manickum and John, 2014)
			0.2–1.9 SW China (n = 3), Korea (n = 120), Germany (n.a.), France (n = 73)	(Bolong et al., 2009; Luo et al., 2014; Vulliet and Cren-Olivé, 2011)
			0.5–230 GW France (n = 73), USA (n.a.)	(Luo et al., 2014; Vulliet et al., 2008; Vulliet and Cren-Olivé, 2011)
17-Beta-estradiol (E2) Estrone (E1)	50-28-2		<1–88 WW China (n = 3), Korea (n = 120), Sweden (n = 3), UK (n.a.), Germany (n.a.)	(Behera et al., 2011; Bolong et al., 2009; Nie et al., 2012; Zorita et al., 2009)
			0.2–10.1 SW China (n = 3), Korea (n = 120), Germany (n.a.), Japan (n = 517), France (n = 71)	(Bolong et al., 2009; Luo et al., 2014; Vulliet and Cren-Olivé, 2011)
			0.3–147 GW France (n = 73), USA (n.a.)	(Luo et al., 2014; Vulliet et al., 2008; Vulliet and Cren-Olivé, 2011)
	53-16-7		<1–220 WW China (n = 3), Korea (n = 120), Sweden (n = 3), UK (n.a.), Germany (n.a.)	(Behera et al., 2011; Bolong et al., 2009; Nie et al., 2012; Zorita et al., 2009)
			0.5–69.1 SW China (n = 3), Korea (n = 120), Germany (n.a.), France (n = 71)	(Bolong et al., 2009; Luo et al., 2014; Vulliet and Cren-Olivé, 2011)
			0.7–79 GW France (n = 73), USA (n.a.)	(Luo et al., 2014; Vulliet et al., 2008; Vulliet and Cren-Olivé, 2011)
Diclofenac	15307-86-5		14.9–4425 WW Spain (n.a.), Italy (n = 3), USA (n.a.), Portugal (n = 4)	(Al Aukidy et al., 2012; Lara-Martin et al., 2014; Pereira et al., 2015; Prieto-Rodriguez et al., 2012)
			0.8–1043 SW Spain (n.a.), Vietnam (n.a), Costa Rica (n = 86), Greece (n = 30)	(Li, 2014; Spongberg et al., 2011; Stasinakis et al., 2012)
			1.17–380 GW Spain (n = 30), France (n = 70)	(Li, 2014; Lopez-Serna et al., 2013; Luo et al., 2014)
2,6-di-tert-butyl-4-methylphenol	128-37-0		49–620 SW USA (n = 19), Sweden (n.a.)	(Bendz et al., 2005; Benotti et al., 2009)
2-ethylhexyl-4-methoxycinnamate	5466-77-3		4.7–505 WW China (n = 17), Norway (n = 5)	(Langford et al., 2015; Li et al., 2007; Tsui et al., 2014)
			12–1040 SW Japan (n = 23)	(Amine et al., 2012)
			770 GW Spain (n = 7)	(Díaz-Cruz et al., 2012)

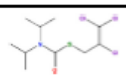
Appendix A

Table 1 (continued)

Name of substance/ group of substances	CAS number	(Substance) structure	Concentration (ng L^{-1}) Matrix Locations (number of samples)	Reference
Macrolide antibiotics	83905-01-5	Azithromycin 	0.4–1220 WW Italy (n = 3), Slovakia (n = 3), USA (n.a.), Portugal (n = 4)	(Al Aukidy et al., 2012; Birosova et al., 2014; Gibs et al., 2013; Pereira et al., 2015)
			0.6–90.8 SW Vietnam (n = 2), China (n = 24)	(Hoa et al., 2011; Tong et al., 2014)
			0.6–1620 GW Spain (n.a.), China (n = 69)	(Lopez-Serna et al., 2013; Ma et al., 2015; Tong et al., 2014)
	81103-11-9	Clarithromycin 	54–1890 WW Spain (n.a.), Italy (n = 3), Slovakia (n = 3), USA (n.a.)	(Al Aukidy et al., 2012; Birosova et al., 2014; Lara-Martin et al., 2014; Prieto-Rodriguez et al., 2012)
			0.01–778 SW Vietnam (n = 2), Spain (n = 18), China (n = 24)	(Gracia-Lor et al., 2011; Hoa et al., 2011; Tong et al., 2014)
			0.2–20.5 GW Spain (n.a.), China (n = 15)	(Lopez-Serna et al., 2013; Tong et al., 2014)
	114-07-8	Erythromycin 	16–147.9 WW Spain (n.a.), Slovakia (n = 3), USA (n.a.), China (n = 3)	(Birosova et al., 2014; Gibs et al., 2013; Prieto-Rodriguez et al., 2012; Yan et al., 2014b)
			0.28–2246 SW Vietnam (n = 2), Spain (n = 18)	(Gracia-Lor et al., 2011; Hoa et al., 2011)
			4.8–154.3 GW Spain (n = 121), China (n = 54)	(Cabeza et al., 2012; Lopez-Serna et al., 2013; Ma et al., 2015)
Methiocarb	2032-65-7		4.73–14.92 WW Spain (n = 55)	(Campo et al., 2013; Masiá et al., 2013)
Neonicotinoids	105827-78-9	Imidacloprid 	2–34.44 WW Spain (n = 55)	(Campo et al., 2013; Masiá et al., 2013)
	138261-41-3		1.1–105 SW Spain (n = 24), USA (n = 35), Greece (n = 89), Portugal (n.a.), Australia (n = 13)	(Gonzalez-Rey et al., 2015; Masiá et al., 2013; Papadakis et al., 2015; Sánchez-Bayo and Hyne, 2014)
	111988-49-9	Thiacloprid 	20–400 SW Australia (n = 13)	(Sánchez-Bayo and Hyne, 2014)
	153719-23-4	Thiamethoxam 	40–1580 SW Brasil (n.a.), Vietnam (n = 11), Australia (n = 13)	(Chau et al., 2015; da Rocha et al., 2015; Sánchez-Bayo and Hyne, 2014)
	210880-92-5	Clothianidin 	20–420 SW Australia (n = 13)	(Sánchez-Bayo and Hyne, 2014)
	135410-20-7 160430-64-8	Acetamiprid 	20–380 SW Australia (n = 13)	(Sánchez-Bayo and Hyne, 2014)
Oxadiazon	19666-30-9		4–1440 SW Canada (n = 8)	(Furtula et al., 2006)

(continued on next page)

Table 1 (*continued*)

Name of substance/ group of substances	CAS number	(Substance) structure	Concentration (ng L^{-1}) Matrix Locations (number of samples)	Reference
Triallate	2303-17-5		n.a.	n.a.

full-scale, some describing the removal of these substances on spiked environmental matrices, and some including the comparison between the real matrix and ultrapure/deionized water. The first step of sample preparation is usually the filtration of the samples, and the works on occurrence take into account this step in the sample preparation protocol of the analytical method. Before such literature overview, the next sections (1.2, 1.3 and 1.4) present a brief description of the most commonly used conventional and advanced treatment processes.

1.2. Treatment by conventional processes

The efficiency of a conventional WWTP varies depending on the characteristics of the pollutant and on the treatment process employed. The main mechanisms for removal of micropollutants occurring during the secondary treatment at WWTPs are biological and/or chemical transformation and sorption (Radjenovic et al., 2009; Verlicchi et al., 2012). The most common employed processes are conventional activated sludge (CAS) and membrane biological reactors (MBRs).

The efficiency of a CAS system depends on the physicochemical characteristics of the substances and on the nature of the microbial community. The most important operational factors affecting the efficiency are the temperature, the hydraulic retention time (HRT) and the sludge retention time (SRT) (Oulton et al., 2010; Petrovic et al., 2009), a higher HRT favoring the removal of more refractory compounds and a higher SRT allowing a higher diversity of microorganisms (Petrovic et al., 2009). The usual SRT in the CAS systems is 7–20 days and the biomass concentration 3–5 kg m^{-3}, with a HRT typically ranging from 2 to 24 h (Verlicchi et al., 2012).

MBRs emerged as an alternative to CAS, integrating aerobic biodegradation and membrane separation, modestly more efficient than CAS in the extent of removal of several CECs (Oulton et al., 2010). MBR treatment differs mainly in the SRT that is normally longer (15–80 days) and the commonly higher biomass concentration (8–10 kg m^{-3}), HRT being often between 7 and 15 h (Verlicchi et al., 2012). Other important difference is the final stage using ultrafiltration (UF) or microfiltration (MF) membranes to separate the liquid from sludge. Therefore, MBR overcome the constraints of CAS treatment related to the sludge retention and settling characteristics, by applying these membranes to retain the biomass (Petrovic et al., 2009), decreasing the chemical oxygen demand while enhancing the removal of suspended solids and pathogens. Unlike the reports related to CAS, studies focusing on the performance of MBR processes to remove CECs are limited and difficult to compare due to the different operation conditions and target pollutants (Kim et al., 2014). Verlicchi et al. (2012) reviewed extensively the occurrence and removal of pharmaceutical compounds in municipal wastewater, comparing the effectiveness of the secondary treatment by CAS and MBR, with much more studies employing CAS and using generally 24 h composite water samples, avoiding diurnal variability and favoring the inter-studies comparison. Pharmaceuticals and hormones that are now included in the watch list of Decision 495/2015 were referred in that review, where it was concluded that average removals found in the literature were superior employing MBR than CAS, namely between 26 and 44% for CAS and higher than 60% for MBR, except for azithromycin (Verlicchi et al., 2012).

1.2.1. Intermediates formation

Overall, most studies on both CAS and MBR have been focusing on the parent compounds and little attention has been given to the produced intermediates. It is noteworthy that biological or chemical reactions occurring in the secondary clarifiers might lead to the accumulation of metabolites/by-products (Oulton et al., 2010). There are also some compounds (e.g., pharmaceuticals, hormones, drugs of abuse that are excreted by humans and/or animals) that can be found at higher concentrations in the WWTPs effluents than in the respective influents, due to their excretion as conjugates that are broken in the WWTPs. These conjugates are generally metabolized during biological treatment and the parent compound is released, often increasing the concentrations of the parent compounds at the outlet of the WWTPs. For example, E1 can be detected in the secondary effluent of a WWTP at a higher concentration than that found in the raw influent, due to the oxidation of E2 that enters into the WWTP. This fact explains the occasional negative removal efficiencies, sometimes at high extents, with the greatest contribution of the biological transformation (Verlicchi et al., 2012). There are other causes for negative removals occurring during the WWTP treatment. In most cases, the sampling protocol does not consider the HRT and/or SRT and as consequence effluent does not correspond to the same plug of influent (Campo et al., 2013). Sometimes the compounds can be released from particulate matter during treatment (e.g. macrolide antibiotics released from feces particles) (Kim et al., 2014). There are already some reports investigating the occurrence and removal of metabolites and/or intermediates; however, it is crucial to develop more studies on this matter, comprising the parent compounds, the possible by-products and the known metabolites in a broader and more comprehensive approach.

1.3. Separation by membrane technologies

Membrane filtration is mostly used for the removal of microorganisms and salts from water/wastewater. The most common membrane technologies include relatively low-pressure systems, such as MF and UF operating at pressures up to 5 and 10 bar, respectively, or high-pressure systems, namely nanofiltration (NF) operating at nearly 50 bar or reverse osmosis (RO) up to 70 bar (or 150 bar for high pressure RO systems) (Coday et al., 2014; Oulton et al., 2010; Peters, 2010). Among these types, the high pressure systems are more suitable for rejection of organic micropollutants, considering the size exclusion mechanism, but larger pores can be employed if electrostatic repulsion or adsorption are the main mechanisms involved in the process (Oulton et al., 2010). The parameters affecting the efficiency of the process include the molecular weight cut-off (MWCO), some membrane properties (e.g., hydrophobicity, surface roughness and charge) and

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physicochemical characteristics of the compounds to be rejected (e.g., molecular weight, pKa,K_{ow} and polarity), among others (Oulton et al., 2010). Regarding the high pressure systems, the main characteristic of NF is the ion selectivity, where monovalent ions can pass through the membrane and multivalent anions are retained (Peters, 2010). The rejection rates are high for organic compounds with molecular weights above 100–200 g mol^{-1} (Hillis, 2000). This process is typically applied for dye/color removal, but recent studies focused on the removal of emerging micropollutants from drinking water and wastewater (Baker, 2012). In the case of RO, the organic and inorganic molecules are separated from the feed solution by their molecular weight (normally, less than 200 g mol^{-1}), size, charge and inability to permeate the active surface of the RO membrane (Lee et al., 2012). The applications range from the production of ultrapure water, to the desalination of seawater for drinking water production and the treatment of industrial wastewater (Peters, 2010). More recently, RO was also applied for the removal of micropollutants, the process depending on complex interactions (e.g., steric, electrostatic/repulsion and hydrophobic) between the contaminants, the solution and the membrane (Dolar et al., 2012). Among the membrane processes, RO was considered as the ultimate treatment step yielding highest pollutant rejection efficiencies (Theepharaksapan et al., 2011).

Forward osmosis (FO) and membrane distillation (MD) are some alternatives to the membrane processes exclusively based on hydraulic pressure. FO is an osmotically driven membrane process that consists on the osmotic pressure difference between the draw solution and the feed solution. Recently, FO has been more intensively investigated for water/wastewater treatment, as a single treatment or coupled to other membrane processes (Coday et al., 2014; Liu et al., 2015). MD (mainly developed for desalination) is based on a vapor pressure gradient across a porous hydrophobic membrane and can operate under different possible configurations (e.g., direct contact, vacuum, air gap and sweep gas MD) (Drioli et al., 2015; Silva et al., 2015; Wang and Chung, 2015). MD has also been studied to reject organic compounds in water treatment (Alkhudhiri et al., 2012) since a complete rejection of inorganic ions and non-volatile substances is theoretically expected.

One of the major disadvantages in this type of processes is the production of a concentrate containing all the retained compounds (Bagastyo et al., 2011; Justo et al., 2014). The disposal of the concentrate can be performed by sewer disposal, evaporation ponds and deep well injection (Umar et al., 2015), but direct discharge to water bodies (oceans, surface and groundwater) is common and constitute potentially serious threat to ecosystems (Justo et al., 2015; Pérez-González et al., 2012). Thus, careful environmental practices are recommended to handle such a concentrated waste before discharging into the aquatic environment (Westerhoff et al., 2009). Different approaches for the treatment of membrane concentrates have been investigated, mainly using AOPs, but also coagulation/flocculation and adsorption with activated carbon were reported (Bagastyo et al., 2011; Justo et al., 2013b, 2014). However, most of these emerging technologies have been developed at laboratory or pilot plant scale (Pérez-González et al., 2012). Good results have been achieved by AOPs for the removal of organic pollutants and persistent compounds, but the cost of these processes can limit their wide implementation at full-scale (Pérez-González et al., 2012; Westerhoff et al., 2009).

1.4. Degradation by advanced oxidation processes (AOPs)

Advanced oxidation processes (AOPs) are conceptually based on the production of highly reactive oxidizing species, such as hydroxyl radicals ($HO^{\bullet}$). AOPs are able to degrade unselectively organic pollutants (Hoigné, 1997) and can be used as pre- or post-treatment of a biological process. As pre-treatment, the aim of a single or a sequence of complementary AOPs is to obtain a more biodegradable effluent able to be treated by a conventional biological process. AOPs can be used as post-treatment to remove micropollutants and their by-products, ideally yielding as final products CO_2, H_2O and inorganic ions, if the aim is the direct discharge in natural water courses. One shortcoming often found in the application of AOPs for wastewater treatment is the frequent presence of radical scavengers in the wastewater, limiting the attack of the radicals to the organic pollutants. Commonly employed AOPs to investigate the treatment of micropollutants in real matrices, include the Fenton and photo-Fenton processes, (catalytic) wet peroxide/air oxidation, (catalytic) ozonation, heterogenous photocatalysis, electrochemical oxidation or combination of them. For the catalytic processes, different catalysts have been identified as the most active depending on the reaction system, including metal oxides (based on Ti, Cu, Zn, Mn, Fe, Co and Bi, among others), supported noble metals (e.g., Ru, Pt, Pd, Ir and Rh), or even metal-free carbon materials such as activated carbons, carbon xerogels, carbon nanotubes, carbon foams and fibers and graphite (Ribeiro et al., 2015).

Briefly, the Fenton process, based on the Fenton reagent (Fenton, 1894), employs H_2O_2 and a precursor of iron, generating $HO^{\bullet}$ at atmospheric pressure and room temperature. High efficiency, relatively cheap reagents, no need of energy to activate H_2O_2 and the consequent easy implementation and operation are the advantages of such treatment. Some disadvantages are the generation of a secondary waste (sludge) and the narrow range of optimal pH (2.5 – 3.0). The photo-assisted Fenton process can be more efficient than Fenton alone, mainly due to the faster regeneration of Fe^{2+} (Pastrana-Martínez et al., 2015). Other related options are electro-Fenton, where Fe^{2+} is produced from sacrificial cast iron anodes (Nidheesh and Gandhimathi, 2012), or even photo-electro-Fenton (Umar et al., 2010).

The concept of catalytic wet peroxide oxidation is similar to that of the Fenton process, but in this case any catalyst can be used (not only iron species) and slightly higher temperatures (50–70 °C) are typically employed (the operating pressure and temperature dramatically increasing in the case of wet air oxidation).

Regarding ozonation, this process involves the direct attack of ozone (quite selective for electron-rich organic molecules) mainly at low pH and/or indirect reactions through $HO^{\bullet}$ more prone at high pH (Ikehata and El-Din, 2004; Munter, 2001). The main handicap of ozonation is the typical low efficiency to mineralize the organic pollutants, while natural organic matter (NOM) and carbonate ions can have a significant interference with the ozone decomposition rate (Saquib et al., 2010). For this reason, different heterogeneous catalysts are under investigation to improve the process (Faria et al., 2008, 2009; Gonçalves et al., 2013; Gonçalves et al., 2010; Orge et al., 2012; Restivo et al., 2012).

Heterogeneous photocatalysis is other process that has been extensively investigated for water/wastewater treatment and is based on the use of wide band-gap semiconductors which generate electrons and holes (and subsequent chain reactions including $HO^{\bullet}$) when irradiated with photons of energy higher than the semiconductor band-gap (i.e., $h\nu \geq E_G$) (Frank and Bard, 1977; Fujishima and Honda, 1972). TiO_2 is the most widely used reference photocatalyst due to the outstanding activity, photochemical stability, good band gap energy, low cost and relatively low toxicity (Hoffmann et al., 1995; Kabra et al., 2004). The possible use of sunlight and the intrinsic anti-microbial ability of heterogeneous photocatalysis (Marin et al., 2011; McCullagh et al., 2007; Monteiro et al., 2015; Rincón and Pulgarin, 2004) are counterbalanced by its main shortcomings, such as the fast recombination of electron–hole pairs and the limited usage of solar light when bare TiO_2 is

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Table 2
Some examples of studies dealing with the removal of E1, E2 and/or EE2. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
E1	ng L^{-1} level	3 pilot WWTPs, one employing CAS; Average flow rate: 107 $dm^3 d^{-1}$; SRT 3 d; HRT 7 h; 24 h composite samples; Burlington Skyway municipal WWTP; Ontario, Canada.	Removal efficiency of CAS was greater than 65% for E1.	(Ogunlaja and Parker, 2015)
E2 EE2 E1	14.5 ± 4.5 ng L^{-1} n.d. 3.2 ± 4.1 ng L^{-1}	Municipal WWTP with biological and chemical treatment; Average flow rate: 20,000 $m^3 d^{-1}$; Kristianstad; South Sweden.	Removals of 78% and >47% were observed for E1 and E2, respectively.	(Zorita et al., 2009)
E2	10 μg L^{-1} (spiked wastewater)	lab-scale MBR and CAS; Industrial-municipal mixed wastewater before secondary treatment.	E2 was almost completely removed (99%) applying both treatments.	(López-Fernández et al., 2013)
E1 E2 EE2	n.d./n.d.; up to 23.2/21.2 ng L^{-1}; up to 22.2/29.2 ng L^{-1}.	WWTPs: CAS or MBR coupled with UF or MF; 24 h composite samples composed by 4 h-aliquots collected by an automatic device; Granada, Spain.	The concentrations after the CAS and MBR treatments were respectively: up to 0.81 and 4.9 ng L^{-1} for E2 and up to 6.62 and 6.92 ng L^{-1} for EE2. MBR system was shown as good alternative to provide high-quality water for reuse. MBR with MF was more efficient for E2 removal.	(Camacho-Munoz et al., 2012)
EE2	140 ng L^{-1} (after primary clarifier)	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 $m^3 d^{-1}$; Gwinnett County, GA, USA.	After CAS and MF, the concentration of EE2 decreased by more than 90%. Ozonation oxidized the remaining compounds by more than 60%.	(Yang et al., 2011)
EE2 E1	8.73 ng L^{-1} 20.69 ng L^{-1}	Pilot-scale combination of MBR and NF or RO; MBR permeate flux: 10.5 L $m^{-2} h^{-1}$ (constant flux mode); 4-L samples of the influent and effluents of each MBR, NF and RO process.	Removal efficiencies higher than 70% (based on the detection limits) were verified for E1 and EE2 with each treatment process.	(Lee et al., 2008)
E1 E2 EE2	150 μg L^{-1} (spiked surface water)	lab-scale UF prior to NF; NF experiments were conducted at 10 bar and 3.6 cm s^{-1} of cross-flow velocity; Surface water from Tagus river, Portugal.	High rejections (higher than 90%) were obtained for E1, E2 and EE2.	(Sanches et al., 2012)
E2 EE2	0.2 μg L^{-1} (spiked wastewater)	MF and RO or MF prior to a pilot-scale UV/H_2O_2; LP-UV lamp; H_2O_2: 3 mg L^{-1}.	Removal of 99% was achieved in both cases.	(James et al., 2014)
E2 EE2 E1	1 mg L^{-1} (spiked surface water)	Multi-barrier approach; lab-scale NF followed by LP-UV (λ_{max} = 245 nm) or indirect (H_2O_2-assisted) LP-UV; H_2O_2: 0, 20, 40, 60, 80 or 100 mg L^{-1}; Surface water.	A rejection of 71% was verified using NF (for all the compounds). Direct photolysis led to high E1 removal, while a removal >74% was obtained by indirect (H_2O_2) photolysis. The multi-barrier approach led to higher overall removals (80, 90 and 95% for E2, EE2 and E1, respectively).	(Pereira et al., 2012)
E1 E2 EE2	1.65–3.59 μg L^{-1} (treated wastewater from the secondary clarifier)	Pilot plant O_3, O_3/UV, O_3/H_2O_2 and O_3/UV/H_2O_2; O_3: 3.15 g h^{-1}; 5% of ozone in gas mixture.	A removal higher than 99.7% was observed for the 3 estrogens.	(Pesoutova et al., 2014)
E1	3 μg L^{-1} – 5 mg L^{-1} (spiked wastewater)	lab-scale O_3, UV, UV/H_2O_2, O_3/UV, O_3/H_2O_2 and O_3/UV/H_2O_2; Annular reactor (750 mL); LP-UV lamp (λ_{max} = 253.7 nm); O_3: 0.33–1.31 mg L^{-1}; H_2O_2: 20, 40 and 60 mg L^{-1}; Municipal wastewater (London, OR, Canada).	A complete removal after 30 min was achieved, employing all processes, except for UV (75 min). Ozonation achieved the higher removal rates of E1. Low TOC removal was observed for all the AOPs tested, with the degradation rate decreasing with higher TOC values.	(Sarkar et al., 2014)
E2 EE2 E1	0.035 mg g^{-1} (dw) 0.150 mg g^{-1} (dw) 0.125 mg g^{-1} (dw)	lab-scale UV, H_2O_2 and UV/H_2O_2; Reactor with continuous recirculation (800 mL); 75 W LP Hg lamp (λ_{max} = 253.7 nm); H_2O_2: 0.5 mol L^{-1}; pH 3; Spiked waste activated sludge.	E2, EE2 and E1 were removed respectively by 92%, 95% and 97%, after 2 min. UV/H_2O_2 was more efficient than UV or H_2O_2 alone. The sludge matrix influenced the degradation rate.	(Zhang and Li, 2014)
EE2	10 mg L^{-1} (spiked wastewater)	Catalytic ozonation; O_3: 20 mg L^{-1}; Catalysts: 5 g of commercial γ-Al_2O_3 or synthesized Co_3O_4/Al_2O_3; Ultrapure water and secondary effluents pre-treated to remove its carbonate/bicarbonate content by stripping; Municipal wastewater from a WWTP; Badajoz, Spain.	EE2 was removed in less than 10 min, regardless the matrix or the presence of catalyst. Comparing with single ozonation, catalytic ozonation enhanced the COD and TOC removals, especially in the presence of the Co_3O_4/Al_2O_3 catalyst.	(Pocostales et al., 2011)

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Table 2 (continued)

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
EE2 E1	<4.3–7.4 ng L^{-1} 1.6–2 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86–153 g Nm^{-3}; O_3 consumption: 0.6 and 0.9 g O_3 g DOC_0^{-1}; Wastewater; Austria.	The application of 0.6 g O_3 g DOC^{-1} increased the removal of these compounds (to not detected).	(Schaar et al., 2010)
E2 EE2 E1	10–250 ng L^{-1} (spiked river water)	Ozonation; O_3: 3–4 mg L^{-1}; River water.	High removal (98–99%) after 10 min was achieved by ozonation process for all estrogens.	(Westerhoff et al., 2005).
EE2 E2 E1	391.4 ± 59.3 ng L^{-1} 110.4 ± 55.4 ng L^{-1} 20.2 ± 3.3 ng L^{-1}	Lab-scale photolytic ozonation, ozonation and photocatalysis; O_3 flow rate: 150 Ncm^3 min^{-1}; O_3: 50 g Nm^{-3}; MP Hg vapor lamp (UV/Vis $\lambda > 300$ nm); TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.	Complete removal by photocatalytic ozonation was achieved for all estrogens, while EE2 was not completely removed using ozonation (77.2% only) and E1 was not completely removed using photocatalysis (61.8% only).	(Moreira et al., 2015)
EE2	2.0 μM (spiked surface water)	Quartz photolysis tubes (1.4 cm i.d. × 20 cm) at a 45° angle were used in photodegradation experiments; Lake water from Lake Quinsigamond.	EE2 showed very high resistance to microbial degradation while rapid photodegradation under sunlight irradiation occurred (half-life of 2.3 h).	(Zuo et al., 2013)

AOP, advanced oxidation process; CAS, conventional activated sludge; COD, chemical oxygen demand; DOC, dissolved organic carbon; dw, dry weight; GAC, granular activated carbon; HRT, hydraulic retention time; LP, low pressure; MBR, membrane biological reactor; MF, microfiltration; MP, medium pressure; n.a., not available; n.d., not detected; NF, nanofiltration; RO, reverse osmosis; SRT, sludge retention time; TOC, total organic carbon; UF, ultrafiltration; WWTP, wastewater treatment plant.

employed (i.e. only the UV fraction, near 3–5% of the overall spectrum) (Andreozzi et al., 1999). A recent approach is the hybridization of photocatalysis with membrane processes, with emphasis in the preparation of new filtration membranes with photocatalytic properties (Athanasekou et al., 2015; Pastrana-Martinez et al., 2015).

Sonolysis, supercritical water oxidation, γ-ray irradiation, microwaves and pulsed electron beam are less commonly applied AOPs (Ribeiro et al., 2015).

2. The watch list: occurrence and removal

This section aims to overview the substances and group of substances of the watch list for European Union monitoring, defined in the Decision 2015/495/EU, regarding their occurrence in aqueous matrices as well as their removal by using the above-mentioned treatments. Scopus database was used and the keywords were the name of each substance and the following treatments: CAS, MBR (conventional processes); RO, MF, UF, NF, FO or MD (membrane technologies); and UV- and peroxide-based, Fenton-based, heterogeneous photocatalysis or ozonation-based processes (AOPs). The studies selected for this review were performed using realistic matrices. Considering the huge amount of literature available for estrogens (EE2, E2 and E1) and for diclofenac, only some examples of studies related to treatment processes for these particular substances (Tables 1 and 2) are included in this review.

2.1. EE2, E2 and E1

Steroid hormones include highly active biological compounds able to induce the therapeutic effect at very low doses. Within this group, estrogens are the most usually found in the aquatic environment, existing either as natural or synthetic substances and acting as endocrine-disrupting compounds (EDCs) (Barreiros et al., 2016; Rocha et al., 2008). Estriol, E1 and E2 are natural estrogens mainly excreted from humans whereas EE2 is the most used oral contraceptive, also excreted by humans, causing injurious effects to the ecosystems such as feminization of male fishes, DNA and immunity alterations (Li, 2014). The effects of EDCs toward animals are well reported, for example, a 7-year experiment was developed (Kidd et al., 2007) and it was concluded that the chronic exposure of fathead minnow to 5–6 ng L^{-1} of EE2 led to feminization of male fish and altered oogenesis in females. Some studies suggested that the effect of EDCs exposure on human health includes a decrease in male sperm count, an increase in testicular, prostate, ovarian and breast cancers and reproductive malfunctions (Joffe, 2001). The major concern is related to fetuses and newborn babies, because of their higher vulnerability (Sharpe and Irvine, 2004). Recently, Kabir et al. (2015) reviewed extensively the mechanism of action and harmful effects of EDCs on human health; and Futran Fuhrman et al. (2015) highlighted the EDCs risk assessment, namely issues related to long-term and combined exposure, transgenerational and mixture effects. Due to the potential deleterious effects that can arise from their release into the environment, their occurrence is well described and reviewed by several authors (Khanal et al., 2006; Li, 2014; Teske and Arnold, 2008). Table 1 summarizes some studies on the occurrence of E1, E2 and EE2 (concentration, matrix and location), which are frequently found in water matrices, namely wastewater, surface and groundwater, at ng L^{-1} levels.

The removal of these hormones are reported in several studies (Fig. 2), varying depending on the processes (Table 2). Biological treatments coupled with membrane processes are reported as effective mean for elimination of these types of compounds (Camacho-Munoz et al., 2012). As example, more than 90% of EE2 was removed in an advanced wastewater reclamation plant employing a biological treatment and MF (Yang et al., 2011). Few studies were developed using other membrane technologies to remove E1, E2 and EE2 (Table 2), being highly removed by NF and/or RO (Cheng et al., 2010; Lee et al., 2008). AOPs are promising to remove this type of pollutants, with ozonation having the highest efficiency (Table 2). Data regarding these compounds can be consulted in article reviews that have been published in the last few years and that already encompass a significant amount of information dealing with their removal from water (Basile et al., 2011; Jung et al., 2015; Kaplan, 2013; Liu et al., 2009; Luo et al., 2014; Yu et al., 2013). Concerning the studies on the removal of the substances of the watch list, it can be concluded that E1, E2 and EE2 were the most studied in the last decade, employing all the types of processes herein referred (Fig. 2).

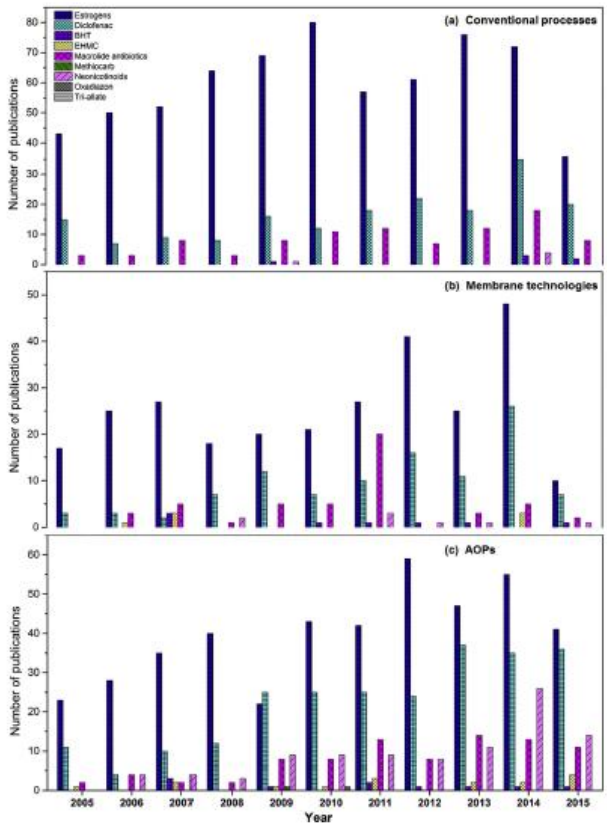

Fig. 2. Number of publications dealing with the removal of the 10 substances/groups of substances included in the first watch list for European Union monitoring (Decision 2015/495/EU). The search comprised publications since 2005 in Scopus database, using as keywords each substance and the treatments reported in the previous sections, namely (a) conventional processes (CAS or MBR); (b) membrane technologies (RO, MF, UF, NF, FO or MD); and (c) AOPs (UV-and peroxide based, Fenton based, heterogeneous photocatalysis or ozonation-based processes). In this particular search, any type of matrix (realistic and non-realistic) was considered.

2.2. Diclofenac

Regarding the non-steroidal anti-inflammatory drug (NSAID) diclofenac, it is considered harmful to several species at environmental concentrations, as indicated by Vieno and Sillanpää (2014), who overviewed its occurrence, fate and transformation processes during treatment in WWTPs. Diclofenac is often detected in WWTP influents and effluents, surface water and groundwater. Table 1 describes some studies on its occurrence in these aquatic compartments, with diclofenac found up to 4.4 μg L^{-1}. Information concerning the removal of diclofenac can be checked in article reviews that have been published in the last few years and which already included systematized data of its removal from water (Barra Caracciolo et al., 2015; Cherik et al., 2015; Fatta-Kassinos et al., 2011; Petrie et al., 2013; Ziylan and Ince, 2011). Diclofenac can be partially adsorbed on sludge and is usually poorly biodegradable, which means low removal rates during biological wastewater treatment (Table 3) (Vieno and Sillanpää, 2014; Zhang et al., 2008). Membrane technologies to remove diclofenac have been used, but more research is needed (Table 3). Concerning AOPs, some studies dealing with heterogeneous photocatalysis and/or photo-Fenton are described in Table 3, with a moderate diclofenac removal, most using a pilot compound parabolic collector (CPC) plant and a high reaction time. Ozonation as single process, or

Appendix A

Table 3
Some examples of studies dealing with removal of diclofenac. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial diclofenac concentration	Treatment and sampling conditions	Concluding remarks	Reference
Up to 12.7 ng L^{-1} (CAS); up to 38 ng L^{-1} (MBR).	WWTP with CAS or MBR coupled to UF or MF; 24 h composite samples composed by 4 h aliquots collected by an automatic device; Granada, Spain.	Mean removal rates were between 54 and 71% for MBR and approximately 79% for CAS.	(Camacho-Munoz et al., 2012)
30 mg L^{-1} (spiked surface water)	Photocatalysis and solar photolysis; Advanced lab-scale reactor immersion-well (UV-RS-1) made of Pyrex glass (cut-off <290 nm); Solution volume: 400 mL; MP Hg vapor lamp: TQ 150 W Heraeus, Germany; TiO_2 load: 0.1 g L^{-1}; pH 6.2.	Water quality showed high influence in the treatment efficiency. For river water, solar photolysis showed higher removal compared to TiO_2 photocatalysis, with 66% and 82% diclofenac removal for photocatalysis and direct sunlight, respectively.	(Kanakaraju et al., 2014)
0.05 μg L^{-1} (spiked surface water)	Kagithane Drinking Water Treatment Plant; Flow rate: 700,000 m^3 d^{-1}; Lab-scale NF; MF: cross-flow rate of 3 L min^{-1} and water flux of 137 L m^{-2} h^{-1}; Raw water sources: Terkos lake and Alibeyköy Dam.	Diclofenac overall rejection was approximately 61%.	(Vergili, 2013)
220 ng L^{-1} (after primary clarifier)	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 m^3 d^{-1}; Gwinnett County, GA, USA.	Diclofenac was removed by CAS, between 51 and 80%, achieving the LOQ.	(Yang et al., 2011)
WWTP1: 507 ng L^{-1}; WWTP2: 1450 ng L^{-1}.	WWTP1: parallel CAS and MBR serving 28,000 inhabitants and treating 5544 m^3 d^{-1} by CAS and 7237 m^3 d^{-1} by MBR; WWTP2: CAS serving 100,000 inhabitants and treating 20,846 m^3 d^{-1}; 24 h composite samples; Lede, Belgium.	Removal was not observed in both treatments of WWTP1 employing parallel CAS and MBR. Almost no removal occurred in WWTP2, with 1391 ng L^{-1} in the effluent of CAS.	(Vergeynst et al., 2015)
Up to 2400 ng L^{-1}	15 WWTPs designed for 6850 to 756,000 population equivalents; Flow rates: 349–140,000 m^3 d^{-1}; Discharge points: main Portuguese rivers and Atlantic Ocean; 24 h composite influent and effluent samples; Portugal.	Concentration of diclofenac was quantified up to 670 ng L^{-1} in the effluent. The mean removal of this substance was 45.6%.	(Pereira et al., 2015)
Up to 0.49 μg L^{-1}	4 WWTPs impacted by effluents from mid-size hospitals, corresponding to a WWTP inflow between 1 and 30%; Flow rates: 1300–103,000 m^3 d^{-1}; WWTP 1/3 – SBR and UV-tertiary treatment; WWTP 2 – CAS; WWTP 4 – oxidation ditch activated sludge process; 24 h composite samples; New York, USA.	WWTP 2 and 4, employing CAS or oxidation ditch activated sludge, were more efficient than the others for the removal of diclofenac.	(Oliveira et al., 2015)
Up to 6.34 ng L^{-1}	4 WWTPs; Chongqing, China; 2 WWTPs: anaerobic/anoxic/oxic (A/A/O) activated sludge process; 1 WWTP: CAST; 1 WWTP: OD.	Although diclofenac was quantified up to 4.7 ng L^{-1}, the removal was not assessed due to its detection in some cases, below than LOQ.	(Yan et al., 2014a)
n.a.	MBR pilot plant in continuous operation (ca. 1% of diurnal hospital sewage); 2 h composite influent and effluent samples; Luxembourg.	Diclofenac was removed at an extent between 40 and 50%. UV was evaluated as post-treatment; degradation was improved by applying H_2O_2.	(Kohler et al., 2012)
6.01 ng L^{-1}	4th largest WWTP in China, serving 1,540,000 equivalent inhabitants; 600,000 m^3 d^{-1}. CAST followed by chlorination; Grab samples collected according to the residence time in each treatment; Southwest China.	The removal obtained after secondary treatment was 41.8% for diclofenac. Chlorination led to a reduction of 8.6%.	(Yan et al., 2014b)
361–911 ng L^{-1}	Pilot-scale MF followed by RO; MF: flow rate of 2 m^3 h^{-1} and flux of 323 L m^{-2} h^{-1}; Residence time 3 min; RO: flow rate of 1 m^3 h^{-1} and permeate flux of 34 L m^{-2} h^{-1}; Residence time 50 min; Treated effluent; Girona, Spain.	High removal of diclofenac was observed. RO reduced the concentration of diclofenac in the MF permeate to levels below the limit of detection.	(Rodriguez-Mozaz et al., 2015)
57–131 ng L^{-1}	Pilot-scale NF and RO; NF: Water flux between 12 and 62 L m^{-2} h^{-1}, depending on the type of membranes; RO: Water flux of 23.5 L m^{-2} h^{-1}; Treated effluent; Sydney, Australia.	RO was the most efficient treatment for the rejection of diclofenac, reaching concentrations lower than 5 ng L^{-1}.	(Shanmuganathan et al., 2015)
104.1 ng L^{-1}	Pilot-scale UF followed by a RO; UF permeate flux: 227 m^3 d^{-1}; RO permeate flux: 82 m^3 d^{-1}; Ansan, Gyeonggi-do, Korea.	Concentration of diclofenac considerably decreased by UF (permeate concentration: 69.7 ng L^{-1}). RO completely removed diclofenac.	(Chon et al., 2013)

(continued on next page)

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Table 3 (continued)

Initial diclofenac concentration	Treatment and sampling conditions	Concluding remarks	Reference
750 ng L^{-1}	Pilot-scale NF; Flux: 1–2 L m^{-2} h^{-1}; 24 h composite sample; Giessen, Germany.	Diclofenac decreased by at least 65%.	(Röhricht et al., 2010)
605 ng L^{-1}	2-L reactors at 25 °C; UV/H_2O_2: 3 LP Hg lamps (λ_{max} = 254 nm); H_2O_2 consumed ranged from 0.04 to 0.72 mg H_2O_2 mg TOC^{-1}; Ozonation: 10 g O_3 Nm^{-3}; RO concentrates from a municipal WWTP	UV/H_2O_2 exhibited higher performance than ozone in the removal of diclofenac, which had one of the lowest initial observed kinetic constants probably due to the matrix effects on the process.	(Justo et al., 2013a)
935 ng L^{-1}	2-L reactors at 25 °C; UV/H_2O_2: 3 LP Hg lamps (λ_{max} = 254 nm); H_2O_2 consumed ranged from 0.01 to 0.90 mg H_2O_2 mg TOC^{-1}; RO concentrates from a municipal WWTP in a coastal area of Catalonia, Spain.	Diclofenac was completely decomposed by UV, after the first minutes of treatment; it was also removed by UV/H_2O_2 and ozonation process.	(Justo et al., 2014)
283 ng L^{-1}	Biological activated carbon (BAC) process to treat municipal wastewater RO concentrate; lab scale during 320 days of operation; BAC, combined UV/UV/H_2O_2—BAC and ozone—BAC.	54% of diclofenac was removed by the BAC filter. The integration of the UV/H_2O_2 or the ozonation processes was necessary to obtain a complete removal of diclofenac.	(Justo et al., 2015)
>750 ng L^{-1}	Photocatalysis in a pilot-scale CPC plant under natural solar irradiation; TiO_2 load: 20 mg L^{-1}; Effluents of the biological treatment of El Ejido WWTP; Almería, Spain.	Complete diclofenac removal was achieved after 480 min.	(Prieto-Rodriguez et al., 2012)
671–4941 ng L^{-1}	Photo-Fenton in a pilot-scale CPC plant; Fe^{2+}: 5 mg L^{-1}; pH: 3 and 10; H_2O_2: 50 mg L^{-1}; Complexing agents (humic acid and ethylenediamine-N,N′-disuccinic acid); Effluents of the secondary treatment in a municipal WWTP; Almería, Spain.	Diclofenac was removed by 97% in the photo-Fenton process (pH 3), after 50 min. Photo-Fenton with humic acids at neutral pH resulted in a longer treatment time required to reach a similar degradation.	(Klamerth et al., 2013)
≈70 ng L^{-1}.	Bench-scale UV and UV/H_2O_2 (λ_{max} = 254 nm); H_2O_2: 7.8 mg L^{-1}; Volume and HRT: 35 L and 5 min, respectively; Capacity: 10 m^3 d^{-1}; Municipal WWTP; Japan.	A complete removal of diclofenac was observed for both processes.	(Kim et al., 2009)
10 μg L^{-1} (spiked surface water)	UV/H_2O_2 in a pilot plant with three parallel reactors with MP, LP or dielectric barrier discharge UV lamps. Pre-treated surface water (by coagulation, flocculation and sedimentation in a natural reservoir, micro-straining and dual layer rapid sand filtration) from Meuse River (Netherlands), spiked with a mixture of 15 compounds.	The degradation of diclofenac was higher than 80%.	(Lekkerkerker-Teunissen et al., 2013)
n.a.	Sulfate radical based homogeneous photo-Fenton involving peroxymonosulfate as oxidant, ferrous iron (Fe(II)) as catalyst and simulated solar irradiation as light source; Biologically treated domestic wastewater effluents;	PMS/Fe(II)/UV–Vis advanced oxidation system using simulated solar irradiation has demonstrated better kinetic performances over TiO_2/UV–Vis system for diclofenac.	(Ahmed et al., 2014)
0.1 mg L^{-1} (spiked wastewater)	Heterogeneous photocatalysis and Photo-Fenton; Pilot-scale CPC solar plant at the Plataforma Solar de Almería (Spain); A: Photo-Fenton (pH 2; 5 mg L^{-1} of Fe^{2+}; 50 mg L^{-1} of H_2O_2; 5 mg L^{-1} of TiO_2); B: no pH adjustment; 50 mg L^{-1} of H_2O_2; 5 mg L^{-1} of Fe^{2+} (demineralized water); 5, 15 and 55 mg L^{-1} of Fe^{2+} (standard freshwater); 5 mg L^{-1} of Fe^{2+} (standard fresh water without $NaHCO_3$).	Solar TiO_2 photocatalysis showed complete diclofenac degradation. 20–50% of degradation in demineralised water was achieved in the dark (Fenton process) and photo-Fenton was the most effective treatment with a complete removal observed after 20 min. In standard fresh water, diclofenac was removed by Fenton process.	(Klamerth et al., 2009)
0.276 μg L^{-1}	Heterogeneous photocatalysis: Solardetox Acadus-2006 CPCs with 3.0 m^2 irradiated surface and 24 L of irradiated volume; TiO_2 load: 0.2 g L^{-1}; Effluent of a WWTP from the South East of Spain.	High diclofenac removal (≈ 88%) was observed after 3 h of treatment (bellow LOQ) applying solar TiO_2 photocatalysis.	(Bernabeu et al., 2011)
10 mg L^{-1} (spiked wastewater)	Catalytic ozonation; O_3: 20 mg L^{-1}; Catalysts: 5 g of commercial γ-Al_2O_3 or synthesized Co_3O_4/Al_2O_3; Ultrapure water and secondary effluents pre-treated to partially remove its carbonate/bicarbonate content by stripping; Wastewater from a municipal WWTP; Badajoz, Spain.	Diclofenac was removed in less than 10 min, regardless the matrix or the presence of catalyst. Comparing with single ozonation, catalytic ozonation enhanced the COD and TOC removals, in particular with a Co_3O_4/Al_2O_3 catalyst.	(Pocostales et al., 2011)

Table 3 (continued)

Initial diclofenac concentration	Treatment and sampling conditions	Concluding remarks	Reference
30–80 mg L^{-1} (spiked wastewater)	UVA, O_3, O_3/UVA, O_3/TiO_2, O_3/UVA/TiO_2; O_3: 5–30 g m^{-3}; HP Hg lamp; TiO_2 load: 0.5 and 2.5 g L^{-1}; Ultrapure water and urban wastewater from a municipal WWTP; Badajoz, Spain	Complete removal of diclofenac was verified by applying photocatalytic ozonation within 6 min (60–75% TOC reduction after 60 min, regardless the water matrix used). Photocatalytic ozonation showed the lowest ozone consumption compared to the other ozonation processes.	(Aguinaco et al., 2012)
30 mg L^{-1} (spiked surface water)	Single ozonation and catalytic ozonation; O_3: 10 g m^{-3}; pH = 7; Catalysts: 1 g L^{-1} of lab-prepared Mn–Ce–O or a commercial (N-150) catalyst; Synthetic effluent and river water collected from Mondego River; Portugal.	The catalysts had no significant effect on diclofenac removal when compared with single ozonation. However, both catalysts increased the COD removal per mg of ozone applied.	(Martins et al., 2015)
n.a.	Bench-scale photolysis; 150 W MP Hg lamp, which emits radiation between 200 and 450 nm; Municipal wastewater of secondary effluent of a biological WWTP; Portugal.	The degradation rate constants obtained for diclofenac in a filtered wastewater matrix were lower than in a pure water matrix.	(Salgado et al., 2013)
2.5 mg L^{-1} (spiked wastewater)	Lab-scale TiO_2 photocatalysis; 125 W black light fluorescent lamp (300–420 nm); Catalyst load: 0.2–0.8 g L^{-1}; Urban WWTP effluent.	TiO_2 photocatalysis showed a high removal of diclofenac (≈ 98%).	(Rizzo et al., 2009)
100 μg L^{-1} (spiked wastewater)	Solar photo-Fenton in a pilot-scale solar CPC reactor; H_2O_2 dose = 0–50 mg L^{-1}; Fe^{2+} = 5 mg L^{-1}; Municipal wastewater.	Diclofenac was completely removed (<LOQ) after 34 min.	(Klamerth et al., 2010)
464.8 ± 64.7 ng L^{-1}	Lab-scale photolytic ozonation, ozonation and photocatalysis; O_3: 50 g Nm^{-3}; O_3 flow rate: 150 Ncm^3 min^{-1}; MP Hg vapor lamp (UV/Vis λ > 300 nm); TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.	For all processes, the complete removal was achieved.	(Moreira et al., 2015),
13.5–52.0 μg L^{-1} (spiked wastewater)	Lab-scale ozonation; O_3: 5.5–8.5 mg L^{-1}; O_3 flow rate: 0.39 Ndm^3 min^{-1}; Urban wastewater samples from the secondary clarifier of two WWTPs from West-Alcalá and Alcázar de San Juan; Spain.	High diclofenac removal (>90%) was observed.	(Rodriguez et al., 2012)
970–2300 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86–153 g Nm^{-3}; O_3 consumption: 0.6 and 0.9 g O_3 g DOC_0^{-1}; Municipal wastewater; Austria.	The application of 0.6 g O_3 g DOC_0^{-1} increased the removal of diclofenac (to values < LOQ).	(Schaar et al., 2010)
5–20 mg L^{-1} (spiked wastewater)	UV-A/TiO_2 photocatalysis: 9 W lamp; Catalyst load: 50–1600 mg L^{-1}; H_2O_2 = 0.07–1.4 mM; Treated municipal effluent from Limassol; Cyprus.	UV-A/TiO_2 was efficient for the degradation and mineralization of diclofenac in treated municipal effluents.	(Achilleos et al., 2010)

BAC, Biological activated carbon; CAS, conventional activated sludge; CAST, cyclic activated sludge technology; COD, chemical oxygen demand; CPC, compound parabolic collector; DOC, dissolved organic carbon; GAC, granular activated carbon; HRT, hydraulic retention time; LOQ, limit of quantification; LP, low pressure; MP, medium pressure; MBR, membrane biological reactor; MF, microfiltration; n.a., not available; n.d., not detected; NF, nanofiltration; OD, oxidation ditch; RO, reverse osmosis; SBR, Sequential Batch Reactor; TOC, total organic carbon; UF, ultrafiltration, WWTP, wastewater treatment plant.

combined with photolysis and/or photocatalysis, has been widely investigated showing a high performance for diclofenac removal. Overall, diclofenac is the second most studied substance of the watch list in the last 10 years, employing all the types of processes (Fig. 2).

2.3. 2,6-di-tert-butyl-4-methylphenol

The anti-oxidant 2,6-di-tert-butyl-4-methylphenol (BHT) has been used as a common anti-oxidant to preserve and stabilize the freshness, nutritive value, flavor and color of food and animal feed products, since the 1950s (Fries and Püttmann, 2002; Tombesi and Freije, 2002). BHT can also improve the stability of pharmaceuticals and cosmetics and increase the durability of rubber and plastics. Approximately 40 countries allow the use of BHT as a direct or indirect food additive (Fries and Püttmann, 2002). The use of BHT as a food additive does not appear to pose a public health risk. However, in the natural environment, BHT is degraded biologically to 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO), reported by generating peroxides in mice and rats and inducing cellular DNA damage (Fries and Püttmann, 2004). The occurrence of the anti-oxidant BHT in the aquatic environment has been demonstrated (Table 1), with studies conducted in Sweden (Bendz et al., 2005) and USA (Benotti et al., 2009) reporting the presence of BHT in surface water up to 620 ng L^{-1} and 49 ng L^{-1}, respectively. In other studies, BHT was detected in wastewater (between 22 and 258 ng L^{-1}) (Fries and Püttmann, 2004), whereas higher values were quantified in surface water (up to 1560 ng L^{-1}) and groundwater (up to 2156 ng L^{-1}) in Greece and Germany (Fries and Püttmann, 2002, 2004; Papadopoulou-Mourkidou et al., 2001).

Table 4
Studies dealing with removal of 2-ethylhexyl-4-methoxycinnamate (EHMC). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial EHMC concentration	Treatment and sampling conditions	Concluding remarks	Reference
n.a.	5 WWTPs equipped with different treatment levels: preliminary screening, primary sedimentation, secondary treatment; UV-disinfection, chlorination, sand filtration and RO; 24 h composite or grab samples, depending on the plant; Hong Kong.	Removal of EHMC varied depending on the season in the range of 30–50%.	(Tsui et al., 2014)
Up to 234 ng L^{-1}	Ozonation: nominal capacity of 3000 m^3 h^{-1}; 5 L glass reactor operating in semi-batch mode, at a temperature of 25 °C and pH 8.5; WWTP located in Madrid, which treats a mixture of domestic and industrial wastewater.	The UV filter EHMC was completely refractory to ozone.	(Rosal et al., 2010)
23.6 ± 8.1 ng L^{-1}	UV, visible light, photocatalysis (visible light), O_3; 15 W LP Hg vapor lamp (λ_{max} = 254 nm); Xe 150 Xe-arc lamp with spectral emission in the visible region; Photocatalyst: ceria-doped TiO_2 at 0.5 g L^{-1}; O_3: 22 g Nm^{-3}; Mixture of domestic and industrial wastewater from the secondary clarifier of a 3000 m^3 h^{-1} WWTP placed in Alcalá de Henares; Madrid, Spain	EHMC was removed up to 50% after 15 min of UV-photolysis, mainly during the first 2 min. Visible light Xe-lamp driven photolysis led to an EHMC removal near 20% after 15 min. Removal was not enhanced, applying visible light Ce/TiO_2 photocatalysis. EHMC was not significantly removed by ozone.	(Santiago-Morales et al., 2013)

n.a., not available; LP, low pressure; RO, reverse osmosis; WWTP, wastewater treatment plant.

Additional data are needed to support assessments of human health risks associated with the exposure to this compound in the aquatic environment and to establish possible pathways of removal in aquatic systems. Considering the lack of studies on its removal (Fig. 2), it is urgent to study its elimination from water matrices.

2.4. 2-ethylhexyl-4-methoxycinnamate

Organic UV filters are chemical filters used in many personal care products, alone or in formulations containing a physical filter like ZnO or TiO_2 nanoparticles (Kaiser et al., 2012a). Their occurrence in the environment has been described in several papers that have been given a great attention to the aqueous matrices. These CECs reach the environment by two pathways, wash off from skin or through wastewater or swimming pool water. Organic UV filters are likely to be present in sediments (Kaiser et al., 2012b), where they might induce toxicological effects. Their known estrogenic effects on biota and humans was recently reviewed by Ramos et al. (2015), who highlighted not only the recognized *in vivo* and *in vitro* estrogenic activity to fish and mammals, but also other non-estrogenic hormonal targets in such organisms. The UV filter 2-ethylhexyl-4-methoxycinnamate (EHMC), included in the watch list for Union-wide monitoring, is an EDC and was reported at concentrations levels of hundreds of μg kg^{-1} in diverse organisms including macroinvertebrates and fish (Kaiser et al., 2012a). Lake and rivers sediments are well characterized regarding this contaminant, which is usually present at μg kg^{-1} levels (Kaiser et al., 2012a, 2012b; Langford et al., 2015). This compound was also detected up to 260 ng L^{-1} in tap water from Barcelona (Spain), one of the most frequently found of a group of five UV filters included in that study (Díaz-Cruz et al., 2012). Little is known about the removal of EHMC in the aquatic environment (Table 4, Fig. 2), only three studies reporting its removal. The removal of EHMC varied (30–50%), depending on the respective treatment applied at the WWTP and season (Tsui et al., 2014). This UV filter was refractory to ozonation, without any degradation being observed after 15 min (Rosal et al., 2010) or after 22 min, but could be removed by UV treatment (Santiago-Morales et al., 2013).

2.5. Macrolide antibiotics

Among the different classes of pharmaceuticals present in the environment, particular importance has been given to antibiotics, which are the most often discussed pharmaceuticals due to their potential role in the development of resistant mechanisms by bacteria (Xekoukoulotakis et al., 2010). Macrolide antibiotics, such as clarithromycin, azithromycin and erythromycin are widely used in human and veterinary medicine, as well as in aquaculture, for the purpose of preventing or treating serious infections induced by pneumococci, staphylococci and streptococci (Lange et al., 2006; Xekoukoulotakis et al., 2010). The conventional municipal WWTPs do not fully eliminate these drugs, which are found in WWTP effluents (Lange et al., 2006) and in other aquatic systems (Gracia-Lor et al., 2011; Hoa et al., 2011; Lopez-Serna et al., 2013; Tong et al., 2014). These antibiotics have been extensively detected in wastewater, surface and groundwater in several countries at ng L^{-1} levels, with some studies reporting antibiotics at several μg L^{-1} (Table 1). For instance, azithromycin, erythromycin and clarithromycin were found in effluents of a WWTP in Slovakia at ng L^{-1} levels (Birosova et al., 2014). Clarithromycin and erythromycin were reported in surface water in Spain and Vietnam (Gracia-Lor et al., 2011; Hoa et al., 2011). Lopez-Serna et al. (2013) also reported the occurrence of the three macrolide antibiotics in groundwater (Spain) in the range 1.6–1620 ng L^{-1}.

Elimination of this class of antibiotics in the environment has been reported in the last decade, for all the types of processes here discussed (Fig. 2). Biological treatments occurring at WWTPs are normally insufficient to remove such recalcitrant pharmaceuticals (Table 5). The combination of biological with advanced treatments can be fruitful, as example MBR and RO led to elimination rates above 99% (Dolar et al., 2012) for the macrolides included in the watch list. Hence, advanced methods should be applied to deal with this environmental concern. Membrane technologies alone are not enough for the complete removal of such micropollutants (Table 5). Studies reported in the literature employing AOPs for the removal of this type of antibiotics in environmental samples are focused only on photocatalysis (Bernabeu et al., 2011; Xekoukoulotakis et al., 2010), revealing a lack of knowledge regarding the efficiency of other AOPs to remove this compounds in real scenarios. In fact, some studies with other AOPs were already published considering these compounds, but not using real matrices and, thus, they are out of the scope of the present review; for instance, UV/TiO_2 and ozonation were studied for the removal of clarithromycin and erythromycin, ozonation apparently being more effective for the parent compounds (complete degradation), while catalytic ozonation improving the mineralization of erythromycin

Appendix A

Table 5
Studies dealing with removal of macrolides (azithromycin, clarithromycin and erythromycin). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Erythromycin	2600 ng L^{-1} (after primary clarifier)	WWTP with CAS-MF-GAC-ozonation; Average flow rate: 227,000 m^3 d^{-1}; 24 h composite samples; Gwinnett County, GA, USA.	Erythromycin was recalcitrant to the biological treatment, but it was removed at an extent of 74% by GAC adsorption. Ozonation oxidized the remaining compounds by more than 60%.	(Yang et al., 2011)
Azithromycin Clarithromycin Erythromycin	118 ng L^{-1} 2020 ng L^{-1} 49 ng L^{-1}	MBR coupled to RO; Coastal WWTP Castell-Platja d'Aro, Spain.	The combination of MBR and RO led to removal rates above 99% for the target pollutants, with RO showing removal rates always higher than 99%. MBR removed 75–85% of the antibiotics, and the remaining non-degraded macrolides were removed by RO.	(Dolar et al., 2012)
Azithromycin Clarithromycin Erythromycin	232.5–876.9 ng L^{-1} >0.1 μg L^{-1} 4.11–42.01 ng L^{-1}	WWTP1 with secondary treatment (anaerobic/anoxic/oxic (A/A/O) treatment + moving bed biofilm reactor (MBBR) + secondary clarifier) and tertiary treatment (rotary fiber disc filters (RFDFs)). WWTP2 with secondary treatment (C-Orbal OD process + secondary clarifier) and tertiary treatment (UV disinfection and RFDFs); 24 h composite wastewater samples at different sampling points; Wuxi City, Jiangsu Province, China.	Removal efficiencies were generally higher in the WWTP1 employing the A/A/O-MBBR process than those obtained by the conventional WWTP2 adopting the C-Orbal OD process, except for clarithromycin. The type of biodegradation process was the predominant factor in this study, the better performance being obtained with WWTP1.	(Yuan et al., 2015)
Erythromycin	0.2 μg L^{-1}	1 WWTP serving 500,000 population equivalent, with an industrial inlet lower than 10% of the total load; with biological treatment, final clarification and tertiary treatment by phosphorus precipitation; 2 h composite influent and effluent samples, during 24 h Nancy, France.	No elimination was reported for erythromycin in the liquid phase. This antibiotic was also not adsorbed on the particulate matter or the sludge.	(Pasquini et al., 2014)
Azithromycin, Clarithromycin Erythromycin	406–611 ng L^{-1} 785–941 ng L^{-1} 164–210 ng L^{-1}	1 WWTP equipped with MBR and UV treatment, serving 24,000 inhabitants; Membrane modules made of hollow-fiber membranes; Average flow rates: 8800 m^3 d^{-1}; 24 h composite influent and effluent samples; Canada.	The degraded fraction of azithromycin was approximately 49% and that of erythromycin was negligible. Clarithromycin was not removed during MBR treatment, being even formed during treatment.	(Kim et al., 2014)
Azithromycin	up to 719 ng L^{-1}	15 WWTPs, designed for 6850 to 756,000 population equivalents; Average flow rates: between 349 and 140,000 m^3 d^{-1}; Discharge points: Portuguese rivers and Atlantic Ocean; 24 h composite influent and effluent samples; Portugal.	The concentration of azithromycin in the effluent was up to 200 ng L^{-1}, with a mean removal of 94.6%.	(Pereira et al., 2015)
Clarithromycin Erythromycin	up to 0.33 μg L^{-1} up to 0.13 μg L^{-1}	4 WWTPs impacted by effluents from mid-size hospitals (250–600 beds) corresponding to a WWTP inflow ranging between 1 and 30%; Average flow rates: between 1300 and 103,000 m^3 d^{-1}; WWTP 1/3 – SBR and UV-tertiary treatment; WWTP 2 – CAS; WWTP 4 – OD activated sludge process; 24 h composite samples; New York, USA.	WWTP 2 and 4, employing CAS or OD activated sludge process were more efficient than the others for the removal of clarithromycin and erythromycin.	(Oliveira et al., 2015)
Azithromycin Erythromycin	up to 661.9 ng L^{-1} up to 338.2 ng L^{-1}	2 municipal WWTPs with anaerobic/anoxic/oxic (A/A/O) activated sludge process, one of them employing a cyclic activated sludge technology (CAST) whereas the other having an OD; Chongqing, China.	WWTP using the OD biological treatment process had the higher efficiency to remove the macrolide antibiotics.	(Yan et al., 2014a)
Clarithromycin Erythromycin	n.a.	MBR pilot plant in continuous operation ca. 1% of diurnal hospital sewage; 2 h composite influent and effluent samples; luxembourg.	Erythromycin was almost totally removed by MBR, while clarithromycin was removed at extents between 40 and 50%. UV was evaluated as post-treatment, with improved degradation obtained by adding H_2O_2.	(Kohler et al., 2012)
Azithromycin Erythromycin	330.27–376.5 ng L^{-1} 238.6–275.4 ng L^{-1}	4th largest WWTP in China, serving 1,540,000 equivalent inhabitants and treating 600,000 m^3 d^{-1}. CAST (anaerobic/anoxic/aerobic (A/A/A) treatment secondary clarifier) followed by chlorination; Grab samples collected according to the residence time in each treatment; Southwest China.	The removal obtained after secondary treatment was 75.6% for azithromycin and 42.8% for erythromycin. Chlorination led to a reduction of 8.0% for azithromycin. Erythromycin was not removed during chlorination.	(Yan et al., 2014b)
Azithromycin Clarithromycin	160–279 ng L^{-1} 1129–1570 ng L^{-1}	Samples were collected in winter from four WWTP located in Kyoto and Shiga prefecture (Japan); WWTPs employed a wide variety of secondary treatment processes: CAS; anaerobic/anoxic/aerobic (A/A/A) and anoxic/aerobic (A/A).	Removal efficiency of the macrolide antibiotics were higher using CAS (39–83%) and A/A (34–86%) processes than using A/A/A (41–53%) process.	(Ghosh et al., 2009)

(continued on next page)

Appendix A

Table 5 (*continued*)

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Clarithromycin	up to 27.4 μg L^{-1}	MBR followed by NF and RO; Membrane surface of NF and RO modules: 2.5 m^2; Operation: cross flow membranes; NF/RO modules: maximum flux between 20 and 36 L m^{-2} h^{-1}; Hospital wastewater, Germany.	Clarithromycin was completely removed by RO and NF treatments (<LOQ).	(Beier et al., 2010)
Erythromycin Clarithromycin	337 ± 19.2 ng L^{-1} 377 ± 30.9 ng L^{-1}	Pilot-scale UF and RO treatments in sequence; UF flux range of 25–47 L m^{-2} h^{-1}; RO flux range of 22–31 L m^{-2} h^{-1}; Municipal WWTP; Tel-Aviv, Israel.	High removal rates were achieved after RO (99% for macrolides antibiotics).	(Sahar et al., 2011)
Azithromycin Erythromycin	187–367 ng L^{-1} 180–191 ng L^{-1}	Pilot-scale MF followed by RO; MF: flow rate of 2 m^3 h^{-1} and flux of 323 L m^{-2} h^{-1}; Residence time 3 min; RO: flow rate of 1 m^3 h^{-1} and permeate flux of 34 L m^{-2} h^{-1}; Residence time 50 min; Municipal treated effluent; Girona, Spain.	High removals were observed for these pharmaceuticals compounds. Even though the pharmaceuticals were present in the MF permeate at levels higher than 100 ng L^{-1}, RO filtration reduced their loads to the low ng L^{-1} range or to below the method LOQ.	(Rodriguez-Mozaz et al., 2015)
Clarithromycin	77 ng L^{-1}	2-L reactors at 25 °C; UV/H_2O_2: 3 LP Hg lamps (λ_{max} = 254 nm); H_2O_2 consumed ranged from 0.01 to 0.90 mg H_2O_2 mg TOC^{-1}; RO concentrates from a municipal WWTP in a coastal area of Catalonia, Spain.	Clarithromycin was completely removed by ozonation, but it was recalcitrant to UV (removal of 60%) and UV/H_2O_2 (removal of almost 80%).	(Justo et al., 2014)
Clarithromycin	46 ng L^{-1}	Biological activated carbon (BAC) process to treat municipal wastewater RO concentrate; lab scale during 320 days of operation; BAC, combined UV/UV/H_2O_2–BAC and ozone–BAC.	70% of clarithromycin was removed by the BAC filter. Pretreatment of RO brine with UV/H_2O_2 or ozonation led to the removal of the pharmaceutical.	(Justo et al., 2015)
Clarithromycin Erythromycin	<750 ng L^{-1}	Pilot-scale photocatalysis: CPC plant under natural solar irradiation; TiO_2 load: 20 mg L^{-1}; Municipal effluents collected downstream of the secondary biological treatment of El Ejido WWTP; Almería, Spain.	Using a low TiO_2 load (29.2 mm photoreactor), the treatment was not effective due to the slow reaction rate; 85% of the pollutants were degraded after 480 min. Increasing the light-path of the reactor, the performance was enhanced (90% of the pollutants removed after 300 min).	(Prieto-Rodriguez et al., 2012)
Clarithromycin Erythromycin	≈0.0275 μg L^{-1} <0.05 μg L^{-1}	Pilot-scale photocatalysis: Solardetox Acadus-2006 CPCs; 3.0 m^2 irradiated surface; 24 L irradiated volume; TiO_2 load: 0.2 g L^{-1}; Wastewater.	Removal was high for all the compounds after 3 h of treatment (bellow LOQ).	(Bernabeu et al., 2011)
Azithromycin	1653.84 ng L^{-1}	O_3/H_2O_2; O_3: 24 g O_3 Nm^{-3}; Gas flow: 0.36 Nm^3 h^{-1}; H_2O_2: 0.15 mL of a 30% (w/v) solution; Wastewater from the secondary clarifier from a sewage treatment plant of Alcalá de Henares; Madrid, Spain.	The removal for azithromycin was 89.6% after 5 min.	(Rodriguez et al., 2011)
Clarithromycin	up to 0.1 μg L^{-1}	Bench-scale ozonation: at pH 8.5 (original) and at pH 7.0 (adjusted by adding H_2SO_4); O_3 doses (g O_3/g DOC): 0.25, 0.5, 1.0, and 1.5; H_2O_2/O_3 molar ratio = 0, 0.25, and 0.5; 24 h composite samples of hospital wastewater effluents from a pilot MBR; Baden, Switzerland.	The elimination of clarithromycin was efficient when the ratio of O_3/DOC was higher than 0.5. The LOQ was achieved and the removal was higher than 92% at both pH conditions. The average removal was 80% using a ratio of O_3/DOC of 0.25.	(Lee et al., 2014)
Azithromycin Clarithromycin Erythromycin	n.d. 228 ng L^{-1} 150 ng L^{-1}	Ozonation of secondary effluent; O_3: 3 mg L^{-1}; Samples were collected from a municipal sewage treatment plant; Tokyo.	The removal efficiencies of all the target macrolides antibiotics were up to 80%.	(Nakada et al., 2007)
Clarithromycin	363–469 ng L^{-1}	lab-scale UV, UV/H_2O_2, solar irradiation, Fenton, solar photo-Fenton; UV-C irradiation (λ_{max} = 254 nm); H_2O_2: 25 mg L^{-1}; Fenton: 25 mg H_2O_2 L^{-1} and 5 mg Fe^{2+} L^{-1}; Photo-Fenton: 2.5 mg H_2O_2 L^{-1} and 5 mg Fe^{2+} L^{-1}; Municipal wastewater from Vidy WWTP; Lausanne, Switzerland.	From the five different treatments applied, only the UV-based processes were able to remove 80% of clarithromycin. After 30 min of treatment, the oxidation was significant, verified by COD and TOC removals. For the cases of solar light, Fenton and photo-Fenton processes, the degradation rates were lower.	(Giannakis et al., 2015)
Clarithromycin	469 ng L^{-1}	Solar Fenton treatment (natural solar driven oxidation) in a pilot-scale CPC plant; H_2O_2: 50 mg L^{-1}; Fe^{2+}: 5 mg L^{-1}; Municipal wastewater from the El Ejido municipal WWTP; Almería, Spain.	Clarithromycin was completely degraded, applying photolytic and solar Fenton experiments, with a removal of 77% at the end of the treatment time (250 min), when present at low concentrations and at low Fenton reagent dosages.	(Karaolia et al., 2014)
Erythromycin	170 ng L^{-1}	Pilot-scale ozonation plant; O_3: 86 –153 g Nm^{-3}; O_3 consumption: 0.6 and 0.9 g O_3 g DOC^{-1}; Wastewater; Austria.	The application of 0.6 g O_3 g DOC^{-1} increased the removal of erythromycin (to values < LOQ).	(Schaar et al., 2010)

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Table 5 (continued)

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Azithromycin Clarithromycin Erythromycin	139.9 ± 6.2 ng L^{-1} 116.4 ± 2.7 ng L^{-1} 27.0 ± 2.5 ng L^{-1}	lab-scale photolytic ozonation, ozonation and photocatalysis; O_3: 50 g Nm^{-3}; O_3 flow rate: 150 Ncm^3 min^{-1}; MP mercury vapor lamp (UV/Vis λ > 300 nm); TiO_2 photocatalyst: 0.5 g L^{-1} load; Urban wastewater from the secondary treatment of a WWTP; North of Portugal.	It was verified a complete removal by photocatalytic ozonation for all macrolide antibiotics, while by ozonation only erythromycin was totally eliminated. Photocatalysis was the less efficient process in study.	(Moreira et al., 2015)
Erythromycin	0.7–0.9 μg L^{-1} (spiked wastewater)	lab-scale ozonation; O_3: 5.5–8.5 mg L^{-1}; O_3 flow rate: 0.39 Ndm^3 min^{-1}; Urban wastewater samples (spiked) from the secondary clarifier of two treatment plants from West-Alcalá and Alcázar de San Juan; Spain.	High removal of erythromycin (>90%) was observed for both wastewaters studied.	(Rodriguez et al., 2012)

BAC, Biological activated carbon; CAS, conventional activated sludge; CAST, cyclic activated sludge technology; CPC, compound parabolic collector; DOC, dissolved organic carbon; GAC, granular activated carbon; LOQ, limit of quantification; MBR, membrane biological reactor; MF, Microfiltration; n.a., not available; n.d., not detected; OD, oxidation ditch; RFDFs, rotary fiber disc filters; RO, Reverse osmosis; SBR, sequential batch reactor; UF, ultrafiltration; WWTP, wastewater treatment plant.

(Derrouiche et al., 2013; Lange et al., 2006).

2.6. Methiocarb

Regarding pesticides, their use plays an important role in harvest quality and food protection, providing enormous benefits to increase production, as pests and diseases are usually responsible to damage up to one-third of crops (Herrero-Hernández et al., 2013). As consequence of massive global consumption, pesticides and their degradation products spread through the environment and can contaminate water resources. Surface and groundwater located in intensive agricultural areas are more susceptible to pesticide contamination, which is a major concern if the water is used for human consumption (Masia et al., 2013). The impact of these contaminants in the environment and to the wildlife is demonstrated by several injurious effects, including the enhancement of the incidence of cancer, birth defects, genetic mutations, or other problems such as damage in the liver or in the central nervous system (Dabrowski et al., 2014). The occurrence of pesticides in aquatic compartments and their possible effects to public health are a topic of considerable environmental interest.

Methiocarb (also known as mercaptodimethur, mesurol, 3,5-dimethyl-4-(methylthio)phenyl methylcarbamate) is one of the most commonly used carbamate pesticides worldwide. This pesticide has been applied since 1960s for a variety of invertebrate pests and also as a bird repellent on fruit crops (Altinok et al., 2006; Qiang et al., 2014). The detected concentrations of methiocarb in wastewater and groundwater are generally low (Table 1); however, it poses a serious health threat to aquatic life and humans considering its high toxicity (Qiang et al., 2014). A negative removal of methiocarb was reported in a Spanish sewage treatment plant (Table 6), probably due to the limitations on the sampling procedure, where both HRT (24–72 h) and SRT (7.5–25 days) were not taken into consideration, consequently higher concentrations were found in the effluents than in influents (Campo et al., 2013). Recent studies related to the removal of this compound by advanced treatment options were not found in the literature.

2.7. Neonicotinoids

In the last decade, the neonicotinoid group of insecticides has been one of the most broadly adopted conventional management tools to deal with insect pests of annual and perennial cropping systems. Benefits of the neonicotinoids include flexibility of application, a wide range of active ingredients and broad spectrum activity (Huseth and Groves, 2014; Morrissey et al., 2015). This group includes imidacloprid, thiacloprid, thiamethoxam, clothianidin and acetamiprid, which are extremely toxic to all aquatic arthropods, except water fleas (Sánchez-Bayo and Hyne, 2014). However, as a result of structural differences in the polypeptide subunit containing the neonicotinoid-binding region of the vertebrates' nicotinic acetylcholine receptors, neonicotinoids pose a relatively low risk to fish and mammals (Sánchez-Bayo and Hyne, 2014). Neonicotinoids are systemic insecticides and are applied as seed dressings by sprays, owing to their solubility in water. Therefore, the main sources of this class of herbicides in the environment are the runoff from agriculture areas and leaching into groundwater, with the consequent subsurface discharge into wetlands and other surface water (Morrissey et al., 2015). As a result of their high water solubility and persistence in soil, neonicotinoids cause a threat for water contamination, mainly after storm events that produce runoff pulses (Sánchez-Bayo and Hyne, 2014). Other sources of these compounds are soluble or insoluble fractions transported via snowmelt, decay of treated plants in water bodies, and deposition of treated seeds or soil into water bodies (Morrissey et al., 2015). Recent studies from Spain, Portugal, USA, Australia and other countries (Table 1) have confirmed the occurrence of this group of pesticides in the aquatic ecosystems (Campo et al., 2013; Chau et al., 2015; da Rocha et al., 2015; Gonzalez-Rey et al., 2015; Masiá et al., 2013; Papadakis et al., 2015; Sánchez-Bayo and Hyne, 2014).

There is a lack of literature concerning the removal of this class of pesticides in the environment (Fig. 2). The majority of the reports refers to the performance of AOPs, dealing with their degradation at laboratory or pilot-scale conditions and mostly using spiked water or spiked simulated water (Pena et al., 2011). Photolysis,

Table 6
Studies dealing with removal of methiocarb. Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Initial methiocarb concentration	Treatment and sampling conditions	Concluding remarks	Reference
3.77–5.74 ng L^{-1} (2010); 1.26–105.31 ng L^{-1} (2011).	Sewage treatment plants monitored in the four River Basins of Ebro River; Spain.	The removal of methiocarb was negative. The higher concentrations in effluents than in influents were attributed to the sampling limitations: influent and effluent samples were collected at the same day, without considering the HRT (24–72 h) and SRT (7.5–25 days).	(Campo et al., 2013)

HRT, hydraulic retention time; SRT, sludge retention time.

Table 7
Studies dealing with removal of neonicotinoids (imidacloprid, thiacloprid, clothianidin and acetamiprid). Pollutants included in these studies that are out of the scope of 495/15/EU Decision are not discussed.

Compound	Initial concentration	Treatment and sampling conditions	Concluding remarks	Reference
Acetamiprid	<0.05 μg L^{-1}	Pilot-scale photocatalysis: Solardetox Acadus-2006 CPCs 3.0 m^2 irradiated surface; 24 L of irradiated volume; TiO_2 load: 0.2 g L^{-1}; Water taken from the outlet of a WWTP from the South East of Spain.	High removal was obtained for all the emerging contaminants after 3 h of treatment (bellow LOQ).	(Bernabeu et al., 2011)
Thiacloprid	0.05 −0.38 mM (spiked spring water)	Photocatalysis: Lab-scale reactor operated in a circular 'closed-loop' mode; Six 18 W UV lamps (λ_{max} = 366 nm); ZnO load: 0.5–3.0 g L^{-1}; Thermal water collected from the spring of Kistelek, Hungary.	Very low degradation was verified by direct photolysis. A removal of 86.6% was observed for thiacloprid, with a ZnO load of 2 g L^{-1} and pH 6.8. The efficiency of the thiacloprid removal in filtered and un-filtered thermal water was about two times lower than from the distilled water, indicating that the removal was due to the dissolved substances.	(Abramović et al., 2013)
Thiacloprid	0.32 mM (spiked river water)	Lab-scale UV and UV/H_2O_2; 125 W HP Hg lamp (emission bands λ = 304, 314, 335, 366 nm) (λ_{max} = 366 nm); H_2O_2 concentration: 0–162 mM; pH: 2.8–9; Spiked water from Begej river at Itebej, Serbia.	The removal rate of thiacloprid was influenced by the presence of HCO_3^-. Very low degradation rates were observed for single UV and H_2O_2. High removal of thiacloprid was achieved after 120 min of UV/H_2O_2. The removal rate for natural water was lower compared with distilled water (45 mM H_2O_2) at pH 8.2. However, the removal in natural water adjusted at pH 2.8 was higher than in distilled water due to the naturally occurring photosensitizers, i.e. dissolved organic matter.	(Abramovic et al., 2010)
Clothianidin	n.a.	Sulfate radical based homogeneous photo-fenton involving peroxymonosulfate as an oxidant, ferrous iron (Fe(II)) as a catalyst and simulated solar irradiation as a light source; Biologically treated domestic wastewater effluents.	PMS/Fe(II)/UV–Vis advanced oxidation system using simulated solar irradiation has demonstrated better kinetic performances over TiO_2/UV–Vis system for clothianidin.	(Ahmed et al., 2014)
Acetamiprid	100 μg L^{-1} (spiked wastewater)	Pilot-scale photocatalysis: CPC; Wastewater of Almería, Spain.	The removal of acetamiprid was poor in the wastewater matrix.	(Jiménez et al., 2015)
Imidacloprid	60 mg L^{-1}	Pilot-scale CPC plant (60 L); Fe(III)-EDDS as complexing agent; Spiked tap water from the groundwater well of Plataforma Solar de Almería, Spain.	Photolysis of the complexing agent generated radical species able to act independently of carbonate scavengers that are present in natural water.	(Papoutsakis et al., 2015)

CPC, compound parabolic collector; EDDS, ethylenediamine-N,N′-dissucinic acid; HP, high pressure; LOQ, limit of quantification; n.a., not available; WWTP, wastewater treatment plant.

photocatalysis and photo-Fenton were applied to study the removal of these compounds from water, photocatalysis being the most applied (Table 7). Studies dealing with real water and other treatment processes should be performed to bring a more realistic overview of the elimination of this group of pesticides. Some other studies with these substances were already published, but not using real matrices and, therefore, they are out of the scope of the present review; for instance the degradation of imidacloprid (Peng et al., 2015; Tang et al., 2011; Wang et al., 2014; Zabar et al., 2012; Zarora et al., 2010), thiamethoxam (Mir et al., 2013; Šojić et al., 2012; Zabar et al., 2012), clothianidin (Zabar et al., 2012) and acetamiprid (Mitsika et al., 2013) were studied with photo-assisted and ozonation processes.

2.8. Oxadiazon

The oxadiazole herbicide oxadiazon [5-tert-butyl-3-(2,4-dichloro-5-propan-2-yloxyphenyl)-1,3,4-oxadiazol-2-one] has been habitually used to combat weeds in various agricultural crops such as rice, cotton, soybean, potato, peanut and onion. Oxadiazon is an organic contaminant causing a great environmental concern due to its relatively long half-life (Rahman, 2010). Previous studies on the leaching of oxadiazon in soils indicated that, the strong adsorption of the herbicide to soils reduces the displacement towards the sub-surface layers (Pinilla et al., 2008). However, oxadiazon was found in surface water in Canada (Table 1) at ng L^{-1} levels (Furtula et al., 2006). In contrast, the removal of oxadiazon in aquatic matrices is still unknown.

2.9. Triallate

Triallate (S-2,3,3-trichloroallyl di-isopropyl thiocarbamate) is a carbamothioate herbicide widely used to control annual and perennial grasses in wheat, barley, legumes and a number of other crops (D'Orazio et al., 1999; Volpe et al., 2004). This pesticide is often used in mixture with other chemicals (chloridazon, isoproturon, metoxuron) and its use, in the last decades, has exceeded 500 tons per year in some European countries (Volpe et al., 2004). Triallate has a high hydrophobic partitioning (Hornsby et al., 1996), therefore it adsorbs to loam and clay soils and is not readily dissolved in water (Bernal et al., 1996). This information indicates that this herbicide is not likely to move through the soil, even though it has a long soil half-life (82 days). Nevertheless, if there is significant moisture and/or low levels of organic matter in the soil, triallate may become desorbed from soil particles (D'Orazio et al., 1999). Leaching and consequent groundwater contamination would be possible in such situations, but Environmental Protection Agency (EPA) suggests that triallate leaching does not cause a threat to the environment, since it is usually used where the water table is relatively low (Kamrin, 1997). A lack of knowledge exists about its occurrence and removal in the aquatic environment due to its chemical nature.

3. Conclusions

Despite the considerable amount of studies reported on the occurrence and removal of E1, E2, EE2, diclofenac and macrolide antibiotics (azithromycin, clarithromycin and erythromycin), a lack

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of knowledge exists concerning the pesticides (methiocarb, neonicotinoids, oxadiazon and triallate), the UV filter (EHMC) and the antioxidant (2,6-di-tert-butyl-4-methylphenol), which are included in the watch list of Decision 2015/495/EU for European Union monitoring. Thus, more investigation is needed regarding the occurrence and removal of neonicotinoids, EHMC and 2,6-di-tert-butyl-4-methylphenol and the performance of different treatments to remove the substances included in the watch list under realistic conditions. These compounds are usually present at residual concentrations, as mixtures in the different environmental compartments (e.g., municipal wastewater, surface water, groundwater, solid matrices) and comprehensive works considering it are scarce. As shown by different studies, the efficiency of the treatment processes can decrease considerably when realistic water matrices are used instead of simulated ones. For example, the presence of carbonates and bicarbonates can decrease the efficiency of AOPs, principally due to competition by HO•. Since multiple factors can affect the efficiency of the treatments, experiments should be performed as close as possible to the real conditions. Additionally, the formation of intermediates should be attempted in this type of studies, considering that the produced by-products might be more toxic and/or persistent than the parent compounds. Toxicological studies are needed to determine the deleterious effects on the ecosystems and human health of parent compounds and by-products formed in real matrices. Considering the scale up of the treatment option, these processes can be expensive both in the implementation and maintenance, therefore it is of major importance to perform cost effectiveness analysis for each of them under a common base of comparison.

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Appendix B

Original version and supplementary material of Chapter 3:

Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal

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RESEARCH PAPER

Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal

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Abstract Organic micropollutants present in drinking water (DW) may cause adverse effects for public health, and so reliable analytical methods are required to detect these pollutants at trace levels in DW. This work describes the first green analytical methodology for multi-class determination of 21 pollutants in DW: seven pesticides, an industrial compound, 12 pharmaceuticals, and a metabolite (some included in Directive 2013/39/EU or Decision 2015/495/EU). A solid-phase extraction procedure followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (offline SPE–UHPLC–MS/MS) method was optimized using eco-friendly solvents, achieving detection limits below 0.20 ng L^{-1}. The validated analytical method was successfully applied to DW samples from different sources (tap, fountain, and well waters) from different locations in the north of Portugal, as well as before and after bench-scale UV and ozonation experiments in spiked tap water samples. Thirteen compounds were detected, many of them not regulated yet, in the following order of frequency: diclofenac > norfluoxetine > atrazine > simazine > warfarin > metoprolol > alachlor > chlorfenvinphos > trimethoprim > clarithromycin ≈ carbamazepine ≈ PFOS > citalopram. Hazard quotients were also estimated for the quantified substances and suggested no adverse effects to humans.

Keywords Drinking water · Priority substances · Contaminants of emerging concern · Solid-phase extraction · Ultra-high-performance liquid chromatography–tandem mass spectrometry

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Introduction

Many micropollutants are not completely removed during conventional domestic wastewater treatment and are discharged into water bodies (such as rivers) that are then used to supply drinking water treatment plants (DWTPs) providing tap water. Amoxicillin, naproxen, metoprolol, phenacetin, indomethacin, sulfamethoxazole, and caffeine are some of these refractory micropollutants, and despite their low concentrations in DW, they are of increasing public health concern [1, 2]. Moreover, even if public health effects are not expected, chemical compounds may cause ecotoxicological adverse effects after long-term exposure, particularly when present as complex mixtures [3, 4].

Some regulations on water pollution have been published in recent years. In the particular case of the European Union (EU), the requirements for a good chemical status of groundwater have been set out in Directive 2006/118/EC [5] and the values for wholesome and clean water for human consumption in Directive 1998/83/EC [6]. Moreover, the EU identified surface water protection as one of the top work priorities due to the increasing demand for water protection and treatment by environmental organizations and the general public. Directive 2000/60/EC [7] was the first mark in the European water policy, which set up a strategy to define high-risk substances to be prioritized. A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by Directive 2008/105/EC [8]. In 2013, Directive 39/2013/EU [9] recommended attention to the monitoring and the progress of

innovative water/wastewater treatment technologies, identifying 45 PSs to meet requirements for the protection of the aquatic compartments and human health. More recently, a set of substances for EU monitoring in surface water bodies was defined in the Watch List of Decision 2015/495/EU [10]. The occurrence and removal of these substances was already reviewed [11]. However, reports focused on the determination of organic micropollutants in DW, and in particular regarding contaminants of emerging concern (CECs), are still scarce and most countries do not have monitoring programs to routinely determine these micropollutants. In fact, the analytical challenge of measuring pollutants at low concentrations in environmental matrices, such as sludge and wastewater [12, 13], has been a major research focus for scientists in recent decades, but much less attention has been given to DW [14]. In this context, it is crucial to develop sensitive and reproducible analytical methods that enable the determination of organic micropollutants belonging to different classes in DW.

The employment of an accurate and precise sample preparation as well as analytical techniques with high standards of sensitivity and reproducibility, such as ultra-high-performance liquid chromatography (UHPLC), is required to assess the occurrence and respective removal of micropollutants after water treatment. Hyphenated chromatography–mass spectrometry techniques are presently the methods of choice for DW analysis (Electronic Supplementary Material (ESM) Table S1), with only few works dealing with both pharmaceuticals and pesticides [15, 16], some with pesticides and/or their metabolites [17–19], and most referring only to pharmaceuticals and/or their metabolites [14, 20–27]. Considering the resources and time consumed in these tasks, new analytical methods should incorporate multi-residue and environmentally friendly approaches, being able to determine trace levels of a wide range of chemically heterogeneous compounds and simultaneously reduce the cleanup and extraction steps using green solvents [28, 29].

Green chemistry principles were introduced in the 1990s, aiming to reduce the environmental impact of diverse chemical activities, including those used in research [30, 31]. In this scenario, green analytical chemistry (GAC) plays an important role, e.g., by reducing hazardous wastes, using reusable materials, and/or employing "eco-friendly solvents" or "green solvents". The last two terms refer to solvents that have a lower environmental impact resulting from their production, use, and disposal (life cycle assessment), and/or that allow health and safety impacts to be minimized [32]. The main goals of GAC include the multi-analyte determination and the development of new (or modification of) analytical methodologies through the replacement of toxic reagents by smaller amounts of safer reagents, preferentially obtained from renewable sources [29, 32]. Several strategies have been used in LC–MS/MS, such as the reduction of the internal diameter and particle size (sub-2 μm) of chromatographic columns (to diminish eluent consumption), and the replacement of conventional mobile phases (consisting of acetonitrile and/or methanol) by environmental friendly alternatives like water, ethanol, and carbon dioxide in the particular case of supercritical fluid chromatography [30, 33].

The aim of this work was the optimization and validation of an eco-friendly analytical method based on offline SPE-UHPLC–MS/MS for the multi-class determination of organic micropollutants (12 pharmaceuticals, one metabolite, seven pesticides, and one industrial compound) in DW from northern Portugal. The targeted organic contaminants (ESM Table S2) were selected on the basis of their inclusion in EU regulations; some of the compounds are specified in Directive 2013/39/EU or in the Watch List of Decision 2015/495/EU. The selected micropollutants were previously reported as toxic and frequently found in the aquatic environment [14, 20, 34]. The occurrence of the multi-class contaminants was investigated for the first time in DW samples from different sources (tap, fountain, and well waters) and locations in northern Portugal, and the related hazard quotients (HQs) were determined. The HQs evaluation for these micropollutants could be a predictive way to assess the human health risk of exposure to CECs, but only a few reports focused on this approach for organic contaminants in DW [2, 14, 34–37]. The efficiency of two processes (UV and ozonation) typically employed for DW disinfection and/or degradation of organic pollutants in DWTPs was also verified using the analytical strategy proposed.

Experimental

Chemicals and materials

All reference standards (diclofenac sodium, tramadol hydrochloride, azithromycin dihydrate, clarithromycin, trimethoprim, warfarin, clopidogrel hydrogen sulfate, metoprolol tartrate, carbamazepine, citalopram hydrobromide, venlafaxine hydrochloride, fluoxetine hydrochloride, norfluoxetine oxalate, alachlor, atrazine, simazine, isoproturon, chlorfenvinphos, pentachlorophenol, clofibric acid, and perfluorooctanesulfonic acid; >98 % purity) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of approximately 1000 mg L^{-1} were prepared in methanol, ethanol, or acetonitrile, depending on the solubility of each analyte. Two working standard solutions containing all the target analytes at 200 μg L^{-1} and 20 μg L^{-1} were prepared by diluting each stock solution in ethanol. Surrogate standards (ketoprofen-d_3, fluoxetine-d_5 solution, and atrazine-d_5) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of 1000 mg L^{-1} of the isotopically labeled internal standards ketoprofen-d_3 and atrazine-d_5 were prepared in methanol, the same solvent as the fluoxetine-d_5 solution. An ethanolic working solution containing 1 mg L^{-1} of each isotopically labeled internal standard was prepared.

Methanol and acetonitrile (MS grade) were obtained from VWR International (Fontenay-sous-Bois, France). Ethanol (HPLC grade) and ethylenediaminetetraacetic acid (EDTA) (99 %) were acquired from Fisher Scientific UK Ltd. (Leicestershire, UK). Sodium thiosulfate and L-ascorbic acid (99 %) were purchased from Sigma-Aldrich (Steinhein, Germany). Ammonium acetate, ammonium hydroxide 25 %, sulfuric acid, and formic acid were obtained from Merck (Darmstadt, Germany). Ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ cm, at 25 °C). HPLC-grade solvents were filtered with 0.22-µm nylon membrane filters (Membrane Solutions, TX, USA). Oasis® HLB (Hydrophilic-Lipophilic-Balanced), Oasis® MCX (Mixed-mode Cation eXchange), and Oasis® MAX (Mixed-mode Anion-eXchange) cartridges (150 mg, 6 mL), obtained from Waters (Milford, MA, USA), were tested for SPE optimization. A pHenomenal® pH 1100L pH meter (VWR, Germany) was used for the pH adjustments.

Sample preparation

Tap water samples were collected from the water supply network for use as matrix for the SPE optimization and method validation. The vacuum extraction and drying devices LiChrolut® used for SPE procedure were acquired from VWR (Merck Millipore, Billerica, MA, USA). In order to assess the best performance of SPE cartridges to extract the overall compounds, SPE optimization was performed by comparing Oasis® HLB, MCX, and MAX cartridges. Oasis® MAX and MCX cartridges were conditioned sequentially with 4 mL of methanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. For HLB cartridges, the conditioning was performed at the same flow with 4 mL of methanol or ethanol and 4 mL of ultrapure water. The sample pH was optimized for HLB cartridges using methanol as conditioning solvent by comparing the recoveries achieved with initial sample pH adjusted to 3, 7, and 9. For MAX and MCX SPE procedures, samples were respectively alkalinized to pH 9 or acidified to pH 3, before loading. The pH adjustments were done with ammonium hydroxide or sulfuric acid. Sample loading was carried out with 250 mL of blank and spiked (35 ng L^{-1}) tap water samples at a constant flow rate of 10 mL min^{-1}, using the vacuum manifold unit connected to a vacuum pump. The washing step was performed with 4 mL of ultrapure water, 5 % ammonium hydroxide aqueous solution, or 2 % formic acid aqueous solution for HLB, MAX, and MCX, respectively. After the washing steps, the cartridges were dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of methanol or ethanol for Oasis® HLB cartridges, 4 mL of methanol to extract the neutral compounds and weak bases in the case of Oasis® MAX, and neutrals and weak acids in the case of Oasis® MCX. A second elution was performed for mixed-mode cartridges Oasis® MAX and MCX with a 2 % formic acid methanolic solution (elution of acids) or 5 % ammonium hydroxide methanolic solution (elution of basic compounds), respectively. The LiChrolut® drying device was coupled to the vacuum extraction unit to evaporate the extracts to dryness with a gentle nitrogen stream. The dry residues were reconstituted in 300 µL of ethanol and the ethanolic extracts were filtered using 0.22-µm polytetrafluoroethylene syringe filters (Membrane Solutions, TX, USA). To assess the breakthrough volume, sample loading was tested with three volumes of non-spiked (blanks) and 35 ng L^{-1} spiked tap water samples, namely 250, 500, and 1000 mL, using the optimized SPE procedure. In order to improve the recovery rates, the chelating agent EDTA (100 mg L^{-1}) was tested as well as two dechlorination agents, ascorbic acid (10 mg L^{-1}) and sodium thiosulfate (30 mg L^{-1}). Analysis of reuse efficiency for the optimized SPE protocol was performed in three consecutive days.

UHPLC–MS/MS

A Kinetex™ 1.7 µm XB-C18 100 Å column (100 × 2.1 mm, i.d.) (Phenomenex, CA, USA) was used and different mobile phases were tested (acetonitrile, ethanol, or methanol as organic phase and ammonium acetate, formic acid aqueous solutions, or water as aqueous phase). The optimized mobile phase was ethanol/water (70:30, v/v), pH 7.0, performed in isocratic mode using a flow rate of 0.20 mL min^{-1}. Column oven and autosampler temperatures were set respectively at 35 and 4 °C, and the volume of injection was 5 µL. An electrospray ionization source was used operating in both positive and negative ionization modes. The precursor ion and the two most abundant fragments were used for quantification by selected reaction monitoring (SRM) and identification (ESM Table S3). The mass spectrometer parameters declustering potential, collision energy, and collision cell exit potential of each analyte are described elsewhere [38]. The optimized conditions for MS parameters, using argon at 230 kPa as CID gas, were 2.5 dm^3 min^{-1} for nebulizing gas flow, 10 dm^3 min^{-1} for drying gas flow, 0.5 kV for capillary voltage, 450 °C for source temperature, and 200 °C for desolvation temperature.

Quality assurance/quality control

The offline SPE–UHPLC–MS/MS method validation was performed according to the international guidelines [39] and previous works [38, 40, 41], through the evaluation of the following parameters: selectivity, linearity and range, limits of detection and quantification, accuracy, precision, and recovery. Chromatograms of non-spiked tap waters (blank extracts), standards extracted from the spiked tap waters at 35 ng L^{-1}, and an ethanolic solution containing all the standards at a concentration corresponding to the theoretical concentration after SPE were compared to assess the selectivity. For

recovery experiments, three quality control (QC) standard solutions were prepared in triplicate in three consecutive days by extracting tap water samples spiked with three different concentrations (3.5, 15, and 35 ng L^{-1}). The peak areas of the standards extracted from the spiked tap waters were compared with those of ethanolic solutions containing all the standards at the theoretical concentration of recovered extracts to assess the recovery of each SPE procedure. For target compounds detected in the blank matrix, the peak areas were subtracted from those obtained with the spiked matrix.

The internal standard calibration method was used to define the linearity and range for each target analyte. Triplicates of 250 mL tap water samples spiked with seven different standard concentrations (0.75, 1.5, 2.0, 4.0, 8.0, 20, and 40 ng L^{-1}) were prepared, the pH was adjusted to 3, and sodium thiosulfate solution was added to obtain a concentration of 30 mg L^{-1}. Then 10 μL of a working internal standards solution of 1 mg L^{-1} was added to each sample. These standard solutions were extracted by the optimized SPE procedure and reconstituted in 300 μL of ethanol to create the calibration curves by injecting 5 μL into the UHPLC apparatus. Method detection (MDL) and quantification (MQL) limits were determined as described elsewhere [38, 41], spiking water samples prior to the SPE procedure with ethanolic standard solution to achieve successively diluted samples. The minimum detectable amount of each compound giving a signal-to-noise (S/N) ratio of 3.3 and 10 gave MDL and MQL, respectively. The three triplicate QC solutions, described above, were also used to evaluate the accuracy of the method as well as the precision (intra- and interbatch). The concentrations of the analytes in the SPE extracts calculated using the calibration curves were compared with the nominal concentration, in percentage, to determine the accuracy. The relative standard deviation (RSD) of the intra- and interbatch replicate analyses expressed the precision of the method [42, 43]. In order to evaluate the possible carry-over effect, ethanol was injected after each set of triplicates. The stability of the compounds was assessed by calculating the RSD of the three QC extracts stored at 4 °C in the autosampler 24 and 48 h after reconstitution.

Matrix effect

The post-extraction addition method was used to assess the matrix effect [38, 41, 43]. The method was carried out on tap water samples, by comparison of three post-spiked extracts of blank samples and three extracts of non-spiked blank samples, using the optimized SPE procedure. The matrix effect (ME) was calculated as the ratio of the peak areas obtained for blank extracts spiked after SPE, subtracting those of the non-spiked blanks (A) and the peak areas of the standards solution with a similar concentration as the post-spiked extracts (B) through the following equation: ME (%) = $A/B \times 100$ [41, 43]. The absence of matrix effect, the ionization enhancement, and the ionization suppression are given respectively by values of 100 %, >100 %, or <100 %.

Application to drinking water samples and chemical treatment

Grab DW samples from different sources, namely tap water ($n = 13$), fountain water ($n = 5$), and well water ($n = 5$), were collected at the end of May 2015 from various locations in northwest Portugal and analyzed by the proposed method. Samples were immediately stored at 4 °C until extraction, which was performed within 24 h. Before SPE, samples were acidified with sulfuric acid (pH 3), and sodium thiosulfate was added to each sample (30 mg L^{-1}) to reduce any residual chlorine that might be added as a disinfectant.

Tap water samples collected from the water supply network were spiked with the target analytes at 30 ng L^{-1} to assess the applicability of the present UHPLC–MS/MS method to assess the removal of the target micropollutants by chemical processes. UV and ozonation experiments were performed as described elsewhere [44], and the removal of the target micropollutants was evaluated after 30 min using a 1 L reactor loaded with 750 mL of the spiked samples under magnetic stirring at 350 rpm.

Human health risk assessment

For those substances found in DW, a preliminary human health risk assessment was performed through the estimation of the HQ according to previous works [35, 45]. HQ is given by the quotient of the estimated daily intake (EDI) and the acceptable daily intake (ADI):

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{ADI}} \quad (1)$$

where EDI values were calculated for the higher concentration of each substance quantified in tap, fountain, or well water as follows:

$$\mathrm{EDI} = \frac{\text{Concentration} \times \text{Ingestion rate}}{\text{Body weight}} \quad (2)$$

by considering an average body weight of 70 kg for adults based on the average life expectancy at birth of the global population in 2013 of the World Health Organization and a water intake of 2 L day^{-1} [35]. ADI for each pesticide was based on the Australian ADI list [46], whereas the values for pharmaceuticals were calculated from Eq. 3:

$$\mathrm{ADI} = \frac{\mathrm{ADD}}{\mathrm{AF}} \quad (3)$$

where ADD is the average daily dose and AF is an assessment factor of 1000, which accounts for 10 from intraspecies variability, 10 for sensitivity in susceptible population groups, and 10 for the differences between the ADD and the no observed effect concentration [35, 37].

Results and discussion

UHPLC–MS/MS optimization

Chromatographic separation was optimized using a sub-2-μm-particle Kinetex™ column, allowing short and high resolution chromatographic runs. Since the present work deals with different groups of compounds with a vast range of physicochemical characteristics (ESM Table S2), the ideal mobile phase for certain target compounds might lead to low sensitivity for many other analytes. The mobile phase consisting of ethanol and ultrapure water gave the best signal intensity and symmetric peaks as previously found for a wastewater matrix [38]. The variation of organic/aqueous phase proportion and flow rate was optimized, and a mixture of ethanol and ultrapure water (70:30, v/v) was used with a flow rate of 0.20 mL min^{-1} in isocratic mode. The column oven temperature was set at 35 °C, thereby improving the resolution and peak shape of the analytes and reducing the analysis time to 15 min because raising the temperature reduces the viscosity of the mobile phase.

MS/MS optimization

The tandem MS detection using a triple quadrupole enabled the simultaneous quantification of the 21 analytes at trace levels, as well as confirming their identity. The precursor ions of each compound were selected through the flow injection analysis of each target analyte in full scan mode, under both positive and negative modes. From all the compounds studied in this work, 18 compounds and two internal standards had a higher intensity under positive mode of ionization, with the protonated molecular ion of each compound $[M+H]^+$ chosen as precursor ion, whereas four substances (three compounds and one internal standard) were more intense in the negative ionization mode using the deprotonated molecular ion of each compound $[M-H]^-$ as precursor ion. Most compounds presented two or more SRM; the most abundant product ion from each precursor ion (SRM1) was selected for quantification and the second most abundant (SRM2) was monitored for identity confirmation (ESM Table S3), with a scan time of 100 ms per transition. In order to confirm the identity of the compounds, both the retention time (Table 1) and the ion ratio (SRM1/SRM2) of each analyte were used, according to European Commission Decision 2002/657/EC. Two pharmaceuticals and one pesticide (tramadol, fluoxetine, and pentachlorophenol) had a poor fragmentation and only one SRM was monitored, a drawback overcome by the internal standard calibration using the respective surrogate standard.

SPE optimization

A detailed optimization study was carried out on the most relevant parameters that affect recovery rates and matrix effects, namely the sample pH, the extraction solvents, the type of cartridges, the sample volume, and the addition of chelating and dechlorination additives. Preliminary studies were performed to evaluate the performance of different sample pH, by extracting 250 mL of tap water samples through the versatile Oasis® HLB cartridges. The water samples were adjusted to different pH (3, 7, and 9) and extracted using a conventional solvent, i.e., methanol, as conditioning and eluting solvent. Acidic pH provided higher recoveries for acidic compounds, and in particular for pesticides and some pharmaceuticals (ESM Fig. S1); whereas, basic analytes were recovered better at higher pH, but a lower influence of pH on the extraction efficiencies was found for these compounds. Thus, the best compromise was to adjust the sample pH to 3 in order to get the best recovery for as many analytes as possible.

Recoveries of Oasis® MCX cartridges useful for extraction of basic compounds and Oasis® MAX adequate for extraction of acidic compounds were then compared to Oasis® HLB cartridges. A recovery higher than 70 % was achieved using Oasis® MCX for the antidepressants (citalopram, venlafaxine, fluoxetine) and for trimethoprim (Fig. 1). These results were expected owing to the high pK_a of these compounds (near 9). Clofibric acid and diclofenac were better recovered when extracted by Oasis® MAX cartridges (Fig. 1), owing to their acidic nature (pK_a values of approximately 4). However, the versatile Oasis® HLB cartridges suitable for most compounds (acidic, basic, and neutrals) provided higher recoveries for most analytes (Fig. 1), as observed in other works [15, 20]. Thus, Oasis® HLB was the adsorbent selected for the next recovery experiments, using sample pH adjusted to 3.

Different sample volumes were tested (250, 500, and 1000 mL) using Oasis® HLB cartridges and sample pH adjusted to 3 to determine the breakthrough volume, the volume that allows the maximum extraction efficiency and from which extraction efficiency declines [41]. A sample volume of 250 mL provided the highest recoveries for the majority of the compounds, except for fluoxetine and norfluoxetine, and was therefore selected as the optimized sample volume (data not shown). Although a higher volume would give a theoretical higher enrichment factor, the results showed that recovery rates for most compounds decreased using higher sample volumes because of the aforementioned phenomenon of decrease of extraction efficiency above the so-called breakthrough volume, as previously described [47]. Although a clean matrix was studied in the present work, it is reported in other studies

Table 1 Retention time, range, linearity, method detection (MDL) and quantification (MQL) limits, accuracy, precision (intra- and interbatch), and matrix effect for each target analyte

Class and subclass	Analyte	Retention time (min)	Range ($ng\,L^{-1}$)	r^2	MDL ($ng\,L^{-1}$)	MQL ($ng\,L^{-1}$)	Accuracy (%)	Intrabatch precision RSD (%)	Interbatch precision RSD (%)	Matrix effect (%)
Pharmaceuticals										
Anti-inflammatories	Diclofenac	1.27	0.75–40	0.9982	0.17	0.52	106.3 ± 10.5	1.67–8.48	10.1	22.2 ± 2.3
	Tramadol	5.65	0.75–40	0.9976	0.07	0.22	103.7 ± 9.3	2.28–3.55	12.9	117.1 ± 0.1
Antibiotics	Azithromycin	8.08	0.75–40	0.9969	0.20	0.61	93.4 ± 13.3	7.93–9.75	9.38	23.7 ± 8.4
	Clarithromycin	8.47	0.75–40	0.9957	0.11	0.32	104.1 ± 6.1	7.75–10.0	11.2	26.4 ± 11.5
	Trimethoprim	4.00	0.75–40	0.9993	0.07	0.21	97.1 ± 15.7	2.99–5.80	7.21	64.9 ± 13.3
Anticoagulant	Warfarin	1.28	0.75–40	0.9965	0.17	0.52	97.6 ± 15.1	7.67–15.2	10.6	193.4 ± 1.7
Antiplatelet agent	Clopidogrel	2.11	0.75–40	0.9982	0.01	0.04	112.1 ± 6.6	2.75–8.24	6.89	77.4 ± 10.3
Beta-blockers	Metoprolol	6.29	0.75–40	0.9984	0.05	0.15	109.3 ± 0.7	3.26–14.0	13.2	113.1 ± 5.6
Psychiatric drugs	Carbamazepine	1.32	0.75–40	0.9966	0.19	0.59	100.6 ± 3.5	9.76–15.0	8.38	30.4 ± 8.4
	Citalopram	6.06	0.75–40	0.9961	0.09	0.26	86.6 ± 6.4	5.09–11.4	14.5	113.2 ± 11.7
	Venlafaxine	6.84	0.75–40	0.9978	0.10	0.32	105.2 ± 5.4	1.11–4.60	14.5	108.9 ± 2.1
	Fluoxetine	8.86	0.75–40	0.9963	0.04	0.13	118.1 ± 0.3	0.77–3.96	5.19	95.2 ± 6.4
Metabolite	Norfluoxetine	8.93	0.75–40	0.9975	0.05	0.16	119.0 ± 0.2	3.04–6.79	6.99	95.2 ± 4.4
Pesticides										
Chloroacetanilide	Alachlor	1.65	0.75–40	0.9975	0.09	0.28	98.8 ± 0.3	6.39–14.9	8.97	99.2 ± 10.3
Triazine	Atrazine	1.33	0.75–40	0.9945	0.12	0.37	92.3 ± 2.8	2.60–6.47	7.86	52.5 ± 15.4
	Simazine	1.21	0.75–40	0.9983	0.15	0.46	84.9 ± 4.6	3.86–9.23	8.35	49.8 ± 2.4
Organophosphorus	Chlorfenvinphos	1.62	0.75–40	0.9971	0.18	0.54	98.6 ± 6.2	5.01–14.7	14.8	96.9 ± 2.0
Phenylurea	Isoproturon	1.34	0.75–40	0.9968	0.04	0.12	99.2 ± 3.4	2.00–4.10	5.02	34.4 ± 9.4
Organochlorine	Pentachlorophenol	1.55	0.75–40	0.9986	0.20	0.60	94.1 ± 6.8	7.75–13.2	8.65	57.5 ± 9.0
Herbicide	Clofibric acid	1.23	0.75–40	0.9995	0.14	0.42	92.7 ± 5.5	6.20–11.0	6.57	19.1 ± 6.5
Industrial compound	PFOS	1.07	0.75–40	0.9957	0.06	0.19	80.6 ± 6.2	5.30–13.5	4.51	48.7 ± 1.4

MDL method detection limit, *MQL* method quantification limit

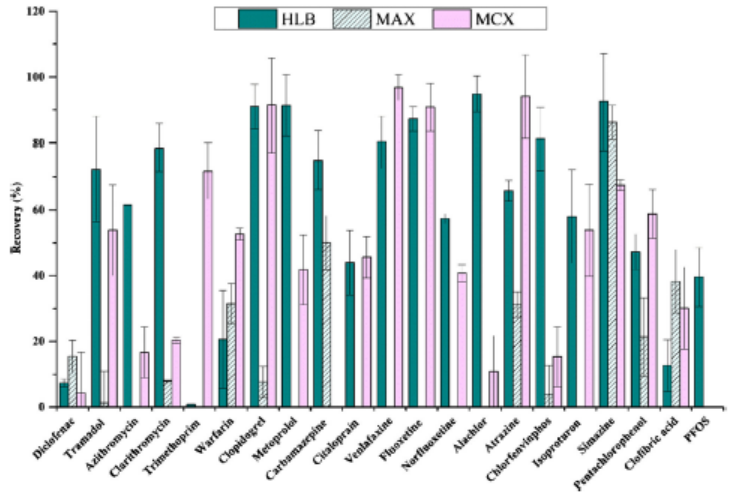

Fig. 1 Recoveries obtained for the target analytes with the following SPE conditions: HLB, MAX, and MCX using methanol and extracting 250 mL of tap water samples, adjusted to pH 3 for HLB and MCX and pH 9 for MAX cartridges

dealing with different matrices that even when using the same method, the recovery is not always better for matrices that are supposed to be cleaner [20, 48].

Afterwards, Oasis® HLB cartridges were employed to extract 250 mL of tap water samples at pH 3 (optimized for methanol) using ethanol as conditioning and elution solvent because of the known toxicity of methanol, usually used for SPE. Ethanol (Fig. 2) gave recoveries slightly higher than methanol (Fig. 1) for the majority of compounds. Moreover, ethanol is considered a "green" solvent, i.e., minimizes the environmental impact resulting from the use of solvents, and follows the guidelines of GAC [28, 29]. In fact, several methods reported in the literature employ solvents such as methanol or acetonitrile, presenting high toxicity [14, 15, 20, 22, 23, 27]. Thus, ethanol was selected as solvent for the next experiments. This is the first SPE procedure proposed for extraction and cleanup of DW samples that employs ethanol as extracting and eluting solvent.

Subsequently, the chelating and dechlorination effects were studied. Whilst a solution of EDTA was added to the water samples to test the chelating effect, ascorbic acid or sodium thiosulfate was added to assess the dechlorination effect. Regarding to the addition of EDTA, it was possible to verify a slight improvement in the extraction efficiency of a few compounds (Fig. 2), compared with the results obtained for samples without additive, namely for chlorfenvinphos, clofibric acid, trimethoprim, and diclofenac. This could be explained by the fact that these compounds might bind to residual metals present in the sample matrix, resulting in lower extraction recoveries [20]. By adding EDTA, soluble metals bind to the chelating agent, increasing the extraction efficiency of some compounds that are available to be extracted and detected [20]. This phenomenon was previously observed in DW by several authors [14, 20, 23]. Concerning the dechlorination agents, the addition of sodium thiosulfate increased the overall extraction recoveries (Fig. 2), probably because it reduced the residual chlorine that had been added as a disinfectant in the DW supply [22]. The effects of filtering and/or aeration of the water samples and the simultaneous addition of EDTA and sodium thiosulfate were also studied; however, the recovery efficiency was not improved. Therefore, sodium thiosulfate was used before SPE to enhance the recovery rates.

The main objective of the optimization of the sample preparation methodology was the development of a single SPE procedure, allowing the extraction of a large group of compounds with different physicochemical characteristics. As a result, and according to the higher recoveries obtained for most of the target compounds, the selected conditions were Oasis® HLB cartridges, ethanol as conditioning and eluting solvent, and 250 mL of water samples (pH 3) with sodium thiosulfate at 30 mg L^{-1} as dechlorination agent.

The recoveries obtained for reuse performance assessment of the cartridges showed that each reuse led to a loss of retention capacity of the cartridges, reflected by the decrease of the

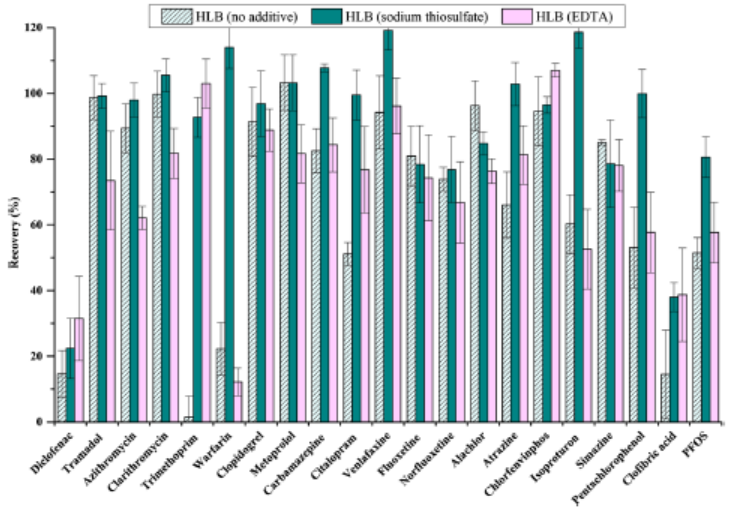

Fig. 2 Recoveries obtained for the target analytes with the following SPE conditions: HLB cartridges using ethanol, extracting 250 mL of tap water samples, adjusted to pH 3, without additives, with sodium thiosulfate, or EDTA as additives

recovery of the compounds. The first reuse of the cartridges led to an average decrease of 14 % in the recovery efficiency. The loss was higher for the second reuse, with a decrease of approximately 50 % in the recovery rates. Here, it was verified that although claimed by the supplier, reuse of cartridges is not a good practice for analytical purposes that require a high reproducibility.

Matrix effect

The matrix effect was determined by the post-extraction addition method to assess the influence of the matrix in the ionization process occurring in the ionization source of the mass spectrometer [38]. The percentage ratio between the post-spiked blank extracts and ethanolic standard solutions was between 19.1 % and 193 %. Although DW is considered a clean and simple matrix, a wide range of values was found for the matrix effect. Cotton et al. [49] also reported high matrix interferences for many compounds; only less than half of the analytes had matrix effect values within 80–120 %. When LC–MS/MS methods are developed to determine various micropollutants in different matrices, e.g., DW, surface water, and wastewater, matrix effects are usually calculated for only one of these matrices. Most compounds presented signal suppression, i.e., matrix effect < 100 %, namely diclofenac, azithromycin, clarithromycin, trimethoprim, clopidogrel, carbamazepine, atrazine, simazine, isoproturon, pentachlorophenol, clofibric acid, and PFOS (Table 1). Tramadol, metoprolol, citalopram, and venlafaxine had a slight ionization enhancement (matrix effect > 100 %) while the signal of warfarin was highly increased. Compounds with almost no matrix effect, under the conditions of the current work, were fluoxetine, norfluoxetine, alachlor, and chlorfenvinphos.

Quality assurance/quality control

The trends of GAC were applied in the chromatographic optimization, namely the use of low volumes of non-toxic solvents [28, 29]. Enhanced productivity and reduced cost are the main objectives for routine analysis, both being possible using stationary phases with reduced column length and diameter [30, 33]. Also the new instruments operating at higher pressure allow the use of more viscous solvents such as ethanol, which is less volatile than acetonitrile and has less toxicity and lower disposal costs than both acetonitrile and methanol, complying with the trends of GAC. The short run time and the low volume of a non-toxic organic phase such as ethanol are a great achievement in the method development, in comparison to chromatographic methods for DW analysis using methanol [15, 20, 21] or acetonitrile [14, 22, 23, 27] as organic mobile phases, as well as methanol as solvent for conditioning and eluting the SPE cartridges

[14, 15, 20, 22, 23]. In the present work, 21 compounds with diverse chemical nature (seven pesticides, one industrial compound, 12 pharmaceuticals, and one metabolite) were determined in a single run (ESM Fig. S2a, b). In the limited literature for DW analysis, the number of compounds analyzed by LC–MS/MS varies up to ca. 80, most reports deal with pharmaceuticals only [14, 20–23, 27], and a couple of them deal with both pharmaceuticals and pesticides [15, 16].

The offline SPE–UHPLC–MS/MS method was validated according to the international guidelines [39] and works published elsewhere [38, 41, 50], regarding recovery, accuracy, intra and inter-batch precision (Table 1). The recovery of the target analytes using the optimized SPE procedure was assessed after preconcentration of blank samples and 35 ng L^{-1} spiked samples. The recoveries evaluated for the DW matrix were reproducible and between 22.4 % and 139 % (Fig. 2). Peak areas of the target analytes found in the DW blank matrix were deducted for recovery rate evaluation. The dissimilar recoveries are due to the wide chemical nature of the target compounds and were taken into account, using the matrix-matched calibration curves and addition of internal standards before SPE. For instance, Gros et al. [20] developed a multi-residue analytical method, with similar recoveries values for DW, namely for cimetidine (24 ± 16.5 %). In that work, recovery values for the same compounds were higher in other matrices such as surface and wastewaters. López-Serna et al. [48] also reported some low values of recovery (<10 %) for groundwater, and higher recoveries for matrices presumably more affected by interferents. Accuracy and intra- and interbatch precision were evaluated by analysis of the QC extracts. The accuracy ranged from 80.6 % to 119 % (Table 1), which is within the range of 80–120 %, according to the international criteria [39]. RSD of the triplicate measurements of the three QC was used to guarantee the precision of the method (Table 1), with intrabatch precision less than 15.2 % and interbatch precision less than 14.8 %, meeting the international guidelines (RSD lower than 15 % or 20 % for the lower concentration QC) [39]. RSD of the triplicate analysis of the three QC samples after 24 and 48 h of reconstitution was lower than 5 %. The calibration curves were generated using the internal calibration method through spiking samples with isotopically labeled internal standards before SPE extraction. Three internal standards were used for three sets of compounds that were defined depending on the acid/basic nature (see ESM Table S3), as in other published works dealing with multi-class determination [14, 20, 27], which use an internal standard for each set of compounds owing to the high cost for routine environmental monitoring and difficulty in finding suitable internal standards for each compound in a series of compounds with distinct properties. The coefficients of determination of the calibration curve extracts were higher than 0.99 in the range of 0.75–40 ng L^{-1} for all compounds (Table 1). The MDL and MQL were 0.01–0.20 ng L^{-1} and 0.04–0.61 ng L^{-1}, respectively, allowing one to detect the target contaminants at residual concentrations (few nanograms per liter levels).

Quantification of micropollutants in DW

The developed offline SPE–UHPLC–MS/MS method was applied to DW samples collected at the end of May 2015, from various locations of northwest Portugal and from different sources (Table 2), namely tap water ($n = 13$) (ESM Fig. S2c), fountain water ($n = 5$), and well water ($n = 5$). Of the 21 investigated chemicals, 13 were detected in DW samples at nanogram per liter levels, which is consistent with concentrations reported in other studies [14, 15, 20–23, 27, 51]. The most common chemicals observed were diclofenac, trimethoprim, warfarin, metoprolol, norfluoxetine, atrazine, and simazine.

Regarding tap water, diclofenac, warfarin, norfluoxetine, atrazine, and simazine were the compounds most frequently detected. The micropollutants found at the highest concentrations were diclofenac and the pesticide chlorfenvinphos considered a PS, although well below the 0.1 μg L^{-1} required for single pesticides in Directive 1998/83/EC [6]. Concerning fountain water samples, diclofenac and atrazine were the most common micropollutants, being also found at the highest concentrations. The results obtained for well water samples showed that diclofenac was quantified in all the samples. Diclofenac, carbamazepine, and the PS simazine were those found at the highest concentrations.

The comparison of the results obtained in this work with similar studies conducted by other authors (ESM Table S4) is difficult, since the consumption of pharmaceutical compounds as well as the intensity of agricultural and industrial activities vary among different regions. Carbamazepine, caffeine, ibuprofen, and sulfamethoxazole were often reported in DW, with carbamazepine being the most frequently found up to 40 ng L^{-1} [14, 15, 20–23, 27, 51]. Other compounds such as atenolol, clofibric acid, azithromycin, erythromycin, fluoxetine, and diclofenac were also detected but at very low levels [15, 20, 21, 23, 27]. It is important to emphasize the need for revision of the European policy regarding tap water, considering that Directive 1998/83/EC is outdated in view of the studies reported in the last decade. The more recent Directive 2013/39/EU regulates surface waters, demanding more rigorous acceptable values than Directive 1998/83/EC [6] regulating water for human consumption. The same issue should be considered for groundwater regulated by Directive 2006/118/EC [5], considering that fountain and well waters used for human consumption can be sourced from this type of water.

Human health risk assessment

The maximum values of each micropollutant in DW were used to estimate the respective HQ. This prediction gives insights about the human health risk assessment by evaluating the

Table 2 Concentrations of micropollutants (ng L^{-1}) detected in tap, fountain, and well water samples analyzed

Class and sub-class	Analyte	Tap water ($n = 13$)		Fountain water ($n = 5$)		Well water ($n = 5$)	
Pharmaceuticals		Concentration (ng L^{-1})	Frequency	Concentration (ng L^{-1})	Frequency	Concentration (ng L^{-1})	Frequency
Anti-inflammatories	Diclofenac	<MQL–7.87	7/13	3.95–7.66	4/5	1.60–36.20	5/5
	Tramadol	ND	ND	ND	ND	ND	ND
Antibiotics	Azithromycin	ND	ND	ND	ND	ND	ND
	Clarithromycin	<MQL	1/13	ND	ND	1.14	1/5
	Trimethoprim	<MQL	1/13	<MQL	1/5	0.86	1/5
Anticoagulant	Warfarin	0.39–3.89	5/13	4.07	1/5	11.2	1/5
Antiplatelet agent	Clopidogrel	ND	ND	ND	ND	ND	ND
Beta-blockers	Metoprolol	<MQL	5/13	ND	ND	<MQL	1/5
Psychiatric drugs	Carbamazepine	3.34	1/13	ND	ND	58.8	1/5
	Citalopram	<MQL	1/13	ND	ND	ND	ND
	Venlafaxine	ND	ND	ND	ND	ND	ND
	Fluoxetine	ND	ND	ND	ND	ND	ND
Metabolite	Norfluoxetine	<MQL	13/13	<MQL	1/5	<MQL	1/5
Pesticides							
Chloroacetanilide	Alachlor	<MQL	4/13	ND	ND	3.07	1/5
Triazine	Atrazine	1.14–2.24	6/13	1.59–103	3/5	1.66	1/5
	Simazine	<MQL–1.45	4/13	<MQL–2.20	2/5	2.84–28.40	2/5
Organophosphorus	Chlorfenvinphos	2.46–6.50	2/13	0.49–3.89	2/5	ND	ND
Phenylurea	Isoproturon	ND	ND	ND	ND	ND	ND
Organochlorine	Pentachlorophenol	ND	ND	ND	ND	ND	ND
Herbicide	Clofibric acid	ND	ND	ND	ND	ND	ND
Industrial compound	PFOS	<MQL	1/13	ND	ND	11.7	1/5

MQL method quantification limit, *ND* not detected

probability of adverse effects: HQ values below 0.1 indicate no expected adverse effects; values between 0.1 and 1.0 suggest potential for adverse effects that should be considered, despite the low risk; HQ values ranging from 1.0 to 10 indicate adverse effects or mild risk; a high risk is assumed only for HQ values above 10 [35]. The maximum measured concentrations observed for the targeted chemicals found in DW (Table 2) were used to calculate EDI, predicting the worst case scenario. Even so, the HQs for all micropollutants found in DW samples were between 4.56×10^{-6} and 4.49×10^{-3}, i.e., well below 0.1, so adverse effects are not likely to be expected at such concentrations. Risks assessment of simultaneous exposure to multiple contaminants was not considered, although some of these compounds are already recognized to trigger several additive, synergistic, or antagonist effects [34, 36].

Removal of micropollutants in DW using UV radiation or ozonation

Tap water samples collected from the water supply network were post-spiked with the target micropollutants at nanogram per liter level and exposed to UV radiation or ozonation to assess the removal of the target micropollutants using the eco-friendly analytical method (Fig. 3), since these processes are often applied in DWTPs.

Only seven pharmaceuticals were completely removed by these water treatments: (i) tramadol, venlafaxine, and azithromycin by both processes; note that azithromycin was recently included in the first Watch List by the EU Decision 2015/495; (ii) clopidogrel, carbamazepine, and isoproturon by ozonation; and (iii) the metabolite norfluoxetine by UV. Regarding the other micropollutants, the efficiency of the processes varied according to the substance. The results showed that, in general, UV radiation was more effective than ozonation for the removal of pesticides and for the industrial compound, whereas ozonation performed slightly better for pharmaceuticals. The feasibility of this UHPLC–MS/MS analytical method for monitoring chemical processes used to improve the quality of DW was shown.

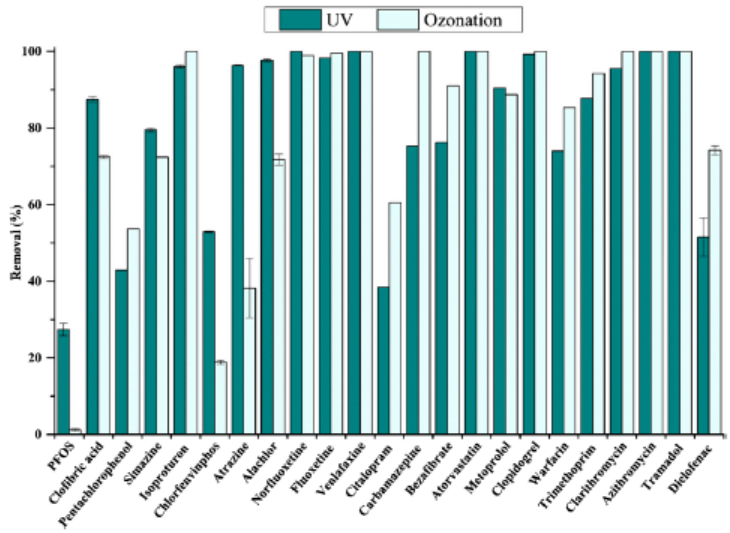

Fig. 3 Removal percentage of the micropollutants in spiked DW after the bench-scale UV or ozonation treatments

Conclusions

The offline SPE–UHPLC–MS/MS method that was developed and validated in this work for the assessment of the occurrence and removal of 21 multi-class micropollutants in DW has the great advantage of using an eco-friendly solvent (ethanol) for both SPE procedure and UHPLC analysis, according to the recent concerns about GAC applied to environmental analyses. Additional advantages of the method are (i) low detection limits (below 1 ng L^{-1}); (ii) short run time; (iii) low volume of eluent employed for each analysis; (iv) the use of a single cartridge/SPE procedure to extract all the target analytes; (v) and the low volume of sample used. The potential of the offline SPE–UHPLC–MS/MS method for monitoring programs and evaluation of advanced treatment options (UV and ozonation) was demonstrated in the selected case studies. For instance, analysis of tap, fountain, and well water samples from different locations of northwest Portugal showed the widespread occurrence of micropollutants in such matrices at nanogram per liter levels. Among the 13 micropollutants detected in DW samples, the most common were diclofenac, trimethoprim, warfarin, norfluoxetine, atrazine, and simazine; the feasibility of the method for monitoring DW treatment processes was also validated.

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Compliance with ethical standards

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

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Supplementary material

Eco-friendly LC-MS/MS method for analysis of multi-class micropollutants in tap, fountain and well water from northern Portugal

Material and methods

Text B-S3.1. Instrumentation

A Shimadzu Corporation (Tokyo, Japan) equipment was employed to perform the chromatographic analysis, specifically a UHPLC equipment (Nexera) with two pumps (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a degasser (DGU-20A 5R) and a system controller (CBM-20A), coupled to a triple quadrupole mass spectrometer detector (Ultra Fast Mass Spectrometry series LCMS-8040), with a LC Solution Version 5.41SP1 software. Nitrogen used as source gas was provided by a nitrogen generator (Peak Scientific, Bedford, MA, USA). The collision induced dissociation gas (CID) was argon at 230 kPa.

Capillary voltage, drying and nebulizing gas flows, desolvation and source temperatures were optimized for the set of analytes herein studied, through the injection of a working standard solution with the target compounds at 50 µg L^{-1}.

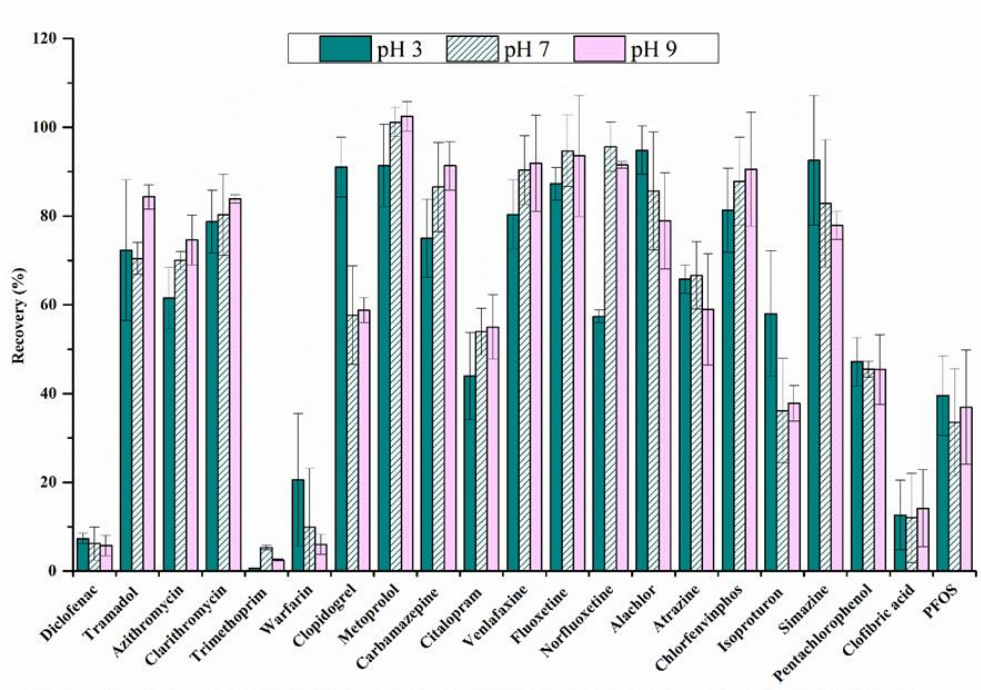


Fig. B-S3.1. Recoveries obtained for the target analytes with the following SPE conditions: HLB cartridges using methanol, extracting 250 mL of tap water samples, adjusted to pH 3, 7 or 9.

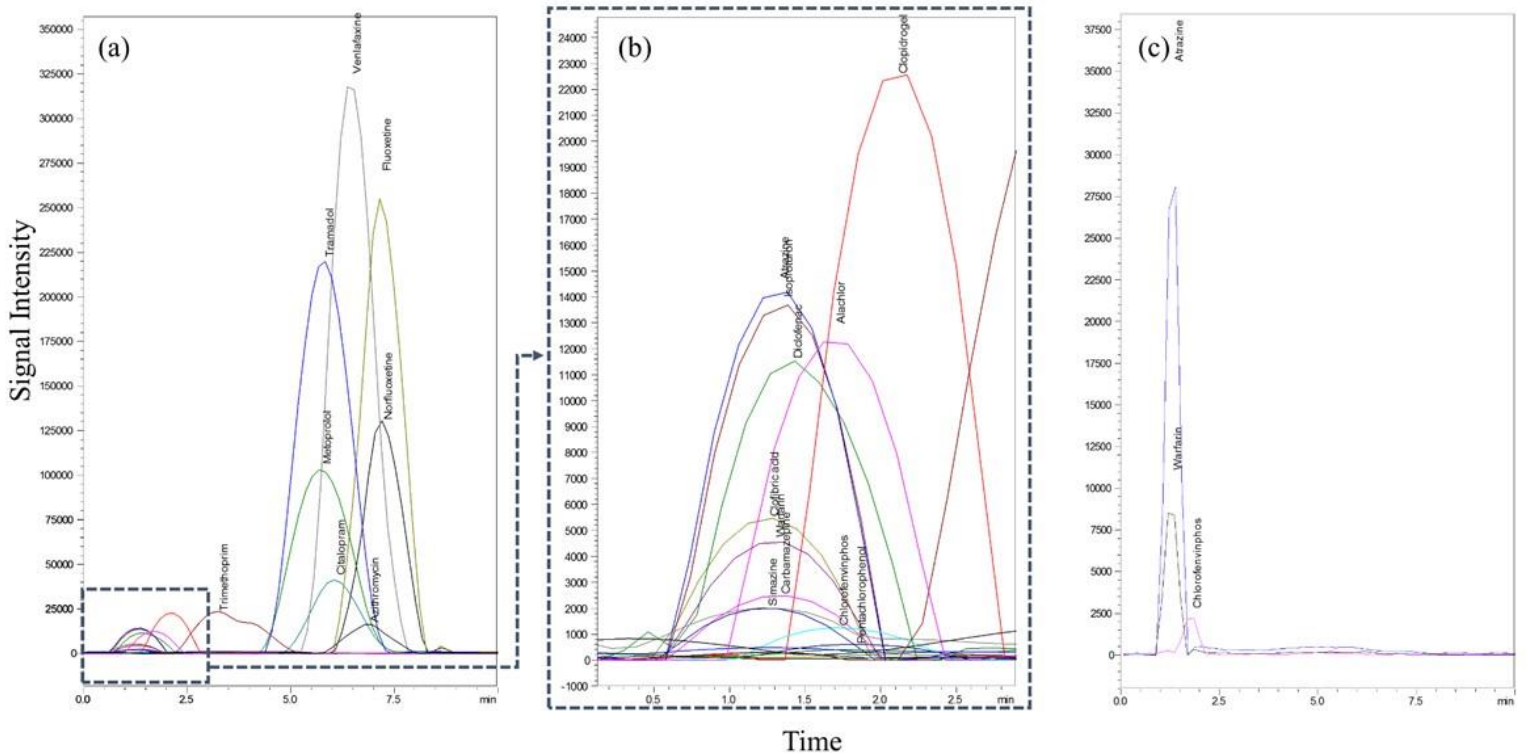


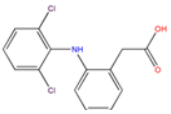
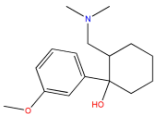
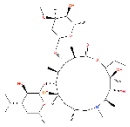
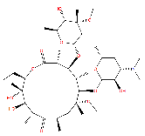
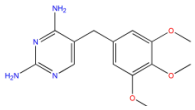
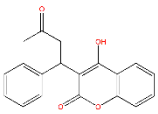
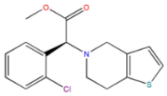
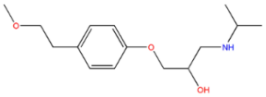
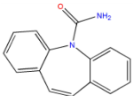
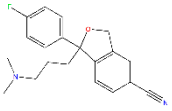
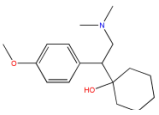
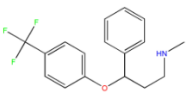
Fig. B-S3.2. Total ion chromatograms of **(a, b)** QC sample spiked with the targeted chemicals at 15 ng L^{-1}; **(c)** tap water sample contaminated with atrazine, chlorfenvinphos and warfarin.

Table B-S3.1. Hyphenated chromatography-mass spectrometry techniques for DW analysis.

Analytes	Sample analysis	Concentration (ng L^{-1})	Location	Ref.
Azithromycin, Carbamazepine, Clarithromycin, Trimethoprim	SPE (using methanol as solvent) followed by LC/MS–MS	n.d. – 5	Colorado (USA)	[1]
Clofibric acid, Diclofenac, Simazine, Atrazine	SPE (using methanol as solvent) followed by LC/MS–MS	n.d. – 81	Campinas (Brazil)	[2]
Diclofenac, Clofibric acid, Carbamazepine	SPE (using methanol as solvent) followed by LC/MS–MS	n.d.	Coruña (Spain)	[3]
Simazine	SPE (using acetonitrile as solvent) followed by LC/MS–MS	4100	Burriana (Spain)	[4]
Diclofenac, Carbamazepine, Venlafaxine, Fluoxetine, Norfluoxetine, Metoprolol, Azithromycin, Clarithromycin, Trimethoprim	SPE (using methanol as solvent) followed by LC/MS–MS	n.d. – 2	Girona (Spain)	[5]
Carbamazepine, Clofibric acid, Diclofenac	SPE (using acetone as solvent) followed by LC/MS–MS	n.d. – 100	Netherlands	[6]
Carbamazepine, Clofibric acid, Trimethoprim	SPE (using methanol as solvent) followed by LC/MS–MS	n.d. – 8.7	Missouri (USA)	[7]
Carbamazepine, Clofibric acid, Diclofenac, Fluoxetine	SPE (using methanol as solvent) followed by LC/MS–MS	n.d – 96 % (frequency of detection)	Lisbon (Portugal)	[8]

Carbamazepine, Diclofenac	SPE (using acetonitrile as solvent) followed by LC/MS–MS	n.d. – < MQL	France	[9]
Carbamazepine, Clarithromycin, Diclofenac, Trimethoprim	On-line SPE followed by LC/MS–MS	n.d. – 41	Dordogne (France)	[10]
Diclofenac, Azythromycin, Clarithromycin, Trimethoprim, Clofibric acid	SPE (using methanol as solvent) followed by LC/MS–MS	n.d.	Catalonia (Spain)	[11]
Clofibric acid, Fluoxetine	SPE (using methanol as solvent) followed by LC/MS–MS	n.d. – 300	New Jersey (USA)	[12]

Table B-S3.2. Target analytes, describing their class, sub-class, chemical structure, molecular weight (Mw) and p*K*a.

Class and sub-class	Analyte	Chemical structure	Mw (g mol^{-1})	p*K*a
Pharmaceuticals				
Anti-inflammatories	Diclofenac		296.14	4.15
	Tramadol		263.38	9.41
Antibiotics	Azithromycin		748.51	8.74
	Clarithromycin		747.95	8.99
	Trimethoprim		290.32	7.12
Anticoagulant	Warfarin		308.33	4.50
Antiplatelet agent	Clopidogrel		321.06	5.14
Beta-blockers	Metoprolol		267.36	9.67
Psychiatric drugs	Carbamazepine		236.27	13.94
	Citalopram		324.16	9.78
	Venlafaxine		277.40	9.40
	Fluoxetine		309.33	9.80

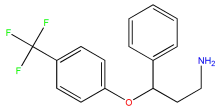
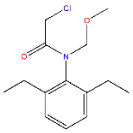
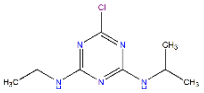
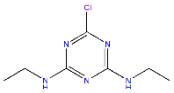
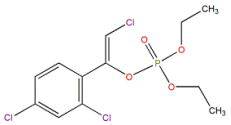
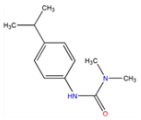
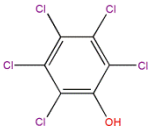
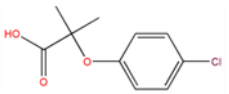
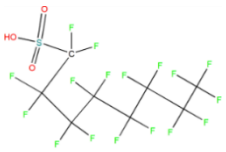
Metabolite	Norfluoxetine		295.3	9.77
Pesticides				
Chloroacetanilide	Alachlor		269.77	0.62
Triazine	Atrazine		215.68	1.70
	Simazine		201.66	1.62
Organophosphorus	Chlorfenvinphos		359.57	-
Phenylurea	Isoproturon		206.28	-
Organochlorine	Pentachlorophenol		266.34	4.73
Herbicide	Clofibric acid		214.65	3.00
Industrial compound	Perfluorooctane-sulfonic acid (PFOS)		500.13	0.14

Table B-S3.3. Optimized mass spectrometer parameters for SRM analysis of the target analytes.

Class and sub-class	Analyte	IS set[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Product ion (m/z) (SRM1)	CE [d] (V) (SRM1)	Product ion (m/z) (SRM2)	CE [d] (V) (SRM2)	Ion ratio (±SD)
Pharmaceuticals									
	Diclofenac [f]	1	NI	294.1	250.10	12	214.05	21	19.70 ±0.09
Anti-inflammatories	Tramadol	2	PI	264.0	57.70	-25	-	-	n.a. [h]
	Ketoprofen-d3 (1)		NI	256.2	212.10	8	-	-	n.a. [h]
	Azithromycin [f]	2	PI	749.5	83.15	-52	116.10	-47	1.14 ±0.11
Antibiotics	Clarithromycin [f]	2	PI	748.4	158.15	-30	590.30	-21	3.22 ±0.07
	Trimethoprim	2	PI	290.5	230.00	-24	123.05	-26	1.26 ±0.09
Anticoagulant	Warfarin	1	PI	309.0	163.00	-16	251.05	-21	1.32 ±0.10
Antiplatelet agent	Clopidogrel	1	PI	321.6	212.05	-17	184.00	-23	1.67 ±0.07
Beta-blockers	Metoprolol	2	PI	267.8	116.15	-20	74.15	-23	1.18 ±0.06
	Carbamazepine	2	PI	236.9	194.10	-20	192.10	-22	4.22 ±0.18
	Citalopram	2	PI	324.5	109.10	-27	262.00	-20	4.22 ±0.18
Psychiatric drugs	Venlafaxine	2	PI	277.8	58.10	-22	260.15	-12	2.90 ±0.11
	Fluoxetine	2	PI	310.0	44.15	-14	-	-	n.a. [h]
	Fluoxetine-d5 (2)		PI	315.0	44.15	-14	-	-	n.a. [h]
Metabolite	Norfluoxetine	2	PI	296.0	134.15	-8	30.25	-13	1.52 ±0.04
Pesticides [e]									
Chloroacetanilide	Alachlor [g]	3	PI	270.0	238.10	-11	162.05	-20	2.07 ±0.09

Triazine	Atrazine [g]	3	PI	215.9	174.05	-18	68.15	-37	2.44 ±0.10
	Simazine [g]	3	PI	201.9	124.10	-18	131.95	-20	1.35 ±0.20
	Atrazine–d5 (3)		PI	221.0	179.05	-19	-	-	n.a. [h]
Organophosphorus	Chlorfenvinphos [g]	3	PI	360.5	155.10	-40	99.10	-15	1.49 ±0.14
	Isoproturon [g]	3	PI	206.9	72.10	-21	46.15	-18	2.19 ±0.07
Organochlorine	Pentachlorophenol [g]	3	PI	265.1	35.15	48	-	-	n.a. [h]
Herbicide	Clofibric acid	3	NI	213.1	127.00	13	85.00	11	8.42 ±0.31
Industrial compound	PFOS [g]	3	NI	498.7	79.95	50	99.00	46	3.15 ±0.13

[a] IS is internal standard.

[b] NI is negative ionization mode.

[c] PI is positive ionization mode.

[d] CE is the collision energy.

[e] Included in the chemical parameters of the quality of water intended for human consumption (Part B of Annex I of the Directive 1998/83/EC) and in the groundwater quality standards (Annex I of the Directive 2006/118/EC).

[f] Included in the Watch List for the intent prioritization process at EU level (Annex of the EU Decision 2015/495).

[g] PSs of the Directive 2013/39/EU.

[h] n.a. is not applicable.

Table B-S3.4. Comparison of occurrence data for the targeted pollutants in DW samples (ng L^{-1}), observed in the present study and reported in others.

Analyte	Concentration (ng L^{-1}) Present study	Concentration (ng L^{-1}) Other studies	Ref.
Carbamazepine	3.34 – 58.8	5	[1, 5]
		2	[8, 13]
		n.d. – ca. 5	
		n.d. – 40	
Venlafaxine	n.d.	<MQL	[13]
Diclofenac	<MQL – 36.20	n.d.	[2, 8]
		n.d. – 14	
Atrazine	1.14 – 103	9.3 – 81	[2]
Simazine	<MQL – 28.40	n.d.	[2]
Trimethoprim	<MQL – 0.86	20 – 60	[13]

MQL, method quantification limit; n.d., not detected.

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Appendix C

Original version and supplementary material of Chapter 4:

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

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Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

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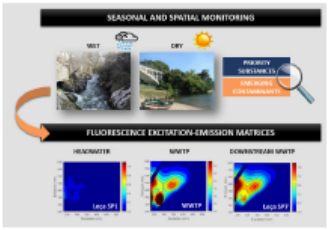
HIGHLIGHTS

- Spatial and seasonal variations of multi-class micropollutants were studied.
- Four stressed rivers in Portugal were monitored in dry and wet seasons.
- Ketoprofen, tramadol, enrofloxacin and thiacloprid were measured at highest levels.
- The fluorescence EEMs of surface water matched the distribution of micropollutants.
- Correlation of fluorescence signatures found for azithromycin, carbamazepine, EHMC.

GRAPHICAL ABSTRACT



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Fluorescence excitation-emission matrix

ABSTRACT

The European Union (EU) has recommended the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2015/495) in surface waterbodies. The present study provides spatial distributions and temporal variations of a wide range of multi-class PSs and CECs in four stressed rivers in Portugal (Ave, Leça, Antuã, and Gêrtima). Thirteen micropollutants were found in all four rivers, including the priority pesticide isoproturon (up to 92 ng L^{-1}), various pharmaceuticals (up to 396 ng L^{-1}), and the UV-filter 2-ethyl-hexyl-4-methoxycinnamate (EHMC, up to 562 ng L^{-1}) identified in Decision 2015/495. The industrial priority compound perfluorooctanesulfonic acid (PFOS) was found in three rivers (Antuã, Gêrtima, and Leça) below the method quantification limit, together with four pharmaceuticals not included in these EU guidelines. The already banned priority pesticide atrazine was detected in Ave, Antuã, and Leça (up to 41 ng L^{-1}) and simazine in Gêrtima and Leça (up to 26 ng L^{-1}). Acetamiprid and imidacloprid (included in Decision 2015/495) were only detected during the dry season in the Ave. Leça river was selected as a waterbody case study for assessment of fluorescence excitation-emission matrices (EEMs). These results matched the spatial distribution trend of micropollutants along the river, with stronger fluorescence response and higher concentrations being found downstream of industrial areas and urban wastewater treatment plants (WWTPs). Moreover, the fluorescence signature of surface water collected downstream of an urban WWTP aligned very well with that obtained for the respective WWTP effluent. Thus, actions are needed to preserve a good environmental status of these stressed European waterbodies.

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1. Introduction

Environmental contamination of aquatic compartments by organic micropollutants is a subject of major concern in the last two decades (Cho et al., 2014; Tousova et al., 2017). Surface waters are constantly exposed to such contaminants, which mainly originate from agricultural runoff and discharge of effluents from industrial and municipal wastewater treatment plants (WWTPs), with the latter considered the major source of some classes of micropollutants found in river waters (Cho et al., 2014; Barbosa et al., 2016). The occurrence of organic micropollutants in rivers at residual concentrations (Yan et al., 2013; Robles-Molina et al., 2014; Tsui et al., 2014; Campanha et al., 2015; Dai et al., 2015; Ccanccapa et al., 2016; González-Alonso et al., 2017; Wilkinson et al., 2017; Sousa et al., 2018) can lead to adverse effects for aquatic wildlife and human health, limiting the use of water for recreation, irrigation, and consumption (Vasconcelos Ferreira et al., 2010; Gorito et al., 2017).

To tackle these problems, current European Union (EU) recommendations suggest the regular monitoring of an extensive range of chemical and biological parameters in surface waters (Directive, 2000), including a list of 45 substances for priority action (priority substances, PSs) with environmental quality standards (EQS) set up for some compounds (Directive, 2008; Directive, 2013) and a Watch List of contaminants of emerging concern (CECs) (Decision_495, 2015). Moreover, Directive, 2013/39/EU set the Maximum Allowable Concentration-EQS (MAC-EQS), corresponding to the concentration that should not be exceeded at any representative monitoring point for any given surface water body. In this context, the monitoring of PSs and CECs in surface waters is a useful tool not only to assess pollution sources, but also to ensure efficient management of water resources and the protection of aquatic flora and fauna (Tousova et al., 2017).

The occurrence of particular PSs and CECs in Portuguese rivers has been reported in Ave river (Ribeiro et al., 2016a; Rocha et al., 2013), Leça river (Rocha et al., 2012), Douro river (Ribeiro et al., 2016b), Ria de Aveiro (Rocha et al., 2016), and Guadiana river (Palma et al., 2014). However, an integrated study comprising the spatial distributions and temporal variations of a wide range of PSs and CECs belonging to different classes has not been concurrently conducted in multiple rivers. Therefore, the purpose of the present study was to perform two seasonal monitoring campaigns of 39 organic micropollutants in four stressed Portuguese rivers: i) Ave; ii) Leça; iii) Antuã; and, iv) Cértima. Contamination levels of the target compounds in these rivers were investigated during dry and wet seasons, and the water samples, collected at different points on each river, were analyzed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), after sample preparation by solid phase extraction (SPE).

Quantitative PSs/CECs analysis require significant costs, which often limit the scope of the sampling plan for specific projects. For this reason, quick, inexpensive screening tools that provide insights into PSs/CECs occurrence and concentration would not only allow optimization of sampling strategies, but also elicit new research questions on the occurrence and fate of PSs/CECs in surface water systems. Excitation-emission matrix (EEM) analyses are increasingly being used to describe the fluorescence properties of dissolved organic matter (DOM) for characterization (Yamashita et al., 2008), source-tracking (Cuss et al., 2016), and fate/transformation (Mangalgiri et al., 2017) purposes. We posit that EEM analysis may serve as a useful screening tool for representative PSs/CECs given their chemical similarity with selected molecules in the DOM matrix. This concept has been previously explored with respect to CEC occurrence (Yang et al., 2013) and transformation (Yan et al., 2017). For example, Yang et al. (2013) found significant correlations for caffeine, sulfamethoxazole, acetaminophen, and ciprofloxacin concentrations with the summed volume from regions I, II, and IV of the EEMs of water samples collected from the Pearl river (China). Nevertheless, the correlation of other PSs/CECs with specific EEM regional volumes needs to be explored to develop location-based screening tools for other watersheds, especially as the DOM matrix and PSs/CECs use vary by watershed. In this study, potential correlations between EEMs and PSs/CECs occurrence and concentration were investigated in the Leça river. This report is the first to evaluate the spatiotemporal distribution of PSs/CECs and fluorescence EEMs, as well as the correlations between these water quality parameters, in a Portuguese river, and the results have important implications for other water systems around the world.

2. Materials and methods

2.1. Chemicals and materials

All reference standards (i.e., acetamiprid, alachlor, atenolol, atorvastatin, atrazine, azithromycin dihydrate, bezafibrate, carbamazepine, ceftiofur, chlorfenvinphos, citalopram hydrobromide, clarithromycin, clindamycin, clofibric acid, clopidogrel hydrogen sulfate, clothianidin, diclofenac sodium, diphenhydramine, 2-ethylhexyl-4-methoxycinnamate (EHMC), enrofloxacin, erythromycin, fluoxetine hydrochloride, hydrochlorothiazide, imidacloprid, isoproturon, ketoprofen, methiocarb, metoprolol tartrate, norfluoxetine oxalate, ofloxacin, perfluorooctanesulfonic acid (PFOS), propranolol, simazine, thiacloprid, thiamethoxam, tramadol hydrochloride, trimethoprim, venlafaxine hydrochloride, and warfarin; >98% purity) and surrogate standards (i.e., acetamiprid-d3, azithromycin-d3, atrazine–d5, diclofenac-d4, fluoxetine–d5, ketoprofen–d3, methiocarb-d3, and ofloxacin-d3) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol (MS grade) and ethanol (HPLC grade) were acquired from VWR International (Fontenay-sous-Bois, France) and Fisher Scientific (Leicestershire, UK), respectively. Formic and sulfuric acid were obtained from Merck (Darmstadt, Germany), and ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ cm at 25 °C). Oasis® HLB (Hydrophilic-Lipophilic Balanced) cartridges (150 mg, 6 mL), used for sample preparation, were purchased from Waters (Milford, MA, USA).

2.2. Sampling area

Two sampling campaigns were performed in the dry (September 2016) and wet seasons (February 2017). During the sampling period, the weather was characterized by a mean atmospheric temperature of 23 °C in September 2016 and 11 °C in February 2017. The average precipitation was 24.3 and 113.5 mm in September and February, respectively (www.ipma.pt accessed on May 2018). The selection of Ave, Leça, Antuã, and Cértima rivers was based on the following: recognized contamination due to adjacent land-use patterns (i.e., residential, agricultural, and industrial areas); the existence of tributaries and WWTPs that can have a negative impact on the quality of these water courses; and, the presence of drinking water treatment plants (DWTPs) that may be affected by surface water pollution. Sample collection was performed along the whole course of the four target rivers (8 sampling points (SPs) for Leça and Cértima rivers and 9 SPs for Ave and Antuã rivers, Fig. 1), comprising locations near the source and mouth, as well as strategic areas subjected to impacts from urban, agricultural, or WWTP activities. The GPS coordinates of the SPs for each river are given in Table S1, Supplementary Material.

2.2.1. Ave river

The Ave river, which is situated in the North of Portugal, has an extension of about 100 km and a drainage basin area of 1340 km^2. The headwaters are located in Cabreira Mountain (1260 m above mean sea level, a.m.s.l.), and the estuary is located in Vila do Conde, along the Atlantic coast. The most important tributaries are the Este and Vizela rivers at the right and left banks, respectively. The average flow in the Ave river was 2.96 $m^3 s^{-1}$ in the dry season (September 2016) and

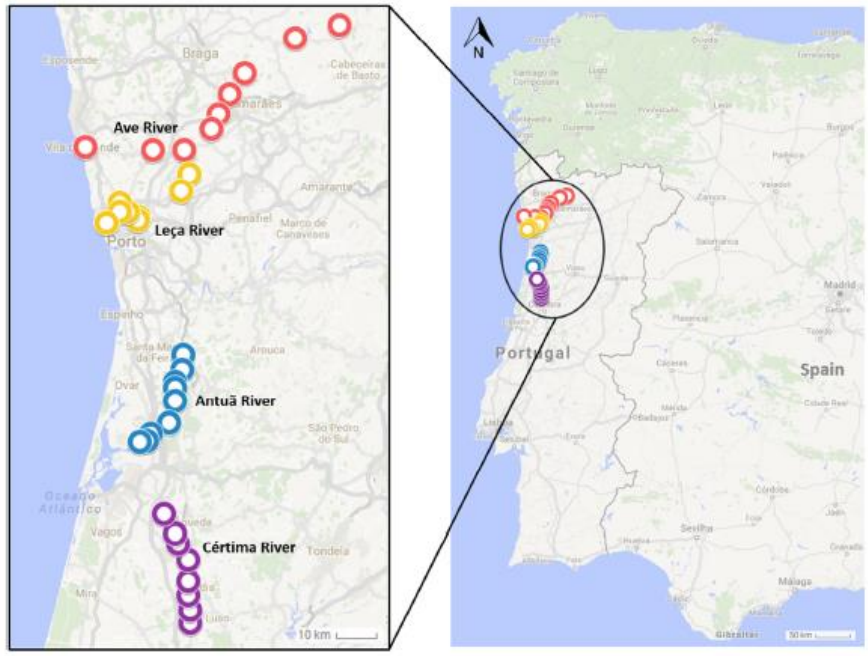


Fig. 1. Ave, Leça, Antuã, and Cértima rivers (Portugal) and the location of each sampling site.

63.08 $m^3 s^{-1}$ in the wet season (February 2017) (www.snirh.pt accessed on May 2018). The water resources are used for manufacturing and irrigation of rural activities. Most of the river basin area is used for agricultural and livestock activities (Ribeiro et al., 2016a). Water quality problems observed in this area are associated with high industrial density, including the textile sector (largest industry), leather tanning, rubber manufacture, and plastic production. Some industrial effluents are still illegally discharged into the water courses without treatment (Rocha et al., 2013).

2.2.2. Antuã river

Antuã river (extension of 38 km) has its source at Romariz, Santa Maria da Feira (400 m a.m.s.l.) and drains into the Atlantic Ocean through the Ria de Aveiro. Antuã basin is one of the sub-basins of the Vouga river with a total area of approximately 149 km^2. The main tributaries are Pintor stream, Cercal stream, and Ínsua river on the left bank and Arrifana stream on the right bank. Antuã river is characterized by an average flow of 4 $m^3 s^{-1}$, with the monthly average ranging between 0.6 $m^3 s^{-1}$ in August and 10 $m^3 s^{-1}$ in February (www.cesam.ua.pt/files/8_Congresso_Agua.pdf accessed on May 2018). Low water quality in this river stems from industrial and urban discharges, runoff from agricultural fields, and discharges from livestock farms. Agriculture is the principal activity within the borders of the Antuã river basin, which is situated near the city of Estarreja (Cerqueira et al., 2008).

2.2.3. Cértima river

The 43 km long Cértima river is located in North-Central Portugal and serves as a sub-tributary of the Vouga river, which drains into the Atlantic Ocean through the Ria de Aveiro coastal lagoon. The river source is at Buçaco Mountain (380 m a.m.s.l.), and the basin drains an area of 538 km^2. In its lower section, the river valley opens widely to form Pateira de Fermentelos lake, a sensitive wetland classified as a *Ramsar* site (i.e., wetlands of international importance designated under the *Ramsar Convention*). The river narrows again at Requeixo, where the Cértima discharges into the Águeda river (Serpa et al., 2014). The Cértima river has a flow rate of 7.17 $m^3 s^{-1}$ at SP8 in the wet season and 0.13 $m^3 s^{-1}$ during the dry season (Sena, 2007). The main tributaries are the Serra and Levira rivers and Ribeira do Pano (Cerqueira et al., 2005). Agriculture, domestic discharges, and industrial activities are the major sources of chemical pollution in the Cértima river basin (Vasconcelos Ferreira et al., 2010).

2.2.4. Leça river

On its flow towards the Atlantic Ocean, Leça river has an extension of 45 km and drains an area of 190 km^2 (Rocha et al., 2012), with an average flow of 3.4 $m^3 s^{-1}$ (http://maretec.mohid.com accessed on May 2018). The source of Leça river is located at Monte Córdova, Santo Tirso (475 m a.m.s.l.) and its mouth is located at Leixões Harbor basin, an important international harbor having dock facilities for commercial, cruise, and fishing vessels and an oil terminal. The main tributaries are

Ribeira do Arquitecto and Ribeira do Leandro, both on the right bank. The river receives effluents from several industries, some of which are untreated, and urban WWTPs (Gomes et al., 2014). Leça river was selected as a case study to evaluate the correlation of fluorescence EEMs to PSs and CECs concentrations since it is located between highly urbanized and industrialized regions belonging to the Porto metropolitan area, which represents the largest Port in Northern Portugal. One sample collected after the secondary biological treatment stage of an urban WWTP was also analyzed for comparison with surface samples.

2.3. Sample collection and preparation

Surface water samples were collected in the middle of each river, using a bottle sampler. Subsequently, samples were transferred to 1 L amber glass bottles and stored at 4 °C until extraction, which was performed within 24 h. Leça river surface water and wastewater effluent samples were frozen at −20 °C until analysis of the respective fluorescence EEMs. Several parameters, such as pH, conductivity, oxidation-reduction potential, temperature, salinity, dissolved oxygen, and total dissolved solids, were analyzed on site using a HI98194 Multiparameter Meter (HANNA® instruments; Woonsocket, RI, USA). Before SPE, all samples were filtered through 1.2-μm glass-fiber filters (47 mm GF/C, Whatman™; Maidstone, United Kingdom) and the pH was adjusted to 3 using sulfuric acid.

2.4. SPE–UHPLC–MS/MS method

An offline SPE–UHPLC–MS/MS method was applied for quantification of the target organic micropollutants according to previous works (Ribeiro et al. 2015; Barbosa et al., 2016). Briefly, Oasis® HLB cartridges were sequentially conditioned with 4 mL of ethanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. Sample loading of 500 mL of surface water samples was carried out at a constant flow rate of 10 mL min^{-1}, using a vacuum manifold unit. The washing step was performed with 4 mL of ultrapure water, and the cartridges were then dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of ethanol and the extracts were evaporated to dryness in a Centrivap Concentrator® device (LABCONCO® Corporation, Kansas City, MO, USA). The dried extracts were reconstituted in 250 μL of ethanol, and the resulting solutions were filtered through 0.22 μm polytetrafluoroethylene syringe filters (Membrane Solutions, Kent, WA, USA).

Surface water sample analysis was performed by UHPLC-MS/MS, using a Shimadzu Corporation apparatus (Tokyo, Japan), consisting of an UHPLC (Nexera) with two pumps (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a degasser (DGU-20A 5R), and a system controller (CBM-20A) with proper software (LC Solution Version 5.41SP1) coupled to a triple quadrupole mass spectrometer (Ultra Fast Mass Spectrometry series LCMS-8040). Analytical separation occurred along a Kinetex™ XB-C18 100 Å column (100 × 2.1 mm i.d.; 1.7 μm particle diameter) supplied by Phenomenex, Inc. (Torrance, CA, USA). The mobile phase consisted of (A) 0.1% formic acid aqueous solution and (B) methanol operated in gradient mode. Column oven and autosampler temperatures were set at 35 °C and 4 °C, respectively, and the injection volume was 5 μL. Selected reaction monitoring (SRM) transitions between the precursor ion and the two most abundant fragment ions were evaluated to quantify and confirm the identity of each compound. SRM1 was used for quantification purposes and the ratio between SRM1 and SRM2 was used for qualitative confirmation, along with the analyte retention time. Detailed analytical parameters and method selectivity, linear range, and limits of detection and quantification are described in the Supplementary Material (Tables S2 and S3).

2.5. Fluorescence excitation-emission matrices (EEMs)

Fluorescence EEMs of Leça river and wastewater samples collected during the wet season were measured using a Horiba Aqualog fluorescence spectrophotometer (Horiba Scientific; Edison, NJ USA). For all samples, 3-mL aliquots were added to a 1-cm quartz cuvette for analysis. Excitation wavelengths were incrementally increased from 209 to 620 nm using 3-nm steps, and the emission spectrum was recorded at 244–822 nm with 2.33-nm steps. Fluorescence EEMs of environmental samples were blank-corrected using LC-MS grade water. Inner-filter effects were corrected using the controlled dilution approach (Luciani et al., 2009; Kothawala et al., 2013). The 1st and 2nd order Rayleigh scattering lines were removed using the Horiba masking tool. A sealed Raman water fluorescence standard (Agilent Technologies; Santa Clara, CA USA) was used to convert all data to Raman Units (Timko et al., 2015). Corrected EEMs were plotted in Matlab (Mathworks; Natick, MA, USA), and regional volumes were calculated according to Chen et al. (2003). The corrected EEMs were considered in terms of tyrosine (region I), tryptophan (region II), fulvic acid (region III), soluble microbial product (region IV), and humic acid (region V)-like fluorescence. Pearson correlations were conducted to assess relationships between CECs concentrations for the compounds detected at all sampling sites (i.e., azithromycin, carbamazepine, and EHMC) and regional/total volumes from the EEM analysis. The correlations were considered statistically significant at a 95% confidence interval (p-value < 0.05). All statistical analyses were performed in R-studio 3.5.0.

3. Results and discussion

3.1. Physicochemical characterization

To assess the water quality and anthropogenic impacts in the four rivers, physicochemical parameters, namely pH, temperature, dissolved oxygen, conductivity, salinity, total dissolved solids, and turbidity, were measured at all sampling sites in both seasons (Table S4). The pH values ranged between 5.0 and 8.1 in the Ave, between 6.3 and 7.2 in the Leça, between 6.7 and 7.4 in the Antuã, and between 6.9 and 8.0 in the Cértima. This parameter affects the solubility of nutrients, and the values measured in all rivers (between 5 and 8) are optimal for plankton growth and nutrient availability (Ribeiro et al., 2016a, 2016b). The pH was generally higher during the wet season in comparison to the dry season. The pH was constant during each season along the Leça and Cértima rivers. In the Ave, pH increased from SP1 to SP9 (close to the mouth of the river), whereas pH increased slightly from SP1 to SP5 and then decreased from SP5 to SP9 in the Antuã. Dissolved oxygen concentrations varied slightly in each season but were generally higher during the wet season, except in the case of the Ave. In Ave and Leça, a gradient of conductivity, salinity, and total dissolved solids was detected from SP1 until the last SP, where the values were higher by at least one order of magnitude, since these SPs were located in the estuary. The same increasing trend was not observed between SP5 and SP9 of Antuã river or for SP2 to SP3 and SP5 to SP9 of Cértima river. These three physicochemical parameters (i.e., conductivity, salinity, and total dissolved solids) were typically higher during the dry season in all rivers.

3.2. Distribution and seasonal variation of target micropollutants in four Portuguese rivers

Thirteen micropollutants, namely azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, EHMC, fluoxetine, isoproturon, metoprolol, thiacloprid, tramadol, and venlafaxine, were found in all four rivers (Table S5). Many of these ubiquitously detected compounds are pharmaceuticals, and their occurrence is related to overall consumption and recalcitrance to wastewater treatment with domestic and hospital effluents being the

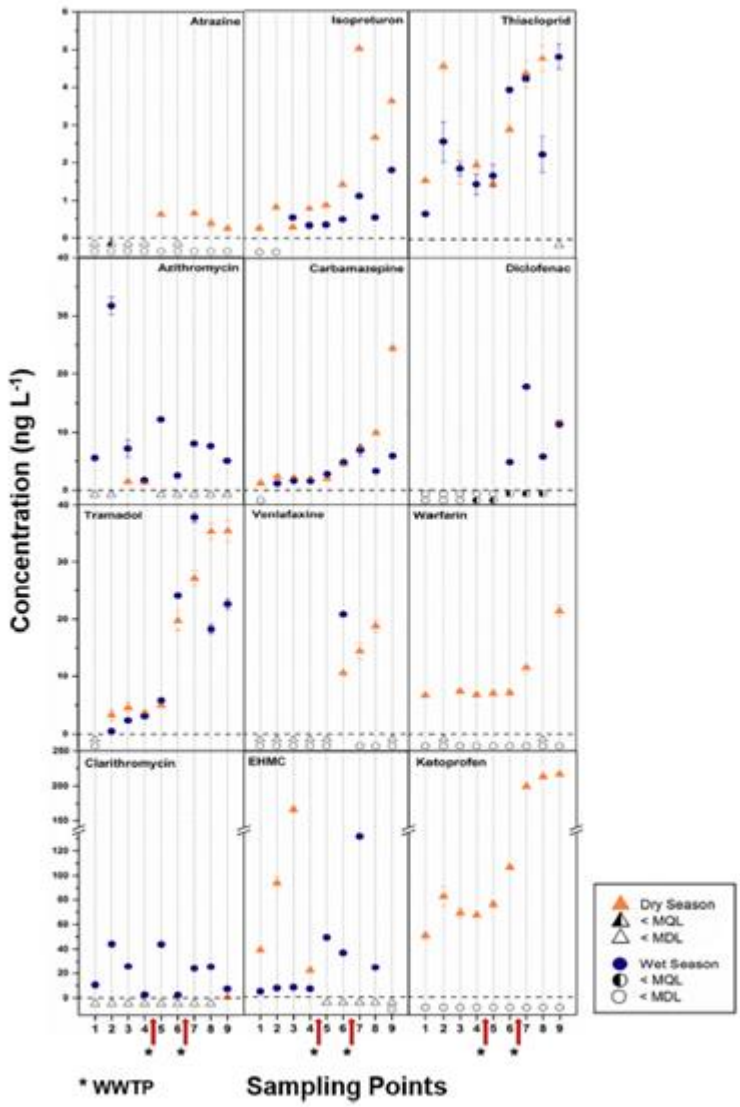


Fig. 2. Spatial distribution and concentrations of micropollutants in Ave river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Ave river, please see Fig. S1).

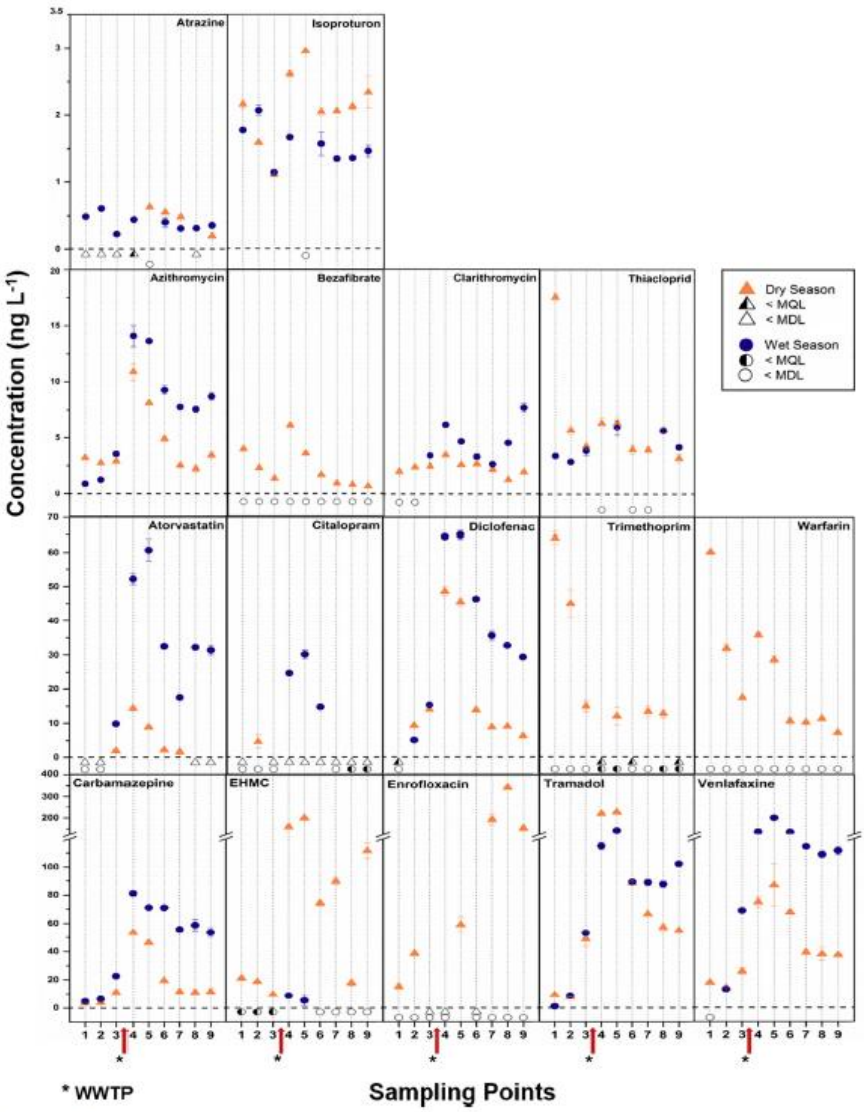


Fig. 3. Spatial distribution and concentrations of micropollutants in Antuã river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Antuã river, please see Fig. S2).

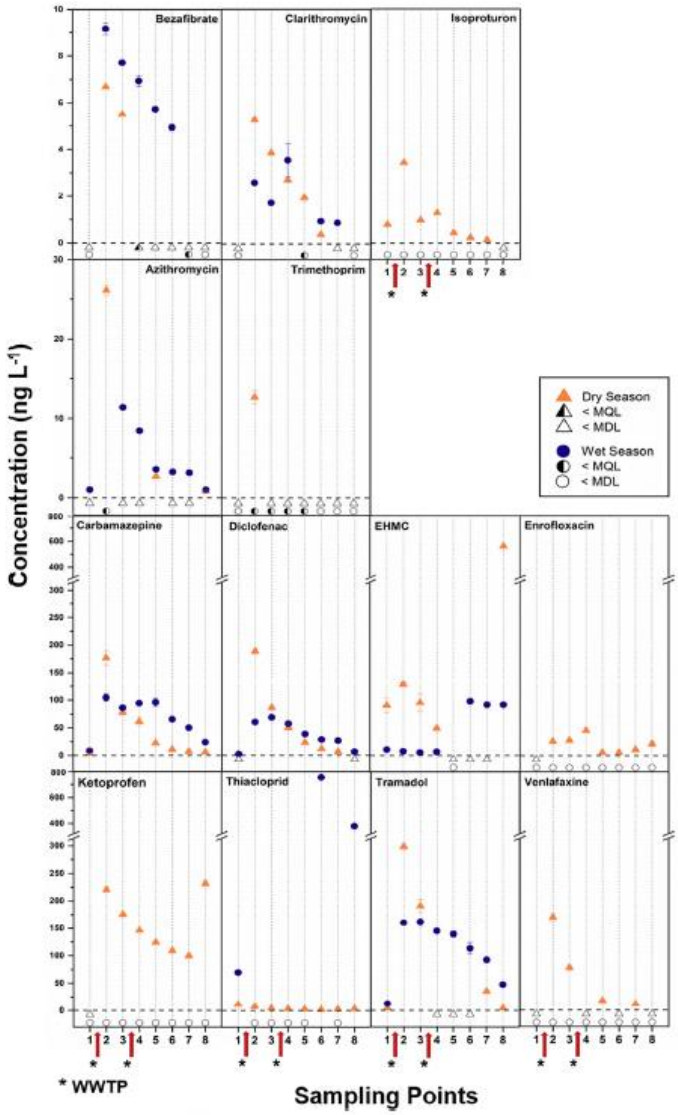


Fig. 4. Spatial distribution and concentrations of micropollutants in Cértima river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in Cértima river please see Fig. S3).

main sources (Chitescu et al., 2015). For pesticides, isoproturon was found below 5.0 ng L^{-1}, except in Leça river, where it was detected at higher concentrations (9.42–92.5 ng L^{-1},), but still below the 1.0 μg L^{-1} MAC-EQS set in Directive, 2013/39/EU and the maximum admissible concentration for pesticides in drinking water, established by Directive 98/83/EC as 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for their sum (Directive, 1998). On the contrary, thiacloprid was quantified above the maximum value allowed for individual pesticides in drinking water at two SPs of Cértima river. Bezafibrate, enrofloxacin, PFOS, propranolol, and trimethoprim were also identified in the Antuã, Cértima, and Leça rivers. PFOS was always below the MAC-EQS set in Directive, 2013/39/EU. Acetamiprid and imidacloprid were only detected in the Ave in the dry season, being acetamiprid below the method quantification limit (MQL) at three SPs and imidacloprid quantified at SP6 and below the MQL at SP9. In this river, the pharmaceuticals, ketoprofen (also in Cértima) and warfarin (also in Antuã), were quantified at almost all SPs in the dry season. Atrazine was detected in the Ave, Antuã, and Leça rivers. This banned triazine pesticide was present at levels <1.58 ng L^{-1}, except at SP9 of Leça, where it reached 41 ng L^{-1}. Simazine, which is also a triazine pesticide, was detected in Cértima and Leça rivers, with the highest concentration (26 ng L^{-1}) measured at Leça SP9. Both triazine pesticides were always below their MAC-EQS (2.0 μg L^{-1} for atrazine and 4.0 μg L^{-1} for simazine). The sum of all pesticides was below the maximum admissible concentration (0.5 μg L^{-1}) for pesticides in drinking water, defined in Directive 98/83/EC (Directive, 1998). Atorvastatin was identified in the wet and dry seasons in the Antuã (up to 61 ng L^{-1}) and Leça (up to 24 ng L^{-1}) rivers. The antidepressant drug citalopram was only quantified at 30 ng L^{-1} in the Antuã river. Overall, the concentrations of the micropollutants were generally lower in the wet season, which can be attributed to dilution effects associated with the higher flow rates in all rivers. For some compounds, the opposite trend was observed, namely higher concentrations were determined in the wet season. These findings may be attributed to seasonal differences in consumption (e.g., antibiotics), as reported in other works (Nannou et al., 2015). The lower temperatures and shorter daylight hours of the winter season may also impede biodegradation and phototransformation mechanisms resulting in less environmental transformation (Meierjohann et al., 2016).

3.2.1. Ave river

Eighteen of the thirty nine target compounds were detected in the Ave. Figs. 2 and S1 show the spatial distribution and concentrations of micropollutants in dry and wet seasons. Some target compounds, namely acetamiprid, atrazine, clindamycin, diphenhydramine, imidacloprid, ketoprofen, metoprolol, and warfarin, were only quantified in the dry season, which may be related to the lower precipitation and flow rates observed. From the target micropollutants, those with frequency of occurrence higher than 50% in the Ave were as follows: clindamycin, diphenhydramine, fluoxetine, ketoprofen, and warfarin in the dry season; the macrolide antibiotics (i.e., azithromycin and clarithromycin) and EHMC in the wet season; and isoproturon, thiacloprid, carbamazepine, and tramadol in both seasons. The highest concentrations determined in this river corresponded to the anti-inflammatory ketoprofen, which was found at all SPs during the dry season at concentrations between 50 and 217 ng L^{-1}. This anti-inflammatory compound was also determined at high concentrations in the Llobregat river (Spain) (Osorio et al., 2012), due to its broad use in human medicine. EHMC was detected during the dry season at four SPs (SP1–4) at concentrations up to 168 ng L^{-1}. This UV-filter was also identified at almost all SPs during the wet season, with a maximum concentration of 132 ng L^{-1}. Similar EHMC concentrations were reported in other studies from Brazil (n.d. to 150 ng L^{-1}), Spain (mean: 24.2 ng L^{-1}), Japan, China, USA, and Arctic (up to 150 ng L^{-1}) (Tsui et al., 2014; da Silva et al., 2015; Aparicio et al., 2017). The occurrence of EHMC in Hong Kong river water samples was reported at higher levels (4043 ng L^{-1}) (Tsui et al., 2014). Although UV-filters are widely used in personal care products to protect human skin from UV radiation, they are also applied in several materials, such as rubber, plastics, and paints, to prevent degradation (Tsui et al., 2014). The occurrence of EHMC in both seasons may be related to these applications. Downstream of SP6 in the Ave, a marked increase was observed for the concentration of many micropollutants (except for azithromycin, clarithromycin, and EHMC), which can be explained by the presence of two urban WWTPs, and other anthropogenic activities (e.g., agriculture and industry). Overall, the concentration of pollutants generally increased from the headwaters to the mouth of the Ave.

3.2.2. Antuã river

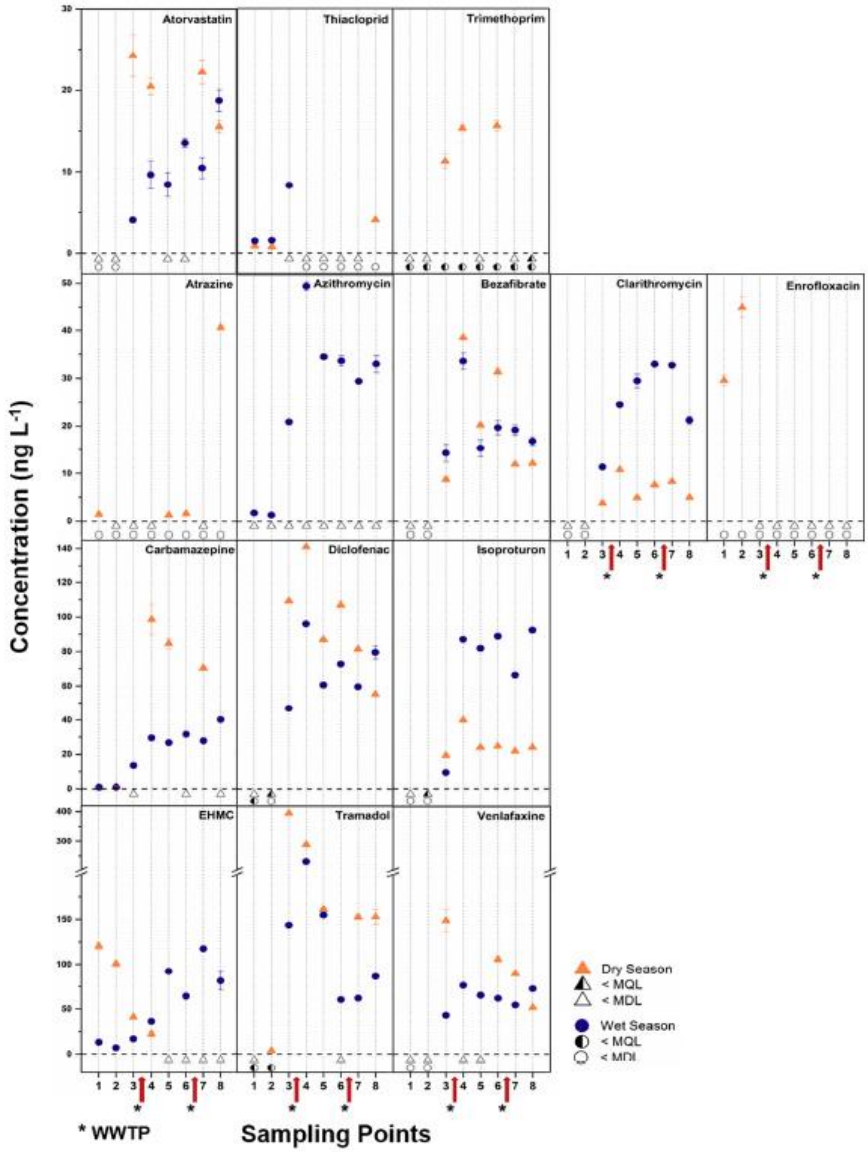
Twenty-two target organic micropollutants were found in the Antuã, including seventeen pharmaceuticals, three pesticides, a UV-filter, and an industrial compound (Figs. 3 and S2). Some of these analytes occur only in the dry season, namely the antibiotics enrofloxacin and trimethoprim, bezafibrate, metoprolol, and warfarin. The most frequently detected compounds (>50%) in Antuã river samples during the two campaigns were the pesticides isoproturon and thiacloprid and the pharmaceuticals atorvastatin, azithromycin, carbamazepine, clarithromycin, clindamycin, diclofenac, diphenhydramine, fluoxetine, tramadol, and venlafaxine. The highest concentration in this river was found for enrofloxacin in the dry season (343 ng L^{-1}), which may be due to the livestock production in the surrounding areas. In the wet season, venlafaxine registered the highest concentration at SP5 (199 ng L^{-1}). A significant increase in the concentrations of many contaminants was recorded downstream of Salgueiro WWTP (SP4). Several factors contribute to the variation of micropollutant concentrations along this river and between the different rivers investigated here. For instance, enrofloxacin and PFOS were determined in Antuã river and not detected in Ave river. Overall, flow rate, environmental factors (e.g., temperature, sunlight, nutrients), and fate/distribution mechanisms, such as adsorption to sediments or particulate matter, biodegradation, photodegradation, other abiotic processes, and uptake by biota, affect the concentrations of these compounds along the rivers of interest (Paíga et al., 2016).

3.2.3. Cértima river

In Cértima river (Figs. 4 and S3), twenty compounds were determined at concentrations up to 755 ng L^{-1} during the wet and dry season monitoring campaigns. The high concentrations observed in this river can be related to its low flow rate (ca. 0.13 m^3 s^{-1}) during the dry season. In fact, some target compounds, namely the pharmaceuticals diphenhydramine, propranolol, metoprolol, trimethoprim, enrofloxacin, ketoprofen, and venlafaxine and the pesticide isoproturon, were quantified only in the samples collected during the dry season. The most frequently detected (>50%) micropollutants in the Cértima varied by season: in the dry season, diphenhydramine, isoproturon, ketoprofen, the antibiotic enrofloxacin, and the neonicotinoid thiacloprid; in the wet season, azithromycin, bezafibrate, fluoxetine, and the pesticide simazine; and across both seasons, clindamycin, carbamazepine, diclofenac, EHMC, tramadol, and clarithromycin. The highest concentrations in this river were recorded for the neonicotinoid thiacloprid in the wet season (755 ng L^{-1}), a finding which may be due to agricultural leaching caused by precipitation events, and for the anti-inflammatory ketoprofen in the dry season (702 ng L^{-1}). With the exception of thiacloprid and PFOS, the concentrations of organic pollutants increased at SP2. The higher concentrations of most micropollutants in this area can be explained by the presence of Mealhada WWTP.

3.2.4. Leça river

The levels of organic micropollutants determined in the eight SPs of Leça river are reported in Figs. 5 and S4. Twenty-one compounds were observed, including the antibiotics clindamycin, enrofloxacin, and trimethoprim, metoprolol, and the pesticides atrazine and simazine


Atorvastatin
Thiacloprid
Trimethoprim
Atrazine
Azithromycin
Bezafibrate
Clarithromycin
Enrofloxacin
Carbamazepine
Diclofenac
Isoproturon
EHMC
Tramadol
Venlafaxine
Concentration (ng L-1)
Dry Season
< MQL
< MDL
Wet Season
< MQL
< MDL
* WWTP
Sampling Points

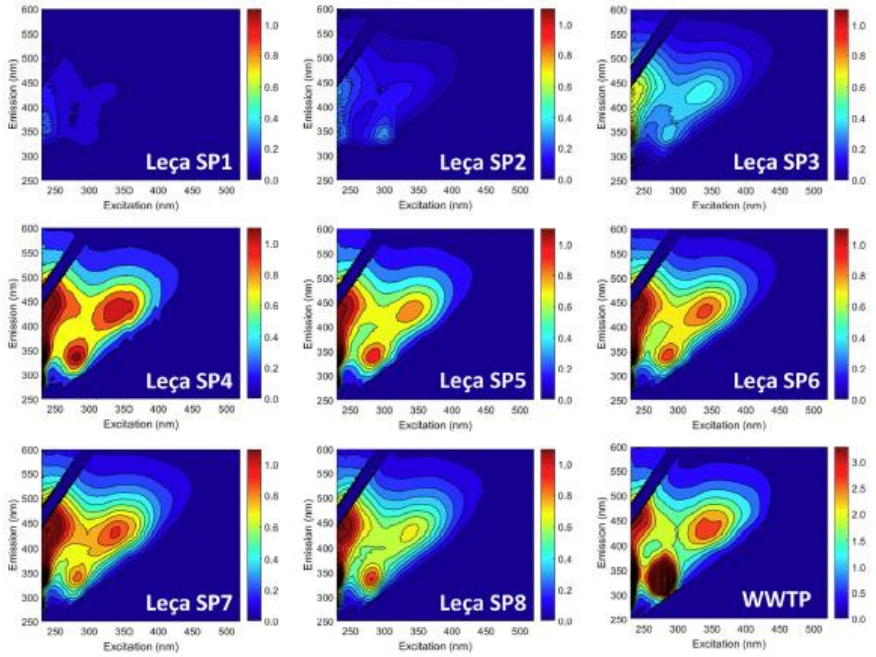


Fig. 6. Fluorescence EEMs for the eight Leça river samples and one WWTP sample.

quantified only during the dry season, and azithromycin found only in the wet season. Twelve analytes, namely atorvastatin, diphenhydramine, fluoxetine, propranolol, bezafibrate, clarithromycin, carbamazepine, diclofenac, isoproturon, EHMC, tramadol, and venlafaxine, were detected in >50% of the river samples across both seasons. During the dry and wet seasons, tramadol was found at the highest concentration, with a maximum of 396 ng L^{-1} and 233 ng L^{-1}, respectively. This analgesic was frequently detected in all four rivers up to hundreds of ng L^{-1}, as also recently reported by Burns et al. (2018) for two rivers in York (UK). EHMC, isoproturon, carbamazepine, diclofenac, and venlafaxine were also found at high concentrations during the two sampling campaigns and, together with tramadol, these micropollutants exhibited the highest detection frequencies and concentrations.

Parada and Ponte de Moreira WWTPs (upstream of SP4 and downstream of SP6, respectively) and the associated industrialized areas (SP4 and SP7) seemed to directly influence the levels of organic micropollutants in Leça river, increasing the concentration of most compounds. These data were well correlated with the fluorescence EEMs of the surface water and WWTP effluent samples (Fig. 6). The WWTP effluent sample exhibited a strong fluorescence response in all regions, and the fluorescence signature was similar to previous reports for wastewater (Baker, 2001; Sgroi et al., 2017). The fluorescence signatures of SP1 and SP2 were minimal. SP3 showed minor fluorescence in regions III (fulvic acid-like) and V (humic acid-like). The fluorescence response increased in all regions at SP4, downstream of the first WWTP. The SP5–8 samples showed similar fluorescence signatures in all regions, although the signal was slightly lower than at SP4. However, slight increases in the fluorescence response were observed at SP6, which is influenced by a WWTP, and SP7, which is surrounded by a highly industrialized area.

To better highlight the changes in fluorescence with sample location, the regional volumes of the environmental samples were normalized by the corresponding regional volumes from the WWTP sample. As indicated in Fig. 7, the relative presence of aromatic protein-, fulvic acid-, soluble microbial product-, and humic acid-like fluorescence increased between SP3 and SP4. Downstream of SP4, the fluorescence response associated with regions I and II decreased, presumably due to biotic/abiotic degradation mechanisms. The fluorescence in regions III, IV, and V remained fairly consistent from SP5 to SP8.

The similarity of CEC concentrations and regional EEM volumes along the Leça river is shown in Fig. 7. Azithromycin and carbamazepine concentrations were significantly, positively correlated with the EEM volumes for regions I–V; furthermore, significant correlations were observed for EHMC concentrations with fluorescence signatures from regions III, IV, and V (Table S7 in the SI). Significant correlations were also observed for all three CECs with the total fluorescence response,

Fig. 5. Spatial distribution and concentrations of micropollutants in Leça river for dry and wet seasons, determined above 30 ng L^{-1} at least in one of the four rivers (for other micropollutants in leça river, please see Fig. S4).

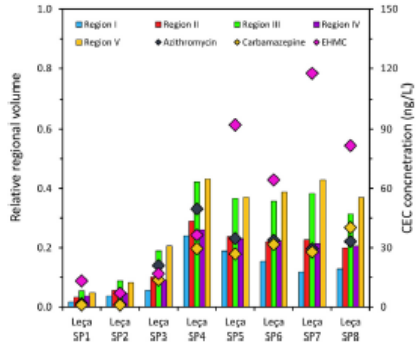


Fig. 7. The relative regional volume of environmental samples (Leça SP1–8) as a function of the WWTP sample for region I (tyrosine-like fluorescence), region II (tryptophan-like fluorescence), region III (fulvic acid-like fluorescence), region IV (soluble microbial product-like fluorescence), and region V (humic acid-like fluorescence). The average azithromycin, carbamazepine, and EHMC concentrations are plotted to highlight the correlation in EEM results and CEC concentrations.

which was calculated as the sum of EEM volumes from regions I–V. Based on these results, the fluorescence intensity in regions III, IV, and V may serve as useful screening tool to inform the presence of PSs/CECs in the Leça river. As DOM signatures and sources vary in other river systems, the EEM-based screening tool needs to be evaluated and refined for other watersheds.

While previous reports Yang et al. (2013) have found correlations between the total and summed (e.g., region I, II, and IV) EEM volumes with particular CECs, the more specific correlations with particular regions identified in this study may better reflect CECs fate and transport along spatiotemporal gradients. In particular, strong correlations were observed for azithromycin, carbamazepine, and EHMC concentrations with the region V fluorescence response, suggesting that these CECs exhibit similar fate and transport behavior as humic acid-like substances. Yan et al. (2017) highlighted the use of parallel factor (PARAFAC) analysis to not only deconvolute fluorescence EEMs into a finite number of components, but also predict CEC degradation. This approach may provide further insights into the fate and transport of PSs/CECs in Portuguese rivers.

3.3. Occurrence of target micropollutants in surface waters

The comparison of many target micropollutants found in this study with results from other reports on seasonal surface water monitoring (Table S6) is complex since the production and usage of industrial products, the application of pesticides in agricultural activities, and the consumption of pharmaceutical compounds is different between locations. However, the macrolide antibiotic azithromycin, the anti-inflammatory chemicals diclofenac and ketoprofen, and the antidepressants fluoxetine and venlafaxine have been reported in surface water at levels similar to those found in the present work. For instance, azithromycin, fluoxetine, and venlafaxine were found in Lis river (Portugal) up to 30 ng L^{-1}, 20 ng L^{-1}, and 159 ng L^{-1}, respectively (Paíga et al., 2016). Diclofenac was quantified in surface water collected from China at a maximum of 170 ng L^{-1} (Dai et al., 2015). Ketoprofen was detected in Spain up to 225 ng L^{-1} (Moreno-González et al., 2014). These findings indicate that a similar consumption pattern of these pharmaceutical compounds may occur in different regions of the world.

The pesticides atrazine and simazine were quantified in other studies at maximum levels well above those determined here. For example, in three different monitoring studies performed in Spain (Herrero-Hernández et al., 2017), Brazil (Machado et al., 2017), and Thailand (Sangchan et al., 2014), atrazine was found at maximum concentrations of 333, 320, and 800 ng L^{-1}, respectively. These concentrations are more than one order of magnitude higher than those determined in the present study (e.g., 41 ng L^{-1}). Simazine was found in Australia at concentrations between 50 and 670 ng L^{-1} (Allinson et al., 2014) and in Spain up to 207 ng L^{-1} (Herrero-Hernández et al., 2017). The intense agricultural activity in those regions may explain the presence of triazine herbicides in surface water even after these chemicals were phased out since they can be illegally acquired and/or released from existing sediments/soils. In contrast, the other target pesticide, isoproturon, was determined at higher concentrations in the present study compared to values found in the literature (Palma et al., 2014; Papadakis et al., 2015).

The UV-filter EHMC was determined in this work at a maximum value of 562 ng L^{-1} and a similar level was reported in Brazil (669 ng L^{-1}) (da Silva et al., 2015). This compound was also detected in surface water samples in Hong Kong at concentrations one order of magnitude higher, i.e., 4043 ng L^{-1} (Tsui et al., 2014), than those determined in the present study. Importantly, this compound has also been shown to accumulate in aquatic and marine organisms (He et al., 2017), raising concerns about the high aqueous-phase concentrations detected here.

The concentrations of bezafibrate, carbamazepine, clarithromycin, and thiacloprid described in the literature are slightly higher than those reported in the target Portuguese rivers. The beta-blocker metoprolol was found at a maximum concentration of 25 ng L^{-1} in Ave river, while it was determined up to 448 ng L^{-1} in Beiyun river of Beijing, China (Dai et al., 2015; Ma et al., 2017). The maximum concentration of the antibiotic trimethoprim in the current study was 64 ng L^{-1} in Antuã river in the dry season, which was comparable to the maximum concentration (36 ng L^{-1}) found in Llobregat river, Spain (Osorio et al., 2012) and lower than the maximum concentration (180 ng L^{-1}) reported in a monitoring study performed in the Los Angeles and San Gabriel rivers (Sengupta et al., 2014).

4. Conclusions

In this first simultaneous survey of specific PSs and CECs defined by EU documents in four stressed rivers (i.e., Ave, Leça, Antuã, and Cértima) in Portugal, 26 out of 39 target micropollutants were found at least in one of the selected rivers. Of the detected compounds, thirteen were consistently determined in all four rivers: azithromycin; carbamazepine; clarithromycin; clindamycin; diclofenac; diphenhyhydramine; EHMC; fluoxetine; isoproturon; metoprolol; thiacloprid; tramadol; and, venlafaxine. The highest concentrations were verified for ketoprofen in Ave river, tramadol in Leça river, enrofloxacin in Antuã river, and thiacloprid in Cértima river. These data highlight the different land-use patterns and contaminant sources found in the targeted rivers, with the occurrence and concentration distributions along particular rivers depending on location and seasonal variations. The increase in fluorescence response profiles for specific locations of the Leça river matched the distribution of micropollutants along this river. Although some of these compounds are already prioritized or defined in the Watch List, larger monitoring programs are needed for further prioritization and risk assessment of such contaminants. Given the significant correlations found for EEM regional volumes with CEC concentrations in the Leça river, preliminary EEM analysis may help to inform the design of future monitoring studies.

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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.2018.06.355.

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Supplementary material

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices

Table C-S4.1. Sampling points of Ave, Leça, Antuã and Cértima Rivers and the respective GPS coordinates.

River	Target Point	GPS coordinates	
		Latitude (°N)	Longitude (°W)
Ave	A1	41.602806	-8.048497
	A2	41.576678	-8.167964
	A3	41.505453	-8.306579
	A4	41.462017	-8.345558
	A5	41.420383	-8.377358
	A6	41.389508	-8.396708
	A7	41.346214	-8.470378
	A8	41.345131	-8.556364
	A9	41.351132	-8.739358
Leça	L1	41.295462	-8.455716
	L2	41.261419	-8.478392
	L3	41.207586	-8.593489
	L4	41.202336	-8.596394
	L5	41.218038	-8.624068
	L6	41.236006	-8.647019
	L7	41.217806	-8.646578
	L8	41.195417	-8.683078
Antuã	AT1	40.922548	-8.473395
	AT2	40.889828	-8.475903
	AT3	40.865931	-8.494225
	AT4	40.853750	-8.495064
	AT5	40.825844	-8.495789
	AT6	40.780139	-8.510508
	AT7	40.756622	-8.561378
	AT8	40.743714	-8.573203
	AT9	40.741856	-8.591586
Cértima	C1	40.366286	-8.455169
	C2	40.392678	-8.454639
	C3	40.424764	-8.458111
	C4	40.452272	-8.458767
	C5	40.496672	-8.458561
	C6	40.530744	-8.486222
	C7	40.552161	-8.495519
	C8	40.591714	-8.525628

Table C-S4.2. Selected reaction monitoring (SRM) instrument parameters for tandem mass spectrometry analysis of target analytes.

Analyte	IS[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Quantification (SRM1)				Confirmation (SRM2)				Ion ratio
				Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)	Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)	
Acetamiprid [h]	1	PI	222.70	126.00	-15.0	-20.0	-23.0	56.10	-15.0	-16.0	-22.0	2.56
Acetamiprid – d3 (1)		PI	226.10	126.00	-24.0	-21.0	-23.0	_	_	_	_	n.a. [g]
Alachlor [i]	2	PI	270.00	188.00	-13.0	-11.0	-24.0	147.00	-13.0	-20.0	-15.0	1.89
Atenolol	5	PI	266.80	145.10	-30.0	-27.0	-26.0	190.10	-30.0	-19.0	-18.0	5.56
Atorvastatin	5	NI	557.30	278.20	20.0	47.0	30.0	397.25	20.0	30.0	27.0	1.59
Atrazine [i]	2	PI	215.90	174.05	-23.0	-18.0	-30.0	68.15	-23.0	-37.0	-24.0	2.70
Atrazine – d5 (2)		PI	221.00	179.05	-11.0	-19.0	-18.0	_	_	_	_	n.a. [g]
Azithromycin [h]	3	PI	749.40	158.15	-36.0	-52.0	-13.0	591.35	-36.0	-47.0	-21.0	1.02
Azithromycin – d3 (3)		PI	752.40	158.05	-38.0	-47.0	-14.0	_	_	_	_	n.a. [g]
Bezafibrate	6	NI	360.20	274.15	17.0	17.0	19.0	154.05	17.0	31.0	29.0	2.86
Carbamazepine	5	PI	236.90	194.10	-28.0	-20.0	-19.0	192.10	-28.0	-22.0	-19.0	5.00
Ceftiofur	8	PI	524.10	241.00	-26.0	-19.0	-25.0	210.10	-26.0	-24.0	-20.0	2.99
Chlorfenvinphos [i]	2	PI	360.50	155.10	-25.0	-40.0	-16.0	99.10	-25.0	-15.0	-15.0	1.43

Citalopram	5	PI	324.50	109.10	-24.0	-27.0	-19.0	262.00	-24.0	-20.0	-27.0	2.70
Clarithromycin [h]	3	PI	748.40	158.15	-40.0	-30.0	-15.0	590.30	-40.0	-21.0	-28.0	3.03
Clindamycin	8	PI	425.00	126.15	-20.0	-30.0	-23.0	377.10	-20.0	-21.0	-25.0	33.33
Clofibric acid	2	NI	213.10	127.00	10.0	13.0	13.0	85.00	10.0	11.0	30.0	6.25
Clopidogrel	5	PI	321.60	212.05	-27.0	-17.0	-22.0	184.00	-27.0	-23.0	-17.0	1.54
Clothianidin [h]	1	PI	249.90	132.00	-29.0	-15.0	-23.0	169.05	-29.0	-13.0	-16.0	1.02
Diclofenac [i]	4	NI	294.10	250.10	14.0	12.0	17.0	214.05	14.0	21.0	23.0	25.00
Diclofenac – d4 (4)		NI	297.90	254.05	14.0	12.0	28.0	_	_	_	_	n.a. [g]
Diphenhyhydramine	5	PI	255.80	167.00	-28.0	-13.0	-30.0	165.00	-28.0	-40.0	-29.0	3.85
2-Ethyl-hexyl-4-trimethoxycinnamate (EHMC) [h]	7	PI	291.20	179.10	-14.0	-9.0	-18.0	161.10	-14.0	-19.0	-15.0	1.01
Enrofloxacin	8	PI	360.20	316.15	-17.0	-21.0	-21.0	342.20	-17.0	-23.0	-23.0	2.63
Erythromycin [h]	3	PI	734.40	158.15	-36.0	-34.0	-30.0	576.35	-36.0	-21.0	-28.0	2.08
Fluoxetine	5	PI	309.90	44.15	-15.0	-14.0	-16.0	_	_	_	_	n.a. [g]
Fluoxetine – d5 (5)		PI	315.00	44.15	-16.0	-14.0	-15.0	_	_	_	_	n.a. [g]
Hydrochlorothiazide	4	NI	296.10	269.00	14.0	19.0	28.0	205.00	14.0	23.0	21.0	1.22
Imidacloprid [h]	1	PI	255.70	209.05	-30.0	-15.0	-21.0	175.05	-30.0	-18.0	-17.0	1.05

Isoproturon [i]	2	PI	206.90	72.10	-22.0	-21.0	-27.0	46.15	-22.0	-18.0	-16.0	2.22
Ketoprofen	6	NI	253.00	209.15	16.0	7.0	22.0	_	_	_	_	n.a. [g]
Ketoprofen – d3 (6)		NI	256.20	212.10	12.0	8.0	22.0	_	_	_	_	n.a. [g]
Methiocarb [h]	7	PI	226.10	169.10	-24.0	-9.0	-17.0	121.10	-24.0	-19.0	-21.0	1.04
Methiocarb – d3 (7)		PI	229.10	169.10	-25.0	-11.0	-30.0	_	_	_	_	n.a. [g]
Metoprolol	5	PI	267.70	116.10	-20.0	-20.0	-20.0	74.15	-20.0	-23.0	-28.0	1.08
Norfluoxetine	5	PI	296.00	134.15	-30.0	-8.0	-13.0	30.25	-30.0	-13.0	-30.0	1.23
Ofloxacin	8	PI	362.00	318.15	-28.0	-19.0	-21.0	261.15	-28.0	-29.0	-26.0	1.56
Ofloxacin – d3 (8)		PI	365.1	321.15	-18.0	-20.0	-21.0	_	_	_	_	n.a. [g]
Perfluorooctanesulfonic acid (PFOS) [i]	2	NI	498.70	79.95	18.0	50.0	14.0	99.00	18.0	46.0	18.0	1.82
Propranolol	5	PI	259.70	116.10	-29.0	-19.0	-20.0	183.00	-29.0	-19.0	-18.0	1.61
Simazine [i]	2	PI	201.90	124.10	-22.0	-18.0	-11.0	131.15	-22.0	-20.0	-23.0	1.06
Thiacloprid [h]	1	PI	252.90	126.00	-28.0	-21.0	-21.0	99.00	-28.0	-44.0	-17.0	6.25
Thiamethoxam [h]	1	PI	291.90	211.10	-30.0	-14.0	-21.0	181.05	-30.0	-24.0	-17.0	2.08
Tramadol	5	PI	296.20	268.90	-30.0	-25.0	-30.0	204.90	_	_	_	n.a. [g]
Trimethoprim	8	PI	290.50	230.00	-30.0	-24.0	-24.0	123.05	-30.0	-26.0	-21.0	1.23

Venlafaxine	5	PI	296.20	268.90	-30.0	-22.0	-22.0	204.90	-30.0	-12.0	-27.0	3.13
Warfarin	6	PI	309.00	163.00	-15.0	-16.0	-28.0	251.05	-15.0	-21.0	-26.0	1.43

[a] IS is internal standard.
[b] NI is negative ionization mode.
[c] PI is positive ionization mode.
[d] DP is the declustering potential.
[e] CE is the collision energy.
[f] CXP is the collision cell exit potential.
[g] n.a. is not applicable.
[h] Included in the Watch List for the intent prioritization process at European Union level (Annex of the EU Decision 2015/495).
[i] PSs of the Directive 2013/39/EU.

Table C-S4.3. Retention time, range, instrument and method detection and quantification limits for each target analyte.

Analyte	Retention time (min)	Range (ng L^{-1})	IDL[a] (µg L^{-1})	IQL[b] (µg L^{-1})	MDL[c] (ng L^{-1})
Acetamiprid	0.921	3.30 – 100	2.18	6.60	1.09
Alachlor	2.468	8.71 – 100	5.74	17.42	2.87
Atenolol	0.758	6.61 – 100	4.36	13.22	2.18
Atorvastatin	2.279	0.65 – 100	0.42	1.30	0.21
Atrazine	1.470	0.14 – 100	0.10	0.28	0.05
Azithromycin	0.945	0.44 – 100	0.30	0.88	0.15
Bezafibrate	1.795	4.55 – 100	3.00	9.10	1.50
Carbamazepine	1.240	0.08 – 100	0.06	0.16	0.03
Ceftiofur	0.930	0.39 – 100	0.26	0.78	0.13
Chlorfenvinphos	2.890	3.22 – 100	2.12	6.44	1.06
Citalopram	0.774	12.44 – 100	8.22	24.88	4.11
Clarithromycin	1.047	0.39 – 100	0.30	0.78	0.15
Clindamycin	0.876	0.48 – 100	0.32	0.96	0.16
Clofibric acid	1.825	2.42 – 100	1.60	4.84	0.80
Clopidogrel	2.549	0.32 – 100	0.22	0.64	0.11

Clothianidin	0.937	0.65 – 100	0.65	0.21	0.21
Diclofenac	2.716	3.75 – 100	2.48	7.50	1.24
Diphenhyhydramine	0.836	0.05 – 100	0.04	0.10	0.02
EHMC	4.437	4.60 – 100	3.04	9.20	1.52
Enrofloxacin	0.836	4.79 – 100	3.16	9.58	1.58
Erythromycin	0.954	0.39 – 100	0.26	0.78	0.13
Fluoxetine	0.876	0.30 – 100	0.20	0.60	0.10
Hydrochlorothiazide	0.860	2.54 – 100	1.68	5.08	0.84
Imidacloprid	0.916	1.21 – 100	0.80	2.42	0.40
Isoproturon	1.472	0.13 – 100	0.08	0.26	0.04
Ketoprofen	1.642	9.88 – 100	6.52	19.76	3.26
Methiocarb	1.816	1.25 – 100	0.82	2.50	0.41
Metoprolol	0.700	0.90 – 100	0.60	1.80	0.30
Norfluoxetine	0.838	4.88 – 100	3.22	9.76	1.61
Ofloxacin	0.834	21.58 – 100	14.24	43.16	7.12
PFOS	3.941	2.77 – 100	1.82	5.54	0.91
Propranolol	0.829	3.57 – 100	2.36	7.14	1.18
Simazine	1.217	2.01 – 100	1.32	4.02	0.66

Thiacloprid	0.918	0.64 – 100	0.42	1.28	0.21
Thiamethoxam	0.862	5.10 – 100	3.36	10.20	1.68
Tramadol	0.698	0.49 – 100	0.32	0.98	0.16
Trimethoprim	0.801	10.94 – 100	7.22	21.88	3.61
Venlafaxine	0.788	0.11 – 100	0.08	0.22	0.04
Warfarin	1.875	1.13 – 100	0.74	2.26	0.37

[a] IDL is the instrument detection limit.
[b] IQL is the instrument quantification limit.
[c] MDL is the method detection limit.

Table C-S4.4.1. Physicochemical parameters measured at each sampling point (SP) of the **Ave River**, in the wet and dry seasons.

Parameter	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8		SP 8	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
pH	5.7	5.0	5.8	5.1	6.2	5.3	6.4	5.2	6.5	5.7	6.8	5.3	7.8	6.1	8.1	6.6	7.6	6.6
Temperature (°C)	8	22	11	22	11	22	11	22	12	22	12	22	14	22	13	22	13	22
Dissolved oxygen (mg L^{-1})	3.6	5.4	3.9	5.7	3.7	5.7	4.4	5.8	4.2	5.7	3.9	5.7	4.0	5.8	3.7	5.5	3.7	5.0
Conductivity (µS cm^{-1})	24	389	54	446	83	454	101	454	109	400	186	535	241	609	287	685	2420	11290
Salinity (PSU)	0.01	0.22	0.02	0.21	0.04	0.22	0.05	0.19	0.05	0.19	0.09	0.22	0.11	0.29	0.14	0.33	1.26	6.15
Total dissolved solids (mg L^{-1})	12	226	26	223	42	228	50	199	55	200	93	248	120	305	144	341	1211	5408

PSU, Practical Salinity Unit.

Table C-S4.4.2. Physicochemical parameters measured in at each sampling point (SP) of the **Leça River**, in the wet and dry seasons.

Parameter	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
pH	7.0	6.3	7.2	5.9	6.7	6.6	6.8	6.9	6.8	6.9	6.8	7.1	6.8	6.9	6.6	6.8
Temperature (°C)	14	22	14	22	14	22	14	22	14	22	13	22	14	22	15	22
Dissolved oxygen (mg L^{-1})	12.3	5.6	13.1	5.7	11.3	5.6	12.7	5.3	11.5	5.5	13.9	5.5	13.1	5.3	9.4	5.5
Conductivity (µS cm^{-1})	121	454	156	424	335	799	420	789	421	738	455	799	531	816	9203	3049
Salinity (PSU)	0.06	0.22	0.07	0.20	0.16	0.38	0.20	0.39	0.20	0.36	0.22	0.39	0.26	0.40	5.18	7.15
Total dissolved solids (mg L^{-1})	61	227	78	212	168	385	210	395	211	369	228	399	267	408	4600	6205

PSU, Practical Salinity Unit.

Table C-S4.4.3. Physicochemical parameters measured in at each sampling point (SP) of the **Antuã River**, in the wet and dry seasons.

Parameter	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8		SP 9	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
pH	6.7	6.8	6.7	6.7	6.9	7.1	7.1	7.2	7.3	7.4	7.2	7.0	7.1	7.0	7.1	6.9	7.1	7.1
Temperature (°C)	19	19	19	19	19	19	19	19	19	19	20	22	19	21	19	21	19	21
Dissolved oxygen (mg L^{-1})	9.0	6.3	8.4	5.7	8.3	6.0	7.2	4.5	7.4	4.5	8.0	5.3	8.1	5.8	8.4	5.3	7.9	5.6
Conductivity (µS cm^{-1})	123	522	124	534	156	567	205	701	248	791	190	622	190	624	195	615	187	607
Salinity (PSU)	0.06	0.25	0.06	0.26	0.07	0.28	0.10	0.34	0.12	0.39	0.09	0.30	0.09	0.30	0.09	0.3	0.09	0.29
Total dissolved solids (mg L^{-1})	62	281	62	266	78	283	103	350	124	395	95	311	95	312	97	308	97	303

PSU, Practical Salinity Unit.

Table C-S4.4.4. Physicochemical parameters measured in at each sampling point (SP) of the **Cértima River**, in the wet and dry seasons.

Parameter	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
pH	7.3	7.0	7.7	7.2	7.6	7.1	7.6	7.2	7.7	7.0	7.4	6.9	7.9	7.0	8.0	6.9
Temperature (°C)	18	20	18	21	18	20	18	21	18	21	18	21	17	21	18	20
Dissolved oxygen (mg L^{-1})	9.0	6.6	6.9	5.5	7.6	6.8	8.6	6.5	7.5	6.5	7.6	6.4	7.9	7.2	8.4	6.9
Conductivity (µS cm^{-1})	517	775	652	1028	607	881	590	1061	617	1091	568	754	569	748	525	756
Salinity (PSU)	0.25	0.36	0.32	0.50	0.30	0.42	0.29	0.51	0.30	0.53	0.28	0.36	0.28	0.36	0.25	0.36
Total dissolved solids (mg L^{-1})	258	364	326	509	303	427	295	517	308	534	284	369	284	364	262	308

PSU, Practical Salinity Unit.

Table C-S4.5.1. Concentration (ng L^{-1}) of each target compound in different sampling points (SP) of the **Ave River**, in the wet and dry seasons.

Target compound	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8		SP 9	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Acetamiprid	n.d.	n.d.	n.d.	< MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MQL	n.d.	< MQL	n.d.	n.d.	n.d.	n.d.
Alachlor	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.d.	n.d.	n.d.	n.d.
Atenolol	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.d.	n.d.	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.	n.d.	n.d.	n.d.	n.d.
Atrazine	n.d.	n.d.	n.d.	< MQL	n.d.	n.d.	n.d.	n.d.	n.d.	0.62 ± 0.01	n.d.	n.d.	n.d.	0.62 ± 0.00	n.d.	0.38 ± 0.13	n.d.	0.25 ± 0.00
Azithromycin	5.51 ± 0.44	n.d.	31.74 ± 1.48	n.d.	7.12 ± 1.47	1.50 ± 0.08	1.72 ± 0.18	1.43 ± 0.07	12.21 ± 0.02	n.d.	2.51 ± 0.14	n.d.	7.95 ± 0.06	n.d.	7.54 ± 0.13	n.d.	5.02 ± 0.15	n.d.
Bezafibrate	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.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	1.23 ± 0.15	1.13 ± 0.02	2.33 ± 0.35	1.61 ± 0.03	2.09 ± 0.17	1.60 ± 0.09	1.85 ± 0.03	2.75 ± 0.09	2.01 ± 0.04	4.78 ± 0.39	4.47 ± 0.08	6.81 ± 0.95	7.27 ± 0.11	3.27 ± 0.24	9.93 ± 0.35	5.84 ± 0.10	24.50 ± 0.58
Ceftiofur	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.d.	n.d.	n.d.	n.d.
Chlorfenvinphos	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.d.	n.d.	n.d.	n.d.
Citalopram	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.d.	n.d.	n.d.	n.d.
Clarithromycin	10.56 ± 2.04	n.d.	43.90 ± 0.82	n.d.	25.75 ± 1.64	n.d.	2.46 ± 0.11	n.d.	43.72 ± 0.33	n.d.	2.34 ± 0.02	n.d.	24.10 ± 0.26	n.d.	25.30 ± 0.53	n.d.	7.30 ± 0.30	0.82 ± 0.03

Clindamycin	4.18 ± 0.11	n.d.	n.d.	n.d.	3.93 ± 0.00	n.d.	3.73 ± 0.05	n.d.	3.77 ± 0.02	n.d.	3.70 ± 0.03	n.d.	3.79 ± 0.12	n.d.	4.48 ± 0.27	n.d.	3.89 ± 0.04	n.d.
Clofibric 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.	n.d.	n.d.	n.d.	n.d.
Clopidogrel	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.d.	n.d.	n.d.	n.d.
Clothianidin	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.d.	n.d.	n.d.	n.d.
Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MQL	n.d.	< MQL	n.d.	4.75 ± 0.18	< MQL	17.78 ± 0.31	< MQL	5.71 ± 0.34	< MQL	11.66 ± 0.48	11.34 ± 0.24
Diphenhyhydramine	n.d.	1.05 ± 0.17	n.d.	1.42 ± 0.22	n.d.	1.21 ± 0.11	n.d.	1.10 ± 0.02	n.d.	0.93 ± 0.04	n.d.	1.05 ± 0.02	n.d.	1.20 ± 0.03	n.d.	2.70 ± 0.12	n.d.	2.56 ± 0.01
EHMC	4.67 ± 0.47	38.88 ± 0.03	7.27 ± 0.31	93.95 ± 5.56	8.00 ± 1.25	167.53 ± 4.64	6.68 ± 0.46	21.79 ± 1.75	48.92 ± 1.11	n.d.	36.44 ± 1.32	n.d.	131.80 ± 0.90	n.d.	24.28 ± 1.83	n.d.	n.d.	n.d.
Enrofloxacin	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.d.	n.d.	n.d.	n.d.
Erythromycin	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.d.	n.d.	n.d.	n.d.
Fluoxetine	n.d.	n.d.	n.d.	0.54 ± 0.14	n.d.	0.65 ± 0.06	n.d.	0.63 ± 0.02	n.d.	0.40 ± 0.00	n.d.	n.d.	n.d.	0.74 ± 0.00	n.d.	27.45 ± 1.66	0.55 ± 0.01	10.01 ± 0.09
Hydrochlorothiazide	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.d.	n.d.	n.d.	n.d.
Imidacloprid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.92 ± 0.49	n.d.	n.d.	n.d.	n.d.	n.d.	< MQL
Isoproturon	n.d.	0.27 ± 0.02	n.d.	0.84 ± 0.03	0.55 ± 0.01	0.30 ± 0.03	0.34 ± 0.08	0.80 ± 0.01	0.36 ± 0.07	0.89 ± 0.03	0.50 ± 0.02	1.43 ± 0.01	1.13 ± 0.05	5.02 ± 0.01	0.55 ± 0.06	2.67 ± 0.05	1.81 ± 0.02	3.62 ± 0.00

Ketoprofen	n.d.	50.53 ± 2.74	n.d.	82.48 ± 8.21	n.d.	68.95 ± 4.06	n.d.	67.11 ± 0.20	n.d.	75.83 ± 2.54	n.d.	106.68 ± 1.45	n.d.	199.74 ± 0.66	n.d.	213.68 ± 0.09	n.d.	216.82 ± 3.56
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.	n.d.	n.d.	n.d.	n.d.
Metoprolol	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.	5.55 ± 0.13	n.d.	n.d.	n.d.	n.d.
Norfluoxetine	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.d.	n.d.	n.d.	n.d.
Ofloxacin	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.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.	n.d.	n.d.	n.d.	n.d.
Propranolol	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.d.	n.d.	n.d.	n.d.
Simazine	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.d.	n.d.	n.d.	n.d.
Thiacloprid	0.66 ± 0.06	1.56 ± 0.04	2.57 ± 0.54	4.54 ± 0.10	1.86 ± 0.19	1.88 ± 0.40	1.45 ± 0.27	1.95 ± 0.13	1.68 ± 0.28	1.46 ± 0.08	3.93 ± 1.07	2.90 ± 0.10	4.22 ± 0.07	4.36 ± 0.35	2.23 ± 0.47	4.75 ± 0.34	4.80 ± 0.33	n.d.
Thiamethoxam	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.d.	n.d.	n.d.	n.d.
Tramadol	n.d.	n.d.	0.50 ± 0.27	3.31 ± 0.93	2.37 ± 0.03	4.55 ± 0.94	3.07 ± 0.08	3.70 ± 0.16	5.79 ± 0.09	4.96 ± 0.05	24.07 ± 0.16	19.72 ± 1.72	37.71 ± 0.96	27.16 ± 1.32	18.26 ± 0.84	35.25 ± 1.36	22.59 ± 0.93	35.33 ± 1.92
Trimethoprim	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.d.	n.d.	n.d.	n.d.
Venlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.77 ± 0.01	10.51 ± 0.22	n.d.	14.44 ± 1.43	n.d.	18.76 ± 1.07	n.d.	n.d.
Warfarin	n.d.	6.71 ± 0.30	n.d.	n.d.	n.d.	7.43 ± 0.41	n.d.	6.79 ± 0.44	n.d.	7.03 ± 0.62	n.d.	7.17 ± 0.62	n.d.	11.50 ± 0.82	n.d.	n.d.	n.d.	21.43 ± 0.93

MQL, method quantification limit; n.d., not detected.

Table C-S4.5.2. Concentration (ng L^{-1}) of each target compound in different sampling points (SP) of the **Leça River**, in the wet and dry seasons.

Target compound	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Acetamiprid	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.d.	n.d.
Alachlor	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.d.	n.d.
Atenolol	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.d.	n.d.
Atorvastatin	n.d.	n.d.	n.d.	n.d.	4.07 ± 0.31	24.25 ± 2.55	9.65 ± 1.72	20.48 ± 1.04	8.42 ± 1.50	n.d.	13.55 ± 0.53	n.d.	10.50 ± 1.30	22.22 ± 1.43	18.74 ± 1.32	15.56 ± 0.74
Atrazine	n.d.	1.44 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.25 ± 0.01	n.d.	1.58 ± 0.03	n.d.	1.58 ± 0.03	n.d.	40.63 ± 0.43
Azithromycin	1.72 ± 0.04	n.d.	1.26 ± 0.03	n.d.	20.98 ± 0.54	n.d.	49.57 ± 0.86	n.d.	34.63 ± 0.45	n.d.	33.78 ± 1.09	n.d.	29.49 ± 0.31	n.d.	33.12 ± 1.77	n.d.
Bezafibrate	n.d.	n.d.	n.d.	n.d.	14.28 ± 1.85	8.75 ± 0.54	33.63 ± 1.70	38.56 ± 0.29	15.29 ± 1.81	20.21 ± 0.37	19.70 ± 1.52	31.41 ± 0.80	19.16 ± 1.08	11.93 ± 0.04	16.81 ± 0.87	12.12 ± 0.18
Carbamazepine	0.98 ± 0.01	0.82 ± 0.00	1.01 ± 0.02	1.45 ± 0.00	13.64 ± 0.38	n.d.	29.60 ± 1.45	98.59 ± 8.71	26.83 ± 1.20	84.66 ± 3.17	31.67 ± 0.33	n.d.	27.87 ± 0.13	70.50 ± 0.20	40.26 ± 0.68	n.d.
Ceftiofur	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.d.	n.d.
Chlorfenvinphos	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.d.	n.d.
Citalopram	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.d.	n.d.
Clarithromycin	n.d.	n.d.	n.d.	n.d.	11.38 ± 0.04	3.79 ± 0.00	24.54 ± 0.11	10.79 ± 0.42	29.48 ± 0.11	4.91 ± 0.21	32.98 ± 0.56	7.62 ± 0.40	32.76 ± 0.24	8.32 ± 0.05	21.25 ± 0.80	4.98 ± 0.03
Clindamycin	n.d.	n.d.	n.d.	n.d.	n.d.	5.09 ± 0.22	n.d.	4.77 ± 0.01	n.d.	4.35 ± 0.07	n.d.	4.81 ± 0.07	n.d.	5.90 ± 0.05	n.d.	4.17 ± 0.11
Clofibric 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.	n.d.	n.d.
Clopidogrel	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.d.	n.d.
Clothianidin	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.d.	n.d.
Diclofenac	< MQL	n.d.	n.d.	< MQL	46.94 ± 1.00	109.46 ± 1.13	96.10 ± 1.02	141.15 ± 0.78	60.71 ± 1.60	87.10 ± 0.05	72.79 ± 0.69	107.05 ± 2.34	59.63 ± 0.93	81.46 ± 0.80	79.60 ± 3.66	55.25 ± 0.90

Diphenhyhydramine	n.d.	0.75 ± 0.00	n.d.	n.d.	1.79 ± 0.09	3.75 ± 0.34	3.49 ± 0.29	6.26 ± 0.78	2.57 ± 0.24	3.74 ± 0.19	2.94 ± 0.10	3.72 ± 0.09	2.49 ± 0.05	3.54 ± 0.05	3.09 ± 0.05	2.56 ± 0.23
EHMC	13.19 ± 1.51	120.39 ± 4.10	7.01 ± 0.31	100.88 ± 3.03	16.83 ± 1.60	41.08 ± 0.68	36.40 ± 0.18	22.41 ± 3.10	92.12 ± 0.84	n.d.	64.29 ± 0.63	n.d.	117.49 ± 1.00	n.d.	81.61 ± 10.15	n.d.
Enrofloxacin	n.d.	29.60 ± 1.10	n.d.	44.99 ± 2.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Erythromycin	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.d.	n.d.
Fluoxetine	n.d.	1.27 ± 0.11	n.d.	n.d.	4.18 ± 0.05	5.18 ± 0.47	12.20 ± 0.08	9.38 ± 0.13	9.64 ± 0.39	7.32 ± 0.24	9.63 ± 0.08	5.37 ± 0.30	7.54 ± 0.48	3.75 ± 0.27	8.11 ± 0.32	4.21 ± 0.34
Hydrochlorothiazide	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.d.	n.d.
Imidacloprid	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.d.	n.d.
Isoproturon	n.d.	n.d.	n.d.	< MQL	9.42 ± 0.17	19.45 ± 0.32	87.18 ± 0.20	40.04 ± 0.23	81.88 ± 1.38	24.12 ± 0.59	88.90 ± 1.74	24.78 ± 0.19	66.39 ± 0.82	21.96 ± 0.21	92.45 ± 1.57	24.16 ± 0.20
Ketoprofen	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.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.	n.d.	n.d.
Metoprolol	n.d.	n.d.	n.d.	n.d.	n.d.	15.00 ± 3.38	n.d.	13.40 ± 1.87	n.d.	n.d.	n.d.	10.52 ± 0.28	n.d.	n.d.	n.d.	9.26 ± 0.19
Norfluoxetine	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.d.	n.d.
Ofloxacin	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.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MQL	< MQL	n.d.	< MQL	n.d.	< MQL	n.d.	< MQL
Propranolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.65 ± 0.03	7.56 ± 1.24	3.65 ± 0.09	4.90 ± 0.26	4.23 ± 0.18	n.d.	3.80 ± 0.26	4.51 ± 0.09	4.61 ± 0.51	3.74 ± 0.03
Simazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.43 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	25.76 ± 0.04
Thiacloprid	1.49 ± 0.12	0.89 ± 0.05	1.55 ± 0.17	0.78 ± 0.01	8.30 ± 0.20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.06 ± 0.26
Thiamethoxam	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.d.	n.d.
Tramadol	< MQL	n.d.	< MQL	4.22 ± 0.27	143.91 ± 1.72	396.11 ± 3.20	232.75 ± 0.94	289.76 ± 5.13	155.00 ± 0.05	161.45 ± 1.69	60.56 ± 1.15	n.d.	62.33 ± 1.20	152.92 ± 2.77	86.66 ± 0.45	153.14 ± 8.17

Trimethoprim	< MQL	n.d.	< MQL	n.d.	< MQL	11.31 ± 0.90	< MQL	15.33 ± 0.41	< MQL	n.d.	< MQL	15.63 ± 0.66	< MQL	n.d.	< MQL	7.37 ± 1.11
Venlafaxine	n.d.	n.d.	n.d.	n.d.	43.29 ± 1.24	149.19 ± 12.27	76.70 ± 1.11	n.d.	65.58 ± 1.63	n.d.	62.31 ± 0.03	106.37 ± 3.31	54.80 ± 0.93	90.08 ± 0.21	72.90 ± 1.59	52.05 ± 1.51
Warfarin	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.d.	n.d.

MQL, method quantification limit; n.d., not detected.

Table C-S4.5.3. Concentration (ng L^{-1}) of each target compound in different sampling points (SP) of the **Antuã River**, in the wet and dry seasons.

Target compound	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8		SP 9	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Acetamiprid	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.d.	n.d.	n.d.	n.d.
Alachlor	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.d.	n.d.	n.d.	n.d.
Atenolol	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.d.	n.d.	n.d.	n.d.
Atorvastatin	n.d.	n.d.	n.d.	n.d.	9.87 ± 0.27	1.92 ± 0.41	52.17 ± 1.64	14.31 ± 0.46	60.66 ± 3.27	8.89 ± 0.59	32.51 ± 0.30	2.13 ± 0.05	17.50 ± 0.45	1.57 ± 0.00	32.24 ± 0.73	n.d.	31.42 ± 1.51	n.d.
Atrazine	0.49 ± 0.00	n.d.	0.61 ± 0.03	n.d.	0.23 ± 0.02	n.d.	0.44 ± 0.01	< MQL	n.d.	0.63 ± 0.02	0.40 ± 0.07	0.55 ± 0.01	0.31 ± 0.01	0.48 ± 0.02	0.32 ± 0.00	n.d.	0.36 ± 0.04	0.20 ± 0.05
Azithromycin	0.91 ± 0.08	3.21 ± 0.07	1.26 ± 0.00	2.76 ± 0.05	3.56 ± 0.23	2.91 ± 0.07	14.08 ± 0.93	10.87 ± 0.75	13.62 ± 0.14	8.14 ± 0.03	9.26 ± 0.38	4.89 ± 0.26	7.77 ± 0.19	2.54 ± 0.04	7.56 ± 0.28	2.24 ± 0.28	8.70 ± 0.32	3.46 ± 0.28
Bezafibrate	n.d.	3.99 ± 0.02	n.d.	2.30 ± 0.03	n.d.	1.39 ± 0.04	n.d.	6.13 ± 0.09	n.d.	3.62 ± 0.15	n.d.	1.71 ± 0.05	n.d.	0.96 ± 0.02	n.d.	0.83 ± 0.01	n.d.	0.71 ± 0.03
Carbamazepine	4.59 ± 0.07	3.43 ± 0.01	6.21 ± 0.23	3.73 ± 1.03	22.63 ± 0.01	11.02 ± 0.87	81.13 ± 1.86	53.53 ± 0.82	71.18 ± 0.54	46.25 ± 1.44	71.07 ± 1.25	19.43 ± 0.25	55.71 ± 0.19	11.48 ± 0.10	58.80 ± 4.11	11.10 ± 2.07	53.61 ± 3.27	11.55 ± 2.57
Ceftiofur	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.d.	n.d.	n.d.	n.d.
Chlorfenvinphos	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.d.	n.d.	n.d.	n.d.
Citalopram	n.d.	n.d.	n.d.	4.66 ± 2.02	n.d.	n.d.	24.72 ± 0.07	n.d.	30.20 ± 1.18	n.d.	14.76 ± 0.23	n.d.	n.d.	n.d.	< MQL	n.d.	< MQL	n.d.
Clarithromycin	n.d.	2.02 ± 0.07	n.d.	2.41 ± 0.07	3.43 ± 0.18	2.50 ± 0.21	6.20 ± 0.16	3.50 ± 0.02	4.69 ± 0.07	2.64 ± 0.04	3.33 ± 0.25	2.68 ± 0.16	2.68 ± 0.18	2.20 ± 0.02	4.56 ± 0.16	1.32 ± 0.02	7.71 ± 0.38	1.99 ± 0.11

Clindamycin	3.35 ± 0.06	10.17 ± 0.26	3.60 ± 0.00	6.82 ± 0.12	3.40 ± 0.02	5.34 ± 0.06	3.65 ± 0.03	4.28 ± 0.04	3.65 ± 0.01	4.30 ± 0.14	3.48 ± 0.00	4.31 ± 0.05	3.52 ± 0.04	4.75 ± 0.06	3.57 ± 0.01	5.94 ± 0.00	3.65 ± 0.00	4.06 ± 0.04
Clofibric 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.	n.d.	n.d.	n.d.	n.d.
Clopidogrel	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.d.	n.d.	n.d.	n.d.
Clothianidin	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.d.	n.d.	n.d.	n.d.
Diclofenac	n.d.	< MQL	5.16 ± 0.07	9.35 ± 0.14	15.34 ± 0.47	14.07 ± 0.11	64.5 ± 0.02	48.64 ± 1.34	65.02 ± 0.02	48.64 ± 1.34	46.35 ± 0.20	45.55 ± 1.07	35.65 ± 1.35	13.91 ± 0.11	32.76 ± 0.04	8.90 ± 0.09	29.39 ± 0.10	6.37 ± 0.14
Diphenhyhydramine	n.d.	2.23 ± 0.00	1.04 ± 0.07	2.04 ± 0.47	2.28 ± 0.22	2.00 ± 0.30	2.89 ± 0.07	2.67 ± 0.25	3.00 ± 0.09	2.66 ± 0.17	2.43 ± 0.24	1.63 ± 0.01	2.08 ± 0.11	1.30 ± 0.02	2.30 ± 0.12	1.98 ± 0.56	2.08 ± 0.14	1.89 ± 0.68
EHMC	< MQL	21.09 ± 0.08	< MQL	18.66 ± 0.14	< MQL	9.48 ± 0.08	8.65 ± 0.69	159.49 ± 2.82	5.19 ± 3.95	200.32 ± 8.76	n.d.	74.27 ± 1.70	n.d.	89.69 ± 22.56	n.d.	17.83 ± 2.02	n.d.	111.62 ± 5.52
Enrofloxacin	n.d.	15.02 ± 1.71	n.d.	38.66 ± 1.77	n.d.	n.d.	n.d.	n.d.	n.d.	59.08 ± 5.38	n.d.	n.d.	n.d.	192.88 ± 23.73	n.d.	343.28 ± 8.21	n.d.	153.69 ± 10.70
Erythromycin	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.d.	n.d.	n.d.	n.d.
Fluoxetine	n.d.	3.22 ± 0.08	n.d.	5.17 ± 0.09	1.34 ± 0.03	2.43 ± 0.15	9.74 ± 0.34	8.32 ± 0.51	9.19 ± 0.46	8.26 ± 0.21	3.80 ± 0.35	2.71 ± 0.08	2.72 ± 0.01	0.64 ± 0.01	2.63 ± 0.07	1.55 ± 0.16	2.70 ± 0.09	1.33 ± 0.32
Hydrochlorothiazide	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.d.	n.d.	n.d.	n.d.
Imidacloprid	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.d.	n.d.	n.d.	n.d.
Isoproturon	1.77 ± 0.00	2.16 ± 0.06	2.07 ± 0.08	1.59 ± 0.01	1.15 ± 0.05	1.11 ± 0.01	1.67 ± 0.03	2.61 ± 0.05	n.d.	2.96 ± 0.03	1.57 ± 0.17	2.05 ± 0.06	1.35 ± 0.03	2.06 ± 0.00	1.36 ± 0.03	2.13 ± 0.04	1.46 ± 0.09	2.34 ± 0.24
Ketoprofen	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.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.	n.d.	n.d.	n.d.	n.d.

Metoprolol	n.d.	7.00 ± 0.23	n.d.	4.79 ± 0.02	n.d.	9.14 ± 2.79	n.d.	9.86 ± 0.66	n.d.	10.86 ± 0.73	n.d.	7.39 ± 0.29	n.d.	n.d.	n.d.	6.90 ± 1.42	n.d.	7.36 ± 1.41
Norfluoxetine	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.d.	n.d.	n.d.	n.d.
Ofloxacin	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.d.	n.d.	n.d.	n.d.
PFOS	n.d.	< MQL	n.d.	< MQL	< MQL	< MQL	< MQL	< MQL	n.d.	< MQL	< MQL	< MQL	< MQL	n.d.	< MQL	n.d.	< MQL	n.d.
Propranolol	n.d.	< MQL	< MQL	n.d.	n.d.	n.d.	4.39 ± 0.32	4.44 ± 0.00	5.38 ± 0.04	4.09 ± 0.06	3.72 ± 0.05	< MQL	< MQL	< MQL	3.61 ± 0.03	n.d.	< MQL	n.d.
Simazine	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.d.	n.d.	n.d.	n.d.
Thiacloprid	3.40 ± 0.20	17.56 ± 0.34	2.87 ± 0.01	5.67 ± 0.38	3.86 ± 0.39	4.26 ± 0.06	n.d.	6.34 ± 0.48	5.91 ± 0.70	6.32 ± 0.56	n.d.	3.96 ± 0.41	n.d.	3.94 ± 0.26	5.63 ± 0.10	5.72 ± 0.30	4.18 ± 0.23	3.19 ± 0.37
Thiamethoxam	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.d.	n.d.	n.d.	n.d.
Tramadol	1.02 ± 0.08	9.24 ± 0.11	8.63 ± 0.20	7.76 ± 0.29	53.16 ± 0.25	48.89 ± 15.12	114.96 ± 2.38	220.88 ± 1.30	142.12 ± 2.38	227.37 ± 4.22	89.35 ± 1.57	88.64 ± 0.96	88.57 ± 2.27	66.67 ± 0.39	87.62 ± 2.26	57.06 ± 2.91	97.38 ± 0.69	54.82 ± 0.81
Trimethoprim	n.d.	64.14 ± 7.80	n.d.	45.06 ± 3.90	n.d.	14.90 ± 1.61	< MQL	< MQL	< MQL	12.07 ± 2.56	n.d.	< MQL	n.d.	13.40 ± 1.62	< MQL	12.77 ± 1.15	< MQL	< MQL
Venlafaxine	n.d.	17.98 ± 0.28	13.17 ± 0.69	14.14 ± 0.32	68.92 ± 0.31	25.74 ± 2.32	135.31 ± 19.07	74.88 ± 3.71	199.07 ± 3.39	86.85 ± 14.93	124.19 ± 5.86	67.74 ± 0.82	114.41 ± 9.21	39.15 ± 0.97	108.79 ± 2.39	37.98 ± 4.44	111.47 ± 10.75	37.41 ± 1.04
Warfarin	n.d.	60.23 ± 0.44	n.d.	31.93 ± 1.16	n.d.	17.38 ± 0.44	n.d.	35.83 ± 0.51	n.d.	28.61 ± 1.11	n.d.	10.63 ± 0.74	n.d.	10.29 ±0.24	n.d.	11.36 ± 0.24	n.d.	7.34 ± 0.20

MQL, method quantification limit; n.d., not detected

Table C-S4.5.4. Concentration (ng L^{-1}) of each target compound in different sampling points (SP) of the **Cértima River**, in the wet and dry seasons.

Target compound	SP 1		SP 2		SP 3		SP 4		SP 5		SP 6		SP 7		SP 8	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Acetamiprid	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.d.	n.d.
Alachlor	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.d.	n.d.
Atenolol	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.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.	n.d.	n.d.
Atrazine	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.d.	n.d.
Azithromycin	1.02 ± 0.02	n.d.	< MQL	26.08 ± 0.60	11.42 ± 0.09	n.d.	8.48 ± 0.02	n.d.	3.57 ± 0.14	2.70 ± 0.01	3.25 ± 0.01	n.d.	3.15 ± 0.32	n.d.	1.01 ± 0.01	0.89 ± 0.02
Bezafibrate	n.d.	n.d.	9.17 ± 0.26	6.69 ± 0.03	7.71 ± 0.06	5.50 ± 0.01	6.94 ± 0.22	< MQL	5.73 ± 0.10	n.d.	4.94 ± 0.13	n.d.	3.82 ± 0.04	n.d.	n.d.	n.d.
Carbamazepine	8.60 ± 0.02	3.96 ± 0.42	104.56 ± 6.53	177.01 ± 13.28	86.05 ± 0.42	77.56 ± 2.11	94.48 ± 1.49	61.57 ± 7.61	95.95 ± 7.31	22.61 ± 2.74	65.37 ± 1.87	10.77 ± 0.31	50.38 ± 1.55	7.46 ± 0.06	23.83 ± 0.36	6.39 ± 0.02
Ceftiofur	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.d.	n.d.
Chlorfenvinphos	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.d.	n.d.
Citalopram	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.d.	n.d.
Clarithromycin	n.d.	n.d.	2.60 ± 0.07	5.28 ± 0.10	1.74 ± 0.07	3.87 ± 0.06	3.56 ± 0.71	2.72 ± 0.03	< MQL	1.96 ± 0.17	0.96 ± 0.10	0.39 ± 0.05	0.88 ± 0.02	n.d.	n.d.	n.d.
Clindamycin	n.d.	n.d.	3.98 ± 0.01	5.13 ± 0.08	3.89 ± 0.08	5.94 ± 0.12	3.63 ± 0.06	5.73 ± 0.02	4.09 ± 0.00	3.76 ± 0.03	3.69 ± 0.01	3.55 ± 0.02	3.72 ± 0.08	3.56 ± 0.04	3.69 ± 0.05	3.51 ± 0.06
Clofibric 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.	n.d.	n.d.
Clopidogrel	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.d.	n.d.
Clothianidin	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.d.	n.d.

Diclofenac	2.44 ± 0.44	n.d.	60.38 ± 1.70	188.73 ± 4.57	68.92 ± 1.58	86.20 ± 2.66	57.23 ± 1.62	50.29 ± 0.62	38.68 ± 1.03	23.67 ± 0.50	28.84 ± 1.62	12.34 ± 0.09	26.77 ± 0.16	7.03 ± 0.08	6.70 ± 0.46	n.d.
Diphenhyhydramine	n.d.	n.d.	n.d.	7.42 ± 0.22	n.d.	1.93 ± 0.13	n.d.	1.84 ± 0.28	n.d.	1.42 ± 0.07	n.d.	0.93 ± 0.04	n.d.	0.80 ± 0.08	n.d.	n.d.
EHMC	10.91 ± 1.51	90.80 ± 12.80	7.73 ± 0.33	128.17 ± 2.67	5.37 ± 0.21	95.40 ± 15.89	6.81 ± 0.26	49.44 ± 1.05	n.d.	n.d.	97.76 ± 0.39	n.d.	91.75 ± 0.17	n.d.	91.75 ± 0.91	561.74 ± 0.39
Enrofloxacin	n.d.	n.d.	n.d.	25.87 ± 0.01	n.d.	27.97 ± 1.83	n.d.	45.53 ± 0.66	n.d.	5.89 ± 0.20	n.d.	5.55 ± 0.09	n.d.	10.89 ± 0.42	n.d.	21.66 ± 4.94
Erythromycin	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.d.	n.d.
Fluoxetine	n.d.	n.d.	6.40 ± 0.13	12.34 ± 0.21	2.88 ± 0.09	1.62 ± 0.03	1.67 ± 0.01	1.19 ± 0.03	1.39 ± 0.10	< MQL	0.73 ± 0.37	n.d.	0.41 ± 0.09	n.d.	n.d.	n.d.
Hydrochlorothiazide	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.d.	n.d.
Imidacloprid	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.d.	n.d.
Isoproturon	n.d.	0.79 ± 0.01	n.d.	3.44 ± 0.03	n.d.	0.98 ± 0.01	n.d.	1.28 ± 0.06	n.d.	0.43 ± 0.03	n.d.	0.23 ± 0.02	n.d.	0.13 ± 0.00	n.d.	n.d.
Ketoprofen	n.d.	n.d.	n.d.	220.66 ± 4.56	n.d.	176.05 ± 2.79	n.d.	146.19 ± 1.81	n.d.	124.40 ± 2.49	n.d.	108.90 ± 0.88	n.d.	99.91 ± 1.19	n.d.	231.98 ± 5.40
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.	n.d.	n.d.
Metoprolol	n.d.	n.d.	n.d.	24.63 ± 0.37	n.d.	15.84 ± 0.37	n.d.	13.28 ± 1.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norfluoxetine	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.d.	n.d.
Ofloxacin	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.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.	< MQL	n.d.	< MQL	n.d.	< MQL

Propranolol	n.d.	n.d.	n.d.	9.07 ± 0.09	n.d.	3.81 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Simazine	2.98 ± 0.04	n.d.	3.81 ± 0.03	n.d.	3.54 ± 0.04	3.24 ± 0.00	3.70 ± 0.02	n.d.	3.19 ± 0.02	n.d.	3.45 ± 0.03	< MQL	< MQL	< MQL	< MQL	n.d.
Thiacloprid	69.79 ± 2.36	11.61 ± 1.01	n.d.	7.80 ± 0.88	n.d.	4.32 ± 0.42	n.d.	3.69 ± 0.43	n.d.	2.75 ± 0.03	755.25 ± 9.26	2.04 ± 0.26	n.d.	35.28 ± 3.21	379.38 ± 0.46	3.60 ± 0.41
Thiamethoxam	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.d.	n.d.
Tramadol	12.51 ± 0.29	4.27 ± 1.04	160.42 ± 4.40	299.03 ± 6.17	161.72 ± 7.09	191.37 ± 11.63	144.97 ± 4.44	n.d.	139.26 ± 5.54	n.d.	113.26 ± 10.61	n.d.	92.57 ± 1.46	35.28 ± 3.21	47.30 ± 0.14	4.36 ± 0.01
Trimethoprim	n.d.	n.d.	< MQL	12.68 ± 0.79	< MQL	n.d.	< MQL	n.d.	< MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Venlafaxine	n.d.	n.d.	n.d.	170.24 ± 6.83	n.d.	77.84 ± 4.53	n.d.	n.d.	n.d.	17.58 ± 2.28	n.d.	n.d.	n.d.	11.12 ± 1.70	n.d.	n.d.
Warfarin	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.d.	n.d.

MQL, method quantification limit; n.d., not detected.

Table C-S4.6. Comparison of occurrence data for the targeted pollutants in surface water samples (ng L^{-1}) from this study with previous reports.

Compound	Concentration range (ng L^{-1})		Ref.
	Present study	Other studies	
Atrazine	< MQL – 12	10 – 800	[1-5]
Azithromycin	< MQL – 50	< MQL – 30	[6-8]
Bezafibrate	< MQL – 34	< MQL – 73	[9-11]
Carbamazepine	0.8 – 177	1 – 330	[5, 6, 11-14]
Clarithromycin	< MQL – 44	21 – 97	[6, 7, 9, 14]
Diclofenac	< MQL – 189	2 – 170	[5, 9, 11, 12, 14]
EHMC	< MQL – 562	669 – 4043	[15, 16]
Fluoxetine	< MQL – 12	2 – 20	[6]
Isoproturon	< MQL – 92	29 – 58	[17, 18]
Ketoprofen	51 – 232	58 – 225	[6, 9, 17, 19]
Metoprolol	5 – 25	0.6 – 495	[7, 9, 11]
Simazine	< MQL –26	17 – 670	[1, 2, 18]
Thiacloprid	0.7 – 70	< MQL – 120	[17, 20]
Trimethoprim	< MQL –45	< MQL – 180	[5, 7, 11-13]
Venlafaxine	11 – 199	0.1 – 159	[6, 7, 21]

Table C-S4.7. Statistical relationships between regional EEM volumes and CEC concentrations (ng L^{-1}). The values shown are the correlation coefficients (R2) of the linear regression of the listed regional volume with CEC concentration. The ***, **, and * notations indicate $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

Compounds	Region I	Region II	Region III	Region IV	Region V	Total FI
Azithromycin	0.94 ***	0.97 ***	0.96 ***	0.96 ***	0.94 ***	0.97 ***
Carbamazepine	0.78 *	0.88 **	0.89 **	0.92 **	0.93 ***	0.90 **
EHMC	0.51	0.70	0.75 *	0.74 *	0.78 *	0.71 *

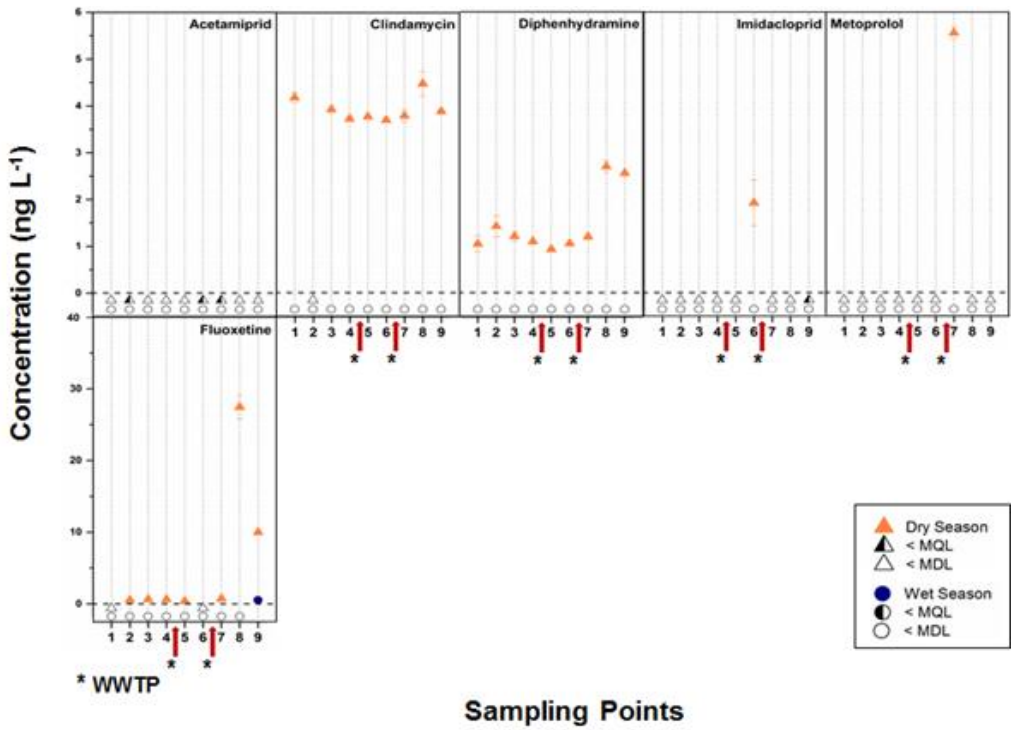

Fig. C-S4.1. Spatial distribution and concentrations of micropollutants determined at concentrations below 30 ng L^{-1} in Ave river for dry and wet seasons.

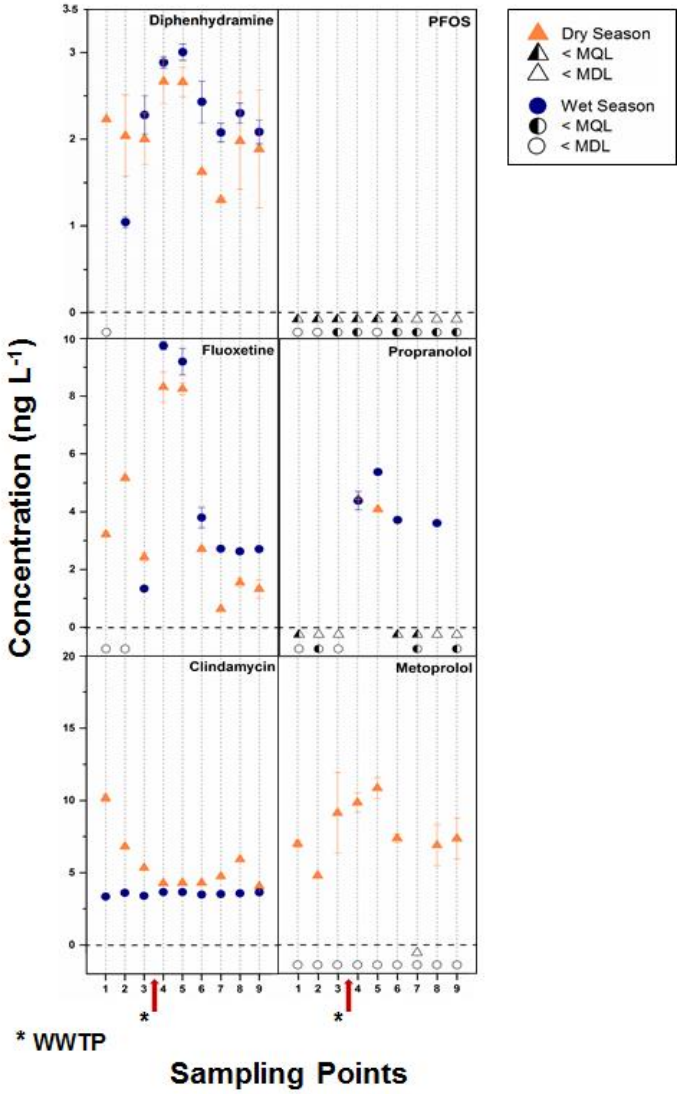

Fig. C-S4.2. Spatial distribution and concentrations of micropollutants determined at concentrations below 30 ng L^{-1} in Antuã river for dry and wet seasons.

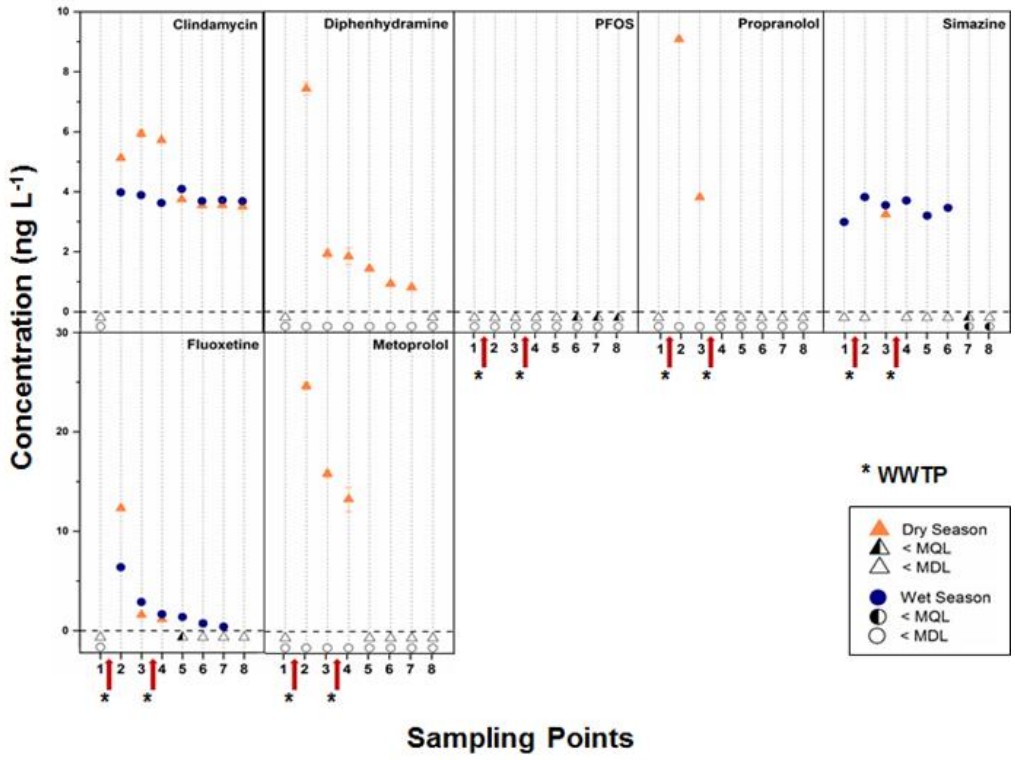

Fig. C-S4.3. Spatial distribution and concentrations of micropollutants determined at concentrations below 30 ng L^{-1} in Cértima river for dry and wet seasons.

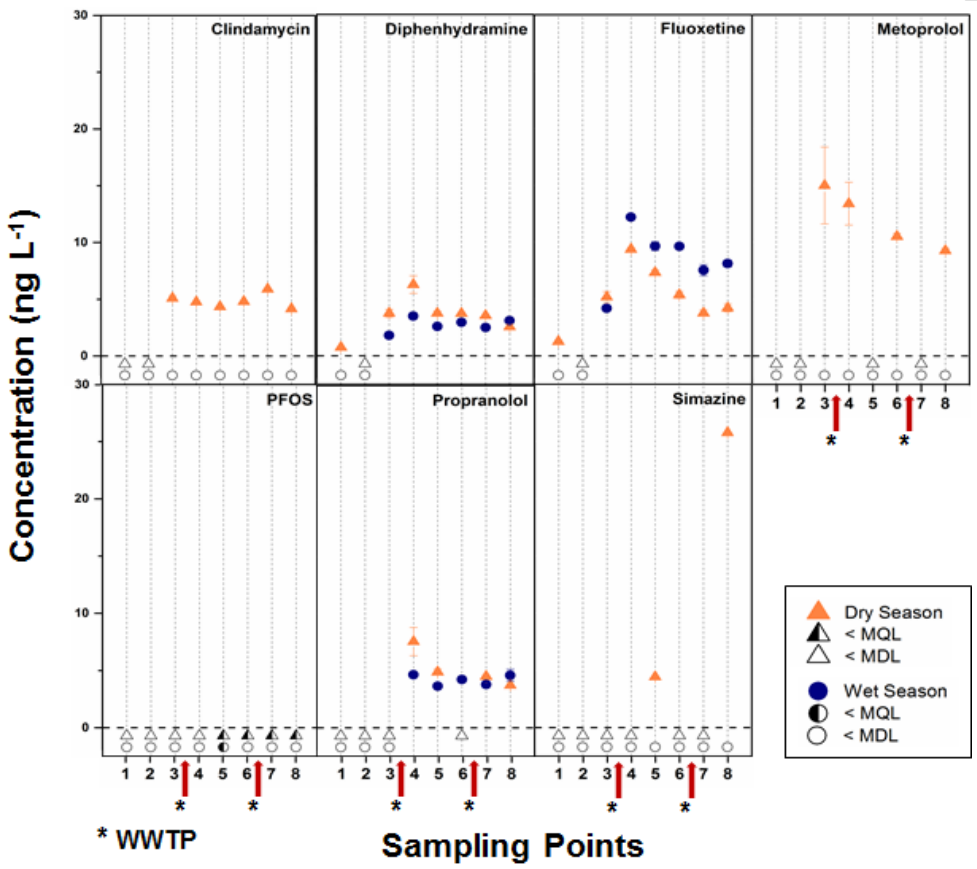

Fig. C-S4.4. Spatial distribution and concentrations of micropollutants determined at concentrations below 30 ng L^{-1} in Leça river for dry and wet seasons.

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Appendix D

Original version and supplementary material of Chapter 5:

Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants

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Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants

Marta O. Barbosa, Rui S. Ribeiro, Ana R. L. Ribeiro, M. Fernando R. Pereira & Adrián M. T. Silva✉

Pristine and functionalized multi-walled carbon nanotubes (MWCNTs) were investigated as adsorbent materials inside solid-phase extraction (SPE) cartridges for extraction and preconcentration of 8 EU-relevant organic micropollutants (with different pKa and polarity) before chromatographic analysis of surface water. The recoveries obtained were > 60% for 5/8 target pollutants (acetamiprid, atrazine, carbamazepine, diclofenac, and isoproturon) using a low amount of this reusable adsorbent (50 mg) and an eco-friendly solvent (ethanol) for both conditioning and elution steps. The introduction of oxygenated surface groups in the carbon nanotubes by using a controlled HNO_3 hydrothermal oxidation method, considerably improved the recoveries obtained for PFOS (perfluorooctanesulfonic acid) and methiocarb, which was ascribed to the hydrogen bond adsorption mechanism, but decreased those observed for the pesticide acetamiprid and for two pharmaceuticals (carbamazepine and diclofenac), suggesting π–π dispersive interactions. Moreover, a good correlation was found between the recovery obtained for methiocarb and the amount of oxygenated surface groups on functionalized MWCNTs, which was mainly attributed to the increase of phenols and carbonyl and quinone groups. Thus, the HNO_3 hydrothermal oxidation method can be used to finely tune the surface chemistry (and texture) of MWCNTs according to the specific micropollutants to be extracted and quantified in real water samples.

In the last decades, a growing interest has been raised about the fate and effects of a large group of organic micropollutants (OMPs) on the aquatic environment. These pollutants found at trace concentrations (ng L^{-1} to μg L^{-1}) can be natural or anthropogenic substances, such as pharmaceutical compounds, pesticides, industrial compounds and steroid hormones[1]. Conventional wastewater treatment plants are not designed to completely remove many of these organic compounds at low concentrations, which are thus discharged into receiving water bodies, including groundwater and surface water (SW), reaching drinking water for human consumption[2]. Other sources of contamination include direct discharge and runoff, namely in the case of industrial compounds, pesticides applied in agriculture, and veterinary pharmaceuticals used for livestock and aquaculture[3,4]. Most of these compounds are pseudo-persistent since their transformation/removal rates are overcome by their continuous release into the environment. Moreover, their recalcitrant character and polarity favours the dispersion and interchange between aquatic compartments[4,5]. The presence of such OMPs in the aquatic environment is considered an important issue in terms of public health safety[6]. Therefore, the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2018/840 and Decision 2020/161) in SW bodies has been recommended within the European Union (EU). The comprehensive identification and quantification of PSs and CECs in freshwater samples is crucial to collect information on their

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sources, distribution and fate in the environment, to study the effects on ecosystems and human health, and to update the water policy in this field. To achieve this goal, it is important to set up fast, sensitive and reliable analytical methods enabling the determination of a wide range of OMPs typically found at residual levels in aquatic compartments.

Despite the shortcomings of solid phase extraction (SPE), such as the high volumes of organic solvents needed in comparison with miniaturized techniques, time consumption and high cost, this sample preparation technique is still the most employed for preconcentration of OMPs in water matrices due to the efficient removal of interferences, consequent reduction of matrix effects and high enrichment factors and recoveries often yielded[1,7]. SPE is an essential preconcentration step prior to analysis by a sensitive and reproducible analytical technique such as ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The type of sorbent, its structure and interactions with the target analytes play an important role in SPE, reason why many carbon materials have been already reported as good candidates as filling materials for this purpose[8,9].

Multi-walled carbon nanotubes (MWCNTs) are the most studied carbon materials for environmental applications of SPE. This fact can be attributed to: (i) the unique structure of MWCNTs that enables strong interactions with organic molecules through non-covalent forces (i.e., hydrophobic interactions, hydrogen bonding, π–π stacking, electrostatic forces and van der Waals forces); (ii) their large surface-to-volume ratio; (iii) good thermal and mechanical stability; and (iv) the possibility to control their affinity towards target compounds upon surface functionalization by chemical or physical methods[10]. MWCNTs have been investigated as SPE sorbents for sample preparation of water matrices and to pre-concentrate and extract OMPs such as pesticides (e.g.[11–16]), polycyclic aromatic hydrocarbons[17,18], industrial compounds[19], macrolide antibiotics and nonsteroidal anti-inflammatory drugs[20,21], with recoveries higher than 62%. However, the number of studies dealing with multi-class PSs and CECs with different physicochemical properties are still very limited[20,21]. Moreover, application of functionalized MWCNTs in SPE for extraction of EU-relevant OMPs is even scarcer in the literature. Only three studies were reported, namely for: (i) pentachlorophenol using MWCNTs oxidized with 8.0 mol L^{-1} of HNO_3[22]; (ii) the industrial compound perfluorooctanesulfonic acid (PFOS) using amino-terminated alkyl-functionalized MWCNTs[19]; and (iii) thirteen pharmaceutical compounds, some of them defined in the EU Decisions (erythromycin, azithromycin and diclofenac), using MWCNTs treated with high concentrations of HNO_3 (4.0 mol L^{-1}), HCl (1.0 mol L^{-1}) and KOH (4.0 mol L^{-1})[21], i.e. having pronounced environmental implications and costs.

In the present work, pristine and modified MWCNTs were investigated as SPE sorbents for the simultaneous extraction of 8 EU multi-class OMPs in SW before UHPLC-MS/MS analysis. The target compounds, namely 5 pesticides (acetamiprid, atrazine, isoproturon, metaflumizone and methiocarb), 2 pharmaceutical compounds (carbamazepine and diclofenac), and one industrial compound (PFOS) were strategically selected due to their high frequency of detection and/or their high levels of concentration in water matrices observed during the monitoring sampling campaigns performed by our research group in the last years[2,6] and, in the specific case of metaflumizone due to its presence in the recently 3rd Watch List (Decision 2020/1161). A set of experiments was performed using pristine MWCNTs to study the parameters that influenced the extraction efficiency of the 8 OMPs spiked in SW samples, namely the sample pH and volume, the elution and extraction solvent and respective volumes, and the amount of MWCNTs packed in the cartridge. After optimizing these parameters, the cartridge packed with MWCNTs and the commercial cartridge Oasis HLB were compared, in terms of extraction efficiency, reusability, and costs. Then, we attempted to investigate a HNO_3 hydrothermal oxidation methodology reported by our group[23] to obtain a series of MWCNTs with meticulously introduced surface oxygen functionalities. This methodology allows the fine control of the type and amount of surface groups introduced on carbon materials by adjusting the concentration of oxidizing agent employed in the treatment (HNO_3 concentration in the range 0.01–0.30 mol L^{-1}), as determined by different characterization techniques. This distinctive feature allowed establishing correlations between both the synthesis conditions and the oxygen-containing surface functionalities introduced on the MWCNTs; and the type and amount of those functionalities and the recoveries obtained for the 8 target OMPs; while employing much lower concentrations of oxidizing agent than those previously reported with similar hydrothermal methodologies[23–26]. Therefore, the novelty of this study relies on (i) the development of a systematic study, upon application of a controlled HNO_3 hydrothermal oxidation methodology to pristine MWCNTs; but also on (ii) the use of ethanol as elution solvent in the SPE procedure, when using MWCNT cartridges; and (iii) the study of metaflumizone for the first time in real water compartments.

Experimental section

Chemicals and materials. MWCNTs (NC3100, powder) with an average diameter of 9.5 nm, average length of 1.5 µm and > 95% purity were obtained from Nanocyl SA (Sambreville, Belgium). All reference standards (acetamiprid, atrazine, carbamazepine, diclofenac sodium, isoproturon, metaflumizone, methiocarb and PFOS; > 98% purity) and deuterated compounds used as internal standards (acetamiprid-d3, atrazine-d5, diclofenac-d4, fluoxetine-d5 and methiocarb-d3) were purchased from Sigma-Aldrich (Steinhein, Germany). The physicochemical properties of the target compounds can be found in Table S1. Methanol and acetonitrile (MS grade), ethanol (HPLC grade), and hydrochloric acid were obtained from VWR International (Fontenay-sous-Bois, France). Individual stock solutions of 1000 mg L^{-1} of each reference and internal standard were prepared in methanol, ethanol or acetonitrile, depending on their solubility. Two ethanolic working solutions containing the 8 target compounds (2.5 mg L^{-1}) and the 5 internal standards (5.0 mg L^{-1}) were prepared by dilution of the individual stocks. Sulfuric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Sodium chloride was purchased from José Manuel Gomes dos Santos. Ultrapure water was supplied by a Milli-Q water system. Oasis HLB (Hydrophilic–Lipophilic-Balanced) cartridges (150 mg, 6 mL) were obtained from Waters (Milford, MA, USA), and the empty SPE cartridges (6 mL) with two frits (20 µm) (Bond Elut) were

purchased from VWR International (Fontenay-sous-Bois, France). pH measurements were performed with a pHenomenal pH 1100L pH meter (VWR, Germany).

Surface functionalization of MWCNTs. Hydrothermal oxidation of the pristine MWCNTs was performed in a Teflon-lined stainless-steel autoclave (Mod. 4748, Parr Instruments, USA) with 125 mL of capacity, following the experimental procedure described elsewhere[27]. 75 mL of a HNO_3 solution (concentration in the range 0.01–0.30 mol L^{-1}) was transferred to a PTFE vessel and 0.2 g of the pristine MWCNTs was loaded. The PTFE vessel was placed into the stainless-steel autoclave, which was sealed and placed in an oven at 200 °C for 2 h. After this time, the autoclave was allowed to cool down until ambient temperature. The recovered material was washed several times with distilled water until a neutral pH of the rinsing water was attained, and then dried overnight at 120 °C. Additionally, a blank hydrothermal treatment with distilled water instead of the HNO_3 solution was performed. The resulting materials were labelled as MWCNT followed by a subscript number corresponding to the concentration of HNO_3 employed in the hydrothermal treatment in mol L^{-1} (i.e., $MWCNT_0$, $MWCNT_{0.01}$, $MWCNT_{0.05}$, $MWCNT_{0.1}$, $MWCNT_{0.2}$, and $MWCNT_{0.3}$).

Characterization of MWCNTs. Temperature programmed desorption (TPD) was performed in a fully automated AMI-300 Catalyst Characterization Instrument (Altamira Instruments), equipped with a quadrupole mass spectrometer (Dymaxion, Ametek), as described elsewhere[27]. Briefly, TPD is a well-established advanced characterization technique assuming that all oxygen-containing surface groups are decomposed into CO_2 and CO upon heating under controlled operating conditions[28]. In this case, a low heating rate of 10° C min^{-1}, and a high helium flow of 25 cm^3 min^{-1} were set to minimize secondary reactions during the experiments[26,28]. The mass signals m/z = 28 and 44 were monitored during the thermal analysis, the corresponding TPD spectra being obtained. CO and CO_2 were calibrated at the end of each analysis with the respective gases. The concentrations of the different oxygen containing surface groups were then obtained by deconvolution analysis of the CO_2 and CO TPD spectra using a procedure established by our group[28,29]. Accordingly, the peaks in the CO_2 TPD spectra were assigned to diverse functional groups, namely strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (CAn), and lactones (Lac). Similarly, the peaks in the CO TPD spectra were assigned to carboxylic anhydrides (Can), phenols (Ph), carbonyls and quinones (CQ), and basic surface groups (Bas), such as pyrones and chromenes. The width at half-height (W) of the peak was taken the same for Can and Lac in the CO_2 spectra, and the same W was considered for Ph and CQ in the CO spectra whenever peak shoulders were unclear. Thermogravimetric analysis (TGA) was performed in a Netzsch STA 490 PC/4/H Luxx thermal analyser, in which the powder sample was heated from 50 to 900 °C at 10 °C min^{-1}, under an inert (N_2) gas flow. Regarding TPD and TGA analysis, selected experiments were performed in duplicate, the standard deviations (SD) never exceeding the values given in the caption of Fig. 3. Textural properties were determined from N_2 adsorption–desorption isotherms at − 196 °C, as described in our previous work[23], and included specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}), micropore volume (V_{micro}) and total pore volume (V_{total}). The pH at point of zero charge (pH_{PZC}) was obtained by pH drift tests[27].

MWCNTs SPE procedure. Commercial cartridges Oasis HLB were used for comparison purposes in this study (Text S1). After preparing the cartridges with 50 mg of each adsorbent (Figure S1), the SPE protocol previously optimized (Text S2) for pristine MWCNTs (NC3100) was performed. Briefly, ethanol (4 mL) and ultrapure water (4 mL) were used to condition and equilibrate the cartridge at a flow rate of 1 mL min^{-1}. 500 mL of blank or spiked (200 ng L^{-1} of each target compound) SW sample previously acidified to pH 3 was loaded at 10 mL min^{-1}. 4 mL of ultrapure water was then added in the washing step, followed by 45 min of vacuum drying. For the elution step, 4 mL of ethanol was used and, after evaporation, the filtered reconstituted ethanolic extracts were analysed by UHPLC-MS/MS. All experiments were performed in triplicate and relative standard deviation (RSD) were estimated. For details on the SPE procedure, please see Supplementary Material (Text S1 and S2; Figure S1).

Evaluation of the SPE recovery efficiency. The recovery efficiency (%) is the most important parameter supporting the selection of the optimal conditions for a given SPE procedure. Therefore, the performance of the off-line SPE method was assessed considering the recovery efficiency for the 8 target analytes under study. The recovery was calculated as the ratio of the peak areas obtained for extracted spiked sample (A) and the peak areas of the post-spiked extracted sample (B), as described in Figure S2 and Eq. (1).

$$Recovery\ efficiency(\%) = 100 \times (A/B) \quad (1)$$

Since the matrix effect is considered the same in both A and B, and thus not accounted for, this approach allows evaluating exclusively the recovery promoted by the adsorbent material. Total Ion Current (TIC) chromatograms of the 8 target OMPs (200 ng L^{-1}) after SPE of a spiked sample and after post-spiking a blank extract using original MWCNT packed cartridges are showed in Figure S3 a and b.

UHPLC–MS/MS method. A Shimadzu Corporation UHPLC-MS/MS (Tokyo, Japan) consisting of a Nexera UHPLC (two chromatographic pumps LC-30AD with a degasser DGU-20A 5R, an autosampler SIL-30AC, an oven CTO-20AC, and a system controller CBM-20A with a Shimadzu LC Solution Version 5.41SP1 software), and a Ultra Fast Mass Spectrometry series LCMS-8040 triple quadrupole mass spectrometer, was used for SW analysis. The chromatographic separation of the target compounds was performed by using a column Kinetex XB-C18 100 Å (100 × 2.1 mm i.d.; particle diameter of 1.7 µm) acquired to Phenomenex, Inc. (Torrance, CA, USA) operating under gradient mode of flow of the mobile phase water/ethanol (50/50, v/v). The column oven

temperature was set at 35 °C. The autosampler temperature was set at 15 °C and the injection volume was 5 µL. The MS settings were: 2.5 $dm^3 min^{-1}$ of nebulizing gas flow, 12.5 $dm^3 min^{-1}$ of drying gas flow, capillary voltage of 0.5 kV, 400 °C and 250 °C for source and desolvation temperatures, argon at 230 kPa as CID gas. The quantification and confirmation of the identity of each analyte was performed by selected reaction monitoring (SRM). Along with the retention time of the analyte, the transition between the precursor ion and the most abundant fragment ion (SRM1) was used for quantification and the ratio between SRM1 and the transition between the precursor ion and the second most abundant fragment ion SRM2 was used for identity confirmation. All the analytical parameters used, namely SRM instrument parameters, retention time, linearity, and limits of detection and quantification, are detailed in the Supplementary Material (Tables S2 and S3).

Sample collection. SW samples (pH = 6.5 ± 0.1) were collected from Cavalum River (tributary of the Sousa River) located in Penafiel (40 km from Porto, Portugal). Samples were stored in amber glass bottles (1 L) at 4 °C until extraction, which was performed within 24 h. Before SPE, all samples were filtered through 1.2-µm glass-fiber filters (47 mm GF/C, Whatmam, Maidstone, United Kingdom) and the pH was adjusted using sulfuric acid or sodium hydroxide solutions, according to the SPE procedure ("MWCNTs SPE procedure" section, Texts S1 and S2).

Results and discussion

***Optimization of SPE procedure with* pristine MWCNTs (NC3100) *cartridges*.** In order to study the performance of pristine MWCNTs (NC3100) as SPE adsorbent for the simultaneous enrichment of the 8 target EU OMPs with different p*Ka* and polarity range, the main experimental conditions affecting the extraction efficiency were optimized, namely the sample pH and volume, the elution and extraction solvent and respective volumes, and the amount of MWCNTs packed in the cartridge.

Regarding the sample pH (3, 7 or 9), it determines the state of the target micropollutants in solution as ionic or molecular form, directly affecting the recovery efficiency of the process. When using ethanol as solvent (Fig. 1a), an acidic pH enabled higher recoveries of acetamiprid, atrazine, methiocarb and the industrial compound PFOS, while the recovery of the pharmaceutical compounds carbamazepine and diclofenac performed better at alkaline pH. Neutral pH led to lower recoveries, except for isoproturon. Methanol and acetonitrile were also tested as solvents (Fig. 1b), but ethanol (as conditioning and elution solvent) and an acidic sample pH allowed similar or slightly higher recoveries for most of the target compounds, in comparison with the other studied conditions. Moreover, ethanol is considered an eco-friendly (and greener) solvent[30], and thus selected for the next experiments. Different amounts of the adsorbent material packed in the SPE cartridge (between 25 and 150 mg) were then investigated (Fig. 1c), and the highest recoveries for the target compounds were obtained when using cartridges packed with 50 mg of MWCNTs (except in the case of the pesticide metaflumizone). Lower recoveries were obtained when using amounts below 50 mg of MWCNTs, which may be due to the limited adsorption capacity of this carbon material at these conditions. Lower recoveries were also obtained for amounts above 50 mg of MWCNTs, which may be explained by a lower desorption of OMPs from MWCNTs during the elution step. Bearing this in mind, 50 mg was considered the optimum amount of MWCNTs, and thus selected for the following experiments.

In what concerns the volume of the SW sample (Fig. 1d), the higher extraction efficiencies were obtained for the majority of the compounds when using a sample volume of 500 mL (except for atrazine and metaflumizone). The sample volume is expected to be directly proportional to the sample preparation enrichment factor (i.e., the ratio between the sample volume and the volume of reconstitution). However, the recoveries obtained for most compounds decreased when the sample volume increased from 500 to 1000 mL. This phenomenon may be ascribed to the SPE breakthrough volume, which is the highest sample volume that allows the maximum extraction efficiency, as observed in previous works[3,6]. Using the optimum volume of SW sample for most OMPs (500 mL), different volumes of eluent (4–10 mL) were then tested (Fig. 1e). The recoveries obtained for the 8 OMPs under study slightly increased with the volume of ethanol used in the elution step, a lower volume of eluent being selected for the next experiments (i.e. lower costs) since the recoveries obtained were quite similar.

The reusability of the MWCNT cartridge is confirmed in Fig. 1g, similar recoveries being obtained in three consecutive cycles. Moreover, the recoveries achieved (> 60%) for 5 of these EU multi-class OMPs analyzed simultaneously (acetamiprid, atrazine, carbamazepine, diclofenac and isoproturon), using a low amount of adsorbent (50 mg of MWCNTs for 500 mL of SW samples at pH 3) and a conditioning and elution solvent considered "green" (ethanol—4 mL), were comparable to those reported in the literature using more toxic solvents and a single compound or specific class of compounds (Table S5). Thus, the next step was to functionalize the MWCNTs in order to investigate the influence of the surface chemistry on the performance of this analytical tool.

Comparison of optimized SPE procedures for MWCNT and commercial cartridges. The comparison of enrichment performance of the MWCNT cartridge previously optimized and the commercial cartridge Oasis HLB was performed with SW samples. The optimized SPE methodology was applied and the recoveries of the 8 target micropollutants (spiked at 200 ng L^{-1} each) were obtained (Fig. 1f). A recovery higher than 60% was achieved for 3 pesticides (acetamiprid, atrazine and isoproturon) and the 2 pharmaceutical compounds (carbamazepine and diclofenac) when using both MWCNTs and commercial cartridges. However, the commercial cartridge Oasis HLB gave recovery values also higher than 60% for the industrial compound (PFOS) and the other 2 pesticides (metaflumizone and methiocarb). Except for metaflumizone, the overall recovery of the other 7 micropollutants was similar.

The textural properties of MWCNTs and Oasis HLB were investigated through N_2 adsorption–desorption isotherms, as described in "Characterization of MWCNTs" section. The results revealed that these materials have

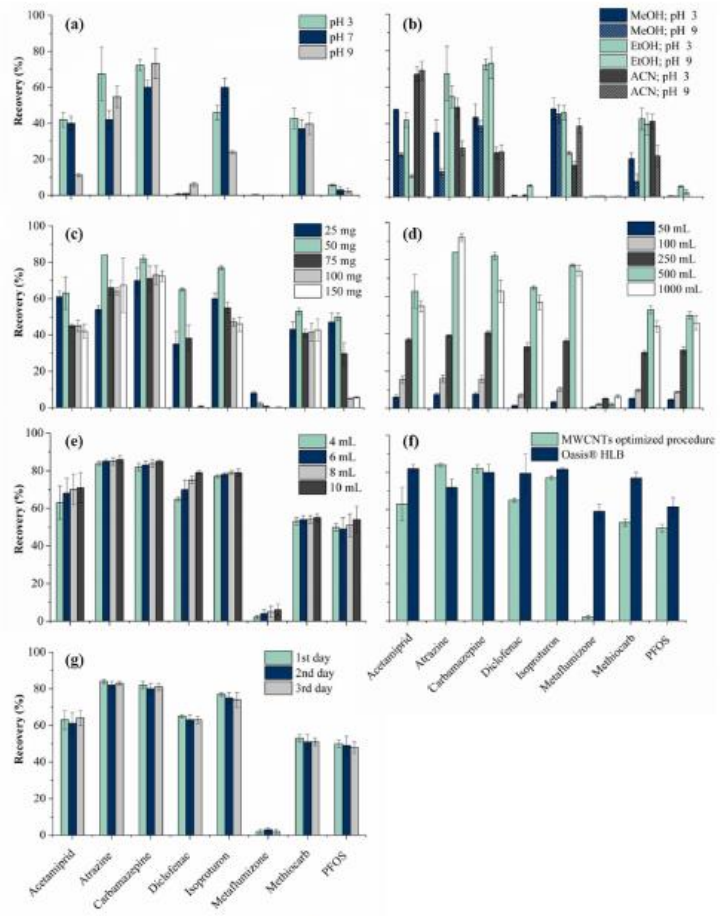

Figure 1. Recoveries obtained for micropollutants (200 ng L^{-1} each), using: (**a**) different pH (3, 7 and 9) (fixed conditions: cartridges packed with 150 mg of MWCNTs, 500 mL of SW and 4 mL of ethanol as a solvent); (**b**) different solvents (4 mL of methanol, ethanol or acetonitrile) and pH (3 and 9) (fixed conditions: cartridges packed with 150 mg of MWCNTs, 500 mL of SW and 4 mL of solvent); (**c**) cartridges packed with different amounts of MWCNTs (25–150 mg) (fixed conditions: pH 3, 500 mL of SW and 4 mL of ethanol as a solvent); (**d**) different volumes (50–1000 mL) of SW (fixed conditions: cartridges packed with 50 mg of MWCNTs, pH 3 and 4 mL of ethanol as a solvent); (**e**) using different volumes (4–10 mL) of ethanol as elution solvent (fixed conditions: cartridges packed with 50 mg of MWCNTs, 500 mL of SW, pH 3); (**f**) MWCNT optimized cartridge (50 mg) and commercial cartridge Oasis HLB (experiments performed with 500 mL of SW samples (pH 3) and using ethanol as solvent (4 mL); and (**g**) recoveries obtained for micropollutants (200 ng L^{-1} each), extracting 500 mL of SW (pH 3) with 4 mL of ethanol as solvent, during consecutive reuse cycles performed with the same cartridge packed with MWCNTs (50 mg); n = 3 (RSD is represented as error bars).

different porous characteristics (Table S4). Although the total pore volume (V_{total}) is similar for both adsorbents (1.264 and 1.284 $cm^3 g^{-1}$ for MWCNTs and Oasis HLB, respectively), the specific surface area (S_{BET}) of Oasis HLB (756 $m^2 g^{-1}$) is ca. 3.8-fold higher than that of MWCNTs (198 $m^2 g^{-1}$). Consequently, the average pore diameter (d_{pore}) of MWCNTs (25.5 nm) is almost fourfold higher than that of Oasis HLB (6.8 nm). Interestingly, the sorbent load in each cartridge Oasis HLB is 3 times higher than that of the cartridges packed with MWCNTs, which have a S_{BET} 3.8-fold lower. However, the SPE performances obtained for MWCNTs (Fig. 1f) cannot be explained by one unique parameter. Instead, the adsorption followed by elution/desorption of the organic micropollutants result from the interplay of many factors: the textural properties of the adsorbent material (Table S4); the functional groups of the adsorbent and of the organic pollutants; the hydrophobic interactions between the target micropollutants (log K_{OW} of each target compound can be found in Table S1) and MWCNTs, and other adsorption mechanisms; the morphology of the adsorbent material; and the sample characteristics (for example, the dissolved organic matter present in the water matrix)[10].

In addition to the analytical performance of the SPE procedure, it is important to take into consideration the cost of the adsorbent material. Considering 2020 retail prices, each commercial cartridge Oasis HLB costs around 8 euros, while the whole cost associated to each cartridge packed with 50 mg of MWCNTs amounts to ca. 2 euros (including the empty polypropylene cartridge and two frits). This represents a possible cost reduction of 75%. Furthermore, these MWCNT cartridges are reusable, while commercial cartridges often are single-use disposable devices[4,20].

Textural and surface chemistry characterization of MWCNTs. The type and overall amount of oxygen-containing surface groups were determined by TPD analysis, as described in "Characterization of MWCNTs" section. The CO_2 and CO TPD spectra of the hydrothermally treated MWCNTs (HNO_3 concentration in the range 0.01–0.30 mol L^{-1}) are shown in Fig. 2a,b, respectively. For comparison, the TPD profiles determined for the pristine MWCNTs (original) and for MWCNTs after hydrothermal treatment with water (i.e., $[HNO_3] = 0$) are also included. The total amount of surface groups (released as CO_2 and CO) and the corresponding oxygen content (calculated from the total amounts of CO_2 and CO) are summarized in Table 1.

The amounts of CO_2 and CO increase as the concentration of the oxidizing agent increases (up to 0.30 mol L^{-1}), confirming that MWCNTs are suitable to the inclusion of oxygenated functional groups through hydrothermal oxidation under mild conditions. The high level of oxidation in this type of carbon material can be associated to their structure, which provides a great number of defects where the oxidation process can be started[27]. Nevertheless, only a slight increase in the amount of oxygenated surface groups is observed when the HNO_3 concentration is incremented from 0.20 to 0.30 mol L^{-1} (Fig. 3a,b; Table 1). This phenomenon was already reported in a previous work on HNO_3 hydrothermal oxidation of carbon xerogels[23], and suggests that there is a maximum extent of surface functionalization achievable through this mild hydrothermal methodology. A prevalence of surface groups released as CO was found in contrast to those released as CO_2, the $[CO]/[CO_2]$ ratio being higher than one for all the MWCNTs under study (Table 1). The amount of oxygen follows the same trend of the surface groups released as CO_2 and CO, as expected. Comparing the pristine MWCNTs and those hydrothermally treated with water (blank), both have similar (and low) amounts of oxygen surface groups, indicating that the hydrothermal treatment without addition of HNO_3 has no effect on the surface chemistry of MWCNTs.

Correlations between the amount of oxygenated groups introduced on the surface of the MWCNTs (released as CO_2 and CO) and the concentration of HNO_3 employed in the hydrothermal treatment were obtained (Fig. 3a). Likewise, the contents of oxygen and volatiles (determined by TGA under inert atmosphere; Table 1) are also given as function of the HNO_3 concentration (Fig. 3b). As observed, the evolution of all the parameters under study can be described as function of the HNO_3 concentration by single exponential functions (r^2 in the range 0.972–0.999), which is in accordance with our previous results on hydrothermally treated MWCNTs[24], single-walled carbon nanotubes (SWCNTs)[25] and carbon xerogels[23–25]. These correlations are very useful to fine tune the surface chemistry of MWCNTs, as they allow a given amount of oxygenated surface groups to be obtained by setting the proper concentration of HNO_3 in the hydrothermal treatment.

Deconvolution analysis of the CO_2 and CO TPD spectra was performed in order to identify and quantify the amounts of the different functionalities (Fig. 4a,b, respectively, and Figure S4, and corresponding results detailed in Tables S6 and S7). As a representative example, Fig. 4a,b show the deconvoluted CO_2 and CO spectra of $MWCTN_{0.3}$ (i.e., the material obtained after hydrothermal treatment with 0.30 mol L^{-1} HNO_3). As observed, the surface groups released as CO_2 were mainly assigned to strongly acidic carboxylic acids (SA, 172 μmol g^{-1}), followed by carboxylic anhydrides (CAn, 84 μmol g^{-1}) and lactones (Lac, 84 μmol g^{-1}), and less acidic carboxylic acids (LA, 53 μmol g^{-1}). Regarding the CO spectrum, the main contribution was assigned to carbonyls and quinones (CQ, 388 μmol g^{-1}), followed by phenols (Ph, 148 μmol g^{-1}) and carboxylic anhydrides (CAn, 84 μmol g^{-1}). Moreover, a minor contribution was found at high temperature, as revealed by the shoulder observed at around 900 °C (Fig. 4b), which can be attributed to basic surface groups (Bas, 64 μmol g^{-1})[29]. Considering the results shown in Tables S6 and S7, the concentration of the oxygen functional groups generally increases with the concentration of HNO_3 employed in the hydrothermal treatment, as previously observed for the total amounts of CO_2 and CO.

The effect of the hydrothermal treatment on the overall surface charge (assessed though pH_{PZC} measurements) of the resulting materials, was also studied (Table 1). As observed, materials with a more pronounced acidic character are gradually obtained as the HNO_3 concentration increases (i.e., pH_{PZC} is 6.9 for pristine MWCNTs and 5.0 for the MWCNTs treated with 0.30 mol L^{-1} HNO_3). This continuous decrease of the pH_{PZC} values can be ascribed to the increasingly significant amounts of carboxilic acids (CA) introduced on the carbon surface, as summarized in Table 1. A similar conclusion was achieved in a previous publication of our group, upon a thorough analysis of results obtained by Raman spectroscopy[31]. As shown in Figure S5, the intensity ratio of

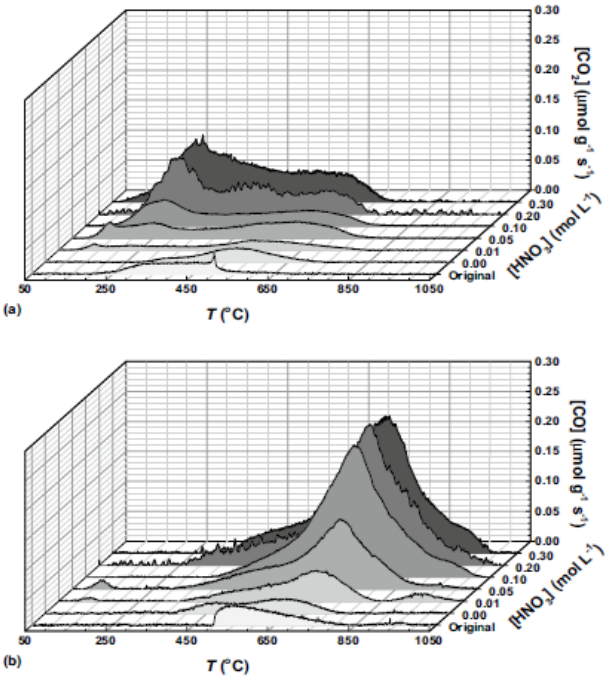

Figure 2. TPD spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations: (**a**) CO_2 and (**b**) CO evolution with temperature.

	Parameters									
$[HNO_3]$ (mol L^{-1})	$[CO_2]$ (μmol g^{-1})	[CO] (μmol g^{-1})	O (wt%)	$[CO]/[CO_2]$	Volatiles (wt%)	[CA] (μmol g^{-1})	pH_{PZC}	S_{BET} (m^2 g^{-1})	S_{meso} (m^2 g^{-1})	V_{total} (cm^3 g^{-1})
Original MWCNT	59	72	0.3	1.2	2.87	n.d.	6.9	198	198	1.264
0 (Blank)	59	72	0.3	1.2	2.92	5	6.9	188	188	1.581
0.01	71	150	0.5	2.1	3.41	20	6.7	202	202	1.148
0.05	156	327	1.0	2.1	3.75	54	6.1	229	204	1.115
0.10	178	590	1.5	3.3	4.41	93	5.5	250	237	1.566
0.20	304	656	2.0	2.2	4.80	152	5.2	261	255	1.669
0.30	392	671	2.3	1.7	5.12	225	5.0	262	262	2.193

Table 1. Properties of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations: amounts of CO_2 and CO released by TPD, $[CO/CO_2]$ ratio, percentage of oxygen obtained from the analysis of the TPD spectra (assuming that all the surface oxygen is released as CO and/or CO_2), amount of volatiles (determined by TGA), amount of carboxylic acids (CA; corresponding to the sum of SA and LA, as determined by TPD), pH at the point of zero charge (pH_{PZC}), specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}) and total pore volume (V_{total}). n.d.: Not determined.

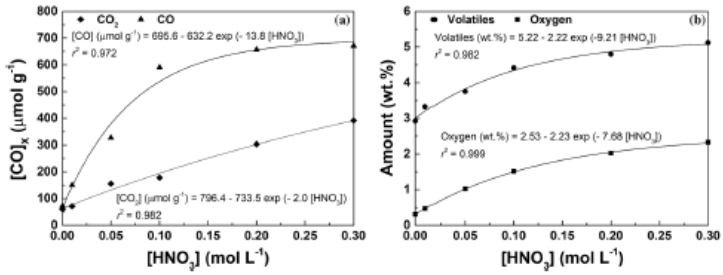

Figure 3. (**a**) Amounts of CO_2 ($SD \leq 39$ μmol g^{-1}) and CO ($SD \leq 48$ μmol g^{-1}) released by TPD and (**b**) contents of oxygen ($SD \leq 0.19$ wt%) and volatiles ($SD \leq 0.49$ wt%) as function of the concentration of HNO_3 employed in the hydrothermal treatment of MWCNTs. Points represent experimental data, while lines represent non-linear fittings.

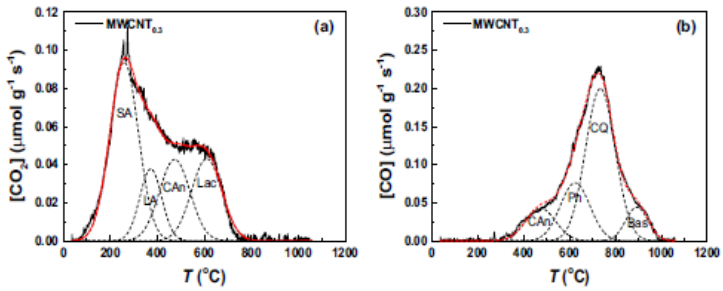

Figure 4. Deconvolution results of (**a**) CO_2 and (**b**) CO TPD spectra of MWCNTs subjected to hydrothermal treatment with 0.30 mol L^{-1} HNO_3 ($MWCNT_{0.3}$). Dashed lines represent peaks assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (CAn), lactones (Lac), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes. Red lines represent cumulative peak fitting.

the D band relative to the G mode (ID/IG) obtained by Raman spectroscopy plotted as a function of the total amount of functional groups determined by TPD revealed a linear function when characterizing single-walled carbon nanotubes treated with different HNO_3 concentrations. Thus, data obtained by TPD correlate well with data obtained by Raman spectroscopy or other techniques such as water adsorption/desorption[31].

The effect of the hydrothermal treatment on the textural properties of the MWCNTs was evaluated through N_2 adsorption–desorption isotherms. All the MWCNTs possess negligible microporosity (as revealed by the low adsorption obtained at low N_2 pressures); on the contrary, the prevalence of mesopores is revealed by the high adsorption observed at higher N_2 pressures (Figure S6). The mesoporous nature of the MWCNTs is confirmed by the results given in Table 1. As observed, the surface area (both S_{BET} and S_{meso}) and pore volume (V_{pore}) increase as the concentration of HNO_3 employed in the hydrothermal treatment increases. The average diameter of the MWCNTs is 9.5 nm (technical description provided by the manufacturer) and the average internal diameter of the tubes is around 4 nm[31], i.e., almost 2-fold higher than the maximum diameter of micropores (2 nm). Therefore, as expected, micropores were not found in the original sample ($V_{micro} = 0$) and the values were very low for the oxidized ones (i.e., within the error of the analysis).

Our results are in line with those previously reported in a study performed with SWCNTs, in which it was concluded that functionalization with HNO_3 causes the opening of the nanotube caps with no significant defects being additionally produced[32]. The progressive increase of S_{BET}, S_{meso} and V_{pore} can thus be ascribed to the opening of the nanotube caps, which enhances the accessibility to the inner part of the MWCNTs, rather than defect creation. As stated in a previous work, a gradual increase of both S_{BET} and the amount of oxygenated functional groups is observed when the hydrothermal oxidation treatment is performed with increasing HNO_3 concentrations[24]. This methodology leads to a slight increase of the S_{BET}, around 32% when comparing

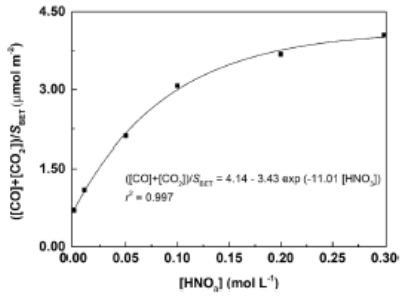

Figure 5. $([CO_2]+[CO])/S_{BET}$ as a function of HNO_3 concentration.

the pristine and the $MWCNT_{0.3}$ sample, i.e. from 198 $m^2 g^{-1}$ in the pristine MWCNTs to 262 $m^2 g^{-1}$ in the sample treated with 0.30 mol L^{-1} HNO_3. This increase of S_{BET} as consequence of the hydrothermal oxidation treatment with HNO_3 is similar to that obtained in a previous study of our group performed under similar conditions, i.e., 27%[33]. In that study, both pristine and oxidized (0.3 mol L^{-1} of HNO_3) MWCNTs were characterized by scanning electron microscopy (SEM) (Figure S7), allowing to conclude that both samples consist of agglomerated carbon nanotubes, with no significant morphological changes being perceptible as a consequence of the HNO_3 hydrothermal oxidation[33]. When the total amounts of CO_2 and CO released by TPD are normalized by the S_{BET} (i.e., $([CO_2]+[CO])/S_{BET}$), and represented as a function of HNO_3 concentration (Fig. 5), two distinct stages in the exponential curve are observed: (i) in the first stage, the pronounced increase is associated with the functionalization of the accessible surface area of the original MWCNTs; while (ii) the final part of the curve corresponds to the functionalization of new surface area made accessible during the HNO_3 hydrothermal oxidation. The correlation obtained in Fig. 5 (with $r^2=0.997$) can be extended to other carbon materials obtained through the same hydrothermal functionalization methodology.

Application of functionalized MWCNTs for extraction of EU multi-class OMPs. The applicability of the original and hydrothermally treated MWCNTs as sorbents for SPE of the 8 target OMPs was studied, as well as the influence of both textural and surface chemistry properties of these materials in the adsorption/desorption process. For that purpose, the recoveries of the target compounds were determined as described by Eq. (1) ("Evaluation of the SPE recovery efficiency" section). The recoveries obtained revealed different behaviors for the OMPs under study (Fig. 6) due to their distinct classes and physicochemical properties. For instance, similar recoveries were achieved for the pesticides atrazine and isoproturon (around 80 and 70%, respectively) with all the samples of MWCNTs that were tested, indicating that the adsorption/desorption process is not affected by the oxygenated surface groups introduced by the HNO_3 hydrothermal treatment. The SPE cartridges packed with the original MWCNTs (recoveries > 60%) performed better than the materials treated with HNO_3 for the pharmaceutical compounds diclofenac and carbamazepine and the neonicotinoid pesticide acetamiprid (recoveries < 60%). In the case of metaflumizone, the recovery obtained is ineffective with the original and treated MWCNTs packed in the SPE cartridges. On the other hand, performing SPE with $MWCNT_{0.30}$ leads to a significant improvement of the recoveries obtained for methiocarb and the industrial compound PFOS, when compared to the original MWCNTs.

Several mechanisms may simultaneously control the adsorption/desorption process of the organic pollutants on MWCNTs, including (i) π–π interactions, i.e., the interactions between bulk π systems present on the surface of MWCNTs and organic molecules with their benzene rings or C=C double bonds; (ii) hydrogen bonds with functional groups on the surface of the sorbent material; and (iii) electrostatic interactions due to of the charged carbon material surface[10,34]. However, each mechanism could be affected differently by the environmental conditions, which makes the application of MWCNTs for SPE of different organic compounds from aqueous matrices a challenging research topic. The obtained results (Fig. 6) suggest that the HNO_3 hydrothermal treatment applied to MWCNTs affects the SPE efficiency of the target OMPs in two distinct ways. In the case of acetamiprid, diclofenac and carbamazepine, the dominant adsorption mechanism seems to be π–π dispersive interactions, which decrease with the increase of the oxygen-containing functional groups, most of them with electron-withdrawing properties. In contrast, for methiocarb and PFOS, the HNO_3 functionalization leads to higher recoveries, possibly due to the predominance of the hydrogen bond adsorption mechanism favoured by the increase of the oxygen surface groups.

In the case of the carbamate pesticide methiocarb, it is interesting to observe the continuous increase in the recovery values with the increase of the acid concentration. Thus, the recoveries obtained for methiocarb were plotted as a function of the total amount of functional groups introduced ($CO+CO_2$) divided by the respective S_{BET} (Fig. 7). A good linear correlation ($r^2=0.995$) was obtained, i.e., the total amount of CO and CO_2 divided

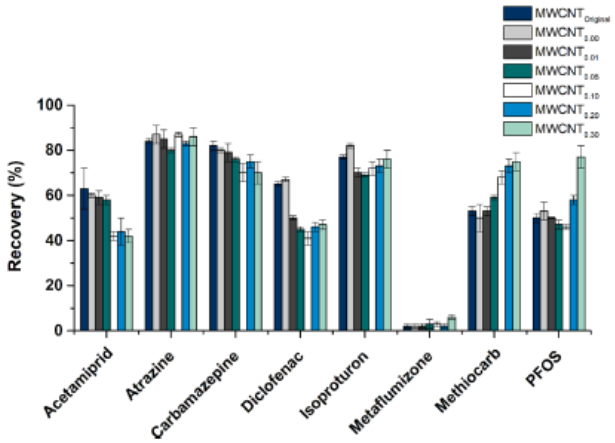

Figure 6. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with MWCNTs (50 mg) obtained after hydrothermal treatment with different HNO_3 concentrations (0–0.30 mol L^{-1}). Experiments performed with 500 mL of sample (SW; pH 3) and using ethanol as solvent (4 mL); n = 3 (RSD is represented as error bars).

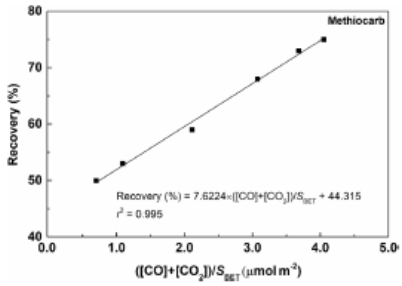

Figure 7. Recovery obtained for methiocarb as a function of $([CO_2] + [CO])/S_{BET}$.

by the S_{BET} has proved to be a good predictor of methiocarb recovery. For this carbamate pesticide, the HNO_3 functionalization of MWCNTs led to a continuous and significant increase in the SPE efficiency. In order to understand if this correlation was associated with any specific functional group previously determined by TPD, a similar analysis was made but with the amount of each surface group (SA, LA, CAn, Lac, Ph, CQ and Bas), instead of the total amount released as CO and CO_2. Good correlations with Ph ($r^2 = 0.916$) and CQ groups ($r^2 = 0.918$) were also obtained (Figure S8a and b), suggesting that the presence of higher amounts of these functional groups on the MWCNT surface increases the affinity for methiocarb.

In the literature, there are several studies reporting the possible mechanisms of adsorption of some target compounds on MWCNTs. For example, in the case of atrazine, π–π dispersive and polar interactions were appointed as responsible for the adsorption on MWCNTs[35]. Regarding the industrial compound PFOS, a study conducted by Li, et al.[36] concluded that hydrophobic interactions are the main mechanism of adsorption of PFOS. The pharmaceutical compound diclofenac was already studied, and diverse types of interactions were suggested, such as electrostatic and hydrophobic interactions and hydrogen bonding[34,37]. In the case of carbamazepine, the π–π electron–donor–acceptor interactions, hydrogen bonding and hydrophobic interactions had a key role

in the adsorption on MWCNTs[34,37]. Therefore, the mechanism and the extraction performance result from the interplay of the characteristics of each pollutant and the properties of the sorbent material.

Conclusions

Pristine and modified MWCNTs were applied as adsorbent materials in conventional SPE for enrichment of 8 EU multi-class OMPs in SW samples and analysis by UHPLC-MS/MS. The optimized SPE procedure with pristine MWCNTs has the great advantage of using an eco-friendly solvent (ethanol) for both conditioning and elution steps. Additional advantages of this carbon-based cartridge are the small amount of adsorbent that is needed (50 mg), representing a ~ 75% cost reduction in comparison with the commercial cartridge (while obtaining similar recoveries), and the ability to be reused at least three times without substantial impact on the retention capacity of the adsorbent. The oxidation of the MWCNTs surface (and thus the introduction of oxygenated functional groups) can affect the SPE recoveries in different ways. The dominant adsorption mechanism seems to be π–π dispersive interactions in the case of acetamiprid, diclofenac and carbamazepine (i.e. the recoveries were higher when using the original MWCNTs), whereas the hydrogen bond adsorption mechanism (favoured by the increase of the oxygen surface groups) seems to be predominant in the case of methiocarb and PFOS. Moreover, a very good correlation between the recovery of methiocarb and the functionalities created on the MWCNTs was found, which was attributed to the phenol and carbonyl and quinone groups. The fine control of the surface chemistry and texture of MWCNTs, with the purpose of improving the selectivity and specificity of these materials, opens a window of opportunity for the development of more efficient and eco-friendly analytical tools for the analysis of EU-relevant OMPs, for instance by mixing MWCNTs with different textural and surface chemistry properties in the same SPE cartridge.

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Author contributions

M.O.B. performed most of the research work, data analysis and wrote the first version of the manuscript. R.S.R. was involved in the application of the defined methodology for the synthesis and characterization of the materials, respective data analysis, and revised the manuscript. A.R.L.R., M.F.R.P. and A.M.T.S. were responsible for the conceptualization and supervision of the work done, for acquisition of resources and funding, and revision of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary material

Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants

Text D-S5.1. Reference SPE protocol.

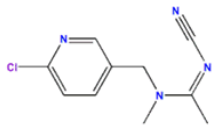
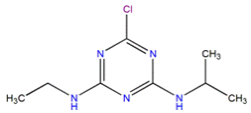
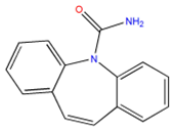
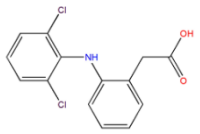
An offline SPE method with commercial Oasis HLB cartridges was used as reference protocol, according to our previous works [1, 2]. Briefly, Oasis HLB cartridges were successively conditioned with 4 mL of ethanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. Sample loading was carried out with 500 mL of acidified (pH 3) blank and spiked (200 ng L^{-1} of each target compound) SW samples at a constant flow rate of 10 mL min^{-1}, using a vacuum manifold unit connected to a vacuum pump. The washing step was performed with 4 mL of ultrapure water. The cartridges were then dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of ethanol and the extracts were evaporated to dryness in a Centrivap Concentrator device (LABCONCO Corporation, Kansas City, MO, USA). The dried extracts were reconstituted in 250 µL of ethanol and the resulting ethanolic extracts were filtered through 0.22 µm polytetrafluoroethylene syringe filters (Membrane Solutions, Kent, WA, USA) to be injected into the UHPLC-MS/MS system.

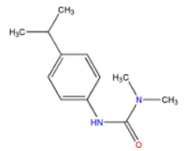
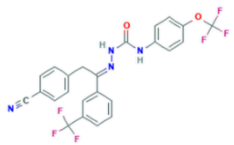
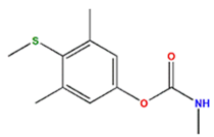
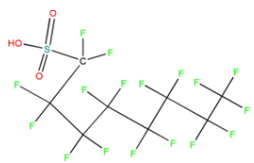
Text D-S5.2. MWCNTs SPE optimization.

In order to study the performance of functionalized MWCNTs as SPE adsorbents for enrichment of the target OMPs, the main experimental conditions affecting the extraction efficiency of pristine MWCNTs (NC3100) were optimized in detail. All experiments carried out during the optimization were performed in triplicate. The pristine MWCNTs cartridges were prepared manually, using a lab-scale packing device specifically designed for that purpose (Figure S1). This procedure involved four sequential steps: (i) one polyethylene frit (20 µm) was positioned on the bottom of an

empty cartridge (6 mL); (ii) a certain amount (25, 50, 75, 100 or 150 mg) of pristine MWCNTs (NC3100) was then introduced; (iii) the sample was covered with another polyethylene frit; and (iv) slightly compressed until a specific bed height was reached. Cartridges packed with 150 mg of MWCNTs were used in preliminary studies to assess the effect of different sample pH in SPE. The SW samples (500 mL) were adjusted to different pH (3 and 9) and extracted using ethanol, methanol or acetonitrile (4 mL) and then ultrapure water (4 mL) was passed through the SPE cartridge at a flow rate of 1 mL min^{-1}. A sample pH of 7 was also tested, using ethanol as extraction solvent. After selecting the best sample pH and extraction solvent, the effect of the amount of the adsorbent material packed in the SPE cartridge was studied in the range 25 – 150 mg. Sample loading was carried out with 50, 100, 250, 500 or 1000 mL of blank and spiked SW samples (200 ng L^{-1} of each target compound). After washing and drying, the retained analytes were eluted with 4, 6, 8 or 10 mL of ethanol, as this volume can also influence the enrichment efficiency and the overall cost of the SPE method. The elution volume should be enough to elute the analytes from the sorbent material; however, excessive amounts represent waste and avoidable costs. The resulting extracts were reconstituted and filtered as the reference protocol.

Table D-S5.1. Target compounds, class, structure, relative molecular mass (M_r), p*K*a and log K_{OW} values and solubility in water.

Compound	Class and sub class	Structure	M_r	p*K*a	log K_{OW}	Solubility in water (mg L^{-1})
Acetamiprid[a]	Pesticide *Neonicotinoid*		222.68	0.70	0.80	4250 (25 ºC)
Atrazine[b]	Pesticide *Triazine*		215.69	1.60	2.61	33.0 (25 ºC)
Carbamazepine	Pharmaceutical *Psychiatric drug*		236.27	13.9	2.45	17.7 (25 ºC)
Diclofenac[c]	Pharmaceutical *Anti-inflammatory*		296.14	4.15	4.51	2.5 (25 ºC)

Isoproturon[b]	Pesticide *Phenylurea*		206.28	n.a.	2.87	70.0 (20 ºC)
Metaflumizone[a]	Pesticide *Insecticide*		506.41	n.a	n.a	n.a
Methiocarb[a]	Pesticide *Insecticide*		225.31	14.8	2.92	27.0 (20 ºC)
Perfluorooctanesulfonic acid (PFOS)[b]	Industrial compound		500.13	-3.30	4.49	3.2×10^{-3} (25 ºC)

[a] Contaminant of emerging concern of Decision 2018/840/EU; [b] Priority substance of Directive 2013/39/EU; [c] Contaminant of emerging concern of the former Decision 2015/495/EU; n.a. - not available.

Table D-S5.2. Selected reaction monitoring (SRM) instrument parameters for tandem mass spectrometry analysis of target analytes.

Analyte	IS set[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Quantification (SRM1)				Confirmation (SRM2)			
				Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)	Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)
Acetamiprid	1	PI	222.70	126.00	-15	-20	-23	56.10	-15	-16	-22
Acetamiprid-d3 (1)	-	PI	226.00	126.00	-25	-20	-23	-	-	-	-
Atrazine	2	PI	216.00	174.00	-24.0	-16.0	-30.0	68.05	-24.0	-36.0	-10.0
Atrazine-d5 (2)	-	PI	221.10	179.05	-11.0	-18.0	-17.0	-	-	-	-
Carbamazepine	4	PI	237.00	194.00	-12.0	-20.0	-30.0	192.00	-12.0	-25.0	-30.0
Diclofenac	3	NI	293.90	250.00	21.0	11.0	17.0	214.05	21.0	20.0	22.0
Diclofenac-d4 (3)	-	NI	297.95	254.05	21.0	12.0	28.0	-	-	-	-
Fluoxetine-d5 (4)	-	PI	315.10	44.00	-15.0	-20.0	-15.0	-	-	-	-
Isoproturon	2	PI	206.80	72.00	-15.0	-20.0	-29.0	46.00	-15.0	-18.0	-16.0
Metaflumizone	3	NI	505.00	302.05	36.0	18.0	21.0	116.95	36.0	43.0	22.0
Methiocarb	5	PI	226.10	169.10	-24.0	-9.0	-17.0	121.10	-24.0	-19.0	-21.0
Methiocarb-d3 (5)	-	PI	229.10	169.10	-25.0	-11.0	-30.0	-	-	-	-
Perfluorooctanesulfonic acid	2	NI	498.70	79.95	18.0	50.0	14.0	99.00	18.0	46.0	18.0

[a] IS is internal standard; [b] NI is negative ionization mode; [c] PI is positive ionization mode; [d] DP is the declustering potential; [e] CE is the collision energy; [f] CXP is the collision cell exit potential.

Table D-S5.3. Retention time, range, linearity, instrument and method detection and quantification limits for each target analyte.

Analyte	Retention time (min)	Range (ng L^{-1})	r^2	IDL[a] (µg L^{-1})	IQL[b] (µg L^{-1})	MDL[c] (ng L^{-1})	MQL[d] (ng L^{-1})
Acetamiprid	1.19	3.06 – 200	0.999	16.1	48.7	1.01	3.06
Atrazine	1.83	4.61 – 200	0.993	8.79	26.6	1.52	4.61
Carbamazepine	1.52	2.09 – 200	0.999	1.61	4.90	0.69	2.09
Diclofenac	3.86	3.74 – 200	0.998	3.62	11.3	1.23	3.74
Isoproturon	2.07	11.7 – 200	0.999	9.24	28.0	3.85	11.7
Metaflumizone	24.5	1.35 – 200	0.999	2.02	6.13	0.45	1.35
Methiocarb	2.68	2.69 – 200	0.997	0.74	2.24	0.89	2.69
Perfluorooctanesulfonic acid	1.47	11.7 – 200	0.997	25.4	77.1	3.87	11.7

[a] IDL is instrument detection limit; [b] IQL is instrument quantification limit; [c] MDL is method detection limit; [d] MQL is method quantification limit.

Table D-S5.4. Studies dealing with the application of MWCNTs in conventional SPE: target analyte and respective spiked level (ng L^{-1}), matrix, sample loading (mL), amount of MWCNTs packed in the cartridge (mg), conditioning and elution solvents, and recoveries (%) obtained. Pollutants included in these studies that are out of the scope of EU legislation are not discussed.

Target analyte (spiked level)	Matrix	Sample loading (mL)	Amount of MWCNTs (mg)	Conditioning and elution solvents	Recovery (%)	Ref.
Atrazine and simazine (100 ng L^{-1})	Tap and groundwater	700	300	Conditioning: 10 mL ACN + 10 mL of triply distilled water; Elution: 8 mL MeOH	85–95	[3]
Atrazine (300, 1000 and 5000 ng L^{-1})	Surface and underground water	1000	500	Conditioning: 3 mL MeOH + 5 mL water; Elution: 4 mL ethyl acetate	86–110	[4]
Atrazine and simazine (20000 ng L^{-1})	River water	500	100	Conditioning: 5 mL ACN + 3 mL aqueous solution (pH 3); Elution: 5 mL mixed solution of 90% ACN and 10% H_2O	87–97	[5]
Atrazine and simazine (800 ng L^{-1})	River, tap, reservoir and wastewater	500	100	Conditioning: 5 mL ACN + 5 mL water; Elution: 4 mL ACN	83–104	[6]
Atrazine (50000 ng L^{-1})	Tap, reservoir and stream water	50	200	Conditioning: 5 mL ACN + 5 mL water; Elution: 5 mL ACN	81–108	[7]
Chlorpyrifos (100, 300 and 800 ng L^{-1})	Mineral water, groundwater, and agricultural run-off water	800	40	Conditioning: 10 mL ACN + 10 mL Milli-Q water; Elution: 20 mL DCM	70–100	[8]
Chlorpyrifos (1000000, 5000000 and 10000000 ng L^{-1})	Well, tap and river water	500	100	Conditioning: 5 mL DCM + 5 mL water; Elution: 3 mL DCM	94–98	[9]

Atrazine (500 ng L^{-1})	Tap and reservoir water	600	300	Conditioning: 10 mL ACN + 10 mL of triply distilled water; Elution: 8 mL MeOH	74–>99	[10]
Methiocarb (5000 ng L^{-1})	Tap and SW	200	100	Conditioning: 5 mL MeOH + 5 mL Milli-Q water; Elution: 8 mL dichloromethane	92–96	[11]
Alachlor (1000 ng L^{-1})	Tap and river water	500	100	Conditioning: 2 mL ethyl–acetate + 2 mL MeOH + 2 mL of distilled water; Elution: 7 mL ethyl–acetate	82–85	[12]
Thiamethoxam, acetamiprid and imidacloprid (800 ng L^{-1})	Tap, ground and reservoir water	200	100	Conditioning: EtOH + Milli-Q water; Elution: 4 mL MeOH	88–110	[13]
PFOS (500 ng L^{-1})	Tap and river water	500	200	Conditioning: 5 mL MeOH + 5 mL ultrapure water + 5 mL ultrapure water (pH 3)	88–90	[14]
Pentachlorophenol (PCP) (200000 ng L^{-1})	River water	100	20	Conditioning: 5 mL of deionized water + 5 mL MeOH + 5 mL of deionized water; Elution: 5 mL acetone	62–98	[15]
PCP (5000 ng L^{-1})	Tap and river water	200	300	Conditioning: 5 mL MeOH + 5 mL ultrapure water; Elution: 6 mL MeOH (pH 10)	97–109	[16]
Polycyclic aromatic hydrocarbons (PAHs) (200 ng L^{-1})	Tap, river and seawater	500	150	Conditioning: 10 mL n-hexane + 10 mL MeOH + 10 mL water; Elution: 15 mL n-hexane	67–127	[17]
PAHs (80 – 20000 ng L^{-1})	River, tap and wastewater	500	500	Conditioning: 5 mL MeOH + 5 mL water; Elution: 4 mL ACN	79–118	[18]
Erythromycin, azithromycin and diclofenac	Surface and groundwater	100	50	Conditioning: 5 mL MeOH-dichloromethane (1:1, v/v) + 5 mL	93–112	[19]

(1000 ng L^{-1})				deionized water; Elution: 15 mL MeOH-dichloromethane (1:1, v/v)		
Diclofenac (50 and 100 ng L^{-1})	River water	100	20	Conditioning: 2 mL MeOH + 2 mL ultrapure water (pH 8); Elution: 7 mL MeOH containing 10% (v/v) of ammonium hydroxide (25% purity)	79–94	[20]

Table D-S5.5. Textural properties of MWCNTs (NC3100) and Oasis HLB: specific surface area (S_{BET}), non-microporous specific surface area (S_{meso}), total pore volume (V_{total}) and average pore diameter (d_{pore}).

Adsorbent used in the SPE cartridge	**Parameters**			
	S_{BET} ($m^2\ g^{-1}$)	S_{meso} ($m^2\ g^{-1}$)	V_{total} ($cm^3\ g^{-1}$)	d_{pore} (nm)
MWCNTs (NC3100)	198	155	1.264	25.5
Oasis HLB	756	607	1.284	6.8

Table D-S5.6. Results obtained from the deconvolution of the CO_2 spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. T_M, W and A represent the temperature at the peak maximum, the width of the peak at half-height and the integrated peak area, respectively. Peaks were assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (Can) and lactones (Lac).

[HNO_3] (mol L^{-1})	Peak #1			Peak #2 (SA)			Peak #3 (LA)			Peak #4 (CAn)			Peak #5 (Lac)		
	T_M (°C)	W (°C)	A (μmol g^{-1})	T_M (°C)	W (°C)	A (μmol g^{-1})	T_M (°C)	W (°C)	A (μmol g^{-1})	T_M (°C)	W (°C)	A (μmol g^{-1})	T_M (°C)	W (°C)	A (μmol g^{-1})
0 (Blank)	-	-	0	-	0	0	352	71	5	484	146	30	580	146	25
0.01	131	37	3	230	167	16	352	95	4	507	161	26	639	161	21
0.05	137	39	8	234	150	48	360	90	6	472	173	40	626	173	55
0.1	-	-	0	231	117	60	353	163	33	492	166	35	631	166	49
0.2	-	-	0	244	109	123	370	141	29	435	171	76	609	171	75
0.3	-	-	0	259	143	172	372	110	53	474	153	84	610	153	84

Table D-S5.7. Results obtained from the deconvolution of the CO spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. T_M, W and A represent the temperature at the peak maximum, the width of the peak at half-height and the integrated peak area, respectively. Peaks were assigned to carboxylic anhydrides (Can), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes.

[HNO_3] (mol L^{-1})	Peak #1			Peak #2 (CAn)			Peak #3 (Ph)			Peak #4 (CQ)			Peak #5 (Bas)		
	T_M (°C)	W (°C)	A (µmol g^{-1})	T_M (°C)	W (°C)	A (µmol g^{-1})	T_M (°C)	W (°C)	A (µmol g^{-1})	T_M (°C)	W (°C)	A (µmol g^{-1})	T_M (°C)	W (°C)	A (µmol g^{-1})
0 (Blank)	-	-	-	484	146	30	614	102	24	692	102	18	-	-	-
0.01	131	37	2	507	161	26	565	153	17	691	153	93	950	85	13
0.05	137	39	6	472	173	40	572	144	52	716	144	197	870	160	28
0.1	-	-	-	492	166	35	585	152	81	716	152	390	880	153	85
0.2	-	-	-	435	171	76	611	145	120	726	145	390	880	136	82
0.3	-	-	-	474	153	84	624	152	148	734	152	388	893	113	64

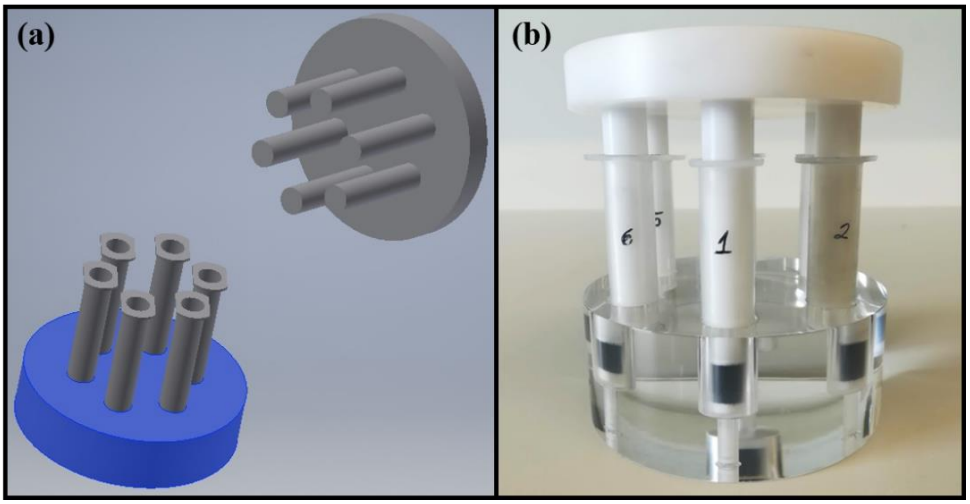


Fig. D-S5.1. Schematic representation **(a)** and photograph **(b)** of the lab-scale packing device designed to prepare MWCNT cartridges (6 at a time).

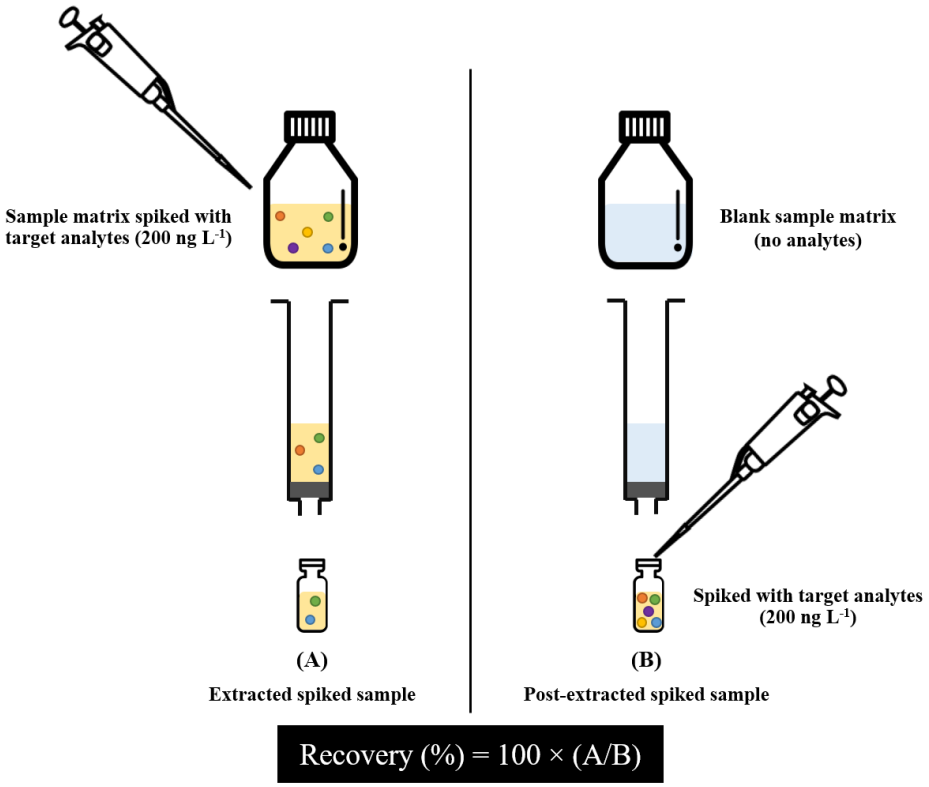


Fig. D-S5.2. Schematic representation of the experimental procedure carried out to evaluate the extraction efficiency (i.e. recovery in %) of each SPE method.

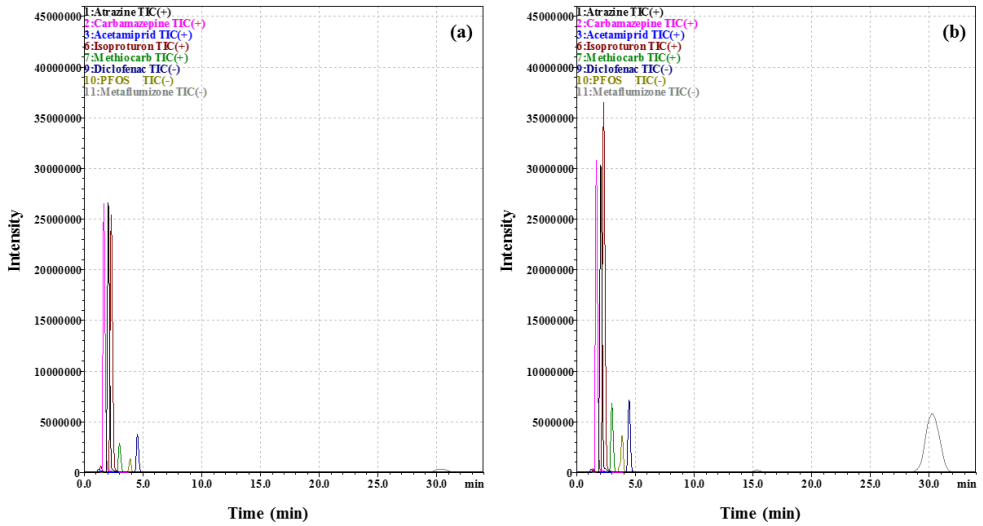
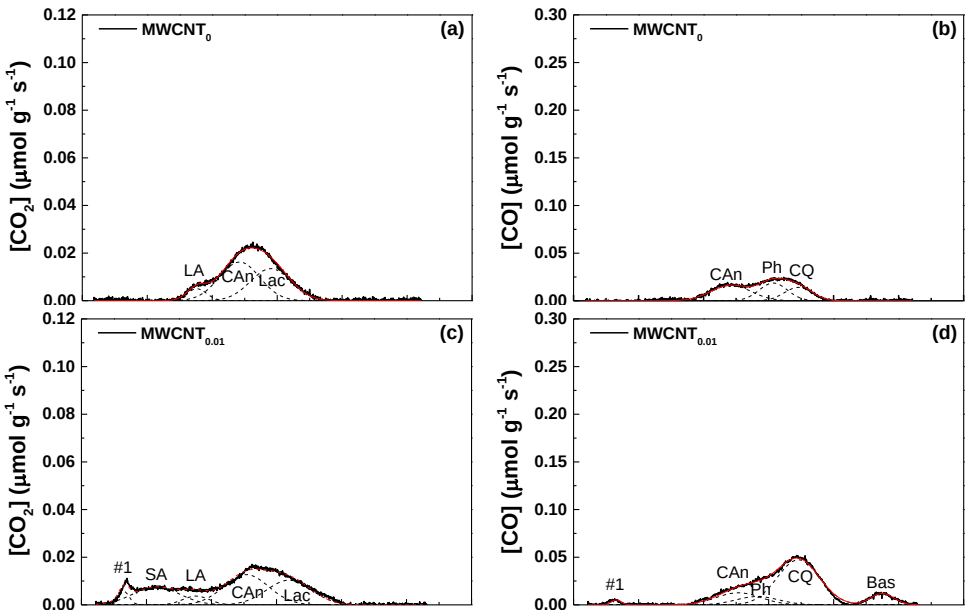


Fig. D-S5.3. Total Ion Current (TIC) chromatograms of the 8 target OMPs (200 ng L^{-1}) in: **(a)** a SPE extract of a spiked sample; and **(b)** a post-spiked blank extract, using cartridges packed with MWCNTs (50 mg).



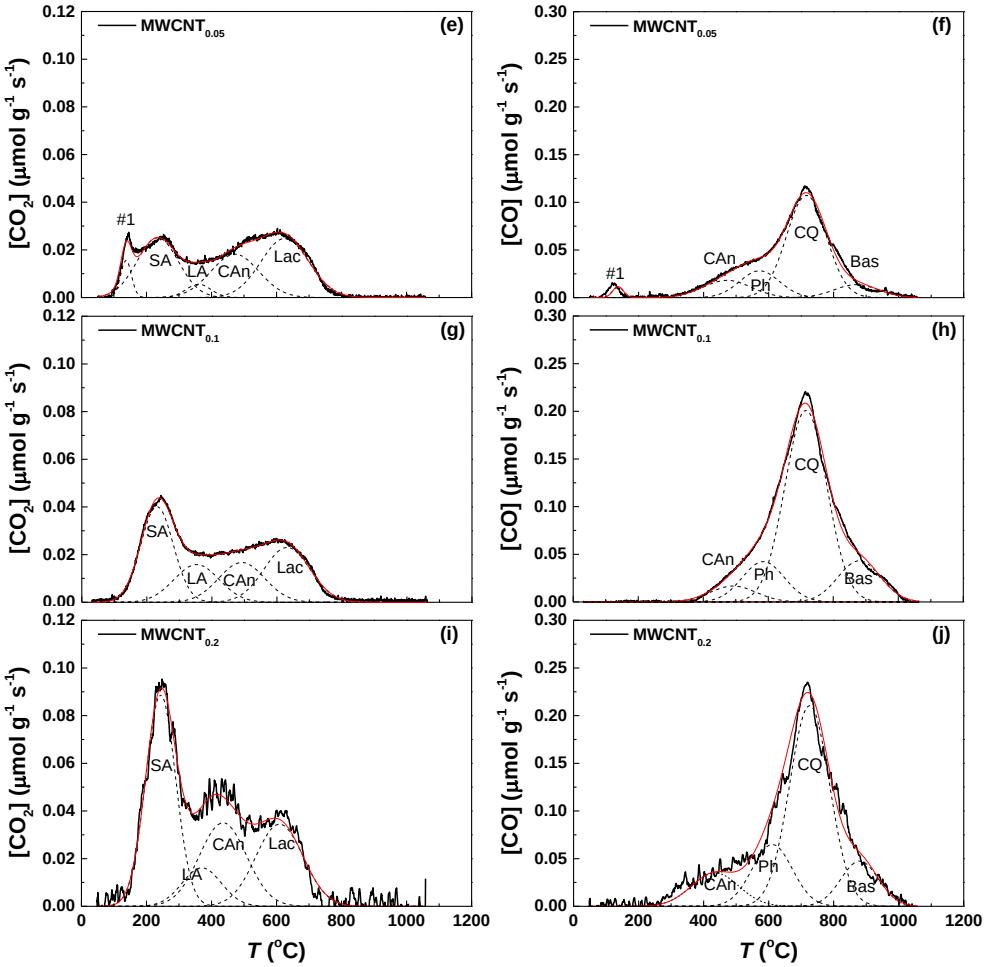


Fig. D-S5.4. Deconvolution of **(a, c, e, g, i)** CO_2 and **(b, d, f, h, j)** CO TPD spectra of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations. Dashed lines represent peaks assigned to strongly acidic carboxylic acids (SA), less acidic carboxylic acids (LA), carboxylic anhydrides (Can), lactones (Lac), phenols (Ph), carbonyls and quinones (CQ) and basic surface groups (Bas), such as pyrones and chromenes. Red lines represent cumulative peak fitting.

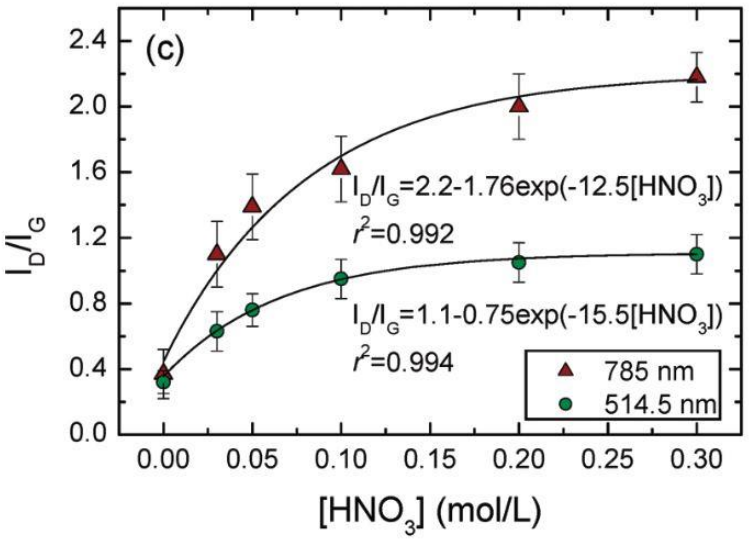


Fig.D-S5.5. Intensity ratio of the D band relative to the G mode (I_D/I_G) obtained by Raman spectroscopy, as function of the total amount of evolved CO and CO_2 determined by TPD when functionalizing single-walled carbon nanotubes with different HNO_3 concentrations in the hydrothermal treatment. Lines designate the linear fit. Reprinted (adapted) with permission from The Journal of Physical Chemistry C, Vol. 115, G.E. Romanos, V. Likodimos, R.R.N. Marques, T.A. Steriotis, S.K. Papageorgiou, J.L. Faria, J.L. Figueiredo, A.M.T. Silva, P. Falaras, Controlling and Quantifying Oxygen Functionalities on Hydrothermally and Thermally Treated Single-Wall Carbon Nanotubes, 8534-8546. Copyright 2011, with permission of American Chemical Society.

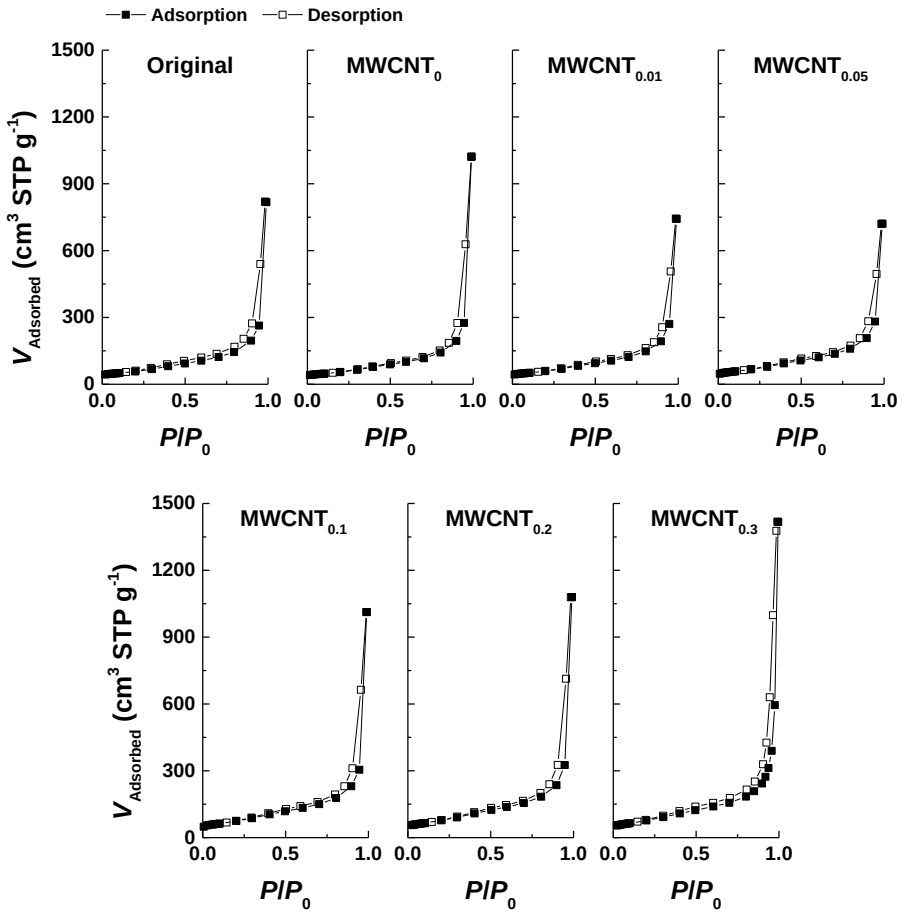


Fig. D-S5.6. N_2 adsorption-desorption isotherms at -196 ºC of MWCNTs subjected to hydrothermal treatment with different HNO_3 concentrations.

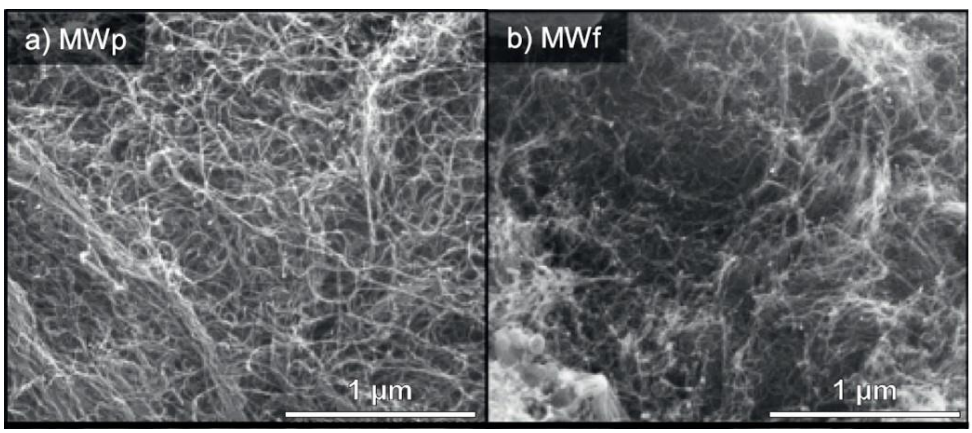


Fig. D-S5.7. SEM micrographs of (a) pristine (MWp) and (b) functionalized (MWf) MWCNTs. Reprinted from The Journal of Membrane Science, Vol. 520, Sergio Morales-Torres, Carla M.P. Esteves, José L.Figueiredo, Adrián M.T. Silva, Thin-film composite forward osmosis membranes based on polysulfone

supports blended with nanostructured carbon materials, 326-336, Copyright 2016, with permission from Elsevier [License number: 4945340204301].

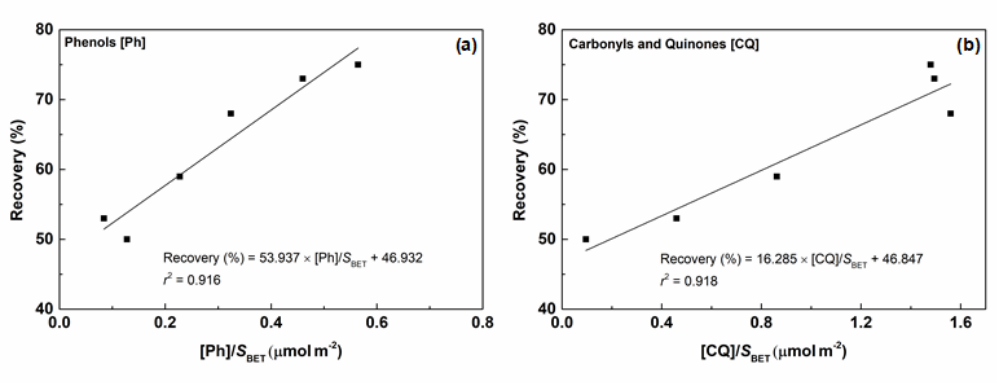


Fig.D-S5.8. Recoveries obtained for methiocarb as function of **(a)** [Ph]/S_{BET} and **(b)** [CQ]/S_{BET}.

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Appendix E

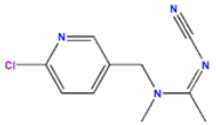
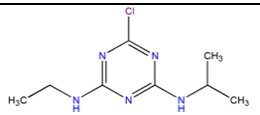
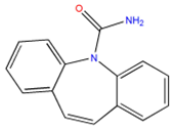
Supplementary material of Chapter 6:

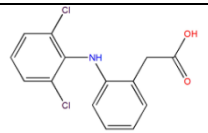
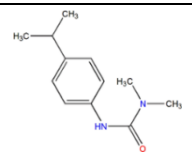
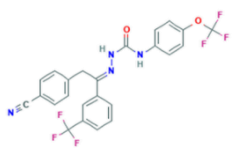
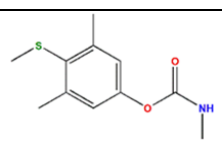
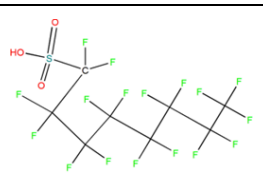
Carbon xerogels combined with carbon nanotubes as an innovative sorbent in solid-phase extraction cartridges to assess the occurrence of organic micropollutants in surface and drinking water

Supplementary material

Carbon xerogels combined with carbon nanotubes as an innovative sorbent in solid-phase extraction cartridges to assess the occurrence of organic micropollutants in surface and drinking water

Table E-S6.1. Target compounds, class, structure, relative molecular mass (M_r), p*K*a, and log K_{OW} values and solubility in water.

Compound	Class and sub class	Structure	M_r	p*K*a	log K_{OW}	Solubility in water (mg L^{-1})
Acetamiprid [a]	Pesticide *Neonicotinoid*		222.68	0.70	0.80	4250 (25 ºC)
Atrazine [b]	Pesticide *Triazine*		215.69	1.60	2.61	33.0 (25 ºC)
Carbamazepine	Pharmaceutical *Psychiatric drug*		236.27	13.9	2.45	17.7 (25 ºC)

Diclofenac [c]	Pharmaceutical *Anti-inflammatory*		296.14	4.15	4.51	2.5 (25 ºC)
Isoproturon [b]	Pesticide *Phenylurea*		206.28	n.a.	2.87	70.0 (20 ºC)
Metaflumizone [a,d]	Pesticide *Insecticide*		506.41	n.a	n.a	n.a
Methiocarb [a]	Pesticide *Insecticide*		225.31	14.8	2.92	27.0 (20 ºC)
Perfluorooctanesulfonic acid (PFOS) [b]	Industrial compound		500.13	-3.30	4.49	3.2×10^{-3} (25 ºC)

[a] Contaminant of emerging concern of Decision 2018/840/EU; [b] Priority substance of Directive 2013/39/EU; [c] Contaminant of emerging concern of the former Decision 2015/495/EU; [a] Contaminant of emerging concern of Decision 2020/1161/EU; n.a. - not available.

Table E-S6.2. Selected variables and respective levels investigated in the definitive screening design (DSD) and Box-Benkhen design (BBD).

	Variables	Selected levels		
		-1	0	+1
Definitive Screening Design (DSD)	(A) mass of sorbent material (mg)	25	75	150
	(B) sample volume (mL)	250	500	1000
	(C) sample pH	3	7	11
	(D) type of solvent	methanol	-	ethanol
	(E) solvent volume (mL)	4	7	10
Box-Behnken Design (BBD)	(A) mass of sorbent material (mg)	50	100	150
	(B) sample volume (mL)	500	750	1000
	(C) sample pH	3	7	11

Table E-S6.3. Selected reaction monitoring (SRM) instrument parameters for tandem mass spectrometry analysis of target analytes.

Analyte	IS set[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Quantification (SRM1)				Confirmation (SRM2)			
				Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)	Product Ion	DP[d] (V)	CE[e] (V)	CXP[f] (V)
Acetamiprid	1	PI	222.70	126.00	-15	-20	-23	56.10	-15	-16	-22
Acetamiprid-d3 (1)	-	PI	226.00	126.00	-25	-20	-23	-	-	-	-
Atrazine	2	PI	216.00	174.00	-24.0	-16.0	-30.0	68.05	-24.0	-36.0	-10.0
Atrazine-d5 (2)	-	PI	221.10	179.05	-11.0	-18.0	-17.0	-	-	-	-
Carbamazepine	3	PI	237.00	194.00	-12.0	-20.0	-30.0	192.00	-12.0	-25.0	-30.0
Diclofenac	3	NI	293.90	250.00	21.0	11.0	17.0	214.05	21.0	20.0	22.0
Diclofenac-d4 (3)	-	NI	297.95	254.05	21.0	12.0	28.0	-	-	-	-
Isoproturon	2	PI	206.80	72.00	-15.0	-20.0	-29.0	46.00	-15.0	-18.0	-16.0
Metaflumizone	4	NI	505.00	302.05	36.0	18.0	21.0	116.95	36.0	43.0	22.0
Methiocarb	4	PI	226.10	169.10	-24.0	-9.0	-17.0	121.10	-24.0	-19.0	-21.0
Methiocarb-d3 (4)	-	PI	229.10	169.10	-25.0	-11.0	-30.0	-	-	-	-
PFOS	2	NI	498.70	79.95	18.0	50.0	14.0	99.00	18.0	46.0	18.0

[a] IS is internal standard; [b] NI is negative ionization mode; [c] PI is positive ionization mode; [d] DP is the declustering potential; [e] CE is the collision energy; [f] CXP is the collision cell exit potential.

Table E-S6.4. Retention time, range, linearity, instrument and method detection, and quantification limits for each target analyte.

Analyte	Retention time (min)	Range (ng L^{-1})	r^2	IDL[a] (µg L^{-1})	IQL[b] (µg L^{-1})	MDL[c] (ng L^{-1})	MQL[d] (ng L^{-1})
Acetamiprid	1.06	36.1 – 400	0.999	283	857	11.9	36.1
Atrazine	1.94	13.8 – 400	0.998	46.3	140	4.55	13.8
Carbamazepine	1.59	7.86 – 400	0.999	27.7	83.9	2.59	7.86
Diclofenac	2.67	5.54 – 400	0.999	31.7	96.2	1.83	5.54
Isoproturon	2.18	15.5 – 400	0.998	79.2	240	5.13	15.5
Metaflumizone	27.9	13.3 – 400	0.996	134	423	4.37	13.3
Methiocarb	2.73	53.5 – 400	0.999	255	771	17.6	53.5
PFOS	1.88	4.83 – 400	0.997	16.4	49.7	1.59	4.83

[a] IDL is instrument detection limit; [b] IQL is instrument quantification limit; [c] MDL is method detection limit; [d] MQL is method quantification limit.

Table E-S6.5. Recovery, accuracy, precision (intra- and inter-batch), and matrix effect for each target analyte.

Analyte	Recovery (%)	Accuracy (%)	Intra-batch precision RSD (%)	Inter-batch precision RSD (%)	Matrix effect (%)
Acetamiprid	27.3	96.5 ± 12.3	3.62 – 7.61	7.68	-38.4
Atrazine	52.0	92.5 ± 9.4	10.2 – 11.3	13.4	-24.4
Carbamazepine	40.6	86.2 ± 6.3	6.03 – 7.65	11.8	-7.6
Diclofenac	31.7	106 ± 5	2.87 – 3.87	4.60	-27.4
Isoproturon	34.6	83.4 ± 9.5	6.30 – 6.54	8.33	-25.2
Metaflumizone	20.5	101 ± 7	7.86 – 9.26	11.2	-38.8
Methiocarb	47.1	100 ± 6	5.31 – 6.04	5.81	-41.2
PFOS	34.3	84.3 ± 9.0	7.48 – 11.3	14.5	13.5

Table E-S6.6. Design of trial runs (in coded form) for DSD and corresponding recovery values for the 8 target compounds. All the experiments were performed with pristine CX samples.

Experimental number	Variables					Recovery (%)							
	A	B	C	D	E	Atrazine	Carbamazepine	Acetamiprid	Isoproturon	Methiocarb	Diclofenac	PFOS	Metaflumizone
1	+	-	-	+	0	9	6	2	6	7	7	2	1
2	+	+	-	-	+	29	29	0	21	19	23	11	8
3	+	-	+	-	+	11	8	7	6	0	2	13	5
4	0	0	0	+	0	15	16	7	9	6	3	8	20
5	+	0	+	-	-	13	9	6	6	0	2	23	5
6	0	+	+	+	+	18	10	0	7	3	3	27	54
7	-	+	+	-	0	4	3	3	4	0	1	10	60
8	-	+	-	+	-	1	0	0	2	0	2	1	55
9	-	-	+	+	-	1	1	0	0	0	0	2	17
10	+	+	0	+	-	18	13	11	8	6	3	15	36
11	0	-	-	-	-	7	9	0	8	5	2	1	1
12	-	0	-	+	+	9	4	3	12	2	4	2	4
13	-	-	0	-	+	7	7	5	3	1	3	5	22
14	0	0	0	-	0	13	13	14	8	4	2	10	26

Table E-S6.7. Design of trial runs (in coded form) for BBD and corresponding recovery values for the 8 target compounds. All the experiments were performed with pristine CX samples.

Experimental number	Variables			Recovery (%)							
	A	B	C	Atrazine	Carbamazepine	Acetamiprid	Isoproturon	Methiocarb	Diclofenac	PFOS	Metaflumizone
1	0	-	+	15	6	5	6	0	4	17	13
2	+	0	-	20	18	5	11	17	13	3	16
3	0	+	+	23	11	0	10	0	9	34	28
4	+	-	0	41	28	13	18	6	2	7	8
5	-	+	0	14	8	5	7	5	3	5	69
6	0	-	-	9	9	3	8	12	8	2	7
7	-	0	-	9	7	4	6	5	7	1	28
8	-	-	0	6	5	3	3	3	1	4	47
9	0	0	0	14	8	5	6	5	3	8	22
10	0	0	0	15	9	5	6	6	4	9	23
11	-	0	+	12	11	5	8	0	4	15	62
12	+	+	0	39	23	10	19	10	3	10	25
13	0	0	0	26	18	8	14	8	3	9	21
14	0	+	-	11	12	5	17	32	14	5	12
15	+	0	+	36	15	9	15	0	6	41	15

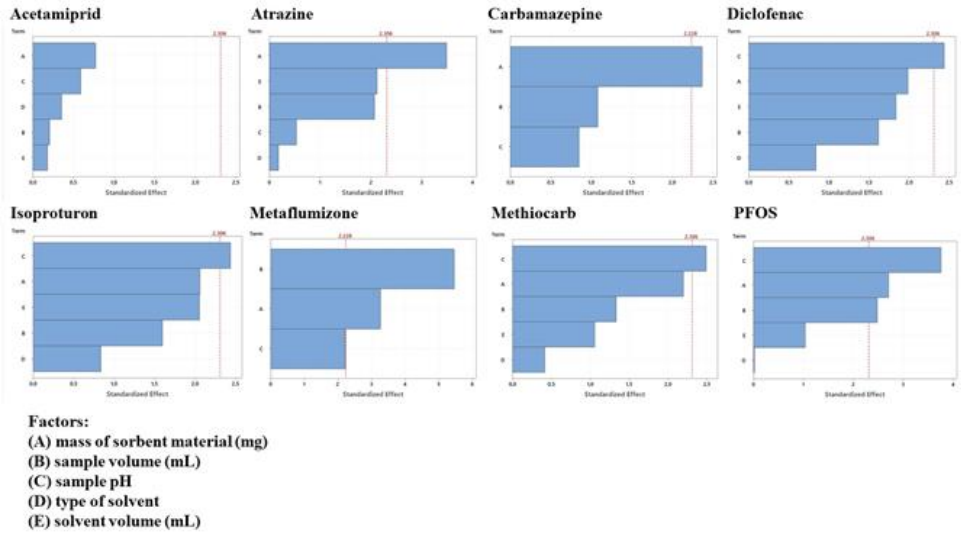


Fig. E-S6.1. Pareto charts representation of the standardized effects originated by the main factors (A, B, C, D and E) for each target compound; response is recovery (%); α = 0.05. All the experiments were performed with pristine CX samples.

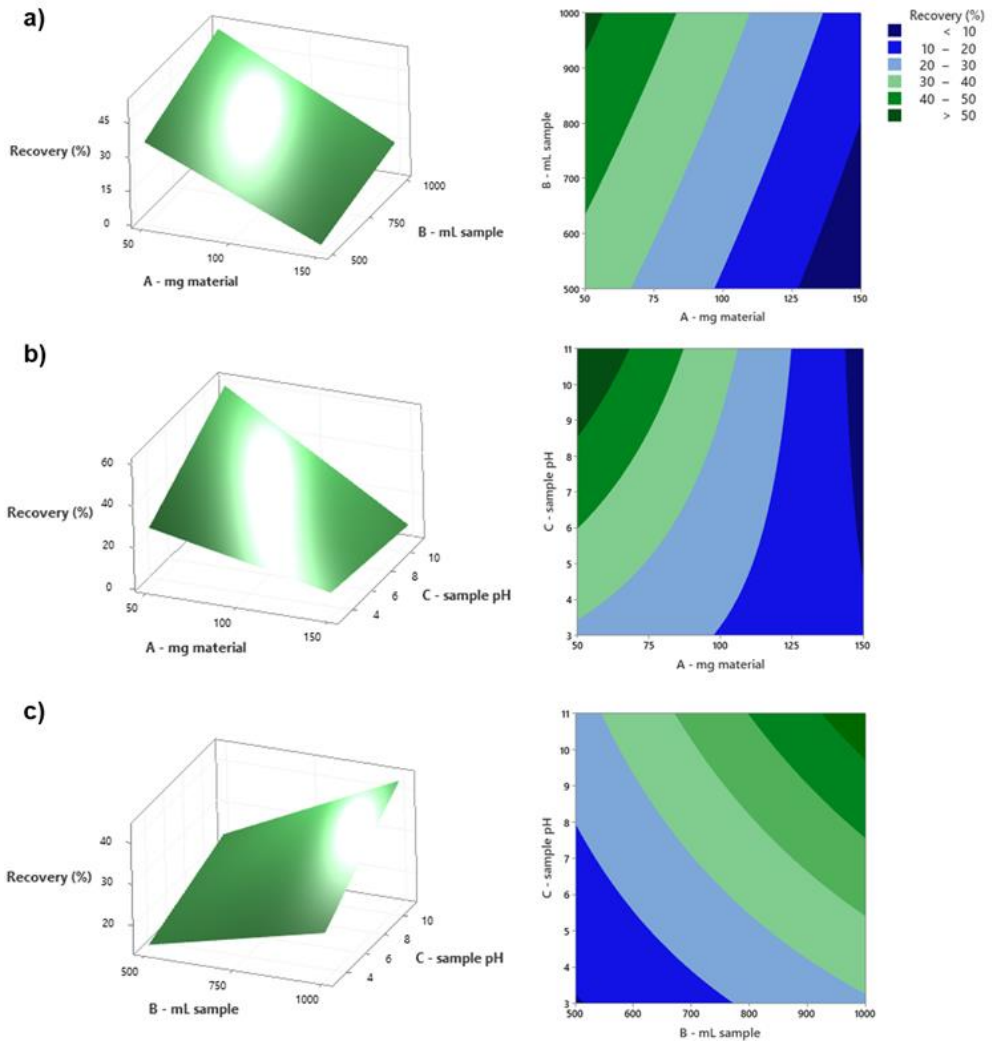


Fig. E-S6.2. Response surface and contour plots showing the effect of the mass of sorbent material (mg) (factor A), sample volume (mL) (factor B), and sample pH (factor C) on the recovery of metaflumizone: **(a)** Response surface and contour plots of recovery as a function of factors A and B (hold value: C = 7); **(b)** Response surface and contour plots of recovery as a function of factors A and C (hold value: B = 750 mL); **(c)** Response surface and contour plots of recovery as a function of factors B and C (hold value: A = 100 mg). All the experiments were performed with pristine CX samples.

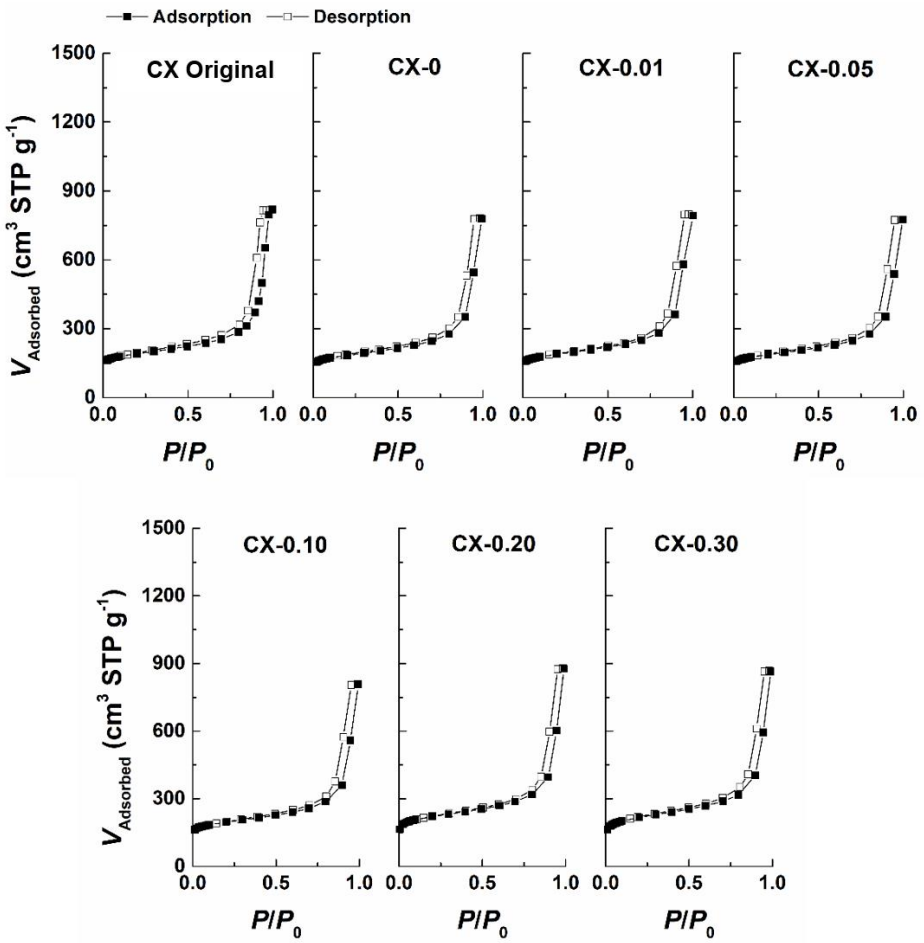


Fig. E-S6.3. N_2 adsorption-desorption isotherms at -196 ^oC of CXs subjected to hydrothermal treatment with different HNO_3 concentrations: the subscript number in CX_0, $CX_{0.01}$, and $CX_{0.05}$ corresponds to the concentration of HNO_3 (mol L^{-1}).

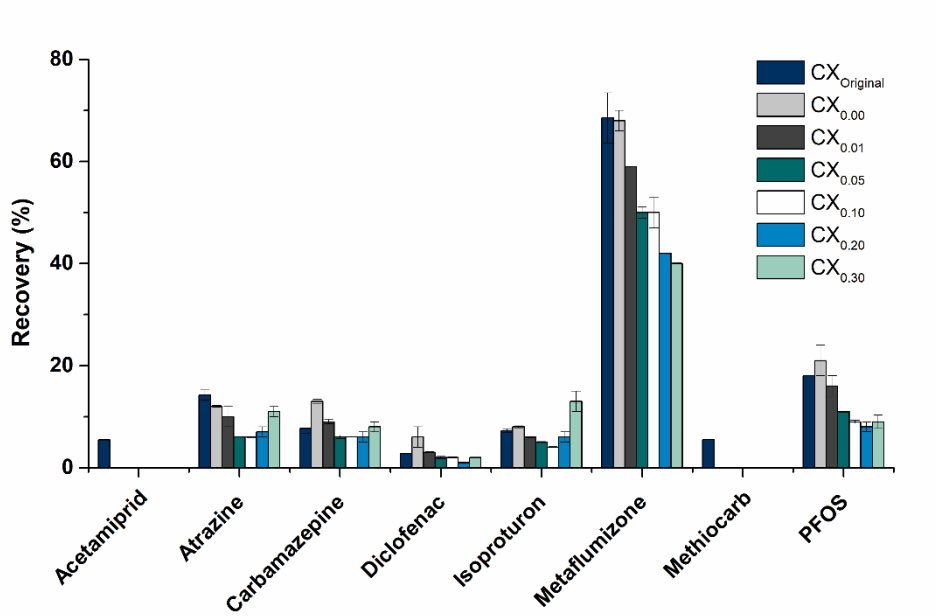


Fig. E-S6.4. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with carbon xerogel (CX; 50 mg) obtained after hydrothermal treatment with different HNO_3 concentrations (0-0.30 mol L^{-1}). Experiments performed with 1000 mL of surface water (SW; pH 7) and using ethanol as solvent (8 mL); n = 3 (RSD is represented as error bars).

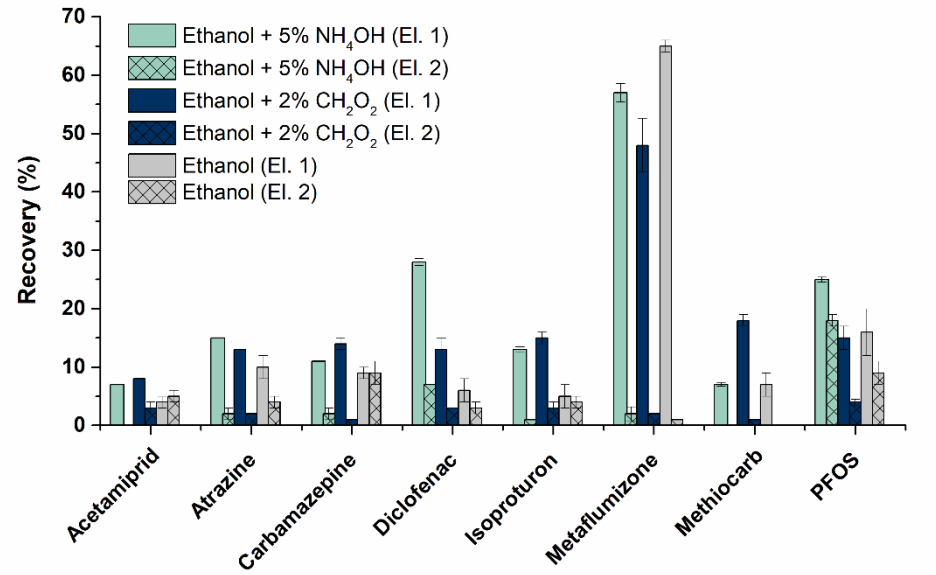


Fig. E-S6.5. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with CXs (50 mg) with different solvents (ethanol + 5% of NH_4OH, ethanol + 2% of CH_2O_2, and ethanol) and two elution steps (8 + 8 mL). Experiments performed with 1000 mL of SW (pH 7); n = 3 (RSD is represented as error bars).

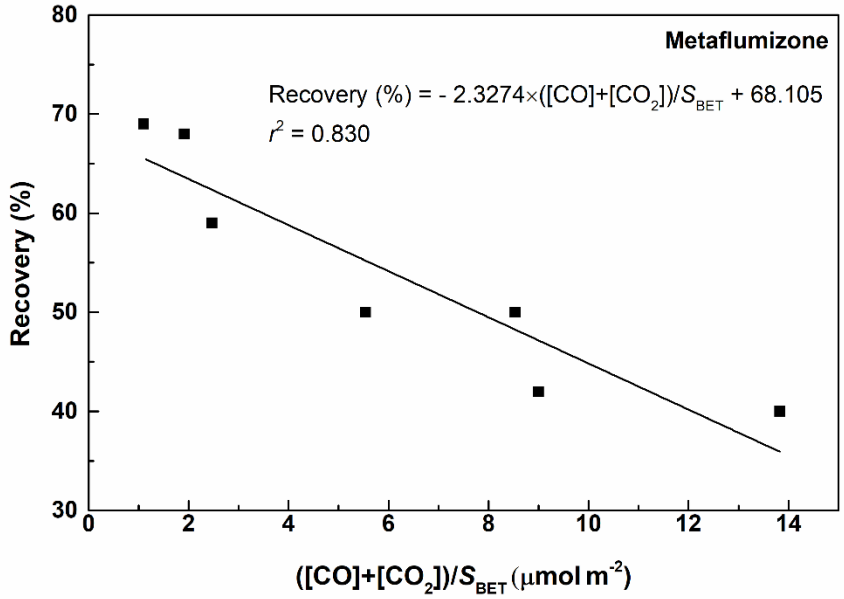


Fig. E-S6.6. Recovery obtained for metaflumizone as a function of ($[CO_2]$ + [CO])/S_{BET}.

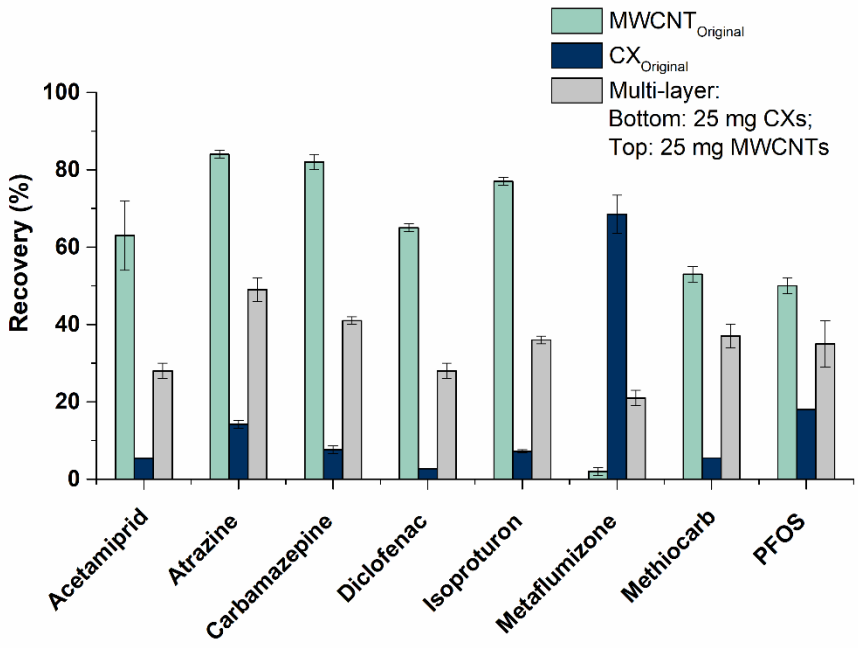


Fig. E-S6.7. Recoveries obtained for the target micropollutants (200 ng L^{-1} each), when using cartridges packed with different carbon materials: multi-walled carbon nanotubes (MWCNTs), CXs and multi-layer (bottom: 25 mg CXs; top: 25 mg MWCNTs). Experiments performed with the optimized procedures for each type of sorbent; n = 3 (RSD is represented as error bars).

Appendix F

Co-authors authorization regarding the full agreement of the inclusion of each manuscript in the present thesis

Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

2021.01.14

To Whom it May Concern

Nuno F.F. Moreira, PhD, Associate Laboratory LSRE-LCM and LEPABE, Faculty of Engineering – University of Porto (FEUP), hereby authorize the PhD student Marta Oliveira Barbosa from the Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), Portugal, to include the following publication in her doctoral Thesis entitled "*Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants*", as a result of her research activity:

Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495, Water Research 94 (2016) 257-279, doi: 10.1016/j.watres.2016.02.047

(Authors: Marta O. Barbosa, Nuno F.F. Moreira, Ana R. Ribeiro, Manuel F.R. Pereira, Adrián M.T. Silva)

Nuno Filipe Figueiredo Moreira

October 20th, 2020

To Whom it May Concern

Nuno Ratola, PhD, LEPABE, Faculty of Engineering – University of Porto (FEUP), Portugal, hereby authorizes the PhD student Marta Oliveira Barbosa from the Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), Portugal, to include the following publication in her doctoral Thesis entitled "*Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants*", as a result of her research activity:

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices, Science of the Total Environment (2018) 644: 1128–1140, doi: 10.1016/j.scitotenv.2018.06.355

(Authors: Marta O. Barbosa, Ana R. Ribeiro, Nuno Ratola, Ethan Hain, Vera Homem, Manuel F.R. Pereira, Lee Blaney and Adrián M.T. Silva)

(Nuno Ratola)

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Vera Homem, PhD, LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering – University of Porto (FEUP), Portugal, hereby authorize the PhD student Marta Oliveira Barbosa from the Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), Portugal, to include the following publication in her doctoral Thesis entitled "*Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants*", as a result of her research activity:

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices, Science of the Total Environment (2018) 644: 1128–1140, doi: 10.1016/j.scitotenv.2018.06.355

(Authors: Marta O. Barbosa, Ana R. Ribeiro, Nuno Ratola, Ethan Hain, Vera Homem, Manuel F.R. Pereira, Lee Blaney and Adrián M.T. Silva)

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Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices, Science of the Total Environment (2018) 644: 1128–1140, doi: 10.1016/j.scitotenv.2018.06.355

(Authors: Marta O. Barbosa, Ana R. Ribeiro, Nuno Ratola, Ethan Hain, Vera Homem, Manuel F.R. Pereira, Lee Blaney and Adrián M.T. Silva)

Ethan Hain

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November 20, 2020

To Whom it May Concern,

I, Lee Blaney (PhD, University of Maryland Baltimore County), hereby authorize the PhD candidate Marta Oliveira Barbosa from the Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), Portugal, to include the following publication in her doctoral Thesis entitled "*Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants*", as a result of her research activity:

Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices, Science of the Total Environment (2018) 644: 1128–1140, doi: 10.1016/j.scitotenv.2018.06.355

(*Authors: Marta O. Barbosa, Ana R. Ribeiro, Nuno Ratola, Ethan Hain, Vera Homem, Manuel F.R. Pereira, Lee Blaney and Adrián M.T. Silva*)

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LABORATORY OF CATALYSIS AND MATERIALS

To Whom It May Concern

Rui S. Ribeiro, Doctoral Researcher at Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), hereby authorizes the PhD student Marta Oliveira Barbosa from Associate Laboratory LSRE-LCM, Faculty of Engineering – University of Porto (FEUP), Portugal, to include the following publication in her doctoral Thesis entitled "*Multi-layer carbon cartridges for determination of EU multi-class organic micropollutants*", as a result of her research activity:

Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants, Scientific Reports 10 (2020) 22304, doi: 10.1038/s41598-020-79244-8

(*Authors: Marta O. Barbosa, Rui S. Ribeiro, Ana R. Ribeiro, Manuel F.R. Pereira, Adrián M.T. Silva*)

Porto, January 7th, 2021

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Appendix G

Publications and Communications

Scientific publications in peer reviewed international scientific journals

1. **Barbosa, M.O.**; Ribeiro, R.S., Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., “Carbon xerogels combined with carbon nanotubes as an innovative sorbent in solid-phase extraction cartridges to assess the occurrence of organic micropollutants in surface and drinking water”, Science of the Total Environment, *submitted for publication*.
2. **Barbosa, M.O.**; Ribeiro, R.S., Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Solid-phase extraction cartridges with multi-walled carbon nanotubes and effect of the oxygen functionalities on the recovery efficiency of organic micropollutants". Scientific Reports 10:22304 (2020).
3. Kumar, V.R.[1]; **Barbosa, M.O.**[1]; Ribeiro, A.R.; Morales-Torres, S.; Pereira, M.F.R.; Silva, A.M.T., "Advanced oxidation technologies combined with direct contact membrane distillation for treatment of secondary municipal wastewater". Process Safety and Environmental Protection 140:111-123 (2020). [1] The authors contributed equally to this study.
4. Sousa, J.C.G.; **Barbosa, M.O.**; Ribeiro, A.R.L.; Ratola, N.; Pereira, M.F.R.; Silva, A.M.T., "Distribution of micropollutants in estuarine and sea water along the Portuguese coast". Marine Pollution Bulletin 154: 111120 (2020).
5. Gorito, A.M.; **Barbosa, M.O.**; Almeida, C.M.R.; Pereira, M.F.R.; Silva, A.M.T.; Ribeiro, A.R.L., "Quenchers in advanced oxidation technologies for analysis of micropollutants by liquid chromatography coupled to mass spectrometry: Sodium sulphite or catalase?". Science of the Total Environment 692: 995-1004 (2019).
6. Sousa, J.C.G.; Ribeiro, A.R.; **Barbosa, M.O.**; Ribeiro, C.; Tiritan, M.E.; Pereira, M.F.R.; Silva, A.M.T., "Monitoring of the 17 EU Watch List contaminants of

emerging concern in the Ave and the Sousa Rivers". Science of the Total Environment 649:1083-1095 (2019).

7. **Barbosa, M.O.**; Ribeiro, A.R.; Ratola, N.; Hain, E.; Homem, V.; Pereira, M.F.R.; Blaney, L.; Silva, A.M.T., "Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices". *Science of the Total Environment* 644:1128-1140 (2018).
8. Sousa, J.C.G.; Ribeiro, A.R.; **Barbosa, M.O.**; Pereira, M.F.R.; Silva, A.M.T., "A review on environmental monitoring of water organic pollutants identified by EU guidelines". *Journal of Hazardous Materials* 344:146-162 (2018).
9. **Barbosa, M.O.**; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal". *Analytical and Bioanalytical Chemistry* 408:8355-8367 (2016).
10. **Barbosa, M.O.**; Moreira, N.F.F.; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495". *Water Research* 94:257-279 (2016).

Oral communications in scientific meetings

1. **Barbosa, M.O.**; Ribeiro, A.R.; Ratola, N.; Hain, E.; Homem, V.; Pereira, M.F.R.; Blaney, L.; Silva, A.M.T., "EU multi-class organic micropollutants in Leça River: Spatiotemporal monitoring and fluorescence excitation-emission matrices assessment", *XXIV Encontro Luso Galego de Química*, 21st to 23rd November, 2018, Porto, Portugal (p. 282).
2. **Barbosa, M.O.**; Ribeiro, A.R.; Ratola, N.; Hain, E.; Homem, V.; Pereira, M.F.R.; Blaney, L.; Silva, A.M.T., "Emerging and Priority Micropollutants: Seasonal Occurrence in Portuguese Rivers", *40th International Conference on Environmental & Food Monitoring* (ISEAC-40), 19th to 22nd June, 2018, Santiago de Compostela, Spain (p. 111).
3. Sousa, J.C.G.; Ribeiro, A.R.; **Barbosa, M.O.**; Ribeiro, C.; Tiritan, M.E.; Pereira, M.F.R.; Silva, A.M.T., "Spatiotemporal Monitoring Campaign of the Watch List Compounds in Ave and Sousa Rivers", *18th European Meeting on Environmental Chemistry*, (EMEC18), 26th to 29th November, 2017, Porto, Portugal (p. 96).
4. Ribeiro, A.R.; **Barbosa, M.O.**; Pereira, M.F.R.; Silva, A.M.T., "Eco-friendly UHPLC-MS/MS method for analysis of multi-class micro pollutants in drinking water", *4th World Congress on Mass Spectrometry*, 19th to 21st June, 2017, London, UK (p. 54).
5. Sousa, J.C.G.; Ribeiro, A.R.; **Barbosa, M.O.**; Pereira, M.F.R.; Silva, A.M.T., "Spatial and temporal distribution of contaminants of emerging concern in Ave River", *Symposium on Chemical, Biological and Environmental Engineering, 2nd Doctoral Congress in Engineering*, 8th to 9th June, 2017, Porto, Portugal, (p. 11-12).

6. Ribeiro, A.R.; Sousa, J.C.G.; **Barbosa, M.O.**; Pereira, M.F.R.; Silva, A.M.T., "Contaminants of emerging concern in Portuguese rivers", *253rd American Chemical Society, NATIONAL MEETING & EXPOSITION, Advanced Materials, Technologies, Systems & Processes*, 2nd to 6th April, 2017, San Francisco, California, EUA.

Poster communications in scientific meetings

1. **Barbosa, M.O.**; Ribeiro, A.R.; Ratola, N.; Homem, V.; Pereira, M.F.R.; Silva, A.M.T., "Monitoring of Organic Micropollutants in Environmental Matrices", *2nd International Meeting on New Strategies in Bioremediation Processes* (BioRemid2019), 24th to 25th October, 2019, Porto, Portugal (pp. 136).
2. **Barbosa, M.O.**; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "EU Organic Micropollutants: Their Occurrence in Environmental Matrices", *Ciência 2019 – Science and Technology Summit in Portugal*, 8th to 10th July, 2019, Lisboa, Portugal.
3. Ribeiro, A.R.; Moreira, N.F.F.; **Barbosa, M. O.**; Pereira, M.F.R.; Li Puma, G.; Silva, A. M. T, "Removal of micropollutants by ozonation in wastewater matrices", *XENOWAC II - International Conference on Xenobiotics in the Urban Water Cycle*, 10th to 12th October, 2018, Limassol, Cyprus.
4. **Barbosa, M.O.**; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Organic Micropollutants from the EU Watch List: Their Occurrence in Drinking Waters", *18th European Meeting on Environmental Chemistry*, 26th to 29th November, 2017, Porto, Portugal (p.174).
5. Gorito, A.M.; Ribeiro, A.R.; **Barbosa, M.O.**; Almeida, C.M.R.; Pereira, M.F.R.; Silva, A.M.T., "Constructed wetlands and advanced oxidation processes for removal of organic micropollutants", *XXII Encontro Luso-Galego de Química*, 9th to 11th November, 2016, Bragança, Portugal (p. 203).
6. Sousa, J.C.G.; **Barbosa, M.O.**; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Determination of contaminants of emerging concern in surface water", *XXII*

Encontro Luso-Galego de Química, 9th to 11th November, 2016, Bragança, Portugal (p. 190).

7. Gorito, A.M.; Ribeiro, A.R.; Barbosa, M.O.; Almeida, C.M.R.; Pereira, M.F.R.; Silva, A.M.T., "Coupling constructed wetlands and ozonation to remove organic micropollutants", *16as Jornadas de Engenharia Química*, 15th November, 2016, Porto, Portugal.

8. Sousa, J.C.G.; **Barbosa, M.O.**; Ribeiro, A.R.; Pereira, M.F.R.; Silva, A.M.T., "Contaminants of emerging concern in river water", 16as Jornadas de Engenharia Química, 15th November, 2016, Porto, Portugal.