Escola de Camins
Escola Tècnica Superior d'Enginyeria de Camins, Canals i Ports
UPC BARCELONATECH

TREBALL FINAL DE MÀSTER

ANALYSIS OF POLY- AND PERFLUOROALKYL SUBSTANCES IN ENVIRONMENTAL SAMPLES

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Màster en: **Enginyeria Ambiental**

Barcelona, **23/09/2016**

Departament: **Escola Tècninca Superioi d'Enginyeria de Camins Canals i Ports de Barcelona**

Analysis of poly- and perfluoralkyl substances in environmental samples

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ABSTRACT

Poly- and perfluoralkyl substances (PFASs) are a group of manmade substances synthesized for more than 60 years. Do to their specific properties, PFASs have been widely used for industrial applications. However, it was during the last fifteen years ago that interest for this group of compounds as environmental pollutant was initiated. Due to their high release level into the environment, stability and accumulation, PFASs have been found ubiquitous in the environment and in biota.

In this context, the main goal of this master thesis was to study the current profile of PFASs including short chain and FTCAs in environmental samples: river water, sediments and biota in Catalonia, taken as a case study the area of the Ebro Delta.

Therefore, the first specific objective was to asses 13 PFASs including: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFODA, PFBS, PFHxS, PFOS, PFDS, FOSA by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using the method previously developed with minor revisions. The different physic-chemical properties and environmental behaviour of FTCAs, makes difficult their analysis together with the other PFASs. Therefore, the second specific objective was to develop and validate a new analytical method for the detection of FTCAs (FHEA, FOEA and FDEA) in waters, sediments and biota (fish). Due to the characteristics of these sub-group of compounds gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) was considered as technique of choose. The last objective was to characterize the ecotoxicity of the water samples from Ebro Delta by standardized approaches.

For the analysis of FHEA, FOEA and FDEA by GC-MS/MS an non-inert TRACE TR-5M column was used and derivatization with BF_3 and methanol was carried out derivatization in order to increase the analytes retention a mild derivatization step was performed to increase the interaction between analytes and the column.

For water samples, PFOA is the most ubiquitous while PFPeA shows the highest concentrations. The most polluted sample is coming from the influent of WWTP. For sediment samples, PFOA, PFNA and PFHpA were the most detected compounds among carboxylic acids while PFOS was the most abundant among sulfonates. The most contaminated fish sample is coming from a bay. As regards to fish samples, PFOS was the most accumulated in skin among different species. On the other side, PFHxA was the most detected among carboxylic acids. The concentrations of PFASs are higher in the skin.

FTCAs were analyzed in twelve representative water samples and 2 fish samples by LC-MS/MS and GC-MS/MS. FHEA and FDEA were the most abundant compounds. The most contaminated sample is coming from the influent of WWTP. GC-MS/MS is more appropriate instrumental method for the detection of FTCAs, which are semi-volatile and allows lower limits of detection and quantification, compared to LC-MS/MS.

Only influent WWTP, shore and estuary samples presented toxicity.

Analysis of poly- and perfluoralkyl substances in environmental samples

AGRAÏMENTS

Primer de tot m'agradaria agrair a la meva tutora del treball de final de Màster, la Marinel·la Farré per donar-me l'oportunitat de treballar en el fantàstic equip d'investigació i ensenyar-me cada dia la seva gran dedicació i passió per la ciència que li ha permès formar aquest gran equip. Gràcies per confiar amb mi, dedicar-me tant de temps i ensenyar-me tant..

D'altra banda, i no menys important, mil gràcies a la Marta Llorca per tot el suport que m'ha donat durant aquets mesos i el temps invertit en ensenyar-me. Cada cop que he mostrat símptomes de desanimació ella sempre deia amb el seu positivisme: " Tot sortirà bé Gemma, tot anirà molt bé!". I crec que finalment així ha estat! Encara que jo me'n reia, la veritat esca m'ha anat més bé del que et penses totes les vagades que m'has dit aquesta frase. Gràcies i mil gràcies Marta.

Agrair també els meus companys d'equip, en Josep, la Cris, la Gabriella, l'Emanuela, la Mar i la Marianne per tots els consells que m'heu donat i l'ajuda que m'heu proporcionat.

A tots els companys de l'IDAEA que fèieu que treballar cada dia en el laboratori sigues divertit i apassionant Juan, Monica, Manu, Ester, Carlos, Simon, Dani, Anna Mir, Xaufei i Oscar.

Gràcies a la Sara Insa per ajudar-me durant la meva estada desenvolupant el mètode.

Als meus amics, per el vostre suport i interès en el transcurs d'aquest projecte Paula, Piula i Guille.

El meu estimat Kevin per donar-me tant suport, confiar en mi i entendre la importància que significava per mi passar-me tantes hores "punxant".

Sobretot, gràcies a tota la meva família, els meus pares, que sense ells no hagués arribat a ser qui sóc ara. Gràcies per la vostre dedicació i ajuda durant aquest projecte i tota la meva vida.

Finalment, vull dedicar aquest treball de final de Màster el meu tiet Jordi. Que segur que hauria estat molt orgullós de mi.

Moltes gràcies a tots.

Analysis of poly- and perfluoralkyl substances in environmental samples

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CI: chemical ionization

EC: European Commission

EC_{50}: the effective concentration where 50% of the population is immobile)

ECF: Eclectrochemical fluorination

EFSA: European Food Safety Authority

EI: Electron impact

EPA: United States Environmental Protection Agency

ESI: electrospray ionization source

FDEA: 10:2 fluorotelomer carboxylic acid

FOEA: 8:2 fluorotelomer carboxylic acid

FHEA: 6:2 fluorotelomer carboxylic acid

FTAL: Fluorotelomer aldehyde

FTCAs: Fluorotelomer carboxylic acid

FTs: Fluorotelomers

FTOH: Fluorotelomer alchol

FTSA: Fluorotelomer sulphonate

FTUCA: unsaturated carboxylic acid

GC: Gas chromatography

GC-MS/MS: Gas chromatography coupled to tandem mass spectrometry

HPLC: High performance liquid chromatography

IDAEA-CSIC: Instituto de Diagnóstico Ambiental y Estudios del Agua-Conesjo Superior de Investigaciones Científicas

IP: Identifications points

LC: Liquid chromatography

LC-MS/MS: Liquid chromatography coupled to tandem mass spectrometry

LLE: Liquid-liquid extraction

LOD: Limit of detection

LOQ: Limit of quantification

MPFDA-$^{13}C_2$: $^{13}C_2$-perfluorodecanoic acid

MPFDoA-$^{13}C_2$: $^{13}C_2$-perfluorododecanoic acid

MPFNA-$^{13}C_5$: $^{13}C_5$-perfluorononanoic acid

MPFHxS-$^{18}O_2$: $^{18}O_2$-perfluorohexanesulfonate

MPFHxA $^{13}C_2$: $^{13}C_2$-perfluorohexanoic acid

MPFOS-$^{13}C_4$: $^{13}C_4$-perlfuorooctanesulfonate

MPFOA-$^{13}C_4$: $^{13}C_4$-perfluorooctanoic acid

M8FOSA: $^{13}C_8$-perfluorooctanesulfonamide

MS: Mass spectrometry

OECD: Organisation of Economic Coperation and Development

PCPS: Personal Care products

PFACAs: Perfluoroalkyl carboxylic acids

PFASs: Poly- and per- fluroroalkyl subsatnces

PFOS: Perflurooctane sulphonic acid

PFOA: Perfluoroctanoic acid

POPs: Persistent Organic Pollutants

PTFE: Poly(tetrafluoroethylene)

PVDF: Poly(viylidene fluoride)

PWTP: Potable water treatment plant

PFSAs: Perfluorosulphonates

PBT-chemicals: Persistent, bioaccumulative and toxic

PFPeA: Containing perfluoropentanoic

PFHxA: Perfluorohexanoic

PFHpA: Perfluoroheptanoic

PFNA: Perfluorononanoic

PFDA: Perfluorodecanoic

PFUdA: Perfluoroundecanoic

PFDoA: Perfluorododcanoic

PFBS: Perfluorobutanesulfonate

PFHxS: Perfluorohexanesulfonate

PFOSA: Perfluoroctanesulfonamide

PFAI: Perfluoroalkyl iodide

REACH: Evaluation, Authorization and Restriction of Chemicals

R_t: Retention time

SPE: Solid phase extraction

TFC: Turbulent flow chromatography

UAE: Ultrasonic assisted extraction

UNESCO: United National Educationak, Scientific and Cultural Organization

WHO: World Health Organization

WWTP: Waste Water Treatment Plant

Analysis of poly- and perfluoralkyl substances in environmental samples

1. INTRODUCTION

1.1. EMERGING CONTAMINANTS

Emerging pollutants are defined as compounds that are not currently covered by existing environmental regulations, have not been studied before, and are thought to be potential threats to the ecological and human health [1]. Therefore, these compounds are not necessarily new, in fact, some of them have been used for decades and have been widely disposed in the environment, but only recently their environmental and health significance is under evaluation. In the US recently this definition has been extended by “Pollutants of Emerging Concern” that includes emerging micro-pollutants, emerging contaminants, and new chemicals.

Pollutants of emerging concern comprises industrial products used in large quantities in everyday life, such as human and veterinary pharmaceuticals, personal care products (PCPS), surfactants, plasticizers and various additives. Some of these do not need to be persistent in the environment to cause negative effects, since their high transformation and removal rates can be offset by their continuous introduction into the environment [2].

Conventional wastewater treatments are only partially effective in the removal or degradation, of some pollutants of emerging concern, therefore are continuously entering water cycle. Once in the environment, these contaminants are distributed [3] in the different aquatic compartments, reaching the aquatic food chain and scaling to the human food chain and drinking water.

Among emerging contaminants, poly- and per- fluoroalkyl substances (PFASs) are a diverse group of compounds that have been synthesised for more than 60 years. Due to their specific properties (repellents of grease and water) and their high stability, PFASs have been widely used for industrial applications including: electronics, textile, food, packaging, flame retardant, among others. However, it was during the last fifteen years when the interest for this group of compounds as environmental pollutants was initiated. Due to their high release levels into the environment, stability and accumulation behaviour, PFASs have been found ubiquitous in the environment and biota [4].

During the last years, the development of more sensitive analytical methods allows the detection of the presence of these contaminants in the environment. The study of emerging contaminants is in the research priorities of the main organizations dedicated to protect human health and the environment such as the World Health Organization (WHO), the United States Environmental Protection Agency (EPA) and the European Commission.

1.2. PERFLUORO ALKYL SUBSTANCES AND FLUOROTELOMERS

“Fluorinated substances” is the general, nonspecific name that describes a universe of organic and inorganic substances that contain at least one fluorine atom, with vastly different physic-chemical, and biological properties. A subset of fluorinated substances is the highly fluorinated aliphatic substances that contain one or more C atoms on which all the H substituents (present in the non-fluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1}-. These compounds are here referred to as “ perfluoroalkyl and polyfluoroalkyl substances” and denoted by the acronym PFASs [5].

Because of their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, they have been used for over years in a number of industrial and commercial applications [6].Therefore, these compounds are widely spread in the environment [7] and also in human blood and liver [8]. Some of these compounds can accumulate and biomagnify in food chain [9]. Meanwhile PFASs have been recognized as emerging contaminants in the food chain by the European Food Safety Authority (EFSA) [10].

PFASs can be classified into three broad groups: perfluoroalkyl carboxylic acids (PFACAs), perfluorosulphonic acids and fluorotelomers [4]. The eight C-chain perfluorinated substances, perflurooctane sulphonic acid (PFOS) and perfluorooctanoic acid (PFOA), were the most widely used and produced substances in the past. Also, these were the more recalcitrant and persistent compounds. Therefore, PFOS and PFOA have been for years the more studied compounds. However, some other substances have been less studied, and there are some gaps of information about their occurrence and environmental behaviour.

Under this frame, this master thesis is mainly focused on the **study of the occurrence and distribution of PFASs in different compartments of the Ebro Delta (water, sediments and biota) and the development of an analytical method specifically designed to assess the fluorotelomers (FTs) subgroup**. Three fluorotelomer carboxylic acid (FTCAs): 6:2 fluorotelomer carboxylic acid or 2-Perfluorohexyl ethanoic acid (FHEA), 8:2 fluorotelomer carboxylic acid or 2-Perflurooctyl ethanoic acid (FOEA) and 10:2 fluorotelomer carboxylic acid or 2-Perfluorodecyl ethanoic acid (FDEA). In naming telomers, the compound name is preceded by X:Y, where X is the number of fluorocarbons and Y is the number of hydrocarbons.

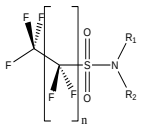
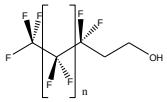
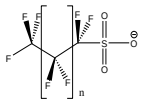
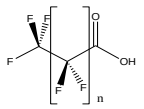
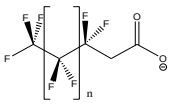
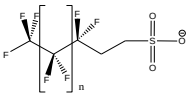
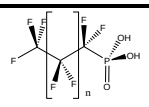
1.2.1. Properties

PFASs are a wide group of compounds varying in their structure and, thus, exhibit different properties, environmental fate, and toxicity, but their common trend is a general high stability by the carbon-chain bond (one of the strongest in nature). The atomic structure of fluorine has a Van der Waals radius of 1.35 Å, lower than the other halogens, and the highest electronegativity of the periodic table, being 3.98 in Pauling scale. As a consequence of the high electronegativity of fluorine, the carbon-fluorine bond is very strong (~110 kcal/mol) [11] and stable, making some of these compounds Persistent Organic Pollutants (POPs).

On the other hand, the high ionisation potentials of fluorine (1^{st}: 1681 kJ/mol, 2^{nd}: 3374 kJ/mol and 3^{rd}: 6147 kJ/mol [12] and its low polarizability leads to weak inter- and intramolecular interactions [13]. Perfluroalkanes present a double character, hydrophobic and oleophobic, and when they are mixed with hydrocarbons and water, from three immiscible phases. However, these compounds are more commonly used with a charged moiety, such as carboxylic acid, sulphonic acid, phosphate or quaternary ammonium group, which decreases their intrinsically hydrophobic character. These functionalised chemicals present surfactant properties and make them suitable to be used as emulsifiers during fluoropolymerisation synthesis [13] among other applications.

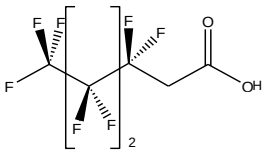
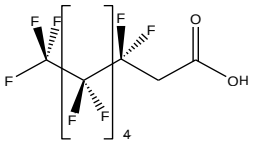
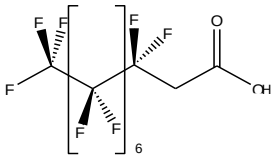
Most common, PFASs, under the industrial point of view, are summarised in Table 1.

Table 1: Perfluoroalkyl chemicals.

Class	Compound	Abbreviation	Formula	Chemical structure
Perfluorinated sulphonamides (FSAs)	*N*-methyl perfluorobutane sulfonamidoethanol	NMeFBSE	$F(CF_2)_4SO_2N(CH_3)CH_2CH_2OH$	
	N-ethyl perfluorobutane sulfonamidoethanol	NEtFBSE	$F(CF_2)_4SO_2N(CH_2CH_3)CH_2CH_2OH$	
	Perfluorooctane sulphonamide	FOSA	$F(CF_2)_8SO_2NH_2$	
	N-methyl perfluorooctane sulfonamide	NMeFOSA	$F(CF_2)_8SO_2N(CH_3)H$	
	N-ethyl perfluorooctane sulfonamide	NEtFOSA	$F(CF_2)_8SO_2N(CH_2CH_3)$	
	N-methyl perfluorooctane sulfonamidoethanol	NMeFOSE	$F(CF_2)_8SO_2N(CH_3)CH_2CH_2OH$	
	N-ethyl perfluorooctane sulfonamidoethanol	NEtFOSE	$F(CF_2)_8SO_2N(CH_2CH_3)CH_2CH_2OH$	
Fluorotelomer Alcohols (FTOHs)	4:2 fluorotelomer alcohol	4:2 FTOH	$F(CF_2)_4CH_2CH_2OH$	
	6:2 fluorotelomer alcohol	6:2 FTOH	$F(CF_2)_6CH_2CH_2OH$	
	8:2 fluorotelomer alcohol	8:2 FTOH	$F(CF_2)_8CH_2CH_2OH$	
	10:2 fluorotelomer alcohol	10:2 FTOH	$F(CF_2)_{10}CH_2CH_2OH$	
	12:2 fluorotelomer alcohol	12:2 FTOH	$F(CF_2)_{12}CH_2CH_2OH$	
Perfluorosulphona -tes (PFSAs)	Perfluorobutane sulphonate	PFBS	$F(CF_2)_4SO_3^-$	
	Perfluorohexane sulphonate	PFHxS	$F(CF_2)_6SO_3^-$	
	Perfluorooctane sulphonate	PFOS	$F(CF_2)_8SO_3^-$	
	Perfluorodecane sulphonate	PFDS	$F(CF_2)_{10}SO_3^-$	
Perfluorocarboxyli -c acids (PFCAs)	Perfluorobutanoic acid	PFBA	$F(CF_2)_4COOH$	
	Perfluoropentanoic acid	PFPeA	$F(CF_2)_5COOH$	
	Perfluorohexanoic acid	PFHxA	$F(CF_2)_6COOH$	
	Perfluoroheptanoic acid	PFHpA	$F(CF_2)_7COOH$	
	Perfluorooctanoic acid	PFOA	$F(CF_2)_8COOH$	
	Perfluorononanoic acid	PFNA	$F(CF_2)_9COOH$	
	Perfluorodecanoic acid	PFDA	$F(CF_2)_{10}COOH$	
	Perfluoroundecanoic acid	PFUnA	$F(CF_2)_{11}COOH$	
	Perfluorododecanoic acid	PFDoA	$F(CF_2)_{12}COOH$	
	Perfluorotridecanoic acid	PFTrA	$F(CF_2)_{13}COOH$	
	Perfluorotetradecanoic acid	PFTeA	$F(CF_2)_{14}COOH$	
	Perfluorohexadecanoic acid	PFHxDA	$F(CF_2)_{16}COOH$	
	Perfluorooctadecanoic acid	PFODA	$F(CF_2)_{18}COOH$	
Fluorotelomer carboxylates (FTCAs, FTUCAs)	6:2 fluorotelomer carboxylate	6:2 FTCA	$F(CF_2)_6CH_2CO_2^-$	
	6:2 fluorotelomer unsatured carboxylate	6:2 FTUCA	$F(CF_2)_6CHCO_2^-$	
	8:2 fluorotelomer carboxylate	8:2 FTCA	$F(CF_2)_8CH_2CO_2^-$	
	8:2 fluorotelomer unsatured carboxylate	8:2 FTUCA	$F(CF_2)_8CHCO_2^-$	
	10:2 fluorotelomer carboxylate	10:2 FTCA	$F(CF_2)_{10}CH_2CO_2^-$	
	10:2 fluorotelomer unsatured carboxylate	10:2 FTUCA	$F(CF_2)_{10}CHCO_2^-$	
Fluorotelomer sulphonates (FTSs)	6:2 fluorotelomer sulphonate	6:2 FTS THPFOS	$F(CF_2)_6CH_2CH_2SO_3^-$	
	8:2 fluorotelomer sulphonate	8:2 FTS	$F(CF_2)_8CH_2CH_2SO_3^-$	
	10:2 fluorotelomer sulphonate	10:2 FTS	$F(CF_2)_{10}CH_2CH_2SO_3^-$	
Perfluoro phosphonic acids (PFPAs)	Perfluorohexa phosphonic acid	PFHxPA	$F(CF_2)_6PO_3H_2$	
	Perfluoroocta phosphonic acid	PFOPA	$F(CF_2)_8PO_3H_2$	
	Perfluorodeca phosphonic acid	PFDPA	$F(CF_2)_{10}PO_3H_2$	

Fluorotelomers are a subgroup of PFASs. They are partially fluorinated, as small carbon-hydrogen chain (generally two carbons) linking the perfluorinated carbon chain to a functional group, such as sulphonate (FTSA), saturated carboxylic acid (FTCA), or unsaturated carboxylic acid (FTUCA) [14]. FTs are used in many industrial applications as surfactants or surface protection products [5]. Due to their carbon-hydrogen chain, FTs can be subjected to degradation in the environment and metabolised to end-stage metabolites, such as PFCASs [15]. Table 2 shows the three FTCAs studied in this master thesis.

Table 2: Fluorotelomer carboxylic acids.

Compound	Abbreviation	Formula	Chemical structure
6:2 fluorotelomer carboxylic acid / 2-Perflurohexyl ethanoic acid	6:2 FTCA / FHEA	$C_8H_3F_{13}O_2$	
8:2 fluorotelomer carboxylic acid / 2-Perfluorooctyl ethanoic acid	8:2 FTCA / FOEA	$C_{10}H_3F_{17}O_2$	
10:2 fluorotelomer carboxylic acid / 2-Perfluorodecyl ethanoic acid	10:2 FTCA/ FDEA	$C_{12}H_3F_{21}O_2$	

1.2.2. Production and industrial applications

Due to these properties, PFASs have found a great variety of industrial applications since the 40s.

PFOA was synthesized for the first time during the 1940s under the frame of the Manhattan Project that produced the first atomic bomb during the World War II [16]. However, the first synthesis process that allowed the large scale manufacture of fluorocarbons was in 1947 by Fowler Process [17]. During the same period, the company 3M discovered the electrochemical fluorination (ECF) or Simon's process [18]. This process is based on the electrolysis of a solution of hydrogen fluoride of an organic compound. Posteriorly, to improve the recovery percentage, or to obtain different functional groups, other syntheses were developed. Moreover, the industrial synthesis of fluorinated vinylidene fluoride, among others, is done by telomerisation because it has greater recoveries than ECF [19, 20].

After the first mass syntheses and their employment to develop new industrial products, their global production increased exponentially. In 2000, the maximum production of PFASs was reached by issuing 5,720 tonnes of the more used compounds by the industry, PFOA, and 3535 tons of PFOS. On the other hand, a production between 5000 and 6000 tonnes of FTs was reached. However, during the same period, other studies have proved the accumulation of PFASs in human tissues. Taves et al., [21] find organic fluorine in human serum (1968), and tentatively PFOA was identified in pooled blood [21]. In 1978, 3M reported that PFOA was found in the blood of 3M workers, and in 1984, PFOA was found to be present in drinking water near Washington Works plant. For these reasons, in 2000 3M announced the phase-out of C8 based PFASs. In 2006 the EPA together with the 8 major companies producing PFAS launched the Stewardship Program, and in 2009, PFOSs and related products were listed under the Annex B of the Stockholm Convention.

On the other hand, FTCAs are degradation products of fluorotelomer alcohol (FTOHs). The FTOHs and other telomere species are synthesized by telomerisation [22]. This process, generate a perfluoroalkyl iodide (PFAI) which can be utilized to produce a variety of fully and partially fluorinated compounds. In Figure 1, the synthesis of FTOH is illustrated. The 8:2 FTOH is one of the most common industrial fluorinated materials.

Under the environmental fate point of view, should be mentioned that FTOHs undergo atmospheric oxidation to produce FTCAs and perfluoro carboxylic acids (PFCAs) [5]. Evidence of FTCAs and PFCAs in precipitation, suggests that this is a likely transport vehicle for FTOH degradation products entering aquatic environments. Substantial evidence also exists for the biological degradation pathway of FTOHs to FTCAs and PFCAs [23, 24]. Hagen et al. [23] first observed 8:2 FTCAs as a biodegradation product of 8:2 FTOH in rat plasma. In general, the first step in biodegradation is aerobic oxidation of the starting FTOH to form the corresponding n:2 fluorotelomer aldehyde (FTAL), a short-lived, highly reactive species. The aldehyde is rapidly oxidized to form the corresponding FTCA. Several other studies have since examined those metabolic pathways in rats, mice and trout [23, 25, 26]. These studies have shown that FTCAs degrade through dehydrofluorination to produce the corresponding FTUCAs [27]. The FTCAs and FTUCAs may further degrade to form environmentally persistent PFCAs, such PFOA. May proposed degradation pathways were studied; however, it is clear that FTCAs and FTUCAs are key intermediates in the transformation of FTOHs to PFCAs [28]. Figure 1 shows a aerobic biotransformation for 8:2 FTOH.

Concerns surrounding persistent perfluorinated materials entering the environment led 3M to case its manufacture of perfluorooctane-based commercial products in 2002 [29]. This might have reduced direct environmental exposure to PFCAs from the manufacture and use of commercial products: however, it does not address the indirect exposure to PCFAs through FTOH degradation. For this reason, the attention is now turning to the precursor compounds such as FTCAs and FTUCAs.

Regarding their common applications, PFASs are employed as fire resistant additives and oil, stain, grease and water repellents. As components of products, they repel water and oil, reduce surface tension much lower than other surfactants, act like catalysts for oligomerisation and polymerisation, and function where other compounds would rapidly degrade [13]. They are used to provide non-stick surface on cookware and waterproof, breathable membranes for clothing, and in many industry segments including the aerospace, automotive, building/construction, chemical processing, electronics, semiconductors, and textile industries [30].

The most relevant application are described in the “Organofluorine Chemistry: Principals and Commercial Application” [31] and includes:

- Textile repellent finishes (GoreTex®).
- Fluorosurfactants providing a predictable wetting, levelling and surface tension reduction properties for use in floor finishes and coatings, sealers/caulks, specially cleaners and personal care products (Masurf®).
- Fluoroplastics as polytetrafluoroethylene (PTFE), perfluorinated copolymers, amorphous perfluoro-plastics, poly(chlorotrifluoroethylene), partially fluorinated plastics as polyvinylidene difluoride, (PVDF) and polyvinyl fluoride, increasing

their resistance and being used in laboratory materials, due also to their chemical and physical inert properties.

- Fluoroelastomers as copolymers of hexafluoropropylene and vinylidene fluoride, terpolymers of tetrafluoroethylene, vinylidene fluoride, hexafluoropropylene and the perfluoromethylvinylether. This synthetic rubber has wide chemical resistance and superior performance due to their high temperature application in different media.
- Flurorpolymer coating as tetrafluoroethylene polymers (Teflon) or PVDF used in non-adherent surfaces, such as frying pans or food packaging materials.
- During the 1980s and 1990s, PFASs were also used for biomedical applications such as in blood diseases treatments, cancer therapy or ophthalmology, among others.

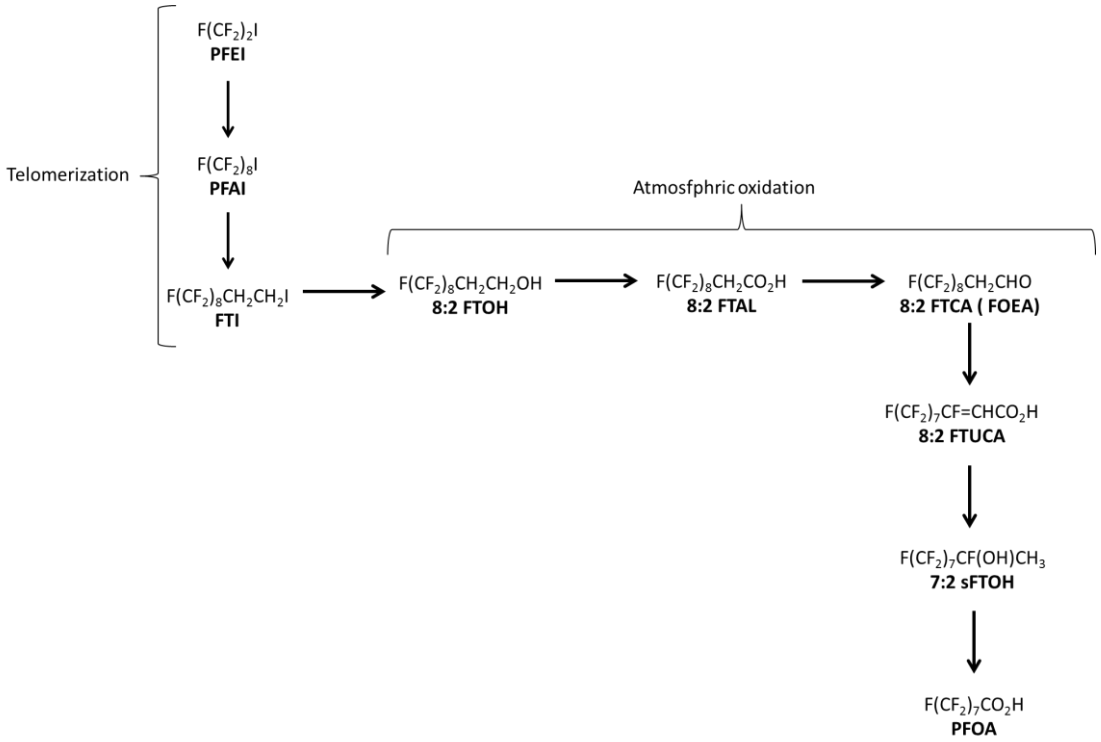


Figure 1: Telomerization and atmospheric oxidation. Adapted from Wang et al. (2009) and Buck et al. (2011).

Concerning the particular group of fluorotelomers have been manufactured since 1970s, with an estimated global production between 2000 and 2002 of 5000 – 6000 tonnes/year [32]. The main fluorotelomers applications are ski wax, medical applications, in particular semifluorinated n-alkanes and alkenes. n:2 fluorotelomer iodiines, oleofins, and alcohols, n:2 polyfluoroalkyl phosphonic acids, n:2 fluorotelomer sulphonic acids, acrylates and methacrylates are the major raw materials used for surfactants production and surface modification products [4].

1.2.3. Occurrence in the environment

PFASs are globally distributed due to their physicochemical characteristics and generally low degradation rates. The presence of these compounds in the environment must be considered in the public interest, since soils, sludge and water are involved in agriculture and cattle industry, and drinking water sources. In this way, PFASs can get to the diet through food and drinking water. Figure 2 shows the origin and source of PFASs in the environment.

Due to the extensive usage, PFASs come in urban and industrial wastewaters at levels of hundreds of ng/L in raw influents [33]. The high stability of these compounds drives only their partial removal in wastewater treatment plants (WWTPs), being redistributed between sewage sludge and effluents. In particular, this is the case of more recalcitrant compounds, such as PFOS and PFOA. While PFOS tends to be absorbed in the active sludge, the PFOA tend to solubilize in water. It has been studied the degradation of the more stable PFASs, longer chain compounds in the different steps of waste water treatments [34, 35]. It has been concluded that one of the main identified routes of PFASs into the environment is effluent of WWTPs. In a study carried out in Catalonia, the concentrations in surface waters of the river before and after of WWTPs discharge were studied. In this work, it was observed that the concentration of PFASs in surface water increased after WWTPs effluent discharges [36]. Once in the water cycle PFASs can reach different environmental compartments exposing wildlife and human health [36-38]. Also, the use of sewage sludge used as fertilizer in agriculture due to the high content of nutrients, can lead to contamination of crops or groundwater [39, 40].

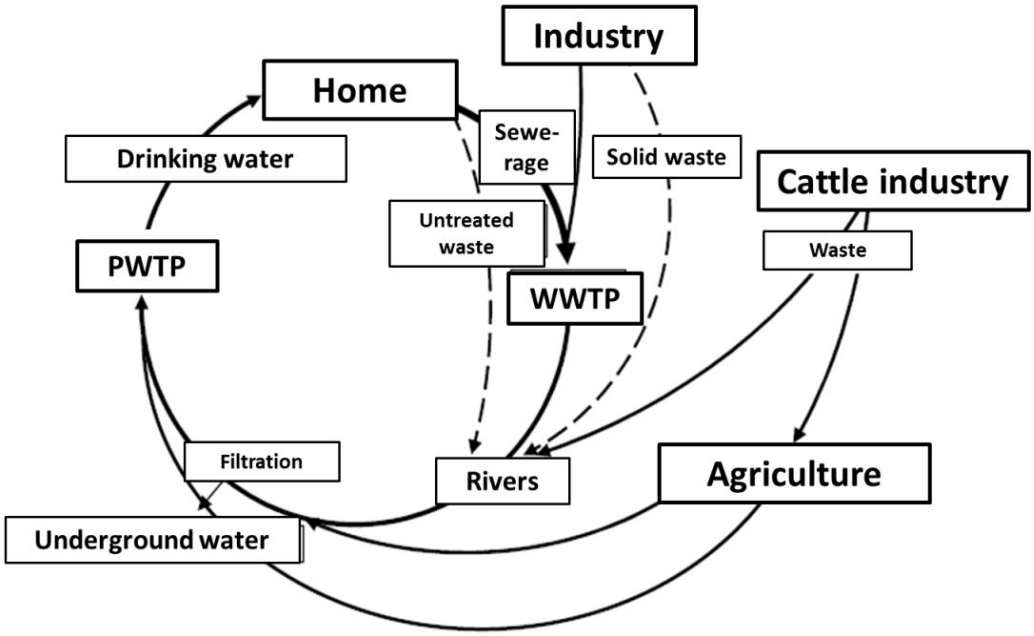


Figure 2: Origin and source of PFASs in the environment. Adapted from Damià Barceló (2011) WWTP: wastewater treatment plant; PWTP: potable water treatment plan.

During the last years, a great effort has been carried out to assess the occurrence of PFASs in the environment. However, in general, these studies were focussed on the investigation of a limited number of compounds as 8 carbon-chain compounds [5]. Besides, some congeners have been much less studied as FTCAs. In spite of only some few studies have carried out but the results have proved their presence in different environmental matrices and biota, such as atmospheric particles [41], indoor dust [42], precipitation [43-47], surface waters [14, 38, 41, 47-49] , sediments [14, 47, 49], WWTP effluent [48, 50], sewage sludge [51], landfill leachates [52], animal biota [53-58], human breast milk [59], and foodstuffs [60].

Regarding PFCAs, FTCAs are often observed at much lower concentration in environmental samples, if at all. This may be due to the relatively short time lifetimes of telomere acids in the environment. The FTUCAs are more likely to be observed in the environmental samples than FTCAs, but levels vary relative to PFCAs [28].

In addition, the telomere acids are of interest not only for their unknown distribution in the environment but also for their potential toxicity and reactivity. A study by Phillips et al. [61] assessed the acute toxicity of FTCAs and FTUCAs to various fresh water invertebrates. Overall, FTCAs and FTUCAs were found to be up to 10,000 times more toxic in aquatic invertebrates than PFCAs themselves. The study found Daphnia magna to be particularly sensitive to telomere acids with a chain length greater than eight [61].

Recently, the change in the industrial PFASs production is shown by the profile of compounds in environmental and biota samples reported in recent studies. In table 3, a summary of major PFASs reported in environmental and human samples are summarized.

Table 3: Occurrence of PFASs in the environment.

COMPAUND	COUNTRY	MATRIX	CONCENTRATIONS	REFERENCE
PFBA	Spain	Surface water	49.87 ng/L	Campo et al. (2016)
PFPEA			0.38 ng/L	
PFHXA			3.82 ng/L	
PFOA			4.36 ng/L	
PFDA			14.21 ng/L	
PFBA		River sediments	5.85 ng/L	
PFPEA			0.93 ng/L	
PFHXA			n.d.	
PFOA			2.47 ng/L	
PFDA			0.23 ng/L	
PFBA		Biota (river fish)	n.d.	
PFPEA			142 ng/g	
PFHXA			n.d.	
PFOA			n.d.	
PFDA			n.d.	
PFBA	USA	WWTP Effluent	16 ng/L	Houtz et al. (2016)
PFPEA			12 ng/L	
PFHXA			26 ng/L	
PFHPA			4.4 ng/L	
PFOA			21 ng/L	
PFNA			8.4 ng/L	
PFDA			3.5 ng/L	
PFBS			2.7 ng/L	
PFHXS			4.8 ng/L	
PFOS			13 ng/L	
PFBA	Spain	WWTP Influent / WWTP Effluent	20.5 ng/L / 13.4 ng/L	Campo et al. (2014)
PFHXA			$1.87 \cdot 10^3$ ng/L / 4.87 ng/L	
PFOA			19.0 ng/L / 16.4 ng/L	
PFDA			36.7 ng/L / 28.1 ng/L	
PFPA			14.3ng/L / 9.58 ng/L	
PFPEA			7.76 ng/L / 8.09 ng/L	
PFBS	Spain	Surface water river	214.3 ng/L	Lorenzo et al. (2016)
PFHXA			4.3 ng/L	
PFDA			1.0 ng/L	
PFBS		River Sediments	10.1 ng/g	
PFHXS			4.1 ng/g	
PFOS			42.6 ng/g	
PFOS		Biota (river fish)	29.7 ng/L	
PFBA	Greece	Sediments(beach)	0.02-0.04 ng/g	Llorca et al. (2014)
PFHXA			0.01 ng/g	
PFOA			0.11-0.15 ng/g	
PFOS			11.17 ng/g	
PFOA	Antarctica	Surface Soils	48 pg/g	Rankin et al. (2016)
PFOS			7 pg/g	

1.2.4. Legislation

Because of their bioaccumulation [62, 63], toxicity [28, 61, 64], and their possible contribution to cancer promotion, non-governmental organizations, national and international authorities have addressed the PFASs issue and legislative actions were proposed. One of the major manufacturers, 3M, started in 2000 the voluntary phasing out of the production of PFOS. In Europe, the hazard assessment of the Organisation for Economic Co-operation and Development (OECD) from the year 2002 identified PFOS as PBT-chemical (persistent, bioaccumulative and toxic).

In 2006, the EPA and eight major PFASs producers companies (Arkema, Asahi, BASF Corporation (successor to Ciba), Clariant, Daikin, 3M/Dyneon, DuPont, Solvay Solexid) in the industry launched the “PFOA Stewardship Program”. The companies committed to phasing out global facility emissions and product content of PFOA by 95% by 2010 and to work toward eliminating emissions and products content by 2015 [65]. During the same year, the OECD investigated production of PFSAs, PFCAs (see Table 1) and products or mixture containing PFASAS and PFCAs [66]. The results reported lower values than in previous studies carried out in 2003 with a decrease from 3000 to 175 tonnes of PFOS containing products (manufactured and/or imported). The values are in agreement with the phasing out of PFOS-based products by 3M Company and the use of related products, and certain products for which no substitutes are available [67].

Finally, PFOS and it salts were included as a POP under the Stockholm Convention for global regulation of production and use [68].

PFASs are now included in different research programs in EEUU, Canada and Europe. The EU-VII European Research Framework Program has funded projects to assess the distribution, toxicity and persistence of these compounds PFASs. On the other hand, currently, the production of short PFASs has increased, because of their use in the industry as a replacement of longer PFSA and PFCA products. An example of the used shorter PFASs is the PFBS-based products [66]

There is not yet legislation for FTs only some PFASs are regulated. In this section is summarized some legislation facts for PFASs.

2. OBJECTIVES

Under this frame the main objectives of Master Thesis is to study the current profile of PFASs including short chain and FTCAs in environmental samples: river water, sediments and biota in Catalonia, taken as a case study the area of the Ebro Delta.

Therefore, the specific objectives were:

1. An environmental study to assess 13 PFASs including: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFODA, PFBS, PFHxS, PFOS, PFDS, FOSA by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using the method previously developed in our group Llorca et al., (2012) [4] with minor revisions.

2. The different physic-chemical properties and environmental behaviour of FTCAs, makes difficult their analysis together with the other PFASs. Therefore, the second specific objective was to develop and validate a new analytical method for the detection of FTCAs in waters, sediments and biota (fish). Due to the characteristics of these sub-group of compounds gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) was considered as technique of choose.

3. Finally, to characterize the ecotoxicity of the water samples from Ebro Delta by standardized approaches.

3. ANALYTICAL METHODS

3.1. CHEMICALS AND REAGENTS

Perfluoroalkyl compounds standards were purchased by Wellington Laboratories Inc. (Canada) and were composed of: (i) a mixture of PFCs (PFAC-MXB, 2 µg/ml in methanol, purity > 98%) containing perfluoropentanoic (PFPeA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), perfluoroundecanoic (PFUdA) and perfluorododcanoic (PFDoA-) acids, and perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluorodecane sulfonate (PFDS); (ii) the perfluoroctanesulfonamide (PFOSA). Surrogate internal standards used for quantification normalization of the samples analyzed in LC-MS/MS were supplied by Wellington Laboratories Inc. (Canada), as well, and included (i) a mixture of labeled PFCs (MPFAC-MXA, 2 µg/ml in methanol, purity > 98%), composed of $^{18}O_2$-perfluorohexanesulfonate (MPFHxS-$^{18}O_2$), $^{13}C_2$-perfluorohexanoic acid (MPFHxA $^{13}C_2$), $^{13}C_4$-perlfuorooctanesulfonate (MPFOS-$^{13}C_4$), $^{13}C_4$-perfluorooctanoic acid (MPFOA-$^{13}C_4$), $^{13}C_5$-perfluorononanoic acid (MPFNA-$^{13}C_5$), $^{13}C_2$-perfluorodecanoic acid (MPFDA-$^{13}C_2$), $^{13}C_2$-perfluorododecanoic acid (MPFDoA-$^{13}C_2$) and (ii) $^{13}C_8$-perfluorooctanesulfonamide (M8FOSA, >99%). Fluorotelomer carboxylic acids solution mixture used to develop the GC-MS/MS method (FTA-MXA, 2 µg/ml in isopropanol, purity > 98%) was purchased by Wellington Laboratories Inc. (Canada) and was composed of 6:2 FTCA, 8:2 FTCA and 10:2 FTCA.

All solvents and reagents were analytical grade. HPLC water, toluene and hexane were obtained from J.T.Baker®Chemicals (Phillipsburg, USA), ammonium acetate (MW: 77.08, purity > 98%), and ammonium hydroxide (MW: 35.05, purity > 98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid fuming 37% (HCl) were obtained from Merck (Darmastadt, Germany) and boron trifluoride-methanol (BF_3-MeOH) was obtained from Sigma-Aldrich (Steinheim, Germany). Sodium chloride (NaCl) was obtained from Carlo Erba Reagents (Barcelona, Spain).

3.2. SAMPLES PRE-TREATMENT AND EXTRACTION PROCEDURES

In order to extract, clean up the extract, eliminate matrix interferences and enrich the sample a series of extraction and clean-up procedures were applied. Different strategies are commonly used, depending on the matrix and on the analysis (LC-MS/MS and GC-MS/MS). In Table 4, the general procedures applied for sample pre-treatment and extraction according to the matrix and posterior analysis type are summarized.

Table 4: General experimental procedure.

Matrices	**Water**	**Sediments**	**Biota (fish)**
Pre-treatment			
	Filtration with 0,7 µm glass microfiber filter	Dried in atmospheric Conditions Homogenization	Homogenization
Extraction			
	PFASs surrogate standards addition SPE / LLE	PFASs surrogate standards addition UAE Centrifugation SPE for GC-MS/MS analysis TFC for LC-MS/MS analysis	PFASs surrogate standards addition Alkaline extraction Centrifugation SPE for GC-MS/MS analysis TFC for LC-MS/MS analysis
Pre-concentration			
	Evaporated under N_2 Reconstitute with hexane for GC-MS/MS analysis Reconstituted with methanol for LC-MS/MS analysis Levelled PFASs internal standards addition for LC-MS/MS analysis	Evaporated under N_2 Reconstitute with hexane for GC-MS/MS analysis Reconstituted with methanol for LC-MS/MS analysis Levelled PFASs internal standards addition for LC-MS/MS analysis	Evaporated under N_2 Reconstitute with hexane for GC-MS/MS analysis Reconstituted with methanol for LC-MS/MS analysis Levelled PFASs internal standards addition for LC-MS/MS analysis
Instrumental analysis			
	LC-MS/MS GC-MS/MS	LC-MS/MS GC-MS/MS	LC-MS/MS GC-MS/MS

3.2.1. Water samples

Water samples were prepared using the procedure previously described by Llorca et al. (2012) [69] with minor modifications. Very briefly, water samples were filtered to remove the suspended particles using 0,7 µm Glass Microfiber filter (Whatman, United Kingdom). Then, the filtered samples (500 ml of sea water, 250 ml of river water and wastewater effluents and 150 ml of wastewater influents) were spiked with 10 µl of a mixture of PFASs surrogate standards at 100 ng/ml and were left to reach the equilibrium for 15 min. All the samples were processed in triplicates.

Extraction and clean-up processes were carried out by solid phase extraction (SPE) using Oasis WAX cartridges (30 cc, 60 mg, 30 µm; Waters Corporation, MA). The extraction procedure was consisting of: first conditioning the SPE cartridges with 4 ml methanol and 4 ml Milli-Q, under gravity conditions. Followed by the sample loading

and cartridges dry for 15 min, both operations under vacuum conditions. And, finally elution with 4 ml of 10% NH_4OH in methanol. The extracts were evaporated under a gentle N_2 stream up at 25 °C using a TurboVap® LV Concentration Evaporator Workstation (Biotage AB, Sweden) to a final approximately volume of 500 µl and split in two vials of 250µl each. Each vial was evaporated until dryness using Reacti-ThermTM III Heating module (Thermo Fisher Scientific, United States); for the analysis by LC-MS/MS samples were reconstituted in 250 µl of a mixture of water and methanol (9:1), while for the analysis by GC-MS/MS, samples were reconstituted in 250µl. Before the LC-MS/MS analysis, the samples were spiked with 10 µl labelled PFASs internal standards at 200 ng/ml. 500 mL of ultra-pure water was employed as procedural blank following the approached described before.

Liquid-liquid extraction (LLE) was also performed to compare the two types of extractions, SPE and LLE. Llorca et al. (2012) [69] already studied the different types of extractions and decided that the one with the best recuperation was SPE. LLE was carried out using two extraction solvents. First was extracted using dichloromethane and after using hexane. One control and a seawater sample were extracted by LLE. The volume extracted was 500 ml. The volume of extraction solvent was 150 ml of dichloromethane and 150 ml of hexane.

3.2.2. Sediments samples

First, sediment samples were dried under atmospheric conditions during one week inside a fume cupboard. Once dried sediments were processed using the procedure previously described by Sanchez-Vidal et al. (2015) [70] with minor modifications. Very briefly, 1 g of dried sediment was spiked with 20 µl of a mixture of subrogate internal standards (100ng/ml) and left to reach the equilibrium for 20 min. After this period, 10 ml of pure methanol was added, and the sediments were extracted by ultrasonic assisted extraction (UAE) using an ultrasonic cleaning bath (J.P Selecta S.A, Spain) for 1 hour. The extracts were then centrifuged using the Centrifuge 5810R (Eppendorf, Germany) for 20 min at 4000 rpm at 17 °C. After the centrifugation, the supernatant was divided in two tubes with 4 ml in each tube.

To carry out the LC-MS/MS analysis, 4 ml of the supernatant were dried with a gentle stream of N_2 and reconstituted in 100 µl of a mixture water: methanol (9:1). The extracts were then directly injected in the on-line clean-up system.

The on-line purification consisted in an on-line method based on turbulent flow chromatography (TFC). TFC was carried on in a Thermo Scientific Aria TLX-1 system coupled to the TurboFlow™ (Thermo Fisher Scientific, Franklin, MA) module. For the purification of PFASs two extraction columns connected in tandem were employed: Cyclone and C18 XL, 50x60 mm, 60µm particle size and 60Å pore size from Thermo Fisher Scientific. In this case TFC was coupled to the chromatographic separation step. The separation of target analytes was achieved using Hypersil GOLD PFP (50x3) analytical column, 3µm (Thermo Fisher Scientific). Hypersil GOLD PFP allows the separation of fluoroisomers. The samples were loaded with acidified water using a turbulent flow and then eluted from the columns with the same solvents for water samples. More details about the loading and eluting conditions are reported in Table 5.

After separation, the detection was carried out using a triple quadrupole analyser TSQ Quantiva^{TM} (Thermo Fisher Scientific) with an electrospray ionization (ESI) source operated in negative conditions.

Table 5: Loading and eluting LC pump conditions used for on-line LC-MS/MS analysis of sediment and fish samples.

	Loading pump						Eluting pump			
Time	**Flow**	**%**				**Step**	**Flow**	**Grad**	**%**	
(min:sec)	***(ml/min)***	**(A)**	**(B)**	**(C)**	**(D)**		***(ml/min)***		**(E)**	**(F)**
00:00	1.5	100	-	-	-	Loading sample	0.4	Step	90	10
00:33	0.2	-	-	100	-	Cleaning matrix effects	0.4	Ramp	90	10
00:50	0.2	70	-	-	30	Transfer step	0.2	Ramp	90	10
01:00	0.4	-	100	-	-	Cleaning column I	0.4	Ramp	20	80
02:50	0.4	-	-	-	100	Cleaning column II	0.4	Ramp	10	90
07:50	0.4	-	-	-	100	Loading loop step	0.4	Step	10	90
08:00	0.4	20	-	-	80	Cleaning column III	0.4	Step	90	10
09:00	0.4	100	-	-	-	Cleaning column III	0.4	Step	90	10
09:50	0.4	100	-	-	-	Cleaning column III	0.4	Step	90	10

Loading pump:	**Eluting pump:**
solvent A: water (pH 3.4, with formic acid) **solvent B**: acetone:isopropanol:acetonitrile (10:45:45) **solvent C**: water **solvent D**: methanol	**solvent E**: water (20 mM NH_4Ac) **solvent F**: methanol (20 M NH_4Ac)

On the other hand, for the analysis of FTCAs by GC-MS/MS, 4 ml of the supernatant were diluted with 46 ml of ultra-pure water and the clean–up was performed by SPE, as previously described for water samples. All the samples were processed in triplicates.

3.2.3. Fish samples

Fish muscles were prepared accordingly with the procedure described by Llorca et al. (2009) [8] with minor modifications. Briefly, 1 g of fish muscle was spiked with 20 µl of a mixture of subrogate internal standards (100 ng/ml) and was left to reach the equilibrium for 20 minutes. Then the sample was extracted by alkaline digestion, consisting on the following procedure: 1g of samples previously spiked with the subrogate internal standard was placed in a glass centrifuge tube with 10 ml of methanol (10 mM NaOH) and the mixture was digested 2 hours in an orbital shaker using Rotabit (J.P Selecta s.a, Spain). Then this mixture was centrifuged at 4000 rpm and 17 °C for 20 minutes using the Centrifuge 5810R (Eppendorf, Germany). After this process, supernatant was divided in two tubes with 4 ml in each tube and the supernatants were processed according to the procedure described before in the sediments samples procedure. All the samples were processed in triplicates.

3.3. ANALYSIS BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

During the last years the technique of choice for the analysis of PFASs has been liquid chromatography coupled to tandem mass spectrometry using an interface of electrospray ionization (LC-ESI-MS/MS). One of the main advantages of this technique is the low limit of detection (LODs) in the nanogram per gram to pico-gram per gram range that can be obtained for most of PFASs. LC-MS/MS performed using triple quadrupole mass spectrometer combined with selected reaction monitoring is one of the widely applied detection system as well as one of the best suited for most of the PFASs compounds [14, 71, 72]. However, in the particular case of FTs and semi-volatile PFASs, their physic-chemical properties makes more suitable analytical methods based on gas-chromatography coupled to mass spectrometry in tandem (GC-MS/MS) [49].

Water samples were analysed using the method described by Llorca et al., (2012)[69] with minor revisions. Briefly, the analytical method consisted in an off-line clean up by SPE according to the protocol above described followed by ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). The chromatographic separation was achieved using an Acquity UPLC® BEH C18 analytical column 2.1x50 mm, 1.7µm particle size (Waters Corporation) using the system ACQUITY UPLC H-CLASS (Waters Corporation). A pre-injection column PFC isolator was used (Waters Corporation). The chromatographic conditions are summarized in Table 5. The flow rate was 0.4 ml/min and the volume injected was 10µl. The detection was carried out using a triple quadrupole mass spectrometer Xevo TQ MS (Waters Corporation) equipped with electrospray ionization in negative mode. The detection was performed by a selected reaction-monitoring mode.

Table 6: Chromatographic conditions used for UPLC-MS/MS analysis of water samples

	Loading pump		
Time	**Flow**	**%**	
(min:sec)	***(ml/min)***	**(A)**	**(B)**
00:00	0.4	20	80
00:10	0.4	20	80
05:00	0.4	80	20
07:00	0.4	90	10
08:50	0.4	90	10
09:50	0.4	20	80
11:00	0.4	20	80

	Loading pump: **solvent A**: methanol (20 mM NH_4Ac) **solvent B**: water (20 mM NH_4Ac)

In the other hand, sediments and fish samples were analysed using the on-line purification method coupled to chromatography described in the section 3.2.2 based on TFC-UPLC [73].

After separation, the detection was carried out using a triple quadrupole analyser TSQ Quantiva™ (Thermo Fisher Scientific) with an electrospray ionization (ESI) source operated in negative conditions.

Acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte according to Commission Decision 2002/657/EC. Xcalibut v 1.4 software was used to control the instrument setup and data acquisition. The main m/z transitions and the experimental conditions of the optimized UPLC-QqQ-MS/MS and TFC-LC-ESI-MS/MS are summarized in Table 7 and Table 8.

Table 7: Experimental conditions of the optimized UPLC-QqQ-MS/MS and TFC-LC-ESI-MS/MS for the analysis of PFASs.

			UPLC-QqQ-MS/MS	**TFC-LC-ESI-MS/MS**
Compound	Precursor ion (m/z)	Product ion (m/z) 1^{st} / 2^{nd}	t_R (min)	t_R (min)
PFPeA	263	69 / 219	3.2	3.21
PFBS	299	80.5 / 99.5	3.49	3.44
PFHxA	313	169 / 269	4.2	3.64
PFHpA	363	169 / 319	4.81	3.83
PFHxS	399	80.5 / 99.7	4.87	3.84
PFOA	413	169 / 369	5.28	4.00
PFNA	463	169 / 219	5.67	4.16
PFOS	499	80 / 99	5.68	4.17
PFDA	513	119 / 469	6.00	4.37
PFDS	599	80 / 99.6	6.28	4.87
PFUnA	563	219 / 519	6.29	4.59
FOSA	498	79 / 119	6.1	4.72
PFDoA	613	269 / 569	6.6	4.79

Table 8: Experimental conditions of the optimized UPLC-QqQ-MS/MS and TFC-LC-ESI-MS/MS for the analysis of FTCAs.

					UPLC-QqQ-MS/MS			TFC-LC-ESI-MS/MS		
Compound	Precursor ion (m/z)	Molecular ion assigment	Prdu ct ion (m/z) 1st / 2nd	Product ion assigment	t_R (min)	Cone voltage (V)	Collision energy (eV)	t_R (min)	Cone voltag e (V)	Collision energy (eV)
FHEA	377	$[C_8H_2O_2F_{13}]^-$	293 / 63	$[C_7F_{11}]^-$ / $[CO_2F]^-$	5.24	10	30 / 10	3.45	32	19.5 /6.5
FOEA	477	$[C_{10}H_2O_2F_{17}]^-$	393 / 63	$[C_9F_{15}]^-$ / $[CO_2F]^-$	6.10	10	30 / 10	3.85	42	19.5 /6.5
FDEA	577	$[C_{12}H_2O_2F_{21}]^-$	493 / 63	$[C_{11}F_{19}]^-$ / $[CO_2F]^-$	6.75	10	30 / 10	4.43	47	19.5 /6.5

3.4. DEVELOPMENT OF AN ANAYTICAL METHOD BASED ON GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY FOR THE ANALYSIS OF FTCAs

Due to the small molecular weight of FTCAs and the results previously obtained by LC-MS/MS an analytical approach based on GC-MS/MS was the second objective this work. However, due to the polar character of these analytes conferred by the carboxyl group different options were initially considered:

- The use of inert GC columns, which in general are a good option for polar low molecular weight analytes without derivatization
- The use of inert GC columns together with a mild derivatization step
- The use a normal GC column and a stronger derivatization step

Use of inert GC column The selected chromatographic capillary column was an Agilent J&W HP-5ms Ultra Inert capillary column (Agilent Technologies). This column was specifically developed for the detection of active analytes, including acidic and basic compounds. With very low bleed characteristics, bonded and cross-linked HP-5ms Ultra Inert is solvent resistant and ideal for GC/MS. Chromatography was carried out in a TRACE™ Ultra gas chromatograph (ThermoFisher Scientific) coupled to a triple quadrupole mass spectrometer TSQ Quantum™ Access Max (ThermoFisher Scientific) using EI mode in positive conditions. Different chromatographic and detection conditions were tried but low analytes retention was always obtained. For these reason the second option was tried.

Use of inert GC column with mild conditions derivatization in order to increase the analytes retention a mild derivatization step was performed to increase the interaction between analytes and the column. In this case, the esterification of carboxylic acids to carboxylic methyl esters was employed, using methanol and HCl as acid catalyst according and to the reaction shown in Figure 3, according to the procedure described by K. Ichihara et al. (1961) [74].

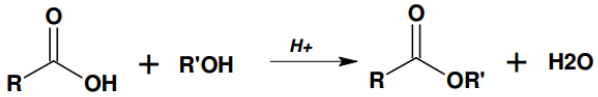


Figure 3: Esterification. R'= CH_3

Very briefly, 0.1 ml of the acid fraction (internal standard of FTCAs at 2 ppm), 0.3 ml of HCl-MeOH (37%) solution, 0.2 ml of toluene, and 1.4 ml of methanol were mixed in a screw-capped tube and shaken with a vortex mixer and then incubated at 45°C for 12 hours and 30 minutes. After the esterification reaction over heat, 1 ml of HPLC water, and 1 ml of hexane were added in the tube. Then the hexane was extracted from the water and it was analysed by GC-EI-MS/MS using the same equipment and properties mentioned before. However, the results were not as expected and low retention rates and low detectability was obtained.

Use of a non-inert TRACE TR-5M column and derivatization with BF_3 and methanol. In this case, a derivatization using BF_3-MeOH (14%) solution according to the procedure described by Metcalfe et al. (1961) [75] was employed. Very briefly, 50µl of the acid fraction (internal standards of FTCAs at 16 ppm) was evaporated under N_2 almost to dryness and 100µl of BF_3–MeOH were added. The vial was degassed with a gentle current of nitrogen and incubated at 70 °C for 1 hour. After this time, the mixture was transferred to a test tube and 100µl of HPLC water-NaCl solution (1%) was added. Then the mixture was extracted with 300 µl of hexane three times. Then the extract was combined and was evaporated and reconstituted until 50 µl. The esterification converts the FTCAs to a non-polar derivative, making it possible to analyze it in GC and having a good detection to the MS. The chromatographic separation was carried out using TRACE TR-5M column (ThermoFischer Scientific) by TRACE^{TM} Ultra gas chromatogram (ThermoFisher Scientific). In this case, the detectability of the selected compounds was possible and the chromatographic conditions were optimized. The better results were obtained with the injector operated in pulsed split-less mode (50psi); injecting 2µl at 250°C. Optimal chromatographic separations was obtained with the oven temperature set as follows: 40°C (2 min); 10°C/min to 130°C; 20°C/min to 280°C (0.5min). Helium was used as carrier gas at a constant flow mode (20ml/min).

For the optimization of the mass spectrometer operating parameters full-scan data acquisition was performed over the range m/z 35-600 at a scan rate of 0.25s/scan. After optimization, acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte according to Comssion Decision 2002/657/EC. In the SRM method, the dwell time was 0.025 seconds in order to obtain 15 points per peak. The mass spectrometer operating conditions were as follow: ion source was 70 eV and emission current 43µl. Xcalibut v 1.4 software was used to control the instrument setup and data acquisition. The main m/z transitions and the experimental conditions of the optimized GC-EI-MS/MS are summarized in Table 9.

Table 9: Experimental conditions of the optimized GC-EI-MS/MS.

Compound	Mass	Precursor ion (m/z)	Precursor ion assigment	Product ion (m/z) 1st / 2nd	Product ion assigment	t_R (min)	Cone voltage (V)	Collision energy (eV)
FHEA	378	361	$[C_{10}H_2OF_{17}]^+$	131 / 69	$[C_3F_5]^+$ / $[CF_3]^+$	4.97	10	23 / 23
FOEA	478	461	$[C_{10}H_2OF_{13}]^+$	131 / 69	$[C_3F_5]^+$ / $[CF_3]^+$	6.56	10	25 / 25
FDEA	578	561	$[C_{10}H_2OF_{21}]^+$	131 / 69	$[C_3F_5]^+$ / $[CF_3]^+$	8.12	10	30 / 30

3.5. QUALITY CONTROL AND QUALITY ASSURANCE

3.5.1. LC-MS/MS

Water samples were collected in polypropylene (PP) or glass bottles pre-cleaned with methanol and acetone and kept in a refrigerator at 4 °C until analysis. The sample containers and the storage procedure were chosen after discarding the possible contamination or adsorbance of selected compounds onto plastic or glass surfaces. To discard the possible cross-contamination from the containers procedural blanks were carried out, using ultrapure-water stored at room temperature during 2 weeks. After this period, the ultra-pure water was extracted using the same protocol as is used for the samples. Prior to the start of the sample enrichment procedure, the blanks and the water samples were allowed to reach room temperature into their initial containers, and then the bottles with the samples were ultra-sonicated for 3 min in order to resolve any possible adsorption onto the surfaces of containers. Also, procedural blanks were prepared in parallel to samples to discard any contamination during sample pretreatment. Sediment samples and biota were keep in foil and freeze until their extraction.

In order to rule out any contamination from the chromatographyc system, instrumental blanks of LC-MS/MS made of methanol:water (1:9) were run every three injections. In addition, standand solutions were analyzed before, during and after samples in order to check sensibility drifts.

3.5.1.1. Selectivity. For identification purposes, retention times of PFASs in the standards and in the samples were compared at a tolerance of ±2.5%. Moreover, in accordance with the 2002/657/EC Decision[76], the relative ion intensities (each product ion area signal versus the base product ion area signal) of the spiked samples were compared with the relative ion intensities of standard solutions, at the same concentration levels as used for the construction of the calibration curve.

3.5.1.2. Limits of detection (LOD) and quantification (LOQ), Recovery and Precision. The LOD was defined as the lowest concentration for which the peak area was at least three times larger than the background noise. Criteria for the LOQ were established as the lowest concentration fulfilling all of the following criteria: (1) bias

from the calibration curve less than 25%, (2) relative standard deviation of four replicates below 19%, (3) peak shapes acceptable, and (4) signal-to-noise ratio at least 10. The LOQs obtained served as the lower limits of the linear range.

According to the 2002/657/EC Decision, since no certified reference materials were available for the analytes and matrices of interest, the recovery from fortified negative samples was measured as an alternative to trueness. Briefly, negative samples of fish tissue (previously analyzed and found to be not contaminated) were spiked in quintuplicate with PFASs at three different levels (LOQ, 10.0, 100.0 µg/kg). Precision, expressed as repeatability, was calculated by repeated analyses on the same sample sets as used for recovery tests, with the only difference that independent samples were re-extracted and analyzed on two other occasions for calculating inter-day repeatability.

Method limits of detection (mLOD) and quantification (mLOQ), as well as recoveries, were experimentally calculated in spiked samples at different concentrations for seawater, river water, wastewaters, sediments and fish. In Table 10 and 11, the results of recoveries and the mLOD and mLOQ are summarized.

3.5.1.3. Matrix effect in fish, sediments and different types of waters The matrix effects were assessed by comparing the response of the analytes in 20 mM ammonium acetate methanol/water (10/90, v/v) solution to the response of the analytes spiked at the same concentration into a blank extracts.

Table 10: Recoveries for waters, sediments and fish samples by LC-MS/MS for PFASs analysis.

	waters			***sediments*** *(16 ng/l)*	***fishes*** *(16 ng/l)*
	sea *(13 ng/l)*	***freshwater*** *(8 ng/l)*	***WWTP*** *(4 ng/l)*		
PFPeA	-	-	-	87.32	47.59
PFBS	70.22	78.97	128.82	89.43	44.19
PFHxA	108.96	38.49	109.51	97.96	64.67
PFHpA	132.27	86.77	112.97	100.55	65.18
PFHxS	81.11	68.27	85.74	107.88	64.61
PFOA	74.26	61.88	102.61	103.31	68.29
PFNA	61.3	51.85	76.01	77.71	51.6
PFOS	83.19	56.37	91.17	98.92	88.18
PFDA	51.44	42.29	63.84	99.64	65.18
PFDS	38.45	34.27	46.25	140.78	148.04
PFUnA	53.88	42.42	90.01	86.51	63.89
FOSA	35.29	33.3	40.48	44.04	59.17
PFDoA	91.7	61.06	54.38	119.86	87.39

Table 11: Method limit of detection (mLOD) and quantification (mLOQ), expressed in ng/l, for waters, sediments and fish samples by LC-MS/MS for PFASs analysis.

	waters						**sediments**		**fishes**	
	sea	**river**	**WWTP**	**sea**	**river**	**WWTP**				
	mLOD *(ng/l)*			***mLOQ*** *(ng/l)*			***mLOD*** *(ng/l)*	***mLOQ*** *(ng/l)*	***mLOD*** *(ng/l)*	***mLOQ*** *(ng/l)*
PFPeA	-	-	-	-	-	-	0.54	1.80	0.91	3.03
PFBS	0.31	0.52	2.70	1.03	1.75	9.00	0.08	0.27	0.09	0.31
PFHxA	1.22	0.18	1.71	4.06	0.60	5.69	0.29	0.97	0.27	0.90
PFHpA	0.68	0.40	0.76	2.25	1.35	2.54	0.23	0.70	0.27	0.91
PFHxS	0.09	0.12	0.23	0.30	0.38	0.75	0.28	0.72	0.38	1.27
PFOA	0.08	0.06	0.11	0.26	0.21	0.37	0.23	0.77	0.29	0.97
PFNA	0.05	0.05	0.08	0.16	0.17	0.26	0.53	1.54	0.76	2.54
PFOS	0.04	0.07	0.08	0.14	0.25	0.27	0.80	1.02	0.64	1.13
PFDA	0.04	0.02	0.10	0.15	0.08	0.33	0.21	0.70	0.21	0.69
PFDS	0.06	0.04	0.30	0.19	0.14	0.99	2.66	8.87	2.69	8.97
PFUdA	0.03	0.02	0.15	0.11	0.08	0.51	0.27	0.91	0.24	0.82
FOSA	0.03	0.04	0.60	0.10	0.13	1.99	0.36	1.19	0.36	1.19
PFDoA	0.02	0.01	0.07	0.07	0.02	0.23	0.48	1.59	0.41	1.38

3.5.2. Quality control for GC-MS/MS

In Figure 4 to 7, the extract ion chromatograms for the 13 PFASs and the 3 selected PFTCAs, respectively, are presented.

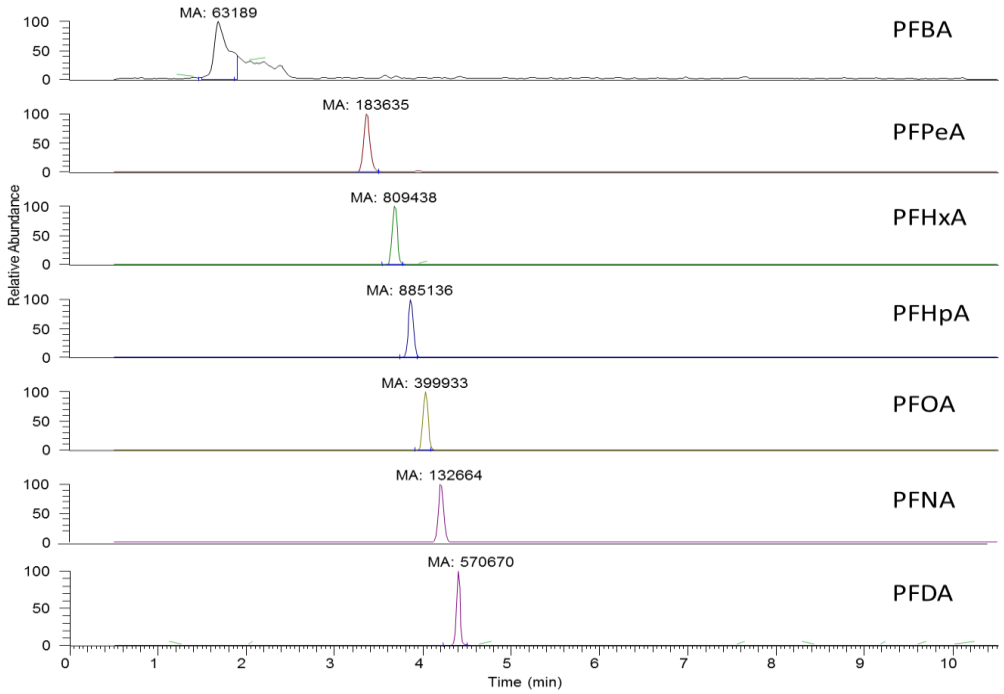


Figure 4: Extract ion chromatograms for on-line LC/MS/MS 5 ppb PFASs (quantificationtransition).

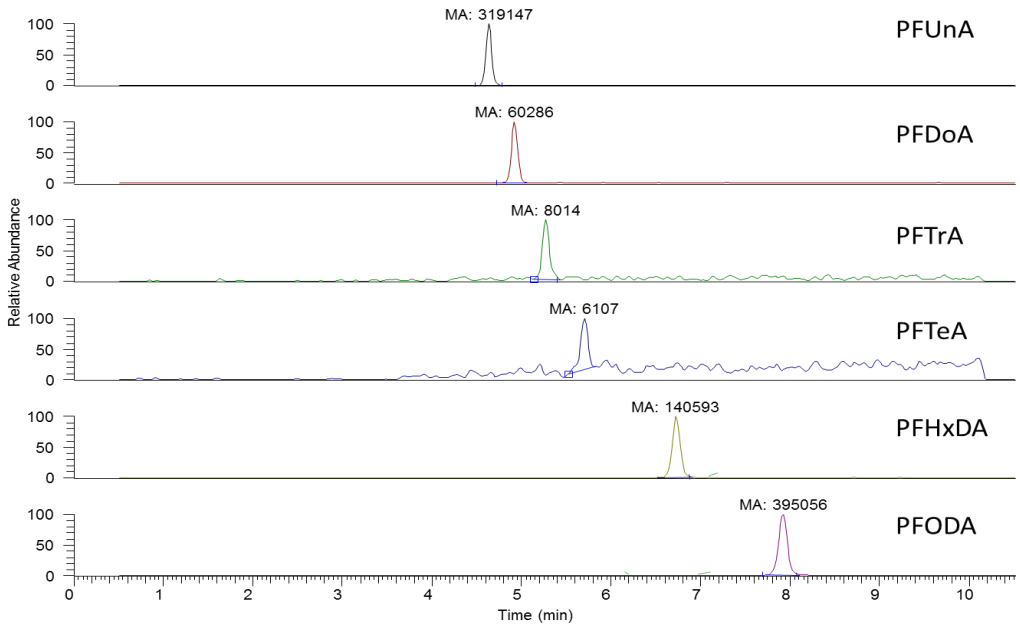


Figure 5: Extract ion chromatograms for on-line LC/MS/MS 5 ppb PFASs (quantification transition).

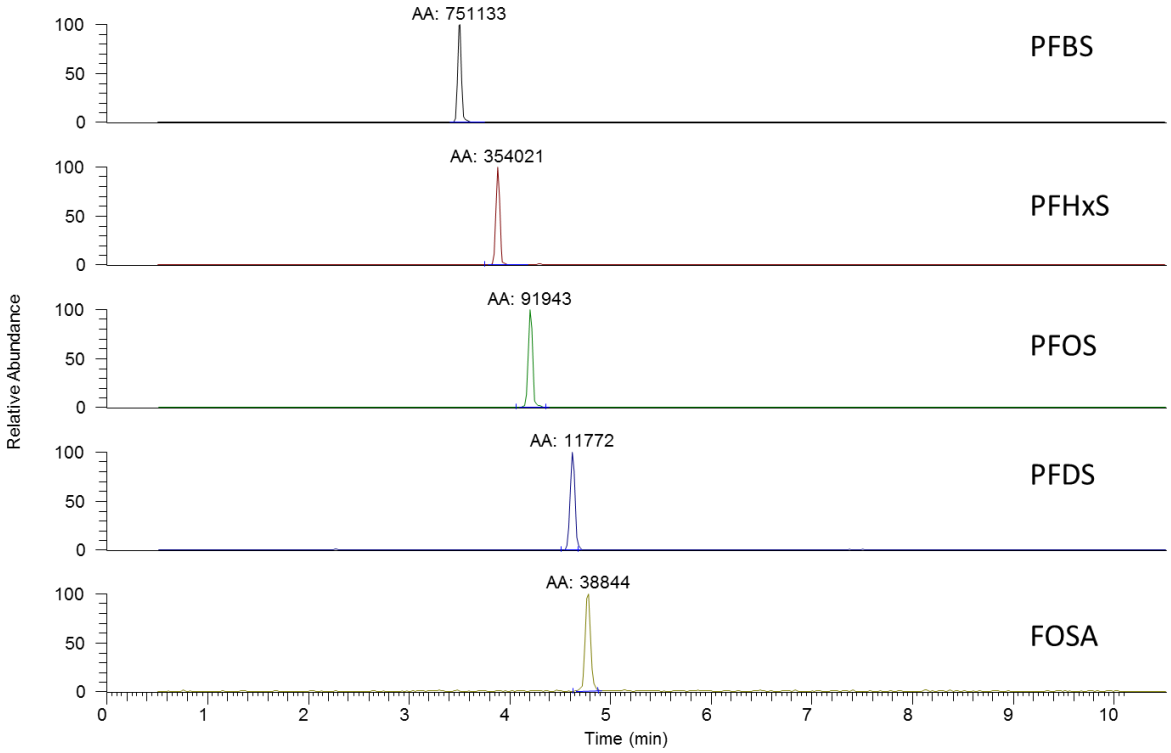


Figure 6: Extract ion chromatograms for on-line LC/MS/MS 5 ppb PFSs (quantification transition).

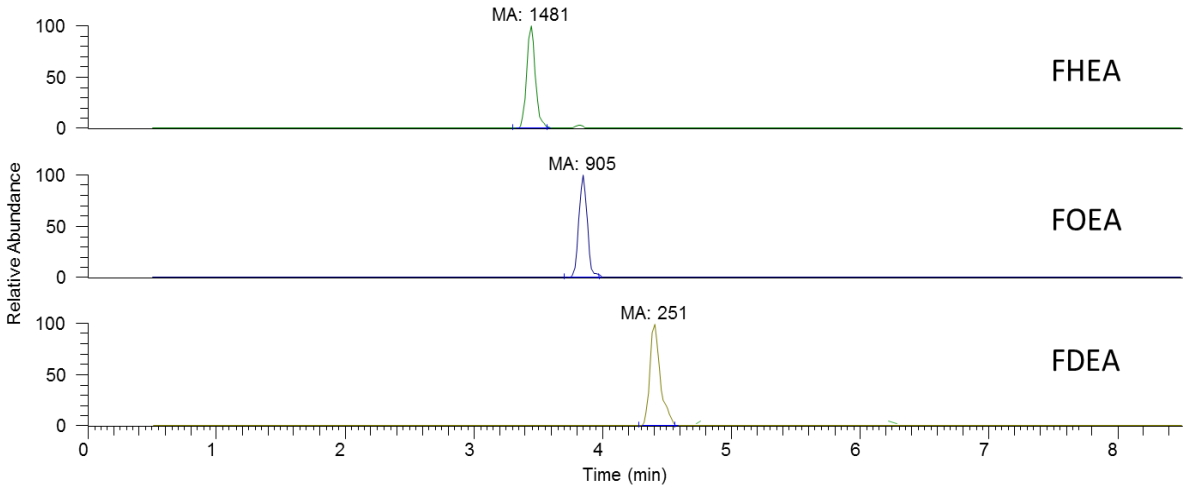


Figure 7: Extract ion chromatograms for on-line LC/MS/MS 5 ppb FTCAs (quantification transition).

To rule out any system contamination, instrumental blanks of GC-MS/MS made of hexane were run every three sample. Standard solutions were analyzed before, during and after samples in order to check sensibility drifts.

In addition, procedural blanks were prepared in parallel to samples to discard any contamination step during sample treatment. Procedural blanks and instrumental blanks were considered.

The instrumental LOD (ILOD) and LOQ (ILOQ), as well as method LOD (mLOD) and LOQ (mLOQ) and recoveries, were calculated by spiking experiments at 20 µl concentrations levels in the different types of studied matrices (seawater, river water, wastewater, river sediment and fish tissue). Results are summarized in Table 12 (recoveries), Table 13 (ILOD and ILOQ) and Table 14 (mLOD and mLOQ).

Table 12: Recoveries of FTCAs from waters, sediments and fish samples

	waters						
	sea *(ng/l)*	***freshwater*** *(ng/l)*	***Influent WWTP*** *(ng/l)*	***Influent WWTP*** *(ng/l)*	***LLE mIlliQ*** *(ng/l)*	***sediments*** *(ng/g)*	***fishes*** *(16 ng/g)*
FHEA	17.19	76.34	118.58	96.94	2.43	1.40	43.79
FOEA	12.70	88.47	64.14	128.52	4.92	10.30	83.89
FDEA	-	48.22	39.97	138.40	26.79	-	49.69

Table 13: Instrumental limits of detection and quantification of FTCAs by GC-MS/MS .

	river	WWTP	river	WWTP	*fish*	
	ILOD *(ng/l)*		***ILOQ*** *(ng/l)*		***ILOD*** *(ng/l)*	***ILOQ*** *(ng/l)*
FHEA	0.2	0.3	0.8	1.7	50	250
FOEA	0.2	0.3	0.8	1.7	50	250
FDEA	0.2	0.3	0.8	1.7	50	250

Table 14: Calculatetd experimentaly ILOQ and ILOD by LC-MS/MS for FTCAs.

	river	WWTP	river	WWTP	**fishes**	
	ILOD *(ng/l)*		***ILOQ*** *(ng/l)*		***ILOD*** *(ng/l)*	***ILOQ*** *(ng/l)*
FHEA	4	6.7	20	33.33	1000	5000
FOEA	4	6.7	40	66.67	1000	1000
FDEA	4	6.7	0.8	1.7	50	250

As can be seen in Table 9, recoveries for freshwater were ranging from 48 and 88 % and between 97 and 138% for wastewater influents. In the case of fish, the recoveries were between 44 and 84%. However, for seawater and sediments the recovery rates were not acceptable, being in both cases below the 20%. Therefore, the purification approach should be changed and improved in the case of seawater and sediments.

Finally, enough good mLOD and mLOQ were obtained for the selected FTCAs by GC-MS/MS in the case of freshwater, river water and wastewater and for biota. In addition, improved instrumental and method LOD and LOQ were obtained in compared with the method by LC-MS/MS for these compounds.

Figures 8 and 9 show the extracted ion chromatogram of the mixture of standards at 1 ng/ml analysed by LC-MS/MS and GC-MS/MS, respectively. As it can be seen, the response for FHEA and FOEA by means of GC are 10-times higher than by LC according to area responses (MA in the chromatograms). In the case of FDEA this compound is not detected by LC-MS/MS as it can be seen in Figure 8. Comparing the same extracted ion chromatograms for a standard mixture at 5 ng/ml (Figures 9 and 10), we can appreciate the same effect in detection intensity for FDEA (20-times higher by GC than by LC).

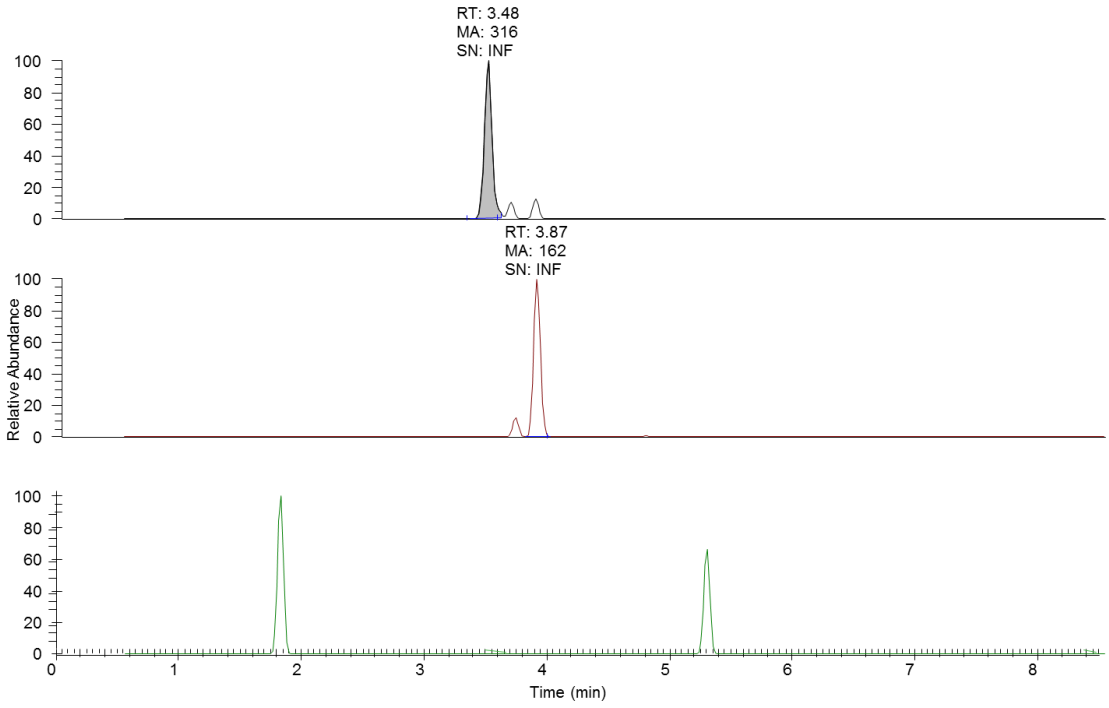


Figure 8: 1ppb FTCASs chromatogram by LC-MS/MS (quantification transitions).

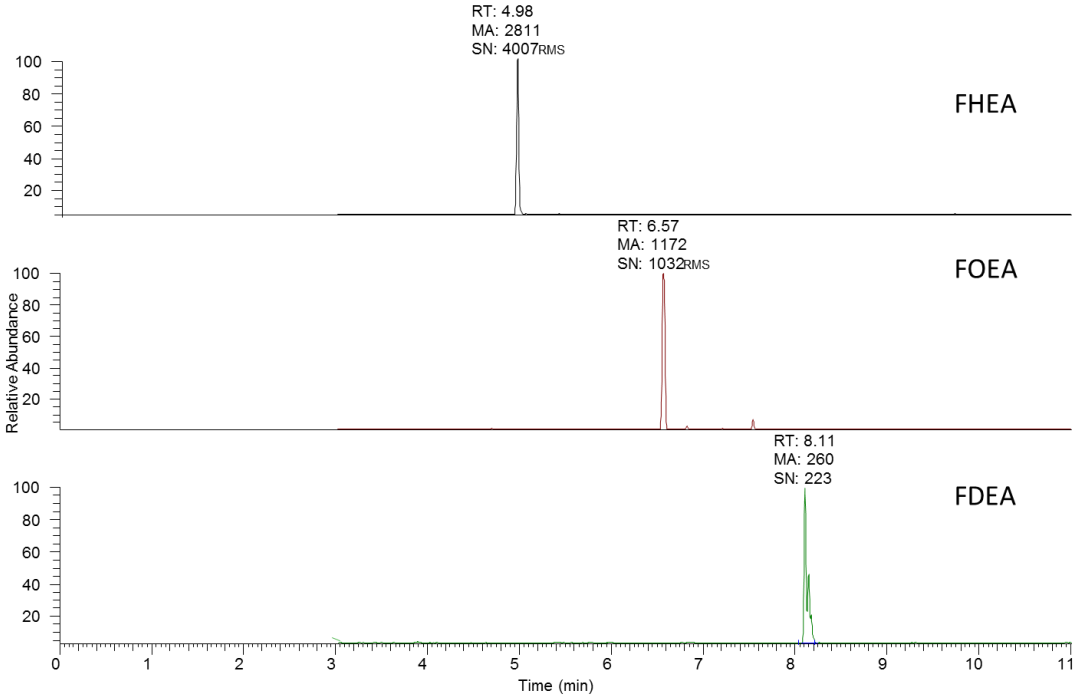


Figure 9: 1ppb FTCASs chromatogram by GC-MS/MS (quantification transitions).

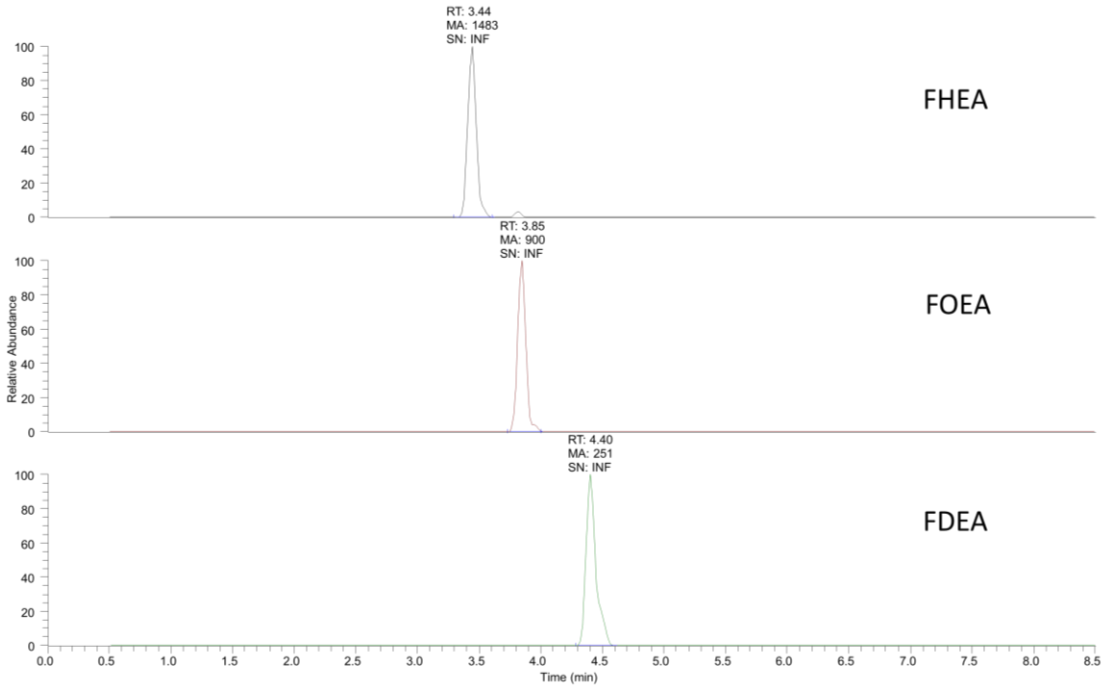


Figure 10: 5ppb FTCAs chromatogram by LC-MS/MS method (quantification transition).

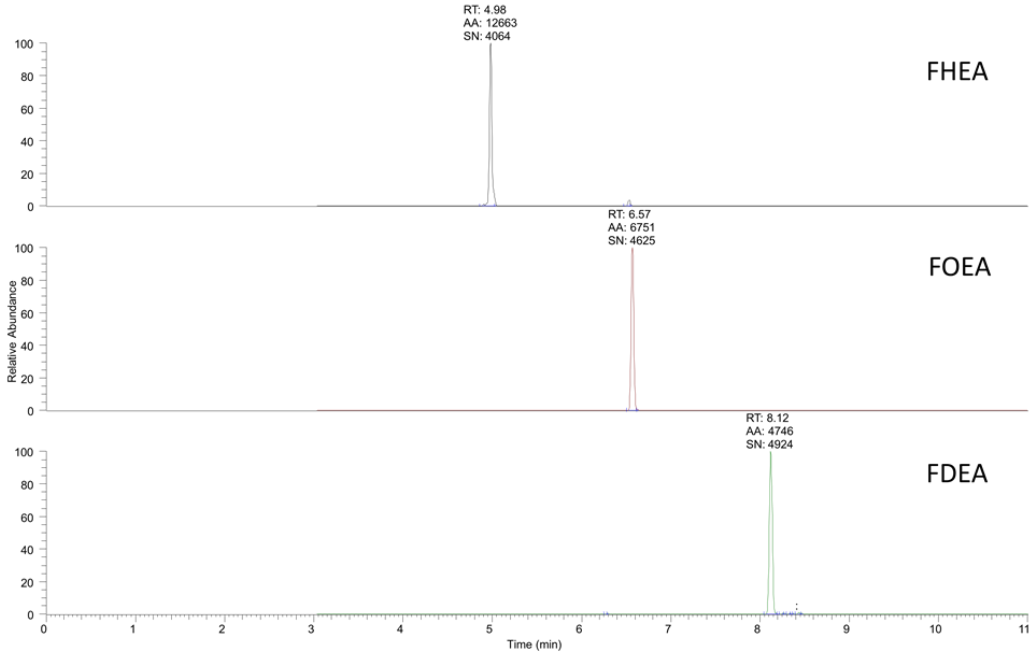


Figure 11: 5ppb FTCAs chromatogram by GC-MS/MS method (quantification transition).

4. PROFILE AND OCCURRENCE OF PERFLUOROALKYL SUBSTANCES IN THE DELTA EBRO CASE STUDY

4.1. AREA OF STUDY AND SAMPLING COLLECTION

The Spanish coastline constitutes a fragile resource of great environmental value with a direct impact on quality of life, human health, and economic development. At present, the environmental pressure of the emerging contaminants requires measures to ensure the sustainable development. However, a series of gaps of information about their occurrence, fate, and behaviour in the aquatic environment, and in particular in estuaries and coastal waters have been identified.

This Master Thesis is under the frame of the Integra-coast project. This project is an integrated study of the fate, behaviour, and the river transportation of emerging contaminants, nanomaterials (NMs) and microplastics (MPLs) in estuaries, wetlands and coastal waters.

The Integra-coast project is focused in Ebro Delta (Catalonia, NE Spain). The Ebro Delta has 320 km^2 of a surface, constitutes the largest wetland in Catalonia, the second most important in Spain (after Doñana National Park) and one of the most important estuarine zone in Europe [77, 78]. This area presents a rich diversity of habitats (river, sea, bays, beaches, dunes, estuaries, river forests and more) and a vast diversity of organisms [79]. Because of its organism diversity, as well for the geological, biological

and cultural aspects is protected as a Natural Park from 1983. Also declared Special Protection Area (SPA) in 1986, Site of Community Importance (SCI) and considered Wetland of International Importance by the RAMSAR convention since 1993. Moreover, in 2000, Ebro Delta was included in the Natura 2000 Network, a European network of nature protection with the aim to assure the long-term survival of the Europe's most valuable and threatened species and habitats [80]. Recently, the United National Educational, Scientific and Cultural Organization (UNESCO) declared the Ebro delta plain as a World Biosphere Reserve (2013) [81].

The river Ebro is the mightiest river of Iberian Peninsula, has a length of 910 km. The source of the river Ebro is in Fontibre, Cantabria, and its basin covers an area of 86,098 km^2 [82]. Figure 12 shows the geographic localization of Ebro Delta.

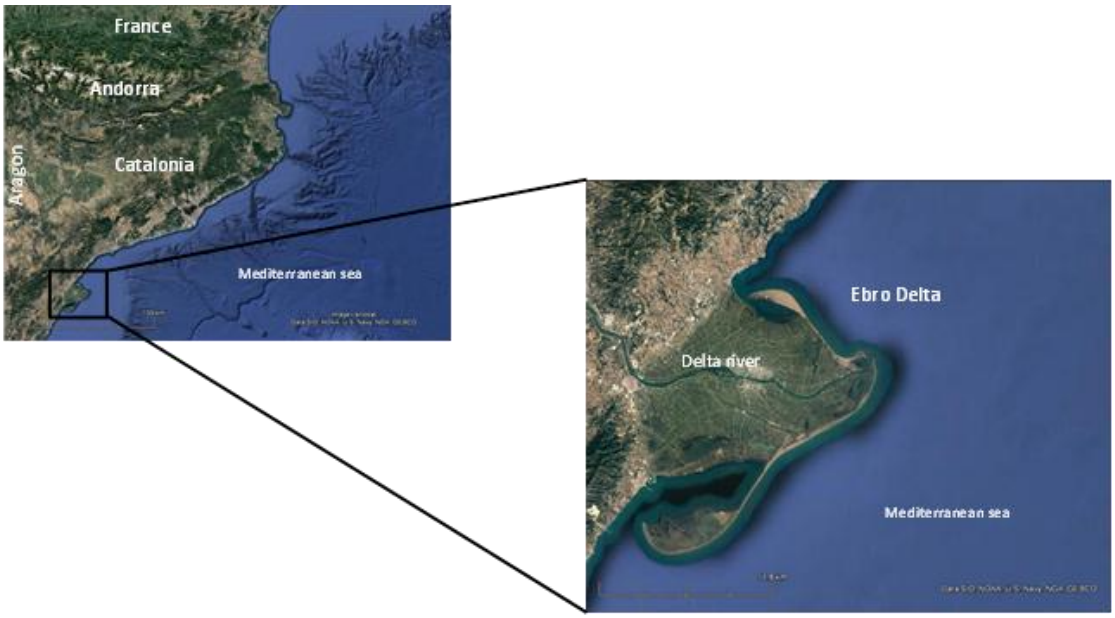


Figure 12: Geographic localization of Ebro Delta (Google Earth).

The topography of Ebro Delta is extremely flat, only 10 % of its surface is over two meters high, 30 % have a height between 1 and 2 m, and the rest, 60 % has an altitude of less than 1 m. The soils are not uniform, and while the river banks and coastal areas have sandy soils, most of the delta land consist of silt soil and also areas dominated by peat.

The Ebro Delta presents small thermal oscillations and high humidity. The winds that predominate are the N-W wind (dry wind) and E wind (wet wind). Rainfall is highly variable. Generally, there are two heavy rain seasons from September to November and April to June [82]. The average temperature detected in Amposta meteorological station in 2015 was 16.6 °C, with a minimum of 12.6 °C and a maximum of 34,9 °C [83].

The Ebro Delta is affected by various types of human pressures. The agricultural activity developed in this region (mainly rice cultivation) has caused important changes and impacts in its hydrological cycle. The geological and hydrological dynamics is altered because of control of river flooding, using upstream barrages and water canalization through the delta. Two main channels, one on each side of the river, bring

the water from Xerta, some 25 km upstream, to the rice cultivation system in the delta, from these two channels, water is carried to the rice fields by a network of irrigation channels [84].

The Ebro Delta present 21.000 ha of rice cultivation and receives upstream waters that flow close to different chemical industries [84-86]. Meanwhile, artisanal fishers were carried out with low surveillance. Moreover, social pressure resulting from the development and expansion of tourism increased in last decades. Thus the Ebro Delta receives human-generated pollutants by two main different ways. First, industrial, agriculture and urban wastes. Secondly, water streams from crops with large amounts of pesticides and other contaminants. All these contaminants may be distributed in water soil and sediments and accumulate in plants and animals [81, 84, 87].

Under this frame, the INTEGRA-COAST project aims to assess the pressure of emerging contamination in the Ebro Delta and their transfer to coastal areas. In the present Master Thesis PFASs were studied in this area as a part of this research project.

Three sampling campaigns were carried out during October-November 2015, February-April 2016 and June-July 2016 in the Delta Ebro. A total number of 175 samples including water, sediments and fish were collected. In Annex I, the information of the sampling points is shown.

Water and sediments were collected during the three campaigns, while fish samples were taken in the second campaign. In Figure 13, the sampling points are detailed and in Annex I (Table 1, Table 2 and Table 3) the features of every sampling point are summarized. Water samples include the influents and the effluent of 2 WWTPs: Sant Carles de la Ràpita and Amposta. 10 surface water samples (2 samples from the Ebro River, 1 sample from the emissary of Sant Carles de la Ràpita and 7 samples from open irrigation channels) were collected. In addition, seawater (2 samples from the beach, 6 samples from the open sea as well as 7 samples from 4 different sites at the Ebro River estuary (Illa de Buda, Llacuna de l'Encanyissada, Llacuna de la Tancada and Canal Vell) were sampled. Sediments were collected at the same sampling points as the water samples with the exception of WWTPs and the emissary of Sant Carles de la Rapita. Finally, 15 fishes were sampled during the second campaign from the Mediterranean Sea (n=8) and the river estuary (n=6). The species include *Mugil cephalus*, *Leuciscus cephalus*, *Cyprinus carpio*, *Anguila anguila*, *Torpedo torpedo*, *Sarpa salpa*, *Trachurus murphyi*, *Boops boops*, *Diplorus anularis* and *Micropterus salmoides*. Annex II shows some characteristics of the collected species.

Water samples were collected in amber polypropylene bottles (0.5 L). Before sampling, the bottles were rinse three times with the same sampling water.

The samples were shipped to Instituto de Diagnóstico Ambiental y Estudios del Agua-Conesjo Superior de Investigaciones Científicas (IDAEA-CSIC) laboratories (Barcelona) refrigerated at 4 °C and the processing was carried out right on their reception.

Sediment samples were collected using a core sampler for the sampling points that present more depth and a shovel for the sampling points that do not present depth. The

sediments were preserved in aluminium trays wrapped with aluminium foil and shipped to IDAEA-CSIC laboratories refrigerated at 4 °C. Samples were stored at -20 °C before analysis.

Fish samples were collected by electric fishing in only three different sampling points. The fish samples were preserved in a portable fridge and shipped to IDAEA-CSIC laboratories refrigerated at 4 °C. Every fish sample was processed in the laboratory right on their reception and the organs, the skin and the muscle was separated and stored with polypropylene falcons at -20 °C before the analysis.

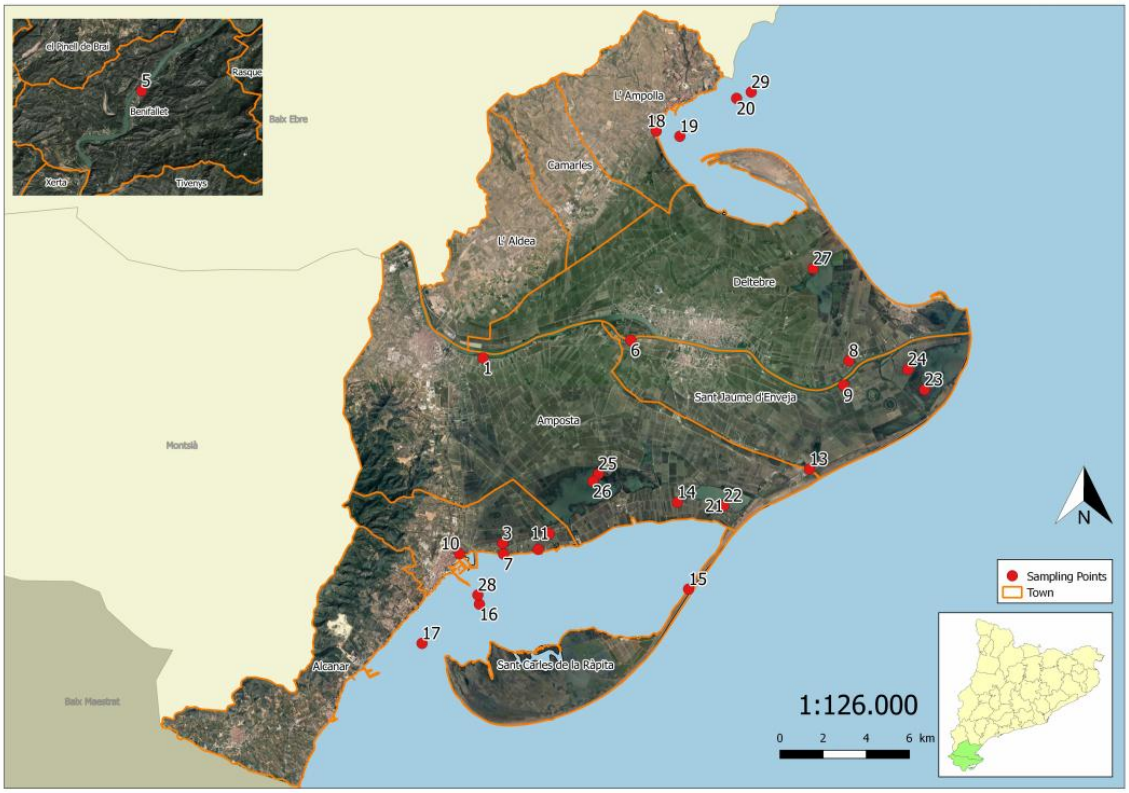


Figure 13: Sampling points in Ebro Delta.

4.2. RESULTS ANALYSIS OF PFASs BY LC-MS/MS

In this section, the results analysis of 13 PFASs by LC-MS/MS are presented and discussed for water, sediment and fish samples.

4.2.1 WATER SAMPLES

Among the 13 selected PFASs, only 6 were detected both in the first (autumn 2015) and the second (winter 2016) sampling campaign, being perfluoro carboxilic acids (PFCAs) the most abundant group. In Table 15, the PFASs levels in both sampling campaigns are provided and compared, along with summary statistics of the analyzed

PFASs. PFOA was the most frequently detected compound in both campaigns with frequencies of 66.7% and 41.7%, respectively. The second compound more frequently found in the samples was PFPeA, with a presence of a 29.6% in autumn and 16.7% in winter, followed by PFNA (22.2% in the first campaign and 20.8% in the second campaign). Among perfluorinated sulfonates (PFSs), in spite of their stop in production, PFOS was the most abundant, being present in the samples with a frequency of the 22.2% in autumn, 4.2% in winter. In addition, PFOS was the unique sulfonate detected, with the exception of the perfluorohexanesulfonate (PFHxS), found only in one sample during autumn period. The fact of the major detection of PFOS and the detection of PFHxS in the samples from autumn can be atributed to a major river turbulency due to the precipitations in this season and the sediment contaminants resuspension.

Perfluorinated sulfonamide PFOSA was not detected in any sample of the Ebro Delta. These results are in agreement with previous studies that also reported that PFOA and PFOS were the most common compounds in river waters among the PFAS class [69, 88, 89]. Moreover, it is worth to be noted that of the longer-chain PFCAs and PFSs, only PFUdA was detected, in two samples. The lower distribution in waters of long-chain PFASs compared to short-chain PFASs is not surprising, and it is mainly dependent on the lower solubility and the current lower production of these compounds in comparison to the shorter PFASs [90].

Table 15: Summary statistics of the selected PFASs in water samples of the first and the second sampling campaigns. Note that for PFPeA mean and standard deviation were calculated removing the two maxima values detected in both campaigns.

	Min (ng/l)		**Max** (ng/l)		**Mean** (ng/l)		**SD** (ng/l)		**n. detected**		**Frequency** (%)	
	1st	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
Perfluorinated carboxilic acids (PFCAs)												
PFPeA	<mLOQ	<mLOQ	2328.51	2774.50	7.67	6.72	2.18	3.98	8	4	29.6	16.7
PFHxA	<mLOQ	<mLOQ	2.47	4.67	-	-	-	-	1	1	3.7	4.2
PFHpA	<mLOQ	<mLOQ	3.27	5.27	2.43	4.29	1.19	1.39	2	2	7.4	8.3
PFOA	<mLOQ	<mLOQ	8.72	4.93	2.62	2.57	1.32	1.42	18	10	66.7	41.7
PFNA	<mLOQ	<mLOQ	3.28	2.98	1.82	1.98	0.80	0.69	6	5	22.2	20.8
PFDA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
PFUdA	n.d.	<mLOQ	n.d.	1.56	-	1.55	-	0.02	-	2	-	8.3
PFDoA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
Perfluorinated sulfonates (PFSs)												
PFBS	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
PFHxS	<mLOQ	n.d.	5.51	n.d.	5.51	-	-	-	1	-	3.7	-
PFOS	<mLOQ	<mLOQ	2.92	4.30	1.77	-	0.77	-	6	1	22.2	4.2
PFDS	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
Perfluorinated sulfonamides (PFSA)												
PFOSA	-	-	-	-	-	-	-	-	-	-	-	-

In general, selected PFASs were detected at low concentrations in the waters of the Ebro Delta (< 10 ng/l), exept for PFPeA, whose concentrations reached up to 2328.51 and 2774.50 ng/l in autumn and winter time, respectively. However, these two values can be considered a local anomalous situations or outliers and removing these two values, the mean concentrations of PFPeA are of the same order of magnitude of the other PFASs (7.67 ± 2.18 ng/l in autumn and 6.72 ± 3.98 in winter). In Figure 14, a bar diagram of the cumulated concentrations of PFASs for the two sampling campaigns is shown. The higher frequency of detection of PFOA above all perfluorinated compounds can be easily noticed; the higher concentrations were found in the influents of the Amposta WWTP (6.84 ng/l) and Sant Carles de la Ràpita WWTP (8.72 ng/l) of the first campaign. However, should be highlighted that the results of the second campaingn the Amposta WWTP influent did not showed PFOA contamination, while, those of Sant Carles de la Ràpita WWTP showed half of the concentration detected in autumn (4.94 ng/l). On the other hand, high concentrations of PFPeA were found in wastewater from Amposta and Sant Cales de la Rapita in both the sampling campaigns. These concentrations were of 1000 times higher than the concentrations found in the sorrounding channels, reflecting the influence of human activities on the occurrence of

this compound in the environment. Should be mentioned that PFPeA is one alternative compound to the longer-chain PFASs, and also is a byproduct of their degradation (Wang et al., 2013). Nevertheless, effluents collected after the WWTPs showed a total efficiency of the two plants in the removal of PFPeA from contaminated waters. On the other hand, the two WWTPs revealed to be ineffective in the removal of the other PFASs. E.g. the effluents of both Amposta and Sant Carles de la Ràpita showed PFOA contamination, because this compound is only partially degraded during wastewater processes (e.g. 6.84 ng/l in influents and 3.49 ng/l in effluents of Amposta WWTP; 8.72 ng/l in influents and 5.99 ng/l in effluents of Sant Carles WWTP detected in autumn 2015).

Also, in the effluent of the WWTP of Sant Carles de la Ràpita an enrichment in PFNA and PFHpA in both sampling campaigns was detected. As well, and a little enrichment in PFOS in the samples collected in autumn (1.29 ng/l) was also detected. Higher concentrations of PFASs in the effluents than in the influents have already been reported in previous studies. The increase of certain PFASs during wastewater treatments is the result of the incomplete degradation of their precursors (such as poly-fluoroalkyl phosphates and fluorotelomer alcohols) during water treatment processes with activated sludges [35, 91]. Detection of PFHpA only in the influents of the Sant Carles de la Ràpita WWTP can be considered as a further evidence of the partial degradation of PFOA in shorter-chain PFASs. It is worth to be noted that the control site “before Amposta” (sample n. 5, Figure 14), located in the Ebro river far from the estuary area and selected as the reference site, reported a slight contamination by PFASs. In particular, PFCAs were at concentrations below 10 ng/l. These low levels of contamination and the low levels of industrialization and urban pressures of this area suggest that the origin can be far from this area and these contaminants are transported via atmospheric transport and deposition. On the contrary, higher levels of concentration of PFASs were found in the final part of the Ebro Delta, as it was registered in the lagoons of La Tancada, L’Encanyissada and Illa de Buda (Figure 14), which collect all the waters from the surrounding irrigation channels and Ebro River. Comparing river water and seawater concentration patterns (Figure 14-15), it is remarkable that samples taken in autumn showed little higher concentrations than those from winter time. Although PFOA still remained the most common compound among all PFASs, its frequency of detection was lower in the second sampling campaign (52% in autumn, 35% in winter, excluding WWTPs data). This behavior could be due to the different weather conditions of the two periods: the first sampling campaign was carried out just after summer period, when the high temperatures that characterize this season can lead to evaporation of river and seawater, with the consequent enrichment of the non-volatile compounds in water. On the contrary, the lower temperatures and higher rainfall rates registered in the two months before the second sampling campaign brought to a pronounced dilution of concentrations of PFASs in waters.

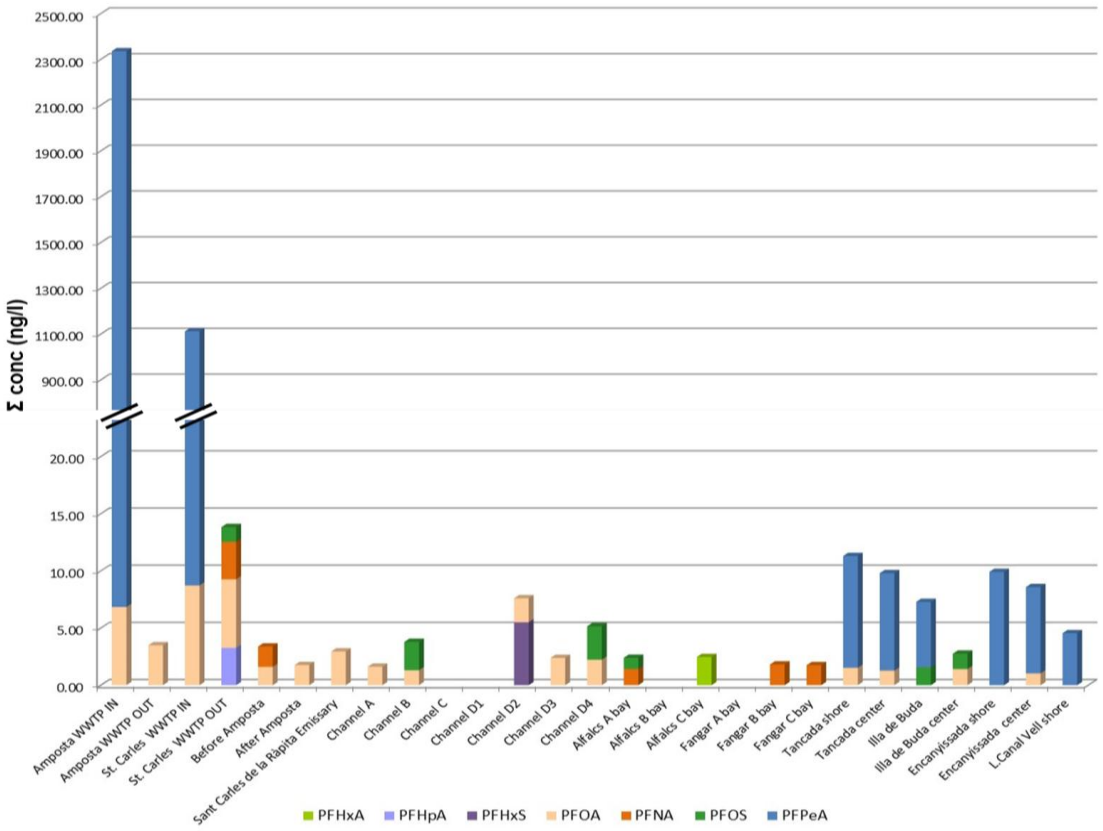


Figure 14: Graph bars reporting the total amount of PFASs (expressed in ng/l) in water samples during the autumn period.

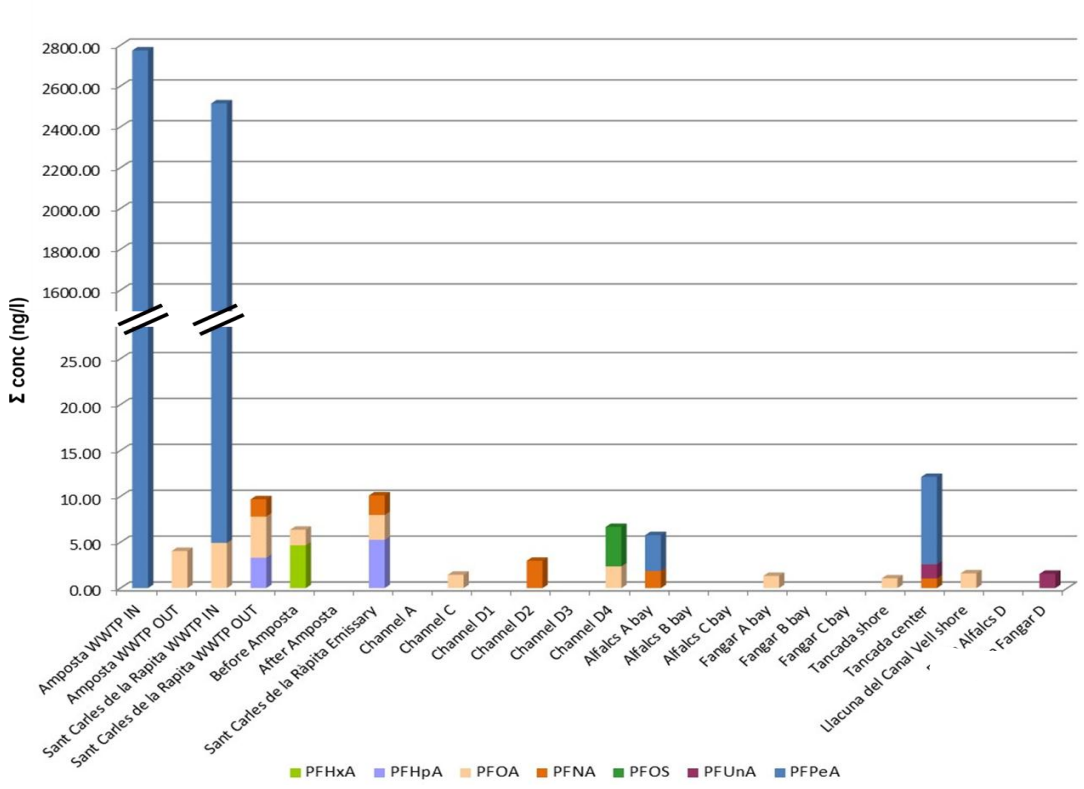


Figure 15: Graph bars reporting the total amount of PFASs (expressed in ng/l) in water samples during the winter period.

4.2.2. SEDIMENT SAMPLES

In Table 16, the concentrations of PFASs in sediments are summarized. The results from the first sampling campaign show that PFOA was the most common compound in sediments, following the same tendency as in waters. PFOA was present with a frequency of detection of a 95.5%, along with PFHxA and PFHpA, occurring in the 40.9% and 36.4% of samples, respectively. PFOS was the most predominant one among sulphonate during the first sampling campaign, and it was found to be the perfluoroalkyl compound with the highest concentration, with a maximum of 22.58 ng/l and mean value of 8.73 ± 8.42 ng/l registered in autumn. Samples collected during the second sampling campaign, on the contrary, showed a very different pattern of concentrations: the only PFASs detected were PFOA and PFOS, at lower concentrations compared to the first sampling campaign and lower frequencies (25% for PFOA, 15% for PFOS). Also, PFDoA was also registered in sediments of both campaigns. The sulfonamide PFOSA was never detected in neither campaign.

Table 16: Summary of PFASs in sediment samples.

	min. (ng/l)		**max** (ng/l)		**mean** (ng/l)		**st dev** (ng/l)		**number of detected**		**frequency** (%)	
	1st	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
Perfluorinated carboxilic acids (PFCAs)												
PFPeA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
PFHxA	<mLOQ	n.d.	3.68	n.d.	2.43	-	0.82	-	9	-	40.9	-
PFHpA	<mLOQ	n.d.	2.06	n.d.	1.24	-	0.58	-	8	-	36.4	-
PFOA	<mLOQ	<mLOQ	12.04	4.60	6.26	2.65	2.68	1.30	21	5	95.5	25.0
PFNA	<mLOQ	n.d.	4.17	n.d.	2.34	-	1.07	-	5	-	22.7	-
PFDA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
PFUdA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
PFDoA	<mLOQ	<mLOQ	3.96	1.72	-	1.69	-	0.03	1	3	4.6	15.0
Perfluorinated sulfonates (PFSs)												
PFBS	<mLOQ	n.d.	4.79	n.d.	2.67	-	1.86	-	3	-	13.6	-
PFHxS	<mLOQ	n.d.	1.49	n.d.	0.97	-	0.45	-	3	-	13.6	-
PFOS	<mLOQ	<mLOQ	22.58	2.51	8.73	1.78	8.42	0.81	6	3	27.3	15.0
PFDS	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-
Perfluorinated sulfonamides (PFSA)												
FOSA	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-

In Figure 16 and 17, the distribution of PFASs concentrations in the sediment samples of first campaign and second campaign are reported, respectively. The two graphs denote an evident difference between the two sampling periods. In autumn, at least one compound was detected in each sample, in addition PFOA was the most frequent compound and PFOS the most abundant one. The most contaminated sites were the two lagoons Illa de Buda and L'Encanyissada, where PFOA and PFOS were accompanied PFHxA, PFHpA, PFHxS, PFBS. Data regarding winter confirm a general depletion of concentrations in sediments, in accordance with their behavior in waters. Figure 18 shows an examples of the extracted ion chromatogram from Illa de Buda first campaign sample.

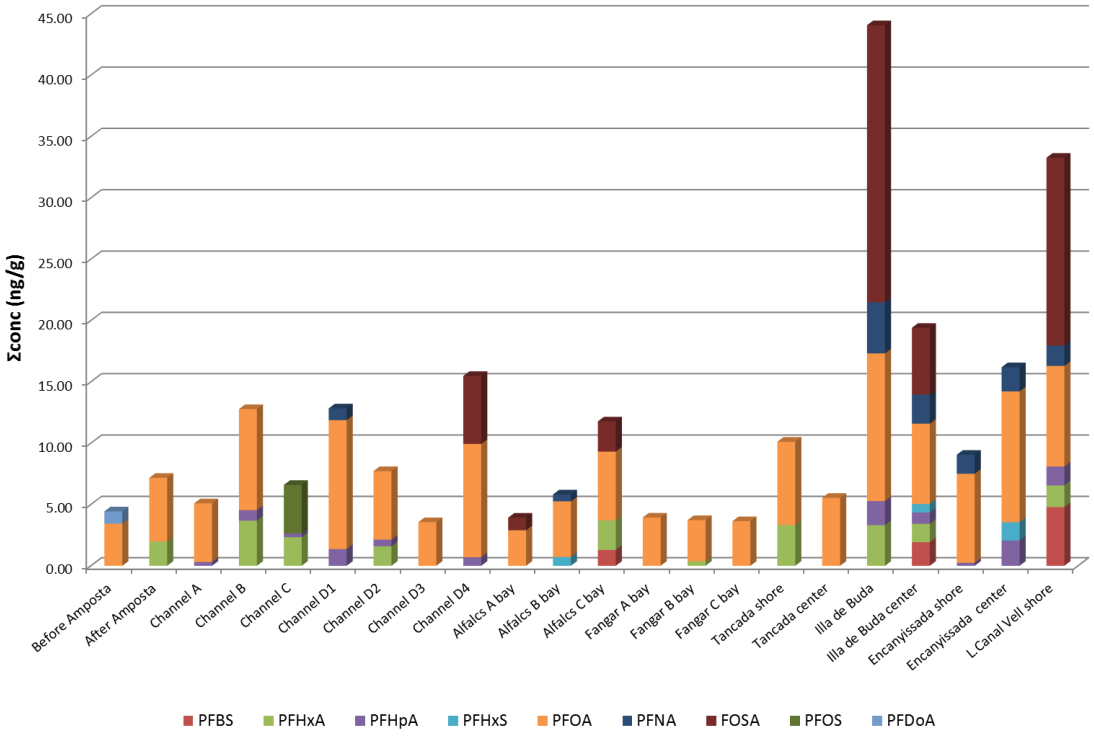


Figure 16: Graph bars reporting the total amount of PFASs (expressed in ng/g dw) in sediment samples collected during the autumn period.

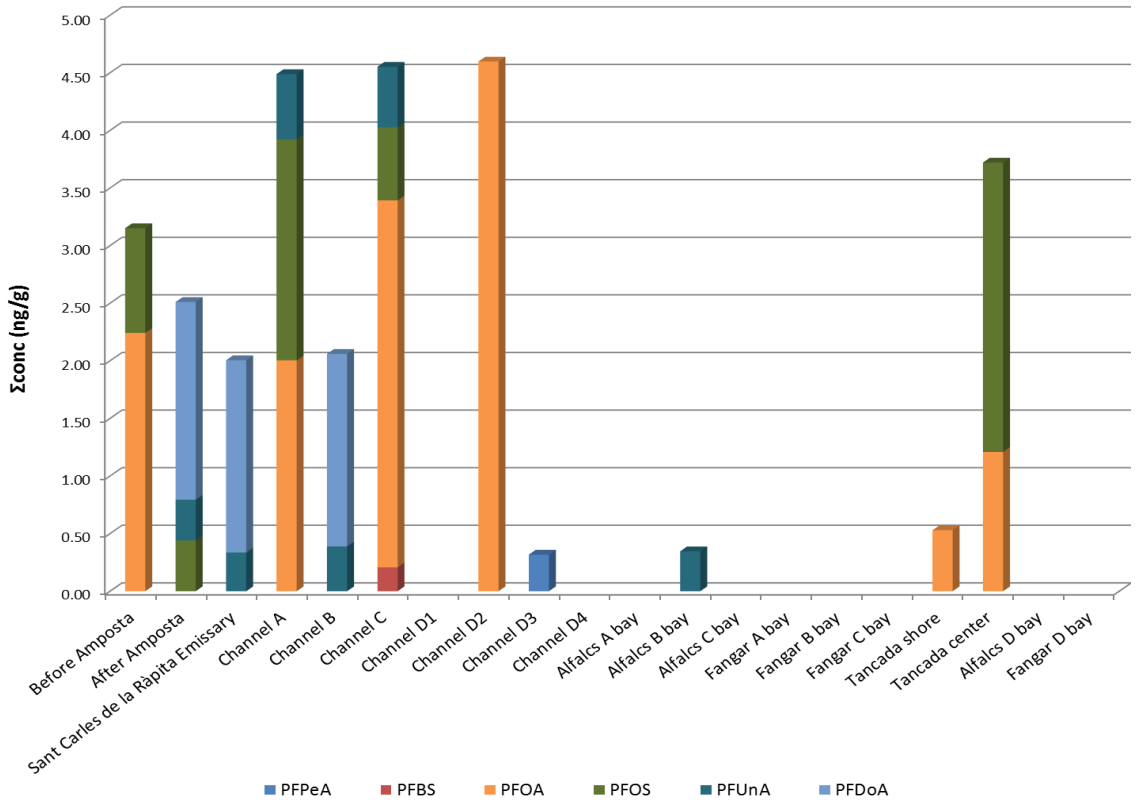


Figure 17: Graph bars reporting the total amount of PFASs (expressed in ng/g dw) in sediment samples collected during the autumn period.

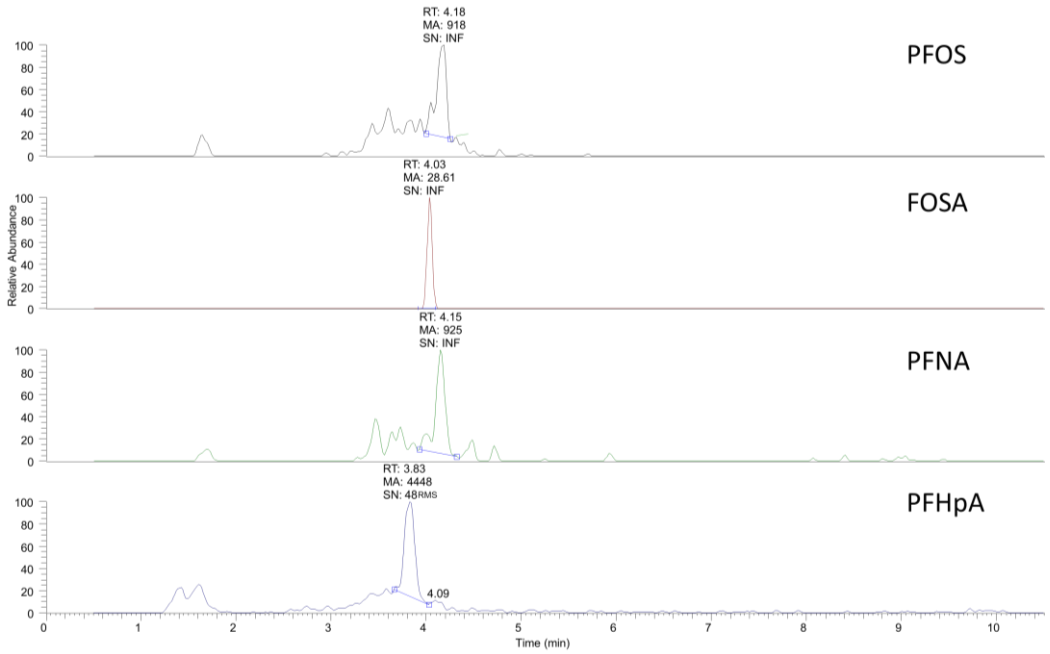


Figure 18: Extract chromatogram from sediment sample 23 (Illa de Buda Estuary) in LC-MS/MS (quantification transitions).

4.2.3. FISH SAMPLES

Figure 19 shows the presence of the detected PFASs in the sampled species in Fangar bay (Figure 19 a,b), Alfacs bay (Figure 19c,d) and Illa de Buda lagoon (Figure 19e,f) and compares data detected in skin (Figure 19, first column) and muscles (Figure 19, second column) of the different fish species.

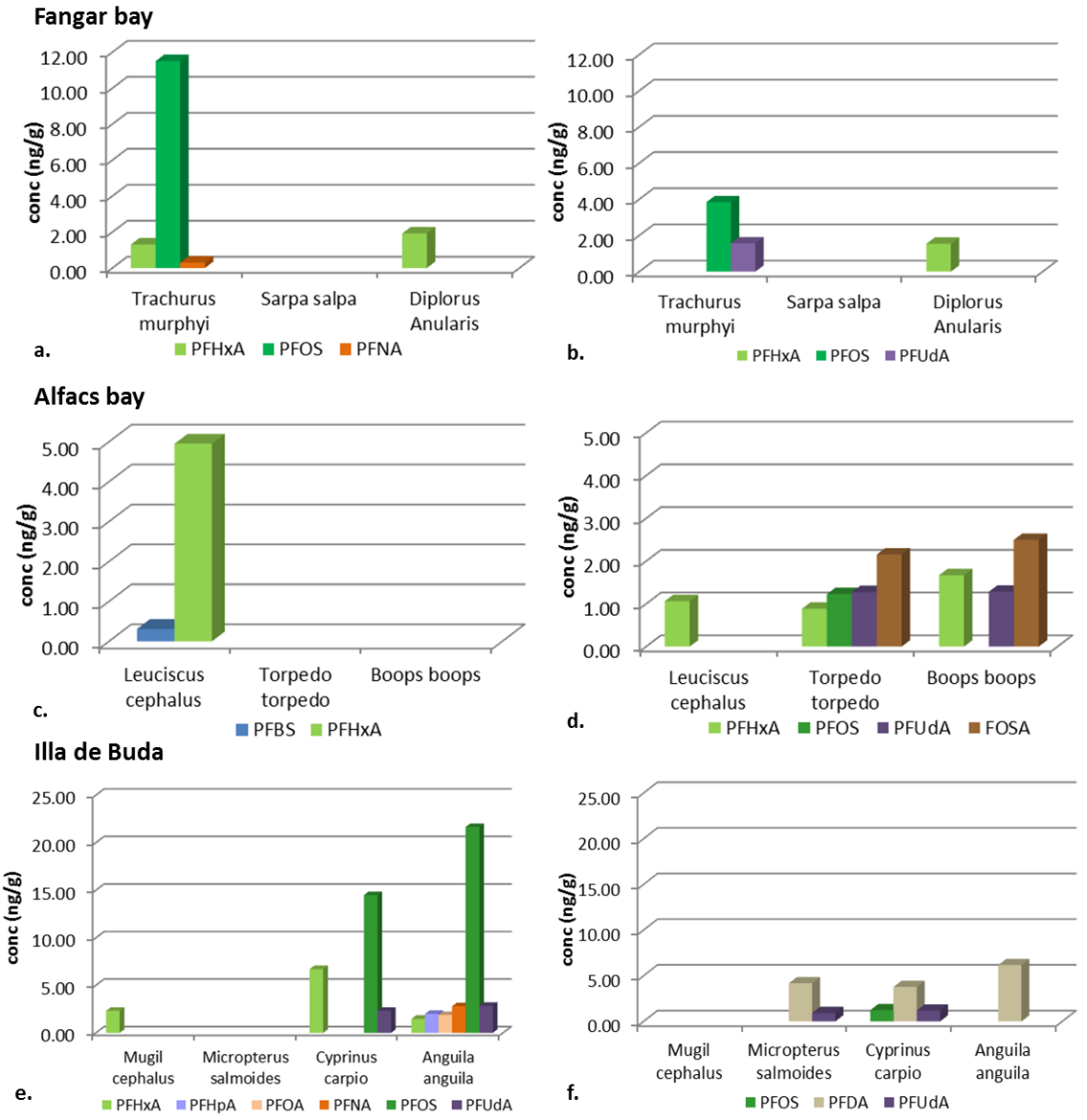


Figure 19: PFASs occurrence on skin (graphs on the first column) and muscle (graphs on the second column), expressed as ng/g ww, of the selected fish species collected in Fangar bay (a,b), Alfacs bay (c,d) and Illa de Buda lagoon (e,f).

As can be seen in Figure 19, PFASs tend to be sorbed in fish skin rather than in muscles. Of all the analyzed fishes, only the species *Sarpa salpa* (commonly known as salema) does not show any kind of contamination by PFASs neither in skin nor in the muscle, whereas all the other species are affected by the presence of at least one of the compound. Should be mentioned that salema is only feeding on green algae. PFOS was the most detected compound, found in 50% of the studied species, at the

highest concentrations (11.50 ng/g ww in the skin of *Tracurus murphii* of Fangar bay; 14.48 ng/g ww in *Cyprinus caprio* and 21.64 ng/g ww in *Anguilla anguilla* of the Illa de Buda lagoon). These results are in agreement with the high potency of bioaccumulation of PFOS [92-94].

Interestingly fishes collected from the open sea (Fangar and Alfacs bays) show an accumulation of perfluorinated compounds not found in waters or sediments (see Figure 15 and 17 for comparison). This fact can be influenced by contamination from marinas in these bays. In addition, some of the fish species from the bays are predators that can be influenced by biomagnification. Of the two investigated bays, fishes taken at Alfacs bay showed higher concentrations of PFASs, with a higher frequency of detection in muscles than in skin. The fish species *Torpedo torpedo* (electric ray) and *Boops boops* (bogue) were found to be very similar in their PFAS accumulation behavior (Figure 19c,d).

The results from the biota samples collected at Illa de Buda presented the highest levels of PFASs. These results were in agreement with the results found in water and sediments from this site (see Figure 14-17). Among the selected species, *Mugil cephalus* (flathead grey mullet) and *Micropterus salmoides* (largemouth bass) showed lower concentrations compared to the other two species, *Cyprinus carpio* (the common carp) and *Anguilla Anguilla* (eel), which were characterized by very high concentrations of PFOS in their skin, as previously assessed, along with PFUdA and shorter-chain PFCAs (PFNA, PFOA, PFHpA, PFHxA), even if at lower concentrations (< 5 ng/g ww). Eel is a bottom dweller species, while carp is an omnivorous fish which preferentially scavenges the bottom looking for insects, crustaceans and benthic worms; due to their habits, they are thus in very close contact with contaminated sediments such as those that characterize Illa de Buda lagoon, and this could be the explanation of such high concentrations found in their skin rather than in muscles.

4.3. RESULTS ANALYSIS OF PFTCAs BY GC-MS/MS

In order to prove the good performance of the developed approach for the analysis of PFCAs, 12 representative samples from the first campaign were analyzed by the new GC-MS/MS method for FTCAs and by LC-MS/MS method (same method used for PFASs analysis): 10 water and 2 fish samples.

Regarding fish samples, only FOEA was detected in a *Leuciscus cephalus* from Alfacs C bay by GC-MS/MS methodology (1.97 pg/g). In contrast, the analysis of these samples by the normal method (LC-MS/MS) didn't allow the detection of this contaminant.

5 over 10 water samples were detected with positive concentrations of FTCAs. FHEA was detected in 4 water samples, FOEA in 3 while FDEA was detected in 5 water samples. The results for water samples are shown in Figure 20 and examples of the extracted ion chromatogram Figures 21 and 22. As it was expected, the most contaminated samples were from the influent and effluent of the WWTP as well as the samples from Tancada center estuary. The higher concentrations were detected in WWTP influent from Amposta (7.40 pg/l of FHEA, 9.08 pg/l of FOEA an 18.25 pg/l of

FDEA). As the results of PFASs analyzed by LC-MS/MS, we can see that the WWTP effluent present lower concentrations of the target compounds. Finally, the comparison of the results from the same samples analysed by GC and LC-MS/MS denoted that the concentrations of the three FTCAs by LC-MS/MS were always lower or even not detected (Figure 11).

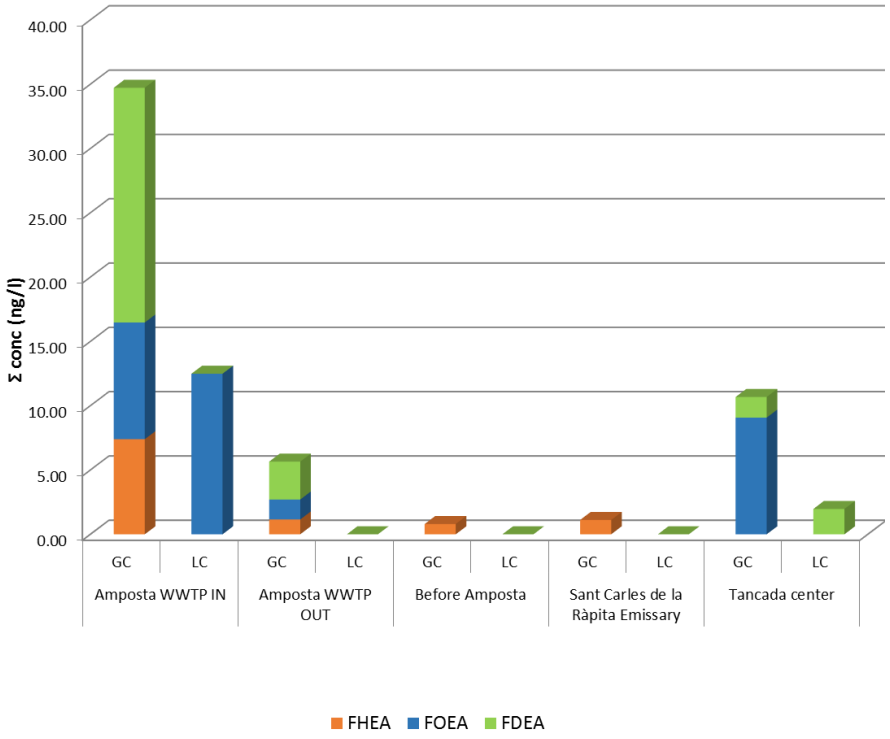


Figure 20: FTCAs occurrence in water samples (first campaign) analysed by GC-MS/MS and LC-MS/MS.

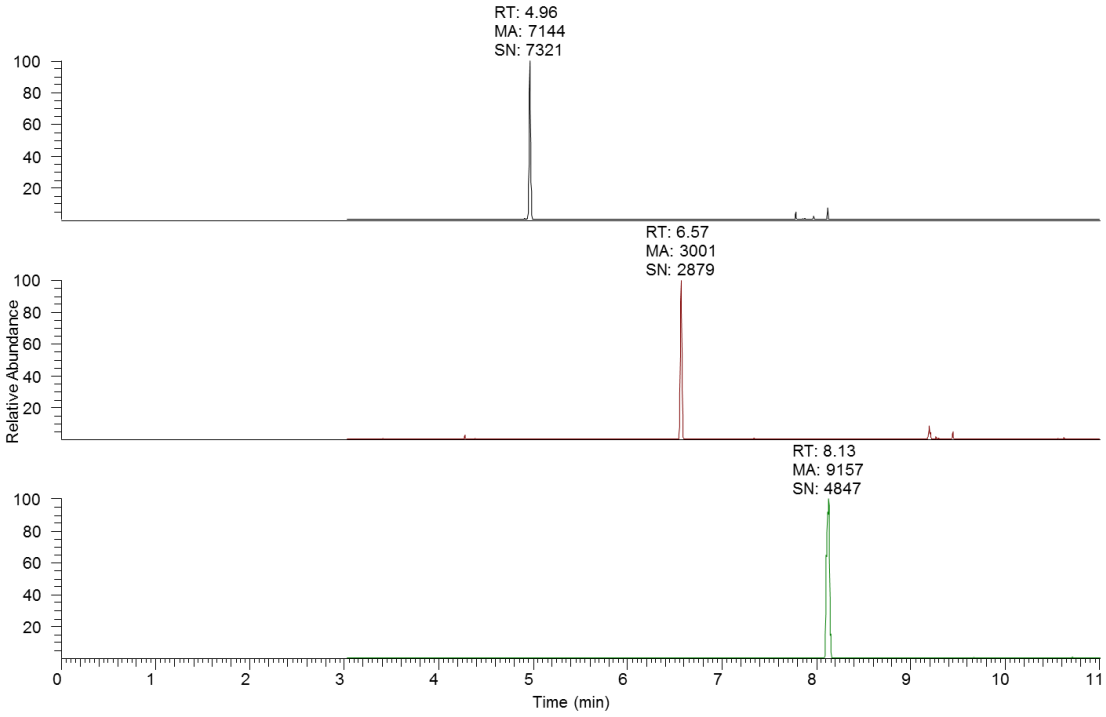


Figure 21: Extract chromatogram from sample 1 (Influent WWTP Amposta) in GC-MS/MS(quantification transitions).

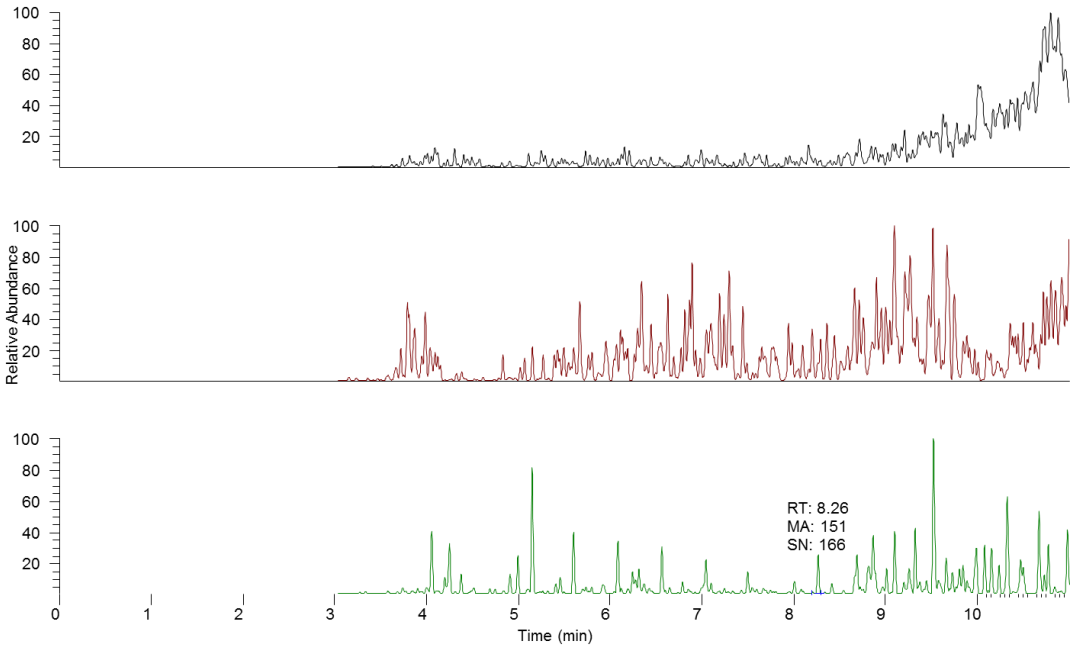


Figure 22: Extract chromatogram from sample 2 (effluent WWTP Amposta) in GC-MS/MS(quantification transitions).

5. ECOTOXICOLOGY

Additional to chemical analysis, ecotoxicological studies provide information about effects produced by complex mixtures of contaminants, their relations and the sum of their interactive effects including synergisms and antagonisms between contaminants and organic material. Therefore, under the frame of the Integra-Coast project a series of ecotoxicological standard tests were selected to include organisms suitable for the different environments, seawater (high salinity), estuarine (medium salinity), and fresh water. Selected organisms were in addition pertaining to different levels of organization.

In this Master Thesis it was included the ecotoxicological studies with the following organisms:

- The fresh water micro crustacean *Daphnia magna*
- The micro-crustacean *Artemia salina sp,* which admits from medium to salty water
- The marine bacteria *Vibrio fischeri*.

These bioassays were employed to assess the ecotoxicity of the water samples of the Ebro Delta and the bays. The information of these studies together with the results of other bioassays and the complete characterization by chemical analysis (not only the results presented here regarding PFASs) have been use to prioritise mixture and group of contaminants presenting major risks for the environment of the Ebro Delta.

5.1. BIOASSAY WITH *Daphnia magna*

Static acute 24–48-72 h assays were conducted according to internationally accepted Standard Methods (OECD and ISO 6341). The tests are performed using neonates which are hatched in about 3 days from the eggs at 20–22 °C, under continuous illumination of 6000 lux. Immobility at 24 h and 48 h is the bioassay endpoint, assumed to be equivalent to mortality. For each test a control solution was measured. Daphnia neonates exposed to the blanks and to the different dilutions are incubated in darkness at 20 °C. After 24 h and 48 h of exposure the number of immobilized organisms is determined. Six dilutions series for each sample (Dilutions: 100%, 80%, 60%, 40%, 20% and 10% of the sample) were tested working in triplicates. The dilution was done with the Standard Fresh water. Five neonates were transferred into each well and the mortality count was performed after 24 h, 48 h and 72 h of exposure. The acute toxicity test with *D. magna* were conducted using a Daphtoxkit F^{TM} Magna obtained from MicroBio Tests Inc. (Gent, Belgium). The Daphtoxkits make use of the dormant eggs of the crustaceans *D. magna*. These eggs are protected by a chitinous capsule called ephippium, and can be stored for long periods of time without losing their viability. When the ephippia are placed in specific environmental conditions and triggers, the eggs develop in bout 3 days of time into neonates which can then be used immediately for the toxicity tests. This bioassay was performed in disposable multiwall test plates.

EC_{50} (the effective concentration (EC) where 50% of the population is immobile) was calculated for the 24h, 48h, and 72h.

5.2. BIOASSAY WITH *Artemia salina*

For *A. salina* bioassay was used a Artoxkit M obtained from MicroBio Tests Inc. (Gent, Belgium). The experimental procedure for *A. salina was* based on Persoone et al. (1987) [95]. The toxicity test was carried out using larvae of the brine shrimp *A.salina* hatched from cysts. For the hatching of the larvae the *A. salina* eggs were in Petri dishes under 4000 lux illumination and 25°C temperature conditions with 10 ml of pre-aerated Standard Seawater during 30 hours. The wells were filled with the water samples (100%) in triplicates and for the control Standard Fresh water was used. It is important to remark that the controls were prepared by diluting Standard Seawater with deionized water depending of the salinity of each sample. In other words, every sample with different salinity had each own control. Ten neonates were transferred into each well and the mortality count was performed after 24 h, 48 h of exposure. In this bioassay was calculated the percentage of inhibition (%I).

5.3. BIOASSAY WITH *Vibrio fisheri*

The experimental procedure for conducting the bacterial bioluminescence assay was based on the ISO 11348 standard protocol [96]. protocol. The analysis is carried out with all dilution and reagents tempered at 15 °C. For the good performance of the tests, the osmolality is adjusted in order to obtain a 2% of saline in each solution or sample. Bacterial reagents are reconstituted just prior to the analysis and the pre-incubation times follow standard protocols. In all measures, the percentage of inhibition (% I) is determined by comparing the response given by a saline control solution to that corresponding to the diluted sample. Each dilution was tested in duplicates. The concentration which causes a 50% of bioluminescence inhibition after exposure for 15 or 30 min is designed as the EC50 value. Tests are performed at 15 °C. The measurements of bioluminescence are made using the luminometer Microtox™ (Carlsbad, CA, USA).

In Annex I (Table 4-6) are reported the selected samples for the bioassays.

5.4. ECOTOXICITY RESULTS

In this section the toxicity assays from *A.salina*, *V.fisheri* and *D.magna* results are shown.

For *A.salina*, *V.fisheri* the %I was determinated. If I% is between 20-40% the samples is considered not toxic. If I% is between 40-60% the sample is considered softly toxic. If the I% is 60% or more, the samples is considered toxic.

The results for *A.salina*, *V.fisheri* are shown in Table 17 and Table 18.

Table 17. Toxicity results for *A. salina* test.

Sample code	Sampling point	I% at 24h (%)	I% at 48h (%)	Toxicity 24h	Toxicity 48h
5	**Before Amposta**	13	37	Non toxic	Non toxic
7	**Sant Carles de la Ràpita Emissary**	3	3	Non toxic	Non toxic
8	**Channel A**	0	11	Non toxic	Non toxic
9	**Channel B**	18	39	Non toxic	Non toxic
10	**Channel C**	7	14	Non toxic	Non toxic
11	**Channel D1**	3	7	Non toxic	Non toxic
12	**Channel D2**	45	52	Softly toxic	Softly toxic
13	**Channel D3**	58	75	Toxic	Toxic
14	**Channel D4**	15	19	Non toxic	Non toxic
21	**Tancada shore**	44	64	Soflty toxic	Toxic
22	**Tancada center**	44	64	Soflty toxic	Toxic
23	**Illa de Buda**	7	7	Non toxic	Non toxic
24	**Illa de Buda center**	3	3	Non toxic	Non toxic
25	**Encanyissada shore**	43	61	Soflty toxic	Toxic
26	**Encanyissada center**	23	34	Non toxic	Non toxic
27	**Llacuna del Canal Vell shore**	0	4	Non toxic	

As we expected,almost all the samples are not toxic. However, Channel D3 and Encanyissada shore presen toxicity afeter 48h of ccontat between the *A.salina* and the sample.

Table 18. Toxicity results for *V.fisheri* test.

Sample code	Sampling point	I% at 15 min (%)	I% at 30 min (%)	Toxicity 15 min	Toxicity 30 min
15	**Alfalcs A bay**	0.0	0.0	Non toxic	Non toxic
16	**Alfalcs B bay**	0.0	0.0	Non toxic	Non toxic
17	**Alfalcs C bay**	0.0	0.0	Non toxic	Non toxic
28	**Alfalcs D bay**	0.0	0.0	Non toxic	Non toxic
19	**Fangar B bay**	18.0	17.5	Non toxic	Non toxic
20	**Fangar C bay**	0.0	0.0	Non toxic	Non toxic
21	**Tancada shore**	0.0	0.0	Non toxic	Non toxic

All the samples were detected as not toxic in V.fishery bioassay.

Table 19 shows *D.magna* toxicity results. The results are expressed using the EC50 the effective concentration (EC) where 50% of the population is immobile and also in toxicity units (TU). TU is 100/EC50. If the TU is higher than 2% the sample is considered toxic.

Table 19. Toxicity results for *D.magna*.

		EC50			TU (%)			Toxicity		
Sample code	**Sampling point**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**
1	**Amposta WWTP IN**	9.70	9.52	9.23	10.31	10.51	10.84	Toxic	Toxic	Toxic
2	**Amposta WWTP OUT**	1186.00	1186.00	722.70	0.08	0.08	0.14	Non toxic	Non toxic	Non toxic
3	**Sant Carles de la Rapita WWTP IN**	0.03	0.04	0.04	3495.28	2665.96	2665.96	Toxic	Toxic	Toxic
4	**Sant Carles de la Rapita WWTP OUT**	107.80	673.50	2789.00	0.93	0.15	0.04	Non toxic	Non toxic	Non toxic
6	**After Amposta**	1193.00	1193.00	7094.00	0.08	0.08	0.01	Non toxic	Non toxic	Non toxic
7	**Sant Carles de la Ràpita Emissary**	1057.00	1057.00	2901.00	0.09	0.09	0.03	Non toxic	Non toxic	Non toxic
9	**Channel B**	387.70	387.70	858.50	0.26	0.26	0.12	Non toxic	Non toxic	Non toxic

In Figure 23 the graphics of the EC50 are shown.

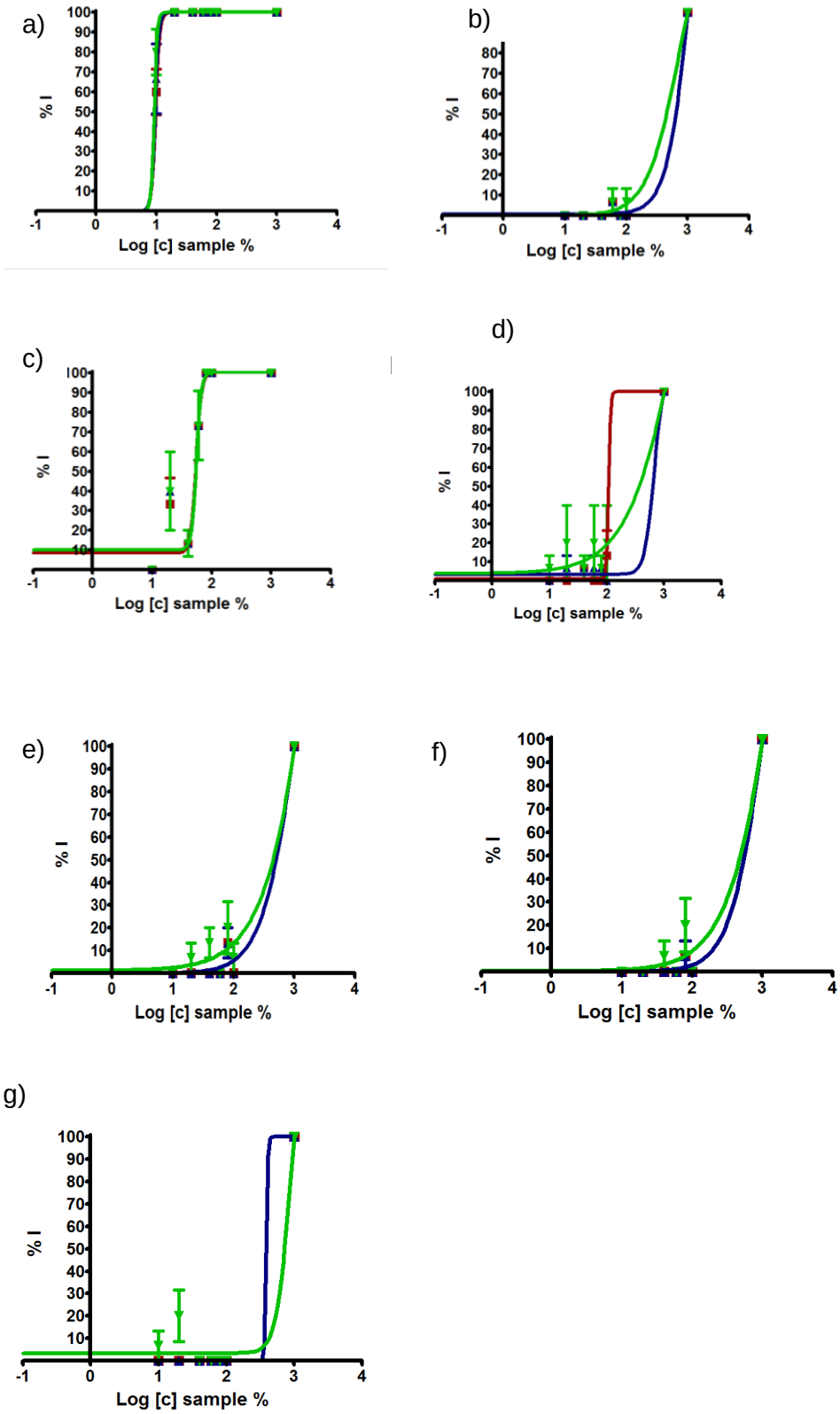


Figure 23: EC50 sigmoidal graphic where b is Amposta WWTP IN, b is Amposta WWTP OUT, c is Sant Carles de la Rapita WWTP IN, d is Sant Carles de la Rapita WWTP OUT, e is After Amposta, f is Sant Carles de la Rapita and g is Channel B.

As was expected, only influent WWTP samples were toxic for *D.magna*. In Figure 14 is shown the EC50 sigmoidal graphic. The samples that are more toxic present a strong sigmoidal profile, that means that the mortality of the organisms when the solution present more volume of sample the mortality increase.

5. CONCLUSIONS

1. In order to assess the occurrence of thirteen PFASs in the Ebro Delta, these compounds were analyzed in water, sediments biota. The samples were collected during two campaigns during autumn and winter 2015. During the first campaign water and sediments were collected in the same points, while during the third campaign also fish samples were collected in some selected sites corresponding to the main river, the estuary area and the open sea. The samples were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using a method previously developed with minor modifications.
2. In general, higher levels of contamination were found during the first sampling campaign. This fact can be attributed to rainfall happening during the first campaign that can drive to the resuspension of contaminants in sediments and the washing of surrounding areas.
3. For water samples, PFOA is the most ubiquitous while PFPeA shows the highest concentrations, which nowadays is used as substitutive compound of PFOA. The most polluted were WWTPs influent as expected.
4. For sediment samples, PFOA, PFNA and PFHpA were the most frequent compounds among carboxylic acids while PFOS was the most abundant among sulfonates. PFOA and PFOS really persist in the environment. The most contaminated sample is coming from Illa de Buda estuary for the first campaign and from Channel D2 for the second campaign.

1. As regards to fish samples, PFOS was the most and PFHxA was the most detected among carboxylic acids. The concentrations of PFASs were higher in the skin. The most contaminated sample were those from Illa de Buda estuary In particular the most polluted ones were *Angila angila* and Cyprius carpio.
2. A new GC-MS/MS method was developed and validated using BF_3-MeOH as derivatizant.
3. FTCAs were analyzed in twelve representative water and two fish samples by LC-MS/MS and GC-MS/MS. FHEA and FDEA were the most abundant FTCAs. The most contaminated sample was the influent of WWTP.
4. GC-MS/MS is more appropriate instrumental method for the detection of FTCAs, which are semi-volatile and allows lower limits of detection and quantification, compared to LC-MS/MS.
5. Finally, a basic ecotoxicity study was performed using standardized tests. Water samples from a third sampling campaign were studied. The test used for the ecotoxicity evaluation were the immobilization of *Artemis salina* and *Daphnia magna* (using the appropriated test according to the original salinity of the samples) and the bioluminescence inhibition of the marine bacteria *Vibrio fischeri*.

Only influent WWTP, channel D3, tancada center estuary and encanyissada shore present toxicity.

6. FUTURE WORK

1. Complete the analysis of PFASs in water, sediments and fish with the analysis of the third campaign, to better understand the seasonal trend and the behaviour of PFASs in different environmental matrices.

2. Complete the analysis of FTCAs by GC-MS/MS in water, sediments and fish samples with the rest of the first, second and third campaign samples.

3. Complete the analysis of ecotoxicity with the first and second campaign.

4. Improve the recuperation of the GC-MS/MS method.

5. Develop a study of different derivatizations approaches for FTCAs, to improve the detection in GC-MS/MS method and increase the time for each derivatization reaction.

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LIST OF TABLES

LIST OF FIGURES

ANNEX I

Taula 1. Water samples

Sample Code	Sampling point	1st sampling campaign	2nd sampling campaign	Water type	Origin
1	**Amposta WWTP IN**	YES	YES	Wastewater	WWTP Influent
2	**Amposta WWTP OUT**	YES	YES	Wastewater	WWTP Effluent
3	**Sant Carles de la Rapita WWTP IN**	YES	YES	Wastewater	WWTP Influent
4	**Sant Carles de la Rapita WWTP OUT**	YES	YES	Wastewater	WWTP Effluent
5	**Before Amposta**	YES	YES	Freshwater	Ebro river
6	**After Amposta**	YES	YES	Freshwater	Ebro river
7	**Sant Carles de la Ràpita Emissary**	YES	YES	Freshwater	Emissary
8	**Channel A**	YES	YES	Freshwater	Channel
9	**Channel B**	YES	NO	Freshwater	Channel
10	**Channel C**	YES	YES	Freshwater	Channel
11	**Channel D1**	YES	YES	Freshwater	Channel
12	**Channel D2**	YES	YES	Freshwater	Channel
13	**Channel D3**	YES	YES	Freshwater	Channel
14	**Channel D4**	YES	YES	Freshwater	Channel
15	**Alfalcs A bay**	YES	YES	Seawater	Shore
16	**Alfalcs B bay**	YES	YES	Seawater	Near to harbor
17	**Alfalcs C bay**	YES	YES	Seawater	Open sea
18	**Fangar A bay**	YES	YES	Seawater	Shore
19	**Fangar B bay**	YES	YES	Seawater	Shore
20	**Fangar C bay**	YES	YES	Seawater	Open sea
21	**Tancada shore**	YES	YES	Estuary	Shore
22	**Tancada center**	YES	YES	Estuary	Middle of lagoon
23	**Illa de Buda**	YES	NO	Estuary	Shore

24	**Illa de Buda center**	YES	NO	Estuary	Middle of lagoon
25	**Encanyissada shore**	YES	NO	Estuary	Shore
26	**Encanyissada center**	YES	NO	Estuary	Middle of lagoon
27	**Llacuna del Canal Vell shore**	YES	YES	Estuary	Shore
28	**Alfalcs D bay**	NO	YES	Seawater	Open sea
29	**Fangar D bay**	NO	YES	Seawater	Open sea

Table 2. Sediments samples

Sample Code	Sampling point	1st sampling campaign	2nd sampling campaign	Water type	Origin
5	**Before Amposta**	YES	YES	Freshwater	Ebro river
6	**After Amposta**	YES	YES	Freshwater	Ebro river
8	**Channel A**	YES	YES	Freshwater	Channel
9	**Channel B**	YES	NO	Freshwater	Channel
10	**Channel C**	YES	YES	Freshwater	Channel
11	**Channel D1**	YES	YES	Freshwater	Channel
12	**Channel D2**	YES	YES	Freshwater	Channel
13	**Channel D3**	YES	YES	Freshwater	Channel
14	**Channel D4**	YES	YES	Freshwater	Channel
15	**Alfalcs A bay**	YES	YES	Seawater	Shore
16	**Alfalcs B bay**	YES	YES	Seawater	Near to harbor
17	**Alfalcs C bay**	YES	YES	Seawater	Open sea
18	**Fangar A bay**	YES	YES	Seawater	Shore
19	**Fangar B bay**	YES	YES	Seawater	Shore
20	**Fangar C bay**	YES	YES	Seawater	Open sea
21	**Tancada shore**	YES	YES	Estuary	Shore
22	**Tancada center**	YES	YES	Estuary	Middle of lagoon
23	**Illa de Buda**	YES	NO	Estuary	Shore
24	**Illa de Buda center**	YES	NO	Estuary	Middle of lagoon
25	**Encanyissada shore**	YES	NO	Estuary	Shore
26	**Encanyissada center**	YES	NO	Estuary	Middle of lagoon
27	**Llacuna del Canal Vell shore**	YES	YES	Estuary	Shore
28	**Alfalcs D bay**	NO	YES	Seawater	Open sea
29	**Fangar D bay**	NO	YES	Seawater	Open sea

Table 3. Fish samples

Sample Code	Sampling point	Water type	Origin	Fish specie	Sex	Stady	Wet weight (g)	Length without caudal fin (cm)	Length with caudal fin (cm)
1	**Fangar C bay**	Sea water	Open sea	*Trachurus murphyi*	Male	Adult	195.52	23.5	29
2	**Fangar C bay**	Sea water	Open sea	*Sarpa salpa*	Male	Adult	174.5	19	22
3	**Fangar C bay**	Sea water	Open sea	*Sarpa salpa*	Male	Adult	157.43	18.5	22
4	**Fangar C bay**	Sea water	Open sea	*Diplorus anularis*	Male	Adult	120.1	16	18.5
5	**Fangar C bay**	Sea water	Open sea	*Diplorus anularis*	Male	Adult	105.6	15	18
6	**Alfacs C bay**	Seawater	Open sea	*Leuciscus cephalus*	Male	Adult	431.05	33	40
7	**Alfacs C bay**	Seawater	Open sea	*Leuciscus cephalus*	Male	Adult	353.2	29	35
8	**Alfacs C bay**	Seawater	Open sea	*Torpedo torpedo*	Male	Adult	187	20	23
9	**Alfacs C bay**	Seawater	Open sea	*Boops boops*	Male	Adult	51.42	14	16.5
10	**Illa de Buda**	Estuary	Shore	*Mugil cephalus*	Female	Adult	566	35	43
11	**Illa de Buda**	Estuary	Shore	*Micropterus salmoides*	Female	Adult	720	34	41
12	**Illa de Buda**	Estuary	Shore	*Mugil cephalus*	Male	Adult	450	33	39
13	**Illa de Buda**	Estuary	Shore	*Cyprinus carpio*	Female	Adult	2000	48	52
14	**Illa de Buda**	Estuary	Shore	*Cyprinus carpio*	Female	Adult	567.17	26.5	32
15	**Illa de Buda**	Estuary	Shore	*Anguila anguila*	-	Adult	295.01	55	-

Table 4. *Artemia salina* Acute Toxicity Test for third campaign water samples.

Artemia salina Acute toxicity Test			
Sample code	Sampling point	Water type	Origin
5	**Before Amposta**	Freshwater	Ebro river
7	**Sant Carles de la Ràpita Emissary**	Freshwater	Emissary
8	**Channel A**	Freshwater	Channel
9	**Channel B**	Freshwater	Channel
10	**Channel C**	Freshwater	Channel
11	**Channel D1**	Freshwater	Channel
12	**Channel D2**	Freshwater	Channel
13	**Channel D3**	Freshwater	Channel
14	**Channel D4**	Freshwater	Channel
21	**Tancada shore**	Estuary	Shore
22	**Tancada center**	Estuary	Middle of lagoon
23	**Illa de Buda**	Estuary	Shore
24	**Illa de Buda center**	Estuary	Middle of lagoon
25	**Encanyissada shore**	Estuary	Shore
26	**Encanyissada center**	Estuary	Middle of lagoon
27	**Llacuna del Canal Vell shore**	Estuary	Shore

Table 5. *Daphnia magna* Acute Acute Toxicity Test for third campaign water samples.

Daphnia magna Acute Immobilisation Test			
Sample Code	**Sampling point**	**Water type**	**Origin**
1	**Amposta WWTP IN**	Wastewater	WWTP Influent
2	**Amposta WWTP OUT**	Wastewater	WWTP Effluent
3	**Sant Carles de la Rapita WWTP IN**	Wastewater	WWTP Influent
4	**Sant Carles de la Rapita WWTP OUT**	Wastewater	WWTP Effluent
6	**After Amposta**	Freshwater	Ebro river
7	**Sant Carles de la Ràpita Emissary**	Freshwater	Emissary
9	**Channel B**	Freshwater	Channel

Table 6. *Vibrio fischeri* Acute Toxicity Test for third campaign water samples.

Vibrio fischeri Inhibitory effect on the light emision Test			
Sample Code	**Sampling point**	**Water type**	**Origin**
15	**Alfalcs A bay**	Seawater	Shore
16	**Alfalcs B bay**	Seawater	Near to harbor
17	**Alfalcs C bay**	Seawater	Open sea
19	**Fangar B bay**	Seawater	Shore
20	**Fangar C bay**	Seawater	Open sea
21	**Tancada shore**	Estuary	Shore
28	**Alfalcs D bay**	Seawater	Open sea