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Grau de Química

Treball Final de Grau

Occurrence of emerging contaminants in Ebro Delta natural parc

Presència de contaminants emergents en el parc natural del Delta de l'Ebre

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June 2019

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The proper use of science is not to conquer nature but to live in it

Barry Commoner

First, I want to express my gratitude to Dra. Marta Llorca and Dra. Marinella Farré for allowing me to work in their team. I am especially grateful to Marta for her dedication, for helping me and for guiding me during the course of this project in which I have learnt a lot of new things.

Also, I want to thanks my tutor Mònica Martínez for her advices, encouragement and for supervising my work.

And finally, but not least, I want to thanks my family and friends for their support in this important stage of my life, specially my parents because without them, none of this would have happened. I am forever grateful to them.

REPORT

CONTENTS

1. SUMMARY

Perfluoroalkyl substances (PFASs) have been widely used since the 1950s and they have a high number of applications in the industry and commerce as a result of their outstanding chemical properties. However, over the last years they have drawn scientific attention due to their occurrence and persistence in the environment as well as their negative effects in the ecosystem and in human health. For example, perfluorooctane sulfonate (PFOS) which is one of the most used in the past, was included in the Stockholm Convention of persistent organic pollutants in 2009 and banned in most of the industrial and commercial applications but is still present in the environment. Because of this, it is important to monitor the occurrence of PFASs in the environment.

This work was executed in the frame of the PLAS-MED project in which the occurrence of 18 PFASs in environmental samples from Ebro Delta, corresponding to two different seasonal campaigns carried out in July 2018 and in February 2019, was evaluated. Previous optimized and validated methods were applied to their determination in seawater, river water and sediments samples by means of Solid Phase Extraction (SPE, waters) or Solid-Liquid Extraction (SLE, sediments) followed by Liquid Chromatography coupled to Mass Spectrometry in tandem (LC-MS/MS). The occurrence of these substances in Ebro Delta was compared with the ones from Mar Menor.

Perfluorocarboxylic acids were the most detected PFASs in all the studied samples. Four PFASs were detected in waters from Ebro Delta area and in samples corresponding to summer season indicating seasonal variation. Comparing these results with the ones from Mar Menor, these last ones showed lower concentrations of PFASs. Regarding sediment samples, these showed similar tendency and only five analytes were detected at quantifiable concentrations. This seasonal variation observed in waters and sediments is an indicative of the influence of the environment (i.e. weather effects) to the presence of PFASs.

Keywords: PFASs, solid phase extraction, LC-MS/MS, Ebro Delta, water, sediments.

2. RESUM

Les substàncies perfluoralquilades (PFASs) han estat àmpliament utilitzades des de la dècada de 1950 i tenen un elevat nombre d'aplicacions a la indústria i al comerç com a conseqüència de les seves excepcionals propietats químiques. No obstant això, en els darrers anys han atret l'atenció de la comunitat científica a causa de la seva aparició i persistència en el medi ambient, així com pels seus efectes negatius en l'ecosistema i en la salut humana. Per exemple, l'àcid perfluorooctanosulfònic (PFOS), un dels més utilitzats en el passat, va ser inclòs a la Convenció d'Estocolm sobre contaminants orgànics persistents el 2009 i prohibit a la majoria de les aplicacions industrials i comercials, però encara és present al medi ambient. Per això, és important fer un seguiment de la presència de PFASs al medi ambient.

Aquest treball es va realitzar en el marc del projecte PLAS-MED, en el qual es va avaluar la presència de 18 PFASs en mostres ambientals del Delta de l'Ebre, corresponents a dues campanyes estacionals diferents realitzades al juliol del 2018 i al febrer del 2019. Es van aplicar mètodes prèviament optimitzats i validats per a la seva determinació en mostres d'aigua de mar, aigua de riu i de sediments mitjançant l'extracció de fase sòlida (SPE, aigües) o extracció en fase líquida (SLE, sediments) seguits de cromatografia de líquids acoblada a espectrometria de masses en tàndem (LC-MS / MS). La presència d'aquestes substàncies al Delta de l'Ebre es va comparar amb les del Mar Menor.

Els àcids perfluorocarboxílics van ser els PFAS més detectats en les mostres estudiades. Es van detectar quatre PFASs en aigües del Delta de l'Ebre corresponents a la temporada d'estiu indicant variació estacional. Comparant aquests resultats amb els del Mar Menor, aquests últims van mostrar concentracions més baixes de PFASs. Pel que fa a les mostres de sediments, aquestes van mostrar una tendència similar i només es van detectar cinc analits en concentracions quantificables. La variació estacional observada en aigües i sediments és un indicador de la influència del medi ambient (efectes meteorològics) en la presència de PFASs.

Paraules clau: PFASs, extracció en fase sòlida, LC-MS/MS, Delta de l'Ebre, aigua, sediments.

3. INTRODUCTION

Technological and scientific advances have made great changes to improve people's live but they also have led to cause negative changes in the biosphere. So, over the last years, emerging contaminants have become an environmental issue that have attracted scientific interest because of their persistent presence, repercussion in the ecosystem and their harmful effects in the human health.

It is so difficult to maintain the equilibrium between the development of science and technology and the preservation of the environment. Therefore, solutions for a sustainable management should be proposed and carried away.

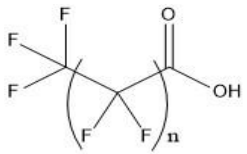
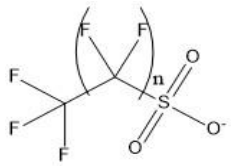
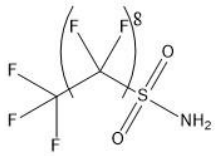
Particular attention has been paid in perfluoroalkyl substances (PFASs). These chemicals have been widely used since the 1950s and present a high number of applications in the industry and commerce as a result of their outstanding chemical properties: these are inert and have a great resistance against chemical, biological and physical degradation. These remain intact in the environment for a long period of time and have potential negative effects on the ecosystem and human health[1]**.** As a result, some of them have been classified as persistent organic pollutants (POPs) under Stockholm Convention[2].

3.1. PERFLUOROALKYL SUBSTANCES

Perfluoroalkyl substances are a group of chemical compounds of anthropogenic origin that have been manufactured for over 70 years. Buck *et al.* (2011)[3] defined them as: "PFASs are aliphatic substances that contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated 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}-".

There are hundreds of types of PFASs, some of them highly volatiles because they have short carbon chains and others with longer chains (>5C) that are more stables. PFASs studied in this project are summarised in Table 1.

Table 1: Perfluoroalkyl substances studied in this project

Class and chemical structure	Compound	Acronym	Molecular formula
Perfluorocarboxylic acids (PFCAs) 	Perfluorobutanoic acid	PFBA	$F(CF_2)_3COOH$
	Perfluoropentanoic acid	PFPeA	$F(CF_2)_4COOH$
	Perfluorohexanoic acid	PFHxA	$F(CF_2)_5COOH$
	Perfluoroheptanoic acid	PFHpA	$F(CF_2)_6COOH$
	Perfluorooctanoic acid	PFOA	$F(CF_2)_7COOH$
	Perfluorononanoic acid	PFNA	$F(CF_2)_8COOH$
	Perfluorodecanoic acid	PFDA	$F(CF_2)_9COOH$
	Perfluoroundecanoic acid	PFUdA	$F(CF_2)_{10}COOH$
	Perfluorododecanoic acid	PFDoA	$F(CF_2)_{11}COOH$
	Perfluorotridecanoic acid	PFTrDA	$F(CF_2)_{12}COOH$
	Perfluorotetradecanoic acid	PFTeDA	$F(CF_2)_{13}COOH$
	Perfluorohexadecanoic acid	PFHxDA	$F(CF_2)_{15}COOH$
	Perfluorooctadecanoic acid	PFODA	$F(CF_2)_1COOH$
Perfluorosulfonates (PFSAs) 	Perfluorobutane sulfonate	PFBS	$(CF_2)_4SO_3^-$
	Perfluorohexane sulfonate	PFHxS	$(CF_2)_6SO_3^-$
	Perfluorooctane sulfonate	PFOS	$(CF_2)_8SO_3^-$
	Perfluorodecane sulfonate	PFDS	$(CF_2)_{10}SO_3^-$
Perfluorinated sulphonamide (FSA) 	Perfluorooctane sulfonamide	PFOSA	$F(CF_2)_8SO_2NH_2$

3.1.1. Properties

PFASs compounds have unique physical and chemical properties. These contain a strong carbon-fluorine bond, one of the strongest in chemistry (~466 kJ/mol) which increases with the number of carbon atoms. Besides, fluorine atoms have a high electronegativity (4.0) and small diameters, and the distance between atomic nuclei in C-F bond is short (133-142 pm)[4].

Therefore, these substances are highly stables, resistant to degradation, and distributed persistently in the environment. Their structures have a polar hydrophilic head and a non-polar hydrophobic tail as is shown in Figure 1. Because of its amphiphilic character, PFASs have excellent surface-active properties[5]. Moreover, these coexist in equilibrium between the anionic and neutral forms and are soluble in water.

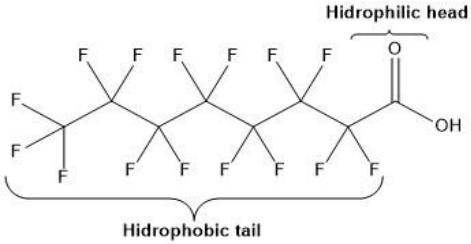


Figure 1: Chemical structure of PFOA

Their main properties make them useful in the industry of lubricants, fire-fighting foams, waterproof textiles and stain repellent coatings, food packaging paper, carpets, insecticides, among many others[6,7]. Because of their extensive usage, industry (direct source) and human activity (indirect source) have been identified as the main sources of PFASs in the environment[8].

3.1.2. Environmental fate

PFASs are highly stable and extensively used. Some of them are soluble in water and have low vapor pressure making them stable in acid, basic and oxidant media and difficult to hydrolyse, biodegrade or photodegrade. In addition, it has been observed that PFASs can be transferred to living organisms not only by ingestion but also by the ingestion of microplastics present in the surrounding waters that absorb them on their surface[9]. As a result, they are widely distributed in the ecosystem and they bioaccumulate and biomagnify though the food chain[10] and some of them have been classified as POPs.

The two most commonly PFASs found in the environment are PFOA and PFOS due to their massive use in the past[3]. Moreover, some polyfluoroalkyl substances are degraded in the environment to most stable PFASs being this source another input of persistent PFASs for the environment. Therefore, the fate of PFASs is the result of their physical and chemical properties.

3.1.3. Toxicity

PFASs accumulate and biomagnify through the food chain specially in aquatic media. Human exposure to PFAS is mainly by ingestion and drinking of contaminated food or water. In general, the toxicity of the other PFASs is proportional to the length of the carbon chain[8].

As mentioned before, PFOS and PFOA are the most PFASs found in the ecosystem, so there is a concern about their harmful effects and, for example, the United States Environmental Protection Agency (U.S. EPA) classifies both of them as possible carcinogens[11].

Experiments testing acute toxicity showed in general that toxicity is negligible because the amounts found in the environment is irrelevant against the concentration that is required to cause a negative effect. Studies in animals shown that PFOS and PFOA are the ones that cause more effects in the thyroid hormones and in the liver[9]. However, the acute toxicity test with the microcrustacean *Daphnia magna* showed that PFOS cause more harmful effects than PFOA[8] or that PFOS suppresses the transport of proteins in zebrafish[8].

In contrast, the experiments testing chronic toxicity evidenced more harmful effects. For example, sub development of the mice offspring, which mother was exposed to PFOA, was observed and that happened because PFOA could pass though the placental barrier of the pregnant mice. Moreover, these compounds can interfere with the endocrine system[8].

3.1.3.1. Bioaccumulation and human exposure

PFASs are bioaccumulated and biomagnified through the food chain increasing the concentration levels of these substances in the ecosystem. Human exposure to PFASs is mainly by ingestion of contaminated water or food being fish identified as the major contributor. The exposure of humans to PFASs have led to detect them in matrices such as human breast milk, blood or seminal plasma[8,12].

3.1.4. Regulation and legislation

Due to the harmful effects of PFASs in the environment, legislation and regulation have been imposed. These have some differences depending on the country. For example, PFASs are included in regulation and legislation programmes in European Union, United States of America (USA) and Canada among others and the production of larger compounds has decreased and substituted with shorter ones due to their easier degradation. Nevertheless, there is no legislation for most of them.

PFOS and PFOA are considered persistent, bioaccumulative and toxic substances. In 2009, PFOS was added in the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation nº1907/2006 with the Annex XVII of Reach Regulation (EC 552/2009) and it was also added into Annex B of the Stockholm Convention on POPs (United Nations Environment Programme (UNEP 2009)[2]. Then, the Persistent Organic Pollutants Review Committee proposed the addition of PFOA and PFHxS and their salts to Annex A/B/C of the Convention in October 2015 and November 2017 (UNEP 2015, 2017). Currently these chemicals are under review[2].

In general, there is a lack of legislation of other substances. For example, in Sweden the National Food Agency recommended to take urgent actions if the concentration of 11 PFASs (PFOA, PFOS, PFBS, PFHxS, PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA and 6:2 FTSA outstrip 0.09 µg/L[13]. The US EPA fix this limit to 0.07 µg/L for PFOA and PFOA together[14].

3.2 ANALYTICAL METHODS FOR THE DETERMINATION OF PFASs

The wide distribution and presence of PFASs in the environment has motivated the development of several fast and robust analytical methods for their determination in matrices such as water or sediments. The most commonly used technique is the liquid chromatography coupled to mass spectrometry in tandem with a previous solid phase extraction[15]. Gas Chromatography coupled to Mass Spectrometry (GC-MS) is also used but this technique is only employed for volatile and semi volatile PFASs that have short carbon chains.

It is important to highlight the problems during analysis related to sorption onto lab material and cross-contamination where each analytical step could be a source of PFASs itself. Because of this, it is important to use the appropriate material like polypropylene (PP) instead of glass recipients to avoid the sorption, and analyse blanks in parallel to avoid contamination and errors[16].

3.2.1. Extraction methods

SPE is the most used extraction technique for the analysis of PFASs in waters. Sometimes a previous filtration is carried out but it can cause analytes loses, so it is not recommended[16]. Two types of SPE can be performed: off-line, the typical one and the most used, and on-line in which the SPE is coupled to LC-MS/MS and the sample manipulation, analysis time and expenses are reduced[12].

SLE it is more used for solid samples. Sediments are complex matrices and agitation or Ultrasound Assisted Extraction (UAE) are usually applied with methanol or acetonitrile as solvents[16]. After extraction step, a centrifugation of the sample is carried out to separate the phases and the supernatant is isolated by decantation[10,16]. Finally, extracts of sediments can be purified by off-line method such as SPE or by an on-line clean-up system based on turbulent flow chromatography (TFC)[10].

3.2.2. Instrumental analysis

3.2.2.1. Liquid chromatography coupled to mass spectrometry in tandem

LC-MS/MS is the most used technique because it can be applied to semi-volatile and non-volatile compounds and provides accurate results with high sensibility and robustness. LC with reversed phase is commonly used as separation technique. After separation, the sample is introduced to the mass spectrometer by Electrospray Ionization (ESI) working in negative mode[16] although ESI and Atmospheric-Pressure Chemical Ionization (APCI) operating in positive mode are employed for some PFASs[16].

Mass spectrometry is the most commonly used technique for the detection of PFASs Different analysers have been used although triple Quadrupole (QqQ) operating in Selection Reaction Monitoring (SRM) mode, is the most used one for PFASs because of its sensibility, selectivity, robustness and low cost even though it offers low resolution[17]. Figure 2 shows how a QqQ works. The analytes that arrive from the LC are ionized by ESI working in negative mode. In the first quadrupole, a filter mass selects the precursor ions. The second one as a collision chamber where the precursor ions are shelled with Argon (Ar) to form new fragment ions which are transferred to the third quadrupole where these fragments are selected. SRM mode allows to obtain the signal of the analyte of interest which m/z transition is well known.

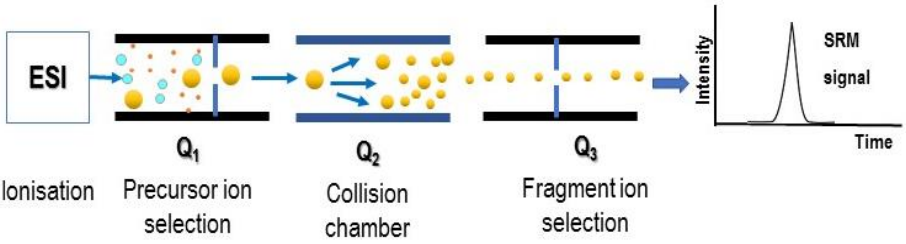


Figure 2: Triple quadrupole analyser operating in SRM mode

3.3. PLAS-MED PROJECT

This work was executed in the frame of PLAS-MED project (Microplastics and microcontaminants in the Mediterranean coast). The aim of this project is the evaluation of the microplastics (MPLs) risks in the environment due to their ability to transfer to the ecosystem other contaminants and propose solutions[18].

4. OBJECTIVES

Ebro Delta is the third largest delta in the Mediterranean Sea. In previous studies, the presence of perfluoroalkyl substances was detected, which can interact with microplastics and then be an extra source of PFASs in the environment.

In this context, the main goal of this project was to assess the occurrence of 18 PFASs in water and sediment environmental samples from two different sampling campaigns carried out in July 2018 and February 2019 by means of:

- off-line SPE followed by LC-MS/MS for the analysis of waters
- SLE followed by on-line TFC coupled to LC-MS/MS for the analysis of sediments.

Furthermore, the results of the occurrence of PFASs in waters were compared with the occurrence of PFASs in Mar Menor, a protected Mediterranean area, in collaboration with Instituto Español Oceanográfico (IEO).

5. EXPERIMENTAL SECTION

5.1. MATERIALS AND METHODS

5.1.1. Reagents and standards

Native perfluoroalkyl compounds standards were supplied by Wellington Laboratories Inc. (Canada) and were composed of a mixture of PFASs. This solution was prepared with PFOSA-I (50 µg/mL in isopropanol, purity >98%) and a mixture of PFASs (PFAC-MXC, 2 µg/mL in methanol, purity >98%) containing thirteen native perfluoroalkylcarboxylic acids and four native perfluoroalkylsulfonates which are summarized in Table 1. This mixture was used for calibration curves.

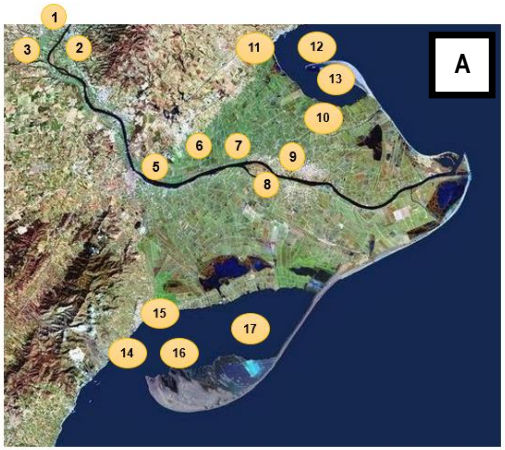
Surrogate internal standards added before the experimental procedure and used for quantification and normalization of the whole analytical process were provided by Wellington Laboratories Inc. (Canada) and were composed of a mixture of labelled PFASs. This solution was prepared with perfluoro-1-[$^{13}C_8$]octanesulfonamide (M8FOSA-I, 50 µg/mL in isopropanol, chemical purity >98%, isotopic purity >99%) and a mixture of mass-labelled PFASs (MPFAC-C-ES, 2 µg/mL in methanol, chemical purity >98%, isotopic purity >99%) containing: (a) ten mass-labelled perfluoroalkylcarboxylic acids (^{13}C): perfluoro-n-[$^{13}C_4$]butanoic (MPFBA), perfluoro-n-[$^{13}C_5$]pentanoic (M5PFPeA), perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic (M5PFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic (M4PFHpA), perfluoro-n-[$^{13}C_8$]octanoic (M8PFOA), perfluoro-n-[$^{13}C_9$]nonanoic (M9PFNA), perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic (M6PFDA), perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic (M7PFUdA), perfluoro-n-[1,2-$^{13}C_2$]dodecanoic (MPFDoA), perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic (M2PFTeDA) acids; and (b) three mass-labelled perfluoroalkylsulfonates (^{13}C): perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate (M3PFBS), perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate (M3PFHxS) and perfluoro-1-[$^{13}C_8$]octanesulfonate (M8PFOS).

Water and methanol (Ultra) Gradient HPLC grade were purchased from J.T. Baker (Netherlands). Ammonium acetate (MW: 77.08 g/mol, purity >98%) and ammonium hydroxide (MW: 35.05 g/mol, purity >98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid (MW: 46.03 g/mol, purity >98% was purchased from Merck (Poland). Acetonitrile

(ACN) was purchased from Fisher Scientific (U.K). Acetone and isopropanol were obtained from Carlo Erba (France).

5.1.2. Sample collection

Two sampling campaigns were carried out by the staff of the Institute of Environmental Assessment and Water Research of the Spanish National Research Council (IDAEA-CSIC) between 2018 and 2019. The first one on July 2018 (summer) and the second one on February 2019 (winter). A total number of 21 samples of sediments and 22 samples of water including seawater and river freshwater were collected from different points of Ebro Delta (Tarragona). From Mar Menor, 9 samples of water were collected. Figure 3 shows the sampling locations.



1. Before Xerta
2. Xerta
3. After Xerta
5. Amposta
6. After Amposta
7. Before Deltebre
8. Deltebre
9. After Deltebre
10. Beach Fangar Bay (Port d'Illa de Mar)
11. Fangar Bay harbor
12. Fangar Bay open sea 1
13. Fangar Bay open sea 2
14. Beach Alfacs Bay
15. Alfacs Bay harbor
16. Alfacs Bay open sea 1
17. Alfacs Bay open sea 2

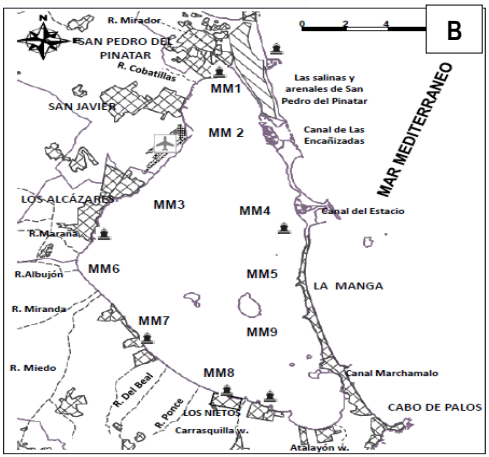


Figure 3: Sampling points A) Ebro Delta and B) Mar Menor

5.1.3. Analysis of water samples

5.1.3.1. Solid Phase Extraction

Extraction and clean-up were carried out by using the method described by Pignotti et al. (2017)[10]. First, 150 mL of river water and 250 mL of seawater were spiked with 6 µL of a mixture of surrogate internal standards in methanol previously prepared at 500 ng/L. The samples were shaken with a vortex and then they were left for 20 minutes for equilibration and then they were extracted by SPE as it is shown in Figure 4.

Figure 4: SPE extraction (loading sample)

The SPE consisted in the conditioning of the cartridges, the sample loading and the elution. Oasis ® WAX cartridges 3cc (30 cm^3, 60 mg, 30 µm; Waters Corporation, Milford, Massachusetts, USA) were the ones used. These are polymeric reversed-phase and weak anion exchange mixed-mode sorbent. These are highly selective for strong acid compounds, and the pH of water samples was between 7.4 and 8.6, so all the analytes were present in their anionic form and they were retained. The cartridges were previously conditioned with 2x2 mL of methanol and 2x2 mL of water under gravity. Then, the samples were loaded under vacuum conditions, the cartridges were dried with vacuum for 20 min and kept in the freezer at -20 ºC until the elution. The analytes were eluted with 4 mL of 0.1% ammonium hydroxide in methanol (2x2 mL) and collected in 15 mL PP tubes. The extracts were evaporated under a gentle nitrogen (N_2) stream near dryness using a Reacti-VapTM III, Pierce (Figure 5). The extracts were transferred using a micropipette (Eppendorf Research® Plus) inside 2 mL vials (Agilent Technologies, Poland) equipped with 250 µL glass inserts (deactivated, Agilent, USA). The remaining volume was evaporated to dryness as explained before and the extracts were reconstituted in 150 µL with a mixture of water and methanol (9:1) so the final concentrations of the surrogate's internal standards were 20 ng/L.

The extracts were kept in the freezer at -20 ºC until the instrumental analysis. Blanks were also extracted in parallel in order to discard any possible contamination in some step of the procedure.

Figure 5: Evaporation under N_2

5.1.3.2. Chromatography separation

The extracts were analysed by Ultra-High Performance Liquid Chromatography coupled to a triple Quadrupole Mass Spectrometer (UHPLC-QqQ-MS/MS). Chromatographic separation was done in Acquity UPLC H-Class system (Waters Corporation, USA) equipped with a reverse phase analytical C18 column Hypersil GOLD PFP (3x50 mm, 3 µm particle size; Thermo Fisher Scientific). The mobile phases consisted in 20 mM ammonium acetate in methanol (solvent A) and 20 mM ammonium acetate in water (solvent B) at a flow rate of 0.4 mL/min. The elution programme was performed as follows: it started with 20% A and 80% B during 0.10 min and then a lineal gradient elution was performed for 5.00 min to achieve 80% A and 20% B. Later, the proportion of the solvent A was increased linearly to 90% for 2.00 min, followed by an isocratic elution at 90% A and 10% B for 1.50 min. In 1.00 min, it was reached again a composition of 20% A and 80% B and it was maintained for 1.50 more minutes. A vial consisting in initial conditions of mobile phase was analysed in parallel through the analytical procedure as instrumental blank to discard any system contamination. So, each injection took 11 min and the injection volume was 10 µL.

5.1.3.3. Mass spectrometry detection

After separation, the detection was carried out using a triple quadrupole analyser Xevo TQ-S (Waters Corporation) with an ESI source operating in negative conditions at 400ºC and with a collision gas flow of 0.15 mL/min. Transitions, retention times and collision energy are summarized in Table 2 while ion source properties are summarized in Table 3.

Table 2: Analytical and instrumental parameters (m/z transitions, *quantification transition, retention time (t_R, water), t_R*(sediment) and collision energy (CE))

Analyte	Precursor ion (m/z)	Daughter (m/z)	t_R (min)	t_R (min)*	CE (V)
PFBA	213	169*	1.2	1.5	10
PFPeA	263	219	2.3	1.6	10
PFHxA	313	269* 169	2.8	3.2	10 20
PFHpA	363	319* 169	4.2	3.5	10 10
PFOA	413	369* 169	4.5	3.7	10 30
PFNA	463	419* 169	4.9	3.8	10 30
PFDA	513	469* 169	5.5	3.9	10 25
PFUdA	563	519* 169	6.0	4.0	10 30
PFDoA	613	569* 169	6.7	4.2	10 50
PFTrDA	663	619* 169	7.4	4.4	10 30
PFTeDA	713	669* 169	8.1	4.6	10 30
PFHxDA	813	769* 169	9.5	5.3	20 30
PFODA	913	869* 269	11.0	6.3	10 30
PFBS	299	80* 99	2.4	3.0	80 80
PFHxS	399	80* 99	3.2	3.5	80 80
PFOS	499	80* 99	4.9	3.8	100 100
PFDS	599	80* 99	6.0	4.0	100 100
PFOSA	498	78* 498	5.7	4.1	80 50

Table 3: Ion source properties of the triple quadrupole analyser Xevo TQ-S

Voltages		Gas Flow	
Capillary (kV)	2.80	Desolvation (L/h)	1000
Cone (V)	20	Cone (L/h)	150
Source Offset (V)	50	Nebuliser (bar)	7.0

Acquisition was performed in SRM mode and the data processing was carried out with the software MassLynx version 4.1 (Waters Corporation). Figure 6 shows the instrument used in the analysis.

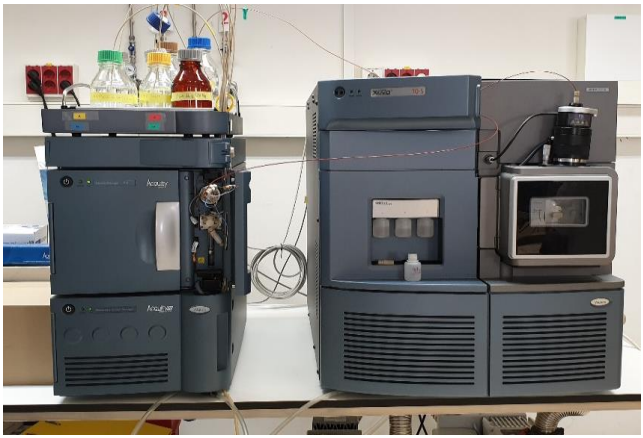


Figure 6: UHPLC-QqQ instrument

5.1.4. Analysis of sediment samples

5.1.4.1. Sample pre-treatment and solid-liquid extraction

Extraction was carried out by using the method described by Pignotti et al. (2017)[10]. Sediment samples were thawed and dried for a week under a fume cupboard at room temperature. After that, samples were grinded with a glass mortar to reduce the particle size. 0.5 g of dried sediment was weighted in a 50 mL PP centrifuge tub with an analytical balance (Mettler Toledo, 5 decimals). The samples were spiked with 4µL of a mixture of surrogate internal standards in methanol previously prepared at 500 ng/L. The samples were shaken with a vortex and then left for 20 min to equilibrate at room temperature. Then, 10 mL of methanol was added and extracted by UAE for 1 h. Afterwards, samples were centrifuged at 4000 rpm for 20 min and at 17 ºC (Centrifuge 5810 R, Eppendorf, Figure 7). A decantation was carried out to separate the solvent (approx. 10 mL) and transferred into 15 mL PP centrifuge tubes.

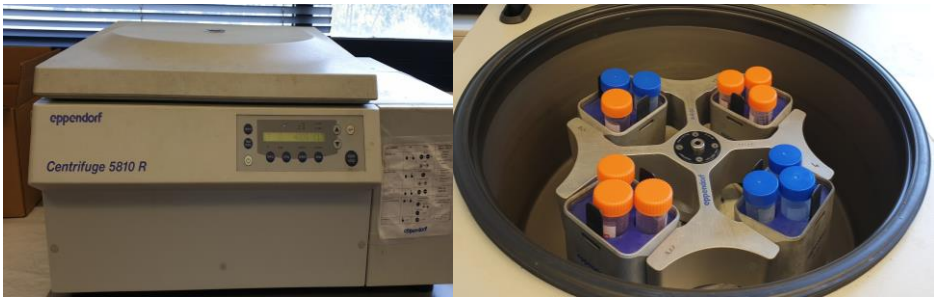


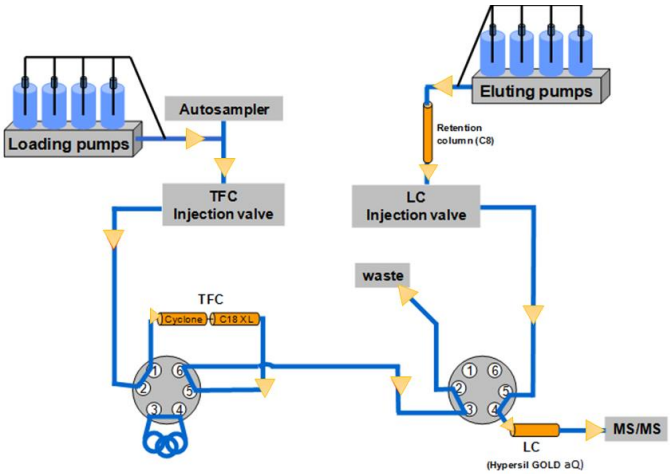
Figure 7: Centrifuge 5810 R, Eppendorf. On the right, the interior

The extracts were kept at 4 ºC until the next step. As before, the extracts were evaporated and then they were reconstituted in 100 µL with a mixture of water and methanol (9:1) so the final concentrations of the surrogate's internal standards were 20 ng/L. They were kept at -20 ºC until the instrumental analysis.

5.1.4.2. On-line turbulent flow chromatography coupled to LC-MS/MS

Extracts of sediments were purified by an on-line clean-up system (Thermo Scientific Aria TLX-1 (Thermo Fisher Scientific, USA)) based on turbulent flow chromatography. Two columns, Cyclone ™ for acids and C18 XL for sulfonates (50 mm x 0.5 mm, 60 µm particle size, 60 Å pore size) connected in tandem were used for the purification. Afterwards, the extracts were directly pumped to the analytical column Hypersil GOLD aQ (2.1 x 50 mm, 12 µm particle size; Thermo Fisher Scientific). The operative mode is shown in Figure 8.

A) Loading mode



B) Eluting mode

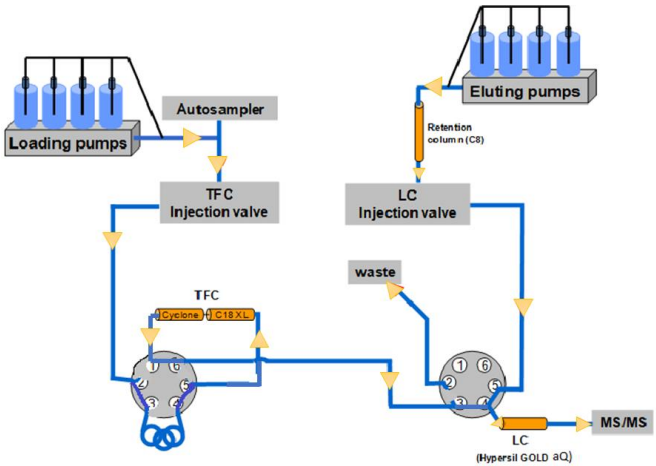


Figure 8: On-line system scheme: A) loading mode, B) eluting mode

Very brief, the samples were introduced to the TFC columns at a high flow-rate (1.5 mL/min) that generated a turbulence inside the columns facilitating the interaction between the active pores of the stationary phase and the analytes while the interfering substances were driven to the waste. Then, the analytes elution was achieved with a mixture of methanol and water and transferred to the LC column where the analytes were separated by reversed phase chromatography as described in Table 4. Again, 20mM ammonium acetate in water (solvent E) and 20mM ammonium acetate in methanol (solvent F) were the two mobile phases used.

In the loading mode, the mix solution acetone: isopropanol: acetonitrile (10:45:45) was used to clean hydrophilic and lipophilic compounds that could be present in the sediment samples These compounds could be retained in the column and could cause errors in the analysis. This way an effective purification was achieved and the system was equilibrated to the initial conditions. The solution of formic acid was at pH= 3.2 in which all the PFASs were in their ionic form in solution. The separation did not depend on the pH because its function was based in the loading sample and the cleaning of the columns. The injection volume was 10 µL. As before, methanol and initial mobile phase conditions were also analysed in parallel. After separation, the detection was carried out using a triple quadrupole analyser TSQ Quantiva (Thermo Fischer Scientific, USA) with an ESI source operating in negative conditions. Analyses were performed in triplicates.

Table 4: Chromatographic conditions for on-line analysis

	Loading pump								Eluting pump			
Step	Start	Sec	Flow (ml/min)	%A	%B	%C	%D	Step	Flow (ml/min)	Grad	%E	%F
1	0.00	20	1.50	100.0	-	-	-	Loading sample	0.40	Step	90.0	10.0
2	0.33	10	0.20	-	-	100.0	-	Cleaning matrix effects	0.40	Step	90.0	10.0
3	0.50	30	0.20	70.0	-	-	30.0	Transfer step	0.20	Step	90.0	10.0
4	1.00	90	0.20	-	-	-	100.0	Cleaning column I	0.40	Ramp	20.0	80.0
5	2.50	300	0.40	-	-	-	100.0	Cleaning column II	0.40	Ramp	10.0	90.0
6	7.50	30	0.40	-	100.0	-	-	Loading loop step	0.40	Step	10.0	90.0
7	8.00	60	0.40	20.0	-	-	80.0	Cleaning column III	0.40	Step	10.0	90.0
8	9.00	30	0.40	100.0	-	-	-	Cleaning column III	0.40	Ramp	90.0	10.0
9	9.50	90	0.40	100.0	-	-	-	Cleaning column III	0.40	Step	90.0	10.0

Loading pump:
Solvent A: water (pH: 3.2 with formic acid)
Solvent B: acetone: isopropanol: acetonitrile (10:45:45)
Solvent C: water
Solvent D: methanol

Eluting pump:
Solvent E: 20mM ammonium acetate in water
Solvent F: 20mM ammonium acetate in methanol

Acquisition was performed in SRM mode. The data processing was carried out with the software Xcalibur™ version 2.1 (Thermo Fischer Scientific Inc), specifically, the QUAN Browser. Figure 9 shows the instrument used in the analysis. Transitions, retention times and collision energy are summarized in Table 2. As it can be seen in this table, retention times of the PFASs in the analysis of waters and sediments are different because different analytical columns were used. Ion source properties are summarized in Table 5.

Figure 9: TFC-HPLC-QqQ instrument

Table 5: Ion source properties of the triple quadrupole analyser TSQ Quantiva

Ion source type	HESI	**Ion transfer Tube Temp (ºC)**	350	
Sheath Gas (Arb)	40	**Vaporizer Temp (ºC)**	300	
Aux Gas (Arb)	15	**Spray Voltage**	Positive ion (V)	3500
Sweep Gas (Arb)	1		Negative ion (V)	2500

6. RESULTS AND DISCUSSION

6.1. QUANTIFICATION METHOD

The LC-MS/MS method used for the determination of PFASs in waters and sediments was already optimized and validated [10,12]. Briefly, MLODs were between 0,01-4,06 ng/L for waters and between 0,08-2,66 ng/g for sediments, while MLOQs range was 0,02-1,75 ng/L for waters and 0,27-8,87 ng/g for sediments. The obtained results shown that MLOQ were lower in the off-line analysis but the cost of the analysis was higher due to the use of cartridges for each sample in the extraction step of the experimental procedure.

The quantification was carried out by calibration curve and isotopic dilution with surrogate internal standards. Solutions at concentrations of natives PFASs mix between 0.01 and 500 ng/L in LC-vial were prepared. Then they were spiked with internal surrogate standards to obtain a final concentration of 20 ng/L, the same as in the samples after extraction and pre-concentration. The correlation factor R^2 of calibration curves were always higher than 0,99 for all the substances. Some points were eliminated from the curve to obtain a bias from the calibration curve less than 29% for all the points.

During the quantification of the samples, different quality parameters should be accomplished: i) retention time of the samples equal to calibration curves; ii) the quotient between the area of the first transition and the second transition should be between the values calculated from the calibration curve; ii) area ratio (A native compound / A surrogate internal standard) obtained in the analysis equal or higher than the lowest point in the calibration curve. This last point is highly important to accomplish when the independent term of the calibration curve is negative since it could drive to an overestimation of the calculated concentration. Moreover, relative standard deviation (RSD) of the replicates must be lower than 30%.

Both in water and sediments samples, acquisition was performed in SRM to obtain two transitions and to confirm the presence of the analytes according to Commission Decision 2002/657/EC[19]. These transitions are summarized in Table 2. For the analyte identification, retention times in the sample and in the calibration curve should be in agreement, and two m/z

transitions should be confirmed. However, for some PFASs, especially the ones with short chains, it was difficult the observe the second transition for some replicates.

6.2. OCCURRENCE OF PFASS IN WATER SAMPLES

In water samples from Ebro Delta, only 4 of 18 studied PFASs were detected at quantifiable concentrations as it can be seen in Table 6. 3 PFCAs and 1 PFSA were detected in 7 river water samples and 4 beach water samples. Specifically, PFHxA was found in 10 samples, PFOA in 11, PFOS in 1 and PFDA in 9. The samples in which they have been detected corresponded to the first sampling campaign carried out in July 2018 (summer). So, seasonal variation was noticed meaning that the environmental conditions had an important influence on these compounds.

Table 6: Results of all the water samples

	Concentration (ng/L) (%RSD)								
Code	**PFBA**	**PFPeA**	**PFBS**	**PFHxA**	**PFHpA**	**PFHxS**	**PFOA**	**PFNA**	**PFDS**
R1 V	<MLOQ	<MLOQ	<MLOQ	7.14(2.86)	<MLOQ	<MLOQ	5.17(17.35)	<MLOQ	<MLOQ
R2 V	<MLOQ	<MLOQ	<MLOQ	2.24(2.60)	<MLOQ	<MLOQ	5.80(9.60)	<MLOQ	<MLOQ
R3 V	<MLOQ	<MLOQ	<MLOQ	0.28(12.57)	<MLOQ	<MLOQ	4.93(9.36)	<MLOQ	<MLOQ
R5 V	<MLOQ	<MLOQ	<MLOQ	7.18(12.18)	<MLOQ	<MLOQ	7.07(4.55)	<MLOQ	<MLOQ
R6V	<MLOQ	<MLOQ	<MLOQ	4.24(1.45)	<MLOQ	<MLOQ	5.33(7.58)	<MLOQ	<MLOQ
R7 V	<MLOQ	<MLOQ	<MLOQ	4.94(6.68)	<MLOQ	<MLOQ	5.93(6.38)	<MLOQ	<MLOQ
R8 V	<MLOQ	<MLOQ	<MLOQ	3.78(9.01)	<MLOQ	<MLOQ	3.83(3.01)	<MLOQ	<MLOQ
B10 V	<MLOQ	<MLOQ	<MLOQ	14.88(1.09)	<MLOQ	<MLOQ	18.79(3.92)	<MLOQ	<MLOQ
B11 V	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	2.82(11.26)	<MLOQ	<MLOQ
B12 V	<MLOQ	<MLOQ	<MLOQ	0,86(12.86)	<MLOQ	<MLOQ	1.77(11.98)	<MLOQ	<MLOQ
B13 V	<MLOQ	<MLOQ	<MLOQ	2.41(18.84)	<MLOQ	<MLOQ	2.19(1.94)	<MLOQ	<MLOQ
B14 V	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
B15 V	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
B16 V	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
B17 V	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R1 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R2 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R3 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R5 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R6 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R7 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
R8 I	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM_{HPLC}	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM1	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	0.15(28.28)	<MLOQ
MM2	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	0.12(1.00)	<MLOQ
MM3	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM4	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	0.18(33.33)	<MLOQ
MM5	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	0.24(25.00)	<MLOQ
MM6	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM7	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM8	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
MM9	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	0.30(20.00)	<MLOQ

Code	PFOS	PFDA	PFUnA	PFOSA	PFDoA	PFTrA	PFTeA	PFHxDA	PFODA
R1 V	<MLOQ	0.53(21.65)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R2 V	<MLOQ	1.17(13.09)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R3 V	<MLOQ	0.75(9.43)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R5 V	<MLOQ	0.95(7.44)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R6V	8.53(1.79)	0.63(18.23)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R7 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R8 V	<MLOQ	0.55(12.86)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B10 V	<MLOQ	4.46(14.80)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B11 V	<MLOQ	1.05(20.20)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B12 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B13 V	<MLOQ	0.15(28.28)	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B14 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B15 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B16 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
B17 V	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R1 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R2 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R3 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R5 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R6 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R7 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
R8 I	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM_{HPLC}	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM1	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM2	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM3	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM4	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM5	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM6	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM7	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM8	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD
MM9	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD

MM: Mar Menor, V: Summer campaign, I: Winter campaign, R: River, B: Seawater

Beach Fangar Bay (Port d'Illa de Mar) corresponding to the sample site 10, was the area with the highest accumulation concentration of PFASs. This sampling site is characteristic since it affords the commercial harbour of "muscleres" (mussels aquaculture), one of the most important aquacultures in Mediterranean Sea. Comparing those results with samples from Mar Menor, these last ones were at lower levels and only PFNA was detected at quantifiable concentrations in 5 samples, possible as a contamination due to the consequence of their fishing tools.

Finally, as it has been explained before, it is important to carry out blank extracts in parallel with the samples in order to monitor cross-contamination. In the case of water samples, a cross-contamination of PFHxA was detected as it can be seen in the chromatogram of blank sample in Figure 10. In this case, it was necessary to subtract their contribution in all water samples.

In figure 11, extract ions chromatograms of some PFASs present in the sample 6 are presented (PFHxA, PFOA and PFDA).

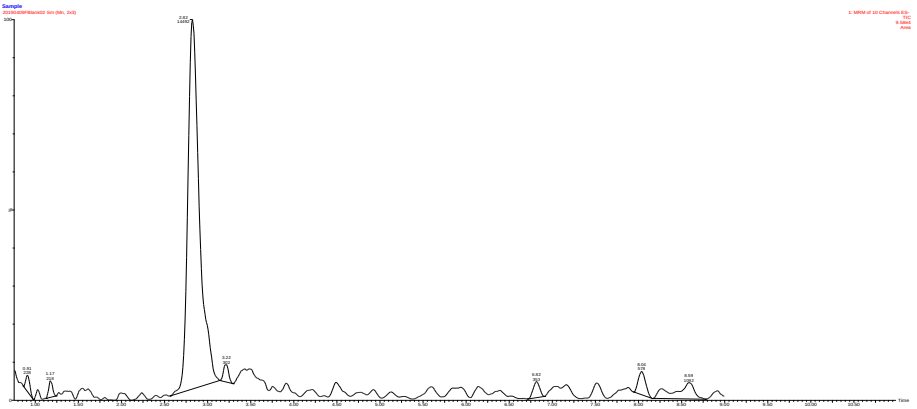


Figure 10: Chromatogram of a blank sample which contains PFHxA

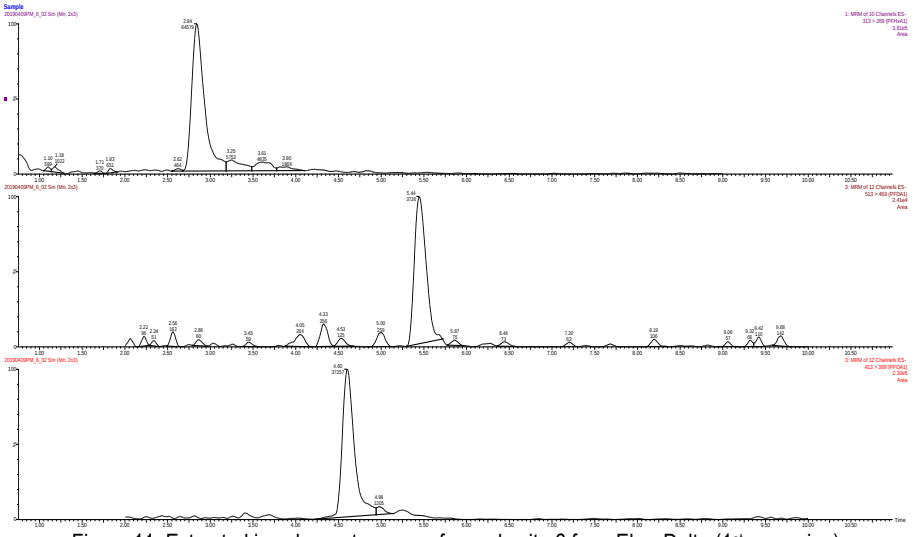


Figure 11: Extracted ion chromatograms of sample site 6 from Ebro Delta (1st campaign)

6.3. OCCURRENCE OF PFASS IN SEDIMENT SAMPLES

Sediments were only studied in Ebro Delta. In this case, only 5 of 18 PFASs studied were detected and quantified while most of the analytes were below MLOD or even MLOQ. Detailed concentrations for each compound in sediments samples are summarised in Table 7. In this

case, similar profile than water samples from Ebro Delta was observed since the major part of samples higher than MLOQ corresponded to the first sampling campaign carried out in July 2018 (summer). Again, seasonal variation was noticed meaning that the environmental conditions had an important influence on the PFASs.

Table 7: Results of all the sediment samples

	Concentration (ng/g) (%RSD)								
Code	**PFBA**	**PFPeA**	**PFBS**	**PFHxA**	**PFHpA**	**PFHxS**	**PFOA**	**PFNA**	**PFOS**
R1 V	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOQ
R5 V	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
R6 V	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOQ
R7 V	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD
R8 V	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD
B10 V	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B11 V	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B12 V	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B13 V	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B14 V	>LOL	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
B15 V	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
B16 V	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD
B17 V	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
R2 I	<MLOQ	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
R5 I	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
R7 I	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
R9 I	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B14 I	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B15 I	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B16 I	>LOL	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B17 I	80.25 (0.68)	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD

Code	**PFDA**	**PFDS**	**PFUnA**	**PFOSA**	**PFDOA**	**PFTrA**	**PFTeA**	**PFHxDA**	**PFODA**
R1 V	<MLOD	<MLOQ	<MLOQ	<MLOD	1.00(13.85)	<MLOQ	1.39(2.45)	<MLOD	<MLOD
R5 V	<MLOD	<MLOD	0.15(2.13)	<MLOD	0.66(0.75)	<MLOD	0.68(29.55)	<MLOD	<MLOD
R6 V	<MLOD	<MLOD	<MLOQ	<MLOD	0.54(8.96)	<MLOQ	<MLOQ	<MLOD	<MLOD
R7 V	<MLOD	<MLOD	0.08(1.59)	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
R8 V	<MLOD	<MLOD	<MLOD	<MLOD	0.92(15.	<MLOD	<MLOD	<MLOD	<MLOD
B10V	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B11V	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B12V	<MLOD	0.61(0.50)	0.12(21.43)	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
B13V	<MLOD	<MLOD	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
B14V	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD
B15V	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
B16V	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B17V	<MLOD	<MLOD	<MLOD	<MLOD	0.45(1.40)	<MLOD	<MLOD	<MLOD	<MLOD
2 I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOD	<MLOD	<MLOD
5 I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
7 I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
9 I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	0.62(6.50)	<MLOD	<MLOD
B14I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B15I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B16I	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
B17I	<MLOD	<MLOD	0.05(5.43)	<MLOD	<MLOD	<MLOD	<MLOQ	<MLOD	<MLOD

V: Summer campaign, I: Winter campaign, R: River, B: Seawater

It is noteworthy to mention that PFBA was above the limit of linearity (LOL) in 9 samples from the first sampling campaign (Table 7) and they could not be quantified because of time limitations. In order to quantify these samples, the final extract would be diluted between 1/2 and 1/10.

Figure 12, shows an example of extracted ion chromatograms of some PFASs present in the sample 1 (PFPeA, PFHxA, PFHxS, PFOS, PDUnA, PFDoA and PFTeA) although just two of them were quantifiable (PFDoA and PFTeA) while the other were below the MLOQ.

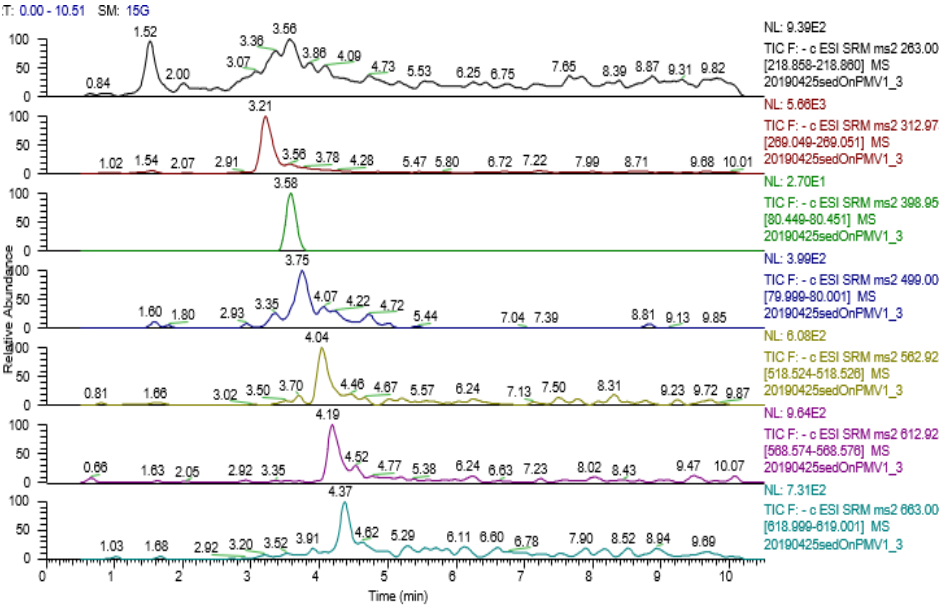


Figure 12: Extracted ion chromatograms of sample site 1 from Ebro Delta (1st campaign)

6.4. COMPARISON OF PFASS IN THE STUDIED ENVIRONMENTAL SAMPLES

Figure 13 shows the accumulation concentration of each analyte in environmental samples from Ebro Delta. PFASs were found specially in the river samples due to the presence of industries that causes pollution. The occurrence of PFASs in the environment was generally higher in water than in sediments. That is because in water, these substances are soluble whereas in sediments they have to be adsorbed so it is easier to find them in water.

Generally, the presence of PFASs in the environment decreased in winter except in Alfacs Bay open sea 2 (17), where an increase of the concentration of PFASs, in this case PFBA, was

observed in sediments. In the case of PFOS, this was only detected in one water sample (6). As mentioned before, in 2009 PFOS was added into Annex B of the Stockholm Convention on POPs because of its harmful effects and high persistency in the environment, and over the years its concentration has been decreasing. In a previous study in Ebro Delta by Pignotti et al. (2017)[10], PFOS was only found in Beach Alfacs Bay (code 14 in present work) during autumn season and at concentration of 1,09 ng/L while during summer season it was detected in almost all sample points but at lower concentrations. During winter season PFOS was not detected in any sample.

In sediments, PFOS was only detected at low concentrations before Amposta during winter season and in Beach Alfacs Bay (code 14) and Alfacs open Bay sea 1 (code 16). Taking these results into account, our hypothesis is that the emission of PFOS in this specific area has been decreasing during the last 3 years and, consequently, its presence in this environment.

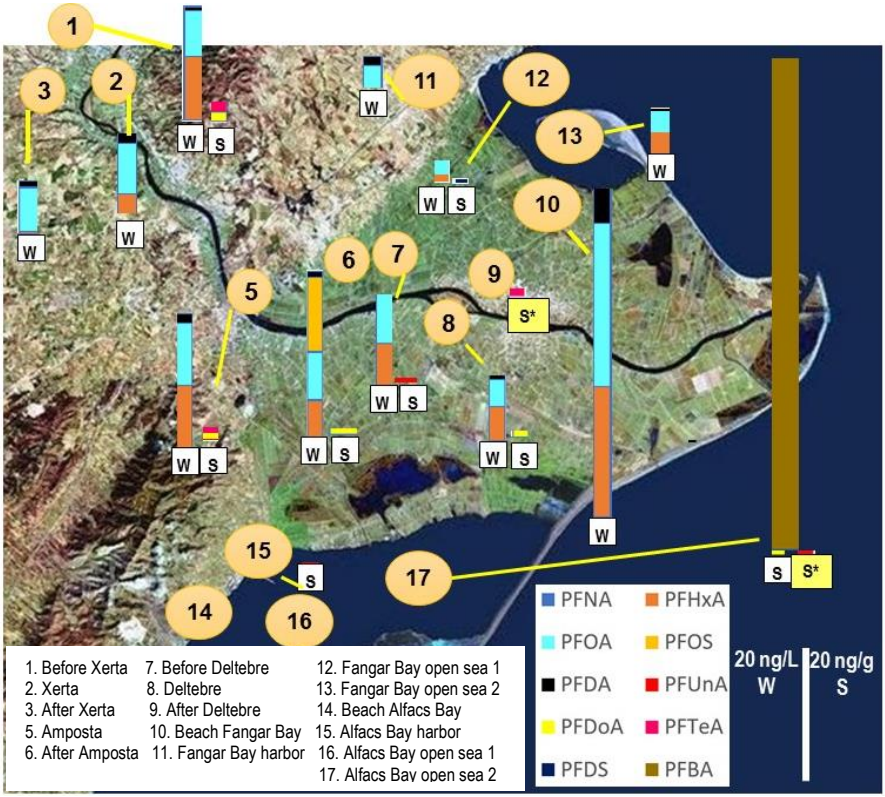


Figure 13: Sample sites and accumulated concentration of PFASs in water (W) and sediments (S) from Ebro Delta. S* correspond to the 2nd sampling campaign.

PFHxA, PFOA, PFDA, with 6, 8 and 10 carbon chains respectively, were substances found in water. PFOA was the most detected one with concentrations between 1.77 and 18.79 ng/L which are in agreement with previous study in the same area[10]. PFDA was the longer chain analyte detected, even though at low concentrations (0.15-4.46 ng/L). And as mentioned before, the amount of PFASs detected in winter season 2019 were lower due to the dilution factor after the rainy season.

In sediment samples, with the exception of PFBA, all the PFASs found had long carbon chains: PFDS, PFUnA, PFDoA and PFTeA although PFDS was only detected in one sample. PFBA was found at high levels probably due to the restriction of PFOA and PFOS and generally the increase of short chain polyfluoroalkyl congeners production that, once in the environment, ends-up in shorter carbon chain PFAS like PFBA after environmental degradation.

In general, the compounds detected in water samples were shorter-chain PFASs compared to the compounds detected in sediments that have longer carbon chain. These results are in agreement with PFASs physicochemical properties since the longest the carbon-chain the higher the sorption capacity on sediments. In addition, heavy rains during autumn and winter seasons can cause the resuspension of sediments and, consequently, it could reduce the concentration of more soluble PFASs from sediments.

In conclusion, PFASs were mainly detected in samples from the summer campaign indicating that the environment conditions have a great influence over the occurrence of these substances. Also, in the summer, touristic human activities and the higher number of inhabitants could cause an increase of the indirect sources of PFASs in the Ebro Delta.

6.5. FUTURE TRENDS

Nowadays, the industry has increased the production of short chain PFASs to replace the ones with long chains due to their lowest persistence in the environment and lower bioaccumulation risks. Over the time, they could cause similar negative effects in the ecosystem, so in the near future it is important to evaluate the occurrence and fate of shorter chain PFASs as well as their related eco-toxicity.

7. CONCLUSIONS

The occurrence of 18 PFASs in environmental samples has been assessed by an off-line SPE extraction followed by LC-MS/MS in the case of waters and LSE extraction followed by on-line TFC coupled to LC-MS/MS in the case of sediments.

In water samples from Ebro Delta, only 4 of 18 PFASs studied were detected and quantified whereas in sediments samples 5 analytes were detected.

In sediment samples, with the exception PFBA, all the PFASs found had long carbon chains: PFDS, PFUnA, PFDoA and PFTeA. The occurrence of PFASs in the environment was generally higher in waters than in sediments but, in general, most of analytes were below MLOD or even MLOQ. It has been seen that in water samples, shorter-chain PFASs were found while in sediments longer-chain were more abundant because the longest ones are less soluble in water.

In both water and sediment samples, seasonal variation was noticed meaning that the environmental conditions had an important influence on the PFASs. The amount of PFASs found in winter were minor maybe due to the dilution after the rainy season.

Comparing with previous works from the same area, the concentrations of PFASs detected in the present work are lower, especially PFOS, which use in the industry was prohibited in 2009.

Lastly, Ebro Delta and Mar Menor waters were compared and it was observed that the occurrence of PFASs in the first one was higher. PFNA was the only analyte detected in Mar Menor sample waters.

8. REFERENCES AND NOTES

1. Kannan, Kurunthachalam. Perfluoroalkyl and Polyfluoroalkyl Substances: Current and Future Perspectives. *Environmental Chemistry*. (2011). 8. 333-338.

2. Stockholm Convention. (n.d). Retrieved from http://chm.pops.int/

3. Buck, Robert C.; Franklin, James; Berger, Urs; Conder, Jason M.; Cousins, Ian T.; de Voogt, Pim; Jensen, Allan Astrup; Kannan, Kurunthachalam; Mabury, Scott A.; van Leeuwen, Stefan P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins, *Integrated Environmental Assessment and Management* (2011), 7(4), 513-541.

4. Lewandowski G., Meissner E., Milchert E. Special applications of fluorinated organic compounds, *Journal of Hazardous Materials* (2016), Volume 136, Issue 3, Pages 385-391.

5. Llorca M, Farré M., Sebastián D. Tavano, Alonso B., Koremblit G., Barceló D. Fate of a broad spectrum of perfluorinated compounds in soils and biota from Tierra del Fuego and Antarctica. *Environmental Pollution*. (2012), 163, 158-166.

6. Olga S. Arvaniti, Athanasios S. Stasinakis. Review on the occurrence, fate and removal of perfluorinated compounds during wastewater treatment. *Science of The Total Environmen*t (2015), volumes 524–525, pages 81-92.

7. Ahrens, L.; Vorkamp, K.; Lepom, P.; Bersuder, P.; Theobald, N.; Ebinghaus, R.; Bossi, R.; Barber, J.L. and McGovern, E. Determination of perfluoroalkyl compounds in water, sediment, and biota. *ICES Techniques in Marine Environmental Sciences* (2010), No. 48, 16pp

8. Wenxiu Liu, Jingyi Wu, Wei He & Fuliu Xu. A review on perfluoroalkyl acids studies: Environmental behaviors, toxic effects, and ecological and health risks, *Ecosystem Health and Sustainability* (2019), 5:1, 1-19.

9. Llorca M., Schirinzi G., Martínez M., Barceló D., Farré M. Adsorption of perfluoroalkyl substances on microplastics under environmental conditions. *Environmental pollution (*2018). 235, 680-691.

10. Pignotti E., Casas G., Llorca M., Tellbüscher A., Almeida D., Dinelli E., Farré M., Barceló D. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). *Science of The Total Environment*. (2017), 607-608, 933-943.

11. U.S. Environmental Protection Agency. (n.d). Retrieved from https://www.epa.gov/

12. Llorca M., Farré M., Picó Y., Müller J., P. Knepper T., Barceló D. Analysis of perfluoroalkyl substances in waters from Germany and Spain. *Science of The Total Environmen*t. (2012). Volume 431, Pages 139-150.

13. National Food Agency, Sweden. PFAS in drinking water and fish - risk management. Reviewed 12/2018. Retrieved from https://www.livsmedelsverket.se/en/food-and-content/oonskade-amnen/miljogifter/pfas-in-drinking-water-fish-risk-management

14. Technical Fact Sheet – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA). EPA (United States Environmental Protection Agency, (11/2017). Retrieved from https://www.epa.gov/sites/production/files/201712/documents/ffrrofactsheet_contaminants_pfos_pfoa_11-20-17_508_0.pdf

15. Matthias Onghena, Y. Moliner-Martinez, Yolanda Picó, P. Campíns-Falcó, Damià Barceló, Analysis of 18 perfluorinated compounds in river waters: Comparison of high-performance liquid chromatography tandem mass spectrometry, ultra-high-performance liquid chromatography–tandem mass spectrometry and capillary liquid chromatography–mass spectrometry, *Journal of Chromatography A*, (2012). Volume 1244, Pages 88-97.

16. Feng H., Ruan Y., Zhang K., K.S. Lam P. Current analytical methodologies and gaps for per- and polyfluoroalkyl substances determination in the marine environament. *TrAC Trends in Analytical Chemistry*. (2019).

17. Llorca M., Farré M., Picó Y., Barceló D. Perfluorinated Compounds in Food: A Global Perspective, *Critical Reviews in Food Science and Nutrition.* (2011). Volume 51, Pages 605-625.

18. Microplastics and microcontaminants in the Mediterranean coast. (n.d). Retrieved from https://www.plas-med.es/

19. 2002/657/EC, C.D., 2002. Council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of European Communities. L221, 8

9. ACRONYMS

PFASs	Perfluoroalkyl substances
PFBA	Perfluorobutanoic Acid
PFPeA	Perfluoropentanoic Acid
PFHxA	Perfluorohexanoic Acid
PFHpA	Perfluoroheptanoic Acid
PFOA	Perfluorooctanoic Acid
PFNA	Perfluorononanoic Acid
PFDA	Perfluorodecanoic Acid
PFUdA	Perfluoroundecanoic Acid
PFDoA	Perfluorododecanoic Acid
PFTrDA	Perfluorotridecanoic Acid
PFTeDA	Perfluorotetradecanoic Acid
PFHxDA	Perfluorohexadecanoic Acid
PFODA	Perfluorooctadecanoic Acid
PFBS	Perfluorobutane Sulfonate
PFHxS	Perfluorohexane Sulfonate
PFOS	Perfluorooctane Sulfonate
PFDS	Perfluorodecane Sulfonate
PFOSA	Perfluorooctane Sulphonamide
POPs	Persistent Organic Pollutants
US. EPA	United States Environmental Protection Agency
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SPE	Solid Phase Extraction

SLE	Solid Liquid Extraction
UAE	Ultrasound Assisted Extraction
TFC	Turbulent Flow Chromatography
APCI	Atmospheric-Pressure Chemical Ionization
GC	Gas Chromatography
EI	Electron Ionization
CI	Chemical Ionization
LC	Liquid Chromatography
HPLC	High Performance Liquid Chromatography
UHPLC	Ultra-High Performance Liquid Chromatography
MS/MS	Tandem Mass Spectrometry
ESI	Electrospray Ionization
QqQ	Triple Quadrupole
SRM	Selection Reaction Monitoring
ACN	Acetonitrile
PP	Polypropylene
MPLs	Microplastics
MLOD	Method Limit of Detection
MLOQ	Method Limit of Quantification