UNIVERSITAT DE BARCELONA

Distribution of perfluoroalkyl substances in the environment

Pere Colomer Vidal

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PhD Thesis

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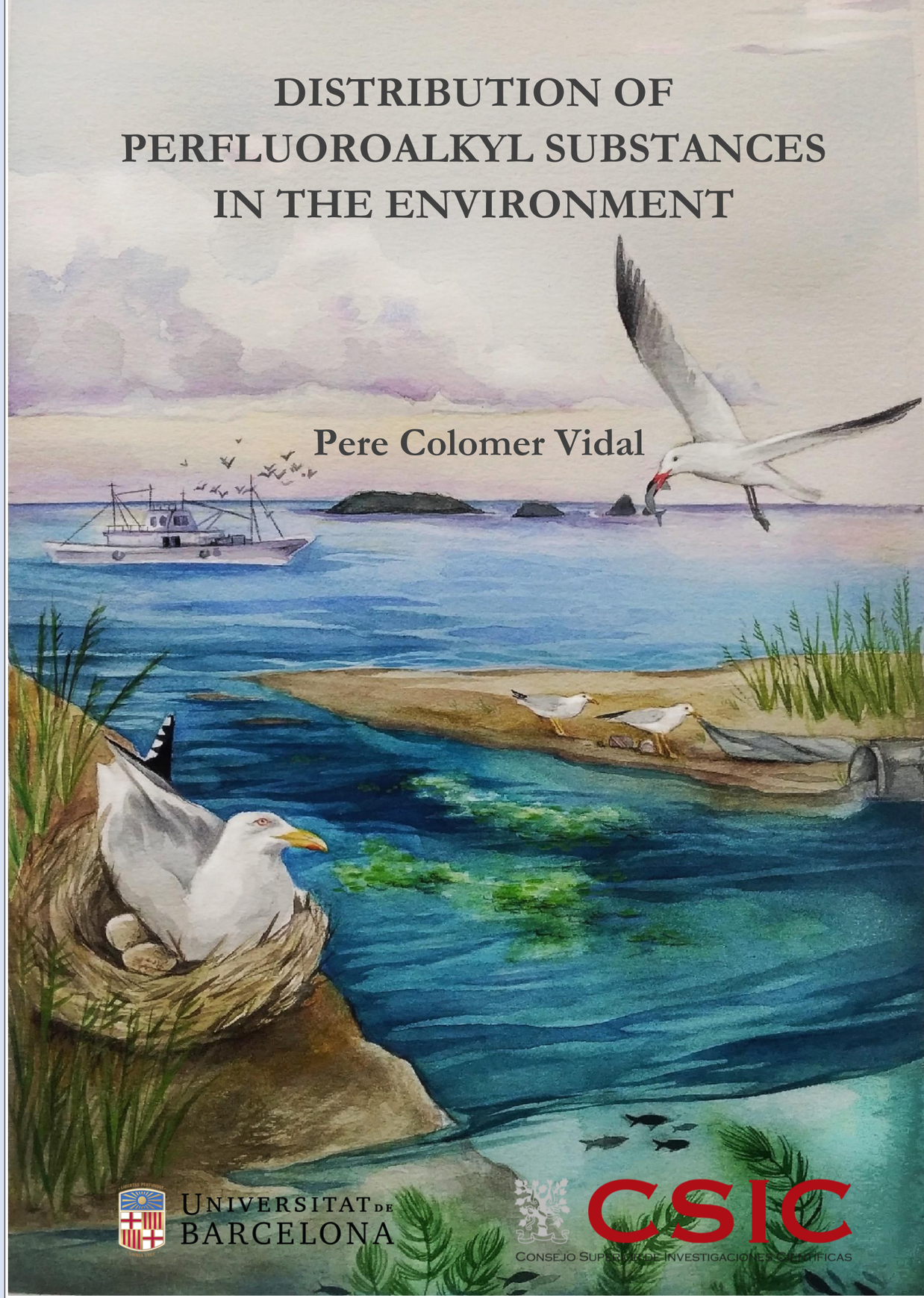
DISTRIBUTION OF PERFLUOROALKYL SUBSTANCES IN THE ENVIRONMENT



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The doctoral program in

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Distribution of perfluoroalkyl substances in the environment

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“Well, I must endure the presence of a few caterpillars if I wish to become acquainted with the butterflies.”

Antonie de Saint-Exupéry, The Litte Prince

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RESUME

Perfluoroalkyl substances (PFASs) are a large group of man-made chemicals that are widely used throughout society and found in the environment. These compounds are characterized by perfluorinated carbon chains of varying lengths with hydrophobic properties and containing an external polar and hydrophilic head. The difference between perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) are found on the polar head of the chemicals. In general, the characteristics of PFASs are based on the length of the perfluorinated carbon chain that they contain. As the number of perfluorinated carbons increases in the molecule, the water solubility decreases and the better the surfactant properties, but will be more toxic for the environment due to their bioaccumulation in the organisms.

In recent years, the interest in PFASs has been growing due to their presence in humans and in wildlife species even from remote locations, which suggests a widespread global distribution of these pollutants. The highest concentration of PFASs has typically been documented in areas with direct industrial emissions. The main purpose of this Doctoral Thesis is to study the distribution and behavior of PFASs in different environmental compartments and to elucidate the interactions among them. In the first study of this Thesis, seventeen PFASs have been analyzed in the water-sediment-plant system along the Dongzhulong and Xiaoqing Rivers in China. The study area is affected by a fluoropolymer facility that belongs to the Dongyue group and is currently one of the major facilities of polytetrafluorethylene production in China. Some studies indicate a presence of PFASs in sediments and soils and these can be a source of pollution for wildlife, and humans. The second study is aimed to evaluate the environmental occurrence of PFASs in sediments, soils, and wildlife in the marine environment surrounding the Chafarinas

Islands (South Spain) and the impact on gulls. The third study that composes this Thesis is aimed to evaluate the occurrence and 10-year temporal trend of seventeen PFAS in eggs of two gull species (*Larus michahellis* and *Larus audouinii*). These species are used as bioindicators of environmental pollution of Spain.

The results of this Thesis show that the fate of the PFASs in the environment is explained by their physicochemical properties and the characteristics of the different study matrices. In freshwater systems, high amounts of PFASs in water and sediments close to the industrial discharge were detected, and concentrations decreased along the river due to dilution. In the water-sediment system, the results suggest that long-chain PFASs accumulated in sediment whereas short-chain PFASs remained in water all along the river. When including plants in the system, PFASs were taken up by plants and translocated in the different plant compartments, and the uptake mechanisms differed among plant species. Floating species show a higher concentration among plants because easily translocate long-chian PFASs direct from the water. Rooted species must compete with the sediment for PFASs uptake. Moreover, long-chain PFASs remain accumulated in the root compartment because of protein affinity while short-chain PFASs are more mobile and can be translocated to shoots. In the marine environment of the Chafarinas Islands, low levels of PFASs detected in soils, sediments, fish, and mussels reflected that the area is not directly impacted by PFASs. In this Thesis we also have estimated the bioaccumulation potential of PFASs, using gulls. We have estimated the intake based on fish-diet in gulls from Chafarinas. We observe that the release of PFOS to the egg is 4.5% of the intake and we provide the basis for using gull eggs as biomonitors. In a final study of this Thesis, we evaluated the presence of PFASs in four main gull colonies in Spain. When comparing gull colonies, eggs from the Ebro Delta and Medes Islands, both located

in the North-Eastern Mediterranean Sea, had a similar distribution of PFASs, while in Chafarinas and Atlantic Islands these PFASs were present at lower concentration levels and variability. In the Ebro Delta colonies, concentrations in eggs from *L. audouinni* were significantly higher than those found in *L. michahellis*, suggesting that fish diet influences PFAS bioaccumulation. Overall, ∑PFAS decreased in the 10-year study period but for individual compounds, trends were colony-species dependant.

This thesis permits to increase the knowledge about the processes that rule the behavior of PFASs in water, sediment, soil, and biota. Also, this thesis demonstrates the advantage of performing systematic monitoring schemes to determine the presence and fate of PFASs in the environment.

LIST OF ABBREVIATIONS

AA-EQS	Annual average - environmental quality qtandards
AFFF	Aqueous fire-fighting foams
APFN	Ammonium perfluorononanoate
APFO	Ammonium perfluorooctanoate
CEPA	Canadian Environmental Protection Act
CLC	Capillary liquid chromatography
CRC CARE	Cooperative Research Centre for Contamination Assessment and Remediation of the Environment
DLLME	dispersive liquid-liquid microextraction
DWTP	Drinking water treatment plants
EC_{50}	Half maximal effective concentration
EPA	Environmental Protection Agency
EQS	Environmental Quality Standards
EQS	Envrionmental quality stardards
ESI	Electrospray ionization
FEP	Perfluorinated ethylene-propylene copolymers
FTI	Fluorotelomer iodide
FTO	Fluorotelomer olefins
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GIG	Guangzhou Instituteo of Geochemistry
HEPA	Heads of the Environmental Protection Agencies
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IDAEA	Institute of EnvironmentalAssessment and Water Research
IPE	Ion-pair extraction
K_{aw}	air-water partition coefficient
K_d	Sediment-water partition coefficient
K_{ow}	Octanol-water partition coefficient
LC	Liquid chromatography
LC_{50}	Half lethal concentration
LC-MS	Liquid chromatography–mass spectrometry

LLE	Liquid-liquid extraction
L_{oa}	Octanol-air partition coefficient
LOAEL	Lowest observed adverse effect level
LOD	Limit of Detection
LOEC	Lower observed effect concentrations
LOQ	Limit of Quantification
LOQ	Limits of quantification
LVI-HPLC	Large volume injection-high performance liquid chromatography
MAC-EQS	Maximum allowed concentration - environmental quality qtandards
MS	Mass spectrometry
NOAEL	No observed adverse effect level
NOEC	No observed effect concentrations
NPCA	Norwegian Pollution Control Authority
OECD	Organization for Economic Co-operation and Development
OSPAR	Oslo-Paris Convention
PCTSR	Prohibition of Certain Toxic Substances Regulation
PFA	Perfluoroalkoxyl polymers
PFAI	Perfluoroalkyl iodide
PFAS	Perfluoroalkyl substances
PFBA	Pentafluorobenzoic acid
PFBS	Perfluorobutane sulfonic acid
PFCA	Perfluorinated carboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFPeA	Perfluoropentanoic acid
PFSAs	Perfluoroalkyl sulfonic acids
PFTrDA	Perfluorotridecanoic acid
PFUnDA	Perfluoroundecanoic acid

PLE	Pressurised liquid extraction
POCF	Perfluorooctane carbonyl fluoride
POPs	Persistent Organic Pollutants
POSF	Perfluorooctane sulfonyl fluoride
PTFE	Polytetrafluoroethylene
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
QqQ	Triple-quadrupole
QTOF	Quadrupole Time-of-flight
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RP-LLE	Reverse phase liquid-liquid extraction
SLE	Solid liquid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TFE	Tetrafluoroethylene
TOF	Total organofluorine analysis
TOP-assay	Total precursor assay
TRV	Toxicological references values
UHPLC	Ultra-high-performance liquid chromatography
UNEP	United Nations Environment Program
UPLC	Ultraperformance liquid chromatography
WA DER	Western Australia Department of Water and Environmental Regulation
WWTP	Wastewater treatment plant

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INTRODUCTION

CHAPTER 1

1.1. PERFLUOROALKYL SUBSTANCES

1.1.1. Origin, use, and production

Perfluoroalkyl substances (PFASs) are synthetic compounds with an anthropogenic origin. PFASs are mainly used as a processing aid to produce polytetrafluoroethylene (PTFE), which is widely known as Teflon [1]. Teflon was accidentally invented in 1938 from the polymerization of tetrafluoroethylene (TFE) by Roy J. Plunkett (1910 – 1994) in DuPont's Jackson Laboratory in Deepwater, New Jersey. In 1949, 3M in Lake Elmo, Minnesota, started with the manufacture of PFOS, which was used as surfactant and in the production of polymers [2], and in 1951 began using perfluorooctanoic acid (PFOA) in its manufacturing process[1]. Due to the health implications of PFOS, from 1975 onward other PFASs as the ammonium salt of perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnDA), and perfluorotridecanoic acid (PFTrDA) also were manufactured [3]. Some decades later, in 1998, the Environmental Protection Agency (EPA) alerted about the risk of PFOS after being detected in human serum from people around the production facilities [4,5]. The article entitled "Global distribution of Perfluorooctane Sulfonate in Wildlife" was published by John P. Giesy and Kurunthachalam Kannan in Environmental Science & Technology. The study measured the levels of PFOS in the tissue of fish, birds, and marine mammals from urbanized areas and remote locations, and concluded that it was widespread in the environment, with higher concentrations in urbanized areas compared to remote locations, and also concentrations of PFOS in predatory animals was greater than the concentration in their

[1] Web page https://www.sciencehistory.org/historical-profile/roy-j-plunkett

diets suggesting bioaccumulation in higher trophic levels. The 3M company stopped manufacturing perfluorooctane sulfonic acid (PFOS) in 2000 and the ammonium salt of PFOA in 2002. Since 2006 the EPA brokered a voluntary agreement with DuPont and eight other major companies to phase-out the use of PFOS and PFOA in the United States (US) and long-chain PFCAs and their precursors from emissions and products by 2015. This initiative replaced the common PFASs for short-chains PFASs. Figure 1.1 illustrate a resume timeline of the production of PFASs.

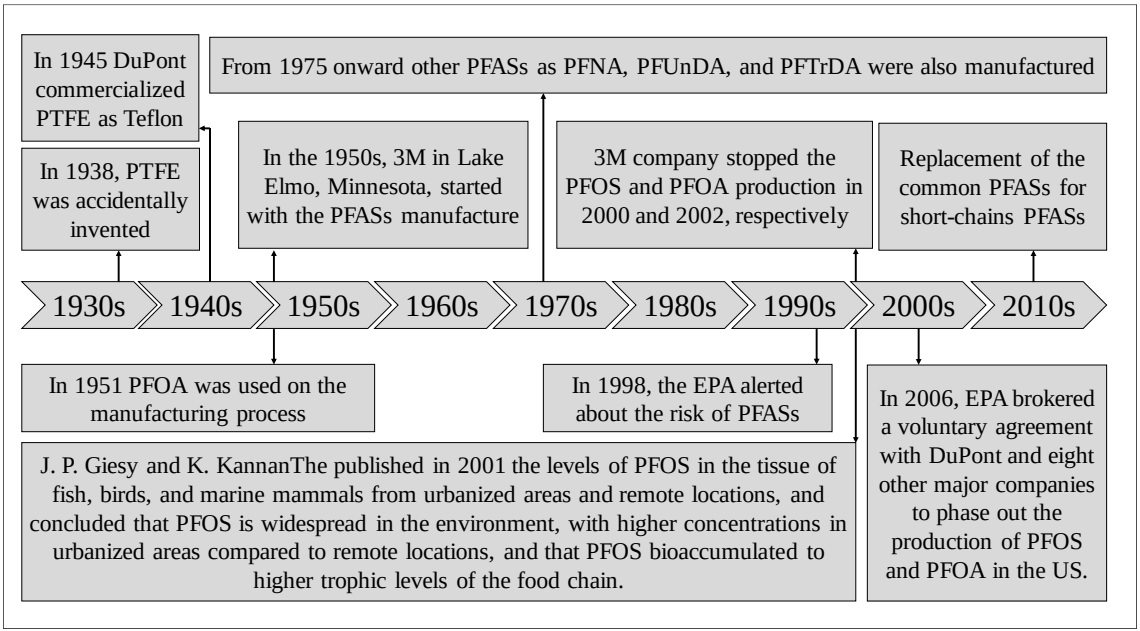

Figure 1.1. Graphical resume of the invention and origin of the PFASs production from the 1930s to 2010s.

PFASs are used as surfactants to reduce the surface tension of a liquid, between two liquids, or between a liquid and a solid [3]. This property is used in the manufacture of plastics, rubber, compression mold release coating, plumbing fluxing agents, fluoroplastic coating, composite resins, and flame retardants for polycarbonate [6]. The chemical industry also uses PFASs as a processing aid in the polymerization of fluoropolymers, the production of chlorine and sodium hydroxide, and other chemicals

including solvents. Fire-fighting foams also contain PFASs for extinguishing fire in inflammable liquids like oil, jet fuel, other non-water-soluble hydrocarbons, alcohols, and acetone [7]. Also, PFASs are used in the textile and leather industry and consumer products in the coating to repel water, oil, and stains in products as umbrellas, tents, sails, architectural material, carpets, and upholstery. The paper industry also uses PFASs as a surface coating to repeal grease and moisture in non-food paper packing and food-contact materials [3]. Other uses of PFASs included metal plating and etching for corrosion prevention, mechanical wear reduction, aesthetic enhancement, and post-plating cleaner; and on biocides as an active ingredient in plant growth regulators or ant bites and coadjuvant in pesticides formulations [8].

The production of PFASs varies with the type of manufacture, where the telomerization process produces primarily or exclusively linear isomers, a mixture of homologs and PFCAs, and the electrochemical fluorination process produces a mixture of branched and linear isomers plus a mixtures of homologs [3]. For example, PFOS has an origin in the electrochemical fluorination process from perfluorooctane sulfonyl fluoride (POSF) and is generally used as a surfactant. PFOA has been produced by electrochemical fluorination from perfluorooctane carbonyl fluoride (POCF), and by telomerization from perfluoroalkyl iodide (PFAI), and used in the production of polytetrafluoroethylene (PTFE), perfluorinated ethylene-propylene copolymers (FEP), and perfluoroalkoxyl polymers (PFA). PFNA has been produced by telomerization from PFAI, fluorotelomer iodide (FTI), and fluorotelomer olefins (FTO), and used in the production of polyvinylidene fluoride (PVDF). PFCAs homologs, especially PFUnDA and PFTrDA are present as ingredients or impurities of the manufacturing processes [6,9]. Figure 1.2

summarize the production process, characteristics, starting material, intermediates, end products, and uses of the major PFASs based on Wang et al., (2014) [9].

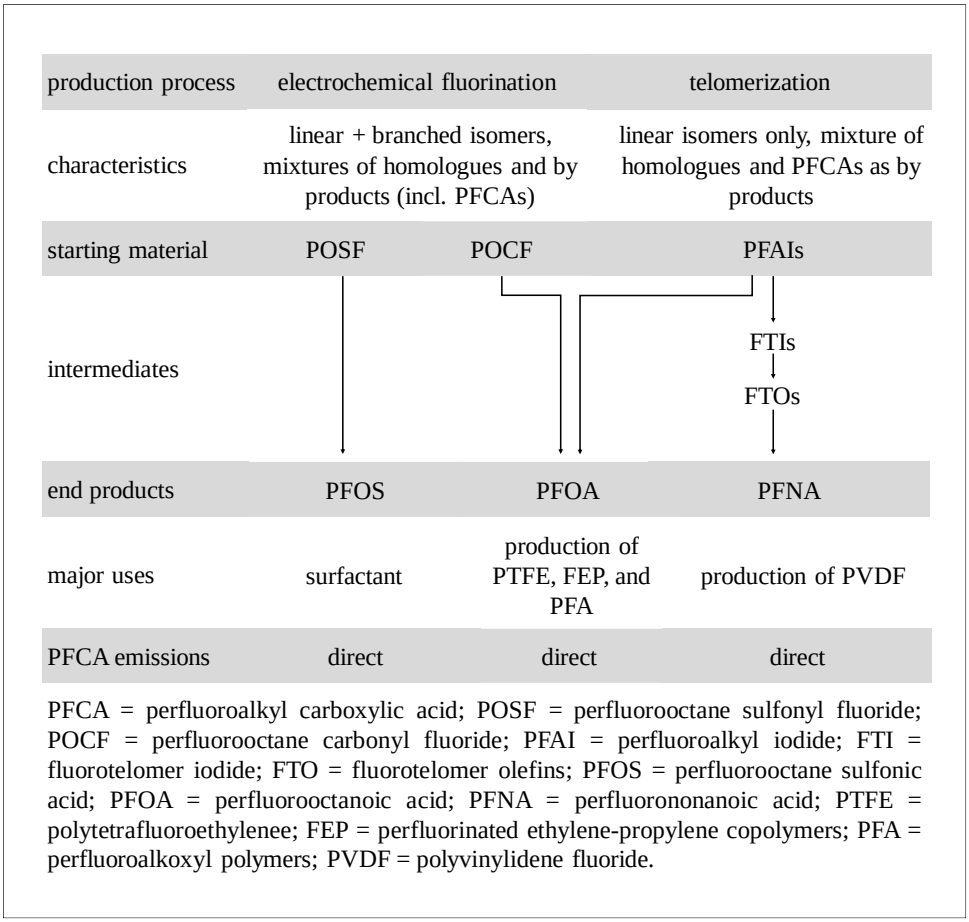

Figure 1.2. Graphical resume of the production process, characteristics, starting material, intermediates, end products, and uses of the major PFASs based on Wang et al., (2014) [9].

PFOS regulation started in Japan, Western Europe, and the US in the early XXI century, where these countries limited the production of PFOS-based, PFOA-based, and PFNA-based products. At the same time, PFOS-based production began in China and India in 2003 to 2008, and PFOA-based production or PFCA-free alternatives started to increase rapidly after 2008. PFOA production in 3M company was 6 tonnes per year (t/yr) between the 1990s to early 2000s, and production of short-chain PFCAs was of 4.54 t/yr [10]. At

a global scale, the production of ammonium perfluorooctanoate (APFO) was approximately 260 t in 1999 and ammonium perfluorononanoate (APFN) manufacture was estimated between 70 and 200 t from 1975 to 2004, calculated based on the production of 2004 that ranged from 15 to 75 t [6]. Global PFOS-based production from 1970 to 2002 increased with an estimated total accumulative production of 105,800 t, whose main uses are in carpets (53,000 t), paper and packaging (26,000 t), apparel (14,000 t), performance chemicals (6,600 t), and aqueous fire-fighting foams (AFFF) (11,000 t) [2]. After the phase-out in the use of PFOS-based products in the US in 2002, in Japan and Europe, a few producers were located with a production volume of 50-160 t in 2003 and 73-162 t in 2005. In China, before 2004 the production volume was of 50 t/yr and increased to 250 t/yr by 2006, but a decline to 100 t/yr took place in 2008. PFOA-based production increased rapidly due to the global demand for PTFE from 6.6 kt/yr in 1999 to around 64 kt/year in 2012. Similarly in India with a production of 2.3 kt/year in 2011 and 7.5 kt/year in 2012. [10].

Emissions occur when the residues of PFASs are discharged to the environment during the production and the indirect emissions of chemical impurities obtained in the manufacture. Also, in the use and discharge of waste at the end of life of the product containing PFASs, and by the degradation of precursors. Most of the PFASs emissions occur with wastewater streams, whereas small amounts are emitted with exhaust gases and solid waste. Figure 1.3 from Wang et al., (2014) [9] shows the annual emissions of PFCA-based which suggests an increase from 1951 to 2002 due to the production in Japan, Western Europe and Us, followed by a short decrease that was a consequence of the cease of the major industries in these countries after 2000. Also, major fluoropolymer producers in these regions start to re-use and recycle wastewater streams to minimize this

pollution. Also, short-chain PFASs and PFCA-free alternatives are new strategies in the polymerization process. The following rapid increase was due to the high emissions from countries as India, Poland, China, and Russia that became the main producers because of their soft regulations [9].

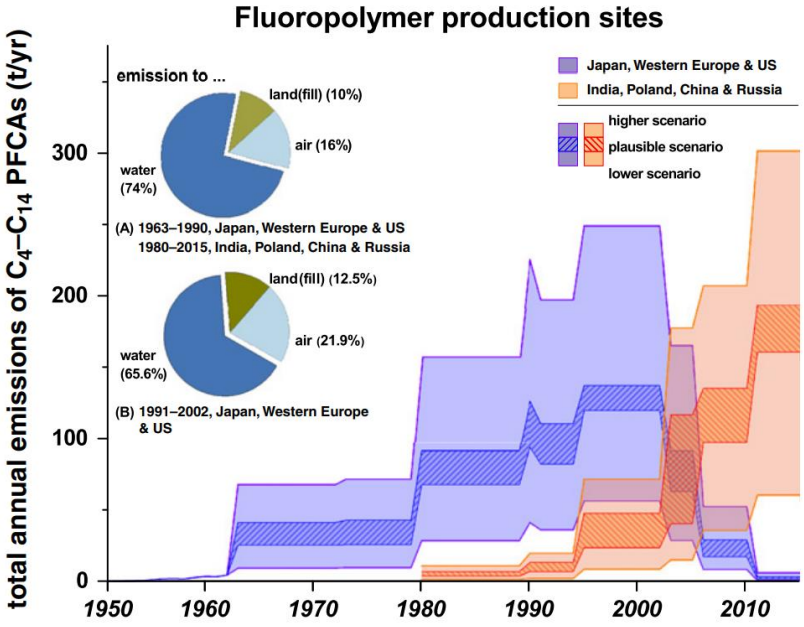

Figure 1.3. Figure from Wang et al., (2014) [9] that shows the estimated annual release of PFCAs from fluoropolymer facilities in the US, Western Europe, and Japan (purple) as well as in China, Russia, Poland, and India (orange). The pie charts show the fractions of emissions to different environmental media. The colored areas represent the estimated ranges of annual emissions in the higher, plausible, and the lower scenario.

Wang et al., (2014) [9] estimated global emissions of 2,610 to 21,400 t of PFCAs from 1951 to 2014 and suggested that from 20 to 6,420 t will be emitted from 2016 to 2030. PFOA and PFNA are the homologs released in the largest amounts, followed by pentafluorobenzoic acid (PFBA) and (pentafluorobenzoic acid) PFPeA while long-chain PFCAs are emitted in relatively low amounts.

1.1.2. Physicochemical properties

PFASs are made up of a perfluorinated carbon chain attached to a charged functional group commonly carboxylic or sulfonic. These two functional groups classify PFASs in perfluoroalkyl sulfonic acids (PFSAs), and perfluoroalkyl carboxylic acids (PFCAs) with perfluorinated carbon chain of different lenght [3]. Figure 1.4 illustrates the PFSAs while Figure 1.5 illustrates the PFCAs studied in this thesis. The Organization for Economic Co-operation and Development (OECD) classified as long-chain PFSAs those with six or more perfluorinated carbons, and short-chain PFSAs those with five or fewer perfluorinated carbons. In the same way, long-chain PFCAs have seven or more perfluorinated carbons, and short-chain PFCAs have six or fewer perfluorinated carbons (OECD/UNEP Global PFC Group, 2013). It is necessary to consider when comparing PFSAs and PFCAs, that on the PFCAs chain one of the carbons is in the functional group, so with the same number of carbons, PFSAs always have one more perfluorinated carbon than PFCAs. As an example, PFOA and PFOS have 8 carbons, but PFOS has the same perfluorinated carbon chain as PFNA that has 9 carbons.

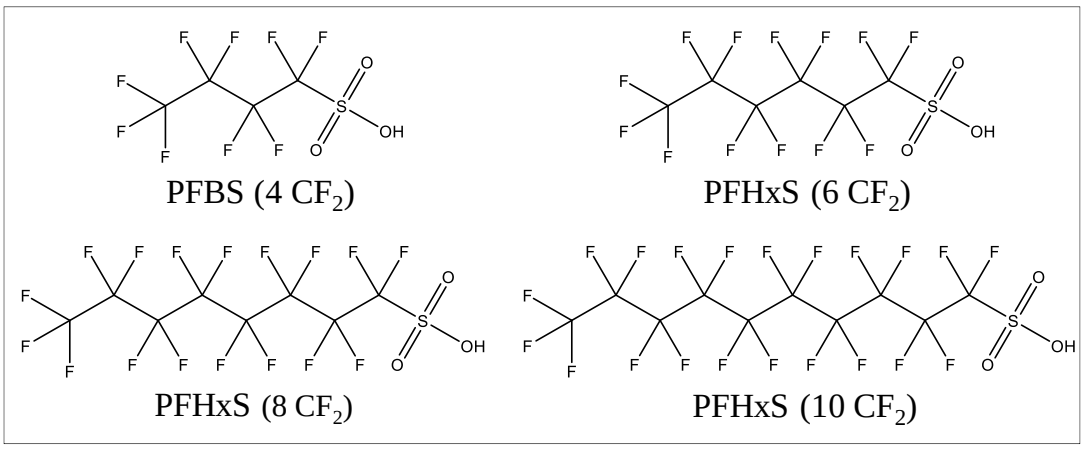

Figure 1.4. Structures, acronyms, and fluorinated carbons (CF_2) of the PFSAs studied

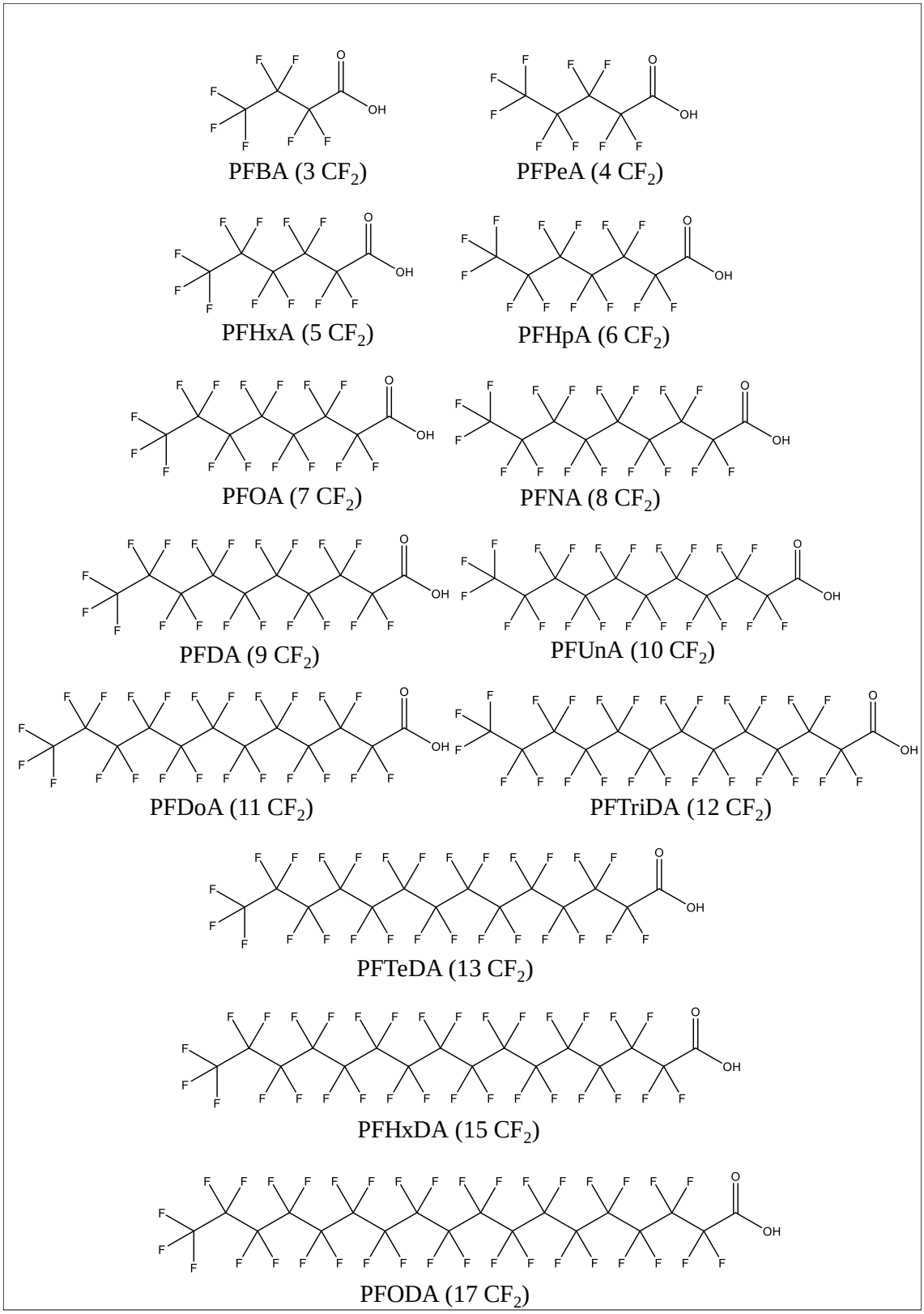

Figure 1.5. Structures, acronyms, and fluorinated carbons (CF_2) of the PFCAs studied.

The molecular structure of PFASs offers a double amphiphilic nature. The functional group of these compounds gives a hydrophilic property to these chemicals, while the perfluorinated carbon chain is completely hydrophobic. Table 1.1 shows some of the relevant physicochemical properties of the individual PFASs.

Table 1.1. Properties of the perfluoroalkyl substances from the databases CompTox Chemicals Dashboard (EPA), EPI Suite, and ChemExper.

Acronym	**Nº CAS**	**Molecular Formula**	**MW[1] (g/mol)**	**WS[2] (mg/L)**	**LogK$_{ow}$[2]**	**LogP[3]**	**LogK$_{aw}$[3]**
PFBA	375-22-4	$C_4HF_7O_2$	214.039	1,373	2.14	2.40	0.30
PFPeA	2706-90-3	$C_5HF_9O_2$	264.047	196	2.81	2.18	0.86
PFHxA	307-24-4	$C_6HF_{11}O_2$	314.054	27.1	3.48	1.96	1.43
PFHpA	375-85-9	$C_7HF_{13}O_2$	364.062	3.65	4.14	1.74	2.00
PFOA	335-67-1	$C_8HF_{15}O_2$	414.07	0.48	4.81	1.51	2.57
PFNA	375-95-1	$C_9HF_{17}O_2$	464.078	6.26E-02	5.48	1.29	3.14
PFDA	335-76-2	$C_{10}HF_{19}O_2$	514.086	8.04E-03	6.15	1.07	3.70
PFUnDA	2058-94-8	$C_{11}HF_{21}O_2$	564.094	1.02E-03	6.82	0.88	4.18
PFDoDA	307-55-1	$C_{12}HF_{23}O_2$	614.102	1.29E-04	7.49	0.62	4.84
PFTriDA	72629-94-8	$C_{13}HF_{25}O_2$	664.109	1.62E-05	8.16	0.22	5.87
PFTreDA	376-06-7	$C_{14}HF_{27}O_2$	714.117	2.02E-06	8.83		
PFHxDA	67905-19-5	$C_{16}HF_{31}O_2$	814.133	8.11E-08	10.2		
PFODA	16517-11-6	$C_{18}HF_{35}O_2$	914.148	4.69E-10	11.5		
PFBS	375-73-5	$C_4HF_9O_3S$	300.095	344	1.82	2.12	1.02
PFHxS	355-46-4	$C_6HF_{13}O_3S$	400.111	6.17	3.16	1.68	2.15
PFOS	1763-23-1	$C_8HF_{17}O_3S$	500.126	0.11	4.49	1.23	3.29
PFDS	335-77-3	$C_{10}HF_{21}O_3S$	600.142	1.68E-03	5.83		

[1]Molecular weight (MW) was extracted from EPA CampTox Chemicals Dashboard

[2]Water solubility (WS) and LogK$_{ow}$ were extracted from EPI Suite Database

[3]Volatility parameter (LogP), and LogK$_{aw}$ were extracted from Kim et al., (2015) [11]

Blank cells indicate that no data are available

The water solubility of PFASs increase with the decreasing carbon chain length, and is higher in carboxylates compared to sulfonates [12], and contrarily, lipid affinity increase with the increasing of the chain length, and is also higher in sulfonates compared to carboxylates due to their octanol-water partition coefficient (K_{ow}) [13]. Due to the low volatility of PFASs, Henry's law constant varies from 0.000014 atm·m^{-3}/mole in PFBA to 0.09 atm·m^{-3}/mole in PFOA, while with the same perfluorinated carbon chain varies from 0.011 atm·m^{-3}/mole in PFOS and 0.477 atm·m^{-3}/mole for PFNA [14]. Kim et al., (2015) [11] estimated the volatility and the air-water partition coefficient (K_{aw}) of PFASs and concluded that as the increase of the chain length of PFASs, decrease the volatility and increase the K_{aw} [6]. The available experimental and calculated pKa values (i.e. PFOA range from 0.5 to 3.8) indicate that PFASs are strong acids that will predominantly be in their dissociated negatively-charged form at environmentally relevant pH values [3].

The physicochemical properties of PFASs are important to define their use as surfactants and polymers in industrial applications because of their chemical and thermal stability, resistance to biological degradation, redox stability, and hydrophobic and lipophobic nature due to the low polarizability of fluorine atoms, and they are also relevant to understand the fate and transport in the environment [15]. The polarity of the functional group makes them highly water-soluble and mobile in an aqueous environment, while the hydrophobicity of the perfluorinated carbon chain suggests their accumulation in organic matter and lipids [16].

1.1.3. Occurrences of PFASs in the environment

PFASs are worldwide distributed from industrial areas in developed countries to remote areas far from human activities as in the Arctic [17] and Antarctic [18] due to atmospheric volatile precursors deposition and sea currents [19]. As a consequence of the PFASs physicochemical properties, these chemicals have been detected in several environmental matrices as air [20–22], fresh and seawater [23,24], sediments [25,26], and soils [27]. Besides, PFASs have been detected in plants [28,29], then bioaccumulated along with the food webs as in plankton [16], larval organisms [30], fish and crustaceans [31], birds and mammals [32] and biomagnified to top predators [33–35] from marine [36] and terrestrial environments [37].

1.1.3.1. PFASs in water

PFASs have been detected at concentrations ranging from pg/L to ng/L throughout the water cycle comprising waste-, river-, lake-, sea-, storm-, snow, groundwater, drinking water [15]. Several reasons indicate the importance to study PFASs in water bodies since they have become main emerging pollutants. PFASs are dispersed in different water bodies, with alarming levels of 120 mg/L in wastewater drained from fire-training areas [38], and in wastewater treatment plants (WWTP) effluents as these facilities are unable to eliminate the loads of PFASs using conventional treatments [24]. In water, generally, the detection frequency of PFCAs is higher compared to PFSAs, while PFASs with shorter carbon chains were frequently more detected than those with longer chains. The relatively high concentrations of short-chain PFASs in seawater found in the bibliography are attributed to their relative high-water solubility and lower octanol/water partition

coefficient compared to long-chain PFASs. Wang et al., (2019) [39] observed that PFASs concentration was higher in surface water compared to bottom water, the results suggest an insufficient mixing of the water due to a stratification process as a consequence of the temperature and salinity along the water column. Seasonal variation was observed in seawaters due to the differences in rainfall-runoff and riverine inputs, as for the wet deposition of PFASs into the oceans [40]. In any case, PFASs profile along the water column depends on the industrial and human settlement impact, the season, and the latitude of the location. The most frequently detected PFASs are PFOA and PFOS because are highly produced and mobile once introduced to the aquatic environment [23]. In addition, PFASs were studied also in rainwater, where PFOA, PFNA, PFUnA, and PFDoA were frequently detected in comparison to PFHpA and PFOS [22]. In Arctic snow, PFOA and PFNA were the most PFASs detected [19], and in ice cores from Arctic glaciers located in Svalbard Archipelago PFBA was detected, followed by PFOA and PFNA [41]. A similar profile was observed in Albany (US), where the authors concluded that the concentration increased with the depth of the snow, suggesting higher rates of scavenging of PFASs from the atmosphere during the initial periods of wet deposition [22] that is considered to be a major pathway of contamination [42].Ten out of twenty-six PFASs were detected in Uganda, where PFOS, PFOA, and PFHxS presented higher levels in the effluent than in the influent due to degradation of PFASs precursors [29]. PFASs levels in surface water from several countries have been compared and countries as China and Canada have one log magnitude higher than other countries as Sweden, Vietnam, Spain, Australia, and Uganda, while France exceeds by three log magnitudes as this country receives the waste stream from AFFF-industry [24], due to AFFF brans in

France that contain a very large amount of PFASs [43]. Table 1.2 shows the concentration range of PFOA and PFOS in different water bodies from different locations.

Table 1.2. Concentration range of PFOA and PFOS in water (ng/L) from literature.

Matrix	n	Location	PFOA	PFOS	Reference
Bottom seawater	33	South China Sea	0.008 - 0.233	0.002 - 0.014	[39]
Lake water	11	Albany	3.27 - 15.8	0 - 9.30	[22]
Port water	16	North Weast Mediterran	0.52 - 2.25	0.03 - 8.38	[44]
Rainwater	11	Albany	0 - 7.27	0 - 1.51	[22]
River water	6	North Weast Mediterran	0.79 - 9.63	1.09 - 9.56	[44]
Seawater	28	Bohai Sea	0.4 - 83.4	0.04 - 6.80	[40]
Seawater	29	North Weast Mediterran	0.08 - 1.86	0.03 - 3.93	[44]
Seawater	41	North Atlantic Ocean	< 0.093 - 0.900	< 0.11 - 0.91	[16]
Snow	21	Albany	0 - 19.6	0 - 1.93	[22]
Surface runoff water	14	Albany	0.51 - 29.3	0 - 14.6	[22]
Surface seawater	227	South China Sea	0.02 - 0.40	0.01 - 0.47	[39]
River water	19	Cantabrian Sea	0.02 - 3.53	0.01 – 6.57	[45]
River water	87	Ebro Delta	0.12 - 8.7	0.04 - 4.3	[46]
River water	15	Jucar River	0.07 - 52.2	0.01 - 128	[47]
River water	35	Shandong Province	0.96 - 4,534	0.4 - 12.78	[48]
River water	133	France	0.08 - 36	0.06 - 173	[49]
WWTP effluent	8	North Weast Mediterran	3.47 - 61.9	0.03 - 72.1	[44]
WWTP effluent	10	Uganda	0.6 - 4.1	0.4 - 3.9	[29]
Tap water	30	Brazil	3.4 - 12	Not detected	[50]
Tap water	27	France	3.9 – 7.4	1.6 – 11	[50]
Tap water	39	Spain	8.3 - 11	Not detected	[50]

In a fluorochemical industrial area in the Shandong province, PFOA contributed 90.1% of the total PFASs with a mean concentration of 3,112 ng/L in river water, followed by PFBA (mean concentration of 49.8 ng/L, 1.4%), PFPeA (mean concentration of 70.9 ng/L, 2.0%), PFHxA (mean concentration of 123 ng/L, 3.5%) and PFHpA (mean concentration of 91.7 ng/L, 2.6%), while the total contribution of long-chain PFCAs and PFSAs was less than 1% [48]. Downstream, in seawater from the Bohai Sea, relatively high concentrations of PFOA (median 4.97 ng/L, 47%), PFHxA (median 0.93 ng/L,

16%), and PFBS (median 0.49 ng/L, 7%) were in July, while in November the profile changed to PFOA (median 0.86 ng/L, 40%), PFOS (median 0.1 ng/L, 14%) and PFHxDA (median 0.59 ng/L, 11%), suggesting a seasonal variation of the PFASs emitted into the Bohai Sea [40]. In the North Atlantic Ocean, eight of twenty-one PFASs were detected in surface seawater samples with a predominance of PFOS (0.11 – 0.91 ng/L), PFHxA (0.155 – 1.00 ng/L), and PFOA (0.093 – 0.90 ng/L) [16]. In the Cantabric coast, the concentrations were detected in ports > WWTP effluents > emissaries, and with a PFASs profile dominated by PFOA, PFOS, and PFNA [45]. In the North Weast Mediterranean, PFASs profile in the river, coastal, and port water was dominated by PFOA and PFOS [44]. In Jucar River, PFOA (53.5%) and PFOS (40%) showed a high frequency of detection, followed by short-chain PFASs (60%) [47].

1.1.3.2. PFASs in sediments and soils

Sediments and soils have an important potential as a reservoir for PFASs and can serve as a long-term contamination source to water, and biota. PFOS and PFOA were commonly the predominant PFASs and the two most well-studied [51]. Table 1.3 gives evidence of the distribution and concentration of PFOA and PFOS in sediments and soils. In the Bohai Sea area, PFOA and PFOS were detected in 40% and 42% of the river samples. PFOA concentration ranged from 0.04 to 76.2 ng/g dw and accounted for 2% to 93% of the total PFASs. Meanwhile, PFOS levels were much lower with a concentration ranged from 0.02 to 1.62 ng/g dw and accounted for 1 to 77%, with high proportions in the river sediments and low in sea sediments [52]. Also in sea sediments from the Bohai Sea, thirteen PFASs were detected with a dominance of PFOA (30 – 40%) [40], while in

the South China sea eleven PFASs with a dominance of PFOA (28.5%) and PFOS (19.9%) [39]. In Juncar River (Spain), eleven PFASs were detected, short-chain PFASs presented the highest mean concentration (5.85 ng/g dw of PFBA and 11.5 ng/g dw of PFBS), while PFOA (2.47 ng/g dw) and PFOS (2.57 ng/g dw) were presented similar concentration [47]. In Ebro Delta sediments (Spain), PFASs profile was dominated by PFOA (mean: 6.0 ± 2.9 ng/g dw) and PFOS (mean: 2.7 ± 56 ng/g dw), with a clear contribution of short-chain PFAS. In the Cantabric coast (Northern Spain), PFOA and PFOS were the dominant contaminants detected in port sediments and sediments receiving WWTP effluents and emissaries [45].

Table 1.3. Concentration range PFOA and PFOS in sediments and soils (ng/g dw) from literature.

Matrix	n	Location	PFOA	PFOS	Reference
Sediment	9	Arctic	0.017 - 0.13	< 0.04	[36]
Costal sediment	26	Bohai Sea	0.07 - 1.8	0.03 - 0.06	[52]
River sediment	26	Bohai Sea	0.04 - 76.9	0.02 - 1.6	[52]
River sediment	47	Yangtze River	0.02 - 1.35	-	[53]
Sea sediment	28	Bohai Sea	0.08 - 0.81	0.02 - 0.74	[40]
Sea sediment	53	South China Sea	2.1 - 22.9	2.6 - 26.7	[39]
Sediment	26	Bohai Sea	0.005 - 29.0	0.027 - 0.435	[52]
River sediment	15	Jucar River	0.15 - 6.69	0.06 - 9.83	[47]
Sea sediment	12	Cantabrian Sea	0.06	0.13	[45]
Soil	21	East China	2.84 - 4.99	0.78 - 4.23	[54]
Soil	20	Norway	0 - 0.403	0 - 0.341	[55]
Soil	18	Uganda	0.25 - 0.91	0.6 - 3.0	[29]

Maximum reported concentration in soils ranged from 0.4 to 460,000 ng/g for PFOS and from 2 to 50,000 ng/g for PFOA in areas directly exposed PFASs manufacturing sites, fire training areas, and other AFFF-associated locations at airports military installations, and a crash site, while in sites where PFASs exposure was indirect as adjacents areas,

concentration ranged from 0.4 to 5,500 ng/g for PFOS and from 0.8 to 2,531 ng/g for PFOA [27]. PFASs concentration in soils depends on the depth profiles. Different studies were summarized and the results suggest that long-chain PFASs reported the majority mass at the shallowest depths, while short-chain PFASs comprise the majority at deeper depths. There is significant retention of PFASs in the vadose zone due to the adsorption by the solid phase of those with higher hydrophobicity that use to be the long-chain PFASs. Also, this adsorption will depend on the soil properties as the organic matter increase the sorption of those with higher hydrophobicity [56]. Specifically, short-chain are more soluble and mobile in the leaching water so will spread to deeper areas and possibly reach groundwater [27]. PFOA and PFOS were also the predominant compounds in soils, accounting for 53.7% and 28.0% of the total of 12 PFASs, respectively, and PFBA was detected with relatively higher concentration, from 0.06 to 10.9 ng/g dw [54]. Thirteen out of twenty-six PFASs analyzed in soil samples from Uganda, PFOS was the most abundant compound (36 - 50%), followed by PFOA (6.2 – 15%) and PFHxA (4.7 - 18%) [29]. In a Nordic Skiing Area, PFASs profile was dominated by PFBA (0.125 – 0.563 ng/g dw) and PFDA (0.053 – 1.96 ng/g dw) and this characteristic PFASs pattern corresponds to PFASs profile in ski wax, suggesting that sky products are an important source of PFASs in the skiing areas [55].

1.1.3.3. PFASs in plants

Due to the partial water solubility of PFASs, water is a relevant vehicle of transfer across the environmental compartments, and field irrigation with contaminated water may be an important source of plant contamination by PFASs but also uptake through roots [28]. In

a study of PFOA and PFOS exposure in different plant species, the author concluded that the accumulation increase with the soil pollutant concentrations and PFOA accumulates more than PFOS [57]. Regarding the fate of PFASs in plants, accumulation is inversely proportional to the perfluorinated chain length, and PFSAs generally having lower accumulation than PFCAs due to different transport mechanisms for PFCAs and PFSAs [58]. Accumulation among plant species is proportional to protein content [59], as well as the surface area of the root system that increases the accumulation of PFASs [60]. In Canada, PFASs levels were higher for PFCAs than for PFOS in vegetation, most plants were dominated by PFOA, and lichen showed dominance by odd carbon chain PFCAs as PFNA, PFTriDA, and PFUnA. Differences are due to plants obtaining nutrients and water via root system in competition with the soil due to the sorption of long-chain PFASs, while lichen absorbs nutrients directly from precipitation [37]. In Arctic environments, PFASs profile was dominated by PFOA, PFNA, PFOS, and PFUnA in macroalgae [36]. In Uganda, only PFBS, PFHpA, PFOA, and PFNA were detected in plant out of twenty-six PFASs, only PFHpA (0.058 – 0.14 n/g dw, 25 – 45%) and PFNA (0.057 – 0.072 ng/g dw, 17 – 36%) were uptaken, and PFOA and PFNA where mostly accumulated in the root [29]. In Tangxun Lake, lotus root contained mean concentration of PFBS and PFBA of 0.5 ng/g ww and 4.66 ng/g ww, respectively, while the other PFASs were all below limits of detection. In two different plant species, PFBS, PFOS, and PFBA were predominant, with mean concentrations of 10.8, 33.7, and 7.62 ng/g ww in common duckweed and 5.96, 5.14, and 11.7 ng/g ww in common water hyacinth, respectively [61]. In artificial wetlands, PFBA was the main compound accumulated in all aquatic plants, followed by PFBS, while PFOA and PFOS were in a relatively lower concentration; PFBA ranged

from 4.63 to 969 ng/g dw, PFBS from 1.44 to 331 ng/g dw, PFOA from 0.46 to 12.6 ng/g dw, and PFOS from 0.31 to 5.45 ng/g dw [62].

1.1.3.4. PFASs in wildlife

PFOS is the predominant PFASs detected in wildlife, followed by long-chain PFCAs [63]. Literature reveals an overall decrease in PFOS levels over time, in contrast with long-chain PFCAs concentration that has a trend to increase [64]. Table 4 gives evidence of the world spread distribution and concentration of PFASs in wildlife.

Table 1.4. Concentration range of PFOA and PFOS in wildlife (ng/g ww) from literature. Species are ordered from primary consumers to top predators.

Matrix	n	Location	PFOA	PFOS	Reference
Plankton	7	North Atlantic Ocean	0.01 - 0.16	0.26 - 3.73	[16]
Caribou	43	Canada	< 0.01 - 0.06	0.01 - 3.35	[37]
Earthworms	26	Norway	0 - 2.47	0 - 1.78	[55]
Ice amphipod	6	Barent Sea	2.07 - 4.33	0 - 7.41	[65]
Mussels	12	Cantabrian Sea	0.01	0.02 - 0.06	[45]
Bank voles	52	Norway	-	0 - 16.0	[55]
Cod	3	Arctic	0.03 - 0.14	0.12 - 5.4	[36]
Fish	55	Ebro Delta	87 - 330	5.5 - 154	[46]
Fish	25	Jucar River	-	0.56 - 8.13	[47]
Polar cod	9	Barent Sea	0 - 1.88	1.07 - 2.85	[65]
Salmon	6	Arctic	0.08 - 1.2	< 0.3 - 1.3	[36]
Eider duck	5	Arctic	< 0.03	2.3 - 25	[36]
White winged scoter	4	Arctic	< 0.03	5.5 - 120	[36]
Northern gannet eggs	105	UK	0.37 - 1.08	24.56 - 109	[66]
Black guillemot	10	Barent Sea	0 - 17.1	0 - 43.8	[65]
Glaucous gull	9	Barent Sea	-	8.49 - 225	[65]
Glaucous gull	75	Svalbard	0.01 - 0.79	1.39 - 508	[35]
Harbor porpoise	11	Wadden Sea	-	89.0 - 534	[67]
Harbor seal	13	Wadden Sea	6.1	430 - 1,284	[67]

Table 1.4 (continued)

Matrix	n	Location	PFOA	PFOS	Reference
Ringed Seal	16	East Greenland	0 - 0.2	51 - 143	[33]
Beluga	13	Arctic	0.14 - 7.0	0.14 - 109	[36]
White-beaked dolphin	7	Wadden Sea	0 - 4.4	126 -540	[67]
Poler bear	35	East Greenland	13 - 38	1,500 - 3,373	[33]
Wolf	27	Canada	< 0.01 - 0.57	0.05 - 2.68	[37]

In plankton samples from the Arctic, fourteen PFASs were detected with the dominance of PFOS (1.70 ± 1.3 ng/g ww, 20%), followed by PFUnA (1.38 ± 0.91 ng/g ww, 19%), PFTriDA (0.83 ± 0.54 ng/g ww, 14%), and PFHxA (0.70 ± 0.26 ng/g ww, 11%) [16]. In mussels from the Cantabric coast, no specific distribution was observed PFOS and PFOA were detected in 5 of 10 samples at a range concentration from 0.01 to 0.06 ng/g ww [45]. In fish from Jucar river, five PFASs were detected and PFPeA was the dominant with a range concentration from 9.84 to 946 ng/g, followed by PFHpA (1.18 - 111 ng/g), PFNA (71.5 ng/g), and PFOS (0.56 - 8.13 ng/g) [47], while in Ebro Delta eight PFASs were detected and PFOA was the most abundant compound in a range concentration from 87 to 330 ng/g ww, followed by PFDA (11.5 – 459 ng/g ww), and PFOS (5.5 – 154 ng/g ww) [46]. In top predators from the North Sea, PFASs profile was dominated by PFOS in the harbor seal (93.2%), in harbor porpoise (89.1%), and white-beaked dolphin (64.8%). On the other side, PFOS has a very low contribution in the harbor seal (0.1%), intermediate in the harbor porpoises (8.3%), and high in the white-beaked dolphin (26%) [67]. Differences among top predators are attributed to different prey preferences, and differences in specific intake on the analyzed compound. As well, species with similar diets as Carnivora and Cetacea show that phylogenetic differences in the ability to transform PFOSA to PFOA play an important role in their accumulation.

1.1.4. PFASs toxicity

Ecological risk studies help to understand the exposure and effects of PFASs on the environment and the impact on wildlife populations. Nowadays, it is important to reduce the risk associated with the global impact of PFASs in the environment. Most of the risk assessment activities focus on PFOS and PFOA, but other PFASs became of the emerging concern due to their increased production and their unknown toxicity. The study of PFASs the distribution and effects in biota is not fully acsessed. Indeed, the monitoring of the fate of PFASs in water, sediment, and the soil is an inherent part of overall risk assessment. In aquatic macrophytes, no observed effect concentrations (NOEC) for PFOS ranged from 0.3 to 29.2 mg/L, and from > 3.2 to 206 mg/L in phytoplankton due to different cell density, respiration, and growth rate [68]. In four different Baltic algae species, half-maximal effective concentration (EC_{50}) for PFOA ranged from 41.6 to 977 mg/L [69]. In marine environments, Mhadhbi et al., (2012) [70] studied the risk assessment in primary producers (*Isochrysis galbana)*, primary consumers (*Paracentrotus lividus* and *Siriella armata*) and secondary consumers (*Psettas maxima*) of the marine food webs, and observed that the risk values were higher at the base of the food chain, while secondary consumers were more sensitive to PFOS and PFOA levels (Table 1.5). In general, the levels reported in the bibliography are far from the observed in marine environments, but the bioaccumulation and biomagnification processes became a way to reach the concerning effects [70].

Table 1.5. Toxicity threshold (mg/L) for PFOS and PFOA in primary producers, primary consumers, and secondary consumers from marine food webs by Mhadhbi et al., (2012) [70].

Compound	Species	Food web stage	NOEC	LOEC	EC10	EC50
PFOS	*Isochrysis galbana*	Primary producer	7.5	15	12.2	37.5
	Siriella armata	Primary consumer	1.25	2.5	3.2	6.9
	Paracentrotus lividus	Primary consumer	1	2	2.6	20
	Psettas maxima	Secondary consumer	0.015	0.03	0.02	0.11
PFOA	*Isochrysis galbana*	Primary producer	25	50	41.6	163.6
	Siriella armata	Primary consumer	5	10	7.8	15.5
	Paracentrotus lividus	Primary consumer	10	20	30.7	110
	Psettas maxima	Secondary consumer	1.5	3	3.9	11.9

NOEC = no observed effect concentration

LOEC = lowest observed effect concentration

EC_{10} = effect observed in 10% of the population of test organisms

EC_{50} = effect observed on 50% of the population of test organisms

In soils, in a 21 days study on onions, NOEC for survival and growth was 15.6 mg/kg PFOS wet weight and EC_{50} was 47 mg/kg. In frogs, after 96 hours of PFOS exposure, half lethal concentration (LC_{50}) was 14 to 18 mg/L, and EC_{50} was 12 to 18 mg/L for developmental malformations, and the growth NOEC was 5.2 mg/L [71]. In embryo and larval fish studies, PFOS exposure produces developmental reproductive effects, impact on the stress response [72,73], reduction of the fecundity at 0.5 mg/L [74], and altered sex ratio of zebrafish in chronic exposure to 0.05 µg/L [75]. In a laboratory study of PFOS in rats, observed effects were related to liver function, reproductive success, and reduced birth weight, with a no observed adverse effect level (NOAEL) of 0.1 mg/kg body weight/day, and the lowest observed adverse effect level (LOAEL) of 0.4 mg/kg body weight/day [76,77]. For PFOA, a decrease of body weight was observed at a NOAEL of 1 mg/kg body weight/day and a LOAEL of 3 mg/kg body weight/day [78]. In avian oral dosing experiments, dietary PFOS exposure of 10 mg PFOS/kg body weight/day (average

daily intake: 0.77 mg/kg body weight/day) showed a statistically significant reduction of the survival of bobwhite quail chicks, but not on mallards [79]. A reduction of hatching success was reported at 100 ng/g of PFOS in leghorn chicken via ovo-injection [80]. In swallows from Minnesota and Wisconsin, hatching success decreased by 80-85% with a PFOS concentration between 120 and 283 ng/g [81].

With the existent bibliography, guidelines for PFAS-contaminated sites have been proposed. These guidelines for site management and assessment help to identify potential adverse effects on non-human biota exposed to the environment. In seawater, Giesy et al., (2010) [82] proposed criteria maximum concentration for most sensitive aquatic species of 0.021 mg/L for PFOS, 25 mg/L for PFOA, and 121 mg/L PFBS, based on acute and chronic toxicity to *Lemna gibba*. Environmental quality standards (EQS) for PFASs were proposed by Valsecchi et al., (2017) [83] to protect pelagic aquatic organisms at levels of 11 mg/L for PFBA, 3.2 mg/L for PFPeA, 3 mg/L for PFOA, and 37 mg/L for PFBS. The Western Australia Department of Environmental Regulation (WA DER) and the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) offer values to complete ecological evaluation of PFOA and PFOS in water quality guidelines using data form five taxonomic groups from 18 studies [84,85]. The Norwegian Pollution Control Authority (NPCA) published guideline values for PFOS in soils of 0.1 mg/kg, and the PNEC for terrestrial and aquatic organisms [86]. The Heads of the Environmental Protection Agencies (HEPA) for Australian and New Zealand have PFASs guideline values based on the lowest concentration (in zebrafish and earthworms) using the precautionary principle which consists in the approach issue that may have environmentally harmful consequences, in aquatic environments (0.00023 μg/kg for PFOS; 19 μg/kg for PFOA) and terrestrial (1,000 μg/kg for PFOS; 10,000 μg/kg

for PFOA) [87]. The guideline values in aquatic environments are over the reported previously in wildlife (Table 4), however, the presence of a substance does not mean it is a toxic effect at that concentration since is a precaution principle.

1.1.5. Legislation

The Stockholm Convention is an international agreement to protect human health and the environment from the exposure to Persistent Organic Pollutants (POPs) that remain in the environment for long periods. POPs are classified as chemicals that can produce serious health effects as cancers, birth defects, dysfunctional immune, reproductive and nervous systems, and endocrine disruption. Because of their physicochemical properties, these chemicals are accumulated on the environment and wildlife and are long-range transported. The convention was adopted on 22 May 2001 with 152 signing parties, including Spain. The treaty aimed to prohibit and/or eliminate the production and use, as well as the import and export of the POPs listed in Annex A, restrict the production and use, as well as the import and export of the POPs listed in Annex B, and reduce or eliminate release from unintentionally produced POPs listed in Annex C. In response, the Stockholm Convention entered in force in 2004 for 184 parties and required its parties to take measures to eliminate and reduce the release of POPs into the environment.

In 2009, the Stockholm Convention included PFOS, its salts, and perfluorooctane sulfonyl fluoride in Annex B (restrict the production and use) with specific exemptions as photo-imaging, semi-conductor, aviation hydraulic fluids, metal plating, fire-fighting foam, electronics, carpets, leather and apparel, coating, rubber, and plastics, among

others. In 2019, PFOA, its salts, and PFOA-related compounds were also included in Annex A (prohibit and/or eliminate the production and use) with no exemptions, and PFHxS, its salts, and PFHxS-related compounds were proposed for Annex A under the Convention to better protect human health and the environment from its harmful impacts.

In 2017, the Government of Canada established the Prohibition of Certain Toxic Substances Regulation (2012; PCTSR) enabled by the Canadian Environmental Protection Act (1991; CEPA) that prohibited the manufacture, use, sale, offer for sale, or import of PFOA, its salts, and its precursors, perfluorocarboxylic acids that have the molecular formula $C_nF_{2n+1}CO_2H$ in wich $8 \leq n \leq 20$, their salts and their precursors and PFOS, its salts, and its precursors.

In Europe, EU Directive 2006/122/EC restricted the use and placement in the market of PFOS and its related substances in 2008. The Directive 2013/39/EU of the European Parliament in the field of water policy established the environmental quality standards (EQS), the annual average-EQS (AA-EQS), and maximum allowed concentration –EQS (MAC-EQS) for PFOS and its derivates. In inland surface water, the AA-EQS is 6.5×10^{-4} μg/L and the MAC-EQS is 36 μg/L, while in other surface waters are 1.3×10^{-4} μg/L and 7.2 μg/L. In biota, the EQS is 9.1 μg/kg wet weight. When comparing EQS with the data reported in Table 1.2 and Table 1.4, the concentration is dependent on the location and the species. Generally, the MAC-EQS in water samples remains below, while in most of the scenarios the data reported exceed the AA-EQS. Controversially, PFOS concentration in wildlife from the bibliography is over the EQS recommended, especially for top predators as a consequence of the biomagnification of PFOS.

The World Health Organization (WHO) recommended recast the Drinking Water Directive (98/83/CW, Directive on the quality of the water consumption) with maxim PFASs value of 0.5 µg/L and 0.1 µg/L for every single compound [15]. In Norway, PFOA was banned from consumer products in 2013, and in Germany, a maximum of 0.1 µg/L was established in drinking water [88]. Several additional PFASs are considered concerning pollutants and are under evaluation for the coming years or have been already evaluated. The aim is to understand the risk to human health and in the environment that manufacturing and using these contaminants could pose.

1.2. DISTRIBUTION AND BEHAVIOR OF PFASs IN THE ENVIRONMENT

The distribution and behavior of PFASs in the environment vary with the physicochemical properties of each compound and hazards move easily with any flow of water. Figure 1.6 illustrates the life cycle of PFASs from the production of the chemicals to the final accumulation in the environment through different pathways. After the production in the PFASs industry, PFASs are directly discharge through drains and sewer systems to the WWTP. The not fully efficient treatment process to remove PFASs in WWTP causes that treated wastewater containing PFASs is discharged into water streams (river and seawater). However, the fate of the different PFASs in the WWTP depends on their physicochemical properties, despite the long-chain PFASs trend to accumulate in sludge, short-chain PFASs remain in the water. After treatment in WWTPs, PFASs are released into the environment hidden in the “cleaned” waters. Once in the environment, PFASs are accumulated in plants due to direct contact in the river banks or in crops due to watering in agriculture. PFASs also accumulate in wildlife, and consequently in food

resources for humans as agricultural and fish products. Another source of pollution in agriculture is the use of sludge from WWTP as fertilizers in agricultural fields. In drinking water treatment plants (DWTP) only activated carbon filtration, reverse osmosis, anion-exchange resin, nanofiltration, and electrochemical treatment could remove mostly long-chain PFASs [89], while other treatments as chlorine disinfection, ozonation, and sand filtration were not fully efficient, anyway, the PFASs detected in tap water would not pose immediate risk in short term exposure [90].

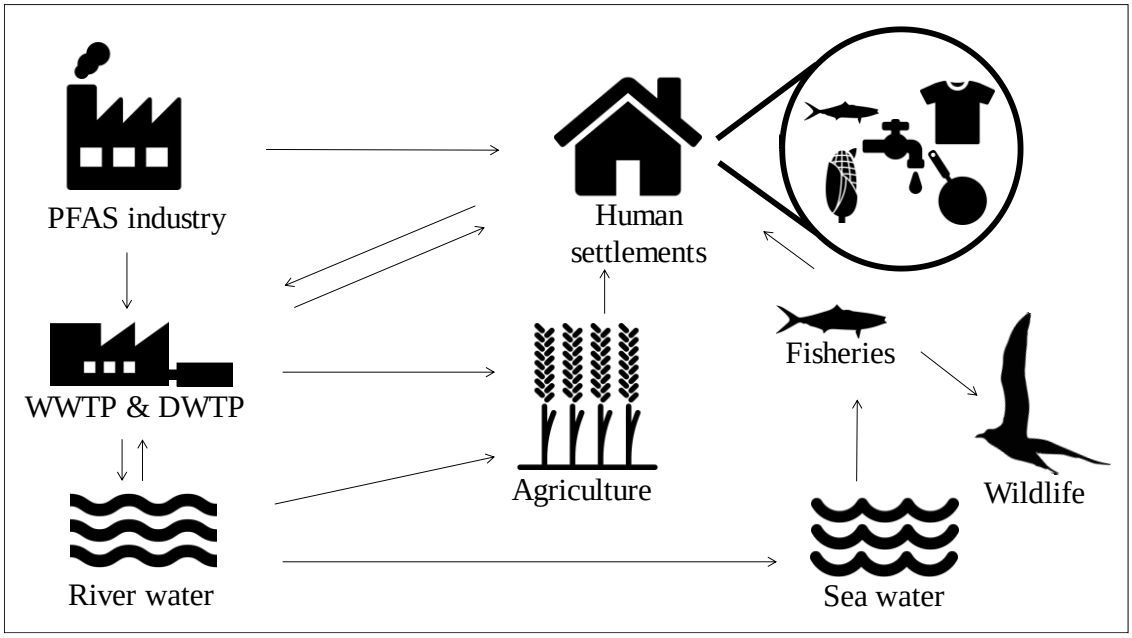

Figure 1.6. The life cycle of PFASs from their production to the environment. WWTPs and DWTPs became a source of pollution because of their inefficiency in the removal of PFASs. The presence of PFASs in agricultural products is due to the contaminated water and the use of sludges in crops and their uptake ability. Once in the environment, wildlife is exposed to PFASs through the diet and exposes humans and top predators.

1.2.1. The fate of PFASs in the aquatic system.

PFASs water solubility depends on the hydrophobic perfluorinated chain length, as well as the hydrophilic functional group. However, the amphiphilic properties and low volatility contribute to their presence in the aquatic environment. PFOS and PFOA are the most common and have been the most well documented and studied [87], nowadays all PFASs from 4 to 18 carbon-chain length are monitored due to their mobility in the aquatic environment [91]. In general, the concentration of short-chain PFASs is at least 50 times larger than the long-chain, reflecting their higher water solubility and the current trend of using short-chain PFASs for manufacturing products [24]. PFBA, PFBS, PFPeA, and PFHxA levels were comparable to PFOS and PFOA, according to some studies [16,39,92].

PFASs concentrations in WWTP effluents depend on the origin of upstream wastewater sources. Lower concentrations are attributed to domestic wastewater, while higher concentrations are linked to textile industrial wastewater [62]. Besides, other sources of PFASs include AFFF, paint, commercial surfactant concentrate, waterproofing agents, and chrome paint. Hospitals also become an input of PFASs to wastewater due to the use of medical devices like radio-opaque, in vitro diagnostic, and color filters [24]. Furthermore, biological treatment both aerobic and anaerobic are only able to break the C-C bond and they lead to the formation of short-chain PFASs, so WWTP became a source of shorter PFASs to surface water and to the general environment [15]. Surface- and groundwater are important sources of drinking water production. A relevant concentration was detected in a groundwater-based water work in Sweden up to 10,000 ng/L in outgoing drinking water [23]. Considering their relative solubility and polarity,

PFAS can be transported via aquatic systems, and oceans became a final global sink [6]. PFASs precursor chemicals are long-range atmospheric transported and can turn into an important indirect source of PFAS into oceans [93].

1.2.2. Distribution of PFASs in the sediment/soil-water system and accumulation in organisms

Sediments and soils are an important sink and reservoir of contaminants and have a large impact on their distribution, transport, and fate in the environment [6,27]. PFASs have been detected in soils and sediments all around the globe and are of increasing concern to understand their impact on the environment [94]. The most common method of estimating contaminant sorption is the solid/liquid partition coefficient (K_d), which is an empirically dimensionless property that describes how a chemical substance distributes itself between sediment/soil and water [95]. The fate of PFASs in the soil/sediment-water system is evaluated with the K_d and is expressed in L/kg.

$$K_d \text{ (L/kg)} = \frac{[PFASs]\ in\ sediment/soil}{[PFASs]\ in\ water} \qquad \text{Equation 1.1}$$

PFASs with low K_d predominantly exist in the aqueous phase and easily can remain in the water, while PFASs with a high K_d are associated with the solid phase and consequently become less mobile and remain linked to the sediment/soil. The K_d can vary in environmental conditions as a result of different factors as substrate characteristics, organic matter, and salinity [13]. Based on the physicochemical properties of PFASs, K_d values depend on one hand of their chain length, where K_d values increase with the

increasing of the perfluorinated carbon chain length of PFASs [96,97]. On the other hand, K_d values are influenced by the organic matter, where the Kd value increases with the increase of the organic carbon [56,98,99].

Sediments and soils play a role as an exposure pathway of PFASs to organisms from different environments. Focusing on sediments, in a study located at Gironde estuary (France), PFASs were evaluated in different invertebrate species (copepods, mysids, and shrimps) and compared to PFASs in suspended sediment, and the authors observed an accumulation of PFASs, where PFOS was the dominant compound followed by long-chain PFASs [98]. Midge larvae were used in laboratory studies to evaluate the accumulation of PFASs from sediments, and both studies observed the accumulation of PFOS and long-chain PFASs [100,101]. In echinoderms (*Holothuria tubulosa*) that frequently are exposed to sediments, PFOS and PFOA were the two most abundant PFASs, while other compounds analyzed (short-chain PFASs) were detected in lower amounts [102]. De Vries et al., (2017) [103] studied the toxic exposure of flamingos to PFASs in an area where AFFF was used after a fire. The preliminary effects of the PFASs exposition were observed on the prey of the flamingo population, that was absent the year after the fire. Afterward, all the flamingo returned and fed on organisms with PFASs levels that exceed the safety threshold, placing the birds and other wildlife at risk [103].

As in sediments, PFASs are easily sorbed in soils [104] and became an important source of contamination and primary consumers. Grønnestad et al., (2019) [55] studied PFASs in earthworms from two ski areas close to Trondheim, PFDA was the predominant in soils, while PFTriDA and PFTrDA were the most predominant in earthworms from Granasen, while in Jonsvatnet PFBA predominated in both soils and earthworms. Munoz

et al., (2020) [105] studied the exposition of PFASs to earthworms from a fire-equipment resting side at a major Canadian airport, where PFOS was detected at 207 ± 13 ng/g dw in soil, followed by PFPeA (49 ± 4.8 ng/g dw), and PFHxS (27 ± 1.6 ng/g dw), while in earthworms was PFOS ranged from 3,600 to 27,000 ng/g ww, PFDA from 62 to 662 ng/g ww, and PFPeA from 38 to 605 ng/g ww. Karnjanapiboonwong et al., (2018) [106] evaluated the PFBS, PFHxS, PFHpA, and PFNA effects in earthworms in spiked soils to assess toxicity in soils, where mortality was observed for exposure to PFBS (1,000 ng/g) and ∑PFASs (100,000 ng/g), and weight loss (29%) for to PFNA (100,000 ng/g).

1.2.3. PFASs fate in plants

Plants are exposed to PFASs through water and soil/sediment that is the main vehicle of PFASs uptake by plants. The study of plants permits to picture the fate of PFASs in the environment. PFASs are uptaken by plants mainly through the roots and the chemicals are translocated and accumulated to the different plant compartments as the wood trunk, shoots, leaves, flowers, and fruits. The accumulation depends on different factors related to plant species and their different uptake mechanisms, the PFASs functional group and chain length, the concentration in the environment (water, sediment, or soil), and the properties of the subtract as organic carbon content, cation exchange capacity, salinity, temperature, and pH [28,107–109]. Among PFASs, PFCAs are better taken up by plants than PFSAs due to the relative higher solubility of PFCAs and the sorption of PFSAs in the sediments and soils [29,104]. So, the accumulation of PFASs on plants reflects a specific partitioning behavior in the sediments/soil-water-plant system [29].

PFOS is the dominant compound detected in soils and sediments, but generally, its concentration is lower than PFOA in vegetables and may be attributed to the strong association of PFOS with soils [110]. Also, short-chain PFASs tend to translocate from the root to the above-ground plant parts, while long-chain PFASs remains accumulated in the roots [57,111] due to their reduced ability to cross the Casparian strip, which consists of hydrophobic suberin and lignin and is impermeable to water and molecules [112]. However, in a nutrient solution study in maize, a U-shaped pattern was observed in the uptake of PFCAs with different perfluorinated carbon chain length. Uptake rates increased from four (PFBA; 2.46 µg/g dw) to seven carbons (PFHpA; 0.12 µg/g dw), and then upward an increasing uptake rate to ten carbon (PFDA; 1.95 µg/g dw) [113]. In wheat, the total concentrations of PFASs in roots, straws, husks, and grains were in the range from 140 to 472 ng/g dw, 36.2 to 78 ng/g dw, 6.15 to 37.8 ng/g dw, and 7.32 to 35.6 ng/g dw, respectively, and the distribution of PFASs followed the order of roots > straws > grains ≥ husks, with a predominance of PFHxS, PFHxA, PFOA, and PFOS [114]. Translocation of PFAS to the different plant compartments as roots, stem, shoots, leaves, or fruits, is reported to be dependent on the protein content of the different tissues [59,104].

Plant uptake of PFASs provides the opportunity for phytoremediation of PFASs contaminated sites [115]. Phytoremediation is a type of bioremediation process that uses plants to remove, transfer, stabilize, and/or destroy contaminants from soil, sediments, or water. Among others, the phytoremediation follows different techniques as rhizosphere biodegradation which enhance biological degradation by the microorganisms in the soil; phytostabilization, where the chemical is immobilized in the soil by the plant; and phytoaccumulation or phytoextraction which consist of the sorption of the contaminants

along with other nutrients and water, and their accumulation in the plant tissue. Juncus was proposed to remove PFASs due to the ability and effectiveness to translocate PFASs from wetlands in long-time periods, mainly PFHxA, PFOA, and PFOS [116]. Submerged and free-floating aquatic macrophytes were suggested as a useful tool in phytoremediation and risk assessment, especially long-chain PFCAs that exhibited higher bioconcentration factors from 865 to 1,280 L/kg, while for short-chain PFAS ranged from 17.3 to 123 L/kg [117]. Huff et al., (2020) [115] evaluated the accumulation potential of six PFASs in different woody and herbaceous plants and tissue concentration followed, in general, the trend PFPeA > PFHxA > PFBS > PFOA > PFHxS > PFOS, suggesting an easy uptake shorter perfluorinated chain lengths and carboxylates over sulfonates. In herbaceous species, the greatest concentration of most compounds ranging from a high of 21,882 ng/g for PFPeA to a low of 131 ng/g for PFHxA, and in hardwood species, PFPeA generally accumulate in foliage at a concentration exceeding 30,000 ng/g. The author suggested that the combination of herbaceous plants with tree species could efficiently maximize phytoremediation efficiency [115].

1.2.4. Impact of PFASs in wildlife

Once in the environment, PFASs are accumulated in wildlife and become a problem of growing concern around the world. The intake and retention of substances in an organism entirely through breathing from water and sediment in aquatic ecosystems or air in terrestrial ones is known as bioconcentration, while the intake of a chemical and its concentration in the organism by all possible means, including contact, respiration, and ingestion is known as bioaccumulation. Another term of relevant importance is

biomagnification, which is the process that occurs when the chemical is passed up the food chain to higher trophic levels, such that in predators it exceeds the concentration to be expected where equilibrium prevails between an organism and its environment [118]. As an example, Figure 1.7 illustrated the bioaccumulation of PFASs in gulls, as well as the biomagnification that shows the increase of the PFASs concentration from the primary consumers to the top predators.

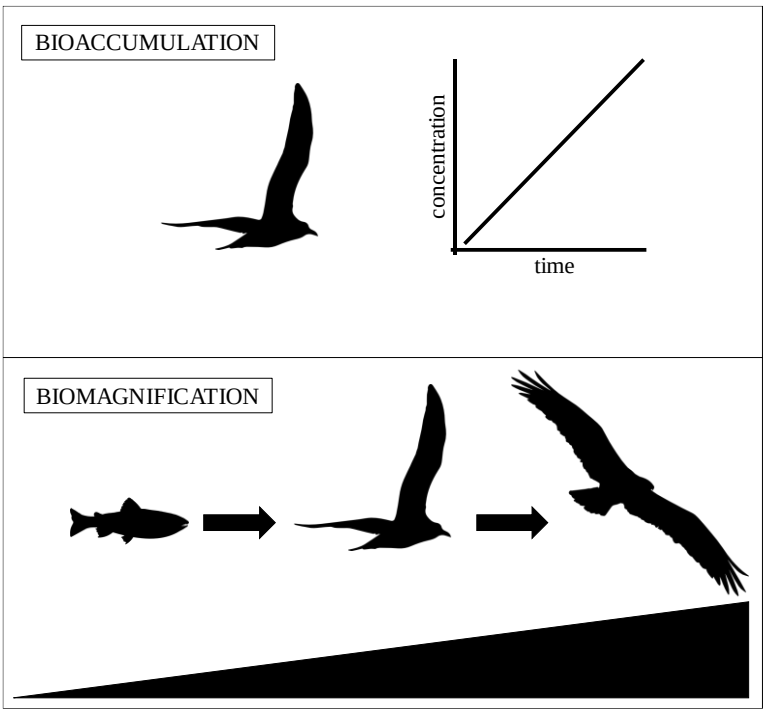

Figure 1.7. Illustration of the different accumulation processes in the natural environment. The upper side, bioaccumulation of contaminants in gulls during a period. On the bottom side, biomagnification of contaminants along the marine-aerial food chain where gulls feed on fish, and eagles feed on gulls and contaminants concentration increases from fish to gull and from gull to eagle.

Bioconcentrations of PFOA and PFOS were studied in different species of larval amphibians and was concluded that the uptake of PFASs was rapid and dependent on the species, explained by functional skin or gills differences in the respiration [30]. Several

studies have revealed bioaccumulation and biomagnification of PFASs, especially PFOS and long-chain PFCAs due to their physicochemical properties (lipophilic and proteinophilic characteristics) and because the accumulation seems to increase with the perfluorinated chain length [63,119]. Bioaccumulation and biomagnification are calculated according to Equation 1.2 and Equation 1.3, respectively, where values >1 indicates bioaccumulation or biomagnification-

$$\text{Bioaccumulation (g/g)} = \frac{[PFASs]\ in\ the\ specie}{[PFASs]\ in\ diet} \qquad \text{Equation 1.2}$$

$$\text{Biomagnification (g/g)} = \frac{[PFASs]\ in\ predator}{[PFASs]\ in\ prey} \qquad \text{Equation 1.3}$$

In East Greenland, PFOS was the predominant among PFSAs in the bear liver (99%), bear fat (89%), seal liver (98%), and PFHxS in blubber (100%). PFUna, PFDoA, PFTriDA, and PFTeDA dominated in bear fat and seal blubber (60–80%), whereas PFNA, PFDA, and PFUnA were the main compounds in the liver (85–90%). The sequence of biomagnification was PFNA > PFDA > PFUnA > PFDoA = PFHxS > PFTriDA, with a decrease in the increase of the chain length due to higher levels in the prey [33]. In bank vole from Granåsen (Norway), PFASs profile was dominated by PFOS (3.30 ± 3.37 ng/g ww), PFTeDA (2.56 ± 9.61 ng/g ww), PFTriDA (2.15 ± 6.25 ng/g ww), and PFDoA (2.11 ± 6.08 ng/g ww), but biomagnification was only observed for PFOS [55]. In a food chain from the Barents Sea, PFOS had the largest relative contribution of the individual PFASs and constituted 52, 41, 80, and 94% o in ice amphipods, polar cod, black guillemot, and glaucous gull, respectively. Biomagnification was calculated for PFHxS, PFOS, and PFNA in different combinations of species and PFOS showed higher bioaccumulation, followed by PFNA and PFHxS [65]. In two remote locations in Canada,

PFASs were analyzed in the liver of caribou and wolf. In caribou liver PFNA (2.2 ± 0.2 and 3.2 ± 0.4 ng/g ww), PFDA (1.9 ± 0.1 and 2.2 ± 0.2 ng/g ww), and PFUnA (1.7 ± 0.1 and 3.2 ± 0.2 ng/g ww) were the predominant PFASs, similar to wolf that were PFNA (4.7 ± 0.9 and 7.4 ± 1.3 ng/g ww), and PFUnA (2.5 ± 0.4 and 6.4 ± 1.2 ng/g ww). Biomagnification was observed for all compounds (from PFOA to PFTriDA, and PFOS) with higher levels in PFNA, PFTriDA, and PFOS [37]. Instead, short-chain PFASs have a low biomagnification degree and may be due to elimination via urinary extraction [120] or respiration extraction [121,122], however, the PFASs elimination through respiration is only observed in water environments via gills (fishes and Mollusca), while in air-breathing animals (birds and mammals) it is negligible due the low volatility of PFASs [36]. Kelly et al., (2009) [36] used the K_{ow} and the octanol-air partition coefficient (K_{oa}) to understand the behaviour of PFASs. PFOA, PFNA, and PFOS have low K_{ow} and high K_{oa} ($K_{ow}<10^5$, $K_{oa}>10^6$) and are expected to biomagnify especially in air-breathing animals, while long-chain PFCAs were characterized with high K_{ow} and high K_{oa} ($K_{ow}>10^5$-10^9, $K_{oa}>10^6$), so were expected to biomagnify in all food webs [36].

1.2.5. Birds as bioindicators of PFASs pollution

To evaluate the presence of PFASs in the environment, sentinel species have been used to monitor the contaminants. Sentinel species are known as a bioindicator and is defined as an organism or biological response that reveals the presence of the pollutants by the occurrences of typical symptoms or measurable responses as physiologically, chemically, or behaviorally [123]. The following criteria were established to identify bioindicators of pollution by Cunha & Guilhermino (2006) [124]:

- Sentinel species should be easy to identify with taxonomic characteristics.
- Species should be representative of the studied area and have a broad geographical distribution.
- Knowledge of the selected species must be good to understand their biology, ecology, physiology, and other relevant aspects of the species.
- Species should be abundant and accessible to avoid effects on population evolution for obvious ecological reasons.
- Sentinel species should be of reasonable size to allow the individual analysis of specimens and specific organs, compartments, or fluids.
- Species should respond to the contaminant but strong enough to survive in the environment.
- Feeding habits of the species must be well known to identify sources of pollution and their trophic position.
- Species should be able to reflect the local conditions and have a life cycle restricted in the study area.

Different taxonomic groups have been used as bioindicators of PFASs pollution in the environment. Among plants, duckweed [125], aquatic macrophytes [117], and junks [116] were suggested for aquatic ecosystems. In the animal kingdom, amphipods were recommended as potential bioindicators because represent the majority of the biomass of benthonic invertebrates even in cases of poor ecological conditions [126]. Phylum Mollusca is also used as bioindicators, especially shellfish [127] as mussels [45]. Fish as trout [126], blue sharks [128], and young-of-the-year fish were found to be suitable bioindicators, and especially the last ones because have several advantages compared to

adult fish [129]. Birds from different species were also recommended, as an example chicken close to industrial areas [130], and glaucous gull [131], and snow bunting from remote areas in Svalbard [132]. Among mammals, wild boar [133] and roe deer [134] were proposed as suitable bioindicators in two different studies in Germany.

Birds are long-lived top predators that are exposed to relatively high levels of environmental contaminants and generally show high site fidelity [135]. Due to their preferable accumulation in protein-rich tissue, PFASs have been studied in many matrices in birds in many matrices as liver [65,136], plasma, and blood [51]. PFASs analysis in these tissues is an invasive method, and for example, to analyze the liver the birds need to be sacrificed. However, more often liver is obtained form dead animals. Other studies have used bird eggs to evaluate the presence of pollutants as a relative non-invasive matrix to monitor the presence of PFASs. PFASs accumulation in female birds is transferred to her entire clutches during the laying period [137] and consequently may lead to toxicological effects on the survival of the chick and having population impacts [138]. Differences in PFASs accumulation patterns among species are due to the dietary habits and the location of each species [139–142], but food accessibility and pollution in the surrounding environment play an important role in the PFASs levels in the same species from different locations [143]. Since the application of the legislation of the PFASs production and use, some studies suggest changes in the PFASs profile in different matrices, with a decrease of PFOS and PFOA and the increase of the short and long-chain PFASs [140,144].

1.3. ANALYTICAL METHODS FOR PFASs DETERMINATION

The first studies in the early 2000s included PFOA and PFOS [51]. Over time, the number of studied PFASs had increased, and in the last 20 years, PFASs have been determined at concentrations from µg/L thanks to the huge advances in analytical techniques [23]. In most of the studies, selective extraction techniques are used and compounds are determined by liquid-chromatography coulpled to mass spectrometry, either using a simple quadrupoe, tandem mass spectrometry, time of flight or Orbitrap. In the following section the methods generally used are indicating according to the different matrices.

1.3.1. Extraction method

1.3.1.1. Water samples

The analysis of water samples can be performed using non-filtered water to determine the total concentration or by applying a pre-treatment as filtration, ultracentrifugation to exclude particulate matter, and/or pH adjustment. Table 1.6 summarizes the different analytical methods used in water samples according to the bibliography. Generally, volumes from 0.5 to 2 L are extracted, but it depends on the water and sampling procedure. Solid-phase extraction (SPE) cartridges are commonly employed for PFASs analysis, and methanol is the most common elution solvent used in SPE cartridges for sample extraction. Other cartridges used are Oasis WAX (Waters, Inc.), Strata-X cartridges (Phenomenex, Torrance, CA, USA), Oasis HLB (WATERS), and SPE cartridges filled with bamboo charcoal.

Table 1.6. Extraction and analysis of PFAS in water.

Matrix	Volume	Extraction	Clean-up	Instrument	LOD/LOQ	QA/QC	Reference
Arctic and Antarctic Water	2 L	SPE (Oasis MAX)	-	HPLC-MS/MS (-ESI)	0.0059-0.051 ng/L	Blank & Recoveries	[145]
Costal water	1 L	SPE (Oasis HLB)	-	UPLC-TQD-MS/MS (-ESI)	0.01-0.04 ng/L	Blank & Recoveries	[44]
Sea and wastewater	1 L	SPE (Oasis WAX)	Filtred (0.2 μm nylon filter)	HPLC-QQQ-MS	0.04-0.24 ng/L	Blank & Recoveries	[40]
Seawater	1 L	SPE (Oasis WAX)	-	LC-MS/MS	-	Blank & Recoveries	[16]
Seawater	1 L	SPE (Oasis WAX)	ENVI-Carb	UPLC-Qtrap-MS	0.001-0.02 ng/L	Blank & Recoveries	[39]
Seawater	1 L	SPE (Oasis HLB)	-	UPLC-TQD-MS/MS (-ESI)	0.0006-0.003 ng/L	Blank & Recoveries	[45]
Surface and wastewater	0.5 L	SPE (Oasis WAX)	-	LC-MS/MS (-ESI)	0.05-1.79 ng/L	Blank & Recoveries	[29]
Water	0.5 L	SPE (Oasis WAX)	-	HPLC-MS/MS (-ESI)	0.05-0.12 ng/L	Blank	[25]
Water	1 L	SPE (Oasis WAX)	-	UPLC-Xevo-TQD-MS/MS	0.005-0.250 ng/L (MDL)	Blank & Recoveries	[146]
Water	1 L	SPE (Oasis WAX)	-	UPLC-TSQ-MS/MS (-ESI)	1.04-20.98 ng/L	Recovery	[147]
Surface, drinking and river water	1 L	SPME)	-	LC--MS/MS (-ESI)	13-0131.9 ng/L	Blank & Recoveries	[148]
Surface and grownd water	1 L	Micro-LLE	-	LC-MS/MS (-ESI)	0.71-67 ng/L (MDL)	Blank & Recoveries	[149]

Clean-up methods, if are required, are generally based on the use of ENVI-Carb (Supelco, Bellefonte, PA, USA), filtration, or column wash [94]. Other extraction techniques such as liquid-liquid extraction (LLE), solid-phase microextraction (SPME), ion-pair extraction (IPE), and dispersive liquid-liquid microextraction (DLLME) have been used to determine PFAS in water and provide enhanced selectivity of target compounds [150].

1.3.1.2. Sediment and soil samples

Pre-treatment in sediment and soil samples is generally freeze-drying, sieving, and homogenization before being analyzed to eliminate background interferences and increase recovery rates. Table 1.7 exposes different analytical methods from the bibliography based on the most common techniques used. Sample weight depends on the accessibility of the sample, usually ranging from 1 to 10 g in dry weight (dw). Sample extraction should be capable of retaining PFAS, and the most used extraction methods in the bibliography are based on Soxhlet extraction, pressurized liquid extraction, and solid-liquid extraction [94] with methanol, acetonitrile or tetrabutylammonium hydrogen sulfate and sodium carbonate, followed by additional clean-up procedures based on ENVI-Carb (Supelco, Bellefonte, PA, USA), SPE cartridge or filtration [94,150]. In some studies, SLE and LEE are performed for extraction, followed by a purification using a nylon filter [40] or direct injection [96]. Activated carbon with glacial acetic acid is used in an Eppendorf (1.5 mL) as a clean-up before the injection [45]. These procedures have been optimized for anionic PFASs measurement, but novel PFASs require different processes to avoid their loss [151,152].

Table 1.7. Extraction and analysis of PFAS in soil and sediment.

Matrix	Weight	Extraction	Clean-up	Instrument	LOD/LOQ	QA/QC	Reference
Soil	3 g dw	SLE	ENVI-Carb	LC-MS/MS (-ESI)	0.02-2.7 ng/g	Blank & Recoveries	[29]
Soil	5 g dw	SLE	ENVI-Carb	HPLC-MS/MS	0.005-0.125 ng/g (MDL)	Blank & Recoveries	[55]
Soil	1 g dw	SLE	-	UHPLC-HRMS Thermo Q-Exactive Orbitrap	0.06-6 ng/g (MRL)	Blank & Recoveries	[105]
Soil	2 g dw	SLE	ENVI-Carb	HPLC-TQD-MS/MS (-ESI)	0.01-0.03 ng/g	Blank & Recoveries	[54]
Sediment	5 g dw	SLE + LLE	Filtred (0.2 μm nylon filter)	HPLC-QQQ-MS	0.2-0.50 ng/g	Blank & Recoveries	[40]
Sediment	10 g dw	SLE	SPE (Oasis WAX)	LC-MS/MS (-ESI)	0.01-0.14 ng/g (MDL)	Blank & Recoveries	[36]
Sediment	5 g dw	SLE	Filtred (0.2 μm nylon filter)	HPLC-MS/MS (-ESI)	0.019-0.027 ng/g	Blank & Recoveries	[26]
Sediment	3 g dw	SLE	ENVI-Carb	HPLC-MS/MS (-ESI)	0.05-1 ng/g	Blank	[25]
Sediment	1 g dw	SLE	LLE	UPLC-Xevo-TQD-MS/MS	0.02-0.20 ng/g (MDL)	Blank & Recoveries	[146]
Sediment	2 g dw	SLE	SPE (Oasis WAX)	UPLC-TSQ-MS/MS (-ESI)	-	Recovery	[147]
Sediment	1.5 g dw	SLE	ENVI-Carb	UPLC-Qtrap-MS	0.001-0.007 ng/g	Blank & Recoveries	[39]
Sediment	1 g dw	SLE	Activated carbon	UPLC-TQD-MS/MS (-ESI)	-	Blank & Recoveries	[153]

1.3.1.3. Biota samples

Biota samples usually are freeze-dried and homogenized before PFASs extraction although some studies use wet extraction. Table 1.8 shows an overview of the different analytical methods performed in biotic samples. The weight analyzed depends on the accessibility of the matrix, for example, earthworms are usually analyzed as an individual organism [105,106], while for plants or animal organs the extraction amount ranged from 1 to 5 g [29,37]. Different extraction methods such as SLE, LLE, IPE, alkaline digestion, and acetonitrile protein precipitation have been used. Methanol, acetonitrile, tetrabutylammonium hydrogen sulfate, and tert-butyl methyl ether are solvents commonly used in the PFASs extraction from biotic samples [150]. Biological matrices from biota and humans are complex matrices, so special care should be taken in the digestion and the extraction of PFASs. IPE methods are performed in biological tissues (muscle, liver, kidney, gall bladder, blood, gill, gonads, and adipose tissue) and biota (fish, mollusks, crustaceans, and benthonic worms), but have shown disadvantages with the co-extraction of lipids, and other disturbing substances [63]. Clean-up usually is performed to purify the extracts before injection using ENVI-Carb (Supelco, Bellefonte, PA, USA), SPE cartridges, active carbon due to their high porosity, and filtration [94]. Low-temperature clean-up with OASIS HLB, OASIS WAX, and Envi-carb SPE cartridge is an effective way for the removal of lipid components in fatty matrices [154]. Quick, Easy, Cheap, Effective, Rugged, and Safe extraction salt (QuEChERS) is used to obtain a well-defined phase separation of the water and the organic supernatant during the extraction [34], as well is used as a clean-up method instead of the traditional SPE [106].

Table 1.8. Extraction and analysis of PFAS in biota.

Matrix	Weight/ Volume	Extraction	Clean-up	Instrument	LOD/LOQ	QA/QC	Reference
Aquatic machrophytes	5 g dw	SLE	SPE (Phenomenex X-AW)	LC-TQD-MS/MS (-ESI)	0.16-1.17 ng/g	Blank & Recoveries	[117]
Caribu and wolf Liver, muscle and kidney	1 g	SLE	SPE	LC-MS/MS (-ESI)	0.01-0.5 ng/g (MDL)	Blank & Recoveries	[37]
Different marine organisms	1-10 g	SLE	SPE (Oasis WAX)	LC-MS/MS (-ESI)	0.01-230 ng/g (MDL)	Blank & Recoveries	[36]
Earthworms	Individual worm	SLE	ENVI-Carb	UHPLC-HRMS Thermo Q-Exactive Orbitrap	0.4-7 ng/g (MRL)	Blank & Recoveries	[105]
Earthworms	Individual worm	SLE	QuEChERS + Filtred (0.2 μm cellulose acetate)	UHPLC-TSQ-MS/MS (-ESI)	1.3-10.3 ng/g (MDL)	Blank & Recoveries	[106]
Earthworms and bank voles	0.5 g	SLE	ENVI-Carb	HPLC-MS/MS	0.021-5.3 ng/g (MDL)	Blank & Recoveries	[55]
Fish	1 g	SLE + QuEChERS	SPE (Oasis WAX)	LC-MS/MS (-ESI)	1 ng/g	Blank & Recoveries	[34]
Fish	1 g ww	SLE	LLE	UPLC-Xevo-TQD-MS/MS	0.025-0.15 ng/g (MDL)	Blank & Recoveries	[146]
Fish and crustaceans	-	SLE	SPE	HPLC-Qtrap-MS/MS	0.3 ng/g (LOR)	Blank & Recoveries	[31]

Table 1.8 (continued)

Matrix	Weight/ Volume	Extraction	Clean-up	Instrument	LOD/LOQ	QA/QC	Reference
Gannet eggs	1 g ww	SLE	Activated carbon	UPLC-TQD-MS/MS (-ESI)	0.015-0.137 ng/g	Blank & Recoveries	[66]
Gull eggs	1 g ww	SLE	Activated carbon	UPLC-TQD-MS/MS (-ESI)	0.09-0.30 ng/g	Blank & Recoveries	[143]
Gull plasma	200 μL	LLE	SPE	UPLC-MS/MS	0.001-0.290 ng/mL	Recovery	[35]
Ice amphipod, polar cod, black guillemots, and glaucous gulls	1 g	SLE	Filtred through Kleenex and Microcon YM-3 centrifugal filter	HPLC-QTOF-MS (-ESI)	0.03-15 ng/g	Blank & Recoveries	[65]
Mussels	1 g ww	SLE	Activated carbon	UPLC-TQD-MS/MS (-ESI)	-	Blank & Recoveries	[45]
Plankton	2 g ww	SLE	-	LC-MS/MS	-	Blank & Recoveries	[16]
Plant	3 g dw	SLE	ENVI-Carb	LC-MS/MS (-ESI)	0.02-2.7 ng/g	Blank & Recoveries	[29]
Plant	1 g	SLE	SPE	LC-MS/MS (-ESI)	0.003-0.076 ng/g (MDL)	Blank & Recoveries	[37]
Seals and polar bear liver	0.3-1.6 g	SPE (Oasis WAX)	VWR centrifugal filter	HPLC-QTOF-MS (-ESI)	0.02-0.59 ng/g	Blank & Recoveries	[33]

1.3.2. Instrumental analysis of PFASs

Different chromatography techniques have been used for PFASs analysis. Liquid chromatography-mass spectrometry (HPLC-MS) is a common technique and is the method of choice for the determination of PFASs. Since most target PFASs are anionic and electronegative, mass spectrometry (MS) is generally operated in negative electrospray ionization (ESI). PFASs are generally separated by a C18 column with an aqueous and methanol/acetonitrile mobile phase containing 5-50 mM ammonium acetate [94]. Some studies used high-resolution mass spectrometry (HRMS) such as Orbitrap- or time of flight mass analyzer (TOF)-MS for quantitative and qualitative analyses [94]. Also, TOF methods have generally lower sensibility leading to higher limits of quantifications. Negative electrospray ionization and Triple-quadrupole (QqQ) MS is a well-known technique used for quantitative analysis [63]. Combustion ion chromatography (CIC) analysis is performed to measure the total organic fluorine and requires proper sample preparation where PTFE should be avoided [155]. Non-ionic, ionic compounds after derivatization and volatile PFASs are determined by gas chromatography-mass spectrometry (GC- MS).

1.4. STUDY AREAS

1.4.1. Gull breeding colonies from the Iberian Peninsula

Gull colonies are distributed along the Iberian Peninsula. Traditional nest sites include sea-cliffs, dunes, islands on the coast and inland, and other inaccessible locations. Among them, some of the most important gull colonies are located in Spanish Natural and

National Parks (Figure 1.8) as Medes Islands, Ebro Delta, Atlantic Islands, and Chafarinas Islands [156].

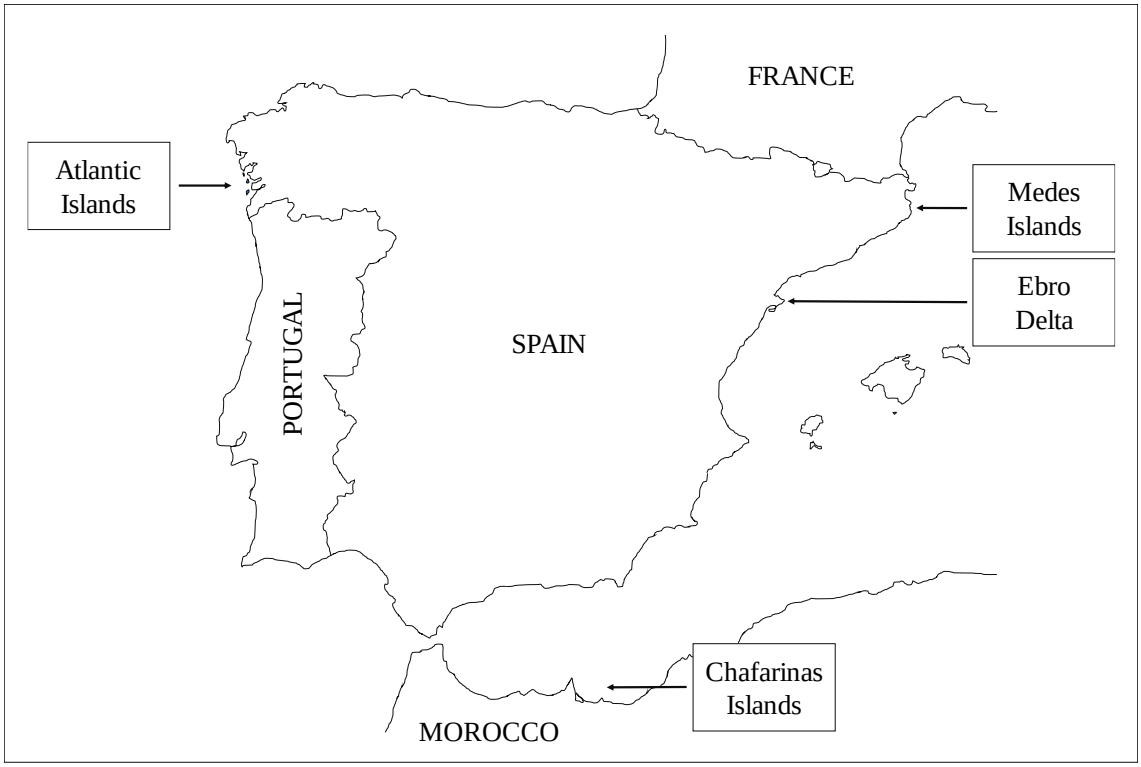

Figure 1.8. Map of the Iberian Peninsula and the location of the studied colonies.

1.4.1.1. Medes islands

Medes Islands are an archipelago of seven islands and some reefs at the Montgrí, Medes, and Baix Ter Natural Park. The biggest island in the archipelago is named Meda Gran, followed by Meda Xica and other rocky outcrops jutting out of from the sea as Cavall Bernat, Tascons Grossos, Medallot, Tascons Petits, and Ferrenelles, with a total area of 0.215 km^2 and an altitude of 75 m (Figure 1.9). The islands have a characteristic asymmetry on the east and west sides, eastern slopes are generally vertical cliffs that

penetrate directly to the sea up to 50 m deep on the water, while the western side penetrates to the water softly[2].

Figure 1.9. Satellite image of Medes Island (Source: Google Earth Pro).

Under a special climate condition, the Medes Islands are covered mainly with halophilic vegetation and without a dense tree layer that offers refuge to the nesting heron colony, European shag, and other bird species. Nevertheless, the yellow-legged gull colony forms the most extensive colony along the island, producing a transformation of the vegetation

[2] Web page http://parcsnaturals.gencat.cat/ca/illes-medes

into more ruderal. Underwater, the environment close to the island is covered by calcareous alga and by a dense cover of more than a hundred algae species. Marine phanerogams, especially *Posidonia oceanica*, form an exclusive hotspot of biodiversity and an important contribution to their biodiversity and ecological values[3].

Outside the Medes Islands, several pressures impact the surrounding environments of the area. Medes Islands receive waters from WWTP effluents of different towns whose economy is based on tourism, and consequently, the discharge of water increases significantly during summer. Also, the Ter River discharges its waters close to the islands and rises in Ulldeter. After 208 km it arrives at the coast and along the journey the river receives water from different agricultural areas, industries, and cities which increases the impact on the archipelago. Due to the south direction of the Mediterranean northwest sea currents, Medes Islands receive also waters from the Gulf of Lion that receives the waters of the Rhone river which is a highly impacted river [157]

The preservation of the Medes Islands started in 1983 when the Government of Catalonia published the Order which forbids the fishing and extraction of natural resources from the area. Thereafter, the regulation was supported by Law 19/1990 that amplified the protected area and focused on the fauna and flora of the islands. In 1992, Decree 328/1992 included the terrestrial environment of the islands in the Plan for Areas of Outstanding Natural Beauty. In 2001, the underwater environment of Medes Islands was included in the list of Special Protected Areas of Importance of the Mediterranean (ZEPIM), and in 2006 the Government of Catalonia designed Medes Islands as a Special Protection Area

[3] Web page (http://parcsnaturals.gencat.cat/ca/illes-medes

for Birds (ZEPA). The Montgrí, Medes and Baix Ter Natural Park were created in 2010 by the Law 15/2010 and remain protected since then[4].

1.4.1.2. Ebro Delta

Ebro Delta Natural Park is the conjunction of the mouth of the Ebro River, coastal area, dunes, bays, brackish waters, riparian forest, coastal lagoons, river islands, tusks, and rice fields (Figure 1.10). Ebro Delta is the largest aquatic habitat in Catalonia with 320 km^2, hosting rich biodiversity in contrast with human settlements and agricultural activities[5].

Figure 1.10. Satellite image of Ebro Delta (Source: Google Earth Pro).

[4] Web page http://parcsnaturals.gencat.cat/ca/illes-medes

[5] Web page http://parcsnaturals.gencat.cat/ca/delta-ebre/

The flat appearance of the region gives a particular aspect to the delta dominated by attractive landscapes with different ecosystems surrounded by water. The aquatic ecosystems of the delta are rich in phytoplankton, invertebrates, bivalves, and fish species. On the seaside, two different environments are related, while open water is directly exposed to the sea, two sand peninsulas conform two bays of important relevance as bird feeding areas. Under the influence of the sea and the wind, dunes are covered by psammophytes, a vegetable community adapted to the changes of the dunes, and a perfect environment for insects, reptiles, and bird nesting. Among the 400 bird species in Ebro Delta, yellow-legged gulls form one of the biggest colonies distributed on the Catalan coast. Also, Audouin's gull nest in this area in one of the biggest breeding colonies of this species endemic from the Mediterranean Sea. Rice fields and lagoons are an excellent feeding area for bird species on their migration[6].

The Ebro delta is the most impacted aquatic environment on the western Mediterranean coast. It is known as an area of intense agricultural activities with 21,000 ha of rice cultivation, and shellfish farming that becomes a continuous input of contaminants (biocides) to the area. Besides, two nuclear stations (Ascó and Vendallós) and a chloro-alkali industry are located upstream waters of the Ebro River. The Spanish national Hydrological Plan enhances a relevant impact on the area, decreasing the water flow that reduces the dilution of the contaminants [158].

The preservation of this area started in 1983 as a consequence of the popular movement against a drying project of the delta. Thereafter, the Government of Catalonia and the

[6] Web page http://parcsnaturals.gencat.cat/ca/delta-ebre/

town hall of Deltebre Decree the Ebro Delta Natural Park on the left bank of the river, and two years later Decree 332/1986 establishes the regulation to the remaining right side of the delta. In 1987 Ebro Delta was declared Special Protection Area for Birds (ZEPA) and in 1993 was included in the List of Wetlands of International Importance (RAMSAR)[7]. In 1997 the European Commission included the Ebro Delta at the European Natura 2000 Network as a Site of Community Importance and Special Area of Conservation because of their rich biodiversity[8], and in 2013 the UNESCO declared *Terres de l'Ebre*, which include Ebro Delta Natural Park, a Biosphere Reserve[9].

1.4.1.3. Atlantic Islands

Atlantic Islands are a conjunction of different groups of islands that all together constitute the Atlantic Islands Maritime-Terrestrial National Park, which includes Cies, Ons, Salvora, and Cortegada archipelagos (Figure 1.11). Cies archipelago is constituted by 26.6 km^2 of marine, 4.33 km^2 of terrestrial, and an altitude of 197 m. Ons with 21.7 km^2 of marine, 4.70 km^2 of terrestrial, and a peek at 119 m. Salvora with 2.31 km^2 of marine, 2.48 km^2 of terrestrial, and an altitude of 73 m. Cortegada with 1.47 km^2 of marine, 0.44 km^2 of terrestrial, and an altitude of 19 m[10].

[7] Web page http://parcsnaturals.gencat.cat/ca/delta-ebre/

[8] Web page https://natura2000.eea.europa.eu/

[9] Web page http://www.unesco.org/

[10] Web page https://www.miteco.gob.es/es/red-parques-nacionales/nuestros-parques/islas-atlanticas

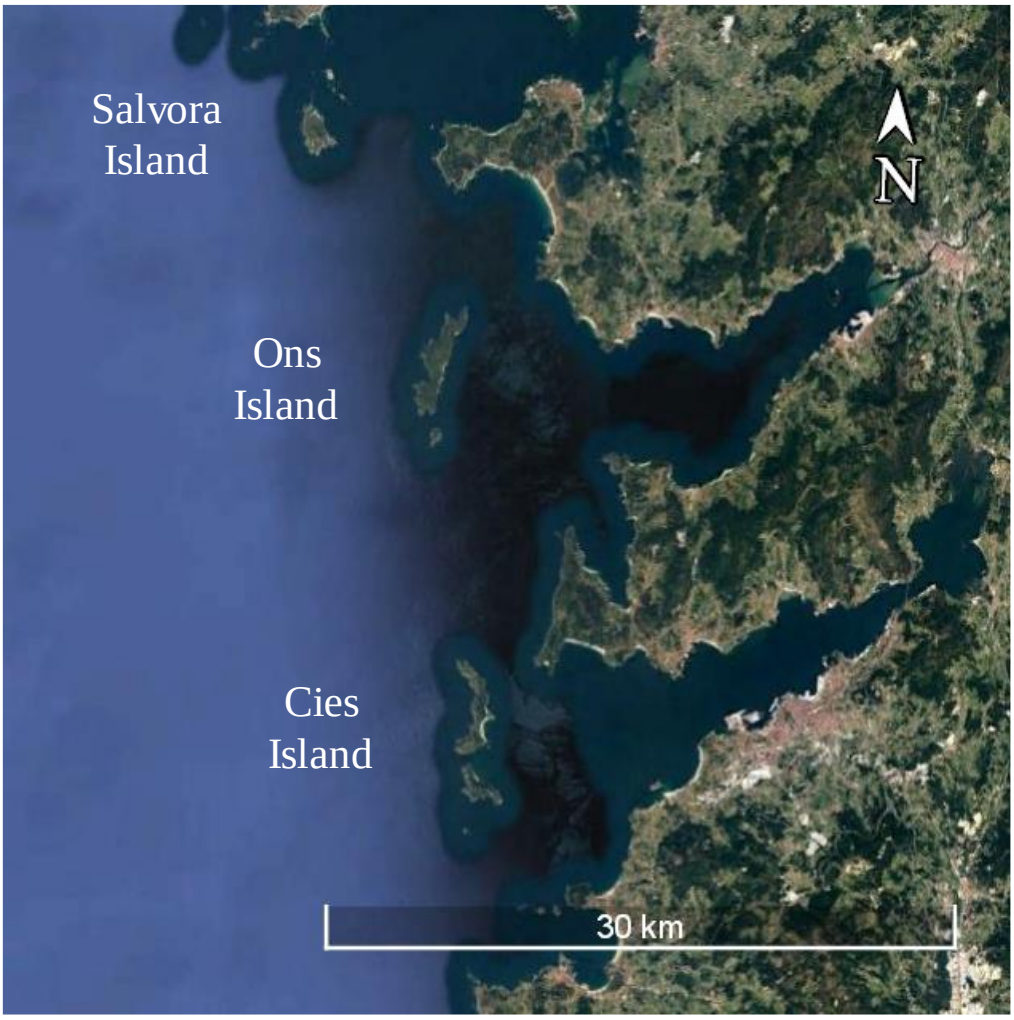

Figure 1.11. Satellite image of the Atlantic Islands (Source: Google Earth Pro).

The Atlantic Islands Maritime-Terrestrial National Park forms a submerged mountain chain that protects the Galician coast from sea storm and receives the water from the characteristic rivers along the coast. These environmental conditions give a special orography to the area, with beaches at the east and sharp cliffs at the west. The terrestrial environment of the islands comprises a wide variety of ecosystems that host many endemic and migrant species. The marine areas of the Atlantic Islands Maritime-Terrestrial National Park are highly relevant because of their ecological value and attractive landscapes. In these islands, the yellow-legged gull colony forms one of the

biggest breeding areas in Europe, however the population decrease during the last years due to the gradual covering of spills from the nearby coast. The European shag is another species that forms colonies in this archipelago. Tides are an important phenomenon in the area and define the existent ecosystems of the islands. The atmospheric conditions generate a perfect environment for the proliferation of the algae community, which is the base of the food chain and host bivalves, cephalopods, and fish species[11].

Atlantic Islands are a landscape highly visited by tourism during the summer season so receive the pressures and sailing activities. The populated coast exerts an impact through the municipal waste and WWTP effluents. Also, the Galician coast is well known for fishing activities and fish and shellfish farming, and consequently, the use of biocides and port activities that are a risk of accidental fuel waste. Besides, Galicia has more than 100 industrial, commercial, and service states spread along the region that enriches the community and at the same that increase the impact of their activities.

In 2002 the Atlantic Islands were designed under the Law 15/2002 Atlantic Islands Maritime-Terrestrial National Park, and Cíes in 1988 and Ons in 2001 were declared Special Protection Area for Birds (ZEPA). Cies, Ons and Salvora were included in the European Natura 2000 Network as a Site of Community Importance, and in 2008 at the agreement of the Oslo-Paris (OSPAR) Commission about the conservation of marine ecosystems, human health, and maritime areas affected by pollution[11].

[11] Web page https://www.miteco.gob.es/es/red-parques-nacionales/nuestros-parques/islas-atlanticas

1.4.2. Chafarinas Islands and the north Moroccan Coast

The Chafarinas Islands (Spain), referred to in Morocco as the Zafarin Islands, are a group of three small rocky islets called Congreso, Isabel II, and Rey Francisco (Figure 1.12). This archipelago with an aggregate area of 0.525 km^2 is placed in the north of the African continent in the Alboran Sea, especially in the Moroccan coast at about 3.3 km off the Moroccan town of Ras Kebdana, 4 km north of Cape Agua, 11 km northwest of the mouth of the Moulouya River, 45 km to the east of Nador (Moroccan City) and 50 km east of Melilla (Spanish City in the African continent).

Figure 1.12. Satellite image of Chafarinas Islands (Source: Google Earth Pro).

Since 1847, the Chafarinas Islands are one of the Spanish territories in North Africa off the Moroccan coast designated as a 'National Hunting Refuge' and managed by the National Parks Autonomous Agency. Also, the archipelago is declared a Special Protection Area for birds in 1989, and became a Site of Community Importance of the Natura 2000 network. Chafarinas Islands is a marine refugee for Yellow-legged gull, Andouin's gull, and Scopoli's shearwater. The islands host population of various species of reptiles [159]. Among the islands, Congreso and Rey Francisco are uninhabited, while Isabel II contains a lighthouse and the Spanish Civil Guard garrison [160]. It is operated under a particular management scheme because a territorial conflict exists between Spain and Morocco and the islands are under military contingent, access control of people - especially for personnel external to the agency- and materials, and administrative authorization is required for some activities such as sampling [161].

The Chafarinas Islands and their natural environment are surrounded by anthropogenic pressures. The archipelago is affected by the waters of the ports of Melilla, Nador, and Ras Kebdana, and human-made water channels which cross Nador and the surrounding agricultural areas and flow to Mar Chica, a natural lagoon with high ecological interest. Moulouya River discharges its waters to the Mediterranean Sea and waters near Chafarinas Islands. Previous studies suggest that Chafarinas Islands receive the impact from lead ore mining areas (Zaida, Morocco) where the Moulouya River flows [162], urban discharges, wastewater discharge, and untreated wastewater, and agricultural run-off [163].

1.4.3. Xiaoqing River Basin in China

The Xiaoqing River is a major river of the Shandong Province on the eastern edge of the North China plain [164]. It emerges from a small spring-fed lake in a limestone outcrops zone near Jinan and flows downstream through the Bohai Rim and empty into Laizhou Bay (Figure 1.13). With a total length of 240 km and a basin area of approximately 10,336 km^2, Xiaoqing River basin host three main cities (Jinan, Zibo, and Weifang) with an approximated total population of 21 million habitants [165].

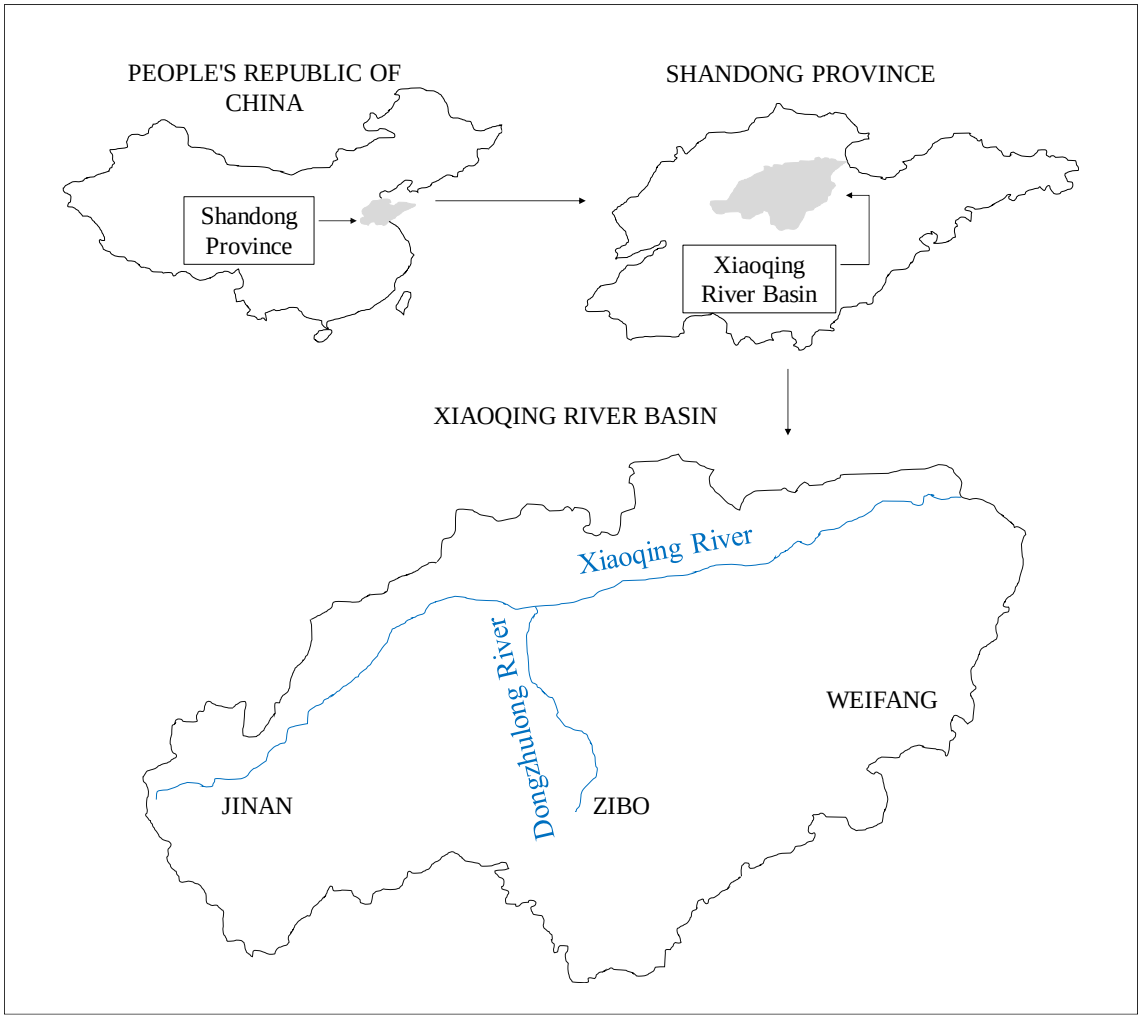

Figure 1.13. Map of the Xiaoqing River Basin in the Shandong Province of the People's Republic of China.

Shandong is China's second most populated province, segmented in an inland zone and a peninsula. The inland zone is bounded by the province of Heibei to the north and west, Henan to the southwest, and Anhui and Jiangsu to the south, and covers two-thirds of the province's total area. This area includes a hilly central region which consists of a much-shattered fault block, mostly composed of archaic crystalline shales and granites and some ancient limestones, and after a fertile and intensively farmed agricultural area on the north, west, and south, which forms part of the Yellow River basin. The peninsula is an upland area extended 320 km seaward with a coastline of 2,535 km and traditionally depends on fishing, mining, and port-related activities. Generally, Shandong has a diversified agricultural and industrial economy for internal consumption and exportation to other provinces and overseas[12].

Natural vegetation in the area remains in the intensively cultivated inland zone with species as reeds, grassy legumes, and several varieties of shrubs, notably tamarisk. Along the coastal area, vegetation is commonly halophytic and they are used for fuel and salt manufacture. Wildlife in the area has suffered a drastic decline because of human settlement, intensive cultivation, and forest destruction. However, mammals as roe deer, mice, mandarin ducks, dollar birds, and large owls are limited, insects, beetles, and moths are still usually diverse and varied in the area [12].

Due to the rapid development of the coastal areas in North China, industrial activities increased to enrich the region and satisfy the needs of the population on a local and global scale. Shandong is one of the fastest developing provinces in China. After intensive

[12] Web page https://www-britannica-com.sire.ub.edu/place/Shandong-province-China

development of urban areas, more industries are moving from urban to rural and/or suburban areas in other to obtain adequate and cheaper land and laborers [166]. Close to the city of Zibo a highly industrialized area is located, especially the chemical industry [167]. The fluoropolymer industry in Shandong province was investigated and the major manufacturing facilities were found located along the Xiaoqing River, and their production began in 2001. The manufacturing history of the profile of PFASs and related products in these facilities is unknown, but until now, fluorinated refrigerants, intermediates for the production of pesticides and medicine, polytetrafluoroethylene, and tetrafluoroethylene have been the main products of these facilities [48].

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CHAPTER 2.

OBJECTIVES

CHAPTER 2

The main purpose of this Doctoral Thesis is to study the distribution and behavior of PFASs in different environmental compartments and to elucidate the fate among them. PFASs have been analyzed in water, sediments, plants, and biota to determine the uptake mechanisms and bioaccumulation patterns in real environmental conditions. This Thesis has the study of a Chinese area directly impacted by a fluoropolymer production facility in the Shandong Province and the study of protected areas in Spain with no direct sources of pollution.

The specific objectives of this thesis can be summarized as follows:

1) To evaluate the partitioning of PFASs in water and sediments collected along the Dongzhulong and Xiaoqing rivers in China and to determine the plant uptake and mobilization of these substances in different aquatic plant species.
2) To evaluate the occurrence and trophic chain magnification of PFASs in the Chafarinas Islands by analyzing soil, sediment, fish, and gull eggs.
3) To study the geographical distribution and 10-year temporal patterns of PFASs in eggs of *Larus michahellis* and *Larus audouinii* breeding colonies of the Iberian Peninsula under a variety of anthropogenic pressures and ecology.

With the aim to reach each specific objective, this thesis was structured into three parts:

- The dynamics of PFASs in a freshwater environment under the pressure of an industrial facility were studied in Chapter 3.
- The bioaccumulation potential of PFASs in wildlife from marine environments was investigated in Chapter 4.
- The PFASs patterns in gull eggs from a protected area were evaluated in Chapter 5.

CHAPTER 3.

PLANT UPTAKE OF PERFLUOROALKYL SUBSTANCES IN A FRESHWATER ENVIRONMENT (DONGZHULONG AND XIAOQING RIVER, CHINA)

CHAPTER 3

3.1. INTRODUCTION

Perfluoroalkyl substances (PFASs) are industrial compounds whose production started in the 1950s for commercial purposes [1]. PFASs have excellent thermal and chemical stability and are used as polymers, surfactants, stain repellents, and flame retardant in several products as carpets, leather, paper, textiles, fire-fighting foams, etc [2]. PFASs are ubiquitous in the environment due to discharges from various point sources such as manufacture and processing industries, use of aqueous film-forming foams, wastewaters discharges, landfills, and air emission [3] and remain in the aquatic system due to their persistent and bioaccumulative properties [4].

Once in the environment, PFASs can remain solubilized in water, sorb to sediments, or be uptaken by plants, depending on the physicochemical properties of the compounds (perfluorocarbon chain length, head of the functional group, water-solubility, volatility, etc.), plant physiology (transpiration rate, lipid and protein content, etc.), and abiotic factors (soil organic matter, pH, salinity, temperature, etc.) [5]. To evaluate the accumulation and mobility of PFAS in the water-sediment-plant system, the estimation partitioning factors (K_d) [6] and the plant uptake rates [5] gain importance as provide information on the distribution and final fate of PFAS. However, these processes have not been widely studied in a river basin scenario considering local discharges of PFASs from a manufacturing plant and their transport along the river.

As a consequence of the fast-economic growth over the last decades, industrial development has expanded to satisfy the global needs of PFASs and related chemicals. The People's Republic of China is one of the main producers of PFASs. The fluoropolymer production facility located in the highly urbanized and industrialized

Bohai Sea Economic Rim in northeastern China in 2012 produced 37,000 tons of polytetrafluoroethylene, 50,000 tons of tetrafluoroethylene, 10,000 tons of hexafluoropropylene, 500 tons of perfluorinated ethylene-propylene copolymers, 300 tons of polyvinylidene fluoride, 40 tons of PFOA ammonium salt, and more than 200,000 tons of different types of fluorinated refrigerants [4]. As a result of this production, the Xiaoqing river and its Dongzhulong tributary are affected by the emissions of PFASs with an input of around 87.3 tons per year to the Bohai Sea [7] and accounting for approximately 10–30% of the global PFOA emissions [8]. As a result of the PFOA manufacturing plant, this compound has been recurrently detected in water and sediments from the Xiaoqing River [9], and in the Laizhou Bay and the Bohai Sea [10,11]. However, the water-sediment partitioning factors and the role of plants in the uptake and remobilization of PFAS have not been elucidated. This information is relevant to determine the fate of PFAS in such an impacted river.

The main objective of the present study was to investigate the distribution and fate of 17 PFASs along the Dongzhulong and Xiaoqing river basin, an area receiving direct PFOA discharges from a manufacturing industry in China. Specific objectives were: (i) to evaluate the occurrence and partitioning of these 17 PFASs in surface waters and surface sediments along the river, (ii) to assess the uptake of PFASs from sediments and water to 4 plant species, both floating and rooted, and (iii) to elucidate the PFASs plant mobilization. Principal Component Analysis (PCA) was used to assess the sources of pollution and distribution trends. Overall, this study provides new information on the occurrence, transport, partitioning and plant uptake of PFASs in an impacted river to better understand the processes that explain the behavior of PFAS in real environmental conditions.

3.2. MATERIALS AND METHODS

3.2.1. Study area and sample collection

The study area comprised the Xiaoqing river basin. Dongzhulong river is a tributary of the Xiaoqing River and receives industrial discharges from the biggest fluoropolymer production facility in China as part of the Dongyue group [3,4,12] and wastewater from major cities (Jinan, Zibo, Binzhou, and Dongying) where petrochemical, chemical, electronic, iron, and steel industries are located [7].

Eleven sampling points along the Dongzhulong and Xiaoqing rivers were sampled in August 2017. The first sampling point was just after the water discharge area of the fluoropolymer production facility (0.16 km) and the last sampling point was just before a dam (38.16 km) (Figure 3.1). Sampling points from 1 to 5 were in the Dongzhulong river, and from 6 to 11 were in the Xiaoqing River. Samples collected in each point included: (i) grab sampling of 1 L of surface water; (ii) freshwater surface sediment (top 0-20 cm) collected with a drag, (iii) 2 plant species floating in the surface water (*Ceratophyllum demersum* and *Lemna minor*) and 2 plant species with roots (*Alternanthera sessilis* and *Eriochloa villosa*), manually collected as well as sediment around the roots. Not all plants were growing in all sampling points, so plants reported in a few sampling points indicate their specific presence in this given area. For each matrix, three samples were collected in each location. The details of the sampling information are listed in Table 3.A1 of Annex.

Lemna minor is a ubiquitous plant and grows in a variety of climates, use to create green structures (fronds) free-floating on the water surface or just below [13], and it is used in water studies to monitor pollutants and heavy metals on the environment because of their

properties [13,14]. *Ceratophyllum demersum* is a perennial and widely distributed submerged plant common from ponds, lakes, and ditches; it does not produce roots, absorbs all nutrients from the water column effectively, and is also used for the remediation of contaminated waters by heavy metals [15,16]. *Alternanthera sessilis* is a green leafy vegetable native from Brazil and inhabits in many tropic and subtropical areas all over the world and in some areas it is used as a diet complement [17]. Several studies reported that Alternanthera sp. was used for the removal of heavy metals from aquatic environments [17,18]. *Eriochloa villosa* is an annual and perennial plant with a high fibrous root system.

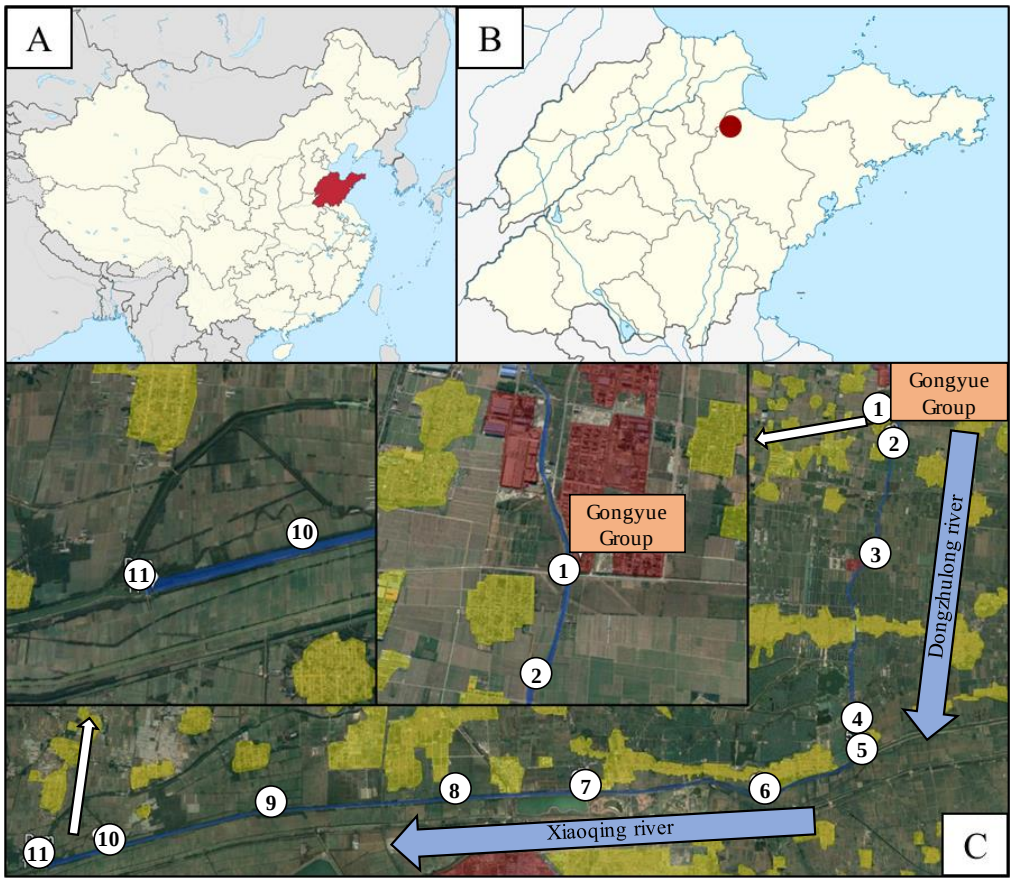

Figure 3.1. Sampling area. A) Map of China with Shandong Province in red, B) red dot indicating Xiaoqing River basin, and C) map showing the river from the fluoropolymer production facility (red) to the dam, indicating urban settlements (yellow), river flow in the arrows (blue), and sampling points in the dots (white).

Water samples were stored in cold conditions (4ºC) until extraction. Surface sediments were freeze-dried during 48 h, homogenized with a blender, and frozen (–20ºC) in bags before analysis. Rooted plants were divided into shoots and roots, and each part was washed sequentially with tap water and Milli-Q water and dried with tissue paper. All plant material was freeze-dried for 48 h, homogenized with a blender, and frozen (–20ºC) in bags before analysis. Sediments taken around the roots were separated manually and processed as river sediments.

3.2.2. Chemicals and reagents

Standards of PFASs mixture were purchased from Wellington Laboratories and contained perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUnDA), perfluoro-n-dodecanoic acid (PFDoA), perfluoro-n-tridecanoic acid (PFTriDA), perfluoro-n-tetradecanoic acid (PFTeDA), perfluoro-n-hexadecanoic acid (PFHxDA) and perfluoro-n-octadecanoic acid (PFODA), potassium perfluoro-1-butanesulfonate (PFBS), sodium perfluoro-1-hexanesulfonate (PFHxS), sodium perfluoro-1-octanesulfonate (PFOS), and sodium perfluoro-1-decanesulfonate (PFDS). A working solution was prepared in methanol at a concentration of 0.2 µg/mL and stored at –18ºC. Mass-labeled Perfluoro-1-[$^{13}C_8$]-octanesulfonamide (M8FOSA-I, 1.2 mL × 50 µg/mL in isopropanol) from Wellington Laboratories, was used as an internal standard (IS). M8FOSA-I was prepared in methanol at a concentration of 1 µg/mL and was stored at –18ºC. Methanol and acetonitrile were supplied by Merck & Co

(New Jersey, Unit States of America), tetrabutylammonium hydrogen sulfonate (TBAHS) from Sigma-Aldrich (Saint Louis, United States of America), and methyl tert-butyl ether (MTBE) from Macklin (Shanghai, China). Supelclean ENVI-Carb SPE Tube cartridges were provided by Supelco (Bellefonte, United States of America).

3.2.3. Extraction and instrumental method

Regarding surface water samples, 10 µL of internal standard (IS) M8FOSA-I at a concentration of 1 µg/mL was added to 1 L of water. The extraction method used was adapted from Heydebreck et al. [19] and briefly consisted of the solid-phase extraction (SPE) of 1 L of non-filtered water at approximately 2 mL/min using Supelclean ENVI-Carb SPE cartridges (250 mg, volume 3 mL, from Supelco). Cartridges were conditioned with 10 mL of acetone, methanol, and methanol with 0.25% ammonium hydroxide, respectively. After sample preconcentration, cartridges were washed with 5 mL Millipore water, then dried using a vacuum pump, and finally eluted with 10 mL of 0.25% ammonium hydroxide in methanol. The extract was reduced under a gentle stream of nitrogen and reconstituted with 1 mL of methanol.

In the case of sediments, 1 g of sample was spiked with 10 ng of IS (10 µL of a solution at 1 µg/mL) and left at 4ºC overnight. Solid-liquid extraction was performed (3 times) with 5 mL of methanol and 5 mL of dichloromethane in an ultrasonic bath for 20 min, centrifuged (10,000 rpm, 30 min), and the supernatant was collected and evaporated under a gentle stream of nitrogen. The extract was reconstituted with 9 mL of water and extracted with SPE cartridges (Supelclean ENVI-Carb SPE Tube, from Supelco), which were conditioned with 4 mL of methanol, and 4 mL of methanol with 0.1% ammonium

hydroxide, and after preconcentration eluted with 4 mL of methanol and 4 mL of methanol with 0.1% ammonium hydroxide. The extract was reduced under a gentle stream of nitrogen and filtered through a 0.22-µm nylon mesh filter and reconstituted with 1 mL of methanol.

Plant extraction was performed according to the method of Wen et al., [20] with some modifications. In brief, 1 g of shoot or root was spiked with 10 ng of IS (10 µL of a solution at 1 µg/mL) and 4 mL of methanol with 0.4 mol/L NaOH were added and left at 4ºC overnight. Solid-liquid extraction was conducted (3 times) adding 4 mL of 0.25 mol/L sodium carbonate buffer, 2 mL of 0.5 mol/L of tetrabutylammonium hydroxide (TBAHS), and 5 mL of methyl tert-butyl ether (MTBE). The mixture was manually shaken for 20 min and centrifuged (4,200 rpm, 10 min). The supernatant was reduced under a gentle stream of nitrogen, filtered through a 0.22-µm nylon mesh filter to a chromatography vial and reconstituted with 1 mL of methanol.

PFASs were analyzed using a Liquid Chromatography system from Agilent Technologies 1220 Series coupled to an Agilent 6410 Triple Quad/Mass Spectrometer (Agilent Technologies, California, USA) (LC-MS/MS). A Synergy Hydro RP 80A column (150 mm × 2 mm, 4 µm particle size) was used (Phenomenex, California, USA). 5 µL of the extract was injected. The mobile phase consisted of water (A) and methanol (B), both with 10 mmol of ammonium acetate. The chromatographic gradient started at 30% B (condition held for 10 min), increased to 70% B in 3 min, and to 90% B in 25 min, and then increased to 100% B in 5 min, the condition that was maintained for 15 min. The flow was set at 0.2 mL/min and the column temperature was 30ºC.

A calibration curve with six points was built over a concentration of 2, 5, 10, 25, 50, and 100 ng/mL. PFASs calibration curve, coefficients of regression, and instrumental

detection limits (IDL) are shown in Table 3.A2 of Annex. Detailed recoveries of each compound can be found for water in Heydebreck et al.[19] and for plants in Wen et al.[20]. Method efficiency was calculated using the recoveries of internal standard M8FOSA-I (10 ng/g level), and ranged from 43% to 121% in water, from 52% to 109% in sediment and from 70% to 115% in plants.

3.2.4. Data treatment and analysis

Pollutants partitioning between water and sediments were studied through the solid-liquid distribution coefficient (K_d, L/kg).

$$K_d = \frac{\text{[PFASs ng/kg dw] in sediment}}{\text{[PFASs ng/L] in surface water}} \qquad \text{Equation 3.1}$$

Transfer of pollutants between plant compartments and the surroundings were analyzed by the Shoot Concentration Factor from water in floating species (SCFw), the Shoot Concentration Factor in rooted species from sediment around the root(SCFs), the Root Concentration Factor (RCF), and the Translocation Factor between shoot and root (TF).

$$\text{SCFw (L/g)} = \frac{\text{[PFASs ng/g dw] in shoots}}{\text{[PFASs ng/L] in surface water}} \qquad \text{Equation 3.2}$$

$$\text{SCFs (g/g)} = \frac{\text{[PFASs ng/g dw] in shoots}}{\text{[PFASs ng/g dw] in sediment around the roots}} \qquad \text{Equation 3.3}$$

$$\text{RCF (g/g)} = \frac{\text{[PFASs ng/g dw] in roots}}{\text{[PFASs ng/g dw] in sediment around the roots}} \qquad \text{Equation 3.4}$$

$$\text{TF (g/g)} = \frac{\text{[PFASs ng/g dw] in shoots}}{\text{[PFASs ng/g dw] in roots}} \qquad \text{Equation 3.5}$$

Each quotient was calculated for those compounds detected simultaneously in the different matrices. The sediments used in the calculation of the RCF and the TF were those surrounding the roots.

Principal Component Analysis (PCA) was performed using R-3.4.4 software (https://www.r-project.org/). We performed PCA on PFASs concentrations detected in all locations by using the covariance matrix to explore the patterns of association among PFASs and sampling points for surface water and sediment, and bioaccumulation factors (SCF and RCF) of rooted species. Compounds omitted because they were not detected were PFTrDA, PFTeDA, PFHxDA, PFODA, and PFDS in water, PFHxDA, PFODA, PFBS, and PFDS in sediment, and PFHxDA, PFODA, PFBS, PFHxS, and PFDS in rooted species. We used the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy for each variable in the model and for the complete model to assess the usefulness of the PCA. KMO ranges from 0 to 1 and should be well above 0.5, considered above 0.6–0.7 adequate so variables are sufficiently interdependent for PCA to be useful [21]. In this study, PFAS have been classified by their functional group and chain length, and include perfluoroalkyl carboxylic acids with seven carbons and lesser (short-chain PFCAs), perfluoroalkyl carboxylic acids with eight carbons and greater (long-chain PFCAs), perfluoroalkane sulfonates with four carbons, and lesser (short-chain PFSAs), and perfluoroalkane sulfonates with six carbons and greater (long-chain PFSAs) [22]. When comparing the fate of PFSAs and PFCAs in the environment, the perfluorinated carbon chain is a characteristic that needs to be considered. On the PFCAs chain one of the carbons is in the functional group, so with the same number of carbons, PFSAs always have one more perfluorinated carbon than PFCAs. As an example, PFOA and PFOS have

8 carbons, but PFOS has the same perfluorinated carbon chain as PFNA that has 9 carbons.

3.3. RESULTS AND DISCUSSION

3.3.1. Occurrence and partitioning of PFASs along the Xiaoqing river basin

The impact of the PFOA manufacturing plant located in the Xiaoqing river and PFASS urban discharges were evaluated by determining the accumulation and trends of PFAS in water and sediment along the basin and specifically assessing the uptake ability of PFAS by different plant species. Figure 3.2 shows the concentration of PFOA and ΣPFASs in water, sediment, floating, and rooted plants along the basin. A clear dilution trend was observed from the 1st sampling point next to the fluoropolymer production facility to the 11^{th} sampling point just before a dam. Consistent with the PFOA production by the Dongyue group, PFOA was the dominant compound in all matrices studied, comprising 74 to 82% of the ΣPFASs in surface water, 91 to 93% in sediments, and 82 to 97% in either floating or rooted plant species (Tables 3.A3, Table 3.A4, and Table 3.A5 of Annex, respectively).

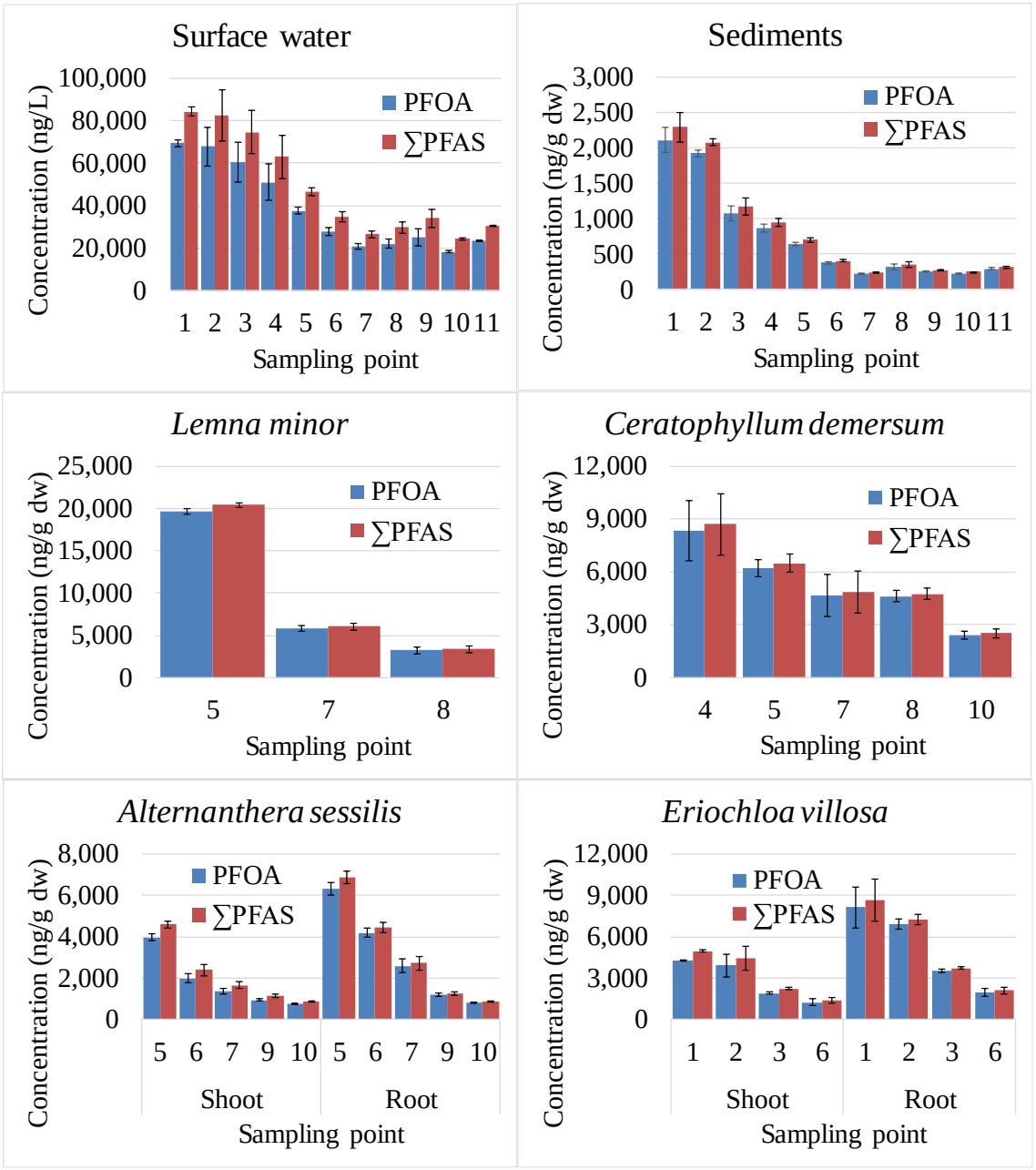

Figure 3.2. Mean concentration of PFOA and ∑PFASs in surface water (ng/L) and sediments (ng/g dw), in shoots and roots of *Alternanthera sessilis* and *Eriochloa villosa* (ng/g dw), and in shoots of *Lemna minor* and *Ceratophyllum demersum* (ng/g dw) along the sampling points in Dongzhulong and Xiaoqing river. Error bars indicate the standard deviation (n=3).

3.3.1.1. PFASs in surface water

In surface water, PFOA concentration ranged from 18,250 ± 480 to 69,500 ± 1,660 ng/L, followed by PFHxA > PFBA > PFHpA > PFPeA which contributed in 2.8-8.4% of ∑PFASs at concentrations from 860 to 4,300 ng/L (Table 3.A6 of Annex). Long-chain PFCAs and PFSAs were found at a concentration one order of magnitude lower, in a range from 0.44 ± 0.04 to 374 ± 4.4 ng/L, or were not detected as PFHxDA, PFODA, and PFDS. Our results are in agreement with previous studies from the Xiaoqing river that report PFOA as the dominant compound in surface water detected at a concentration ranging from 4.06 to 61,900 ng/L, followed by PFHxA and PFHpA [7]. Next to the same fluoropolymer production facility, 106,000 ng/L [19] and 496,000 ng/L of ∑PFASs [8] were reported and dilution occurred when river waters discharged to the Laizhou Bay in the Bohai Sea, with ∑PFASs concentrations of 99.4 ng/L [23], from 3.9 to 118 ng/L[24], and from 4.55 to 556 ng/L [11], and all studies report the predominance of PFOA, suggesting that Xiaoqing river is a significant source of PFOA contamination in this area.

The PCA analysis of surface water samples, (Figure 3.3a) rendered a KMO value of 0.76 indicating that PFASs concentrations were interdependent and significantly intercorrelated with co-predominance of all PFASs. PCA permitted to identify possible sources. The first and second principal components explained 69.2% and 12.5% of the total variance, respectively. PFBA, PFPeA, PFHxA, PFHpA and PFOA had a clear dominance at sampling point 2, and PFNA, PFDA, PFUnA, and PFDoA were segregated with a higher contribution at the first two sampling points.

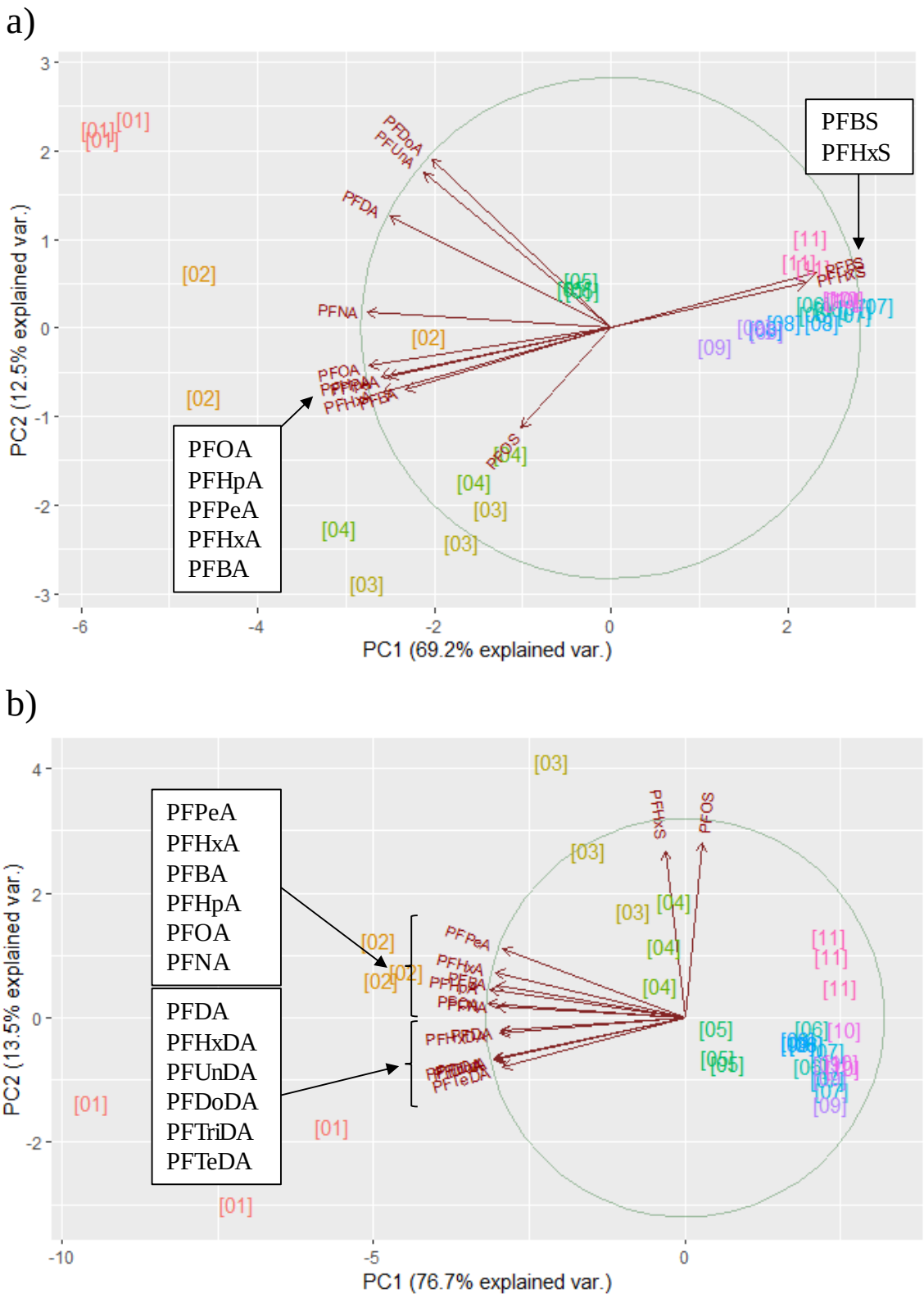

Figure 3.3. PCA analysis of PFASs detected in a) surface water (ng/L) and b) sediments (ng/g dw) along the Dongzhulong and Xiaoqing river. The numbers represent the sampling points.

PFOS showed a high contribution in sampling point 3 located downstream to the cities of Qifengzhen, Boxing City, and Hubinzhen, with mean concentration of 58.4 ± 13.8 ng/L and sampling point 4 receiving waters from Mata Lake (wetland and fishing hotspot) and irrigation channels with mean concentrations of 93.3 ± 10 ng/L. PFOS was neither manufactured nor widely applied in industrial processes in this region [25], but previous studies suggested that its presence is mainly attributed to urban activities, street runoff, and wastewater treatment discharges from the very densely populated area [8]. In sampling point 5, where the Dongzhulong river joins to Xiaoqing river (Figure 3.1), a slight decrease in concentration was observed due to dilution effect of both rivers with lower contribution in both the PC1 and PC2. Thereafter (sampling points 6 to 11) a specific source of pollution of PFBS and PFHxS was observed in PCA and Figure 3.A1 of Annex.

3.3.1.2. PFASs in sediments

In sediments, PFOA was the dominant compound detected at 2,120 ± 182 ng/g dw in sampling point 1 and at 290 ± 17 ng/g dw in sampling point 11, thus showing a clear dilution along the basin. Previous studies in the area detected in the Dongzhulong tributary a PFOA concentration of 3,640 ng/g dw and downstream 382 ng/g dw [8]. The prevalence of PFOA in sediments and the dilution effect along the Xiaoqing river basin is in agreement with previous studies that report decreasing trends at the end of the river close to the river mouth, with 76.9, 2.6, and 2.1 ng/g dw [26], and 29.0, 13.0 and 2.45 ng/g dw [10]. In terms of concentration, other PFAS were detected at levels up to 37.8 ± 8.1 ng/g dw and representing a contribution < 2.4% of ∑PFASs and PFODA, PFDS, and PFBS were not detected (Table 3.A7 of Annex). Long-chain PFASs contributed more to

the total ΣPFASs in sediment than in water due to the affinity for this matrix [7,8]. The PCA analysis shown in Figure 3.3b indicates that most of the PFASs concentrations were interdependent and significantly intercorrelated (KMO = 0.79). The first and second principal components explained 76.7% and 13.5% of the total variance, respectively. The first principal component was predominantly explained by PFCAs which had high concentrations in sampling points 1 and 2, while the second principal component was mainly explained by the presence of PFHxS and PFOS in sampling points 3 and 4, similar to what was observed in water. PCA also showed that at sampling points 5 to 11 the contribution of all PFAS was very low. For all compounds, a decreasing trend was observed along the river, except for PFOS that increased from sampling point 5 to 11 presumably due to urban effluent discharges as observed for water samples (Figure 3.A2 of Annex).

3.3.1.3. PFASs in plant species

There is little information regarding the accumulation of PFAS in aquatic plants, especially under real environmental conditions. The two free-floating species were the macrophyte *Lemna minor* with a single root of 2 cm and *Ceratophyllum demersum*, a submerged, aquatic plant. The rooted species were *Alternanthera sessilis* which has a taproot and *Eriochloa villosa* with an adventitious root that branch as a tap root. Roots are known to play a major role in nutrient uptake and by similarity of contaminants, thus having ecological relevance in freshwater ecosystems [27]. The uptake of PFASs should firstly occur in the root of all four plant species, being absorbed either from water or sediment [28]. The levels and trends of PFAS in plants followed that of water and sediment and indicate that plants are also affected by river pollution. PFOA was the main

PFAS detected in all plant species and reflects once more that the PFOA hot spot discharge has an overall impact all along the river ecosystem.

In floating species, PFOA concentration in *L. minor* ranged from 3,240 ± 430 to 19,600 ± 330 ng/g dw, and in *C. demersum* from 2,390 ± 230 to 6,190 ± 500 ng/g dw, followed by short-chain PFCAs with levels up to 280 ± 10 ng/g dw with very low contribution to ∑PFAS (Table 3.A8 of Annex). Other PFASs were detected in lower concentrations or not detected as PFHxDA, PFODA, PFBS, PFHxS, and PFDS. The literature on PFASs in floating plant species is limited, exemplified by ∑PFASs of 405 ng/g dw in *L. minor* from the Mississippi River (USA) [29] and levels ranging from 4.78 to 7.63 ng/g dw in *C. demersum* pooled with other aquatic plants (*Myriophyllum spicatum* and *Valisneria spiralis*) from Rhone River (France) [30]. These levels are much lower compared to those in our study (∑ PFASs ranging from 2,500 ± 240 to 8,700 ± 1,750 ng/g dw).

In rooted species, PFOA concentration in *A. sessilis* ranged from 570 ± 21 to 3,960 ± 160 ng/g dw in shoots, and from 800 ± 28 to 6,320 ± 310 ng/g dw in roots while in *E. villosa*, values ranged from 740 ± 53 to 4,260 ± 42 ng/g dw in shoots, and from 1,980 ± 290 to 8,140 ± 1,470 ng/g dw in roots. The relatively higher levels in *E. villosa* compared to *A. sessilis* may reflect higher root uptake due to the adventitious root type of the former. However, PFHxS and PFOS had higher levels in roots of *A. sessilis*. Table 3.A9 and Table 3.A10 of Annex shows the concentration of PFASs in plants. Short-chain PFCAs were detected at much lower concentrations in both species, with levels up to 333 ± 35 and 126 ± 21 ng/g dw in shoots and roots, respectively, and representing a contribution < 11% of ∑PFASs (Table 3.A5, Table 3.A9 and Table 3.A10 of Annex). PFODA, PFBS, and PFDS were not detected. In both rooted species, most PFASs showed a general decreasing trend along the river (Figure 3.A3 and Figure 3.A4 of Annex), except for PFDA, PFUnA,

PFDoA, and PFTrDA in *A. sessilis* and shoots of *E. villosa* and PFHxS and PFOS in *E. villosa* due to their low concentrations.

3.3.2. Distribution of PFASs in the water-sediment system

PFASs in surface water and freshwater sediments had a similar profile along the Dongzhulong and Xiaoqing river, and solid-liquid distribution coefficients (K_d) were calculated for each PFASs to assess the relationship between the chain length of PFASs and its distribution in the water-sediment system. As illustrated in Figure 3.4, the K_d values increased with the increasing chain length of PFASs. Thus, short-chain PFCAs had low K_d values (less than 10 L/kg) indicating that these compounds are preferentially partitioned in the water as they have a higher water solubility and mobility. In contrast, long-chain PFCAs, due to their higher hydrophobicity, are expected to be sorbed more readily to the organic matter in the sediment. Thus, PFOA and PFNA showed intermediate values (from 5.6 ± 0.2 to 30.5 ± 3 L/kg), and the PFASs with C-F chain > 10 (PFDA, PFUnA, and PFDoA) presented the highest K_d values (from 51.8 ± 5.1 to 7,800 ± 1,260 L/kg) so they are preferentially accumulated in sediments. Table 3.A11 of Annex shows the individual mean K_d values of PFASs in each sampling point. Previous studies supported our results which confirm that K_d values for PFASs increase with the chain length [31,32]. On the other side, PFHxS and PFOS had higher K_d values than the PFCAs with the same number of fluorinated carbon (i.e., PFHpA and PFNA, respectively), suggesting that the functional group also plays some role in the distribution of PFASs in the water-sediment system. The partition of PFSAs to sediment is favored compared to their PFCA analogs, due to the highest hydrophobicity of PFASs and the

larger size of sulfonate moiety presenting more specific electrostatic interactions with the sediment compared to carboxylate moiety [33].

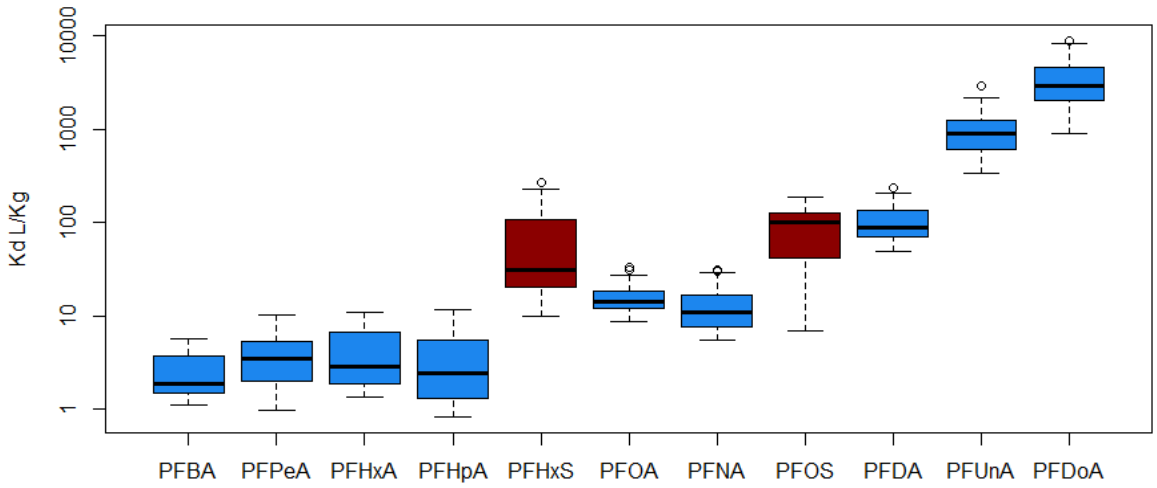

Figure 3.4. Boxplot with partition coefficient (K_d, in L/kg) in Log10 scale for PFCAs in blue and PFSAs in red considering the different sampling points in the Dongzhulong and Xiaoqing river.

3.3.3. Uptake of PFASs in floating and rooted plants

In this study emphasis was given to compare the uptake ability of PFASs in floating vs. rooted plant species and to evaluate the uptake trends along the basin. To do so, SCFw, SCFs, RCF, and TF were calculated to assess the mobility of PFASs in the water-plant system for both floating and rooted species. Table 3.1 shows the mean values considering all sampling points and individual values in each sampling point are indicated in Tables 3.A12, Table 3.A13, and Table 3.A14 of Annex.

Table 3.1. Mean, minimum and maximum (Mean (Min-Max)) of all sampling points of shoot concentration factors for floating species (SCFw , L_{water}/g_{shoot}), shoot concentration factors for rooted species (SCFs, $g_{sediment}/g_{shoot}$), roots concentration factors (RCF, $g_{sediment}/g_{root}$), and transfer factors (TF, g_{root}/g_{shoot}) of PFASs in *Lemna minor* (n=9), *Ceratophyllum demersum* (n=15), *Alternanthera sessilis* (n=15), and *Eriochloa villosa* (n=12) collected along the Dongzhulong and Xiaoqing river. Empty squares are due that the compound was not detected in the matrixes.

	Lemna minor		*Ceratophyllum demersum*		*Alternanthera sessilis*						*Eriochloa villosa*					
	SCFw		SCFw		SCFs		RCF		TF		SCFs		RCF		TF	
	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)	Mean	(Min - Max)
PFBA	0.07	(0.003 - 0.2)	0.03	(0.01 - 0.06)	23	(3 - 39)	6	(0.5 - 21)	12	(1 - 39)	16	(7 - 32)	7	(3 - 14)	2.5	(2 - 3)
PFPeA	0.03	(0.008 - 0.08)	0.01	(0.006 - 0.02)	7	(3 - 11)	2	(0.9 - 4)	5	(1 - 10)	7	(3 - 19)	3	(0.5 - 9)	4	(1 - 6)
PFHxA	0.02	(0.005 - 0.04)	0.01	(0.005 - 0.02)	3	(2 - 5)	2	(0.6 - 3)	2	(1 - 3)	7	(3 - 13)	4	(1 - 7)	2	(1 -4)
PFHpA	0.04	(0.02 - 0.08)	0.02	(0.01 - 0.03)	4	(2 - 6)	4	(2 - 8)	1	(0.5 - 2)	4	(2 - 7)	7	(3 - 11)	0.6	(0.4 - 0.8)
PFOA	0.3	(0.1 - 0.6)	0.2	(0.1 - 0.3)	2.3	(1.7 - 2.7)	3	(2 - 5)	0.7	(0.5 - 1)	3	(2 - 5)	5	(3 - 8)	0.6	(0.4 - 0.9)
PFNA	0.1	(0.02 - 0.2)	0.1	(0.06 - 0.2)	0.7	(0.3 - 1)	2	(1 - 3)	0.4	(0.2 - 0.6)	2	(0.6 - 7)	4	(2 - 8)	0.4	(0.2 - 0.7)
PFDA	0.7	(0.4 - 1)	0.6	(0.3 - 1)	0.6	(0.3 - 0.8)	2	(1 - 3)	0.3	(0.2 - 0.4)	0.9	(0.3 - 2)	3	(1 - 6)	0.3	(0.1 - 0.5)
PFUnA	4	(2 - 6)	3	(1 - 5)	0.5	(0.2 - 0.8)	2	(1 - 3)	0.2	(0.1 - 0.3)	0.9	(0.2 - 3)	2	(1 - 4)	0.3	(0.1 - 0.7)
PFDoA	11	(8 - 16)	7	(3 - 12)	0.3	(0.1 - 0.4)	2	(1 - 2)	0.2	(0.2 - 0.4)	0.8	(0.1 - 2)	2	(0.9 - 3)	0.4	(0.1 - 0.9)
PFTrDA	-[a]	-[a]	-[a]	-[a]	0.7	(0.4 - 1)	3	(1 - 5)	0.3	(0.1 - 0.8)	1	(0.2 - 3)	2	(0.8 - 4)	0.6	(0.2 - 1)
PFTeDA	-[a]	-[a]	-[a]	-[a]	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]	2	(0.2 - 5)	2	(0.9 -5)	0.8	(0.2 - 2)
PFHxDA	-[a,b]	-[a,b]	-[a,b]	-[a,b]	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]	0.3	-[c]	3	(2 - 4)	0.2	-[c]
PFHxS	-b	-b	0.16	(0.09 - 0.3)	3	(1 - 4)	14	(4 - 31)	0.3	(0.1 - 0.4)	6	(4 - 11)	4	(2 - 8)	1	(0.2 - 3)
PFOS	0.5	(0.1 - 1)	0.2	(0.1 - 0.4)	0.9	(0.4 - 2)	6	(2 - 16)	0.2	(0.1 - 0.3)	4	(2 - 7)	5	(2 - 12)	1	(0.4 - 2)

[a] The corresponding compound was not detected in water.
[b] The corresponding compound was not detected in plants.
[c]The corresponding compound was only detected one time in plants.

The uptake of PFASs from water/sediment to plant varies according to the species and the environmental concentration [34], and differences in uptake factors were observed among all four plant species. We found that floating species have a higher concentration of PFASs than rooted species as uptake from water is enhanced compared to sorption from sediments in rooted species, where competition between plant and sediments take place and requires internal transport from roots to shoots [5,28].

Regarding floating species, *L. minor* and *C. demersum* had a SCFw < 1 for PFASs with 10 or less carbon, while PFUnA and PFDoA had SCFw > 1, demonstrating an increase of SCFw with the increasing chain length of PFASs. PFOS and PFHxS also showed higher SCFw values than PFCA with the same chain length, PFNA and PFHpA, respectively. In concordance with our findings in floating species, in a mesocosms experiment it was observed that PFCAs with 10 or more carbons exhibited the highest plant-water bioaccumulation potential in *Echinodorus horemanii* and the lowest bioaccumulation potential were for PFASs with 4 to 9 carbons [28].

Both rooted species had a similar behavior regarding the uptake and translocation of PFAS (Figure 3.5). PCA analysis indicated that SCFs and RCFs were interdependent and significantly intercorrelated (KMO = 0.72). The first two axis explained 51.0% and 17.5% of the total variance, respectively. Short-chain PFASs (i.e., PFBA, PFPeA, and PFHxA) positively contributed to the SCFs, while long-chain PFASs (i.e., PFDA, PFUnA, PFDoA, PFTrDA, PFOS, PFOA, and PFNA) were related to the RCF. Besides, PFHpA had an intermediate position in between these groups.

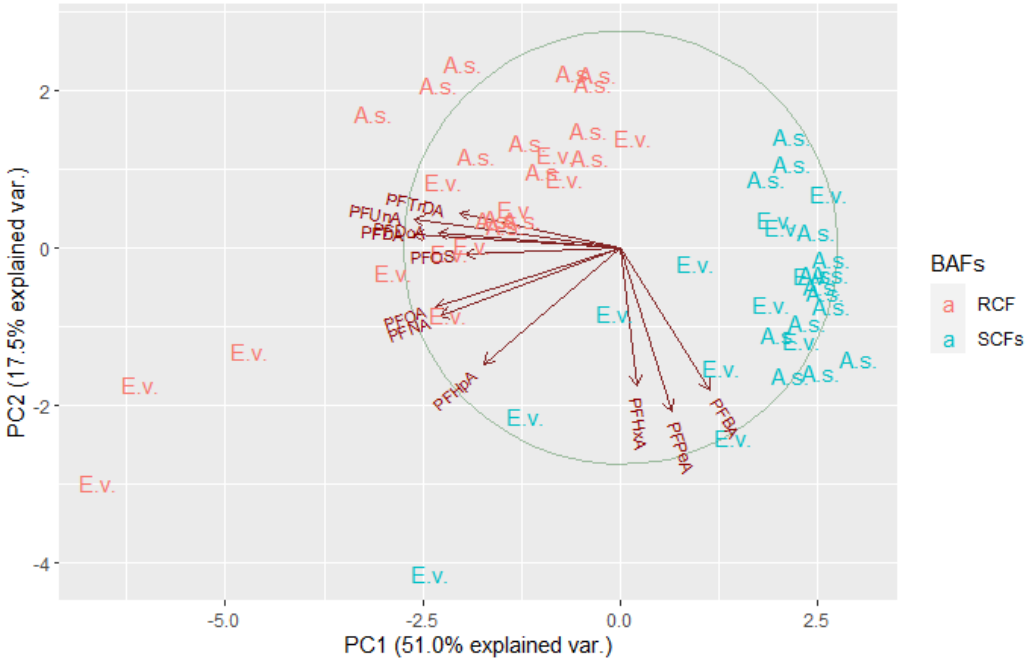

Figure 3.5. PCA analysis of shoot concentration factor (SCFs, $g_{sediment}/g_{shoot}$) and root concentration factor (RCF, $g_{sediment}/g_{root}$) of PFASs in the rooted species *Alternanthera sessilis* (A.s.) and *Eriochloa villosa* (E.v.).

We observed that short-chain PFCA (PFBA, PFPeA, and PFHxA) accumulate more in the shoots than in the roots due to translocation produced by the water potential gradient created by plant transpiration that promotes the upward transport through the xylem of the plant. Contrarily, long-chain PFASs were preferentially accumulated in the roots due to the proteinphylicity linked sorption or retained by the Casparian strip [5,34,35], which is a band of cell wall material around the endodermis of the root which blocks the passive passage of solutes, especially when they might be harmfull [36]. This is also demonstrated by the TF values, also indicated in Table 3.1, that decrease while chain length increases, as was demonstrated in previous studies [16,37]. In maize, PFBA, PFPeA, and PFHxA were more readily translocated from the roots to the shoots, compared with long-chain PFASs which bounded strongly to the surface of the roots [34] presumably because long-chain PFASs are positively correlated with the protein content

[35]. However, environmental concentration, protein, and lipid content of the plant, and different translocation processes may explain the differences among species. Also, chemical properties as polarity, molecular size, and functional group are key to penetrate through the plasma membrane of root cells [34]. No differences in the translocation of PFASs in roots and shoots between the two rooted species were observed and both are recommended as pollutant remediators in freshwater environments [38].

3.4. CONCLUSIONS

Summarizing, this study investigates the environmental impact of the fluoropolymer production facility located next to the Dongzhulong and Xiaoqing River aquatic environment, focusing on the behavior of PFASs in the water-sediment-plant system. A dilution of ∑PFASs was observed along the river in all matrices. According to PCA, PFOA was the main contaminant in water and sediment due to the point source contamination in the Gongyue Group facility, while PFSAs were punctually detected and attributed to urban discharges. K_d values indicated that long-chain PFASs preferentially remain sorbed in the sediment, while short-chain PFASs are mobile in the water column and are uptaken in different plant species. Differences between floating and rooted plants were observed, where floating species easily uptake long-chain PFASs direct from the water, while rooted species must compete with the sediment for the uptake of PFASs. Moreover, in rooted species, long-chain PFASs remain accumulated in the root compartment because of protein affinity while short chain PFASs are more mobile and can be translocated to shoots. Overall, this study provides new information on the occurrence and river-basin distribution of PFAS, sediment accumulation, and plant

uptake under real environmental conditions, which is useful to assess the final fate of PFAS in areas highly affected by direct discharges and environmental pollution.

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3.6. ANNEX

Table 3.A1. Sampling points' coordinates (Location), distance to the fluorochemical industry and detailed samples marked with an X.

Sampling points	Location	Distancie (Km)	Water	Sediments	*Lemna minor*			*Ceratophyllum demersum*			*Alternanthera sessilis*			*Eriochloa villosa*		
					Shoot	Root	Sediments	Shoot	Root	Sediments	Shoot	Root	Sediments	Shoot	Root	Sediments
1	36° 59'42.85"N 118° 2'3.71"E	0.16	X	X										X	X	X
2	37° 0'18.00"N 118° 2'15.00"E	1.31	X	X										X	X	X
3	37° 2'27.52"N 118° 2'41.91"E	5.46	X	X										X	X	X
4	37° 5'32.00"N 118° 2'51.00"E	11.43	X	X				X	X	X						
5	37° 5'51.00"N 118° 2'42.00"E	12.15	X	X	X	X	X	X	X	X	X	X	X			
6	37° 6'42.00"N 118° 4'57.00"E	15.97	X	X							X	X	X	X	X	X
7	37° 6'51.00"N 118° 8'40.99"E	21.63	X	X	X	X	X	X	X	X	X	X	X			
8	37° 7'7.00"N 118°11'22.99"E	25.65	X	X	X	X	X	X	X	X						
9	37° 7'32.00"N 118°15'9.99"E	31.3	X	X							X	X	X			
10	37° 8'24.00"N 118°18'32.00"E	36.53	X	X				X	X	X	X	X	X			
11	37° 8'44.54"N 118°19'31.43"E	38.16	X	X							X	X	X	X	X	X

Table 3.A2. PFASs' calibration curve, coefficients of regression, limits of detection (LOD), and limits of quantifications (LOQ).

Compound	Equation (internal standard)	R2	LOD (ng)	LOQ (ng)
PFBA	y = 335.18x - 155.5	R^2 = 0.996	0.1	0.4
PFPeA	y = 262.48x - 154.79	R^2 = 0.994	0.2	0.6
PFHxA	y = 357.86x - 88.237	R^2 = 0.994	0.1	0.2
PFHpA	y = 555.97x - 36.849	R^2 = 0.998	0.1	0.2
PFOA	y = 603.94x - 8.0444	R^2 = 0.994	0.1	0.2
PFNA	y = 498.64x - 258.78	R^2 = 0.994	0.1	0.2
PFDA	y = 556.97x - 287.53	R^2 = 0.994	0.1	0.3
PFUnA	y = 538.25x - 280.19	R^2 = 0.994	0.1	0.2
PFDoA	y = 542.39x - 228.6	R^2 = 0.996	0.1	0.4
PFTrDA	y = 517.52x - 221.67	R^2 = 0.998	0.2	0.5
PFTeDA	y = 524.22x - 257.5	R^2 = 0.998	0.1	0.3
PFHxDA	y = 259.15x - 94.562	R^2 = 0.999	0.1	0.2
PFODA	y = 165.4x - 69.254	R^2 = 0.995	0.1	0.3
PFBS	y = 52.239x - 43.458	R^2 = 0.998	0.2	0.6
PFHxS	y = 83.443x - 23.497	R^2 = 0.998	0.1	0.5
PFOS	y = 112.76x - 30.778	R^2 = 0.999	0.1	0.4
PFDS	y = 91.23x - 71.021	R^2 = 0.995	0.1	0.4

Table 3.A3. Contribution of PFASs (%) in surface water and sediments.

Matrix	Sampling point	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFODA	PFBS	PFHxS	PFOS	PFDS
Water	1	4	3	5	4	82	0.4	0.2	0	0	0	0	0	0	0	0	0.1	0
	2	5	3	5	5	82	0.4	0.1	0	0	0	0	0	0	0	0	0	0
	3	5	4	6	4	81	0.3	0	0	0	0	0	0	0	0	0	0.1	0
	4	4	3	6	5	81	0.4	0.1	0	0	0	0	0	0	0	0	0.1	0
	5	4	3	6	5	81	0.4	0.1	0	0	0	0	0	0	0	0	0	0
	6	6	3	5	4	80	0.3	0.1	0	0	0	0	0	0	0	0.1	0.2	0
	7	5	3	6	6	79	0.3	0.1	0	0	0	0	0	0	0	0.1	0.2	0
	8	8	4	7	6	74	0.4	0.1	0	0	0	0	0	0	0	0.1	0.1	0
	9	7	5	8	6	74	0.4	0.1	0	0	0	0	0	0	0	0.1	0.1	0
	10	7	5	7	6	75	0.2	0.1	0	0	0	0	0	0	0	0.1	0.1	0
	11	6	4	7	5	77	0.3	0.1	0	0	0	0	0	0	0	0.1	0.1	0
Sediments	1	0.8	0.7	2	1	92	0.4	0.6	0.7	0.7	0.4	0.4	0.1	0	0	0	0.1	0
	2	0.6	0.9	2	2	93	0.5	0.8	0.5	0.5	0.2	0.1	0.1	0	0	0	0.2	0
	3	1	1	2	2	91	0.3	0.3	0.3	0.4	0.3	0.2	0.1	0	0	0.1	0.4	0
	4	0.8	1	2	2	92	0.2	0.4	0.3	0.5	0.2	0.2	0.1	0	0	0.1	0.4	0
	5	1	1	2	1	92	0.3	0.4	0.3	0.6	0.4	0.2	0.1	0	0	0.1	0.3	0
	6	1	0.8	1	1	93	0.2	0.5	0.4	0.7	0.3	0.1	0.1	0	0	0.1	0.6	0
	7	1	0.8	1	0.9	91	0.3	1.0	0.8	1.3	0.5	0.3	0.1	0	0	0.1	1	0
	8	1	0.9	1	0.7	92	0.3	1.0	0.7	0.7	0.5	0.2	0.1	0	0	0.1	0.8	0
	9	1	1	2	0.6	92	0.3	0.6	0.8	0.6	0.4	0.1	0.1	0	0	0.1	0.9	0
	10	1	0.8	1	0.7	92	0.3	0.7	0.7	0.5	0.3	0.2	0.1	0	0	0.1	1	0
	11	0.7	0.7	2	0.7	96	0.2	0.5	0.6	0.5	0.3	0.2	0.1	0	0	0.2	1	0

Table 3.A4. Contribution of PFASs (%) in shoots of *Lemna minor* and *Ceratophyllum demersum*.

Matrix	Sampling point	Compartment	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFODA	PFBS	PFHxS	PFOS	PFDS
Lemna minor	5	Shoot	1	0.5	0.5	0.9	96	0.1	0.2	0.1	0.1	0.1	0	0	0	0	0	0.1	0
	7	Shoot	1	0.1	0.7	0.4	96	0.2	0.3	0.2	0.2	0.2	0.1	0	0	0	0	0.2	0
	8	Shoot	0.3	0.4	0.4	1	97	0.1	0.3	0.2	0.2	0.2	0	0	0	0	0	0.1	0
Ceratophyllum demersum	4	Shoot	1	0.2	0.6	0.9	96	0.3	0.4	0.1	0.1	0.2	0	0	0	0	0	0.1	0
	5	Shoot	2	0.4	0.5	1	96	0.3	0.3	0.1	0.1	0.1	0	0	0	0	0	0.1	0
	7	Shoot	0.8	0.2	0.3	0.7	97	0.2	0.3	0.1	0.1	0.2	0.1	0	0	0	0.1	0.1	0
	8	Shoot	0.6	0.3	0.3	0.7	97	0.2	0.3	0.2	0.1	0.1	0	0	0	0	0.1	0.1	0
	10	Shoot	1	0.3	0.6	0.8	96	0.2	0.3	0.2	0.1	0.1	0.1	0	0	0	0.1	0.2	0

Table 3.A5. Contribution of PFASs (%) in shoots, roots and sediments of *Alternanthera sessilis* and *Eriochloa villosa*.

Matrix	Sampling point	Compartment	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFODA	PFBS	PFHxS	PFOS	PFDS
Alternanthera sessilis	5	Shoot	7	3	2	1	86	0.1	0.1	0	0	0.1	0	0	0	0	0.1	0.1	0
		Root	4	1	1	1	92	0.1	0.2	0.1	0.1	0.1	0	0	0	0	0.3	0.3	0
	6	Shoot	10	3	2	1	83	0	0.1	0.1	0	0.1	0	0	0	0	0.1	0.1	0
		Root	3	0.6	0.7	0.8	94	0.1	0.1	0.1	0.1	0.1	0	0	0	0	0.1	0.2	0
	7	Shoot	11	3	2	2	82	0.1	0.1	0.1	0	0.1	0	0	0	0	0	0.1	0
		Root	2	0.4	0.7	1	95	0.1	0.3	0.3	0	0.3	0	0	0	0	0	0.3	0
	9	Shoot	9	3	2	1	84	0.1	0.1	0.1	0.1	0.1	0	0	0	0	0	0.1	0
		Root	0.5	0.5	0.8	1	95	0.1	0.5	0.4	0.3	0.3	0	0	0	0	0	0.4	0
	10	Shoot	6	3	2	2	87	0.1	0.2	0	0	0	0	0	0	0	0	0.1	0
		Root	0.2	0.4	0.6	1	95	0.2	0.6	0.5	0.4	0.3	0	0	0	0	0	0.7	0
	11	Shoot	8	2	3	2	85	0.1	0.3	0	0	0	0	0	0	0	0	0.2	0
Erioochloa villosa	1	Shoot	5	2	5	1	86	0.1	0.1	0	0	0	0	0	0	0	0	0.1	0
		Root	1	0.3	2	2	94	0.2	0.2	0.2	0.1	0.1	0	0	0	0	0	0.1	0
	2	Shoot	5	2	4	2	88	0.1	0.1	0	0	0	0	0	0	0	0	0.1	0
		Root	0.9	0.2	1	1	96	0.2	0.2	0.1	0.1	0.1	0	0	0	0	0	0	0
	3	Shoot	7	2	4	1	85	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0	0.1	0.1	0
		Root	2	0.2	1	1	95	0.2	0.2	0.1	0.1	0.1	0	0	0	0	0	0.1	0
	6	Shoot	3	2	1	1	92	0.2	0.2	0.2	0.1	0.1	0.1	0	0	0	0.2	0.2	0
		Root	0.8	0.8	0.9	1	95	0.2	0.3	0.2	0.1	0.1	0.1	0	0	0	0.1	0.3	0
	11	Shoot	2	3	3	2	88	0.1	0.4	0.4	0.3	0.3	0	0	0	0	0.4	0.3	0

Table 3.A6. Average concentration (ng/L), and standard deviation (±SD) of PFASs detected in surface water (n=3).

Matrix	Sampling point	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS	∑PFASs[a]
Water	1	3,580	2,820	4,300	3,500	69,500	374	180	21.2	3.90	0.85	0.44	2.36	5.42	57.2	84,400
		±94	±120	±140	±130	±1,660	±4.4	±2	±0.9	±0.07	±0.09	±0.04	±0.09	±0.58	±2.8	±2,100
	2	4,100	2,360	3,700	4,030	67,900	335	98.1	6.67	2.45	0.80	0.54	2.25	4	25	82,600
		±1,250	±550	±910	±1,320	±9,240	±54	±26	±1.59	±0.8	±0.31	±0.2	±0.58	±2.4	±5.8	±12,000
	3	3,800	2,580	4,090	3,310	60,560	194	29.7	1.47	0.55	< LOD	< LOD	2.24	9.26	58.4	74,600
		±170	±540	±470	±370	±9,230	±36	±3.9	±0.28	±0.05			±0.44	±1.64	±13.8	±10,200
	4	2,790	2,090	3,650	3,090	51,040	254	56.9	3.55	0.96	< LOD	< LOD	2.86	3.14	93.3	63,100
		±250	±520	±890	±580	±8,780	±41	±3.5	±0.54	±0.13			±0.32	±1.16	±10	±10,250
	5	1,690	1,540	2,960	2,370	37,580,	175	55.5	6.59	1.62	< LOD	< LOD	2.91	2.44	17.3	46,400
		±200	±104	±10	±140	±1,700	±7.8	±3.8	±0.42	±0.08			±0.27	±0.04	±1.1	±1,900
	6	2,100	980	1,830	1,890	27,780	103	25.1	2.65	1.21	< LOD	< LOD	9.32	27.7	69	34,800
		±170	±81	±150	±140	±1,960	±6.8	±1.7	±0.11	±0.13			±1.37	±2.5	±4	±2,450
	7	1,400	860	1,570	1,480	20,940	87.1	17.6	1.85	0.73	< LOD	< LOD	6.79	19.9	39.9	26,400
		±61	±51	±90	±98	±1,280	±2.5	±0.8	±0.07	±0.08			±0.27	±1.5	±1.8	±1,560
	8	2,490	1,300	2,060	1,690	22,040	108	18.5	1.92	0.83	< LOD	< LOD	6.82	18.3	21.7	29,750
		±350	±43	±170	±200	±2,140	±31	±5.2	±0.52	±0.07			±0.62	±2.5	±7.3	±2,660
	9	2,480	1,700	2,600	1,870	25,200	127	22.6	2.14	0.87	< LOD	< LOD	7.34	18.0	20.5	34,060
		±260	±380	±350	±140	±4,100	±4	±0.7	±0.33	±0.08			±0.66	±2.7	±2.4	±4,400
	10	1,700	1,180	1,780	1,360	18,250	58.2	14.1	1.98	0.93	< LOD	< LOD	5.75	18.6	19.8	24,400
		±62	±13	±16	±23	±484	±2.1	±0.4	±0.16	±0.02			±0.45	±1.3	±0.6	±532
	11	1,840	1,350	2,190	1,540	23,480	88.5	21.9	3.13	1.58	< LOD	< LOD	7.62	24.1	24.6	30,570
		±50	±28	±25	±20	±123	±2.2	±1	±0.16	±0.19			±0.98	±1	±3.4	±237

[a]PFHxDA, PFODA and PFDS were not detected.

Table 3.A7. Average concentration (ng/g dw), and standard deviation (±SD) of PFASs detected in sediments (n=3).

Matrix	Sampling point	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFHxS	PFOS	∑PFASs[a]
Sediments	1	17.7	16.1	37.8	32.9	2,120	8.2	13.7	15	16.6	10.2	8.34	2.9	0.41	1.86	2,300
		±3.2	±3.2	±8.1	±7.1	±182	±2.4	±3.9	±4	±4.7	±1.9	±1.24	±0.7	±0.06	±0.97	±212
	2	13	17.7	34.3	31.6	1,930	9.8	15.8	10.8	9.66	5.1	2.82	1.16	0.48	3.51	2,080
		±0.9	±1.2	±0.9	±0.7	±48	±1	±1.1	±0.8	±0.45	±0.2	±0.13	±0.07	±0.11	±0.1	±51
	3	13.9	15.3	25.6	18.3	1,080	3.6	3.6	3.15	4.2	3.24	2.39	1.28	0.9	4.12	1,170
		±1.5	±1.8	±4.3	±2.2	±108	±0.1	±0.6	±0.69	±0.44	±0.35	±0.51	±0.26	±0.25	±0.55	±120
	4	7.6	11	23.2	16	870	2.3	4	2.58	4.62	2.02	1.45	0.99	0.59	3.48	950
		±0.8	±0.6	±1.2	±1.2	±54	±0.2	±0.3	±0.13	±0.23	±0.2	±0.11	±0.11	±0.07	±0.66	±55
	5	6.8	7.3	15.7	10.1	641	1.9	2.9	2.33	3.87	2.53	1.25	0.55	0.57	1.84	698
		±0.6	±0.5	±0.7	±0.4	±30	±0.1	±0.1	±0.12	±0.15	±0.36	±0.19	±0.01	±0.12	±0.13	±32
	6	4	3.4	5.3	4.6	376	0.7	1.9	1.6	2.71	1.27	0.52	0.28	0.51	2.3	405
		±0.1	±0.2	±0.4	±0.3	±15	±0.03	±0.1	±0.13	±0.05	±0.27	±0.01		±0.09	±0.08	±17
	7	2.4	1.9	2.3	2.1	222	0.8	2.5	2.04	3.26	1.25	0.65	< LOD	0.32	2.76	244
		±0.1	±0.2	±0.1	±0.1	±2	±0.04	±0.2	±0.19	±0.24	±0.09	±0.04		±0.07	±0.2	±3
	8	4	3.1	4	2.4	323	1.1	3.4	2.46	2.6	1.76	0.83	0.39	0.48	2.7	351
		±0.3	±0.1	±0.2	±0.2	±41	±0.02	±0.4	±0.22	±0.16	±0.09	±0.02	±0.03	±0.08	±0.3	±41
	9	3.4	3.2	4.6	1.6	253	0.7	1.7	2.09	1.78	1.1	0.39	LOD	0.31	2.38	276
		±0.1	±0.1	±0.1	±0.1	±9	±0.04	±0.3	±0.15	±0.12	±0.03	±0.04		±0.09	±0.09	±9
	10	2.5	2	2.5	1.7	230	0.9	1.7	1.72	1.23	0.72	0.51	< 0.29	0.35	2.92	248
		±0.1	±0.1	±0.05	±0.03	±4.5	±0.1	±0.1	±0.01	±0.08	±0.03	±0.09		±0.03	±0.34	±4
	11	2.3	2.2	5	2.1	290	0.7	1.7	1.77	1.53	0.87	0.49	0.25	0.73	3.29	312
		±0.3	±0.8	±0.1	±0.2	±17	±0.1	±0.2	±0.06	±0.1	±0.03	±0.11	±0.01	±0.07	±0.335	±19

[a]PFODA, PFBS and PFDS were not detected.

Table 3.A8. Average concentration (ng/g dw), and standard deviation (±SD) of PFASs detected in shoot of *Lemna minor* (n=3) and *Ceratophyllum demersum* (n=3).

Matrix	Sampling point	Compartment	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFASs[a]
Lemna minor	5	Shoot	280 ±10	108 ±16	94.6 ±2.4	181 ±19	19,600 ±330	29.7 ±1	34.2 ±1.4	18.1 ±2.1	15.1 ±1.9	14.7 ±1.5	9.1 ±0.6	< LOD	18.3 ±1.1	20,400 ±300
	7	Shoot	66.6 ±6.5	8.36 ±1.02	44.4 ±16.8	26.1 ±2.9	5,830 ±350	10.5 ±3.9	16.3 ±3	10.4 ±0.9	10.9 ±1.3	10 ±1.9	7.8 ±1.3	< LOD	11.4 ±3.4	6,100 ±400
	8	Shoot	10 ±1.8	13.4 ±4	15 ±3.7	36.1 ±3.5	3,240 ±430	3.5 ±1.3	9.4 ±1.1	7.2 ±0.6	7.5 ±1.1	5.6 ±0.7	< LOD	< LOD	4.6 ±1.7	3,350 ±450
Ceratophyllum demersum	4	Shoot	98.1 ±20.3	14.7 ±3.1	49.9 ±11.6	79.2 ±14.2	8,340 ±1,710	28.1 ±2	31.2 ±2.9	12.9 ±1.9	9.9 ±0.9	13.6 ±0.5	2.3 ±0.2	< LOD	10.5 ±1.6	8,700 ±1,750
	5	Shoot	97.3 ±13.1	25.1 ±3	34.1 ±2.2	62.1 ±8.7	6,190 ±500	18.1 ±1.6	17 ±0.7	8.1 ±0.3	7.3 ±0.4	9.6 ±0.9	2.5 ±0.2	< LOD	5.1 ±0.4	6,500 ±500
	7	Shoot	39.4 ±2	11.3 ±2.7	16.6 ±1	34.9 ±3	4,660 ±1,200	10 ±2.4	14.4 ±1.4	6.7 ±1.1	5.6 ±0.5	12 ±0.9	2.5 ±0.1	2.4	6.5 ±0.8	4,800 ±1,200
	8	Shoot	30.2 ±0.3	12.9 ±1.3	13.5 ±2.6	32.3 ±2.8	4,600 ±340	9.1 ±1.1	12.8 ±0.5	7.2 ±0.6	5.5 ±0.4	6.5 ±0.5	< LOD	4.4 ±1.1	5.6 ±2.1	4,740 ±350
	10	Shoot	23.7 ±6.3	8.61 ±1.35	13.7 ±2.6	20 ±2.2	2,390 ±230	4.2 ±0.6	6.7 ±0.7	4.2 ±0.5	3.6 ±0.8	3.1 ±0.3	2.4 ±0.1	1.9 ±0.6	4 ±0.9	2,500 ±240

[a]PFHxDA, PFODA, PFBS and PFDS were not detected.

Table 3.A9. Average concentration (ng/g dw), and standard deviation (±SD) of PFASs detected in shoot, root, and sediments of *Alternanthera sessilis* (n=3).

Matrix	Sampling point	Compartment	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxS	PFOS	∑PFASs[a]
Alternanthera sessilis	5	Shoot	333	135	81.5	65.1	3,960	2.8	3.4	2.2	1.4	3.9	< LOD	2.6	2.6	4,590
			±35	±28	±3.8	±2.9	±160	±0.6	±0.2	±0.3	±0.2	±1.8		±0.3	±0.2	±180
		Root	246	85.9	68.2	75.6	6,320	7.0	11	8.2	7.0	6.1	< <LOD	19	20.4	6,870
			±35	±12.1	±7.2	±3.8	±310	±0.4	±1.9	±1.1	±0.7	±0.9		±1.6	±3.5	±300
	6	Shoot	239	73.8	51.6	31.7	1,990	1.1	1.8	1.6	1.1	1.6	< LOD	1.3	1.3	2,390
			±30	±12.8	±6.4	±5	±230	±0.2	±0.2	±0.3	±0.2	±0.2		±0.2	±0.2	±290
		Root	127	24.7	33	37.2	4,200	3.4	6.3	5.6	4.0	6.0	< LOD	3.2	9.6	4,460
			±11	±4.6	±3.0	±3.1	±220	±0.6	±0.5	±0.3	±0.5	±0.6		±0.3	±1.5	±240
	7	Shoot	180	53.4	28	24.3	1,370	0.91	1.5	0.9	0.5	0.9	< LOD	< LOD	1.7	1,660
			±14	±8.1	±4	±5.5	±150	±0.09	±0.2	±0.1	±0.2	±0.1			±0.4	±170
		Root	45.4	11.1	18.9	29.3	2,600	3.1	6.9	8.1	LOD	7.3	< LOD	< LOD	7.3	2,740
			±12.8	±3.3	±3.2	±6.8	±310	±0.6	±1.2	±0.5		±1.1			±0.5	±330
	9	Shoot	99	36.9	18.7	14.4	944	0.7	1.11	0.9	0.7	0.6	< LOD	< LOD	0.7	1,120
			±8	±5.1	±1.6	±0.6	±67	±0.2	±0.03	±0.1	±0.1	±0.1			±0.1	±82
		Root	6.1	6.8	9.5	12.7	1,190	1.9	5.7	4.5	3.5	3.6	< LOD	< LOD	5.2	1,250
			±0.6	±0.9	±0.8	±0.8	±80	±0.8	±0.4	±0.5	±0.3	±0.3			±0.5	±83
	10	Shoot	55.2	28.6	15.1	13.4	760	0.57	1.7	< LOD	< LOD	< LOD	< LOD	< LOD	1.3	870
			±3.7	±1.4	±0.9	±0.9	±31	±0.08	±0.2						±0.1	±40
		Root	1.51	3.0	4.9	9.43	800	1.8	5.3	3.79	3.5	2.1	< LOD	< LOD	6.1	840
			±0.03	±0.05	±0.2	±0.93	±28	±0.2	±0.6	±0.475	±0.3	±0.1			±0.3	±30
	11	Shoot	50	14.8	16.7	13.2	570	0.5	1.7	LOD	LOD	LOD	< LOD	< LOD	1.5	670
			±2.0	±2.3	±1.5	±0.8	±21	±0.1	±0.05						±0.4	±25

[a]PFHxDA, PFODA, PFBS and PFDS were not detected.

Table 3.A10. Average concentration (ng/g dw), and standard deviation (±SD) of PFASs detected in shoot, root, and sediments of *Eriochloa villosa* (n=3).

Matrix	Sampling point		PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFHxS	PFOS	∑PFASs[a]
Eriochloa villosa	1	Shoot	242 ±35	105 ±26	263 ±51	60.5 ±7.3	4,260 ±42	4.9 ±0.9	2.8 ±0.5	1.6 ±0.1	1.2 ±0.3	1.1 ±0.2	0.8 ±0.1	LOD	0.8 ±0.1	3.1 ±0.7	4,950 ±80
		Root	109 ±18	24.6 ±2.6	154 ±32	126 ±21	8,140 ±1,470	19.8 ±2.5	18.1 ±1.1	13 ±1.1	7 ±0.6	4.7 ±0.4	3.6 ±0.3	2.6 ±0.1	2.9 ±0.4	4.8 ±1.2	8,630 ±1,540
	2	Shoot	208 ±19	70.4 ±12.2	177 ±44	69.6 ±13.1	3,910 ±810	4.1 ±1.3	2.5 ±0.4	1.7 ±0.1	1.2 ±0.1	1.2 ±0.1	< LOD	< LOD	2 ±0.5	4.2 ±0.5	4,460 ±890
		Root	65.6 ±3.2	15.1 ±2.4	69.6 ±7	101 ±3	6,950 ±370	15.9 ±1.5	16.3 ±1.3	10.8 ±0.5	5.8 ±0.3	3.9 ±0.2	2.6 ±0.2	1.9 ±0.1	LOD	2.6 ±0.4	7,260 ±380
	3	Shoot	162 ±8	40.7 ±5.2	89.4 ±1.7	29.7 ±0.8	1,900 ±73	2.4 ±0.8	2.7 ±0.1	2.5 ±0.3	2.2 ±0.1	2.3 ±0.2	2.3 ±0.02	< LOD	2.5 ±0.02	2.7 ±0.4	2,240 ±80
		Root	62.6 ±8.8	7.1 ±1.6	40.7 ±1.4	52.5 ±0.2	3,540 ±105,	7 ±0.6	7.4 ±0.1	5.4 ±0.4	2.8 ±0.3	2 ±0.1	1.1 ±0.03	< LOD	1.1 ±0.1	3.2 ±1.4	3,740 ±100
	6	Shoot	34.5 ±6.5	31.6 ±7.2	19 ±3.3	13.5 ±3.3	1,260 ±210	2.4 ±0.8	2.8 ±0.4	2.1 ±0.3	1.7 ±0.2	1.3 ±0.2	1.4 ±0.2	< LOD	2.4 ±1.2	3.4 ±0.8	1,370 ±240
		Root	16.2 ±1.3	16.6 ±2.6	17.8 ±3.4	29.9 ±2.3	1,980 ±290	3.6 ±0.8	6.2 ±0.7	3.3 ±0.2	2.9 ±0.3	2 ±0.2	1.5 ±0.1	< LOD	2.4 ±0.6	7 ±0.5	2,090 ±280
	11	Shoot	19.5 ±1.7	24.2 ±7.4	26.6 ±3.7	12.7 ±0.8	740 ±53	1.1 ±0.3	3.8 ±0.5	3.1 ±0.3	2.4 ±0.3	2.2 ±0.1	< LOD	< LOD	3.4 ±0.8	2.8 ±0.3	840 ±65

[a]PFODA, PFBS and PFDS were not detected.

Table 3.A11. Solid/liquid partition coefficients (K_d, L/kg), and standard deviation (±SD) of PFASs (n=3).

Sampling point	PFBA	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	PFUnA	PFDoDA
1	4.9	5.7	8.8	77.3	9.4	30.5	23.6	21.8	76	710	4,230
	±0.8	±0.9	±1.8	±15.2	±1.9	±3	±17.8	±6.7	±21	±220	±1,100
2	3.4	7.9	9.6	155	8.5	28.7	145.5	29.5	167	1,680	4,230
	±1.1	±2.3	±1.9	±87	±2.9	±3.8	±35.2	±2.2	±34	±400	±1,300
3	3.6	6.1	6.2	95.3	5.5	17.9	72.4	18.9	119	2,200	7,800
	±0.3	±1.1	±0.6	±11.4	±0.06	±1.3	±15	±3.6	±9.2	±640	±1,260
4	2.7	5.4	6.7	203	5.4	17.5	37	9.1	71.4	740	4,860
	±0.1	±1.1	±1.7	±66	±1.3	±4.3	±3.2	±2.3	±8.8	±120	±440
5	4	4.7	5.3	215	4.3	17.1	107	11	51.8	350	2,400
	±0.3	±0.2	±0.2	±50	±0.4	±1.4	±6	±0.20	±5.1	±30	±160
6	2	3.5	2.9	18.5	2.5	13.6	33	7.1	74.8	610	2,260
	±0.1	±0.1	±0.2	±5	±0.01	±0.8	±2	±0.6	±6.6	±64	±220
7	1.7	2.2	1.5	16.4	1.4	10.6	69.3	8.7	141.6	1,100	4,510
	±0.1	±0.3	±0.04	±3.9	±0.1	±0.7	±8	±0.6	±3	±140	±740
8	1.7	2.4	2	26.3	1.4	14.9	131	10.4	192	1,380	3,160
	±0.3	±0.1	±0.2	±4.9	±0.2	±3.2	±35	±3.7	±46	±530	±420
9	1.4	1.9	1.8	17.8	0.9	10.2	118	5.6	73.4	1,000	2,040
	±0.20	±0.4	±0.2	±6.9	±0.04	±1.4	±17	±0.2	±13.1	±240	±40
10	1.5	1.7	1.4	18.9	1.3	12.6	147	15	118.8	880	1,320
	±0.05	±0.1	±0.02	±2.7	±0.01	±0.3	±17	±2.4	±1.7	±70	±90
11	1.3	1.7	2.1	30.3	1.4	12.4	137	8	78.6	570	980
	±0.1	±0.6	±0.04	±1.7	±0.1	±0.7	±34	±0.8	±10.8	±30	±70

Table 3.A12. Average shoot concentration factors (SCFw, L_{water}/g_{shoot}), and standard deviation (±SD) of PFASs in *Lemna minor* (n=3) and *Ceratophyllum demersum* (n=3) along the Xiaoqing River.

Matrix	Sampling point	Transfer	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFHxS	PFOS
Lemna minor	5	SCFw	0.17 ±0.02	0.07 ±0.01	0.03 ±0.0007	0.08 ±0.006	0.5 ±0.02	0.17 ±0.01	0.62 ±0.03	2.8 ±0.4	9.3 ±1.1	-	1.1 ±0.09
	7	SCFw	0.05 ±0.003	0.009 ±0.001	0.03 ±0.01	0.02 ±0.002	0.3 ±0.02	0.13 ±0.05	0.94 ±0.2	5.6 ±0.3	15.1 ±1.5	-	0.29 ±0.09
	8	SCFw	0.004 ±0.0006	0.01 ±0.002	0.007 ±0.001	0.02 ±0.004	0.15 ±0.03	0.04 ±0.02	0.53 ±0.15	3.9 ±0.9	9.1 ±0.9	-	0.22 ±0.09
Ceratophyllum demersum	4	SCFw	0.04 ±0.006	0.007 ±0.0004	0.01 ±0.001	0.03 ±0.002	0.16 ±0.02	0.1 ±0.01	0.55 ±0.04	3.7 ±0.6	10.5 ±1.7	-	0.11 ±0.007
	5	SCFw	0.06 ±0.004	0.02 ±0.002	0.01 ±0.0007	0.03 ±0.004	0.17 ±0.02	0.1 ±0.008	0.31 ±0.03	1.2 ±0.1	4.5 ±0.5	-	0.3 ±0.04
	7	SCFw	0.03 ±0.001	0.01 ±0.003	0.01 ±0.001	0.02 ±0.003	0.22 ±0.05	0.12 ±0.03	0.8 ±0.1	3.6 ±0.7	7.8 ±1.6	0.04 ±0.07	0.16 ±0.02
	8	SCFw	0.01 ±0.001	0.009 ±0.001	0.006 ±0.001	0.02 ±0.002	0.21 ±0.007	0.09 ±0.03	0.73 ±0.2	3.9 ±0.9	6.5 ±0.4	0.24 ±0.04	0.27 ±0.11
	10	SCFw	0.01 ±0.003	0.007 ±0.001	0.007 ±0.001	0.01 ±0.001	0.13 ±0.02	0.07 ±0.01	0.5 ±0.07	2.1 ±0.3	3.9 ±0.8	0.1 ±0.02	0.2 ±0.05

SCF was not calculated for PFTrDA, PFTeDA PFHxDA, PFODA, PFBS and PFDS.

Table 3.A13. Average shoot concentration factors (SCFs, $g_{sediment}/g_{shoot}$), roots concentration factors (RCF, $g_{sediment}/g_{root}$), transfer factors (TF, g_{root}/g_{shoot}), and standard deviation (±SD) of PFASs in *Alternanthera sessilis* (n=3) along the Xiaoqing River.

Matrix	Sampling point	Tranfer	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFHxS	PFOS
Alternanthera sessilis	5	SCFs	3	4.9	2.2	2.5	2.05	0.7	0.65	0.59	0.24	0.72	3.2	1.4
			±0.1	±1.5	±0.1	±0.3	±0.14	±0.3	±0.09	±0.19	±0.04	±0.34	±0.8	±0.4
		RCF	2	3	1.8	2.9	3.3	1.7	2.1	2.14	1.2	1.1	23.9	11.4
			±0.3	±0.8	±0.2	±0.1	±0.06	±0.4	±0.2	±0.37	±0.15	±0.2	±7.4	±4.6
		TF	1.4	1.6	1.2	0.9	0.63	0.4	0.31	0.28	0.2	0.62	0.14	0.13
			±0.1	±0.1	±0.08	±0.08	±0.05	±0.08	±0.04	±0.06	±0.04	±0.21	±0.02	±0.02
	6	SCFs	29	4.2	3.4	4.5	2.1	0.6	0.46	0.49	0.34	1	1.9	0.49
			±5	±1.1	±1.4	±0.6	±0.6	±0.2	±0.05	±0.05	±0.04	±0.2	±0.6	±0.13
		RCF	16	1.4	2.1	5.4	4.5	1.7	1.6	1.78	1.2	3.7	4.7	3.7
			±4.5	±0.4	±0.5	±0.7	±0.7	±0.3	±0.2	±0.19	±0.2	±0.3	±1	±0.7
		TF	1.9	3	1.6	0.9	0.5	0.32	0.28	0.28	0.31	0.27	0.4	0.13
			±0.4	±0.1	±0.3	±0.1	±0.05	±0.1	±0.03	±0.06	±0.1	±0.04	±0.03	±0.02
	7	SCFs	35.7	6.7	2.9	5.1	2	0.74	0.41	0.22	0.16	0.44	-	0.69
			±4.5	±1.2	±0.2	±1	±0.2	±0.15	±0.09	±0.001	±0.05	±0.02		±0.3
		RCF	8.9	1.4	2	6.2	4.2	2.5	1.8	2.17	-	3.8	-	2.8
			±1.9	±0.4	±0.7	±1.7	±0.6	±0.4	±0.3	±0.3		±0.7		±0.4
		TF	4.2	5.3	1.5	0.9	0.5	0.3	0.21	0.11	-	0.12	-	0.24
			±1.3	±2.4	±0.4	±0.4	±0.1	±0.07	±0.07	±0.02		±0.02		±0.07
	9	SCFs	29	9.7	2	3.8	2.5	0.74	0.62	0.55	0.31	0.78	-	0.91
			±5	±1.7	±0.3	±0.6	±0.2	±0.32	±0.1	±0.12	±0.02	±0.1		±0.22
		RCF	1.8	1.8	1	3.3	3.2	1.89	3.1	2.9	1.6	4.4	-	7.1
			±0.4	±0.2	±0.1	±0.5	±0.3	±1.13	±0.6	±0.14	±0.2	±0.2		±2.4
		TF	16.5	5.5	2	1.1	0.8	0.43	0.2	0.19	0.2	0.18	-	0.1
			±1.9	±0.7	±0.3	±0.1	±0.06	±0.19	±0.006	±0.04	±0.04	±0.02		±0.02
	10	SCFs	17.6	9.3	2.1	3.1	2.3	0.7	0.66	-	-	-	-	0.77
			±2.2	±0.7	±0.2	±0.1	±0.1	±0.03	±0.09					±0.16
		RCF	0.5	1	0.7	2.2	2.4	2.1	2	1.4	1.7	1.7	-	4.1
			±0.02	±0.06	±0.05	±0.3	±0.02	±0.07	±0.3	±0.3	±0.1	±0.1		±0.8
		TF	36.4	9.7	3.1	1.4	1	0.32	0.3	-	-	-	-	0.21
			±3.1	±0.5	±0.3	±0.2	±0.04	±0.03	±0.07					±0.01

SCF, RCF and TF were not calculated for PFTeDA PFHxDA, PFODA, PFBS, PFHxS and PFDS because were not detected in both matrixes.

Table 3.A14. Average shoot concentration factors (SCFs, $g_{sediment}/g_{shoot}$), roots concentration factors (RCF, $g_{sediment}/g_{root}$), transfer factors (TF, g_{root}/g_{shoot}) and standard deviation (±SD) of PFASs in *Eriochloa villosa* (n=3) along the Xiaoqing River.

Matrix	Sampling point	Transfer	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFHxS	PFOS
Eriochloa villosa	1	SCFs	8.9	4.7	8.3	2.3	2	1.2	0.38	0.23	0.2	0.24	0.23	0.325	-	2.2
			±1.9	±1.8	±2.7	±0.7	±0.2	±0.3	±0.13	±0.02	±0.1	±0.1	±0.03			±0.5
		RCF	4	1.1	4.9	4.9	3.8	5	2.43	1.8	1.3	0.96	0.99	2	-	3.3
			±0.7	±0.2	±2.1	±1.4	±1	±1.1	±0.32	±0.19	±0.4	±0.15	±0.04	±0.3		±0.7
		TF	2.3	4.3	1.8	0.5	0.5	0.3	0.15	0.13	0.17	0.25	0.23	0.19	0.27	0.71
			±0.6	±1.3	±0.6	±0.1	±0.09	±0.07	±0.03	±0.02	±0.02	±0.07	±0.03		±0.09	±0.33
	2	SCFs	11.3	5.9	10.2	5.8	2.7	0.9	0.31	0.3	0.38	0.6	-	-	-	5.4
			±0.5	±1.2	±2.5	±1.1	±0.6	±0.2	±0.02	±0.03	±0.09	±0.05				±0.2
		RCF	3.6	1.3	4	8.4	4.8	3.4	2.07	2.1	1.77	2	1.8	3.8	-	3.3
			±0.2	±0.08	±0.4	±0.4	±0.4	±0.3	±0.15	±0.3	±0.27	±0.1	±0.09	±0.6		±0.5
		TF	3.2	4.7	2.6	0.7	0.6	0.3	0.15	0.15	0.21	0.3	-	-	-	1.7
			±0.2	±0.8	±0.8	±0.1	±0.1	±0.07	±0.02	±0.02	±0.02	±0.03				±0.3
	3	SCFs	19.2	5.5	4.5	2.3	1.6	1	0.84	1.05	1.2	1.5	1.9	-	5.3	2
			±6.03	±2.8	±1.6	±0.6	±0.05	±0.3	±0.32	±0.3	±0.5	±0.4	±0.4		±0.9	±0.4
		RCF	7.3	1	2	4.1	3	3	2.3	2.3	1.6	1.2	1.1	-	2	2.3
			±2	±0.6	±0.6	±1.2	±0.1	±1	±0.99	±0.9	±0.3	±0.3	±0.1		±0.5	±0.6
		TF	2.6	5.9	2.2	0.6	0.5	0.3	0.37	0.47	0.8	1.2	2.2	-	2.5	0.95
			±0.4	±0.6	±0.1	±0.02	±0.03	±0.08	±0.02	±0.08	±0.2	±0.1			±0.6	±0.37
	6	SCFs	26.2	13.4	5.1	4.5	4	4.4	2	2.1	1.6	2.2	3.8	-	6.6	5.4
			±6.1	±4.8	±1.3	±1.4	±0.8	±1.9	±0.5	±0.5	±0.3	±0.5	±1.1		±3.9	±1.6
		RCF	12.3	7	4.8	9.9	6.4	6.4	4.5	3.33	2.4	3.4	4	-	6.7	10.9
			±1.5	±1.8	±1.3	±1.3	±1.4	±2.1	±0.9	±0.5	±0.2	±0.7	±0.8		±1.2	±1.2
		TF	2.1	1.9	1.1	0.45	0.7	0.7	0.45	0.62	0.7	0.7	0.93	-	1	0.48
			±0.3	±0.4	±0.1	±0.09	±0.2	±0.07	±0.06	±0.09	±0.1	±0.07	±0.1		±0.4	±0.11

SCF, RCF and TF were not calculated for PFHxDA, PFODA, PFBS and PFDS

Figure 3.A1. PFASs' concentration (ng/L), and standard deviation (±SD) in surface water along the Xiaoqing River excluding PFOA.

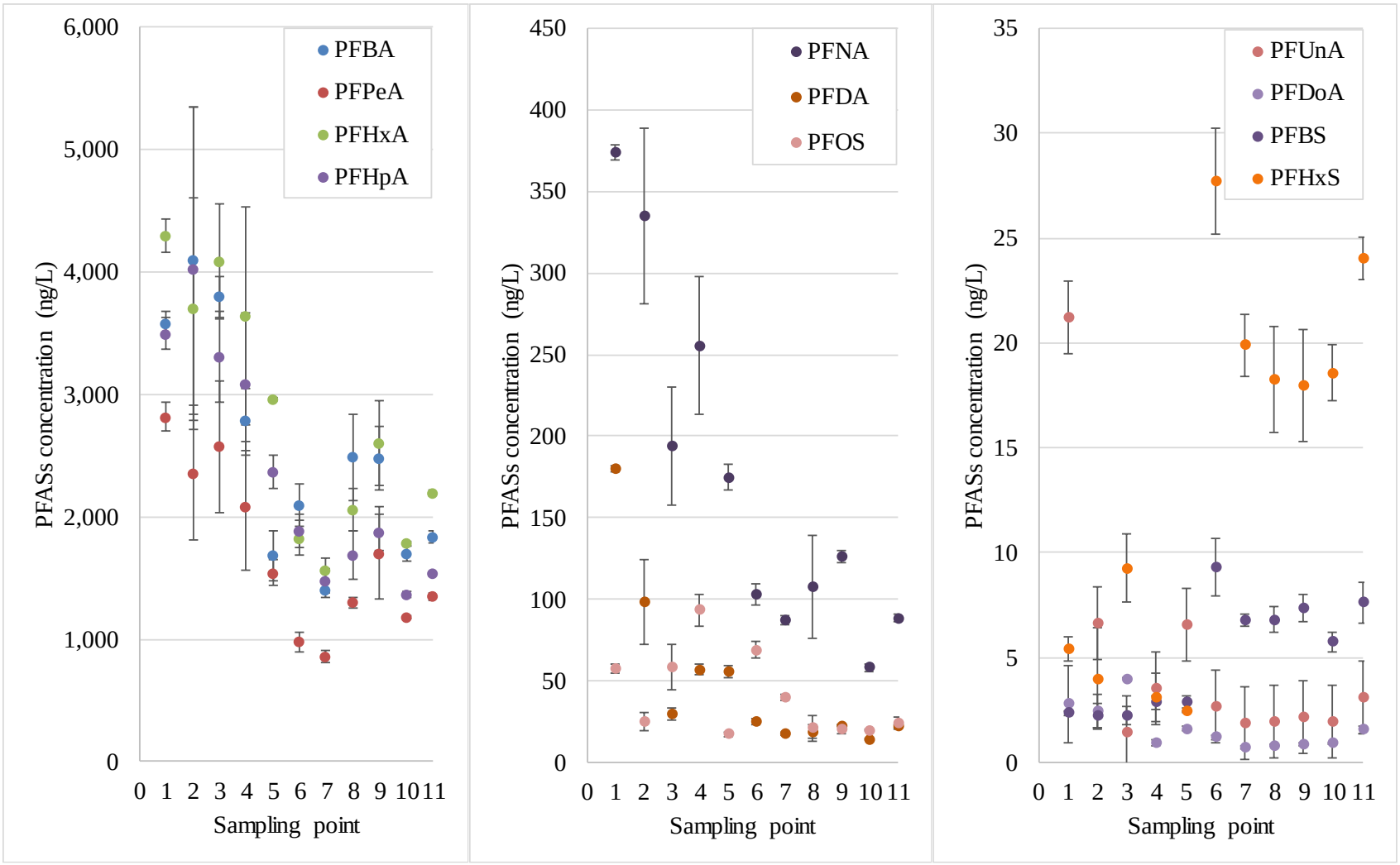


Figure 3.A2. PFASs concentration (ng/g dw), and standard deviation (±SD) in freshwater sediments along the Xiaoqing River.

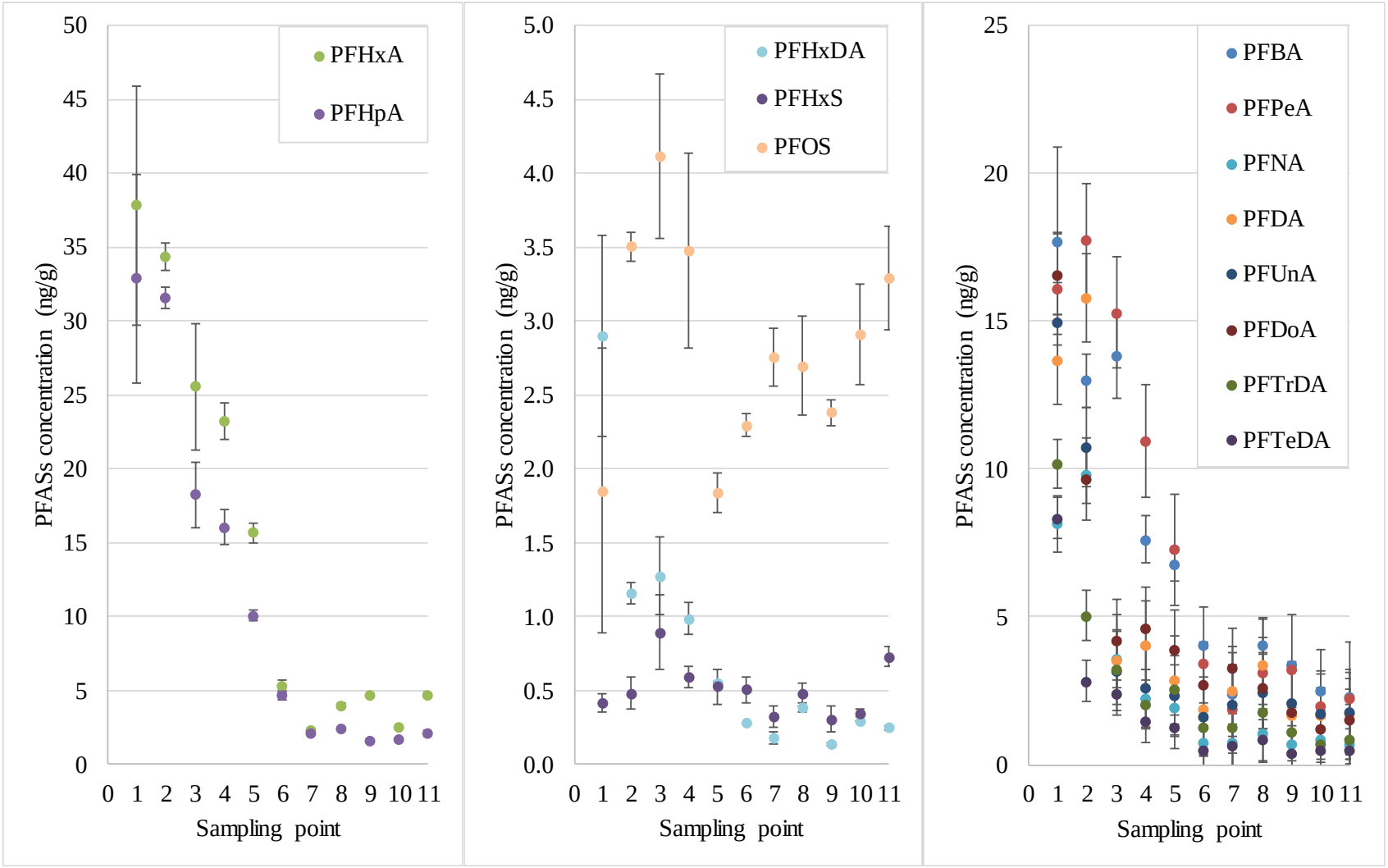
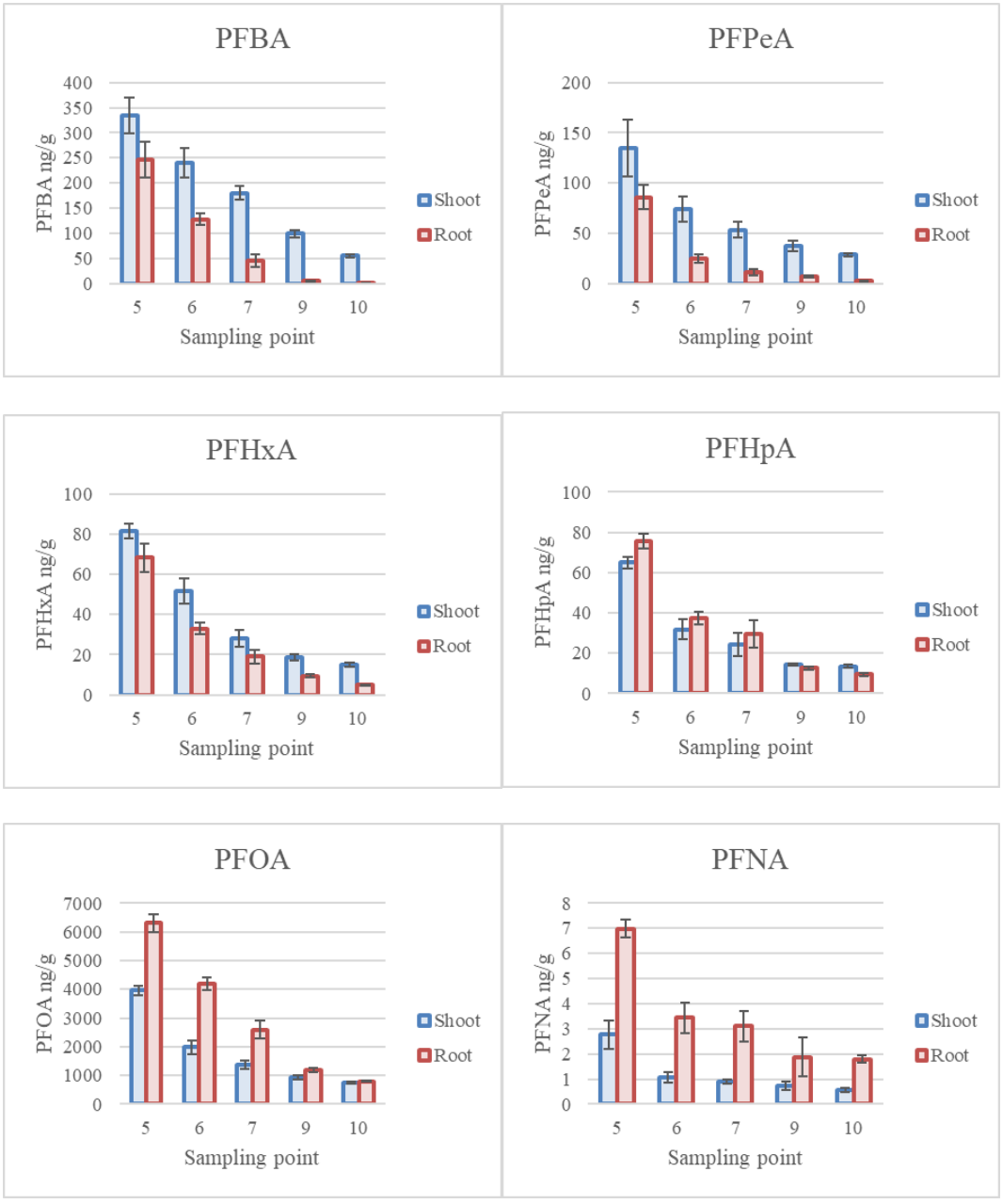


Figure 3.A3. Individual PFASs concentration (ng/g dw), and standard deviation (±SD) in shoots and roots of *Alternanthera sessilis* along the sampling points in Xiaoqing River.



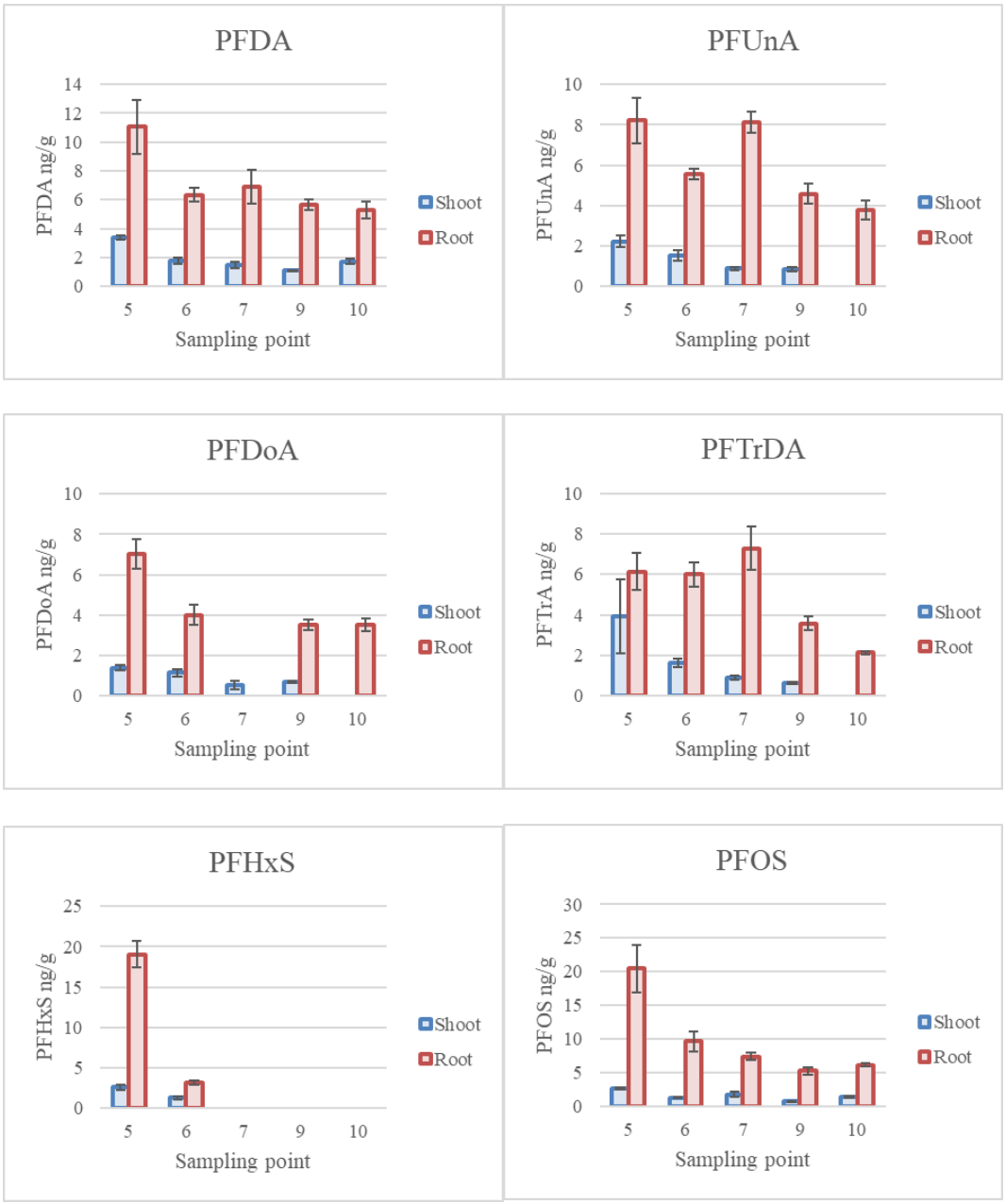
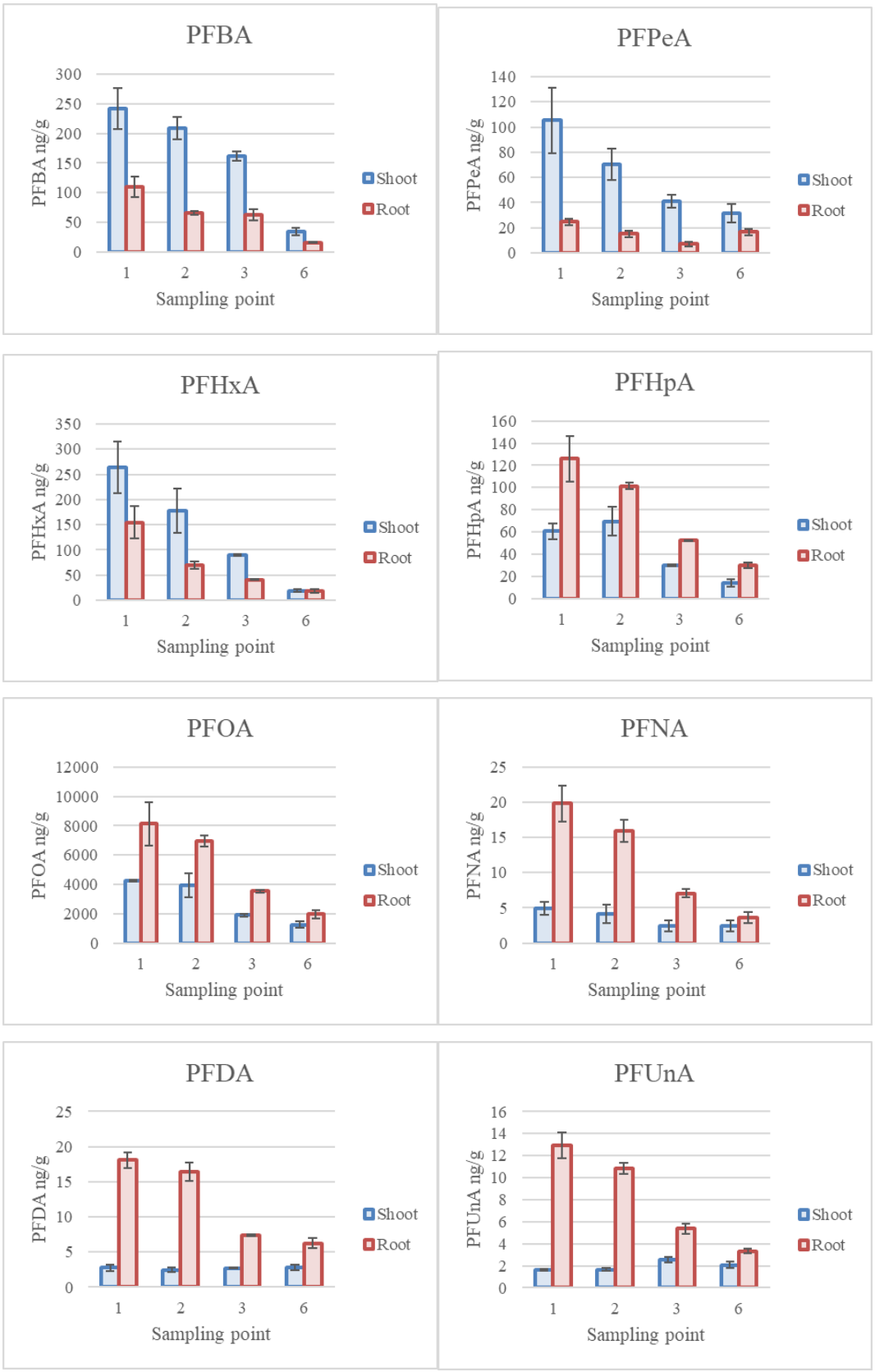
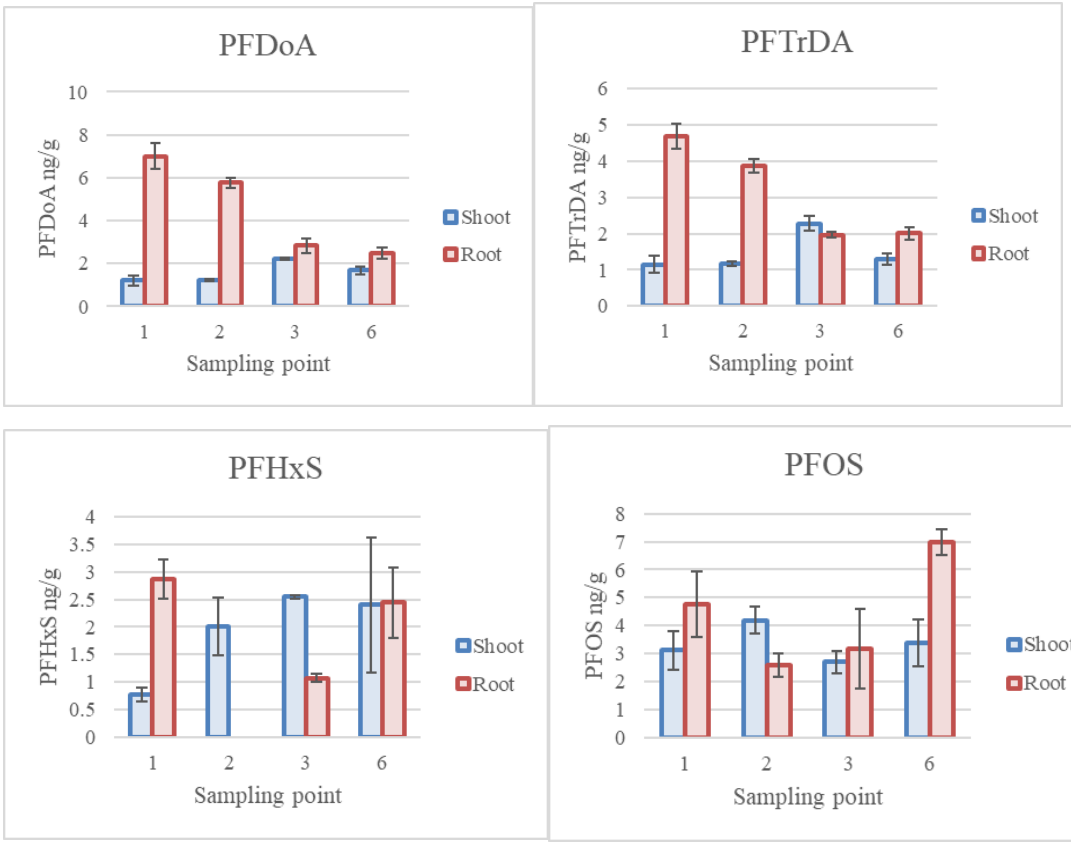

PFDA
PFDA ng/g
14
12
10
8
6
4
2
0
5
6
7
9
10
Sampling point
Shoot
Root
PFUnA
PFUnA ng/g
10
8
6
4
2
0
5
6
7
9
10
Sampling point
Shoot
Root
PFDoA
PFDoA ng/g
10
8
6
4
2
0
5
6
7
9
10
Sampling point
Shoot
Root
PFTrDA
PFTrA ng/g
10
8
6
4
2
0
5
6
7
9
10
Sampling point
Shoot
Root
PFHxS
PFHxS ng/g
25
20
15
10
5
0
5
6
7
9
10
Sampling point
Shoot
Root
PFOS
PFOS ng/g
30
25
20
15
10
5
0
5
6
7
9
10
Sampling point
Shoot
Root

Figure 3.A4. Individual PFASs concentration (ng/g dw), and standard deviation (±SD) in shoots and roots of *Eriochloa villosa* along the sampling points in Xiaoqing River.




PFDoA
PFDoA ng/g
10
8
6
4
2
0
1
2
3
6
Sampling point
Shoot
Root
PFTrDA
PFTrDA ng/g
6
5
4
3
2
1
0
1
2
3
6
Sampling point
Shoot
Root
PFHxS
PFHxS ng/g
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
6
Sampling point
Shoot
Root
PFOS
PFOS ng/g
8
7
6
5
4
3
2
1
0
1
2
3
6
Sampling point
Shoot
Root

CHAPTER 4.

DISTRIBUTION OF PERFLUOROALKYL SUBSTANCES IN THE TROPHIC FOOD CHAIN OF YELLOW-LEGGED GULLS FROM CHAFARINAS ISLANDS

CHAPTER 4

4.1 INTRODUCTION

Perfluoroalkyl substances (PFASs) are chemicals characterized by carbon chains of varying lengths, where hydrogen atoms have been substituted by fluorine atoms. The physicochemical properties of PFASs vary with the chain length that represents the hydrophobic part of the chemical, while the functional group represents the polar part of the chemical that makes PFASs soluble and mobile in the environment [1]. PFASs have been used since the middle of the 20th century in high quantities in the manufacture of surfactants and surface protectors, lubricants, paper coating, stain repellents, food packaging, pharmaceuticals, insecticides, and fire-fighting foams [2] and given their high usage of PFASs-containing products, they have become emerging environmental contaminants [3].

Coastal areas are pivotal to sustain fisheries, to preserve marine biodiversity, and to maintain the ecological equilibrium, but still, they receive high loads of PFASs on a daily basis [4]. Large amounts PFASs are released to coastal waters through wastewater treatment plant effluents and marine emissaries [5], river discharges [4], runoff or use of biosolids [7], desorption from soils [8] or exposure to contaminated sediments [9].

PFASs are bioaccumulated and biomagnified along the marine food webs and impact marine birds [10]. Marine birds exploit the natural resources and reflect the levels of PFASs depending on the geographical area, diet, migratory habits, and local impacts [3]. Globally speaking, PFOS followed by long-chain odd carbons PFASs [11] are the main compounds detected in birds. Levels of PFOS in blood in different species are of 68.9 ng/mL in Scopoli's shearwater (*Calonectris diomedea*) [12], 101.4 ng/mL in Audouin gulls (*Larus audouinii*) [13] both from the Mediterranean, 15.2 ng/mL in black-legged

kittiwake (*Rissa tridactyla*) from Svalbard [14], 60.3 ng/mL in white-tailed eagle (*Haliaeetus albicilla*) [15], and 79.3 ng/g ww in black-backed gull (*Larus fuscus*) [16] both from northern Norway. Accumulation of PFASs in these coastal environments has serious implications as these compounds can affect bird species and impair the ecosystem equilibrium [17] and also because PFASs are regularly detected in edible fish and become a direct source to humans [18].

We have focused this study in the Chafarinas Islands (south Spain), a group of volcanic outcrops placed in the north of the African continent located 4.5 km off the Moroccan coast at about 50 km east of Melilla. The archipelago is designed as a National Hunting Refuge Area since 1982, declared a Special Protection Area for birds in 1989, and became a Site of Community Importance of the Natura 2000 network. Chafarinas Islands is a marine refugee for Yellow-legged gull (therefore YLG), with 2,250 nests in Rey Island and 3,244 nests in Congreso. It also supports a colony of Andouin's gull with 897 nests in Rey, and a colony of Scopoli's shearwater with 400-500 nests in Congreso [19]. The islands host pupulation of various species of reptiles [19], and the marine floor holds soft coral species [20,21]. The area is of interest not only regarding its high biodiversity but also regarding fishery production [22]. The anthropogenic pressures in this area are related to military activities and burning of residues in the Chafarinas Islands itself, the 3 ports (Melilla, Nador and RasKebdana) that serve as cargo, fishing, and passenger ports, agriculture in the Moulouya river basin, which is also a National Park, and urbanization (Nador and Melilla with 170.000 and 68.000 inhabitants, respectively and other medium-sized settlements). However, little is known about the sources and levels of PFASs in this marine environment and how they can impact the marine birds dueling in this area.

The present study is aimed to determine the occurrence and impact of 17 PFASs in the marine environment close to Chafarinas Islands. The specific objectives were to analyse PFASs in (i) agricultural soils and sediment to identify sources of pollution and (ii) fish, mussels and gull eggs to evaluate accumulation patterns. Based on the levels of PFOS detected in fish, we have estimated the daily and yearly intake in gulls according to feeding habits and the percentage of transfer to eggs. With this, we support the idea of the usefulness of gull eggs as bioindicators of pollution in coastal areas.

4.2. MATERIALS AND METHODS

4.2.1. Description of the studied matrices and sampling procedures

4.2.1.1. Sediments and soils

Figure 4.1 shows a map of the sampling locations in different places of the Morrocan coast in June 2015. Agricultural soils were collected in different fields (n=3): citrus (T1), potatoes (T2), and cereal grain (T3). Sediment samples were collected by scuba divers in Melilla's port (n=3): inside of the marina (P1), inside the port near the wastewater treatment plant's (WWTP's) outfall (P2), and near the marine station (P3); *Mar Chica (n=2)*: inside near the channel (BS1) and outside near the channel in open waters (BS2); Moulouya's Delta (n=3) (D1, D2, and D3). Sediment and soil samples were collected with a spade and the first 5 cm were disregarded. Samples were placed inside a glass jar. Each sample consisted of 6 grab subsamples in sites 5-10 m apart and was pooled to make a sample in each point. In this way, we ensured representativeness of the area. Samples were frozen in the Melilla premises and sent frozen to the main laboratory in IDAEA-

CSIC in Barcelona. Sediment and soil samples were dried at room temperature and homogenized with a mortar to grind samples. Samples were sieved through 120 µm.

Figure 4.1. Sampling area. Morocco's northern coast, Melilla and Chafarinas Islands. In yellow, soil samples from agriculture (T1, T2, and T3); in green, sediment samples from, Mar Chica (BS1 and BS2); in pink, sediment samples from Moulouya's delta (D1, D2, and D3); in blue, sediment samples from Melilla's port (PMS1, PMS2, and PMS3).

4.2.1.2. Fish and mussel

Sardina pilchardus (thereafter sardine), *Engraulis encrasicolus* (thereafter anchovy), *Trachurus mediterraneus* (thereafter mackerel), and *Mytilus galloprovincialis* (thereafter mussels) were bought (e.g. 500 g) from the local markets in Melilla and Nador that fish in this area of the southwest Mediterranean Sea. For each species, 12 individuals (full organism, shell-less for mussels) were pooled to make one representative sample.

Samples were frozen and sent frozen to the main laboratory. Samples were homogenized with a blender, freeze-dried, and kept frozen until analysis.

4.2.1.3. Yellow-legged gull eggs

YLG eggs were collected in 2015 in the subcolonies located at Congreso and Rey Islands (n=2). Each sample represents a subcolony that was composed of a pool of 12 YLG eggs.

4.2.2. Chemicals and reagents

Native compounds of perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUnDA), perfluoro-n-dodecanoic acid (PFDoA), perfluoro-n-tridecanoic acid (PFTriDA), perfluoro-n-tetradecanoic acid (PFTeDA), perfluoro-n-hexadecanoic acid (PFHxDA) and perfluoro-n-octadecanoic acid (PFODA), potassium perfluoro-1-butanesulfonate (PFBS), sodium perfluoro-1-hexanesulfonate (PFHxS), sodium perfluoro-1-octanesulfonate (PFOS), and sodium perfluoro-1-decanesulfonate (PFDS) were purchased as a mixture at 2 µg/mL in methanol in Wellington Laboratories (Ontario, Canada). Working solutions were prepared at 1 and 0.1 µg/mL in acetonitrile and stored at –18 ºC. Perfluoro-n-(1,2,3,4-$^{13}C_4$) octanoic acid (m-PFOA) and sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octane sulfonate (m-PFOS) at 50 µg/mL in methanol, also from Wellington Laboratories, were used as internal standards. HPLC grade water and acetonitrile were supplied by Merck (Darmstadt, Germany) and glacial acetic acid from Panreac (Barcelona, Spain). Supelclean Envi-Carb SPE bulk

active carbon (120/400 mesh), and ammonium acetate were provided by Supelco (Bellefonte, United States of America).

4.2.3. The extraction method and chemical analysis

The PFASs were extracted from dry samples for sediments and soils, and wet samples for mussels, fish, and YLG eggs. One g of sample was spiked with internal standards (m-PFOS and m-PFOA) at 50 ng/g and incubated for 18 h at 4 °C. Briefly, for sediment and soils, solid-liquid extraction was with 9 mL of methanol and 10 mL of 1% glacial acetic acid solution, and in mussels, fish, and YLG eggs were extracted with acetonitrile. Clean up was performed adding 25 mg of activated carbon and 50 µL of glacial acetic acid. The analysis of PFASs was performed using an Acquity liquid chromatography (LC) coupled to a TQD (triple quadrupole) mass spectrometer using a negative ionization electrospray. An XBridge C_{18} column (3.5 µm particle size, 4.6 × 50 mm) was used as a residue trap and the chromatographic separation was performed using an Acquity UPLC BEH C_{18} column (1.7 µm, 100 × 2.1 mm I.D.) (from Waters). Detailed information on flow rate, gradient elution, and further analytical conditions are detailed in Supplement 2 of SI. Method details and quality parameters are indicated in previous studies for sediment and soils Gomez et al., 2011 [5], for mussels and fish on Solé et al., (2021) [23], for YLG eggs on Zapata et al., 2018 [24].

4.2.4. Accumulation of PFASs in YLG

The accumulation of PFOS in YLG was calculated with the Estimated Daily Intake (EDI) based on Bertolero et al., (2015) [13] and Newsted et al., (2007) [25]. It was considered

that (i) fish is the main feed in YLG from Chafarinas Islands, (ii) the daily water consumption is negligible, (iii) mussels are part of the diet but in minor proportion compared to fish, and (iii) other feeding contribution as landfills are impossible to quantify but may contribute to PFOS accumulation.

EDI was calculated as:

$$EDI\ (ng/d) = P_{fd} \times C_f \times K_d + P_{md} \times C_m \times K_d \quad \text{Equation 4.1}$$

where**,**

- P_{fd} (*Percentage of fish-based diet*) corresponds to the fish (anchovies, sardines, and mackerel) percentage of the fish diet (77%), considering the different fishing activities and when they operate. The remaining percentage is referred to refuse tips and secondary preys as insects and cephalopods [22].

- C_f (*Concentration in fish*) is PFOS mean concentration in anchovies, sardines, and mackerel (ng/g).

- P_{md} (*Percentage of mussel-based diet*) corresponds to the mussel percentage of the total diet (0.4%) [26].

- C_m (*Concentration in mussels*) is PFOS mean concentration in mussels (ng/g).

- K_d (*Daily Constant*) is the daily food intake, which is assumed to be 200 g food/day based on the YLG from Ebro Deltra reported by Bertolero et al., (2015) [13].

The Estimated Year Intake (EYI) of PFOS was calculated by EDI × 365 days.

4.3. RESULTS AND DISCUSSION

4.3.1. PFASs in sediments and soils

In the present study, PFOS was the only compound detected in soils and sediments (Figure 4.2). In agricultural soils, PFOS levels ranged from 1.14 to 1.17 ng/g dw, and no differences were observed among citrus, potato, and cereal crops.

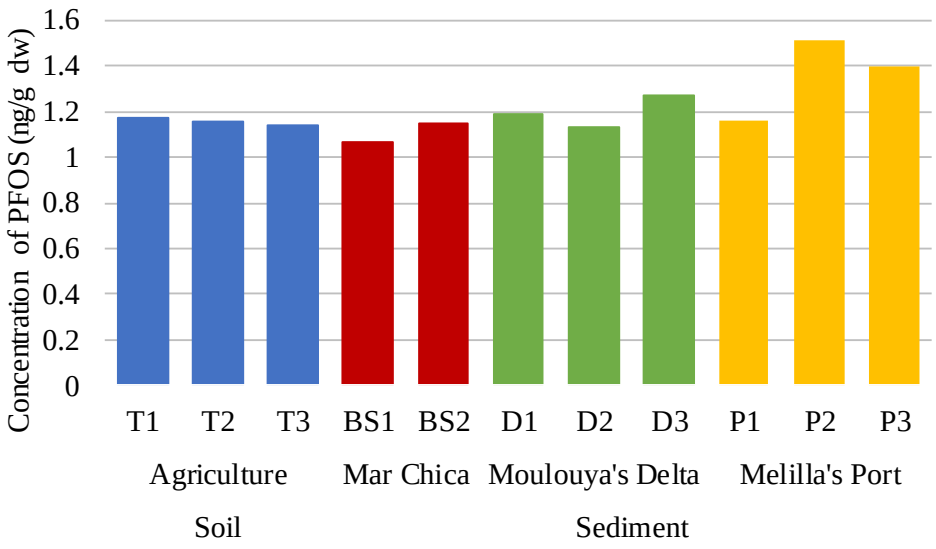


Figure 4.2. The concentration of PFOS in soils from agriculture (Fields) (n=3), in sediments from *Mar Chica* (n=2), Moulouya's Delta (Delta) (n=3), and Melilla's port (Port) (n=3).

The levels detected in this study can be attributed to the irrigation water used in agriculture from the Moulaya river as it has been reported that soils watered with PFASs-contaminated water accumulate PFOS at levels from 0.57 to 12.0 ng/g dw [27]. Also, the use of sludge as fertilizer can account as an additional contamination source as observed in soils from China with 10.4 to 40.8 ng/g dw [28], and from the US with 49.7 to 319.5 ng/g dw [29]. The use of biosolids is a common agronomic practice in Morocco [30]. No bibliography was found related to PFOS in soils in Mediterranean environments. In our study area temperatures are very high which may lead to faster dissipation of soil sorbed

PFASs. In all cases, soil levels detected were well below the 10 ng/g dw proposed by the Federal Environmental Quality Guidelines of the Canadian Environmental Protection Act [31].

In sediments, PFOS levels ranged from 1.07 to 1.51 ng/g dw with similar levels in Melilla port, in Mar Chica, and the Moulouya's Delta. Only slightly higher PFOS levels were detected in Melilla's Port in front of the WWTP outfall (PMS2) and outside the marina (PMS3). Once releases, PFOS are dispersed in open waters in thus, unless there is a direct contamination source, PFOS tends to dilute. The low levels detected are concordance with other studies from the western Mediterranean basin (Table 4.1). The Melilla port receives the direct impact of WWTP effluents that discharge within the port and explains the levels detected. Such effect is also observed in lagoon sediments from Albufera Natural Park in Valencia with levels from 0.10 to 4.80 ng/g dw [32] and from 0.01 to 0.13 ng/g dw in sediments from the Cantabric coast receiving wastewaters from marine emissaries [5]. Chemical industries and a nuclear power plant are other pollution sources, as observed in sediments from the Ebro Delta that contained from 2.50 to 22.6 ng/g dw [33].

Table 4.1. Renge levels of PFOS in sediments from different locations along the Mediterranean and the Iberian Peninsula.

Matrix	n	Location	PFOS (ng/g dw)	Reference
Lagoon sediment	12	Albufera Natural Park	0.1 - 4.80	[32]
Lagoon sediment	2	BS1 and BS2	1.07 - 1.15	**This study**
Delta sediment	71	Ebro	2.50 - 22.6	[33]
River sediment	24	Guadalquivir	0.04 - 0.70	[34]
River sediment	22	Ebro	0.01 - 2.20	[34]
River sediment	3	Cantabria	0.03 - 0.13	[5]
River sediment	3	D1, D2, and D3	1.13 - 1.27	**This study**
Port sediment	4	Cantabria	0.02	[5]
Port sediment	3	P1, P2, and P3	1.16 - 1.51	**This study**

4.3.2. PFASs in fish and mussels

PFOS was also the only compound detected in fish and mussels (Figure 4.3), with levels in anchovy < mackerel < sardine = mussels (1.11, 1.78, 2.59 and 2.59 ng/g ww, respectively).

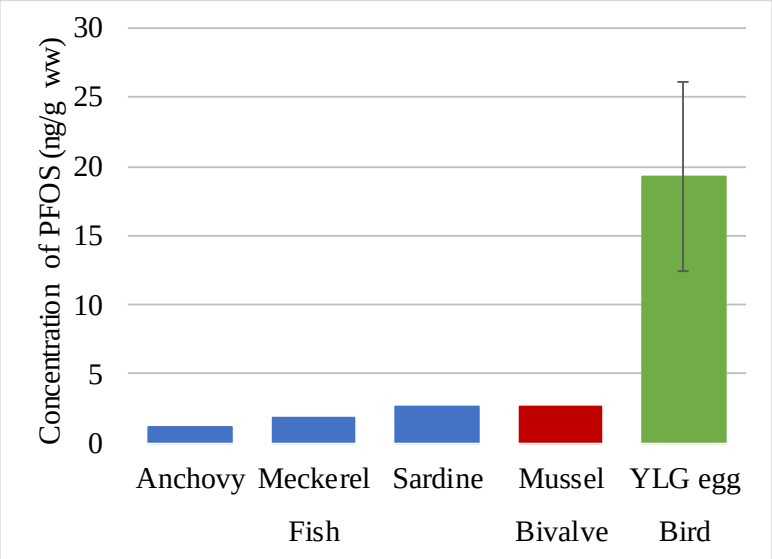


Figure 4.3. The concentration of PFOS in *Engraulis encrasicolus* (Anchovy), *Trachurus mediterraneus* (Mackerel), *Sardina pilchardus* (Sardine), *Mytilus galloprovincialis* (Mussel), and YLG eggs (n=2).

The relatively low levels of PFOS in coastal fish is due to the high dilution of PFASs in marine environments and differences in the bioaccumulation potency that depends on species, dietary habits, and physiology [35]. Similar levels are reported also for sardine and anchovy from the Catalan coast [36], in Mediterranean hake, mullet, mackerel, bogue, and sea bream and other species from the Ebro Delta [33], and from the Aegean Sea [37] and blue-fish from the italic peninsula [38]. In general low concentration levels are reported ranging from a few ng in remote areas to > 100 ng/g ww in several locations

[39]. The levels of PFOS in fish from the Mediterranean and seasides in Europe are indicated in Table 4.2 for the purpose of comparability.

Table 4.2. Range levels of PFOS in mussels and fish from the Mediterranean and Atlantic coast.

Matrix	n	Location	PFOS (ng/g ww)	Reference
Anchovy	1	West French coast	0.34	[35]
Anchovy	37	Italic peninsula	0.93 – 5.96	[38]
Anchovy	4	Greek coast	3.06 ± 0.10*	[37]
Anchovy	5	Catalan coast	0.73 - 1.32	[36]
Anchovy	1	Moroccan coast	1.11	**This study**
Mackerel	37	Italic coast	0.20 - 2.87	[38]
Mackerel	1	Moroccan coast	1.78	**This study**
Sardine	5	Catalan coast	0.54 - 1.14	[36]
Sardine	4	West French coast	1.40	[35]
Sardine	4	Greek coast	<0.49	[37]
Sardine	37	Italic peninsula	0.60 - 0.85	[38]
Sardine	1	Moroccan coast	2.59	**This study**
Mussel	4	French coast	0.007 - 0.173	[40]
Mussel	4	Greek coast	<0.49	[37]
Mussel	9	Italic coast	0.54 - 1.0	[41]
Mussel	13	Italic coast	<2 - 3	[42]
Mussel	10	Cantabri coast	0.02 - 0.06	[5]
Mussel	10	Portugese coast	36.8 - 126	[43]
Mussel	1	Moroccan coast	2.59	**This study**

*Mean and standard deviation.

Filter feeders as mussels have long been used as biomonitors of environmental pollution. Mussels from Chafarinas contained 2.59 ng/g ww. In contrast to other hydrophobic pollutants, mussels show low accumulation of PFOS due to the internal detoxification mechanism [44] although uptake occurs when there is a specific pollution source of PFOS. As an example, mussels from the English Channel and Atlantic accumulated PFOS due to discharges of the Rhone river waters associated with a fluoropolymer facility [45].

Similarly. levels up from 36.8 to 126 ng/g were detected in areas affected by the textile, leather, and paper industry [43]. Contrarily, low levels are detected in open seawaters as in the Cantabric sea (0.02 to 0.06 g/g ww) [5]. PFOS in water affects the mussel larval development at 0.10 mg/L, with the maximal effects observed at 100 mg/L [46], and at 4 mg/L, PFOS cause increased genotoxic damage according to micronucleus assay [47]. Directive 39/2013/EU report PFOS the environmental quality standard with a limit of 9.1 ng/g ww in biota. In all species studied, the levels detected are below this regulated environmental quality standard and it is expected that no effects would appear. These results are below the Federal Environmental Quality Guidelines of the Canadian Environmental Protection Act [31] that are intended to protect avian species that consume fish within a maximum of 8.2 ng/g ww of PFOS in aquatic biota food item.

4.3.3. Accumulation of PFASs in YLG eggs

PFOS has been commonly detected as the predominant PFASs on gull eggs [48]. PFASs are annually transferred to the eggs, as shown for yellow legged gull (*Larus michahellis*) and *L. audouinii* [13]. Thus, gull eggs become excellent bioindicators of PFAS contamination in marine habitats and the levels detected can somehow be extrapolated to other bird species sharing habitat and dietary habits.

Fish represents the main gull diet, so the EDI was assessed with the mean concentration based on the sardine, anchovies, and mackerel detected in this study. The EDI was 283 ± 114 ng/d according to

$$EDI = P_{fd} \times C_f \times K_d + P_{md} \times C_m \times K_d$$

$$= (77/100) \times 200\ g \times (1.83 \pm 0.74\ ng/g) + (0.4/100) \times 200\ g \times (2.59\ ng/g)$$

$= (281 \pm 114) + (2.07)$

$= 283 \pm \text{sqrt}((114^2))$

$= 283 \pm 114$ ng/d.

On a year basin, YLG accumulated 103,433 ± 41,657 ng/y.

This result is higher than the calculated by Bertolero et al., (2015) [13] that reported EDI of 170 ± 48 ng/d for males and 159 ± 42 ng/d for females because PFOS concentrations detected in sardines and anchovies from Ebro Delta were half the levels detected in fish from the Moroccan market. Also, in Ebro Delta was reported the EDI for males and females because the fish consumption varies among sexes (higher in males than in females) [13].

The population of YLG of Chafarinas Islands is closely linked to fisheries (trawlers and purse-seine fisheries) from the Moroccan coast and so the EDI can vary with changes in the fishing activities. As mentioned earlier, the diet of YLG is based on epipelagic fish over 63% of the biomass fish partially collected in association with the purse-seine fisheries (González-Solís, 2003), but can eventually vary when only trawlers operate using human waste from refuse dumps (44% in biomass), epipelagic fish (32%), benthic or mesopelagic fish from trawler discards (20%), and other secondary preys such as insects, and cephalopods [22]. In these cases, the EDI would be of 305 ± 123 ng/d when trawlers and purse-seine fisheries operated, and 193 ± 77.1 ng/d when only trawlers operated.

Considering the concentration of PFOS in eggs and considering that the weight of the egg is 80 g, the concentration in eggs is 1,544 ± 549 ng/egg, that for the clutch of 3 eggs is

4,632 ± 1,646 ng/clutch. This amount represents 4.5% of the yearly intake. Thus, this provides a basis for the accumulative behavior of PFOS in gulls.

Although PFOS was the dominant compound (85% of ∑PFASs), other PFASs detected were PFUnA > PFTriDA > PFDA > PFDoA > PFNA. PFASs concentrations of each compound are found in Table 4.3. Since the PFOS phaseout, production of alternatives moved to long-chain PFCAs, where especially odd chain PFCAs as PFUnA and PFTriDA demonstrated to be highly bioaccumulative and persistent [11]. The presence of long-chain PFCAs in YLG eggs suggests dietary intake [10] but also the accumulation of these chemicals can differ from PFOS since they were not detected in fish species. So, terrestrial prey or refuse tips may be responsible for the long-chain PFCAs concentration. This was previously observed in glaucous-winged gull eggs from Florencia Island where the author suggested that other than marine pray might be the source of PFCAs [49].

Table 4.3. Mean concentration and standard deviation (Mean ± SD) of PFASs in YLG eggs (ng/g ww; n=2) from Chafarinas Islands.

	Concentration (ng/g ww; n=2)
PFNA	0.31 ± 0.07
PFOS	19.3 ± 6.86
PFDA	0.39 ± 0.14
PFUnA	1.49 ± 0.47
PFDoA	0.33 ± 0.10
PFTriDA	0.90 ± 0.05

PFBA, PFPeA, PFHxA, PFHpA, PFTeDA, PFHxDA, PFODA, PFBS, PFHxS, and PFDS were not detected in YLG eggs.

Little is known on the effects that PFASs cause to bird species. Nordén et al., (2016) [50] calculated the median lethal dose (LD50) that is the dose required to kill half of the members of the tested population, the lowest observed effect level (LOEL), and the no observed effect level (NOEL). In chicken embryos, the LD50 was 8.5 µg/g for PFOS, LOEL was 0.9 µg/g, and NOEL 2.73 µg/g, and the embryo survival for herring gull was 59% for 10 µg/g of PFOS, being the wild specie 2.7 times less sensitive than the domestic. Also, bodyweight increased by 11% on gull chicks in the exposition of 10 µg/g of PFOS [50]. Lopez-Antia et al., (2017) [51] evaluated different biomarker parameters in plasma (total protein, albumin, triglyceride, uric acid, and cholesterol) of great tits (*Parus major*) next to a fluoro-chemical plant and concluded no effects by PFOS exposure. Also, the PFOS levels were evaluated in eggs of great tits (*Parus major*), northern lapwing (*Vanellus vanellus*), and the Mediterranean gull (*Larus melanocephalus*), and despite the levels were similar, gulls presented the higher ones [51]. To assess the potential toxicity of PFOS, two concentrations (100 and 200 ng/g of PFOS) were injected in YLG eggs and no effects were observed [52]. In our study, the concentrations detected were some orders of magnitude lower suggesting that the YLG colony from the Chafarinas Islands is below the risk levels. Thus, if extrapolated to other bird species sharing habitat, it is expected that the populations are not at risk due to the presence of PFASs.

4.4. CONCLUSIONS

The Chafarinas Islands receives the impact of different human activities that produce the sorption of PFOS in soil and sediment and accumulation in biota. In soils, PFASs sources

were presumably attributed to water irrigation and the use of sludge. Only PFOS was detected in the different fish species and mussels evaluated in the present study suggesting bioaccumulation of this contaminant in the marine environment of the Chafarinas Islands. The diet of the YLG is partially based on fish and the uptake has been estimated under two scenarios of fishing procedures. Through the diet, YLG accumulated 103,433 ± 41,657 ng of PFOS per year and 4.5% was released annually to the clutches. Other PFAS, namely odd long-chain PFCAs were also detected in gull eggs and maybe incorporated from other sources. Gulls and other species sharing habitat become highly vulnerable, and thus monitoring and protection actions must be implemented.

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CHAPTER 5.

DISTRIBUTION AND 10-YEAR TEMPORAL TRENDS OF PERFLUOROALKYL SUBSTANCES IN GULL EGGS FROM SPANISH BREEDING COLONIES

CHAPTER 5

5.1. INTRODUCTION

Perfluoroalkyl substances (PFASs) are synthetic chemicals with unique properties regarding heat stability, resistance to degradation, and the capacity to repel both water and oil [1]. They have been used since the beginning of the XX century in a myriad of industrial processes and consumer products such as adhesives, lubricants, cosmetics, cleaners, stain-resistant and non-stick coatings, food packaging, electronics, pesticides, fire foams, paper, and photographic products [2]. Wastewater treatment plants effluents, fire-fighting operations at military bases and airports, landfill leachate, and run-off are the main sources of PFASs to the environment [3]. PFASs do not hydrolyze, photodegrade or biodegrade, and are considered persistent and bioaccumulative and are biomagnified along the food webs [4], especially those compounds with a longer carbon length[3,5]. Because of this persistent behavior, PFOS, PFOA, its salts, and related compounds are included in the Stockholm Convention [6] with the purpose to protect human health and the environment, identify contaminated areas and define management action to minimize the occurrence of these compounds.

Despite the restrictions on the production and use of PFASs, these compounds impact wildlife, especially animals on the top of the food webs [7,8]. Fish-eating birds are apex predators exposed to PFASs depending on the habitat, diet, behavior, and life strategy [9], and have become excellent bioindicators of environmental pollution as reflect the levels of pollution of a specific area [10]. Our target species are the yellow-legged gull (*Larus michahellis*; thereafter YLG) and Audouin's gull (*Larus audouinii*; thereafter AG). YLG is a big sized marine gull with an increasing population along the western Mediterranean Sea, west Moroccan coast, and northeast Atlantic coast [11]. YLG has high feeding adaptability, from natural prey to fishing discards, rubbish tips, and nestlings

from other birds [12]. On the other hand, AG is a medium-sized gull that breeds mostly in the western Mediterranean and wintering on the northern and western coast of Africa and is included in the IUCN 2019 Red List as Least Concern [11]. AG is mainly a piscivorous species, feeding preferably on clupeiformes and perciformes [13], but also exploit fishery discards and nocturnal purse seine fisheries [14]. Gulls accumulate PFASs through the diet [15] and are transferred to the entire clutches [16], thus eggs become good bioindicators of environmental pollution.

Natural and National Parks host the main gull colonies and the monitoring of their eggs serves to evaluate the quality of a given habitat and permits to deduce PFASs exposure of other protected or non-protected species sharing a habitat. In addition, systematic annual monitoring can be used to determine temporal patterns of PFASs and diagnose the efficiency of actions to mitigate the pollution impact in vulnerable areas which are breeding grounds of many species. Thus, the objectives of the present study were: (i) to determine the geographical distribution and time trends of 17 PFASs in YLG eggs over the period 2009 to 2018 in four main Spanish breeding colonies; and (ii) to evaluate the different PFASs patterns between YLG and AG that breed sympatrically in the Ebro Delta Natural Park. Overall, we describe the advantages of long-term monitoring schemes using gulls as sentinel species to determine the occurrence of PFASs in areas with a high level of protection still affected by environmental pollution.

5.2. MATERIALS AND METHODS

5.2.1. Study areas and sampling

Gulls' colonies sampled in this study are located in four Spanish Natural and National Parks including the Montgrí, Medes and Baix Ter Natural Park (thereafter Medes), the Ebro Delta Natural Park (thereafter Ebro Delta), the Chafarinas Islands National Hunting Refuge (thereafter Chafarinas), and the Atlantic Islands of Galicia National Park (thereafter Atlantic). Table 5.A1 indicates the location and characteristics of each colony. The sampling protocol was based on Vicente et al., (2012) [17]. Each colony was divided into 3 subcolonies where 12 first-laid eggs were randomly collected, resulting in a total of 36 eggs per colony. The first egg represents the maximum pollutant transferred from female to eggs and permits comparability among results from different colonies [15,18]. Each subcolony was pooled to create a composite sample (12 eggs per pool), and as a result, a total of 3 pooled samples were analyzed per colony per year. YLG eggs were collected during the breeding season from 2009 to 2018, except in Chafarinas where we failed to obtain samples in the period 2012-2014, only one sample was collected in 2011 and two in the 2015-2018 period. In the year 2012, we only obtained one sample in Medes also because of sampling problems. AG's eggs were collected only from Ebro Delta as it was the main AG breeding colony in Spain when we started the study in 2009. As the modal clutch size of both species is three eggs, this kind of sampling does not cause population damage to the colony.

5.2.2. Chemical and reagents

Native compounds of perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorodecane sulfonate (PFDS) were purchased as a mixture at 2 µg/mL in methanol by Wellington Laboratories (Ontario, Canada). Working solutions were prepared at 1 and 0.1 µg/mL in acetonitrile and stored at –18 ºC. Perfluoro-n-(1,2,3,4-$^{13}C_4$) octanoic acid (m-PFOA) and sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octane sulfonate (m-PFOS) at 50 µg/mL in methanol, also from Wellington Laboratories, were used as internal standards. HPLC grade water and acetonitrile were supplied by Merck (Darmstadt, Germany) and glacial acetic acid from Panreac (Barcelona, Spain). Supelclean Envi-Carb SPE bulk active carbon (120/400 mesh), and ammonium acetate were provided by Supelco (Bellefonte, United States of America).

5.2.3. Extraction method and analysis

The PFASs were extracted from wet samples. Briefly, 1 g of sample was spiked with internal standards (m-PFOS and m-PFOA) at 50 ng/g and 9 mL of acetonitrile was added and left overnight. Then, the sample was thoroughly mixed using a vortex mixer and extracted in an ultrasonic bath for 10 min (3 times). Afterward, the sample was

centrifuged at 2500 rpm for 5 min, and the supernatant was transferred to a new vial and cleaned up by adding 25 mg of activated carbon and 50 µL of glacial acetic acid. After centrifugation at 10,000 rpm for 10 min, the supernatant was collected, evaporated under a nitrogen stream and the extract was reconstituted with 500 µL of a mixture of acetonitrile/10 mM ammonium acetate (50/50, v/v).

The analysis of PFASs was carried out on an Acquity UPLC system coupled to a TQD (triple quadrupole) mass spectrometer, equipped with an orthogonal Z-spray-electrospray interface using a negative ionization (Waters, Massachusetts, USA). An XBridge C_{18} column (3.5 µm particle size, 4.6 × 50 mm) was used as a residue trap to remove PFASs contribution from the mobile phase or tubing. Chromatographic separation was performed using an Acquity UPLC BEH C_{18} column (1.7 µm, 100 × 2.1 mm I.D.) (from Waters) at a flow rate of 0.3 mL/min. The initial conditions of the gradient elution were the following: 50 % water and 50 % methanol/acetonitrile (80:20, v/v) buffered with 10 mM of ammonium acetate. These conditions were kept for 3 minutes and the organic mobile phase component increased to 100 % in 7 min. The sample injection volume was 5 µL for the standards and sample extracts. Nitrogen was used as drying gas and nebulizing gas at 30 and 750 L/h N_2, respectively. The capillary voltage was set at 2500 kV and source and desolvation temperature were set at 120 and 350 °C. Experimental conditions for the selected compounds are shown in Table 5.A2. For MS/MS analysis, the collision gas was argon at a pressure of 0.19 mL/min.

Quantification of the target compounds was performed using an internal standard method using m-PFOA and m-PFOS as surrogate standards. Mass Lynx v.4.1 software was used to control the instrument setup, data acquisition, and processing. A calibration curve with five points was built over a concentration range of 5 to 300 ng/mL with the surrogates at

50 ng/mL. Extraction efficiency was evaluated using chicken eggs spiked with native and surrogates PFASs at a concentration of 50 ng/g wet weight (ww). Recoveries of the target compounds ranged from 94 to 116 % and the method detection limits (MDL) were between 0.07 and 1.1 ng/g (Table 5.A3).

5.2.4. Data treatment

Statistical analysis and Principal Component Analysis (PCA) were done considering the detected compounds PFOS, PFNA, PFDA, PFUnA, PFDoA, and PFTriDA but PFOA was excluded because it was only detected in Medes. In all cases concentrations under the MLD were substituted by the half values of the MLD. For YLG eggs, PCA was carried out on log-transformed concentrations by using the covariance matrix to study the patterns of association among PFASs. Varimax rotation was performed at one level of factor analysis to evaluate the relationship among factors. The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was used to assess the usefulness of the PCA. KMO ranges from 0 to 1 and should be well above 0.5 if variables are sufficiently interdependent for PCA to be useful [19].

The time variation of PFASs concentrations in eggs was compared among YLG colonies and between YLG and AG gull species at the Ebro Delta with repeated measures multivariate analysis of variance (RM MANOVA). MANOVA is used when several dependent variables are measured in each sampling unit instead of only one variable (for more details, see Rovira et al., 2012 [20]. Significances were further explored with one-way repeated measures analysis of variance (RM-ANOVA). When comparing YLG colonies, Chafarinas was not included because the data set was incomplete due to

sampling problems (See section 2.1); however, we conducted an additional analysis including the four colonies but excluding the period 2011-2014 (see Annex 5.A1 and Table 5.A4). In addition to *P* values, we used partial eta squared (η_p^2) to measure the importance of factors (effect size), in this case the effect of colony, year or colony x year. Similarly to r^2, partial η_p^2 is the proportion of variation explained for a certain variable and has the advantage over eta squared of not depending on the number of sources of variation used in the ANOVA, thus it can be compared among different designs (Tabachnick and Fidell, 2007). Estimated marginal means (EMM) of dependent variables are the means for each level of the factor, adjusted for the other variables, and were used to describe temporal trends and differences among colonies, and between species. The association of EMM with years (i.e., temporal trend) within each gull colony was analyzed with Spearman's rank correlation coefficient (ρ) using R version 3.4.4 [21]. All other statistical analyses were performed with SPSS 23.0.

5.3. RESULTS

5.3.1. Levels and geographical distribution of PFASs

PFASs were detected in all samples, and the ∑PFASs levels for YLG eggs were Medes ≈ Ebro Delta > Chafarinas ≈ Atlantic. The mean concentrations of both ∑PFASs and individual PFASs compounds detected each year in each colony are shown in Table 5.1. PFOS was the dominant accounting from 61 to 81 % of ∑PFASs (in Chafarinas accounted for the highest proportion)

Table 5.1. Mean ± Standard Deviation (*n* = 3, see footage) concentration of the PFASs (ng/g ww) in gull eggs from Medes Islands, Ebro Delta, Chafarinas Islands, and Atlantic Islands.

Species	Colony	Compound	Years									
			2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Larus michahellis	Medes Islands	PFOA	1.43 ± 0.5	0.71 ± 0.6	0.60 ± 0.4	0.98	1.46 ± 0.2	0.67 ± 0.7	0.29 ± 0.1	0.79 ± 0.3	0.59 ± 0.2	0.38 ± 0.1
		PFNA	6.17 ± 0.7	3.15 ± 0.7	2.35 ± 0.5	3.14	3.53 ± 0.6	2.26 ± 0.3	4.00 ± 0.5	3.71 ± 1.8	2.54 ± 0.3	2.66 ± 1.1
		PFOS	65.5 ± 13	64.7 ± 2.5	56.5 ± 12	71.6	74.0 ± 10	53.4 ± 7.5	80.4 ± 13	76.2 ± 8.4	77.1 ± 16	68.4 ± 4.1
		PFDA	7.33 ± 1.8	3.46 ± 0.2	2.54 ± 0.6	5.63	3.66 ± 0.2	3.41 ± 0.5	3.65 ± 2.6	4.93 ± 2.4	3.96 ± 0.5	1.29 ± 0.6
		PFUnA	29.8 ± 4.4	9.02 ± 7.3	2.96 ± 1.2	3.36	10.2 ± 2.4	3.47 ± 0.3	5.73 ± 1.8	3.53 ± 1.1	5.29 ± 1.0	4.75 ± 1.8
		PFDoA	9.21 ± 0.1	8.03 ± 1.6	4.03 ± 1.8	10.3	8.82 ± 2.7	6.15 ± 2.4	4.42 ± 2.4	6.57 ± 2.3	3.52 ± 0.7	5.29 ± 0.8
		PFTriDA	25.0 ± 5.0	15.5 ± 1.5	8.17 ± 0.6	11.3	10.4 ± 1.5	6.34 ± 1.1	9.62 ± 2.3	4.57 ± 3.0	7.78 ± 5.2	11.9 ± 3.2
		∑PFASs	144 ± 13	104 ± 8.4	77.1 ± 11	106	112 ± 7.3	75.7 ± 8.9	108 ± 18	100 ± 2.6	101 ± 22	94.7 ± 8.4
	Ebro Delta	PFOA	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
		PFNA	3.55 ± 0.8	2.89 ± 0.3	3.94 ± 0.2	3.60 ± 0.5	2.68 ± 0.6	3.34 ± 1.1	3.09 ± 0.8	3.78 ± 0.9	1.84 ± 0.4	1.91 ± 0.5
		PFOS	93.0 ± 6.2	77.4 ± 12	101 ± 9.3	101 ± 15	56.5 ± 8.7	72.2 ± 20	59.2 ± 9.6	48.5 ± 10	57.9 ± 6.1	61.9 ± 7.5
		PFDA	3.16 ± 0.7	2.50 ± 0.4	3.93 ± 0.6	4.65 ± 1.1	5.62 ± 0.4	3.56 ± 1.1	4.81 ± 0.3	3.07 ± 0.6	2.45 ± 1.4	4.28 ± 1.2
		PFUnA	15.7 ± 5.3	8.51 ± 2.3	22.8 ± 3.0	24.0 ± 3.5	16.6 ± 3.3	13.9 ± 3.8	7.52 ± 4.3	6.83 ± 1.5	7.95 ± 3.6	8.31 ± 1.0
		PFDoA	5.05 ± 1.7	5.01 ± 1.6	7.08 ± 1.9	10.9 ± 5.6	6.27 ± 0.4	6.62 ± 4.3	4.86 ± 4.2	5.49 ± 3.0	2.69 ± 0.7	3.13 ± 0.7
		PFTriDA	8.97 ± 1.8	6.83 ± 2.3	14.8 ± 5.8	17.1 ± 2.6	13.6 ± 0.2	6.36 ± 3.5	5.25 ± 2.5	4.92 ± 2.1	6.07 ± 3.7	6.12 ± 0.4
		∑PFASs	129 ± 15	103 ± 11	153 ± 14	161 ± 24	101 ± 13	106 ± 31	84.7 ± 14	72.6 ± 18	78.9 ± 15	85.6 ± 9.8
	Chafarinas Islands	PFOA	< MDL	< MDL	< MDL	n.a.	n.a.	n.a.	< MDL	< MDL	< MDL	< MDL
		PFNA	1.13 ± 0.4	1.01 ± 0.4	0.77	n.a.	n.a.	n.a.	0.31 ± 0.1	0.67 ± 0.2	0.66 ± 0.01	0.45 ± 0.3
		PFOS	19.2 ± 6.2	19.9 ± 4.0	12.8	n.a.	n.a.	n.a.	19.3 ± 6.8	17.8 ± 10	11.5 ± 5.9	11.1 ± 5.1
		PFDA	1.20 ± 0.2	0.89 ± 0.2	0.54	n.a.	n.a.	n.a.	0.38 ± 0.1	0.74 ± 0.5	0.39 ± 0.01	0.51 ± 0.05
		PFUnA	2.28 ± 0.8	1.09 ± 0.7	0.42	n.a.	n.a.	n.a.	1.49 ± 0.5	0.77 ± 0.8	1.75 ± 0.1	0.76 ± 0.1
		PFDoA	0.49 ± 0. 1	0.85 ± 0.6	1.74	n.a.	n.a.	n.a.	0.33 ± 0.1	0.98 ± 0.7	0.19 ± 0.03	0.35 ± 0.1

Table 5.1 (continued)

Species	Colony	Compound	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
		PFTriDA	0.06	< MDL	< MDL	n.a.	n.a.	n.a.	0.89 ± 0.1	LOD/2	1.17 ± 0.1	0.54 ± 0.4
		∑PFASs	24.3 ± 6.1	23.8 ± 5.1	16.3	n.a.	n.a.	n.a.	22.7 ± 7.7	20.9 ± 13	15.6 ± 5.9	13.7 ± 5.9
	Atlantic Islands	PFOA	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
		PFNA	0.50 ± 0.1	0.63 ± 0.1	0.72 ± 0.3	0.84 ± 0.02	0.72 ± 0.2	0.90 ± 0.5	0.99 ± 0.4	0.63 ± 0.2	0.40 ± 0.07	0.57 ± 0.2
		PFOS	12.5 ± 0.2	14.0 ± 1.6	12.6 ± 2.4	11.2 ± 2.6	14.4 ± 3.9	15.0 ± 7.6	16.0 ± 2.7	12.4 ± 2.7	10.5 ± 4.2	11.4 ± 1.6
		PFDA	0.99 ± 0.1	0.97 ± 0.03	1.21 ± 0.3	1.41 ± 0.3	1.44 ± 0.2	1.53 ± 0.7	0.38 ± 0.1	1.06 ± 0.2	0.45 ± 0.06	0.50 ± 0.3
		PFUnA	5.41 ± 2.8	3.33 ± 1.8	2.35 ± 1.5	3.85 ± 0.5	4.30 ± 0.7	3.88 ± 0.9	4.18 ± 1.0	2.50 ± 1.1	2.10 ± 0.8	2.63 ± 0.5
		PFDoA	1.98 ± 0.7	2.28 ± 0.8	0.49 ± 0.04	0.48 ± 0.02	0.80 ± 0.02	0.51 ± 0.01	0.69 ± 0.05	0.51 ± 0.01	0.38 ± 0.1	0.95 ± 0.3
		PFTriDA	2.86 ± 0.6	4.14 ± 1.3	3.08 ± 1.4	1.25 ± 0.8	1.48 ± 0.4	1.46 ± 0.9	1.69 ± 0.3	2.67 ± 0.4	2.06 ± 0.3	2.20 ± 0.3
		∑PFASs	24.3 ± 2.6	25.3 ± 1.4	20.5 ± 4.7	19.0 ± 2.1	23.1 ± 3.5	23.3 ± 9.2	23.9 ± 2.7	19.8 ± 2.2	15.9 ± 4.9	18.2 ± 1.6
Larus audouinii	Ebro Delta	PFOA	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
		PFNA	3.28 ± 0.3	2.72 ± 0.2	3.43 ± 0.5	3.19 ± 0.2	2.47 ± 0.3	3.76 ± 0.5	2.30 ± 0.9	3.77 ± 0.1	1.78 ± 1.0	1.55 ± 0.9
		PFOS	94.6 ± 11	101 ± 6.0	822 ± 12	93.4 ± 7.5	81.5 ± 8.2	91.5 ± 6.8	78.1 ± 8.7	78.0 ± 6.4	94.8 ± 12	74.5 ± 17
		PFDA	4.37 ± 0.3	3.97 ± 0.6	4.86 ± 0.8	4.20 ± 0.2	5.93 ± 0.6	5.20 ± 1.3	5.02 ± 0.04	5.98 ± 0.4	2.79 ± 0.9	4.65 ± 0.8
		PFUnA	26.5 ± 6.7	26.5 ± 5.7	31.9 ± 4.7	29.5 ± 12	32.6 ± 3.2	24.5 ± 7.1	17.6 ± 3.9	17.4 ± 1.8	15.1 ± 4.5	9.49 ± 0.1
		PFDoA	9.20 ± 4.2	6.00 ± 2.7	8.77 ± 1.9	14.4 ± 5.6	10.0 ± 0.9	11.6 ± 8.7	3.27 ± 0.2	16.2 ± 6.4	4.48 ± 1.6	4.23 ± 0.3
		PFTriDA	15.0 ± 3.9	15.4 ± 4.9	19.1 ± 6.2	19.1 ± 3.8	22.0 ± 2.0	17.5 ± 4.8	9.35 ± 0.6	12.0 ± 2.1	8.41 ± 0.6	7.83 ± 0.4
		∑PFASs	153 ± 3.8	156 ± 12	150 ± 16	164 ± 17	155 ± 9.2	154 ± 24	116 ± 7.5	133 ± 12	127 ± 16	102 ± 19

< MDL=method detection limit

n.a.= not analysed

n = 3, except Medes 2+A1:M46012 and Chafarinas 2011 (n = 1), and Chafarinas 2015 to 2018 (n = 2)

Other PFASs varied among colonies, but generally were followed by PFUnA and PFTriDA, as follow: in Medes Islands' colony was PFTriDA > PFUnA > PFDoA > PFDA > PFNA > PFOA; in Ebro Delta (both species) showed PFUnA > PFTriDA > PFDoA > PFDA > PFNA; in Chafarinas Islands was PFUnA > PFTriDA = PFDA = PFNA > PFDoA; and in Atlantic Islands was PFUnA > PFTriDA > PFDA > PFDoA > PFNA. The other analyzed compounds (PFBA, PFPeA, PFHxA, PFHpA, PFTeDA, PFHxDA, PFODA, PFBS, PFHxS, and PFDS), were not detected.

The PCA on PFASs concentrations in YLG eggs showed that all compounds were interdependent and significantly correlated (Pearson's $r > 0.72$; $N = 133$; $P < 0.0001$, for all paired comparisons). The KMO measure of sampling adequacy (0.69) showed the usefulness of the PCA, with the first two axes explaining 51.0 and 39.5 % of the total variation after varimax rotation, respectively (Figure 5.1). The strongest correlations were found between ∑PFASs, PFDoA, PFDA, PFNA and PFOS ($r > 0.84$; $N = 133$; $P < 0.0001$), with a similar contribution to first PCA axis scores, while PFUnA and PFTriDA ($r = 0.72$; $N = 133$; $P < 0.0001$) showed a lower contribution to PC1 but larger to PC 2. The PC1 (Figure 5.1a) summarizes these correlations differentiating the YLG eggs from the Atlantic Islands and Chafarinas colonies (with the lowest levels of all considered compounds) with those from Ebro Delta and Medes Islands colonies (with the highest levels). In contrast, PC2 mainly differentiated Medes, Ebro Delta and Atlantic Islands that had high contribution of PFUnA and PFTriDA, while in Chafarinas these compounds were detected at very low concentration (Figure 5.1b).

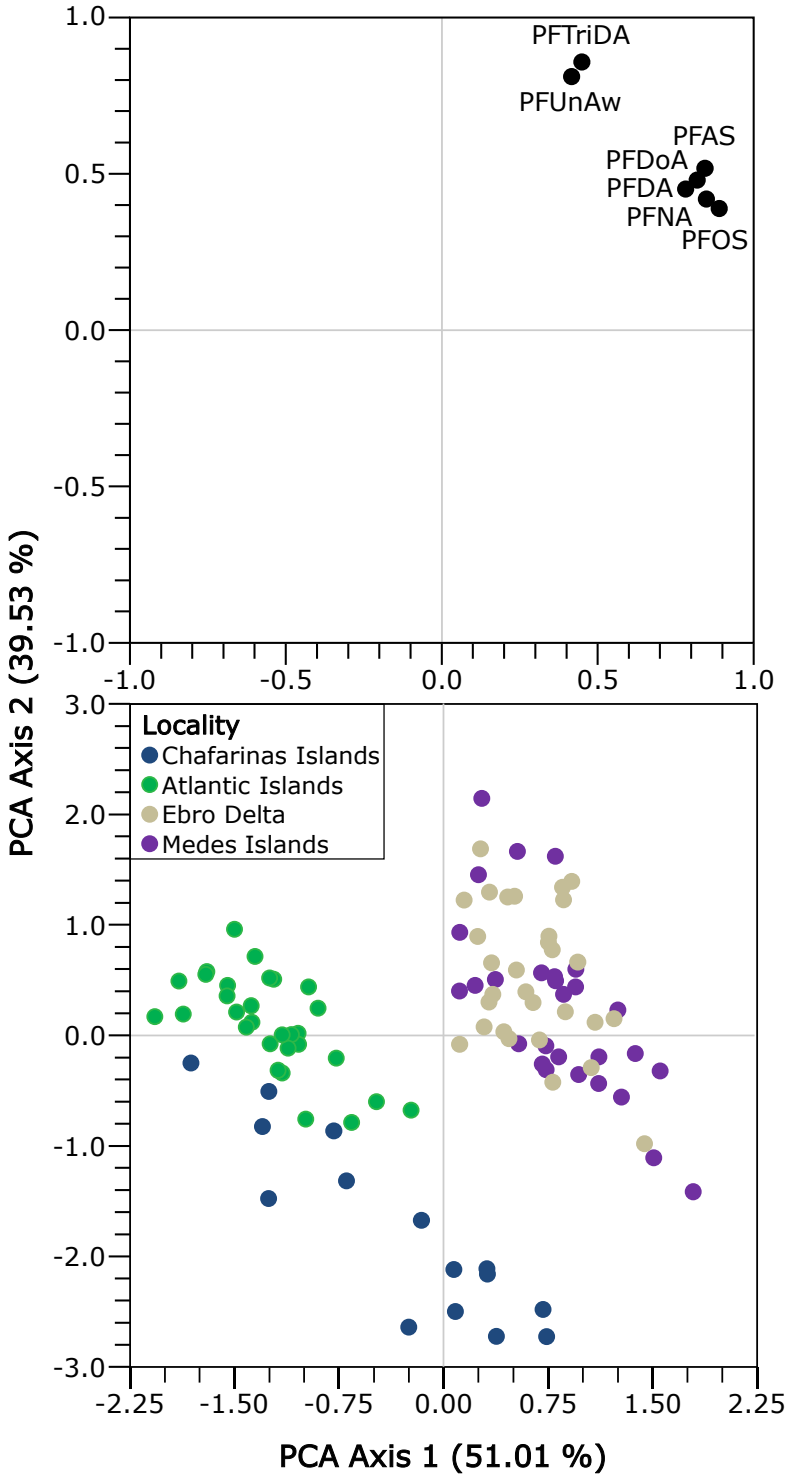

Figure 5.1. Principal Component Analysis (PCA) on log-transformed concentrations of PFASs compounds in yellow-legged gull eggs. Factor loadings of the PFASs compounds (top) and localities scores (bottom) on the first two principal component axes are shown. The percent variation explained by PCA axes 1 and 2 is also shown.

5.3.2. Variation among colonies and temporal trends

The concentration of PFASs in YLG eggs (excluding Chafarinas Islands) significantly varied among years (RM-MANOVA; Wilks's $\lambda = 0.07$, $F_{54.0,\ 162.7} = 4.96$, $P < 0.0001$, $\eta_p^2 = 0.563$), but the temporal pattern differed among colonies (Year × Colony; Wilks's $\lambda < 0.001$, $F_{108.0,\ 184.8} = 4.84$, $P < 0.0001$, $\eta_p^2 = 0.723$). Univariate tests (RM-ANOVAs) confirmed this pattern and is described in Table 5.2. When compared with the other colonies, the Medes Islands presented a significant high concentration for PFDA, PFDoA, and PFTriDA,. ∑PFASs, PFNA, and PFOS did not present differences between the Medes Islands and Ebro Delta. Ebro Delta presented significant high levels for PFUnA. Atlantic Islands showed the lowest levels for all PFASs detected (Table 5.1 and 5.2).

Table 5.2. RM-ANOVAs on the concentrations of PFASs in YLG eggs collected in Medes (MI), Ebro Delta (ED), and Atlantic Islands (AI) from 2009 to 2018. Significance levels; *ns*: not significant; *a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.005$; *d*: $P < 0.001$.

Compound	Within Subjects Effects						Between Subjects Effects		
	Year			Year × Colony			Colony		
	F	*df*	η_p^2	*F*	*df*	η_p^2	$F_{2,\ 4}$	η_p^2	Contrast tests
PFNA	5.64^{c}	5.67, 22.7	0.585	4.63^{d}	11.3, 22.7	0.699	211^{d}	0.991	AI < ED ≈ MI
PFOS	3.75^{a}	3.95, 15.8	0.484	6.24^{c}	7.89, 15.8	0.757	303^{d}	0.993	AI < ED = MI
PFDA	8.65^{d}	5.81, 23.2	0.684	7.73^{d}	11.6, 23.2	0.794	210^{d}	0.991	AI < ED < MI
PFUnA	11.21^{d}	4.39, 17.6	0.737	8.07^{d}	8.79, 17.6	0.801	91.9^{d}	0.979	AI < MI < ED
PFDoA	2.77^{a}	4.90, 19.6	0.409	1.61^{ns}	9.80, 19.6	0.445	373^{d}	0.995	AI < ED < MI
PFTriDA	8.59^{d}	5.80, 23.2	0.682	6.82^{d}	11.6, 23.2	0.773	310^{d}	0.994	AI < ED < MI
∑PFASs	6.76^{c}	3.45, 13.8	0.628	6.50^{c}	6.89, 13.8	0.765	297^{d}	0.993	AI < ED = MI

Figure 5.2 shows the temporal variations of each PFASs in each colony and Table 5.3 shows the Spearman's rank correlations for both YLG and AG from all colonies.

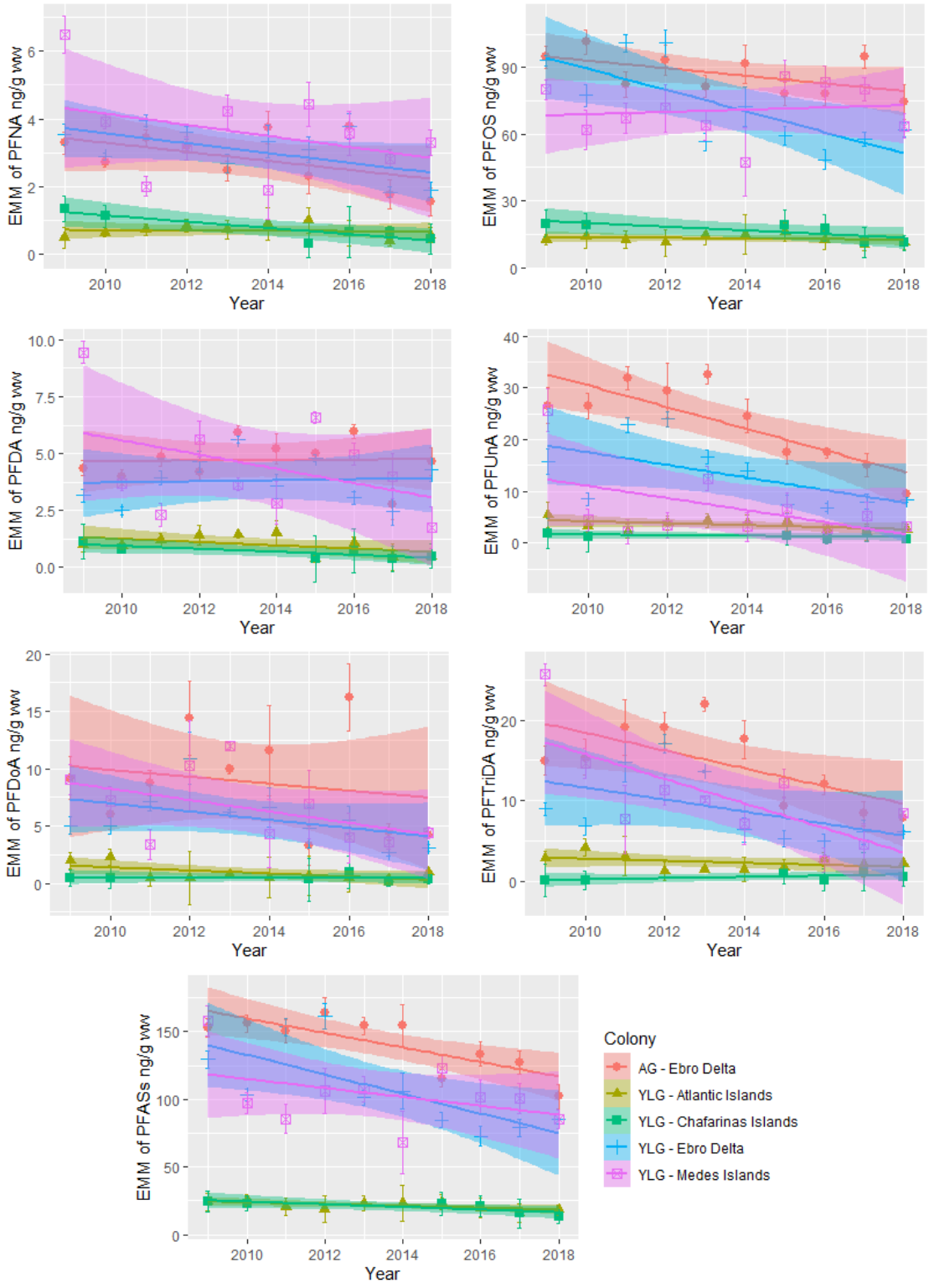

Figure 5.2. Estimated marginal mean (EMM) and standard error (n = 3) of PFASs in eggs of *Larus audouinii* (AG) and *Larus michahellis* (YLG) per locality. YLG EEM from Chafarinas Islands were extracted from RM MANOVA excluding from 2011 to 2014 (see ANNEX Table 5.A4).

Table 5.3. Spearman's rank correlation coefficient between the estimated marginal means (RM-MANOVA) of the PFASs concentrations and year for both YLG and AG from all colonies.

Species	Colony	Compound	ρ	*n*	*P*
Larus michahellis	Medes Islands	PFNA	-0.27	10	0.448
		PFOS	0.21	10	0.559
		PFDA	-0.29	10	0.427
		PFUnA	-0.24	10	0.514
		PFDoA	-0.41	10	0.247
		PFTriDA	**-0.66**	**10**	**0.044**
		∑PFASs	-0.31	10	0.387
	Ebro Delta	PFNA	-0.47	10	0.178
		PFOS	**-0.66**	**10**	**0.044**
		PFDA	0.02	10	0.973
		PFUnA	**-0.65**	**10**	**0.049**
		PFDoA	-0.5	10	0.143
		PFTriDA	**-0.7**	**10**	**0.031**
		∑PFASs	**-0.75**	**10**	**0.018**
	Chafarinas Islands	PFNA	-0.66	6	0.175
		PFOS	**-0.94**	**6**	**0.017**
		PFDA	-0.6	6	0.242
		PFUnA	-0.6	6	0.242
		PFDoA	-0.37	6	0.497
		PFTriDA	0.6	6	0.241
		∑PFASs	**-1**	**6**	**0.003**
	Atlantic Islands	PFNA	-0.15	10	0.682
		PFOS	-0.27	10	0.448
		PFDA	-0.35	10	0.331
		PFUnA	-0.44	10	0.204
		PFDoA	-0.35	10	0.331
		PFTriDA	-0.33	10	0.349
		∑PFASs	**-0.7**	**10**	**0.031**
Larus audouinii	Ebro Delta	PFNA	-0.42	10	0.232
		PFOS	-0.58	10	0.088
		PFDA	0.2	10	0.584
		PFUnA	**-0.78**	**10**	**0.012**
		PFDoA	-0.25	10	0.492
		PFTriDA	-0.64	10	0.054
		∑PFASs	**-0.71**	**10**	**0.028**

A significant lineal negative correlation along the years was observed for PFOS, PFUnA, PFTriDA, and ∑PFASs. In Medes, only PFTriDA showed a significant lineal negative correlation along the years. In contrast, in the Ebro delta YLG of had significant decrease of PFOS, PFUnA and PFTriDA while in AG the decreasing trend was only significant for PFUnA. YLG eggs from Chafarinas Islands presented a significant lineal negative correlation along the years for PFOS. In Atlantic Islands, ∑PFASs had a significant lineal negative correlation along the years and although no differences for individual compounds were observed during the study period, PFNA, PFOS, and PFDA presented slightly higher levels from 2013 to 2015. Similar results were obtained when the Chafarinas Islands colony was included in the analyses (excluding years 2011 to 2014 in all colonies; Table 5.A4).

When comparing YLG and AG eggs in the Ebro Delta colony we found significant differences in PFASs concentrations among years (RM-MANOVA; Wilks's λ = 0.04, $F_{54.0,\ 162.7}$ = 5.90, P < 0.0001, η_p^2 = 0.602), and the temporal pattern differed significantly among gull species (Year × Species; Wilks's λ = 0.104, $F_{54.0,\ 162.7}$ = 1.62, P = 0.007, η_p^2 = 0.314). Univariate tests (RM-ANOVAs) confirmed this pattern and showS that AG eggs have significantly higher concentrations for all PFASs than YLG except for PFNA and PFDoA (Table 5.4). Temporal variation pattern was similar in both species for all PFASs except for PFOS (Year × Species; Table 5.4), but a significant linear negative correlation was observed only for PFUnA and ∑PFASs in AG (Figure 5.2, Table 5.3).

Table 5.4. RM-ANOVAs on the concentrations of PFASs in YLG and AG eggs collected in Ebro Delta from 2009 to 2018. Significance levels; *ns*: not significant; *a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.005$; *d*: $P < 0.001$.

Compound	Within Subjects Effects						Between Subjects Effects	
	Year			Year × Species			Species	
	F	*df*	η_p^2	*F*	*df*	η_p^2	$F_{2,4}$	η_p^2
PFNA	8.86^{d}	9.00, 36.0	0.689	0.42^{ns}	9.00, 36.0	0.095	1.79^{ns}	0.309
PFOS	10.3^{d}	7.12, 28.5	0.720	5.03^{d}	7.12, 28.5	0.557	9.58^{a}	0.706
PFDA	7.58^{d}	7.67, 30.7	0.655	2.27^{ns}	7.67, 30.7	0.362	17.2^{a}	0.812
PFUnA	11.1^{d}	5.03, 20.1	0.735	1.41^{ns}	5.03, 20.1	0.261	115^{d}	0.967
PFDoA	4.91^{c}	6.43, 25.7	0.551	1.38^{ns}	6.43, 25.7	0.257	5.26^{ns}	0.568
PFTriDA	10.3^{d}	9.00, 36.0	0.721	1.34^{ns}	9.00, 36.0	0.251	227^{d}	0.983
∑PFASs	14.3^{d}	7.08, 28.3	0.781	3.32^{a}	7.08, 28.3	0.454	30.8^{b}	0.885

5.4. DISCUSSION

5.4.1. Concentration and patterns among colonies and species

Gulls are exposed to PFASs in all 4 studied colonies and throughout the years, suggesting that National and Natural Parks, despite being areas of a high level of protection, are still affected by environmental pollution caused by humans. The levels of PFASs varied among colonies, and this is relevant because it proves the use of gull eggs as bioindicator of the contamination level of a specific area. It also indicates that the sampling protocol used in this study is representative and that this procedure can be used over the years to determine trends and compare the contamination patterns among colonies. The concentration of PFASs are indicated in Table 5.1, and all of them are long-chain PFCA with high bioaccumulative potential and coincides with previous findings [9].

PFOS was the main compound detected in both YLG and AG eggs and reflects its high bioaccumulation potential through uptake from diet and yearly transfer to eggs [15]. During the years 2009-2018, the mean concentration of PFOS in YLG was 72.8 ± 20.9

ng/g ww in the Ebro Delta, 68.6 ± 12.3 ng/g ww in Medes Island, 16.6 ± 6.08 ng/g ww in Chafarinas Island, and 13.0 ± 3.39 ng/g ww in Atlantic Island, whereas in AG from the Ebro Delta contained 87.0 ± 12.1 ng/g ww. PFUnA and PFTriDA were also ubiquitous in all colonies and its presence indicates their more recent use and high bioaccumulation potential. Other compounds were colony specific and had much lower concentrations (Table 5.1).

PCA revealed differences on PFASs patterns among colonies. Figure 5.1 shows that the 6 detected PFASs co-occurred in gulls, with PFOS, PFDoA, PFDA and PFNA contributing in the PC1 axis, while PFUnA and PFTriDA had a specific contribution towards the PC2 axis. The fact that Medes and Ebro samples are distributed in the right PC1 axis indicates those gull eggs with the highest concentration of all PFASs but also with a high dispersion in the multivariate space. Chafarinas placed in the bottom PC2 groups those samples dominated by PFOS but with small PFUnA and PFTriDA contribution. Finally, eggs from the Atlantic Islands are concentrated in the top PC2 axis and show a very small dispersion, with no significant differences for any compound, indicating that all samples have a very similar PFASs composition. PCA shows that PFASs in YLG eggs from Medes and Ebro delta have a higher variability compared to gull eggs from Chafarinas and Atlantic Islands. This fact is corroborated by the standard deviation of each compound in each colony during the 10 year period (Table 5.1). We observe that higher variability in concentrations in the different colonies reflects changing sources of pollution or a more variable diet.

Regarding the sources of pollution, Spain is an area affected by diffuse PFASs pollution where discharges from rivers, wastewater treatment plants effluents, and harbors affect the coastal areas of the Mediterranean [22] and the Cantabric coast [23]. The higher levels

found in Medes and Ebro colony compared to Chafarinas and Atlantic Islands can be attributed to the more confined and contaminated Mediterranean basin compared to the lower urbanization in NW Spain and the high dilution capacity of the Atlantic Ocean, as described in a previous study [18]. In fact, several studies indicate that the occurrence of PFASs is explained by proximity to industrial and urban areas [24,25]. This may be the reason for the widespread presence of PFOS in gull eggs from different locations. Levels in gull eggs ranging from 24.2 to 170 ± 11 ng/g ww have been reported in Germany [26], from 91 ± 13 to 507 ± 47 ng/g ww in the Great Lakes (USA) [24], and from 361 to 921 ± 431 ng/g ww across the USA. Lower levels are reported in remote areas, with PFOS at 20.0 ± 1.1 ng/g ww in eggs of glaucous gull (*Larus hyperboreus*) from Nunavut [27], and from 55.8 ± 24 to 72.6 ± 31 ng/g ww in eggs of Ivory gulls (*Pagophila eburnea*) from Norwegian and Russian Arctic [28]. High relative abundance of PFOA, PFNA, and PFDA in samples from these remote areas is attributed to atmospheric transport of precursor fluorotelomer alcohols [25].

Also, the Medes Islands was the only colony where PFOA was detected over the years although at low concentrations (Table 5.1). PFOA is used in the production of fluoropolymers [29] and fire-fighting foams [30], but can also be formed by the degradation of fluorotelomer alcohols [31]. PFOA may originate from the use of fire-fighting foam to extinguish several fires that burned more than 1,770 ha in Empordà since 2001, close to Medes Islands. PFOA was reported in gull eggs from Rost (Norway) associated with the cod diet [32]. A similar finding was observed in herring gull eggs from the North Sea [26]. In Canada, elevated concentration and proportion of PFOA was related to the relatively elevated concentration of PFOA in fish [25]. In the Great Lakes

of North America high amounts of PFOA in gull eggs were attributed to PFOA precursors [24].

Regarding the diet as the source of PFASs in gulls, trophic resource availability and feeding habits (reliance on marine or terrestrial food sources, refuse tips or availability of garbage) of gulls play an important role in the differences among colonies and species and the variability observed for PFASs. Stable isotopes analysis indicated that most marine colonies are exposed to PFASs via marine prey [24]. For the Medes, marine prey and garbage was around 40 %, whereas terrestrial invertebrates represented almost 20 % of ingested food, while in the Ebro Delta colony, half of the food was from marine sources, almost a third was freshwater invertebrates, and 15 % from refuse tips [12]. In Chafarinas, YLG exploits epipelagic and benthic prey collected from trawler or purse site discards, and to a lesser extend refuse tips [33] and terrestrial prey account for <5 % of the food. In the Atlantic Islands, the diet was based generally in perciforms, and in a minor proportion with crabs, terrestrial invertebrates, and refuse tips (12-38 %) [34]. Patently, gulls are not unwise and when fish is available, they exploit this resource opposite to garbage. According to feeding habits, the colony with the highest dependence on garbage is Medes followed by Ebro, both showing a relatively high level of dispersion in PFASs concentrations. Contrarily, gulls that exploit fish show less PFASs variability although accumulate PFOS, PFDA, PFUnA, and PFTriDA despite PFASs levels in fish are relatively low [35,36]. This fish-based diet of AG may explain the significantly higher levels of PFOS, PFDA, PFUnA, and PFTriDA compared to YLG from Ebro Delta (Table 5.4). These differences were also observed for dioxins, furans, and planar PCBs [37] and also for indicator PCB congeners and organochlorine pesticides [38].

5.4.2. Temporal trends

We provide evidence of decreasing trends of ∑PFASs in gull eggs from all colonies over the period 2009-2018 (Table 5.3), attributed to a preliminary environmental response on the PFASs phase-out in 2002 by 3M and the restrictions of the Stockholm Convention in 2009 [6] which may have urged the manufacturing of other compounds [40]. Individual PFASs and ∑PFASs temporal trends are shown in Figure 5.2 and show colony specific trends. In several cases, a curvilinear relationship was observed (i.e., PFNA, PFOS and ∑PFASs from Medes and PFDoA and PFTriDA in AG from Ebro Delta). Table 5.3 indicates those PFASs that show a linear decreasing trend along time. In the Medes Islands, a significant decreasing trend was observed only for PFTriDA, while the other PFASs showed no trend although a peak in concentration was observed in the period 2015-2016. In YLG from Ebro Delta a decreasing trend was observed for PFOS, PFUnA, PFTriDA, and ∑PFASs, with the highest PFOS levels in the period 2009-2012 and halved thereafter, whereas PFUnA and PFTriDA had a peak in concentration in the period 2011-2013 and followed by a general decrease towards the end of the study period in 2018.

In Chafarinas Islands, PFOS followed a decreasing trend as well ∑PFASs due to the PFOS influence, while there were no differences along the years for the rest of the compounds, but rather showing a low-level but constant concentration. In the Atlantic Islands no temporal variation was observed for any individual PFASs, although ∑PFASs showed a decreasing trend. In AG gull eggs, a significant decreasing trend was observed only for PFUnA and for ∑PFASs, while for PFOS a slight decrease in concentration over the years was observed, but it was not significant (Table 5.4).

Our results provide evidence that during the period 2009-2018, most PFASs remain stable in gull eggs from the different colonies, except for PFUnA and PFTriA, and in some colonies PFOS that show decreasing trends. Time-variations of PFASs in eggs can be attributed to changes in the emissions rates of PFASs and their precursors, management actions to minimize discharges, direct urban impacts, changes in the feeding habits, and the availability of the food resources that birds exploit [25,39]. However, the degree of reduction change among colonies, depending on the time PFASs were phased out or control measures were implemented.

During the period 2009-2014, gulls from the Great Lakes showed a PFOS decreased except in urbanized areas while PFCA increased in coastal areas and remained constant in the inland areas [25]. In Northern Norway, PFOS levels remained stable from 1993 to 2003, while PFCA levels increased [41], and in the same colony in 2012, PFOS levels were low and PFCA had increased [42].

Our study period is 2009-2018, where PFOS and related compounds had already been phased out for 10 years, and thus the reduction in the levels observed in gulls is slow. In contrast, the levels observed in several species during the period PFASs were in use draw attention for the increasing trends reported, such as in white-tailed sea eagle *(Haliaeetus albicilla)* from marine colonies in Sweden from the 1960s to 2010 [43]; in common murre (*Uria aalge*) from the Baltic Sea during 1968 to 2003 [44]; in herring gull (*Larus argentatus*) from Northern Norway during 1983 to 1993 [41]; in peregrine falcon (*Falco peregrinus*) from the southwest Swedish coast during 1974 to 2007 [45]in herring gull (*Larus argentatus*) from the Baltic Sea during 1988 to 2008 [46]. However, differences related with the species also occur, as described in birds eggs from the Canadian Pacific coast during the period 1990 to 2010, where PFOS decreased in double-crested cormorant

(*Phalacrocorax auritus*) and rhinoceros auklet (*Cerorhinca monocerata*), increased in Leach's storm petrel (*Oceanodroma leucorhoa*), and remain constant in great blue heron (*Ardea herodias*), while PFCA increased only in offshore species [47]. Moreover, in Prince Leopold Island in Nunavut, no-trend was found in PFOS in eggs of northern fulmar *(Fulmarus glacialis)* and thick-billed murre *(Uria lomvia)* from 1975 to 2011, while PFCA had increasing concentration over these years [27]. In UK gannets (*Morus bassanus*) the ∑PFSAs first rose and then fell and ∑PFCAs remained unchanged over the period from 1977 to 2014, although long-chain odd PFCAs concentrations in eggs still increase [48]. All these studies reflect that gulls are still exposed to compound massively produced and discharged to the environment in the past decades and that temporal trends are useful to determine their evolution so that actions to mitigate pollution episodes can be implemented.

5.5. CONCLUSIONS

This study used gull eggs for the long-term biomonitoring of PFASs in Natural and National Parks in Spain to reflect the pollution of a given habitat and identify the geographical differences and time trends. PFASs were detected in all colonies, despite being areas with a high degree of protection. Among 17 PFASs analyzed, only 6 compounds were detected, all of them long-chained PFASs, and PFOS accounted for the main contaminant in the four colonies studied. Differences among colonies depended on location (Mediterranean/Atlantic) and proximity to human settlements and industrial activities, whereas trophic resources availability and diet habits defined geographical and species-related variability. Time trends suggested a significant decreasing concentration of ∑PFASs, exemplified by PFOS, PFUnA, and PFTriDA, after approximately a decade

of the phase-out of PFOS. Longer time-trend biomonitoring studies are needed to confirm the evolution of the presence of PFASs in the environment.

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5.7. ANNEX

Annex 5.A1. Statistical analysis including YLG eggs from Chafarinas (all four colonies) but excluding years 2011 to 2014 in all colonies.

In the YLG eggs we found significant differences in the concentration of PFAS between the four colonies, years and colonies over years (RM MANOVA colony: Wilks' λ = 0.000, $F_{18,6.142}$ = 19.070, p = 0.001, η_p^2 = 0.978; year: Wilks' λ = 0.043, $F_{30,122}$ = 4.869, p < 0.001, η_p^2= 0.467; year*colony: Wilks' λ = 0.003, $F_{90,175.321}$ = 3.405, p < 0.001, η_p^2= 0.612). The univariate test confirmed these differences (Table 5.A3). YLG eggs from Chafarinas and Atlantic Islands did not show significant differences in the concentrations of PFNA, PFOS, PFDA, PFDoA, and ΣPFAS, but eggs from Chafarinas carried lower concentrations of PFUnA and PFTriDA than eggs from Atlantic Islands. On the other hand, YLG eggs from Ebro Delta and Medes did not show significant differences in the concentrations of all detected PFAS and ΣPFAS but showed greater concentration for most compounds than Chafarinas and Atlantic Islands eggs. Although PFOS and ΣPFAS were lower in Chafrinas eggs than in Ebro Delta and Medes, the differences were not statistically significant (post hoc test p > 0.05).

We found a decreasing linear trend over time in all but one PFAS and ΣPFAS. Only PFTriDa showed a quadratic relationship, with a reduction in the period 2009-2016 and an increase in 2017-2018.

The variation in the concentration of most detected PFAS varied in a different way between colonies over the years, but for the ΣPFAS the year variation was similar in all colonies (Table 5.A3).

Table 5.A1. Location, breeding species in the colony, diet, distance from human settlements, human impact of the studied colonies.

Colony and coordinates	Species	Diet	Distance to settlements (Km)	Human impact	References
Medes Islands 3º13'E 42º02'N	*Larus michahellis*	More than 50% on refuse tips and some fisheries discards	0.9	Industry and tourism	[12,49]
Ebro Delta	*Larus michahellis*	Mostly marine food and less 10% of refuse tips	7.5	Agriculture and Chlor-alkaline plant	[12,49]
0º40'E 40º35'N	*Larus audouinii*	Pelagic fish			[13]
Chafarinas Islands 2º25'W 35º10'N	*Larus michahellis*	Mostly epipelagic, benthic and mesopelagic prey mainly form fisheries and refuse tips	3.5	Agriculture	[33]
Atlantic Islands 8º54'W 42º13'N	*Larus michahellis*	Fishery discards, garbage, and urban landfills	2.5	Boating industry and Chlor-alkali plant	[50,51]

Table 5.A2. Analyzed compounds ordered by elution time, parent ion, MRM trasition, collosion energy and cone voltage.

PFAS	Retention time (min)	Parent ion	Transition (m/z)	Collision energy	Cone voltage
PFBA	1.16	213	169	10	20
PFBS	1.66	263	219	10	25
PFPeA	1.89	299	99>80	23>30	45
PFHxA	2.66	313	269>119	10>24	16
PFHxS	4.77	363	319>169	10>14	20
PFHpA	5.57	399	99>80	32>31	55
PFOA	7.37	413	369>169	11>13	18
m-PFOA	7.37	417	372>172	11>12	21
PFNA	8.51	463	419>169	11>23	25
PFOS	8.67	499	99>80	35>35	65
m-PFOS	8.67	503	80>99	36>36	70
PFDA	9.24	513	469>169	9>11	14
PFDS	9.79	563	519>269	10>12	29
PFUnA	9.87	599	99>80	41>40	80
PFDoA	10.26	613	569>319	15>16	20
PFTriDA	10.6	663	619>169	17>24	31
PFTeDA	10.97	713	669>319	10>18	29
PFHxDA	11.46	813	769>169	10>45	35
PFODA	11.82	913	869>169	12>48	45

Table 5.A3. Method detection limits (MDL) and recoveries in percentage (n=3) for all PFAS detected.

Compounds	MDL	Recovery n=3 (%)
PFBA	0.14	90±9.7
PFBS	0.17	107±8.7
PFPeA	0.16	106±8.2
PFHxA	0.05	101±9.6
PFHxS	0.13	105±15
PFHpA	0.13	100±10
PFOA	0.07	98±14
PFOS	0.50	109±11
PFNA	0.23	94±14
PFDA	0.12	116±16
PFDS	0.21	91±16
PFUnA	0.18	98±11
PFDoA	0.20	104±1.8
PFTriDA	0.12	105±6.1
PFTeDA	0.26	102±10
PFHxDA	1.11	80±26
PFODA	0.55	71±12

Table 5.A4. Results of RM MANOVA on log-transformed concentrations of the PFAS found in eggs of yellow-legged gull from the colonies of Chafarinas, Medes, Ebro Delta, and Atlantic Islands (excluding years 2011 to 2014).

	WITHIN SPECIES EFFECTS					WITHIN CONTRAST				
Factor	Compound	df	F	p	η_p^2	relationship	df	F	p	η_p^2
YEAR	PFNA	3.90, 27.3	8.89	**<0.001**	0.560	linear	1, 7	26.8	**0.001**	0.793
	PFOS	5, 35	2.66	**0.039**	0.275	linear	1, 7	11.1	**0.013**	0.612
	PFDA	4.52, 31.7	3.47	**0.015**	0.332	linear	1, 7	10.2	**0.015**	0.593
	PFUnA	3.36, 23.5	19.8	**<0.001**	0.739	linear	1, 7	71.9	**<0.001**	0.911
	PFDoA	2.49, 17.4	3.69	**0.038**	0.346	linear	1, 7	108.4	**<0.001**	0.939
	PFTriDA	5, 35	9.71	**<0.001**	0.581	quadratic	1, 7	30.8	**0.001**	0.815
	ΣPFAS	5, 35	11.0	**<0.001**	0.612	linear	1, 7	71.6	**<0.001**	0.911
YEAR*COLONY	PFNA	11.7, 27.3	3.30	**0.005**	0.586	linear	3, 7	5.78	**0.026**	0.712
	PFOS	15, 35	4.57	**<0.001**	0.662	linear	3, 7	9.40	**0.007**	0.801
	PFDA	13.6, 31.7	4.05	**0.001**	0.634	linear	3, 7	8.62	**0.009**	0.787
	PFUnA	10.1, 23.4	7.73	**<0.001**	0.768	linear	3, 7	26.9	**<0.001**	0.920
	PFDoA	7.46, 17.4	1.05	0.437	0.310	linear	3, 7	18.7	**0.001**	0.889
	PFTriDA	15, 35	6.35	**<0.001**	0.731	linear	3, 7	16.4	**0.002**	0.875
	ΣPFAS	15, 35	2.64	**0.009**	0.531	quadratic	3, 7	9.12	**0.008**	0.796
	BETWEEN SPECIES EFFECT									
COLONY	PFNA	3, 7	81.4	**<0.001**	0.972					
	PFOS	3, 7	171	**<0.001**	0.987					
	PFDA	3, 7	33.2	**<0.001**	0.934					
	PFUnA	3, 7	24.0	**<0.001**	0.911					
	PFDoA	3, 7	154	**<0.001**	0.985					
	PFTriDA	3, 7	71.9	**<0.001**	0.969					
	ΣPFAS	3, 7	208	**<0.001**	0.989					

CHAPTER 6.

GENERAL DISCUSSION

CHAPTER 6

The results of the different studies performed are discussed in this chapter of the Thesis. Firstly, the PFASs methodologies used in the different environmental matrices are reviewed. Secondly, the impact of PFASs according to the sources of pollution studied in this Thesis is evaluated to better understand the behavior, partitioning, and mobilization of PFAS in the environment. Thirdly, a global evaluation of the fate of PFASs integrating all the matrices studied is addressed considering the actual regulation and legislation.

6.1. CHALLENGES IN THE ANALYSIS OF PFASs IN THE ENVIRONMENT

The analysis of PFASs in the matrices considered in this Thesis (water, soil, sediment, plant, and biota) faces some challenges as the methodologies used need to be adapted for each matrix. We discuss the whole analytical methodology, from sampling to analysis and final quantification, as all these steps become relevant in obtaining representative and reliable results. In this section, the different stages of the sampling and analysis of PFASs in each compartment are reviewed and the most relevant aspects are highlighted. The different impediments faced in the analysis of PFAS are also indicated.

6.1.1. Sample collection

Knowledge of the matrices to be studied and the surrounding pressures are important to design a correct sampling strategy. We adopted a sampling procedure in a flexible way capable to adapt to different environmental conditions and situations. Because external contamination of PFAS is possible due to the material used in sampling (e.g. Teflon), we paid attention to avoid contamination from the sampling equipment during collection, as well as to avoid the adsorption of target compounds to sample containers. In all cases,

samples were collected using pre-cleaned study material, then transferred to a container made of glass previously washed with purified water followed by acetone or polypropylene bag. We avoided materials based on Teflon or Gore-tex, which sometimes are used as liners in glass caps.

6.1.1.1. Xiaoqing River basin (Shandong province, China)

In the monitoring study along the Dongzhulong and Xiaoqing River (Chapter 3) the objective was to evaluate the behaivour of PFAS in the water-sediment-plant system. For that we sampled an area directly affected by the discharge of PFOS manufacturing plant A good sampling strategy was adopted to evaluate the processes of sorption and plant uptake of PFASs. Water samples were collected in polypropylene bottles and were sent almost 2,000 km apart to the laboratory of the Guangzhou Institute of Geochemistry (Guangdong province) via messenger service due to the high volume of samples. The long-distance between sampling location and laboratory facilities can become a problem because there may not always be the means for transport and in our case took two days. The mailing of samples needs to be quick and efficient to avoid degradation of target compounds due to bacterial or algae present in freshwater samples, or to prevent cross-contamination, adsorption, or release of chemicals between the sample and the container.

Seasonal variation is a factor that has to be considered when PFASs are analyzed in water bodies. Xiaoqing River was sampled at the end of August when the Shandong province is at the end of the rainy season often produced by marine monsoons. Numerous rainfalls can increase water flow in rivers and water channels and then the concentration in water may change with the dilution of the contaminants. In China, though, the seasonal variation

of PFASs is also influenced by industrial production that is interrupted twice a year due to the Lunar New Year festival (end of January) and the Golden week, during the Mid-Autumn Festival (beginning of October). In Xiaoqing River there is seasonal and yearly production capacity of the major fluorinated chemical manufactures which have the highest total production capacity in summer, followed by autumn and spring, while winter was the lower [1]. For these reasons, we sampled in August to have the worst-case scenario when the production was the highest and represents the season with the highest emissions. Information of sampling season is important to discuss results obtained. Previous studies observed PFASs seasonal variation in water samples from rivers [2], drain outlets, and seawater from the Bohai Sea where the Xiaoqing discharges its waters [3] and in the Yanghe River [4].

In contrast to water, sediments are more stable and not subjected to differences among seasons. Variation and production fluctuation in sediment are less seasonally sensitive than those in water samples due to the relatively low mobility of PFASs in sediments compared to water bodies and the strong affinity of certain PFASs to organic matter [5]. Sediment samples from Xiaoqing River were collected at the same point for water with a shovel, stored in polypropylene bags, and sent to the GIG laboratory. Similar to water, the long way transportation of the samples to the facility needed to be quick to avoid alteration of the content, and avoid bacteria proliferation and the metabolization of PFAS [6]. In the present study, the aim was to evaluate the presence of PFASs in sediment and to evaluate their transport and distribution along the basin. We used grab sampling that provides information on surface sediments. Another possibility is to use core sampling. The composition profiles in core sediments represent the historical substrate overlying along the time or also vertical advective transport through the substrate to groundwater

levels [7]. Core samples were not evaluated in this Thesis because our interest remained on the distribution in the water-sediment-plant system. Besides the sediment samples for the monitoring study along the river, sediments surrounding the roots were collected and analyzed to evaluate the plant uptake of PFASs, and to calculate the translocation factors.

Unlike other chemicals, little is know on the accumulation of PFASs in plants. In fact, most studies are performed under laboratory conditions. In the Xiaoqing River, we had a clear opportunity to sample the plants growing along the basin. Plant samples were hand-collected along the different sampling points and stored in polypropylene bags and sent to the laboratories of GIG to be analyzed. Not all plant species grow or are accessible at all points. Figure 6.1 shows the limited accessibility of some sampling points along the Xiaoqing River basin.

Figure 6.1. Pictures of four different sampling points along the Dongzhulong and Xiaoqing River.

Four different plant species were selected in this study (Figure 6.2), two with roots and two floatings.

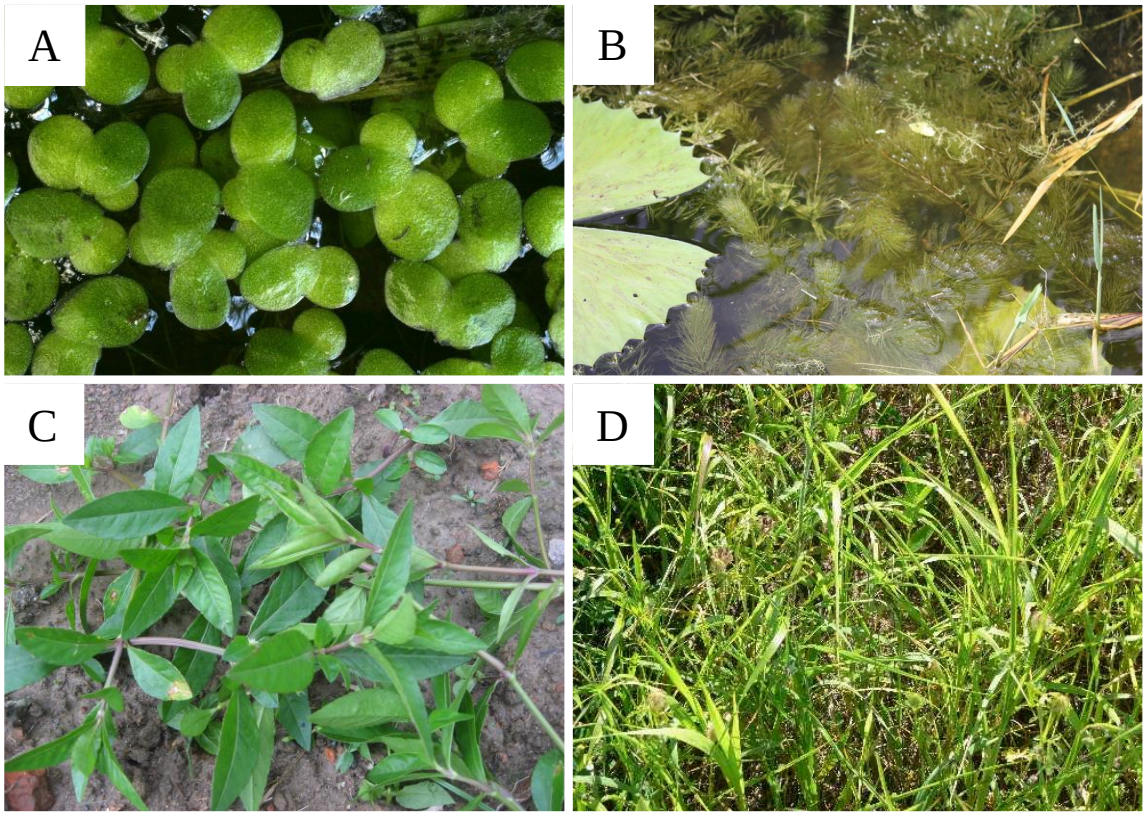

Figure 6.2. Picture of the (A) *Lemna minor* (source: https://eol.org/), (B) *Ceratophyllum demersum* (source: https://eol.org/), (C) *Alternanthera sessilis* (source: https://eol.org/), and *Eriochloa villosa* (http://wisflora.herbarium.wisc.edu/)

Floating species were *Lemna minor* and *Ceratophyllum demersum*. *Lemna minor*, commonly known as duckweed, is a floating freshwater aquatic plant with a maximum of four leaves and a single root hanging in the water, which has a fast growth rate via asexual proliferation that creates green structures on the water surface (fronds). This species was selected in this study due to the large proliferation and presence on the area, their up-take capacity [8,9], and considered a good phytoremediator of PFASs in surface water [10]. *Ceratophyllum demersum*, commonly known as common hornwort, is a submerged macrophyte with the base buried in sandy or silty substrates but does not form

proper roots. The foliar part remains free-floating on the water columns. This species was sampled because was previously used in bioaccumulation studies of PFASs in natural environments [11,12], and it is part of the diet of different herbivorous fish species [13]. Rooted species were *Alternanthera sessilis* and *Eriochloa villosa*. *Alternanthera sessilis,* commonly known as sessile joyweed or dwarf copperleaf, is an aquatic plant and can be observed rooted in marshy areas and wetlands. It is an aerial herb, bearing short petiole, and simple leaves. This plant species was previously used in the phytoremediation of nitrate [14], PCBs [15], and some congeners were used to remove heavy metals [16,17], but no PFASs accumulation studies were previously reported. Regarding rooted species, *Eriochloa villosa*, commonly known as hairy cupgrass, is an abundant rooted plant species that easily grows next to water bodies that in many counties is considered a pest. This species forms a loose tuft of leafy culms that are erect or ascending. The leaf blades are flat or slightly involute and the blade margins have a rough-texture. Each culm terminates in an inflorescence consisting of branches with three to ten clays in the form of spines. Accumulation of PFASs in this species was either reported previously. It was evaluated in an up-take study located at a super-large antimony deposit in China [18]. This species is extended worldwide and could be used to monitor pollution in aquatic environments. The plant species analyzed in this study were perennial or annual (depending on the climate), with a flexible growth habitat and commonly spread around the world. Due to their uptake ability, fast growth, rapid proliferation, and accessibility, these species must be considered as bioindicators of pollution to monitor PFASs in the aquatic environment.

6.1.1.2. National Parks (Spain)

In the Chafarinas Islands, a sampling strategy was adopted to determine the distribution of PFASs in different environmental compartments in an area not directly affected by PFASs pollution. The complexity of sampling in Chafarinas is due to the fact that is a military base with no direct access to scientific personal. So sampling was subcontracted. We did not have permission to sample soil in the island so we sampled agricultural soils in the Moroccan coast. Sediment samples from the northern Moroccan coast (Chapter 4) were collected by scuba divers in different locations along the coast under different anthropogenic pressures. Sediments and soils were collected with a spade and the first 5 cm were disregarded. Samples were placed inside a glass jar. Each sample consisted of 6 grab subsamples in sites 5-10 m apart and was pooled to make a sample in each point. In this way, we ensured the representativeness of the area. Samples were frozen in the Melilla premises and sent frozen to the main laboratory in IDAEA-CSIC in Barcelona.

With the aim to determine the bioaccumulation potential, we studied fish from three species and mussels. The analysis of PFASs in different trophic levels represents potential bioindicators from the marine environment, as well as the bioaccumulation potential of PFASs in the food chain, and a wider vision of their distribution. Mussels, sardines, anchovies, mackerels, and YLG eggs were selected in this Thesis to evaluate the fate of PFASs at different trophic levels. They represent the natural diet of the YLG from Chafarinas Islands and were bought in local markets from Melilla and Nador. Also, mussels have been used previously to monitor PFASs in the environment [19,20]. Whole-body of fish species was used for the analysis of PFASs because gulls feed on the full organism, however, if the aim is to study the bioaccumulation and the body distribution

in fish it is important to consider that PFASs are accumulated preferentially in organs or skin rather than in muscle [21].

Biomagnification was studied using gull eggs from Chafarinas Islands and we observed that the release of PFOS to the eggs was 4.5% of the diet intake. Yellow-legged gull and Audouin's gull from Spanish Natural Parks (Medes Islands, Ebro Delta, Chafarinas Islands, and Atlantic Islands) were proposed in this Thesis to study the accumulation of PFASs in the egg-laying due to the maternal transfer. Yellow-legged gull is a common species in the Iberian Peninsula and nest in colonies mostly along the seaside. Their diet is based on both marine and land resources, other bird species, dumps, and fishery discards. Audouin's gull is an endemic species of the Mediterranean region. This species is mostly piscivorous and also liked to fishery discards. Due to their diet habits and their relatively long life, these gulls were suggested as bioindicators of PFASs pollution in Spain. A bioindicator must be i) easily identifiable, ii) largely distributed along the study area, iii) well-known in terms of biology, physiology, and ecology, iv) abundant and accessible, v) able to reflect local conditions, vi) in a well-defined position in the food chain, and vii) sensible to pollution but not mortal [22]. YLG is omnivorous and its diet consists of fish, invertebrates, and crabs [23], as well as they feed on rubbish tips or robbing chicks of smaller gulls or other seabirds [24]. AG is a fish eater species based on clupeiformas such as sardines and anchovies, and also exploit fisheries discards and crayfish [25]. Eggs represent a non-invasive matrix to monitor PFASs, only the first egg of each nest was sampled since it represents the maximum pollutant transfer from the female to the egg [26]. Figure 6.3 illustrates a YLG nest with complete egg-laying in the Medes Islands.

Figure 6.3. Picture of the YLG nest from Medes Islands.

To evaluate the presence of PFASs in the environment along the time is necessary to get a significant amount of sample to be able to reflect the changes along the time. YLG eggs were collected from 2009 to 2018 in different colonies along the Iberian Peninsula (Medes Islands, Ebro Delta, Chafarinas Island, and Atlantic Islands) to evaluate the patterns of PFASs along time and differences among colonies. AG eggs were sampled in the same years to observe differences among the 2 gull species sharing habitat in the Ebro delta. Colonies are located in protected areas (mostly islands) and special permits are required to perform the sampling. Due to the military restriction accessibility to Chafarinas Islands and administrative protocols, YLG eggs were not collected from 2012 to 2014.

The sampling performed in this Thesis allows to picture the fate of PFASs in different environmental matrices that conform an important representation of the real distribution of these contaminants.

6.1.2. Extraction and analysis

The methodology used in this Thesis differed according to the matrix studied and whether samples were analyzed in the Guangzhou Institute of Geochemistry (GIG) in China or the Institute of Environmental Assessment and Water Research (IDAEA) in Spain. For water, SPE cartridges were used for sample enrichment and clean-up and because it is a high-throughput technique that provides high sensitivity. One of the handicaps we faced was the need to use on some occasions two SPE cartridges because obturation with particles from the surface water was observed. Methanol was used since it effectively eluted target compounds from the SPE cartridge. In GIG (China), the method was based on Heydebreck et al., (2015) [27] that analyzed PFASs in European and Chinese river and estuary systems and recoveries ranged from 49 ± 20% ([13C4]-PFOA) to 98 ± 70% ([13C5]-PFNA). However, it is still difficult to optimize the method for short-chain PFASs because these are more susceptible to matrix effects that cause ionization suppression, resulting in lower analytical sensitivity [28]. This is a concerning topic due to the increased manufacture and use of short-chain PFASs worldwide as an alternative for long-chain PFASs [29]. To determine PFAS in water, the polymer-based SPE cartridges are commonly employed for PFASs analysis, especially Oasis HLB, Oasis WAX, or Strata X [29]. This sorbent permits the analysis of multiple contaminants and utilize less extraction solvent, plastic materials, and decrease the environmental burden. In the water samples of this Thesis, method efficiency was calculated using the recoveries

of the internal standard M8FOSA-I that ranged from 43% to 121%. All calibration graphs over a concentration range from 0.2 ng/mL to 25 ng/mL were linear, and the correlation coefficients were >0.99 for all analytes. These results define the extraction performed as a good method for the analysis of PFASs in freshwater samples.

For the analysis of soil and sediment different extraction methods and clean up were used basically according to the laboratories where the analysis were performed. Sample preparation with freeze-drying, sieve through 120 μm, and homogenizing were used to enhance sample representativity. The differences between the extraction methods and clean-up used in this Thesis were due to the laboratory resources and techniques available. Solvent extraction and SPE cleanup is the most common method used in the bibliography and as well offered better recoveries [29]. Solid-liquid extraction was performed in sediments from the Xiaoqing River with methanol and dichloromethane and sonication. Purification of the sample was performed using ENVI-Carb SPE cartridges. Method efficiency was calculated using the recoveries of the internal standard M8FOSA-I that ranged from 52% to 109%. In sediment and soil samples from the northern Moroccan coast, solid-liquid extraction was performed with acetonitrile and 10 mL of 1% glacial acetic acid solution was used with sonication, followed by a clean-up based on activated carbon and glacial acetic acid Gomez et al., (2011) [30] and the recoveries ranged from 100 ± 26% to 108 ± 18%. Cleanup by activated carbon was frequently used in our research group to eliminate interferences matrix interferences from the sample. A few other studies reported the use of the activated carbon as a cleanup method in biologic samples [21,31], but not in sediments or soils [29].

Extraction of biological matrices faces an additional difficulty that they contain large amounts of lipids and pigments that need to be eliminated to avoid interferences in the

analysis. In this Thesis plants, mussels, fish, and gull eggs were analyzed. Similar to sediments, different extraction methods were used in the analysis of PFASs in the biota samples. Plants samples were extracted in the laboratories of GIG where the methodology was already available in the research group based on Wen et al., (2013) [32]. Shoots and roots were solid-liquid extracted with sodium carbonate buffer, tetrabutylammonium hydroxide, and methyl tert-butyl ether and manual shaking three times. Purification of the extracts was not performed in this method, but samples were passed through a 0.22-µm nylon mesh filter. This method offered good efficiency with internal standard recoveries ranging from 70% to 115%. Similarly, the extraction recoveries obtained by Wen et al., (2013) ranged from 92% to 105% [32]. Biological samples were extracted in the IDAEA-CSIC with a method that was adapted from an already developed method [33], with special modification in mussel and fish [34]. Briefly, the extractions were performed using solid-liquid extraction with vortex and ultrasonic bath and acetonitrile as a solvent, followed by a clean-up with activated carbon and glacial acetic acid to eliminate any lipid or matrix interference from the sample [21]. Due to the complexity of the biological samples, more attention was focused on the efficiency of the extraction and the clean-up methods [29]. In gull eggs, the method detection limits (MDL) ranged from 0.05 (PFHxA) to 1.11 ng (PFHxDA), and reveries from 71 ± 12 (PFODA) to 116 ± 16 (PFDA).

6.1.3. Instrumental analysis

Different chromatographic techniques have been used to analyze PFASs. Liquid chromatography (LC) is the major detection method for ionic PFASs, while gas chromatography is the predominant method for volatile, semi-volatile, and neutral PFASs analysis [35]. LC coupled with mass spectrometer in negative ionization was used to

analyze PFASs in this Thesis. In GIG (China) was used an LC system from Agilent Technologies 1220 Series coupled to an Agilent 6410 Triple Quad/Mass Spectrometer, with a Synergy Hydro RP 80A column for the chromatographic separation. The limits of detection (LOD) and the limits of quantification (LOQ) are the concentration measured by the analytical instrument at a signal-to-noise ratio (S/N) of 3 and 10, respectively. Accordingly, the LOD ranged from 0.1 to 0.2 ng, and the LOQ from 0.2 to 06 ng. In IDAEA (Spain) was used an Acquity UPLC system coupled to a TQD (triple quadrupole) mass spectrometer. An XBridge C_{18} column was used as a residue trap to remove PFASs contribution from the mobile phase or tubing, and the chromatographic separation was performed using an Acquity UPLC BEH C_{18} column. Samples were analyzed with multiple reaction monitoring (MRM) to avoid noise and obtain higher selectivity, as well as different windows were set to increase the sensitivity. The selection of the transitions of each compound was done to have high sensitivity and selectivity and are described in Table 5.A2 of the Annex of Chapter 5. Quantification of PFASs in GIG (China) was performed using Mass Hunter Quantitative Analysis Software, while in the IDAEA-CSIC was used Mass Lynx 4.1 Software. Figure 6.4 shows two chromatograms obtained from surface water and sediment samples for the Xiaoqing River. Characteristically in the water samples, higher peaks are observed at the beginning of the chromatogram when compared to the sediment. These peaks are the short-china compounds, while in the sediment chromatograms long-chain PFASs are better defined.

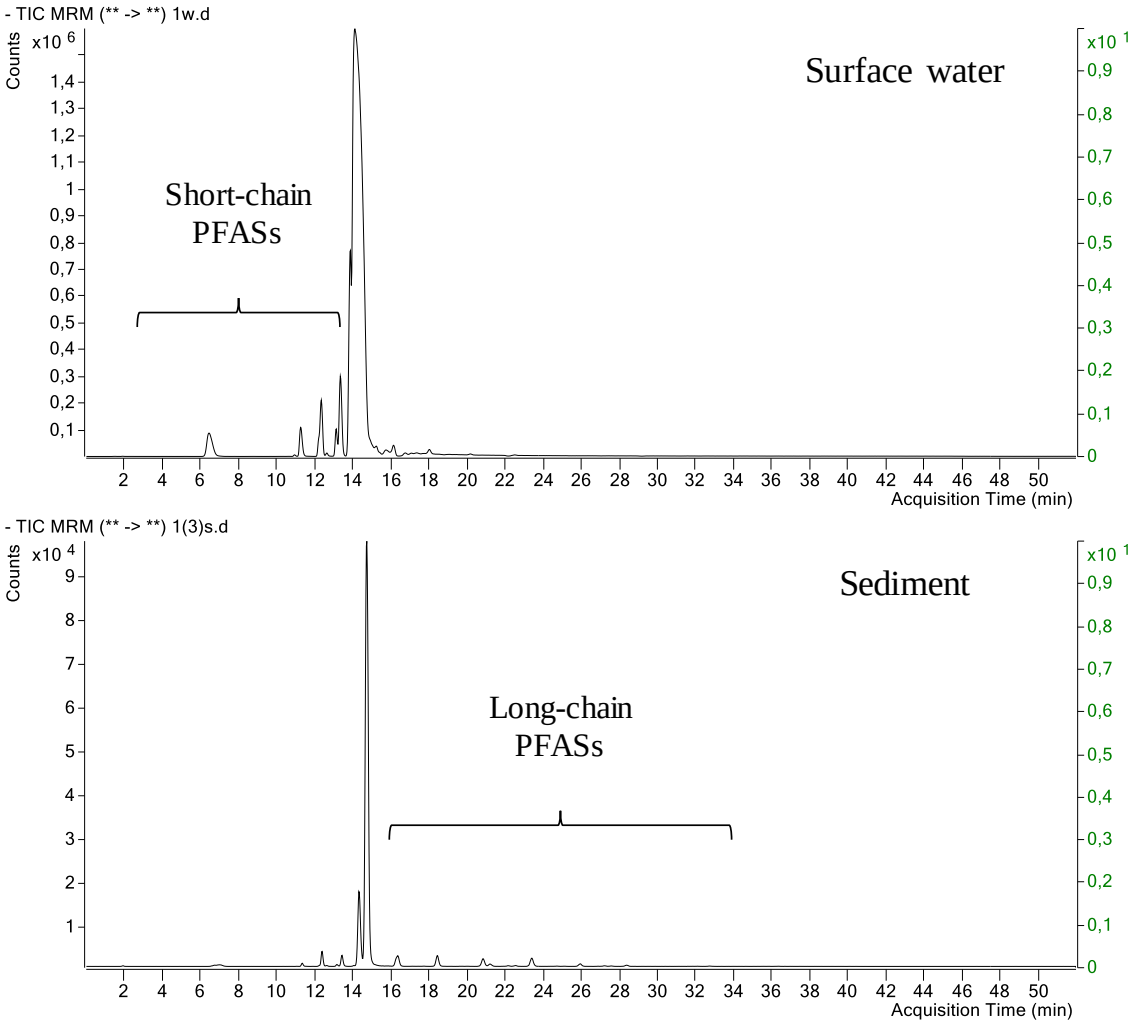

Figure 6.4. Chrogratograms of surface water and sediment of sampling point 1 of the Xiaoring River.

6.2. SOURCES OF PFASs POLLUTION

In this Thesis, we studied a very impacted site with direct discharge of a PFOA manufacturing plant and areas with no direct sources as Natural Parks. Prevedouros et al., (2006) [36] describe direct sources as the result of the production and use of PFASs and include fluoropolymer manufacture and processing that is known as the single largest source, fluoropolymer dispersion, aqueous fire-fighting foams, and consumer and

industrial products. Indirect sources in the environment are those where PFASs are present due to non-point pollution area PFASs precursors degrade to form new PFASs [36]. Chapter 3 of this Thesis is a clear example of a direct source of PFASs due to the presence of a fluoropolymer manufacture facility of the Dongye group (Figure 6.5

). This plant manufactures polytetrafluoroethylene, perfluorinated ethylene-propylene copolymers, polyvinylidene fluoride, and PFOA since 2003, and has a large complex of research and development facilities where fluorinated surfactants are synthesized and emitted to the environment [37].

Figure 6.5. Picture of the wastewater discharge point with a fluoropolymer manufacturing facility of the Dongye group on the back.

This facility is the major in China and uses PFOA as a processing aid during production with certain monomers as raw materials [38] and was estimated to be approximately 10 – 30% of the ongoing global PFOA emissions, with an approximate production of 37,000 tonnes per year (t/y) of PTFE, 500 t/yr FEP, 300 t/yr PVDF, and 40 t/yr of APFO. The authors suggested a theoretical emission calculation from the fluoropolymer production

of 68 t/yr, with a riverine discharge of PFOA from 23 to 67 t/yr in Xiaoqing River in 2014 [39]. In agreement with these findings, emissions of PFASs to the Xiaoqing River were estimated to be 58.0 t/yr of PFOA, 2.04 t/yr of PFBA, 1.53 t/yr of PFHxA, 1.20 t/yr of PFPeA, and 0.73 t/yr of PFHpA per year in 2013 [38]. Furthermore, the emissions previously reported were calculated from the PFASs levels detected in the water. These PFASs levels are in concordance with the results exposed in this Thesis, suggesting similar emissions to the Xiaoqing River when our study was performed. The consequence of these emissions are the contamination of water, sediments, and plants all along the rivers. PFSAs sources were different in the Xiaoqing River due to they were not detected with the same dilution along the river. Previous studies suggest that the origin of PFSAs in Xiaoqing River is attributed to urban activities, street runoff, and water discharges due to the production and use of consumer products of the surrounding human settlements [39]. Despite the river water is not used for municipal drinking water, villagers from this area are exposed to PFASs through the consumption of private well water, irrigation of crops, and fishing activities [37].

In Europe also have been reported different examples of direct sources. In southern France, the Rhône River receives wastewater from two fluoropolymer facilities since the 1960s and 2002 [40]. In the first facility, PFOA was used as a processing aid to synthesize PTFE and in the 80s PFNA was used for PVDF, while the second facility produces fluoropolymers with PFOA as a processing aid, and after was replaced by PFHxA. Bach et al., 2017 [40] estimated that 4,295 kg of PFHxA, 965 kg of PFNA, 307 kg of PFUnDA, and 14 kg of PFOA were discharged into the river by the two facilities in 2013. Also, the composition of the PFASs profile fluctuated significantly along the time, reflecting changes in the production in one of the facilities studied, where PFNA was the

predominant compound followed by PFUnA, and some months after they were not detected. In the other facility, the PFASs profile was dominated by PFHxAand was attributed to the presence of odd long-chain PFCAs to be impurities in the PFNA-based products [40]. The river or location of the facility is not mentioned but probably can be the Rhône River that discharges its water in the northwest of the Mediterranean Sea. Since 1971, one of the largest fluorochemical facility of the 3M company in Europe is located in Antwerp (Belgium) near the Scheldt River [41]. In this area, high levels of PFASs were detected in soils [42], in estuary invertebrates [43], in birds [44,45], and human serum from workers of the facility [46]. In the Netherlands, PFOA was studied in leaves and grass of different plant species near a fluoropolymer manufacturing facility in Dordrecht, and was concluded that the presence of PFOA in/on grass and leaves may imply that these chemicals might be present on the locally grown food in gardens around the factory [47].

Historic use of Aqueous Fire-Fighting Foams (AFFF) is another source of PFASs pollution because are used by the military at aircraft bases, oil and gas production, refining industries, and airports worldwide [36]. A study at the Norwegian firefighting training facility revealed a high concentration of PFAS where PFOS accounted for 96% of the total PFASs in sediment and 71% in groundwater [48]. PFASs were studied in ten United States Air Force fire-training areas and was concluded that the use of AFFF represents a significant source of PFASs at the sites evaluated [49]. In France, run-off water and wastewater from a firefighter training contained and the PFASs contamination and can pose a risk for the environment and humans [50].

Industrial activities and consumer products are other concerning sources of PFASs pollution due to the use of these chemicals as additives in a huge amount of applications [51]. In Zhejiang Province (China), relatively high levels of PFASs were detected in

surface water of the textile, leather, and paper-making industry characterized by the dominance of PFOA, followed by PFHxA and PFBA [52]. PFASs are present in many consumer products as household products (stain- ad water-proofing, non-stick, and cleaning products) of daily use so urban conglomerations can be considered a source of pollution. Total emissions of PFAS from domestic sources in the eastern coastal region of China was estimated at 381 kg in 2010, where the big cities were considered the predominant sources [53]. The levels detected in the gull colonies along the Iberian Peninsula could be attributed to this type of source due to the historical industries located along the Spanish rivers that discharge in the Mediterranean and Atlantic coast. As an example, PFOS was detected in Anoia River at a concentration of 2.71 µg/L and was attributed to the tannery and textile industry located in the area [54]. This river joins the Llobregat River before discharges its water into the Mediterranean Sea. The Ebro River basin was previously studied and levels up to 251 ng/L of PFBA were detected far from industrial activities but surrounded by ski resorts where the high concentrations were attributed to waxes applied on the skis. Downstream, other sources for Ebro River were discharged from the industrial area in the Basque Country and cities like Zaragoza and Lleida [55]. Close to the Ebro Delta, chemical industries and a nuclear power plant are located [56] and become a source of pollution to the area.

WWTPs become another major source of PFASs to surface water due to the discharge of wastewater from industrial and urban origin [57]. PFASs concentration in influents and effluents depends on the wastewater type of upstream sources and the efficiency of the treatment [58]. Mass balance of PFASs in WWTPs was previously evaluated in influent, effluent water, and sludge. On one side, short-chain PFCAs presented a high concentration in the effluent compared to the influent, and it was attributed to the

degradation of fluorotelomers to short-chain PFCAs. On the other side, long-chain PFCAs and PFSAs had a low concentration in the effluent compared to influents but were accumulated in the sludge because are less soluble and have a higher affinity to the solid phase [59]. PFASs levels in WWTPs effluents from the Catalan coast ranged from 3.37 to 132 ng/L and in the river from 2.24 to 21.9 ng/L with dominance in both PFOS and PFOA. Lower levels of PFASs were detected in ports and coastal waters due to the dilution of the sea but levels vary with the proximity of rivers outflows or submarine emissaries [60]. In our study in the Chafarinas Islands (Chapter 4), it was observed that the higher concentrations of PFOS in sediments were found in port sediments in front of a WWTP outfall, suggesting that wastewater was the major source of PFASs in the area. Sludge seems to be the source of soil pollution in soils [61] when it is applied to agricultural lands.

6.3. REGULATION AND CHANGES IN THE PRODUCTION OF PFASs

After the phase-out of PFOS in 2001 by 3M company, the voluntary agreement of EPA with DuPont and eight other major companies, and the implementation of the Stockholm Convention in 2009, the industry of PFASs turned into the production of other PFASs as PFOA and long-chain PFCAs. Ten years later, in 2019, PFOA was included in Annex A of the Stockholm Convention for elimination propose. One of the main issues in the regulation is the absence of international agreement. Countries as Canada restrict the use and production of PFOA-base products, while countries in the EU, USA, and China still import and produce fluoropolymer and fluorotelomer base products in which PFOA is used as a processing aid [38]. Changes in the regulations produce geographical shifts in the production and use of PFASs from European and North American countries to

emerging economies as China where today the major manufacturers are located [39]. Long-chain PFASs are persistent and bioaccumulative [62] and they were proposed to be listed by the Persistent Organic Pollutants Review Committee [63]. Consequently, manufactures have begun producing short-chain PFASs [64], and already in some locations in China higher levels of PFBS and PFBA were detected in comparison to PFOA levels [65]. The technical performance of short-chain PFASs is lower than long-chain PFASs, so much larger quantities are required to substitute long-chain PFASs [66]. Consequences are already predicted because the fluorinated parts of the short-chain PFAss are recalcitrant and can form persistent dead-end transformation products (PFCAs and PFSAs) [67]. These changes in production are reflected in the temporal trends oobseerced in many environmental matrices.

6.4. PFASs DISTRIBUTION IN THE ENVIRONMENT

6.4.1. PFASs in water and sediment system

The concentration of short-chain PFASs in water is generally higher than for long-chain, but this highly depends on the location of the different sources of PFASs pollution along the waterbody. In the Xiaoqing River (Chapter 3), PFOA was the dominant compound detected in water samples (>93%) and short-chain PFCAs> PFOS > long-chain PFCAs were found at minor concentrations. This PFOA dominancy profile is the result of the discharge of wastewater from the fluoropolymer facility located in the area. Due to the efficient transport of short-chain PFCAs in water, these substances will become globally distributed contaminants if the emissions continue.

Electrostatic and, especially, hydrophobic interactions are the key processes of PFASs sorption in sediments, related to PFASs molecular structure and physicochemical

properties [68]. So, once in the environment, long-chain PFASs are accumulated in the sediment of lakes and rivers due to their more hydrophobic character [58]. This was observed in Chapter 3, wherein in terms of percentage long-chain PFASs presented higher values in sediment than in water, and the opposite for short-chain PFASs. So, the relative abundance of long-chain PFASs contributes more to the total PFASs in sediments than in water [39]. Table 6.1 shows the log K_d values for PFASs in comparison with the literature.

Table 6.1. Mean (max-min) log K_d values for PFASs from the Xiaoqing River in comparison with values reported in the literature.

	Xiaoqing River (This Thesis) n=33	Juncar River [69]	Albufera Natural Park [70]	Llobregat River [54]	Gironde estuary* [71] n=12	French rivers* [72] n=11
PFBA	0.35 (0.04 -0.79)	2.40 (0.70-3.23, n=9)		2.33		
PFPeA	0.52 (-0.003-1.09)	3.76 (2.44-4.82, n=6)	1.11	2.40		
PFHxA	0.53 (0.13-1.09)		1.18			
PFHpA	0.43 (-0.08-1.09)	1.97 (n=1)	1.26		2.0 ± 0.4	2.2 ± 0.5
PFOA	1.19 (0.94-1.59)	3.36 (1.71-4.56, n=5)	1.55	2.00	2.4 ± 0.2	1.9 ± 0.6
PFNA	1.06 (0.74-1.49)		2.14		2.9 ± 0.2	2.5 ± 0.5
PFDA	1.98 (1.68-2.39)		2.34	2.51	3.4 ± 0.2	2.6 ± 0.6
PFUnA	2.94 (2.52-3.49)				3.8 ± 0.3	3.0 ± 0.6
PFDoA	3.46 (2.95-3.99)					2.7 ± 0.6
PFTriDA						4.1
PFTrDA		5.14 (n=1)				
PFBS			1.47	2.88		
PFHxS	1.68 (1.00-2.43)	1.68 (n=1)			1.9 ± 0.3	1.8 ± 0.6
PFOS	1.88 (0.83-2.27)	2.45 (1.07-3.70, n=4)	2.15	1.30	3.4 ± 0.2	2.3 ± 0.8
PFDS			3.17			

*K_d values in mean and standard deviation

K_d partition coefficient allows the understanding of the preferential fate of PFASs in water and sediments and supports the previous discussion, showing that K_d values increase with the increase of the chain-length. These K_d values are not set as constant, either the

distribution of PFASs in the environment that varies with the properties of the soils and sediments because of the hydrophobic interaction increase with the increase of the organic matter [73,74]. K_d values were calculated in this Thesis (Chapter 3) in all sampling points along the Xiaoqing River. Levels found in this Thesis are lower than those reported in the literature [69–72] where all PFASs seem to have a preferential partitioning to the sediment. Similar to Pico eet al., (2012) [70], our results show an increase of the K_d value with an increase of the perfluorinated chain length that suggests increasing adsorption in sediments, while short-chain PFASs remain preferentially in the water. When comparing sulfonates and carboxylates with the same perfluorinated chain length (i.e. PFOS and PFNA), sulfonates show higher K_d values suggesting a preferential partitioning to sediment. Similar findings were observed in coastal sediments from the Shandong Peninsula, where $LogK_d$ values were highest in long-chain PFASs compared to short-chain PFASs [75]. PFOS K_d value was 8.0 and 9.7 times greater than the PFOA and PFHxS K_d value in coastal estuary sediments [76].

6.4.2. Uptake of PFASs in plants

Plants that are grown in contaminated areas absorb PFASs from soils [77]. The uptake of PFASs is a complex process where different factors are involved. The physicochemical properties of PFASs (chain length and functional group) play an important role in the plant uptake and their distribution in the different plant tissues. Figure 6.6 describes the different accumulation factors studied in this Thesis.

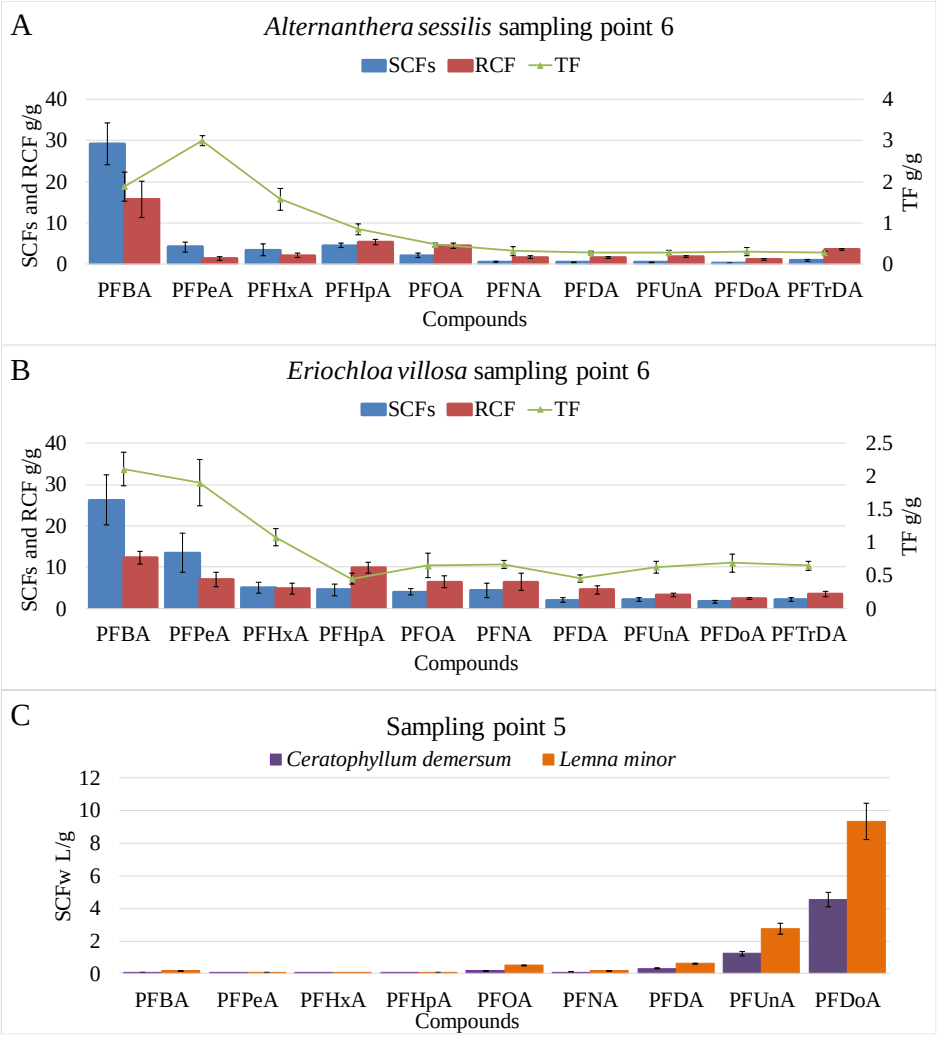

Figure 6.6. Shoot concentration factor of rooted species (SCFs) and root concentration factor (RCF) (left axis); transfer factor (TF) (right axis); and standard deviation (±SD), respectively; for all PFASs in *Alternanthera sessilis* (A) and *Eriochloa villosa* (B) in sampling point 6 from Xiaoqing River. (C) Shoot concentration factor of floating species (SCFw), and standard deviation (±SD) for all PFAs in *Lemna minor*, and *Ceratophyllum demersum* along the sampling points in Xiaoqing River.

In the plant uptake study (Chapter 3) when referring to plants rooted in the sediment, short-chain PFASs are easily uptaken and translocated to the shoot compartment due to their small molecular size and high water solubility that facilitate their penetration through the different structures of the plant roots to the vascular cylinder and the

transpiration stream. Contrarily, long-chain PFASs are mostly accumulated in the roots and are not translocated to shoots due to their large molecular size, their inability to cross the Casparian strip [78], and the sediment competition. Regarding floating species, the higher levels of PFASs in these species are attributed to the direct exposure to the water and long-chain PFASs are the main compound in floating species compared to rooted species due to no competition with the sediment. Rooted species (A and B) show that SCFs is higher than RCF for PFBA, PFPeA and PFHxA, whereas for PFHpA to PFTriDA the RCF is higher than the SCFs, suggesting a preferential accumulation in the root compartment of long-chain PFASs. TF shows a decrease with the increase of the perfluorinated carbon chain due to the capacity of plants to uptake short-chain PFASs and the retention of long-chain PFASs in the sediment. Contrarily, in floating species the SCFw increases with the increasing of the perfluorinated chain length due to their direct exposition from the water. Plant physiological characteristics and species-related differences are suggested as another important factor in the plant uptake, especially the transpiration coefficient that creates a hydrostatic pressure through the intercellular space [79,80] and the protein content [32,81]. Young roots usually contain no apoplastic barriers (Casparian strip, suberin lamellae) between the epidermis and the endodermis so no barrier intercepts the PFASs uptake. Thick taproots could enable large molecules to pass the root epidermis and be retained in the apoplast, while fine roots with a larger root surface enable selective transfer in favor of short-chain PFASs [82]. In this process, the surrounding environment could reduce the PFASs bioavailability to plant roots due to the high affinity of PFASs to sediment organic matter [83]. Concluding, the accumulation of PFASs in plants became a concerning problem due to their role as primary producers in

the trophic chain, a door to the PFASs accumulation into wildlife through primary consumers [84].

6.4.3. Accumulaiton in biota

Bioaccumulation of PFASs has been revealed in different animals in field-based studies. Generally, PFOS is the predominant PFASs detected in wildlife, with also an important contribution of long-chain PFCAs [85]. PFOS was the only PFASs detected in mussels, anchovies, sardines, and mackerels (Chapter 4). Long-chain PFCAs were not detected and could be attributed to the low concentration of these contaminants in the marine environment of Chafarinas Islands; moreover, the dilution of the sea plays an important role in the detection of PFASs in the marine environment. This was supported by a study based on edible fish from the Mediterranean sea, where it was observed a high concentration of PFOS and PFOA in benthonic fish species (including clamps) linked to sediment pollution, while lower concentrations were detected in pelagic species (including mussels) that feed on the water column [86]. Other studies carried out evidenced the low accumulation potential of PFASs in mussels from the Spanish coast [30,87]. PFOS levels ranged from 36.8 to 125.9 ng/g ww in mussels from an area located on the Portuguese coast affected by textile, paper, and leader industries, and a significant correlation was found between shell length and PFOS concentration [88]. Similar to our finding, low levels of PFOS in similar fish species were detected in the Mediterranean [21,89,90].

Bioaccumulation and biomagnification along the trophic chain are affected by different factors like the diet and habitat, the elimination rates, and the biotransformation of PFASs precursors [91]. Biomagnification was observed for PFOS and long-chain PFCAs from

lichen to caribou and from caribou to wolf from Canada [84]. Boisvert et al., (2019) [92] observed a significant and strong correlation with increasing log K_{ow} of PFCAs (PFNA, PFDA, PFUnA, PFDoA, and PFTriDA) and the decreasing of the bioaccumulation factors, suggesting that bioaccumulation factors do not increase with the increase of the chain length. Nevertheless, the accumulation does not occur in all the food chain, in rainbow trout accumulation was not observed for PFOS, PFNA PFHxS, PFOA, and PFBS, and even the PFASs were detected in different organs as liver, blood, kidney, and skin, and these results were attributed to the excretion of the contaminants, for example, the accumulation of PFASs in skin tissues as an intermediate step before extraction via the skin, similarly to the excretion via the urinary and biliary system, or the gills [93]. It was also suggested that long-chain PFASs bind with proteins and are excreted via fecal residues [94,95], while urine is an important elimination pathway for PFOA and PFOS [96,97]. PFASs precursor metabolism may also contribute to PFASs exposure and the accumulation dynamics in prey and predator [92]. Other biological attributes, such as body size, weight, sex, reproduction stage, and growth rate, may also affect bioaccumulation [98]. As an example, when comparing sexes PFASs levels in males are higher than in females in birds due to the discharge PFASs on their clutches [99], and in female mammals due to the milk secretion [100].

Gull eggs were used in this Thesis as bioindicators of PFASs because adults can bioaccumulate contaminants through the diet and transfer them to the eggs [26]. Table 6.2 offers a global vision of the PFASs distribution by comparing PFOS concentration in eggs of several fish-eating bird species from the northern hemisphere.

Table 6.2. Levels of PFOS in eggs of several fish-eating bird species. Ordered according to the species and location.

Species	Location	PFOS (ng/g) min - max	PFOS (%) min - max	Reference
Cepphus grylle	Nunavut	39.8	83	[101]
Cerorhinca monocerata	British Columbia	34.3 - 286	39 - 86	[102]
Synthliboramphus antiquus	British Columbia	16.5	24	[102]
Uria aalge	Baltic Sea	426	91	[103]
		0.62	23	[104]
		400	73	[105]
	North Atlantic Sea	15 - 16	14 - 17	[105]
	Norwegian Coast	85	54 - 80	[105]
Uria lomvia	Nunavut	6.53 - 30.7	35 - 100	[101]
Hydrobates leucorhous	British Columbia	13.3 - 46.6	48 - 82	[102]
Hydroprogne caspia	Great Lakes	387 - 1,395	78 - 87	[106]
Larus argentatus	Baltic Sea	292	73	[107]
	East Coast Canada	8.70 - 277	32 - 91	[108]
	Germany	51.6 - 116	69 - 85	[109]
	Great Lakes	91.0 - 507	56 - 88	[110]
		43.0 - 1,032	31 - 93	[108]
		43.2 - 723	31 - 91	[111]
		103 - 170	79 - 84	[106]
	Manitoba	139 - 322	79 - 84	[108]
	Northwest Territories	8.4 - 41.0	21 - 57	[108]
	Norwegian Coast	15.3 - 42.3	64 - 70	[112]
		21.4 - 41.7	82 - 88	[113]
	Nunavut	9.40 - 33.0	53 - 68	[108]
Larus californicus	Alberta	49.0 - 80.0	70 - 75	[108]
Larus glaucescens	British Columbia	8.30 - 28.0	53 - 75	[108]
Larus hyperboreus	Arctic	104	71	[114]
	Nunavut	20.0	44	[101]
Pagophila eburnea	Artic	55.8 - 72.6	63 - 70	[115]
Rissa tridactyla	Nunavut	9.58	54	[101]
Phalacrocorax aristotelis	Norwegian Coast	36.8	81	[116]
		9.68 - 22.4	51 - 70	[112]
Phalacrocorax auritus	British Columbia	9.60 - 63.8	53 - 89	[102]
	West Coast USA	84.0 - 1,253	76 - 94	[117]
		76.8 - 381	77 - 84	[118]
Phalacrocorax carbo	Baltic Sea	552	76	[107]
	Germany	89.7	88	[109]
Fulmarus glacialis	Nunavut	14.5 - 37.1	39 - 99	[101]
Stercorarius skua	North Atlantic Sea	18.7 - 24.0	52 - 93	[119]

As was mentioned, PFOS was de predominant compound detected followed by long-chain PFCAs in concordance with a review [91]. Concretely, odd carbon chain PFCAs (PFUnA and PFTriDA) in agreement with previous studies in gulls [113,114,120,121]. This characteristic profile was attributed to plastic ingestion in herring gull from the Great Lakes [111]. Whereas short-chain PFASs were not detected in YLG and AG eggs because are not bioaccumulative in birds [62]. PFOS levels discussed in this Thesis were in agreement with the reported in the literature, however, some authors presented levels one order of magnitude higher. Levels up to 1,000 ng/g ww were reported in *Hydropogne caspia* [106], *Phalacrocorax auratus* [117], and *Larus argentatus* [108], while lower levels were reported in *Uria aalge* [104]. Figure 6.7. shows the PFOS and ∑PFCAs concentration in YLG and AG colonies from Natural Parks studied in this Thesis.

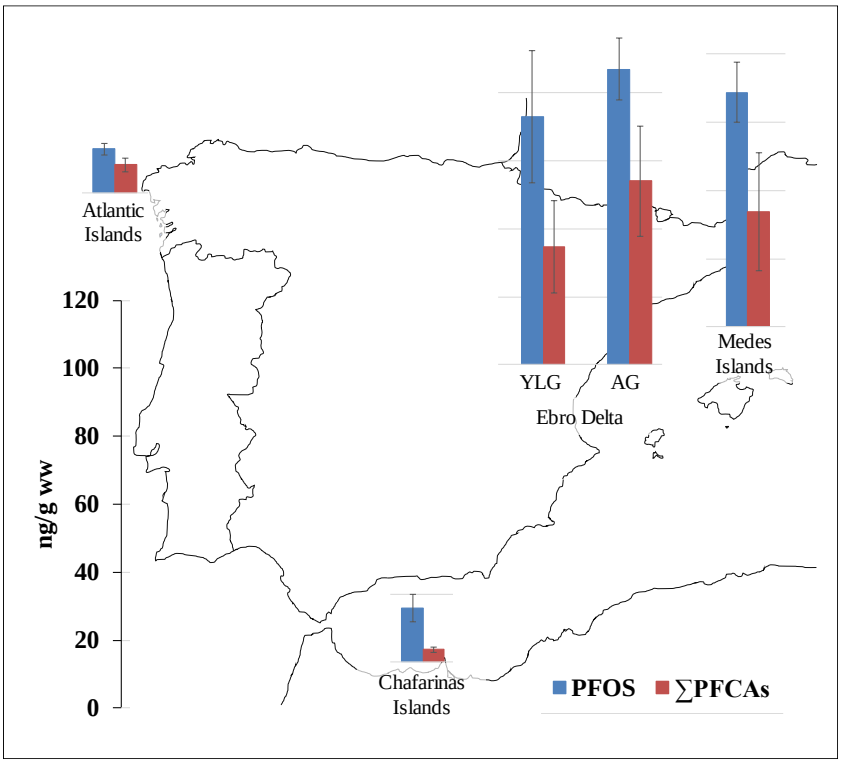

Figure 6.7. PFOS and ∑PFCA levels (ng/g ww; n=3) in eggs of yellow-legged gull (YLG) and Audouin's gull (AG) gull colonies from Natural Parks of the Iberian Peninsula.

Differences in the PFASs levels among bird species are attributed to different reasons. Firstly, the location of the colony where the surrounding area plays an important role in PFASs exposure. YLG eggs from the Ebro Delta and Medes Islands, both located in the North-Eastern Mediterranean Sea, had a similar distribution of PFASs, while in Chafarinas and Atlantic Islands these PFASs were present at lower concentration levels and variability. In the Ebro Delta colonies, concentrations in eggs from AG were significantly higher than those found in YLG, suggesting that fish diet influences PFAS bioaccumulation. Similar to our finding, previous studies also observed differences among colonies located across Canada and US, where the higher levels were located close to urban and industrial areas [108]. Feeding areas are another concerning fact to consider. Miller et al., (2015) [102] found that PFASs concentration in two pelagic bird species was influenced by the time spent close to the Asian shore during the winter season. So, the accumulation of PFASs was a consequence of their long-range distribution to a polluted area, and not from the pollution on the area of the colony is nesting. Feeding habits and accessibility of food are important factors to evaluate the bioaccumulation of PFASs in birds. In Ebro Delta, PFASs levels were measured in eggs of YLG and AG, and differences among species were attributed to their dietary habits. Higher PFASs concentrations were observed in AG that feed mostly on fish and remain closely linked to fisheries activities, while YLG are omnivores and their diet consists of fish, terrestrial invertebrates, crabs, and other seabirds chicks. Due to their high feeding adaptability, this species also feed scavenging in rubbish tips. Similarly, Nordén et al., (2013) [107] observed clear differences in the PFASs profiles of herring gulls and great cormorants from Sweden, where both species had a fish diet but gulls supplement it with human garbage.

In this Thesis, gull eggs were proposed as bioindicators of PFASs as well as to monitor the patterns along time. The PFASs concentration suggests an increasing trend along the time but for some compounds, a linearly colony-dependent decreasing time trend is observed. ∑PFASs decrease linearly in all locations except in Medes, where only PFTriDA decreases. In Ebro Delta, YLG shows a decrease for PFOS, PFUnA, and PFTriDA, whereas AG only for PFUnA. In the Chafarinas Islands, only PFOS shows a decrease. The literature revealed a decrease of PFOS concentration over time, and in contrast, an increasing tendency along the time was observed for PFCAs was in aquatic organisms [91]. Temporal trends of PFAS in bird eggs suggest different behavior of PFAS over time. Several studies conclude an increasing PFOS concentration from the 70s and 80s until the early beginning of the 21st century [113,122,123]. On the other side, other authors detected a constant concentration of PFOS from the 70s and 80s until the early 21st century as well [101,109,118,119,124]. In concordance with our results, some studies observed a declining trend of PFOS after the first and second decade of the 21st century [102,108,125,126]. Contrary to our results, the increase of PFCAs along the time was previously reported in bird eggs [102,113,127,128]. These temporal trends are driven by PFUnA and PFTriDA, the same dominant PFASs detected in gull eggs from this Thesis. These compounds are resulting from impurities of the PFOA and PFNA production [129].

PFASs accumulation in bird eggs have been largely studied for the lasts twenty years. It is observed that PFASs strongly impact bird eggs in areas close to fluorochemical facilities but also in remote locations where industrial discharge is absent. By contrast, the effects of PFASs in adult birds and their eggs are still largely unknown. PFOS levels detected in gull eggs from Natural Parks of the Iberian Peninsula are over the environmental quality standards with a limit of 9.1 ng/g ww that recommend the Directive

39/2013/EU. From a toxicological point of view, Peden-Adams et al., (2009) [130] evaluated the PFOS exposure to leghorn chicken eggs and at a level of 1 µg/g egg and observed significant immunological, morphological and neurological effects. In YLG eggs, a toxicity study was assessed by injecting 100 and 200 ng/g of PFOS, and no sublethal morphological and biochemical effects were observed in embryos [131]. In a *ovo* injection study in herring gulls, the embryo survival was 59% at 10 µg/g of PFOS with a bodyweight increase of 11%. Whereas for PFOA the embryo survival was 46% for 10 µg/g with a decrease of 10% of the bodyweight according to Norden et al., (2016) [132]. Overall, it seems safe to conclude that current environmental levels of PFOS from Natural Parks of Spain do no represent a potential hazard to gull embryos. However, the potential bioaccumulation of PFASs in gulls and the transfer to the eggs remain a concerning topic due to the persistence of PFASs in the environment and toxicity at biomolecular level.

The first step to minimize the pollution of PFASs in the environment is to reduce the consumption of PFASs-based products and to promote the use of green alternatives or those PFASs less persistent. The monitoring of PFASs using bioindicators of pollution is an excellent tool to evaluate their distribution in the environment. In this Thesis gulls were selected as bioindicators of PFASs in Natural Parks of Spain due to their feeding habitats, distribution, and resistance to pollution. However, the definition of bioindicators of pollution should be location-dependent to select the most appropriate species. Fish-eating birds on the top of the food chain seem to be excellent bioindicators of pollution nevertheless other taxons as the plants studied in this Thesis could be used to monitor PFASs pollution in riverine environments. The monitoring aims to evaluate distribution and changes of PFASs in the environment, as a response to changes in production due to

the creation of new regulations. At last, to minimize the pollution of PFASs in the environment the use of bioremediation is highly recommended. Plants species as presented in this Thesis seems to have a good PFASs uptake capacity from water and sediment, but further studies are needed to ensure the use of these plant species as phytoremediators of PFASs pollution.

6.5. REFERENCES

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CHAPTER 7.

CONCLUSIONS

Concerning the emissions of wastewater from a fluoropolymer facility that becomes the main source of pollution of the Xiaoqing River, the following conclusions are derived:

- A dilution of ∑PFASs was observed along the Dongzhulong and Xiaoqing River in all matrices.
- Discharge of wastewater from fluoropolymer facilities becomes the main source of pollution for the aquatic environment. PFOA and other PFCAs were the main contaminants detected in water and sediments due to the impact of the Gongyue Group facility. PFSAs along the river were punctually detected and it is attributed to urban discharge and other activities in the area.
- K_d values indicated that long-chain PFASs preferentially remain sorbed in the sediment, while short-chain PFASs are mobile in the water column.
- Floating species show a higher concentration among plants, especially *Lemna minor*, because easily translocate long-chian PFASs direct from the water.
- In contrast with floating species, the uptake in rooted species followed different mechanisms. Thus, rooted species must compete with the sediment for PFASs uptake. Moreover, long-chain PFASs remain accumulated in the root compartment because of protein affinity while short-chain PFASs are more mobile and can be translocated to shoots.

Regarding the fate of PFASs in the environment surrounding the yellow-legged gull colony grom Chafarinas Islands, the following conclusions were derived:

- PFOS was detected in soils and sediments on the North Moroccan coast, this matrix representing a reservoir of pollution to the surrounding environment of Chafarinas Islands and the gulls.

- PFOS was detected in the natural diet of the yellow-legged gull from Chafarinas Islands and it is suggested as a source of pollution to this species.
- Yellow-legged gull eggs accumulate mostly PFOS, but also odd long-chain PFCAs that are highly accumulative and are suggested to have a different source compared to PFOS.

Regarding the evaluation of PFASs patterns in species suggested as bioindicators of pollution, the following conclusions were drawn:

- Yellow-legged and Audouin's gull accumulate PFASs due to their feeding habits and are transferred to their eggs. Thus, eggs become excellent bioindicators of environmental pollution.
- PFOS accounted for the main contaminant in the four colonies studied, while the the rest of PFASs differed in percentage among colonies.
- Eggs of yellow-legged gull can be considered a suitable biomonitoring matrix, since PFASs concentration is higher in the most industrialized colonies of the Catalan coast (Medes Islands and Ebro Delta) compared to southern Mediterranean colonies (Chafarinas Islands) and Atlantic colonies (Atlantic Islands)
- The differences in PFASs levels between the two gull species from the colonies cohabiting in the Ebro Delta are associated with different feeding habits.
- Time trends suggested a significantly decreasing concentration of PFAS, exemplified by PFOS, PFUnA, and PFTriDA, after approximately a decade of the phase-out of PFOS.