Distribution of Legacy and Emerging Per- and Polyfluoroalkyl substances in Riverine and Coastal Sediments of Southeastern North Carolina, USA

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Abstract

The sediment distribution of per- and polyfluoroalkyl substances (PFAS) along a river to ocean transect was investigated. Samples were collected between September 2017 and October 2019 with targeted quantification of six legacy and replacement PFAS by LC-MS/MS. Total PFAS concentrations ranged from below the LOQ to 7.47 ng/g dry weight with PFOA, PFOS, HFPO-DA and PFMOAA the most frequently detected. Significant correlations ($p < 0.05$) were found between PFOS and HFPO-DA sedimentary concentration and percent organic carbon (%OC); however, PFOA and PFMOAA were not correlated with sediment %OC. This study highlights the occurrence of the replacement PFAS in sediments for the first time. Sediment extracts were screened for 18 additional PFAS compounds by high resolution mass spectrometry. A series of perfluorinated ether carboxylic acid and perfluorinated ether sulfonic acid with either one or two acidic functional groups were detected at various locations in the upper portion of the Cape Fear River. A series of chromatographically resolved isomers ($C_7F_{13}O_5S_1$; M-1) were detected and may be Nafion™ degradation products.

Environmental Significance Statement

The sedimentary distributions and concentrations of legacy and replacement per- and polyfluoroalkyl substances (PFAS) were determined along a river to ocean transect, including two distal locations. Results highlight the variable nature of PFAS sediment partitioning, especially of the replacement HFPO-DA and PFMOAA. Novel PFAS, including a series of perfluorinated ether sulfonic acids, were detected by high resolution mass spectrometry as well.

The results of this study are important because it demonstrates the exposure of benthic organisms and corresponding food web to PFAS.

Introduction

Sediments are an important environmental compartment for many anthropogenically derived pollutants. One class of compounds that have been detected in sediments are per- and polyfluoroalkyl substances (PFAS) [1–3]. PFAS are fluorinated alkyl compounds containing numerous carbon-fluorine bonds and as a result , they exhibit unique traits such thermal stability and surfactant properties leading to many uses in industrial and commercial applications[4,5]. In turn, this had led to the synthesis of many thousands of individual PFAS registered in the Chemical Abstract Services (CAS) database. The biotic degradation of PFAS varies across compound class but as a whole, they are relative resistant to degradation [6,7]. In combination with their high water solubility, these compounds are very mobile in the environment and in turn, this has led to distribution to far reaches of the globe.

The voluntary phaseout of the legacy PFAS (e.g. PFOA and PFOS) has led to the manufacturing of replacements with decreased carbon chain length and ether functionalities with the intent of decreasing half-life within humans and decreasing toxicity [8]. There are currently limited toxicological studies relating to the replacement PFAS, with most of the research done with exposing model organisms to single components and comparing toxicological profiles to a legacy PFAS (e.g., PFOA). The replacement PFAS have been shown to cause a variety of biological responses including increased gestational weight, hepatic damage and other impacts [9–11] but an understanding of the cycling of these compounds in the environment is still lacking.

The Cape Fear River Basin, located in southeastern North Carolina, United States, has been impacted for decades by a fluorochemical facility located along the banks of the river. Discharge from the facility contains legacy and replacement PFAS impacting the river and all communities nearby and downstream[12]. The Cape Fear River serves as the major drinking water supply for the city of Wilmington, NC as well as other municipalities thus exposing many citizens to PFAS[13].

Most research in the Cape Fear River Basin has focused on dissolved phase PFAS distribution and occurrence as it pertains to drinking water supply/treatment and biota[14,15] within the river. The aim of this study was to provide a quantitative analysis of emerging PFAS in Cape Fear River sediments and perform a qualitative analysis for suspect screening of emerging PFAS. Additionally, understanding PFAS distribution in sediment can give insights into the storage and possible release of PFAS back to the water column[16–18]

METHODS

Sample collection

Surface sediment samples were collected along the Cape Fear River (CFR), North Carolina in 2017 and 2018, and outside of CFR (Charleston and Beaufort) in 2019 (see Figure 1 and Table S1 for mapping and sample location longitude and latitude). The upper five cm of sediment was collected from Lock and Dam 1 northward with a shovel and placed in a clean methanol rinsed plastic Ziploc bag. The estuarine transect sediment samples (Horseshoe Bend to mile marker 18) were collected using a Ponar® 6"X6" grab sampler aboard R/V *Cape Fear*. Three samples outside of the CFR (mouth of CFR, Fort Sumter, and Beaufort) were collected using an Ocean Instruments Mark III Box Core aboard R/V *Cape Hatteras*. Sediment samples were stored at 4º C until processed. Sediments were transferred into methanol rinsed high-density polyethylene (HDPE) cups and dried at no more than 40ºC until constant weight. Dried samples were sieved using a methanol rinsed 500-micron mesh polyethylene sieve and transferred into new HDPE cups until extraction.

Chemicals

The targeted legacy and emerging PFAS in this study are PFOA,PFOS, HFPO-DA, PFMOAA, PFMOBA and PFMOPrA/ PMPA with structures in Figure S1. Standards for native PFOA and PFOS were purchased from Synquest (Alachua, Florida). PFMOAA was obtained through a custom synthesis from Zerenex (Greater Manchester, UK). PFMOBA standard was purchased from Aurum Pharmatech (Franklin Park, NJ), and HFPO-DA was purchased from Alfa Aesar (Haverhill, MA). The mass- labeled internal standards, M-PFBA (perfluoro[$^{13}C_4$]butanoic acid), M-PFHxA (perfluoro[$^{13}C_6$]hexanoic acid), M-HFPO-DA (perfluoro[$^{13}C_6$]propoxypropanoic acid), M-PFOA (perfluoro[$^{13}C_8$]octanoic acid), and M-PFOS (perfluoro[$^{13}C_8$]octane sulfonic acid) were all purchased from Wellington laboratories (Guelph, Canada). The targeted PFAS were chosen based upon the historical production at the fluorochemical manufacturer located along the banks of the Cape Fear River. All the standards were individually diluted first in LCMS grade methanol and ammonium hydroxide (NH_4OH) solution (96% methanol, 4% NH_4OH) and stored in clean 30-mL HDPE bottles. The standard mix and internal standard mix were prepared gravimetrically and diluted with one part methanol (2mM ammonium acetate) and 1 part water (2 mM ammonium acetate) prior to LC/MS analysis.

Sediment extraction

Dry sediments were extracted as previously described[19] All labware was methanol rinsed immediately prior to use. Approximately 10 g of dry sediment were weighed and added to pre-weighed 50- mL polypropylene (PP) tubes and spiked with the internal standard mix. Then, 2 mL of 100 mM sodium hydroxide (NaOH) in 20% MilliQ water and 80% methanol were added to the samples. The samples soaked for 30 min at room temperature. Fifteen mL of LCMS grade methanol was added to each sample and shaken for 30 minutes at 400 rpm. Following the shaker, the samples were centrifuged for 15 min at 5000 rpm and then decanted into a new 50-mL polypropylene tube. The extraction process was repeated twice using 1 mL of 100 mM NaOH in 20% MilliQ and 80% methanol and 10 mL of methanol. The supernatants for each sample were combined and acidified with 100 μL of 4 M hydrochloric acid. Following the addition of acid, the extracts were centrifuged for 5 min at 5000 rpm.

For each sample, a 6-mL 250-mg bed weight ENVI-CARB SPE cartridge (SUPELCO) was conditioned with a total of 20 mL of LCMS grade methanol. Following conditioning, pre-combusted beakers were used to pour the supernatants through the SPE cartridges. Filtered eluates were collected in clean pre-weighed 50-mL PP tubes. The SPE cartridges were rinsed with 10 mL of LCMS grade methanol. Extracts were reduced to 0.5 mL under a gentle stream of UHP grade nitrogen stream using a Turbovap LV with water bath setting of 30°C.

Physio-chemical measurement

Percent organic carbon (OC) was measured by elemental analysis of decarbonated sediment [20]. Briefly, approximately 0.5 g of the dried and sieved sediments were weighed into 20-mL glass centrifuge tubes. Standards along with 3-4 blanks were placed into a vacuum desiccator for 24 h to be decalcified using fuming HCl. Approximately 25-65 mg of dried sample were weighed into tin boats. For the sample set, 3 acetanilide (ACE) standards were also included. Tin boats were crimped closed and transferred to a Thermo Quest EA2500 elemental analyzer. Instrument responses were calibrated with 3-4 ACE standards (using the standard weights) and a blank to obtain a linear calibration curve. The sedimentary %OC along with the water column pH at time of collection are in table S2.

Targeted PFAS quantification

PFAS separation and quantification were performed on a Sciex Exion LC equipped with a Luna Omega PS C18 (2.1×50 mm, 1.6 μm, Phenomenex) and AB Sciex 4000 QTRAP. The mobile phase gradient is shown in Table S3. Electrospray ionization (ESI) in negative mode was used, and the ion spray voltage was kept at -4200 V. The ion source temperature was set to 500°C, and the source gas flow was maintained at 60 psi. Transitions for each compound were optimized and have been described previously [21]. PFMOPrA and PMPA are reported as a total sum due to similar molecular and fragment ions as well as lack of chromatographic separation under the LC conditions described. Calibration standards and quality control samples were spiked with isotopically labeled internal standards. Quantification was performed using isotope dilution. Analytes lacking an analogous labeled standard were quantified using the internal standard with the closest retention time (Table S4). The precursor-product ion single reaction monitoring (SRM) transitions are shown in Table S4. A minimum of four points were used to generate calibration curves with linear r^2 values greater than 0.99.

Quality assurance and quality control

A variety of blanks were analyzed with each extraction batch and throughout the analysis. Method blanks were analyzed with each extraction and consisted of pre-combusted sand treated the same as the unknown sediment samples and carried through the extraction. Instrument blanks were analyzed throughout the analytical analysis and consisted of methanol/water. The limit of quantification (LOQ) was defined as the lowest point of the standard curve where the calculated concentration was 70 to 130% of expected using the linear equation[22,23]. The limit of quantification (LOQ) and recoveries for each analyte can be found in detail in Table S5. Recoveries for the targeted compounds ranged from 82 to 106% (n = 6). The variability of PFAS concentration in sediments collected from the same location over multiple trips (n=2-3) is shown in Table S6. The standard deviation was less than 0.5 for the targeted analytes.

Suspect screening and non-targeted analysis

Non-targeted analysis was conducted using an Agilent 1290 LC coupled to a Bruker micro-QTOF II under conditions identical to Saleeby et al. [24]. Briefly, an electrospray ionization source operated in negative mode was used for all analysis with capillary voltage maintained at -4000 V, while the temperature and dry gas flow were maintained at 200ºC and 8 L/min respectively. A Peak Scientific generator produced ultra-high purity nitrogen. Source nebulizer pressure was kept at 14 psi. The column used for chromatographic separation was the same as quantitative analysis. Mobile phase solvents used were the same for quantitative analysis at flow rate of 0.2 mL/min, and 10 µL volume injection of sample. The mobile phase gradient program used during non-targeted analysis was the same as the targeted analysis.

Prior to exploring mass features, the mass axis was calibrated for each LC analysis using the Agilent Low Mass tune mix. Bruker Data Analysis 4.2 software package was used for processing of the data which first involved background subtraction and removal of previously identified and known PFAS with authentic standards. Remaining molecular features with a S/N >3 was compared to a curated suspect list from previous publications (Table S7) by generating extracted ion chromatograms (isolation window 0.05 u) were generated along with mass defect plots. The presence of a suspect compound was tentatively confirmed using retention time and mass error (<5 ppm). The generated molecular formula was compared to the Chemical Abstracts Services (CAS) database for possible matches to aid in tentative structural identification. All but one of the structures are considered level 4, that is a unequivocal molecular formula is possible when taking into account the features (e.g. adducts). [25] The other compound is considered Level 3, tentative candidate, when compared to a previous study[24].

Statistical analysis

Correlation tests and hierarchical clustering were performed using R statistical software (R version 3.6.3). Statistical significance was set at $p < 0.05$. Due to the presence of data below the limit of quantification, functions from the "NADA" package was used to perform non-parametric correlation test, Kendall's tau b [26]. Data displaying more than 80% censored observations (<LOQ) were not included in the analyses [27].

Results and Discussion

Spatial variation of PFAS in surface sediments

The total sum of the PFAS targeted in this study (ΣPFAS) ranged from <LOQ to 6.6 ng/g dw (Figure 2a). The highest total PFAS concentration, 6.6 ng/g dw, was found in sediment collected outside of the fluorochemical facility, with decreasing total PFAS downriver from the fluorochemical facility (Figure 2A). When comparing the occurrence frequency of each compound above the LOQ, legacy PFOA and PFOS were detected in two thirds of the samples analyzed while the replacement PFAS, PFMOAA and HFPO-DA, were detected >50% above the LOQ. The percent composition of each compound is illustrated in figure 2b. Along the upper portions of the river (FBR to HB), PFOS, PFMOAA and HFPO-DA were most prevalent while PFMOAA dominated M61 and M54. Progressing down the river illustrated a shift to HFPO-DA except at the M18 which exhibited a mixture of PFAS detected. HFPO-DA and PFOS were most abundant at Fort Sumter (FS) and Beaufort (BFT).

Concentrations of PFOS/PFOA in CFR sediments in this study are lower compared to other data previously reported in literature (Table 1). Most of the sample locations in these other studies were from areas that are highly populated, urbanized and have intensive industrial activities that contribute more to PFAS contamination [20,28,29,29,30]. Interestingly, PFOS concentrations in our sediment samples are elevated compared to the global median PFOS concentration in sediments of 0.5 ng/g dw [31]. Elevated PFOS concentration in this study suggest that even though PFOS manufacture in the US has been phased out, sediments remain an important sink for these compounds. The distribution of the replacement PFAS in sediments was dominated by HFPO-DA, the most frequently detected compound. The mean HFPO-DA concentration in Cape Fear River sediments was 0.54 ng/g dw and is comparable to the sediment collected along the highly contaminated areas of the Xiaoqing River, China in 2014 (mean concentration 3.09 ng/g dw) and 2016 (mean concentration <LOQ)[32]. Interestingly, coastal marine sediments collected near Beaufort, NC and Fort Sumter, SC (Figure 2a) both had detectable HFPO-DA, 0.4 and 0.2 ng/g respectively. These concentrations are similar to HFPO-DA. For example, sediments from the East China and Yellow Seas contained a mean concentration of 0.119 ng/g dw [30]. The increased global and especially coastal/marine distribution of HFPO-DA suggests potential non-point sources of HFPO-DA, for example, atmospheric deposition [22], groundwater transport [33], and oceanic long-range transport [34].

Spatial and physicochemical factors underlying sediment PFAS distributions

To understand factors controlling targeted PFAS levels and distributions along the Cape Fear River, correlation analysis was performed with sediment organic carbon content and distance along the riverine transect for targeted analytes (Table 2). Kendall's rank correlation was chosen to explore the ordinal relationship between two quantities where τ of +/- 1 is indicative of correlation between two variables. Statistically significant correlations ($p < 0.05$) between PFAS levels and percent sediment organic carbon were observed for PFOS and HFPO-DA with relatively moderate correlation coefficients, Kendall's $\tau = 0.40$ and 0.3 respectively. The correlation of PFOS with sedimentary organic carbon had been reported before in the literature [2,35]. However, the significant correlation between HFPO-DA and sedimentary organic carbon was not observed in a study conducted in the North and Baltic Seas[34], in fact, HFPO-DA was not detected in any of the sediment analyzed by the authors. The exact reason(s) responsible for the differences in HFPO-DA partitioning in this study cannot be answered given the available data, however future studies are warranted to understand the fate and cycling of this compound. PFOA was not correlated with sedimentary organic carbon content ($p = 0.52$). In a previous study, a lack of correlation between PFOA and sedimentary organic carbon content was also observed [2]. A more recent review also noted the partition coefficient (K_d) of PFOA was on average 6.8 times less than the K_d value of PFOS [36] in coastal/estuarine sediments indicating PFOA preferentially partitions to the dissolved phase. Prediction of PFAS sedimentary fate is difficult due to the relatively low concentrations encountered in the sediment as well as the surfactant nature of the chemicals as a class. The fluorochemical facility has historically been a direct discharge source of PFAS to the Cape Fear River. Distance downstream from the fluorochemical manufacture and absolute distance including upstream samples along the Cape Fear River were considered as variables for correlation to the targeted PFAS in this study. PFMOAA sediment distribution had a significant but moderate correlation with downstream distance (Kendall's τ = -0.37, p = 0.013) but not with absolute distance suggesting discharge from the facility is a source to CFR sediments. PFOA concentrations showed a significant albeit weak correlation with downstream (Kendall's τ = -0.28, p = 0.048) and absolute (Kendall's τ = -0.27, p = 0.04) distance parameters. Previous studies of PFAS distribution in Cape Fear River water indicate a similar pattern of PFMOAA and PFOA elevated near the fluorochemical manufacturing facility [38,39]. HFPO-DA and PFOS were not statistically significant in either downstream distance and absolute distance from the fluorochemical manufacturer. This finding is surprising given HFPO-DA is manufactured at this facility and water collected directly adjacent is highest in HFPO-DA concentration decreases moving away, downstream[23]. The parameters considered in this study are important but future work should take into account other sediment characteristics such as grain size, pH and bulk density to unravel PFAS sorption processes in this system[2,40,41].

Suspect screening using high resolution mass spectrometry

Sediment extracts spanning the range from the upper to lower Cape Fear River were analyzed by high resolution mass spectrometry and screened against a list of PFAS not targeted in this study (Table S7). High resolution mass spectrometry yielded molecular features belonging to a variety of PFAS classes including perfluorinated ether carboxylic acid (PFECA) and perfluorinated ether sulfonic acid (PFESA) with either one or two acidic functional groups in sediment extracts (Table 3). The single acidic functional group PFAS were of general formula $CF_3(CF_2)_n(CF2O)_m(R1)$ where the R1 head group is either a R-COOH or R-SO_3H. The PFAS with two acidic functional groups have a general form of R_1-$(CF_2)_n(CF_2O)_m$-R_2, where R_1,R_2 are variable and can be -COOH or SO_3H. The trend in PFAS detection was centered around the upper Cape Fear River, especially at Chemours Creek (CC) and Lock and Dam 1 (LD 1) (Figure 3). Interestingly, the single H-polyfluorinated acids (structures 10-12) were detected in the upper portions of the river and with minimal occurrence in the lower Cape Fear River. The perfluoroether multi-acidic (14 a/b-17) PFAS were only detected at the Chemours Creek location with no occurrence in the lower reaches of the river while the perfluorinated ethers were only detected at the LD 1 station. All of the suspect compounds screened have been detected in the Cape Fear River aqueous phase but the limited occurrence in the sediment phase suggests weak partitioning.

Of particular interest was the occurrence of a series of PFESA detected in sediment from Lock and Dam 1 with monoisotopic mass of 442.9258 u (Figure 4). One of the three detected peaks was confirmed by a standard known as Nafion byproduct 1 (Compound 9; Table S6), that is not commercially available, provided by a fluorochemical manufacturer. Water collected from the Cape Fear River has been shown to contain this compound as well, [42] however it has not been reported in sediments. The structural identify of the remaining three compounds remains unknown but all have molecular formulas of $C_7F_{13}O_5S_1$ (M-1), and there is no other possible formula based on the monoisotopic mass. The origin of these compounds (442.9258 u) is most likely a degradation product of the fluorochemical manufacturing process[43] however, the exact process/mechanism is not certain in sediment. Perfluoroether species with multiple acid groups, compounds 14a/b, 15, 16 and 17 were also detected in sediment closest to the fluorochemical manufacturer facility (Figure 3). The origin is unclear, however, it is postulated they are impurities in fluorochemical production through hydrolysis and other processing reactions in the manufacturing of Nafion™ membranes [43,44].

Conclusions

The study is unique in that sediment was collected along a river transect from the freshwater to oceanic endmembers as well as two distal locations. A mix of legacy and replacement PFAS were detected with PFOA/PFOS the most frequently detected. The high detection frequency of PFOA/PFOS has been reported before in sediments from various locations around the United States. The next frequently detected were the replacement PFAS, HFPO-DA and PFMOAA. While it is clear from the targeted and high resolution mass spectrometric data PFAS do partition to sediment, the underlying physicochemical properties of the sediment and how those control PFAS-sediment distributions remain unknown in this system, especially pertaining to replacement PFAS like HFPO-DA. Ultimately, a better understanding of PFAS-sediment partitioning behavior under various sedimentary conditions (e.g. %OC, mineral composition, surface area), especially for replacement PFAS like HFPO-DA, will improve modeling of PFAS-sediment transport and fate. Interestingly though, the limited detection of PFAS in the lower reaches of the Cape fear River indicate limited transport beyond Lock and Dam 1. However, Lock and Dam 1 appears to be a depositional center for most of the PFAS compounds detected, potentially impacting the drinking water supply for users in municipalities.

Table 1. Comparison of PFOA, PFOS, and HFPO-DA concentrations in the Cape Fear River, NC sediments (ng g^{-1} dw) to previous studies. Ranges and average (in parenthesis) concentration values. n.d: not detected; -denotes compound was not screened for in the study; n/a: information was not available

Location	Year	*n*	PFOA	PFOS	HFPO-DA	Reference
USA						
Cape Fear River, NC	*2017-2019*	29	*0.04-0.51 (0.21)*	*0.08-2.78 (0.91)*	*0.08-1.72 (0.54)*	*Present study*
Charleston, SC	2012	36	0.10-2.51 (0.42)	0.09-7.37 (1.52)	-	White et al., 2015
Baltimore Inner Harbor, MD	2004	3	0.19-0.39 (0.85)	n.d-0.85 (0.39)	-	Higgins et al 2009
Asia						
East China/Yellow Sea	2019	68	0.045-2.79 (0.792)	0.002-0.558 (0.139)	0.018-0.450 (0.119)	Zhong et al. 2021
Bohai Bay, China	2017	18	0.44-18.9 (2.69)	0.68-5.50 (1.37)	n.d	Liu et al., 2019
Xiaoqing River, China	2014	24	<LOQ-3.64x10^3 (2.01x10^2)	0.14-1.20 (0.51)	<LOQ-70.1 (3.09)	Song et al., 2018
Xiaoqing River, China	2016	24	<LOQ-8.13x10^3 (3.48x10^2)	<LOQ-0.92 (0.29)	<LOQ-22.3 (<LOQ)	Song et al., 2018
Other						
Water bodies, Sweden	2015	23	n.d	<0.5-64	-	Mussabek et al., 2019
Bering Sea Chukchi Sea & adjacent Arctic area	2010	20	0.08-0.74 (0.37)	0.03-0.20 (0.08)	-	Kahkashan et al., 2019

Table 2: Kendall correlation analysis of select PFAS to parameters. Significant correlations are indicated in bold.

Compound	% OC	Downstream Distance (km)	Absolute Distance (km)
PFMOAA	τ=0.16 ; p=0.27	**τ= –0.37 ; p=0.013**	τ= –0.19 ; p=0.15
HFPO-DA	**τ=0.3 ; p=0.037**	τ= –0.23 ; p= 0.12	τ= –0.25 ; p= 0.06
PFOA	τ=0.093 ; p=0.52	**τ= –0.28 ; p= 0.048**	**τ= –0.27 ; p= 0.04**
PFOS	**τ=0.4 ; p=0.0044**	τ= –0.26 ; p= 0.071	τ= –0.23 ; p= 0.09

Table 3: Occurrence of PFAS in sediment samples from the upper to lower Cape Fear River. Structures can be found in Figure S2 and two numbers indicate isomers in order of elution from left to right. The sample station codes are the following: LD 1 Lock and Dam 1 ; CC Chemours Creek; WHO William O'Huske Dam ; LD 2 Lock and Dam 2 ; CF 35 Cape Fear 35. All stations can be found on the map in Figure 1.

Category	Structure. number	CAS	Confidence	Formula (M-1)	Monoisotopic Mass M-1 (u)	Reference
[a]*Perfluorinated Ether Acids*	2	39492-89-2	4	$C_5F_9O_5$	310.9602	Strynar et al., 2015
	4	39492-90-5	4	$C_6F_{11}O_6$	376.9519	Sun et al. 2016
	9	29311-67-9	4	$C_7F_{13}O_5S$	442.9259	Strynar et al., 2015
[b]*H-Polyfluorinated*	10	801209-99-4	4	$C_4HF_8O_4S$	296.9468	Saleeby et al.,
	11	2416366-21-5	4	$C_6HF_{12}O_4S$	396.9404	McCord and Strynar 2019
	12	749836-20-2	3	$C_7HF_{14}O_5S$	462.9321	Saleeby et al.,
[c]*PerFluoro Ether Multi-Acidic*	14a/b	2416366-18-0/852157-01-8	4	$C_7HF_{12}O_6S$	440.9302	Zhouet al 2007
	15	2416366-19-1	4	$C_7H_2F_{11}O_7S$	438.9346	McCord and Strynar 2019
	16	1235024-21-1	4	$C_8H_1F_{14}O_7S_1$	506.922	Zhou et al 2007
	17	1235024-21-1	4	$C_5HF_8O_6S$	340.9366	Takasaki et al 2013

a= general formula of $CF_3(CF_2)_n(CF_2O)_m(R_1)$ where R is -SO_3H or -COOH

b= where one F is replaced with an H

c= general formula of R_1-(CF_2)n(CF_2O)m-R_2 where R_1 and R_2 are variable acidic groups of -SO_3H or -COOH

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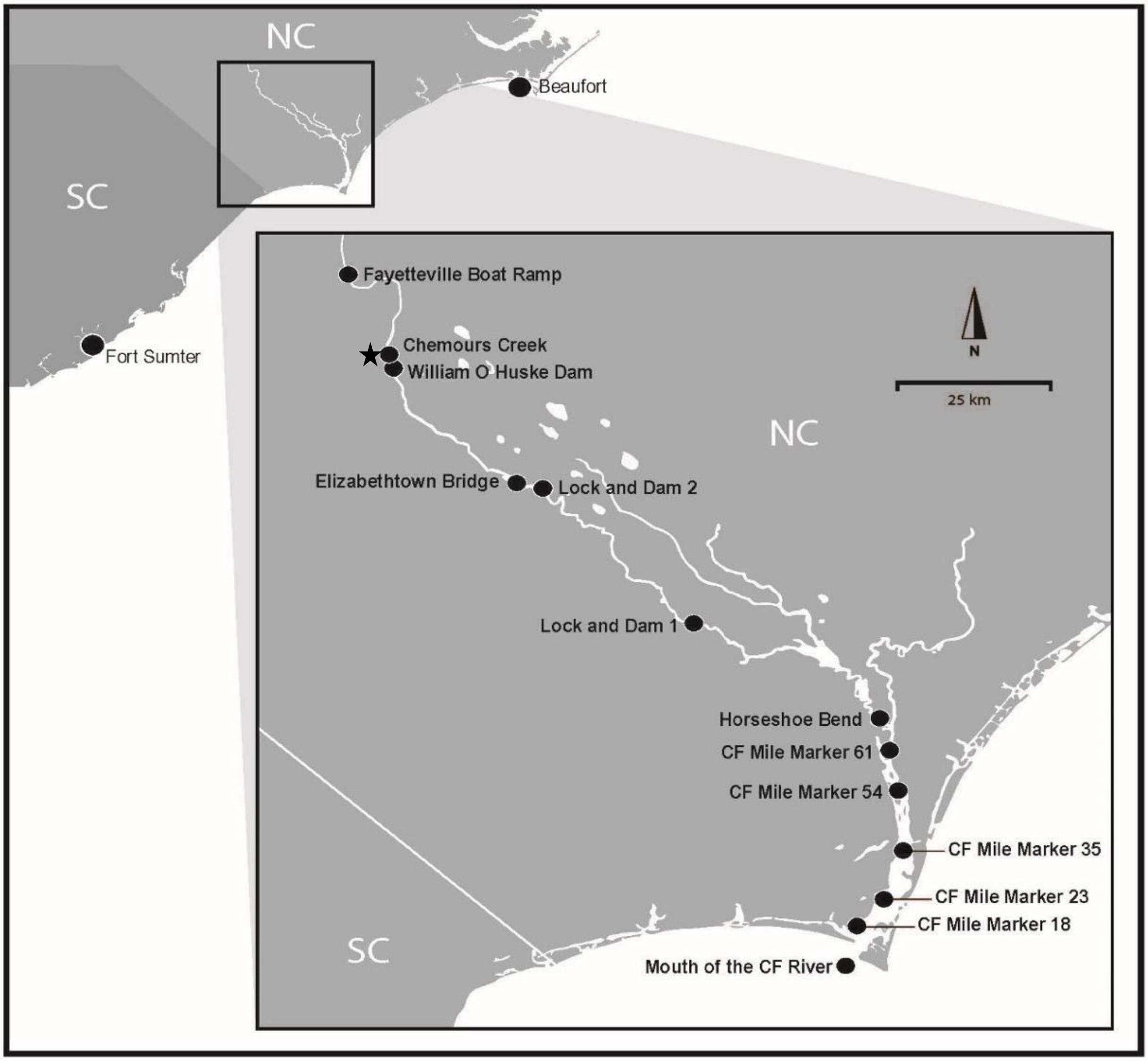

Figure 1. Sampling locations along the Cape Fear River and off-site sampling locations. The star depicts a fluorochemical facility.

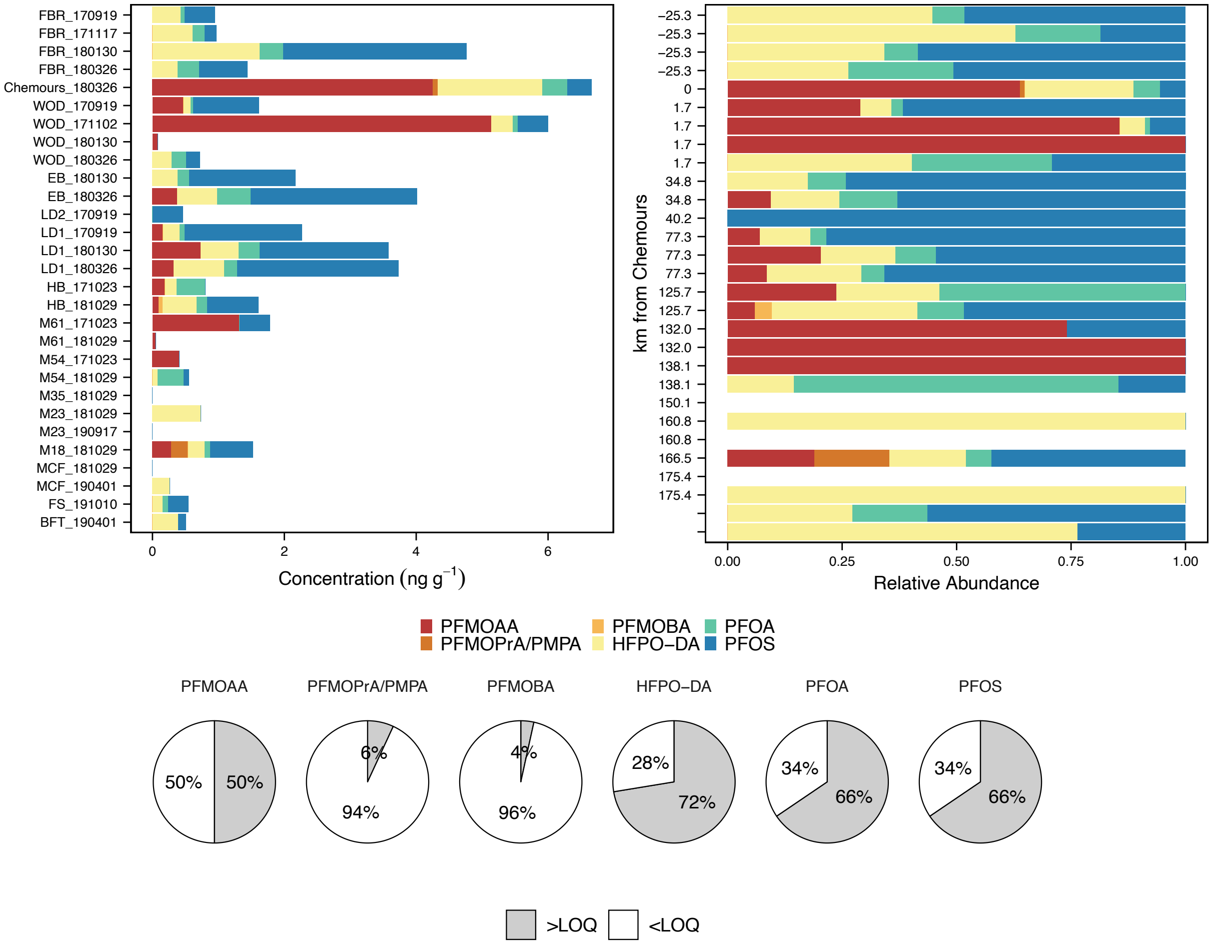

Figure 2. : PFAS concentration (a) and relative abundance (b) from sample stations as well as the distance from the manufacturing facility. The pie charts on the bottoms show the detection frequency of the PFAS quantified in this study.

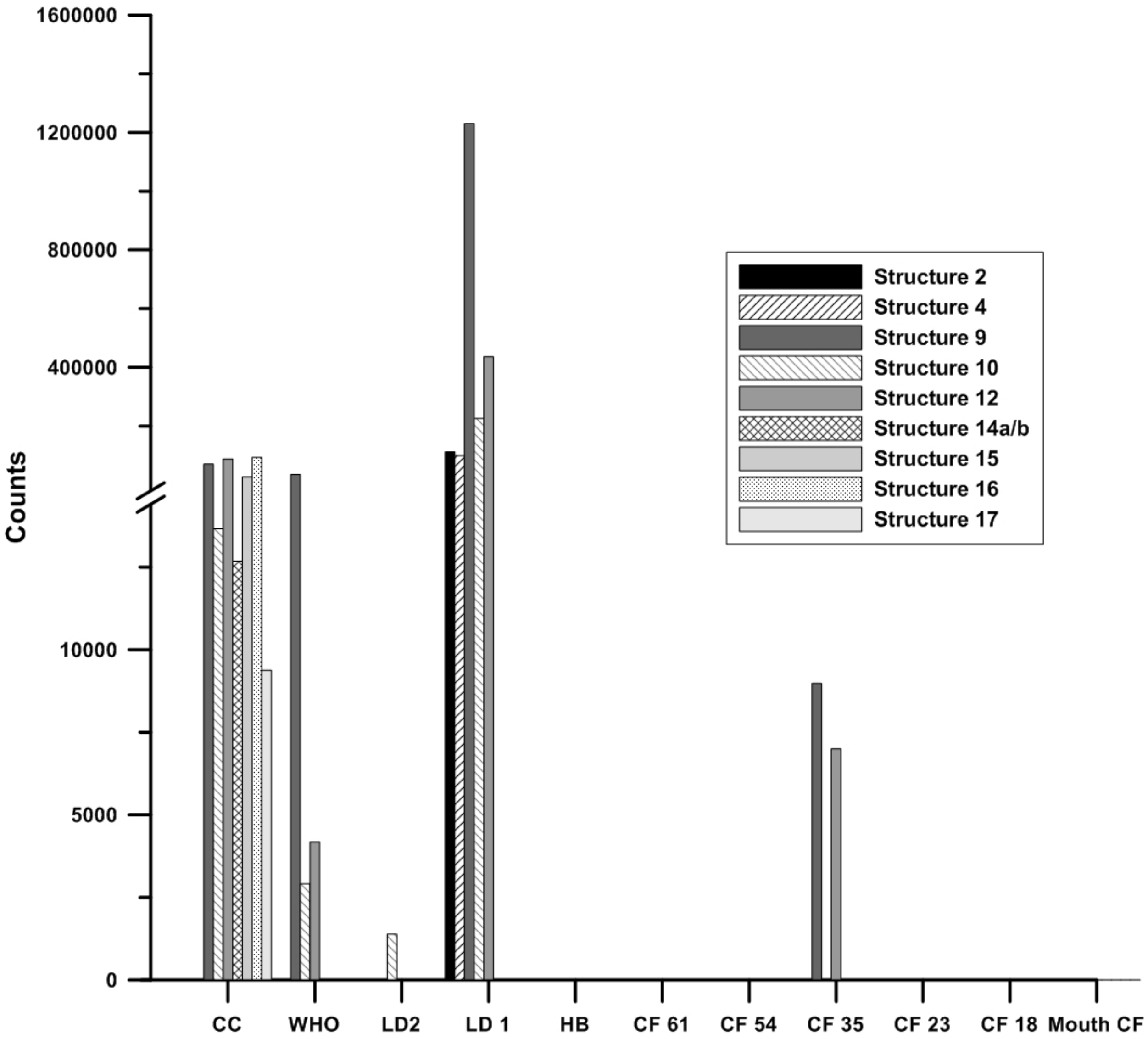

Figure 3: Area counts with S/N >3 of suspect PFAS compounds detected along the Cape Fear River transect. The sample stations can be found on Figure 1 and structures in Figure S2.

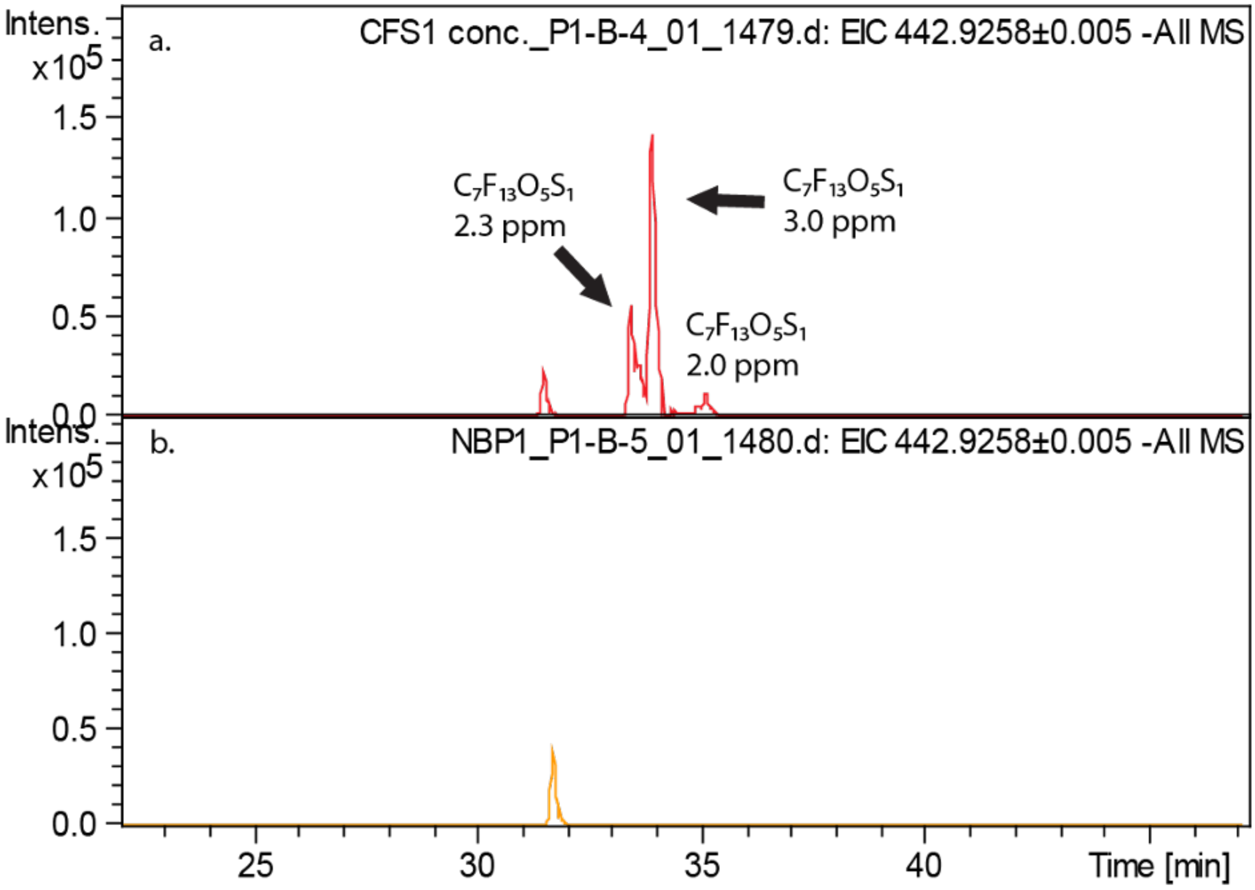

Figure 4: Extracted ion chromatogram (442.9258 u) illustrating several new constitutional isomers (a) and identification of one by authentic standard (b).