Research article

Survey of per- and polyfluoroalkyl substances (PFAS) in surface water collected in Pensacola, FL

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HIGHLIGHTS

- Elevated PFAS levels were quantified in Pensacola Bay surface water.
- ΣPFOS was the compound detected with the highest concentration between all PFAS.
- PFAS diversity increased near sites with industrial effluent and suspected AFFF usage.

ABSTRACT

As the persistence of per- and polyfluoroalkyl substances (PFAS) become a global concern, information about the occurrence and characteristics of PFAS in estuarine and marine ecosystems is poorly represented. In this study, the presence of 51 PFAS were monitored in the Pensacola Bay System (PBS), Florida, USA. Due to the presence of many potential PFAS sources in close proximity to the PBS (e.g., military bases, industries, airports and several firefighting stations), the distribution and concentration of PFAS in this estuarine environment provides insights into the fate of these complex compounds as well as the possible impacts on coastal systems. Surface water was collected and analyzed from 45 different sites via Strata-X-AW cartridge extractions and ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis. Recoveries for many PFAS (13/51) were >60% (mean 77 %), with relative standard deviations below 20%, except for N-methylperfluoro-1-octanesulfo-namidoacetic acid (N-MeFOSAA) (22%). Of the perfluoroalkyl carboxylic acids (PFCAs), which comprised the majority of PFAS detected: perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA) were present in all samples; however, perfluoropentanoic acid (PFPeA) was the individual PFAS with the highest concentration of this group (51.9 ng.L^{-1}, at site 81). The PFAS detected at the highest concentrations were perfluoroalkyl sulfonic acids (PFSA), with perfluorooctane sulfonic acid (PFOS) having the highest detected concentration (269 ng.L^{-1}, at site 81). At all sites, at least eight or more PFAS were quantified. Past and current use of PFAS-containing materials and their fate in areas surrounding military bases, airports, and industries, require more in-depth monitoring efforts to better determine the need for regulation, management, and/or remediation. Here, sites located close to areas suspected of PFAS use had elevated concentrations. For example, one coastal location near an airfield had a ΣPFAS of 677 ng.L^{-1}. Expansion from these ongoing efforts will focus on assessment of PFAS-related effects in local wildlife and evaluating the distribution of PFAS at these "hotspot" sites during large episodic weather events, a critically understudied phenomenon regarding PFAS and vulnerable coastal environments.

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1. Introduction

Emerging contaminants of concern, such as per- and polyfluoroalkyl substances (PFAS), are garnering significant research and media attention, stemming from mounting evidence suggesting adverse environmental and human/wildlife concerns (De Silva et al., 2021; Fenton et al., 2021). PFAS are a class of anthropogenic chemicals comprised in thousands of per- and polyfluorinated aliphatic species, used in a massive array of applications (Buck et al., 2011; Burkhard, 2021; Gluge et al., 2020; OECD, 2018). The unique properties of PFAS (e.g., hydrophobicity) have been widely exploited in many consumer products (e.g., disposable food packaging), cookware, outdoor gear, furniture, and carpet (Gluge et al., 2020; Sunderland et al., 2019; Wang et al., 2017). PFAS are also one of the primary constituents of aqueous film forming foams (AFFF), which are frequently used at airports and military bases for firefighting activities and training exercises (Wang et al., 2017), and thus have subsequently become a significant source of groundwater and drinking water contamination (Banzhaf et al., 2017; Hu et al., 2016). These compounds are considered "forever chemicals" and have become one of the most prevalent and persistent types of contaminants in the world due to their inherently slow environmental degradation (Lewis et al., 2015; Tomy et al., 2004; Wang et al., 2015). Further, due to the strong bond between carbon-fluorine, their amphiphilic properties, and preference to bind with plasma-bound proteins, PFAS have been bioaccumulating within local environments and wildlife since their first production in the 1940s (Armitage et al., 2006; Cui et al., 2020; Prevedouros et al., 2006; Schaider et al., 2017) with relatively little known about the long-term ecological impacts (Ankley et al., 2021). Mixtures of PFAS with different chemical characteristics and biodegradation rates result in different rates of mobility and persistence in aquatic ecosystems (Cui et al., 2020; Guelfo et al., 2021), as well as variable exposure routes and bioaccumulation potential in diverse organisms (Ahrens et al., 2011; Burkhard, 2021; De Silva et al., 2021; Miranda et al., 2021; Savoca and Pace, 2021).

As analytical methods advance and environmental sampling increases, the ubiquity of PFAS in diverse ecosystems and species is apparent (De Silva et al., 2021; Muir and Miaz, 2021). However, there are many potentially impacted locations with minimal PFAS information, specifically coastal environments in North America (Jarvis et al., 2021; Muir and Miaz, 2021). Due to Florida's extensive coastline and vast aquatic ecosystems, including vital estuaries and watersheds, interest toward improving our understanding of the impact of PFAS burden on coastal environments continues to grow. Such environments in Florida are vital and directly linked to recreational, commercial, and industrial activities, many of which are known and/or suspected sources of PFAS. For example, PFAS release can be attributed to the discharge of industrial or municipal wastewater, urban stormwater runoff, and application of chemicals and sprays, e.g., AFFFs, pesticides (Debusk and Box, 2002; Gluge et al., 2020; Lewis et al., 2016). It is worth noting that although perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are currently being considered for designation as hazardous substances, PFAS are not classified as such in the U.S., thus they can enter the waste cycle without any special considerations. In addition to ubiquitous PFAS distribution due to standard use of products and industrial waste (Ahrens et al., 2011), more urbanized areas tend to have higher PFAS densities, as showed in Minneapolis, where concentrations of PFOS ranged between 2.4 to 50.4 ng.L^{-1} in urban areas and between 0.29 to 1.2 ng.L^{-1} in remote areas (Jarvis et al., 2021; Simcik and Dorweiler, 2005). State-level data are currently available for several states, the studies are all corroborative regarding PFAS contamination near areas of industrialization and military and aviation operations (Jarvis et al., 2021). In Colorado, the Department of Public Health and the Environment (2020) measured PFOS in surface water from 71 different sampling locations and the concentration found ranged between 0.42 to 54 ng. L^{-1} (Colorado, 2020; Hu et al., 2016). In 2001, several industrial entities conducted a PFAS study of varied environmental matrices; including water, leachate,

soil, bio-soil and sediment, to determine levels in Florida, with PFOA and PFOS present in two out of the multi-city study: Pensacola and Port St. Lucie (Cui et al., 2020; Jarvis et al., 2021). Additional studies have shown PFAS burdens in groundwater throughout Florida, mostly near areas with military bases and airports, with some contaminated PFAS levels higher than 100,000 ng.L^{-1} (Cui et al., 2020). In 2018, a state-wide investigation was conducted by the Florida Department of Environmental Protection (FDEP) in 45 different locations to evaluate PFAS contamination close to fire training facilities (FDEP, 2020a). Twenty-two out of the 45 facilities detected PFOA and PFOS in surface water, soil and sediment samples (Cui et al., 2020), with three of them located in Pensacola (FDEP, 2020b).

Within the PBS, a multitude of potential sources of PFAS include numerous industrial and aviation-related facilities located in estuarine and coastal locations. Pensacola is surrounded by several military bases and air stations, such as Naval Air Station, Eglin Air Force Base, Hurlburt Field Air Force Base and several diverse industries and wastewater treatment plants. AFFFs are a primary source of PFAS in Florida, and Santa Rosa County is among the counties in FL with the highest detectable levels of PFAS in drinking water, groundwater wells, and surface water (Cui et al., 2020). In a nationwide study, watersheds surrounding Pensacola and Perdido Bay, hereafter Pensacola Bay System (PBS), had detectable levels of PFOA and were among those with the highest levels of PFOS in drinking water (Hu et al., 2016). Drinking water treated locally by Emerald Coast Utilities Authority contained PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS), and perfluoroheptanoic acid (PFHpA) in samples collected between 2013–2015, and PFOS and PFOA were detected in monitoring wells and soil at the Pensacola Fire Department Training Facility (Cui et al., 2020). PFOS and PFOA concentrations were also high in monitoring wells of military bases in the area (Naval Air Station Pensacola, Whiting Field, Saufley Field, and Eglin Air Force Base), and perfluorobutane sulfonic acid (PFBS) was detected at Whiting Field and Eglin Air Force Base (Cui et al., 2020). PFAS have been detected following an extensive legacy use of AFFFs at Tyndall Air Force Base (Moody and Field 1999), and AFFFs were repeatedly discharged and filled hangars at Eglin Air Force Base. Detection of more frequently identified PFAS, as well as the presence of potential sources, motivates further investigation into the PBS. Although common sources and regional high-concentration sites are becoming more widely known, the composition and fate of PFAS mixtures in surrounding surface waters and sediment have not been comprehensively examined. Better identifying the types and concentrations of PFAS relative to the source and mobility will improve capacity to remediate priority sites and reduce contamination by compounds of concern. In this study, we monitored the occurrence and distribution of 51 PFAS to determine region-specific levels and areas of high PFAS burden in the PBS by sampling from 45 different sites. To our knowledge, these are the first baseline concentrations of PFAS in surface water in the Pensacola Bay System.

2. Material and methods

2.1. Chemicals and materials

All PFAS standards were of high purity grade (>90%). A mixture of PFAC-24PAR and individual PFAS standards listed in Table S1 were purchased from Wellington Laboratories Inc (Guelph, ON, Canada). Isotopically-labelled PFAS were used as internal standards (IS), consisting of 23 PFAS (mixture of MPFAC-24ES and individual standards), which were also purchased from Wellington Laboratories Inc (Guelph, ON, Canada). Further details on the nomenclature (and abbreviations) of PFAS can be found in the supplementary material (Table S1). The individual standard solutions, as well as the isotopically-labelled IS solutions, were prepared gravimetrically in methanol. Three primary stock solutions were prepared and used to provide twelve levels of calibration solutions. Table S2 in the supplementary material summarizes the respective concentrations for each level of the calibration curve for both

the non-labeled and isotopically-labeled PFAS (calibration was performed based on isotope dilution method). All solutions were stored at -20 °C. All solvents and chemicals, including water, methanol, ammonium acetate and ammonium hydroxide (all Optima grade) were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). Industrial grade nitrogen, used for drying extracts, was obtained from Airgas (USA).

2.2. Sampling sites and sample collection

Surface water from Pensacola (Florida, USA) and the surrounding region was collected in February 2020, further details concerning each sampling site, including pH, temperature, salinity and dissolved oxygen, are described in Table S3. The monitoring of surface water included 45 sampling sites covering the region in transects across major water bodies (East Bay, Escambia Bay, Perdido Bay, Santa Rosa Sound) both upstream and downstream from potential sources of PFAS, including highly urbanized areas like airports, military bases, and industries, as well as less-developed areas like parks and beaches. Water samples were collected in 500 mL high density polyethylene (HDPE) bottles. Each bottle was rinsed with the surface water three times prior to collection. Samples were kept on ice for transport to a -20 °C freezer until analysis.

Sampling site longitude (X) and latitude (Y) coordinates were obtained using a navigation grade GPS receiver. The sample site identification number and site coordinates were transferred to an Excel worksheet. Corresponding sampling site data analysis results were added to the worksheet and the sheet was formatted for use in a geographic information system (GIS). ArcGIS© v17 spatial software, made by Environmental Systems Research Institute (ESRI), was used to visualize the locations and surface water test analysis results. Test results were visualized using symbol size and colors based upon five natural breaks range classifications.

2.3. Surface water extraction

Surface water samples were thawed prior to solid phase extraction (SPE), as previously described (Robey et al., 2020). Sample pH was adjusted to 4 using glacial acetic acid, followed by spiking of the IS mixture (45 μL). The cartridges used for solid phase extraction were Strata-X-AW (Polymeric Weak Anion Exchange, 500mg, 100 μm, 6 cc) and were purchased from Phenomenex (Torrence, CA, USA). They were fit with reservoirs, and glass filters were added into each reservoir. The glass microfiber filters (GF/F grade, 25mm, 0.7 μm) were purchased from Fisher Scientific (Whatman, USA) and were used for filtration of surface water. Filters were tested prior to sample extraction. For the mentioned test experiment, 500 mL of optima water (n = 6) was spiked with native non-labeled and labeled PFAS at 2000 ng.L^{-1}, before and after the filtering step (each in triplicate). The water passed through the filter and was extracted by following the protocol as noted above. Cartridges were conditioned with 4 mL of 0.3% ammonium hydroxide in methanol followed by the addition of 3 mL of methanol and 4 mL of ammonium acetate/acetic acid aqueous buffer solution (pH 4). Sample loading (500 mL of each sample) was performed using a flow of 1–2 drops per second. Cartridges were then washed with 4 mL of ammonium acetate/acetic acid aqueous buffer and were subsequently dried under vacuum for 5 min. PFAS were eluted using 4 mL of methanol followed by 4 mL of 0.3% ammonium hydroxide in methanol into 15 mL falcon tubes. Samples were evaporated down to ~2 mL and an aliquot of 200 μL was transferred into an autosampler vial for analysis. Each sample bottle, containing surface water, was gravimetrically weighed before and after water removal to obtain a total amount of water extracted (in g).

2.4. Analytical methods – extraction and analysis

The analytical method used in this study has been previously described (Robey et al., 2020). Briefly, samples were analyzed using ultra-high performance liquid chromatography (UHPLC) and tandem

mass spectrometry (MS/MS). The UHPLC was fitted with a Vanquish PFAS Replacement Kit, among which included an Acclaim™ 120 C18 (2.1 × 50 mm, 5 μm, 120Å) as a delay column and UHPLC PFAS-free plumbing and hardware to minimize PFAS background. PFAS were separated using a Gemini C18 column (100 mm × 2 mm; 3μm particle size) preceded by a C18 guard column, both supplied by Phenomenex (Torrence, CA, USA) and water (A) and methanol (B) both containing 5 mM of ammonium acetate as solvents. The gradient elution was set as follows: 0–3 min 10 % B, 3–4.5 min 10–35 % B, 4.5–12.5 min 35–95 % B, 12.5–12.51 min 95–99 % B, 12.51–19 min 99 % and then equilibrated back to initial conditions in 30 min. Temperatures were set at 40 °C (column) and 4 °C (autosampler), flow rate and injection volume (randomized sequence) were 0.5 mL min^{-1} and 10 μL, respectively. A TSQ Quantis triple quadrupole was used in selected reaction monitoring mode (SRM) in negative polarity with the following parameters: ion spray voltage -1500 V with sheath and auxiliary gas set to 50 and 10 arb, respectively. Ion transfer tube temperature was set at 250 °C, while the vaporizer temperature was maintained at 550 °C. PFAS transitions and additional parameters used for these experiments are included in Table S4.

2.5. Quality assurance and quality control

For quality assurance performance, solvent blanks, in triplicate, were analyzed prior to any analysis. Field blank samples (n = 3) were analyzed to investigate PFAS background. The workflow was evaluated using in-house quality control (QC) samples, which were extracted and processed alongside the surface water samples. QC samples were assessed by examining the experimentally derived concentration (and relative error) of a pool of surface water spiked prior to extraction with a known concentration (Ck) of PFAS and comparing the obtained concentration to the theoretical spiked concentration (Cs). The pool was achieved by mixing 200 mL of each sampling site.

$$Relative\ Error\ (\%) = \frac{Cs}{Ck}*100$$

Two different PFAS concentrations were performed in triplicate for the QC test (2000 and 20000 ng.L^{-1}), note this concentration was obtained after preconcentration. Precision was determined by calculating the relative standard deviation (RSD) from the QC tests.

Recovery experiments were also performed by spiking a known concentration of PFAS (non-labeled and labeled compounds) before and after extraction using the pooled surface water (in triplicate). Solvent blanks were included in the sequence after three real samples were analyzed to ensure that no background and/or carry over was detected.

2.6. Data analysis

Data acquisition and peak integration were performed using Xcalibur v.4.1 software (Thermo-Fisher Scientific). SRM transitions were used to detect and quantify PFAS, with the most intense transition used to quantify the PFAS while the second transition was used to confirm the identification (if applicable). For non-labeled PFAS without a labeled analogue, an alternative labeled standard closely related by structure and/or retention time was used. Table S1 summarizes the PFAS and respective IS used for quantification. A linear regression model was used to build the calibration equation for each PFAS, and the intercept, slope and correlation coefficient r^2 were calculated. A total sum of isomers is presented for perfluorohexanoic acid (PFHxS) and PFOS (as ΣPFHxS and ΣPFOS, respectively), as these compounds were monitored as isomeric mixtures in the calibration curves. Extraction recovery was investigated by comparison of the peak area of the replicates of the pooled samples spiked before and after SPE extraction (shown as %). Method detection and quantification limits were defined as the minimum concentration that would yield (visually) a detectable chromatographic peak with

signal-to-noise ratio of 3 (S/N > 3) and a S/N > 10 for limit of quantification (LOQ).

3. Results and discussion

3.1. Method performance parameters

Pensacola surface water samples were heterogeneous and contained suspended solids, which were removed by using glass filters prior to SPE. Recovery experiments, using optima water, PFAS standards and filters, were performed in order to verify the feasibility of the glass filters in the following study. Relative recovery (in percentage) is summarized in Table S5. Glass filter evaluation showed a low capacity of retention for PFAS (recoveries ranging from 71 to 106%), which allowed their use in further experiments. The overall analytical workflow showed satisfactory performance in terms of linearity (correlation coefficients (r^2)), equal to or higher than 0.9952 for all compounds, except for diSAmPAP, which presented 0.9539 (Table S6). Among all 51 target compounds, sensitivity (limits of detection (LODs)) was achieved in the low ng.L^{-1} range (<20 ng.L^{-1}), except for FBSA (80.7 ng.L^{-1}), N-AP_FHxSA (32.5 ng.L^{-1}), NADONA (76.9 ng.L^{-1}), and all FTCA compounds (6:2 FTCA, 8:2 FTCA and 10:2 FTCA), which had values around 35.0 ng.L^{-1}. Table S6 summarizes additional analytical quality control parameters used for monitoring PFAS in the surface water. The limits obtained are related to instrument limits. Isotope dilution was used for a majority of the target PFAS measured in this study. Labeled and native PFAS exhibited reasonable similarity for extraction (as observed in Table S6—recoveries). For mass spectrometric analysis, sequential injections of methanol (n = 3) were performed prior to starting the batch and were continued to be examined throughout the sample queue to assess any carry over and/or background.

A pooled surface water sample (comprised of water from 45 sites) was prepared and spiked with PFAS standards in order to calculate recovery rates. Another set of the same pool (n = 3) was reserved as well (no spiking of non-labeled compounds). Based on the accepted range of recovery (70–120%), 35 out of 51 compounds were recovered in this range. Although some compounds presented recoveries lower than 70% (16 out of 51 compounds), the repeatability (measured by relative standard deviation, RSD) for these compounds was lower than 10%, except to N-EtFOSA-M (15%), PFDoS (12%), PFHxDA (13%), PFTeDA (19%), N-MeFOSAA (23%) and PFTrDA (18%). For this study, accepted values for repeatability were set to be below 20%, which was consistent with the data obtained. N-MeFOSAA presented 23% and was not detected in any of the samples analyzed.

The QC samples were evaluated through the spiked surface water pooled sample. The results are summarized in Table S7. Method precision values were below 20% (33 out of 51 compounds), except for 3 of the 51 compounds in both concentrations, PFTrDA (21%), PFTeDA (22%) and 6:2/8:2diPAP (20%) for QC1 and PFTrDA (25%), PFODA (23%) and diSAmPAP (23%) for QC2. All parameters observed are in accordance with the acceptance criteria for surface water. The most common compounds detected: PFOA, ∑PFOS, PFHxA, PFHpA, 6:2FTS, ∑PFHxS and PFBS all have performance values denoting good accuracy and precision. The exception was PFTeDA, which was out of the range but was detected in three samples. For this reason, this compound was excluded from all the calculations presented in this study.

3.2. Concentration of PFAS in surface water

Of the 51 PFAS monitored, 21 were detected in three or more samples (Table 1). The levels of PFAS detected varied considerably between PFAS classes (Figure S1). ∑PFOS and 6:2FTS were found at higher concentrations, up to 269 ng.L^{-1} and 164 ng.L^{-1}, respectively. The greatest median and maximum concentrations were ∑PFOS, which is representative of PFAS occurrence in aquatic ecosystems worldwide due to the structural properties and extensive use of these compounds (Jarvis et al.,

2021). The third most concentrated compound was ∑PFHxS (maximum = 59.3 ng.L^{-1}), followed by PFPeA (maximum = 51.9 ng.L^{-1}) and PFHxA (maximum = 42.5 ng.L^{-1}). These compounds have been frequently detected in surface water studies in South Florida (Li et al., 2022). The highest concentrations of ∑PFOS, 6:2FTS, ∑PFHxS, PFPeA, and PFHxA were all from the same location. The remaining PFAS were found in much lower levels (low ng.L^{-1} range), except for PFOA (maximum = 19.0 ng.L^{-1}) and PFHpA (maximum = 16.7 ng.L^{-1}). Despite PFDoA being detected in fourteen samples, it was the PFAS detected at the lowest median and maximum concentrations (Figure S1). All PFAS detected in each sample are summarized in Table S8.

∑PFCA was the PFAS class detected at the highest concentrations (median = 7.17 ng.L^{-1}), which corresponded to 34% of total PFAS

Table 1. Summary of the results (ng.L^{-1}) obtained among all 45 sampling sites, in Pensacola area.

Compound Class (Number of analytes)[#]	Analyte	Frequency	Concentration (ng.L^{-1}) Median	Minimum	Maximum
PFCA (13)	PFBA	25	1.24 ± 1.88	0.83	10.5
	PFPeA	19	2.98 ± 11.5	0.53	51.9
	PFHxA	45	0.66 ± 6.39	0.22	42.5
	PFHpA	44	0.51 ± 2.48	0.23	16.7
	PFOA	45	0.97 ± 3.36	0.30	19.0
	PFNA	43	0.21 ± 0.63	0.13	4.32
	PFDA	38	0.09 ± 0.38	0.04	2.19
	PFUdA	6	0.33 ± 0.09	0.29	0.50
	PFDoA	14	0.03 ± 0.02	0.02	0.10
	PFTeDA	3	0.15 ± 0.07	0.14	0.27
PFSA (9)	PFPrS	13	0.19 ± 0.45	0.12	1.83
	PFBS	44	0.74 ± 1.13	0.34	6.03
	PFPeS	26	0.23 ± 1.66	0.08	8.56
	∑PFHxS	45	1.22 ± 8.82	0.27	59.3
	PFHpS	10	0.15 ± 0.48	0.09	1.66
	∑PFOS	45	3.29 ± 39.7	0.68	269
FTS (4)	6:2FTS	45	0.72 ± 24.4	0.20	164
	8:2FTS	3	0.20 ± 2.39	0.12	4.30
FASA (9)	FBSA	3	2.44 ± 2.21	1.31	5.57
	FHxSA	12	2.05 ± 4.89	0.09	16.0
diPAP (4)	6:2diPAP	20	2.75 ± 1.49	0.09	5.75
FTCA (3)	NF				
FTUCA (2)					
PFPi (2)					
Other (6)					

[#] Number of analytes measured from each class in the described method; a full list of abbreviations can be found in supplementary material Table S1.

present in the samples (Figure S2). Of the thirteen PFAS monitored in the PFCA group (Table 1), PFPeA had the highest median concentration (2.98 ng.L^{-1}), followed by PFBA (1.24 ng.L^{-1}), PFOA (0.97 ng.L^{-1}), PFHxA (0.66 ng.L^{-1}) and PFHpA (0.51 ng.L^{-1}). PFSA was the second most abundant PFAS class detected (5.83 ng.L^{-1}), which corresponded to 28% of total PFAS detected. Among the nine compounds present in the PFSA class, $\sum$PFOS was the compound which contributed the most (median of 3.29 ng.L^{-1}). The second highest detected PFAS in the PFSA class was $\sum$PFHxS at 1.22 ng.L^{-1}, followed by PFBS (0.74 ng.L^{-1}). FASA corresponds to 21% (4.50 ng.L^{-1}) of PFAS detected, of which FBSA (2.44 ng.L^{-1}) was the compound with highest concentration. 6:2diPAP (2.75 ng.L^{-1}) represented the diPAP class and corresponded to 13% of PFAS. The remaining 4% of PFAS was detected in the FTS class (0.92 ng.L^{-1}), 6:2FTS (0.72 ng.L^{-1}) being the FTS with the highest concentration. All numbers are presented as a median of concentrations obtained. Further details concerning the contribution of each compound per site can be found in Supplemental Material, Table S8.

3.3. Distribution of PFAS in pensacola, Florida

Among all 45 sites, eight or more PFAS were quantified, with total PFAS ($\sum$PFAS) present categorized in Figure 1. The compounds $\sum$PFOS, 6:2FTS and $\sum$PFHxS were found at all sites analyzed (100% of frequency), and their relative concentrations within the area was similar (Figure S3) with the exception of the relatively higher concentrations of 6:2FTS at sites 8 and 36. The site with the most PFAS detected (by number of species) was site 2 (17 compounds), and the fewest detections were at sites 4, 12 and 28 (8 PFAS detected). Site 81 contained the highest concentrations of total PFAS as well as $\sum$PFOS, 6:2FTS, $\sum$PFHxS, PFPeA, and PFHxA. PFOS was also present in sediment collected at this site (Ahmadireskety et al., 2021).

The highest concentration of total PFAS at Site 81 (677 ng.L^{-1}), was largely driven by a high amount of the individual concentrations at this site, e.g., ΣPFOS (269 ng.L^{-1}), followed by 6:2FTS (164 ng.L^{-1}), $\sum$PFHxS (59.3 ng.L^{-1}), PFPeA (51.9 ng.L^{-1}) and PFHxA (42.5 ng.L^{-1}); Figure 1; Table S8. Site 81 had extensive foam observed on the shoreline as well. The $\sum$PFOS concentration is higher than maximum levels

reported in earlier studies of U.S. surface waters that range between 43-244 ng.L^{-1} (EWG, 2019; SSEHRI, 2020), demonstrating the persistence of these legacy chemicals and/or their varied sources of release, despite PFOS production ceasing in 2002 in North America (Jarvis et al., 2021). The second most concentrated PFAS found at this site was 6:2 FTS, which has been used as a substitute for PFOS in metal plating (Houtz, 2016). Over time, 6:2FTS can degrade into PFPeA and PFHxA (Houtz, 2016), which are also found at high concentrations at this site. Site 81 is in close proximity to Hurlburt Field Air Force Base, offering one possible explanation for the high concentrations of PFAS (e.g., use of AFFFs) at this site (Leeson et al., 2021). According to the data obtained and studies conducted since 1980, high concentration of PFAS are expected in areas surrounding military bases (Hu et al., 2016; Moody and Field 1999).

Site 7 presented the second highest concentration among the sampled sites (Figure 1). The sum of the total PFAS detected at this site was 71.6 ng.L^{-1}, with $\sum$PFOS (20.9 ng.L^{-1}) being the PFAS with the highest concentration, followed by PFOA (19.0 ng.L^{-1}). Site 7 is located in Perdido Bay, designated by the state of Florida as "Outstanding Florida Water" due to its natural attributes, thus highlighting its importance for protection from pollution (Figure 1B). Perdido Bay sites in close proximity to the Perdido River had relatively high concentrations of $\sum$PFAS (sites 7–71.6 ng.L^{-1}, 16–43.2 ng.L^{-1}, 17–20.0 ng.L^{-1}, 18–27.8 ng.L^{-1}, and 19–22.7 ng.L^{-1}), as did sites in the central Perdido Bay area (sites 14–44.5 ng.L^{-1} and 15–35.5 ng.L^{-1}). Together, these sites contain 265.31 ng.L^{-1} (expressed as sum) of total PFAS detected. The primary PFAS contributions to regional contamination were PFOA, PFNA, PFHpA and PFDA (in addition to the presence of PFOS and $\sum$PFHxS at site 7; Figure 2). Surface water in the Perdido Bay is fed by river, creeks, and tributaries. Wastewater and paper mill effluent are a major suspect for possible point of sources of PFAS pollution in this area, along with residential development and runoff (Kirschenfeld et al., 2006). Paper and textile manufacturers can contribute to PFOS pollution, for example N-ethyl perfluorooctane sulfonamido acetic acid (N-EtFOSAA) is often used and it can be degraded into PFOS (Wen et al., 2018). PFAS are also commonly found due to release from wastewater treatment plants, due to manufacturing, industrial and household wastewater (Hamid et al., 2018). Among all the PFAS detected, the most frequently found were
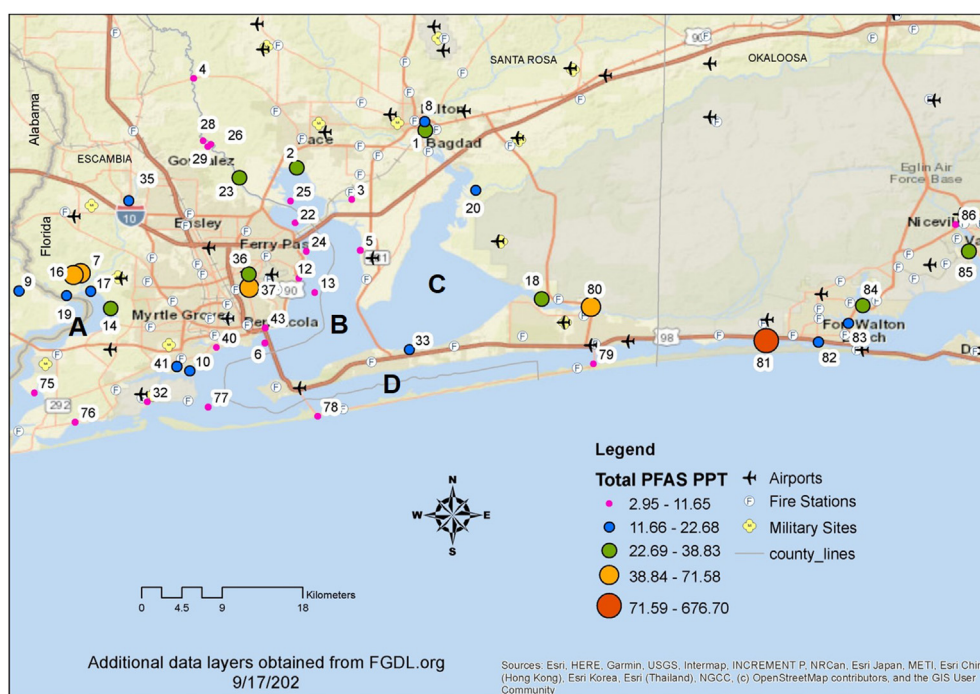

Figure 1. Map of Perdido Bay **(A)**, Pensacola Bay – comprising Escambia Bay **(B)** and East Bay **(C)**, and Santa Rosa Sound **(D)**. Sampling sites were generated using ArcGIS© v17 and the coordinates showed in Table S3. The graphic represents ΣPFAS present at the specific sites collected.

PFOS, PFOA and PFHxA (Rodriguez-Jorquera et al., 2016), which matched with the surface water measured in the present study. Figure 2 illustrates the concentrations of the most frequent PFAS detected in this area. A concurrent study found sediment PFOS and PFBA values, as well as total PFAS, to be relatively high in this area as well (Ahmadireskety et al., 2021).

In eastern Pensacola, there is a large, urbanized area, which contains several parks, industries, airports and military activities (Figure 1). In this central area, two main sites, 36 and 37, contained 34.1 ng.L^{-1} and 42.5 ng.L^{-1} of the total PFAS, respectively. Pensacola has a variety of locations that are popular for many outdoor activities including Bayou Texar, an estuary that is fed by Carpenter's Creek and empties into Pensacola Bay. Sites 36 and 37 are upstream of Bayou Texar, are used industrially as well as recreationally and are also close to Pensacola International Airport. The most concentrated PFAS detected in this area was 6:2FTS (21.2 ng.L^{-1}) at site 36 and $\sum$PFOS (9.88 ng.L^{-1}) at site 37 (Figure S3). Although site 36 has a higher concentration of an individual PFAS, site 37 has higher number of PFAS compounds detected, albeit at lower concentrations. These concentrations are higher than those obtained at site 43 (5.54 ng.L^{-1}), downstream of Bayou Texar, which is the last site prior to entry into Pensacola Bay. This fact could be attributed to the higher volume of water at Pensacola Bay, when compared to sites 36 and 37. Others sites that empty into Pensacola Bay, like sites 40 ($\sum$PFAS: 8.07 ng.L^{-1}), 41 ($\sum$PFAS: 19.5 ng.L^{-1}) and 10 ($\sum$PFAS: 14.6 ng.L^{-1}), had concentrations of $\sum$PFAS higher than those collected directly in Pensacola Bay. For example, sites 6 and 77 were located in the middle of Pensacola Bay and presented the concentration of $\sum$PFAS at 4.01 and 4.20 ng.L^{-1}, respectively. Even with high contributions of each tributary, creek and lagoon, the large volume and size of Pensacola Bay may cause a possible dilution effect that maintains the total PFAS concentration lower. In both sites (6 and 77), $\sum$PFOS was the most abundant PFAS quantified. Pensacola Bay is a tourist destination due to the beaches, parks, restaurants and history. As PFAS levels are highlighted in the area, this study supports the need for more investigations to understand the sources of PFAS, overall burdens, and the potential complications associated with fate and transport within a coastal environment.

Escambia Bay has a total of 7 sites (2, 5, 12, 13, 22, 24 and 25) that were sampled, with low $\sum$PFAS levels. All sites had concentrations of $\sum$PFAS below 10 ng.L^{-1} with the exception of site 2, in which 17 PFAS yielded a total concentration of 28.7 ng.L^{-1} of PFAS. Site 2 is located north of Escambia Bay, surrounded by an urbanized area and is also close to Spencer Naval Outflying Field, which may be a possible contributor to the $\sum$PFOS (12.4 ng.L^{-1}) and 6:2FTS (3.00 ng.L^{-1}) detected at the site. The concentrations of $\sum$PFAS at middle Escambia Bay were 4.46 ng.L^{-1} (site 22), 8.80 ng.L^{-1} (site 24) and 6.00 ng.L^{-1} (site 25), from the west side and 3.74 ng.L^{-1} (site 5) from the east side. At the lower sites 12 and 13, the concentration of $\sum$PFAS were 7.34 and 6.77 ng.L^{-1}. The amount of PFAS in the middle and downstream of Escambia Bay were lower than upstream (site 2), possibly associated with the dilution effect of the higher volume of the water than the related exposure of these contaminants. In Northeast Escambia Bay, the Escambia River empties into the bay around site 25. Upstream Escambia River, several sites were sampled: 26 ($\sum$PFAS = 4.18 ng.L^{-1}), 28 ($\sum$PFAS = 2.95 ng.L^{-1}) and 29 ($\sum$PFAS = 4.79 ng.L^{-1}). In all of these sites, the most concentrated PFAS detected was $\sum$PFOS, with concentrations around 1.00 ng.L^{-1}. Site 23, located at the end of the Simpson River (which empties into Escambia River), contained a higher concentration of PFAS, $\sum$PFAS = 28.1 ng.L^{-1}, with a total of 16 PFAS detected and with 11 out of 16 present at concentrations greater than 1.00 ng.L^{-1}. The highest concentration at this site was $\sum$PFOS (3.72 ng.L^{-1}), followed by 6:2diPAP (3.43 ng.L^{-1}), PFPeA (2.98 ng.L^{-1}) and PFOA (2.86 ng.L^{-1}).

Site 1 presented a total PFAS of 32.6 ng.L^{-1}. Sites 1 and 8 are located in the Blackwater River in Milton, which passes through Blackwater River State Forest and Blackwater River State Park. Site 8 is located near Russell Harber Landing Park and had a total PFAS of 22.1 ng.L^{-1}. The main PFAS present at site 8 were 6:2FTS (11.6 ng.L^{-1}; Figure S3) followed by PFOA (3.39 ng.L^{-1}; Figure 2). Site 1 is located below the Blackwater River Water Management Area and had detectable concentrations of PFPeA (7.59 ng.L^{-1}) and FHxSA (6.23 ng.L^{-1}), both of which were highest at this site. Because $\sum$PFOS was the most abundant PFAS in almost all the areas analyzed, the elevated levels of PFPeA and FHxSA suggests a different source of contamination in this area.
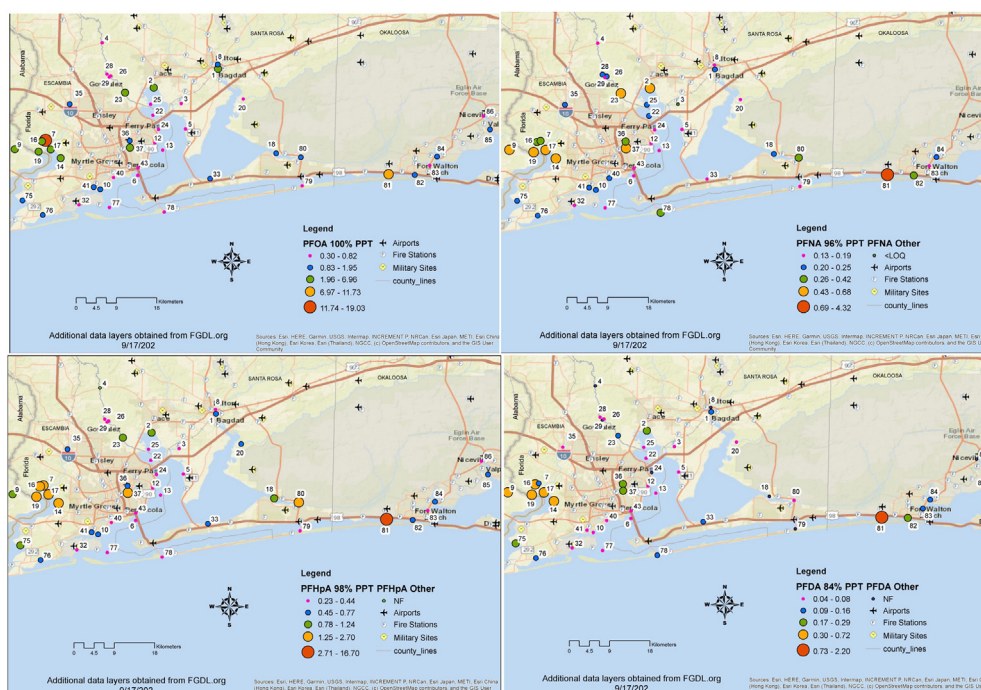

Figure 2. Maps of PFCAs present at sites analyzed and their respective concentrations. Detection frequencies for PFOA were 100% (upper left), for PFNA 96% (upper right), for PFHpA 98% (lower left) and for PFDA 84% (lower right).

The East River is represented by site 80 (east) and 18 (west) and is located in Santa Rosa County, Florida. It flows from east to west, forming near Hurlburt Field and emptying into the eastern portion of the East Bay. Site 80 is one of the most concentrated sites (Figure 1), with a total PFAS of 47.7 ng.L^{-1} from 14 PFAS detected (Table S8). The most abundant PFAS found at this site was $\sum$PFOS (17.6 ng.L^{-1}; Figure S3). This result is in accordance with the findings for site 81 located near Hurlburt Field, which had the highest amount of $\sum$PFOS quantified. As this river begins at the same location and flows west, Hurlburt Field may contribute to the contamination at site 80. The same phenomenon was observed for site 18, which presented a total PFAS of 27.8 ng.L^{-1}, with $\sum$PFOS being the most concentrated PFAS for this site (8.43 ng.L^{-1}).

The remaining two sites highlighted in Figure 1 (which presented concentrations of PFAS higher than 30.0 ng.L^{-1}), are sites 84 and 85. Site 85 (32.7 ng.L^{-1}) is located at Tom's Bayou in Valparaiso, Florida, while site 84 (30.5 ng.L^{-1}) is located due west. Both sites are located close to Destin Fort Airport and Eglin Air Force Base. At both sites, 16 PFAS were quantified, but the most abundant compound found at site 84 was $\sum$PFOS (16.2 ng.L^{-1}) while FHxSA (11.1 ng.L^{-1}) was highest at site 85.

4. Conclusion

Forty-five sites were monitored in the PBS. Of the 51 PFAS monitored, 21 PFAS were quantified, which included 10 PFCA, 6 PFSA, 2 FTS, 2 FASA and 1 diPAP. In all sites, at least 8 PFAS were quantified at every site. The concentrations of individual PFAS ranged from 0.02 ng.L^{-1} (PFDoA) to 269 ng.L^{-1} ($\sum$PFOS) and were comparable with total PFAS concentrations found in surface water for other coastal Florida locations (Li et al., 2022). The sum of total PFAS presented in all sites ranged from 2.95 ng.L^{-1} to 1.47 μg.L^{-1}. $\sum$PFOS, as a known legacy chemical, is highly present within the PBS environment. The sum of total PFAS detected per each sampling site was lower than 50.0 ng.L^{-1}, except at site 81 (677 ng.L^{-1}). The main contribution for the high value detected in site 81 was the presence of $\sum$PFOS (269 ng.L^{-1}). At present, it is challenging to determine the main source of PFAS contamination in any area; however, possible sources of PFAS can be anthropogenic activities associated with military bases, airports, carpet and paper manufacturing, among others. This study showed for the first time the widespread occurrence of PFAS in surface water of Pensacola Bay, which will allow prioritization of sites for future monitoring and perhaps remediation. However, additional information is required for effective remediation techniques as well as determination of the fate of contamination dependent on large episodic storms, seasonal fluctuations, and/or point sources to provide helpful information concerning PFAS contamination.

Declarations

Author contribution statement

Bianca Ferreira da Silva. Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Juan J. Aristizabal-Henao. Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Joe Aufmuth. Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jill Awkerman. Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

John A. Bowden. Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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