FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DEVELOPMENT OF TARGET AND NON-TARGETED ANALYSIS APPROACHES TO CHARACTERIZE EMERGING PFAS AND POTENTIAL PFAS METABOLITES FROM DRINKING AND SURFACE WATER IN FLORIDA

A dissertation submitted in partial fulfillment of

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by

Xuerong Li

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To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Xuerong Li, and entitled Development of Target and Non-targeted Analysis Approaches to Characterize Emerging PFAS and Potential PFAS Metabolites from Drinking and Surface Water in Florida, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: October 24, 2022

The dissertation of Xuerong Li is approved.

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Florida International University, 2022

DEDICATION

I dedicate this dissertation to all my family and friends

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ABSTRACT OF THE DISSERTATION

DEVELOPMENT OF TARGET AND NON-TARGETED ANALYSIS APPROACHES TO CHARACTERIZE EMERGING PFAS AND POTENTIAL PFAS METABOLITES FROM DRINKING AND SURFACE WATER IN FLORIDA

by

Xuerong Li

Florida International University, 2022

Miami, Florida

Professor Natalia Quinete, Major Professor

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic pollutants found ubiquitously in surface and drinking water supply. Due to their persistent nature, bioaccumulative potential, and significant adverse health effects, they pose a concern for human and environmental exposure. The first chapter of the dissertation focused on the development and validation of a target analysis method based on a semi-automated solid phase extraction followed by liquid chromatography-mass spectrometry for the determination of legacy and emerging short-chain PFAS at low parts-per-trillion levels, which was applied to surface waters from Biscayne Bay canals and tap waters from different counties in South Florida (N=36). Total PFAS concentrations of up to 242 ng L^{-1} in tap water and 106 ng L^{-1} in surface water raised human health and ecological concerns due to the elevated levels.

The second chapter of this dissertation expanded the PFAS monitoring study on the occurrence, composition, spatial and seasonal distribution, and potential sources encompassing tap waters from counties on the East coast of South Florida and Central

Florida, and surface waters from Tampa Bay. PFAS were detected in all tap water and surface water samples (N=38), with higher concentrations associated with polluted waterways in Biscayne Bay and sites nearby military airbases and airports. The current findings on PFAS contamination levels from diverse aquatic environments provide additional information for the development of more stringent screening levels that are protective of human health and Florida's environmental resources.

The last chapter of this dissertation focused on developing a non-targeted analysis (NTA) approach based on high-resolution mass spectrometry for a more comprehensive characterization of total PFAS, including degradants and transformation products present in environmental samples that were not measured by target analysis. A total of over 500 PFAS were tentatively identified in drinking and surface waters in South Florida. A semi-quantitation method for NTA (qNTA) was also achieved for the estimation of total PFAS concentration in the samples. A full assessment combining both target and non-targeted approaches play a crucial role in the understanding the diversity of PFAS species in environmental samples, which is needed to better evaluate their toxicological and potential impacts.

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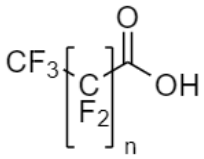
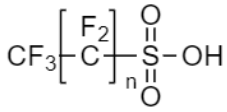
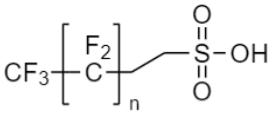
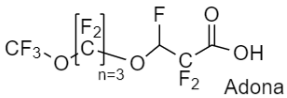
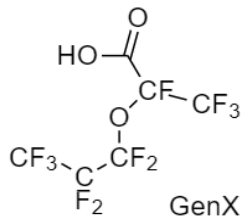
Chapter 1. Introduction

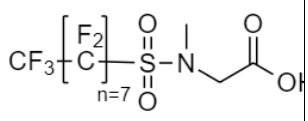
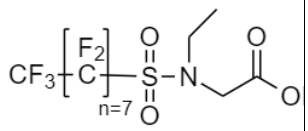
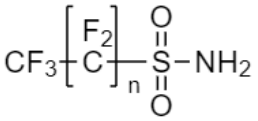
1.1 PFAS and terminology

Polyfluoroalkyl and perfluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been detected globally in the aquatic system, flora, and fauna as one of the chemicals of emerging concern (CECs) that have potentially significant impact on human health and aquatic life (Ahrens & Bundschuh, 2014; Ghisi et al., 2019; Giesy & Kannan, 2001). The terminology of PFAS was defined as chemicals containing hydrophobic alkyl chain with the hydrogen atoms partially (Polyfluoroalkyl substances) or completely (Perfluoroalkyl substances) replaced by fluorine atoms (F) in their chemical structure (Buck, Franklin, et al., 2011). Thus, they usually present the perfluoroalkyl moiety as C_nF_{2n+1}, as well as a functional head group in the structure. PFAS are classified into numerous families based on the functional groups with thousands of individual members. The most common families, perfluoroalkyl carboxylic acids (PFCA), which have varied number of carbon alkyl chain (N≥3) with saturated fluoride and a hydrophilic carboxylate group: perfluorobutanoic acid (PFBA; N=3), perfluoropentanoic acid (PFPeA; N=4), perfluorohexanoic acid (PFHxA; N=5), perfluoroheptanoic (PFHpA; N=6), Perfluorooctanoic acid (PFOA; N=7), etc. The members within the same families only differing in the number of CF_2 units are referred to as homologous series. The common families also include perfluoroalkane sulfonic acids (PFSA), fluorotelomer substances (FTS), perfluoroether carboxylic acid (PFECA), perfluoroalkane sulfonamido acetic acid (FOSAA), perfluoroalkane sulfonamides (PFOSA), etc., as shown in Table 1.1.

Table 1.1 The compound name, chemical formula, abbreviation (Abbr), and chemical structure of some common classes of PFAS species.

Compound Class and Chemical structure n: number of CF2 units	**Compound Name**	**Abbr.**	**Chemical formula**	**n**
Perfluoroalkyl carboxylic acid (PFCA)	Perfluorobutanoic acid	PFBA	C_3F_7COOH	2

	Perfluoropentanoic acid	PFPeA	C_4F_9COOH	3
	Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	4
	Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	5
	Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	6
	Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	7
	Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	8
	Perfluoroundecanoic acid	PFUdA	$C_{10}F_{21}COOH$	9
	Perfluorododecanoic acid	PFDoA	$C_{11}F_{23}COOH$	10
	Perfluorotridecanoic acid	PFTrDA	$C_{12}F_{25}COOH$	11
	Perfluorotetradecanoic acid	PFTeDA	$C_{13}F_{27}COOH$	12
Perfluoroalkyl sulfonic acid (PFSA) 	Perfluorobutane sulfonic acid	PFBS	$C_4F_9SO_3H$	3
	Perfluoropentane sulfonic acid	PFPeS	$C_5F_{11}SO_3H$	4
	Perfluorohexane sulfonic acid	PFHxS	$C_6F_{13}SO_3H$	5
	Perfluoroheptane sulfonic acid	PFHpS	$C_7F_{15}SO_3H$	6
	Perfluorooctane sulfonic acid	PFOS	$C_8F_{17}SO_3H$	7
	Perfluorononane sulfonic acid	PFNS	$C_9F_{19}SO_3H$	8
	Perfluorodecane sulfonic acid	PFDS	$C_{10}F_{21}SO_3H$	9
Fluorotelomer sulfonic acid (FTS) 	4:2 Fluorotelomer sulfonic acid	4-2 FTS	$C_4F_9C_2H_4SO_3H$	3
	6:2 Fluorotelomer sulfonic acid	6-2 FTS	$C_6F_{13}C_2H_4SO_3H$	5
	8:2 Fluorotelomer sulfonic acid	8-2 FTS	$C_8F_{17}C_2H_4SO_3H$	7
Perfluoroether carboxylic acid (PFECA)  	Perfluoro-2-methyl-3-oxahexanoic acid	GenX	$C_6F_{11}O_3H$	-
	4,8-Dioxa-3H-perfluorononanoic acid	Adona	$C_7H_2F_{12}O_4$	-
Perfluoroalkane sulfonamido acetic acid (FOSAA)	N-Methyl perfluorooctane sulfonamido acetic acid	N-MeFOSAA	$C_8F_{17}SO_2N[CH_3]CH_2COOH$	-

 	N-Ethyl perfluorooctane sulfonamido acetic acid	N-EtFOSAA	$C_8F_{17}SO_2N[C_2H_5]CH_2COOH$	-
Perfluoroalkane sulfonamide (PFOSA) 	Perfluorobutanesulfonamide	FBSA	$C_4F_9SO_2NH_2$	3
	Perfluorohexanesulfonamide	FHxSA	$C_6F_{13}SO_2NH_2$	5
	Perfluorooctanesulfonamide	FOSA	$C_8F_{17}SO_2NH_2$	7

Here are some common terminologies often used when describing PFAS:

Non-polymers and Polymers: There are fluorinated polymers containing perfluoroalkyl moieties that may fall under PFAS, however, since polymers have high molecular weight and distinct properties, they were not in the scope of most PFAS studies and are often referred to as "fluoropolymers" (Buck et al., 2011).

Chain length: Long chain PFAS often refers to compounds with 7 or more perfluorinated carbons; Short chain refers to compounds with less than 7 perfluorinated carbons (Buck et al., 2011).

Legacy and emerging PFAS: Legacy PFAS usually refers to the long-chain PFAS that has been phased out of production due to health concerns, such as PFOA, PFOS, and their precursors and degradants. Emerging PFAS are the species with a shorter chain that were introduced to the market as "safer" replacements for the legacy PFAS, such as short-chain PFSA (e.g., PFBS) and PFCA (e.g., PFBA, PFPeA). Some other emerging PFAS are 3H-perfluoro-3-[(3-methoxy-propoxy) propanoate] (ADONA) and hexafluoropropylene oxide (HFPO) dimer acid (GenX) that has ether bond in between the carbon chain, as well as species incorporating cyclic fluorinated carbon chain and H- or Cl- substituted short PFAS (Dhore & Murthy, 2021; Gao et al., 2020).

Nowadays, the universe of PFAS has been encompassing more and more species in this group as many unknown or emerging PFAS are being identified. The Organization for Economic Co-operation and Development (OECD) has recently published a report that defines "Any chemical with at least a perfluorinated methyl group ($-CF_3$) or a perfluorinated methylene group ($-CF_2-$) is a PFAS" with over 4700 PFAS (OECD, 2021)(Buck et al., 2021) (Wang et al., 2021). The United States Environmental Protection Agency (U.S EPA) had consolidated a list of PFAS called PFAS Master List of PFAS substances which also includes partially fluorinated substances, and polymers from various sources with over 12,000 species (EPA, 2021a).

1.2 PFAS chemical properties and applications

PFAS are synthesized and valued in the production of consumer products and industrial applications globally since the 1940s due to their unique chemical properties (Baran, 2001). PFAS are amphiphilic chemicals due to their hydrophobic carbon chain and hydrophilic head groups (Rayne & Forest, 2009). They are also extremely thermal and chemical stable from strong bonding between carbon and fluorine atoms and often refer to as the "forever chemicals". As more hydrogen atoms are substituted by fluorine atoms, the compounds become more chemically inactive to heat, acid, base, reducing agents, and oxidants. PFAS are extensively used as surfactants or surface protections due to their ability to reduce the aqueous surface tension at very low concentrations compared to traditional hydrocarbon-based surfactants (Buck, Murphy, et al., 2011). Especially they are able to function in extreme conditions that require wide temperature and chemical tolerance. Though PFAS are costly to produce and associated with health concerns, they still play an important role in industrial applications and are still unreplaceable for certain uses. They are produced in large volumes as surfactants, wetting agents, emulsifiers, solvents, foaming agents, repellents, and coating in aerospace (hydraulic fluid, coating, etc.), building and construction (cement additive, cable, and wire insulation, etc.), chemical industry (inert reaction media,

solvents), electroplating, solar cells and batteries, mining, oil and gas transport, production of plastic and rubber, automotive (car body coating, engine oil coolers, etc.), aqueous fire-fighting foam (AFFF) and flame retardants, music instruments (guitar strings, pianos), paper and packaging (as water and oil repellent), cosmetics, pesticides, pipes and liners, etc. (Gï et al., 2020 ; Buck et al., 2021). Over 20,000 tons of PFAS were used in the production of plastic and rubber, electronic industry, coating and paints, lubricants and greases, building and construction in Sweden, Finland, Norway, and Denmark between 2000 and 2017 (SPIN database). In the US. thousands of tons of PFAS are used as functional fluids in machinery and appliance manufacturing since 2016 (Chemical Data Reporting under the TSCA).

1.3 PFAS distribution and fate in the environment

PFAS can be introduced into the environment throughout their whole product life cycle, during production, processing, product usage, and disposal (Ahrens & Bundschuhyz, 2014). As illustrated in Figure 1.1, the direct emission sources are released directly from fluorochemical manufacturing facilities, industrial and municipal sewage treatment plants (STP), landfill sites, facilities where PFAS-containing AFFF are used (commercial and military airfields, firefighting training areas, etc.), and the usage of PFAS contained in consumer and industrial products (Banzhaf et al., 2017). Releasing of PFAS into the environment can also be through non-point sources of leaching and runoff from the contaminated area as well as wet and dry atmosphere deposition, such as from rainwater (Ahrens & Bundschuhyz, 2014; Ahrens, 2011; Pfotenhauer et al., 2022; Sammut et al., 2017). Direct emission from STP effluent and indirect emission from runoffs from highly populated or industrialized areas are transported by the river surface water to coastal seawater and then enter the marine environment, as well as partitioning in sediments along the pathway (Ahrens, 2011; Prevedouros et al., 2006). The total emission of PFAS (PFCA, PFOS, and precursors) are estimated to be 10,000-50,000 tons between 1951-2004, with over 95% of the direct emission ending up in

the aquatic environment (Paul et al., 2009). The degradation products of PFAS through transformation and biotransformation are mainly present in water as well. Except for a couple of classes of PFAS (fluorotelomer derivative, perfluoroalkyl sulfonamides derivatives, etc.) that are known to transform to terminal perfluoroalkyl acid (PFAA), the transformation products and pathways for most PFAS species still lack studies (Guelfo et al., 2021). Therefore, the aquatic environment is likely to be the largest reservoir for PFAS.

When PFAS get into the environment, they are not being removed effectively from the water with current conventional water treatment technologies (Belkouteb et al., 2020). Potential treatment technologies such as activated carbon (GAC), nanofiltration, and reverse osmosis (RO) all showed effective removal of PFOS or PFOA in groundwater up to 90%-99% at various concentrations on a laboratory scale (Abunada et al., 2020; Kothawala et al., 2017). However, there are several downsides to these approaches. For example, the saturation issue with RO and nanofiltration membrane can substantially reduce the removal efficacy with a large volume of sample. The waste associated with the process also needs remediation and proper disposal such as contaminated GAC resins, unrecovered water from the nanofiltration process, etc. Other methods such as ozonation, direct UV irradiation, plasma-based processes, and sonolysis/sonochemical treatments show promising efficacy under certain experimental conditions and certain PFAS, however, obstacles remain when it comes to 1) large-scale treatment application and the impact of water quality conditions (dissolved organic matter, presence of other ions and contaminants), 2) costs (e.g. high-energy consuming processes), 3) ineffectiveness on the removal of varied PFAS with different properties, 4) If the eluent after treatment meets the requirements (Abunada et al., 2020; Kucharzyk et al., 2017). Once PFAS get into the environment, they circulate in the water system posing continuous exposure to the terrestrial and aquatic ecosystem.

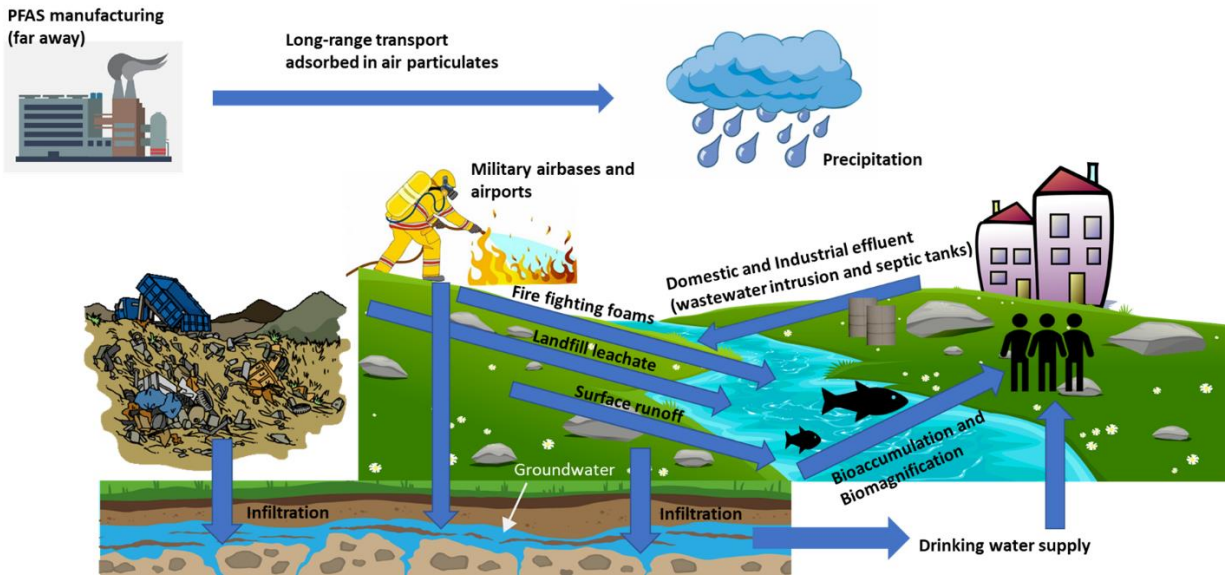


Figure 1.1 The potential sources and transport of PFAS and their exposure pathways in Florida.

1.4 PFAS exposure to animals and humans

As PFAS are highly persistent and mobile, humans and wildlife can be exposed to PFAS in many pathways. Human exposure includes dietary exposure through food and water consumption, indoor exposure through inhalation and dust ingestion, and outdoor air exposure, among others (de Silva et al., 2021). Dietary exposure is considered the main source which includes the consumption of PFAS-contaminated water and food, such as grains, crops, and seafood grown in the contaminated area, and PFAS transferred from non-stick food packaging and cookware (de Silva et al., 2021; Domingo et al., 2012; Jogsten et al., 2009). Due to the large amount of drinking water (up to 4 L) needed and consumed daily, consumption of contaminated tap water is one of the major exposure pathways. There are several studies conducted in the U.S. that found associations between significant high concentrations of PFAS in serum samples of the population and contaminated drinking water in the same area (Herrick et al., 2017; Kate et al., 2011; Kyle et al., 2009; Ryan et al., 2011). PFAS can bioaccumulate through the uptake of all exposures and an increasing effect is observed with fluorinated carbon chain length increase (Martin et al., 2013). Biomagnification of

PFAS through the food chain has been proved for PFAS in eutrophic freshwater and terrestrial food webs (Müller et al., 2011; Xu et al., 2014).

PFAS exposure assessments are performed using two types of approaches: exposure factor approaches and epidemiologic approaches. The exposure factor approach directly measures the concentration of PFAS in any direct exposure media (food, water, air, etc.) and then determines the exposure factor based on the time, frequency, and duration of exposure behaviors (de Silva et al., 2021). The epidemiological approach is based on regressing PFAS concentration in serum/blood against PFAS concentration in exposure media or exposure behavior (e.g., food/water consumption) to evaluate their association with certain exposure pathways (de Silva et al., 2021).

1.5 PFAS toxicity and environmental and human risks

Exposure to PFAS is associated with a variety of adverse health effects, including thyroid disease, increased cholesterol levels, liver damage, kidney cancer, testicular cancer, and developmental effects affecting the unborn child (lower birth weight and delayed development, etc.) with high certainty (Fenton et al., 2021). Epidemiological studies also found high levels of PFAS in serum (maternal, children, and adults) are associated with decreased immunological responses to various vaccinations (Goudarzi et al., 2017; Grandjean et al., 2012; Looker et al., 2014). Studies have reported that PFAS can interact with thyroid hormone transport proteins and interfere with thyroid peroxidase (TPO) enzyme activity (Ren et al., 2016; Song et al., 2012). Women and men with elevated PFOA exposure were reported with clinical hypothyroid diseases in a national survey with over 3000 adult participants (David et al., 2010). With many other relevant evidence and studies, it is concluded that exposure to PFOA has a probable link to thyroid disease (C8 Science Panel 2012). Long-chain PFAS have shown preferred accumulation in the liver, and exposure to long-chain PFAS showed a significant association to disrupt hepatic metabolism and function (Hui et al., 2017; Nian et al., 2019; Valentina et al., 2012). Kidney disease/cancer is also associated with

exposure to long-chain PFAS which may result from renal reabsorption and therefore changes in excretion rates and half-life of such exposure (Mastrantonio et al., 2018; Shankar et al., 2011). Studies show that exposure to PFAS has been associated with impaired human fertility, such as decreased sperm mobility and penetration, and lower sperm concentration and count (Anne et al., 2013; Šabović et al., 2020; Yuan et al., 2020). PFAS can be transferred from mother to child through the umbilical cord and breast milk and PFAS exposure is associated with decreased infant birth weight in many studies (Gyllenhammar et al., 2018; I et al., 2014; Juleen et al., 2014). So far, most toxicological studies have been focused on PFOA, PFOS, and PFHxS. Though short-chain PFAS was brought to the market for their potential less tendency on bioaccumulation and toxicity, toxicological studies on animal models have reported that exposure to PFBA also induces adverse effects on thyroid and liver as well as body weight loss (Chang et al., 2008). Exposure to PFBS has resulted in developmental delays, adverse female reproductive effects as well as hepatic, thyroid, and renal toxicity. PFHxA also showed hepatic and thyroid toxicity in rats (Li et al., 2020). PFBA showed higher accumulation in the lung and kidney than PFOS, whereas PFHxA showed preferred accumulation in human liver and brain tissue (Pérez et al., 2013). Therefore, there is continued concern over the alternative short-chain PFAS since studies have demonstrated that they can be as toxic as the already banned legacy PFAS.

1.6 Environmental occurrence of PFAS in Florida

PFAS occurrence in drinking water has been reported worldwide, including in Europe (France, Germany, Italy, Sweden, etc.), America (USA, Canada, Brazil, etc.), Asia (China, Japan, etc.), Africa, and Australia (Domingo & Nadal, 2019). In Florida, elevated PFAS levels have been reported in effluent and sludge of wastewater plants, landfill leachate, drinking water, surface water, sediment, and groundwater (Cui et al., 2020). The spatial distribution of PFAS in drinking water supplies, surface water, and groundwater wells in Florida reported in a few studies are

summarized in Figure 1.2 (Cui et al., 2020). Recently in 2018, the Department of Defense (DoD) reported PFOS and PFOA in groundwater at military on-base with concentrations up to 1,397,120 ng/L combined (Naval air station, Jacksonville). High concentrations of PFBS, PFHxS, PFHxA, PFHpA, PFNA (2210- 335,000 ng/L) were also reported in MacDill Air Force Base (FDEP, 2020a; Cui et al., 2020)). In 2014, the Environmental Working Group (EWG) investigated drinking water in cities of Florida between 2013 and 2015, finding PFOS, PFOA, PFHxS, PFHpA, PFNA in more than 12 utilities. The highest level of PFOS reported was up to 380 ng/L at Emerald Coast Utilities Authority (Pensacola, FL) (EWG, 2019). In 2018, the Florida Department of Environmental protection (FDEP) conducted a PFAS assessment in surface water, monitoring wells, soil, and sediment near fire training facilities in Florida. In the monitoring wells of seven facilities, the maximum concentration of PFOA and PFOS combined was over 10,000 ng/L. Particularly, the maximum concentration of PFOA and PFOS combined was up to 130,000 ng/g in the soil from Florida State Fire College, which is substantially higher than the soil samples from other facilities (FDEP, 2020). The high concentration of PFAS found in the water environment suggests a potential local source of PFAS in Florida, whereas systematic assessments are still lacking.

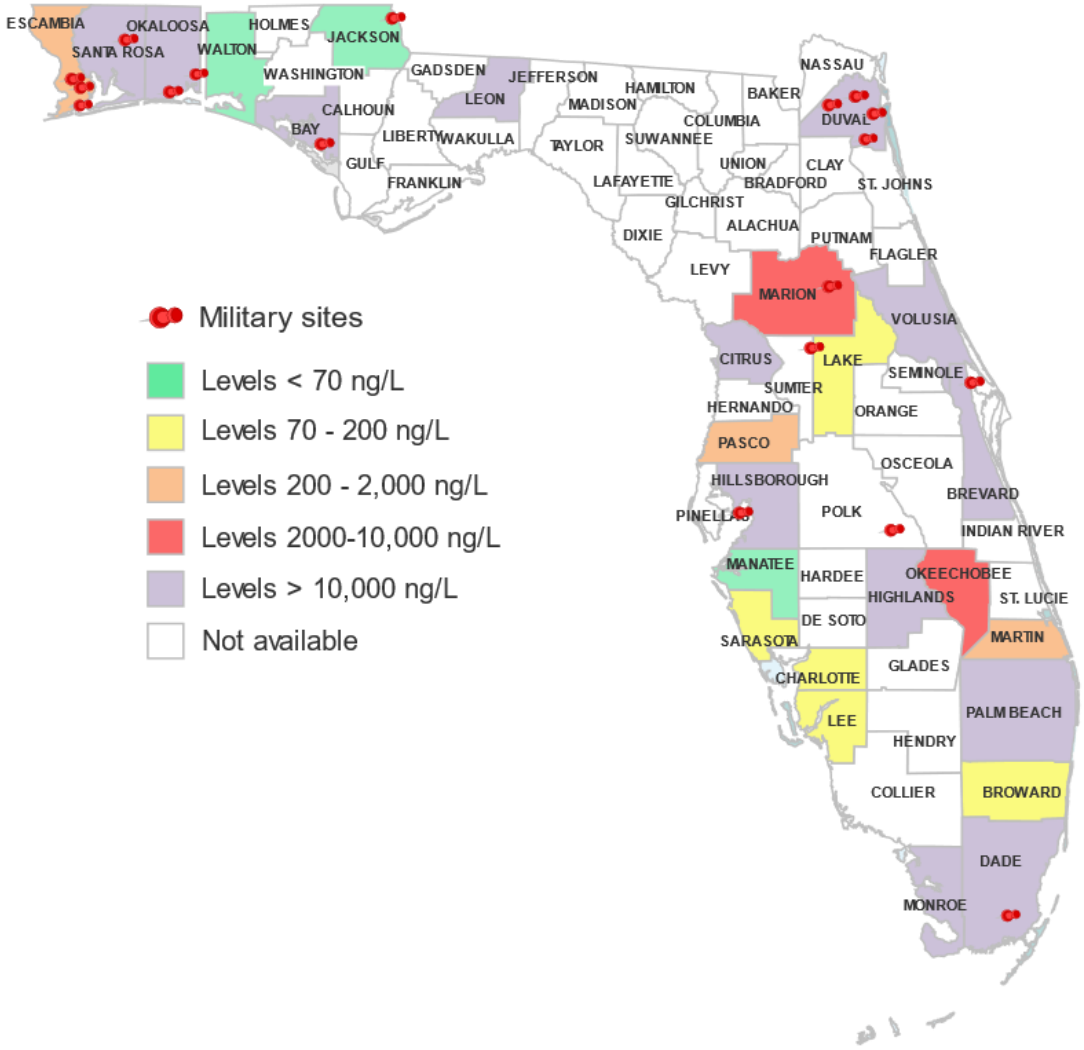


Figure 1.2 Spatial distribution of PFAS in drinking water supplies, surface water and groundwater wells in Florida. Adapted from Cui et al., 2020.

1.7 **The U.S EPA regulation**

The health advisories (HAs) levels refer to the concentration of a contaminant that is anticipated to lead to adverse health effects. The U.S EPA published interim updated health advisories (HAs) for PFOA (0.004 ppt) and PFOS (0.2 ppt) in drinking water on June 2022 to replace the HAs issued in 2016, which was 70 ppt for PFOA and PFOS combined (EPA, 2022). The final lifetime drinking water HAs were issued for GenX (10 ppt) and PFBS (2000 ppt) on June 2022 as well. The interim

HAs are intended to provide current information to the public and agencies related to water usage and they are subject to change until the National Primary Drinking Water regulation for PFAS takes effect.

The HAs were established based on recent human epidemiology studies in population and toxicity assessment according to EPA (EPA, 2021b, 2021c, 2021d, 2021e). The lifetime noncancer health advisory is derived as:

$$Lifetime\ HA = \left(\frac{RfD}{DWI - BW}\right) * RSC$$

RfD refers to chronic reference dose that is likely not to cause the risk of adverse effects through daily oral exposure, in the unit of mg/kg/day. As PFAS can cause various adverse health effects, the most sensitive non-cancer effects among available data were selected to derive the HAs. The RfD for PFOA (1.5 E-9) and PFOS (7.9E-09) were based on developmental immune health outcomes in human epidemiological studies. The RfD (3E-06) for GenX is based on critical liver effects in parental female mice by gavage. The RfD (3E-04) for PFBS is based on the critical effect of decreasing serum total thyroxine in newborn mice after gestational exposure to the mother.

DWI-BW refers to drinking water intake rate adjusted for body weight for direct and indirect (in food or beverages) consumption, which is 0.0701 L/kg-day. RSC refers to the relative source contribution of oral exposure from drinking water, which is 0.2.

1.8 PFAS assessments and screening based on Mass spectrometry: Target and Non-Targeted Analysis

Mass spectrometry (MS) coupled to chromatographic separation techniques are largely applied to PFAS measurement in media and environment. The method often requires an extraction technique that extracts and concentrates PFAS effectively from the media using appropriate sorbents or solvent through reverse phase and/or weak anion exchange (Brumovský et al., 2018). Following

chromatographic separation, such as gas chromatography (GC) for volatile PFAS, and liquid chromatography (LC) for non-volatile and semi-volatiles PFAS, MS is able to detect and quantify PFAS compounds based on their exact mass, retention time, and tandem MS (MS2, fragment/product ions) when compared to their matching certified standards for target analysis. Currently, the U.S. EPA has published analytical methods based on solid phase extraction (SPE) LC-MS/MS for the detection of 30 and 40 PFAS in drinking water. The EPA methods 533, 537, 537.1, and 1633 can be validated and optimized to accommodate different laboratory applications (USEPA, 2020b; USEPA,2021f; Guelfo et al., 2021)).

Though with the current target analysis based on LC/GC-MS, contaminants can be detected with high sensitivity and accuracy, one of the biggest drawbacks is that it requires the use of authentic standards to match with the analytes for the identification and quantification, which greatly limits the detection range due to fact that the reference standards cannot cover every single potential contaminant in a sample. Not only there is a limitation on the number of certified standards that can be encompassed in one analysis, but also the native and labeled standards are not always commercially available. With the advancements of high-resolution MS which has the ability to provide accurate exact mass and distinguish ions with minor differences in mass-to-charge ratio (m/z), non-targeted analysis (NTA) has emerged to overcome the limitations of target analysis (Schymanski et al., 2014). In NTA, no prior information or certified standard are required to obtain the full scan mass spectrum data (MS) and MS2 spectrum (data dependent or independent) for the analyzed samples and generate a list of potentially identified features. Each feature detected (m/z associated with RT) and the information it contains, including the exact mass, isotope, adduct, and fragmentation, are used to identify the potential chemicals that exist in the sample under the experimental condition (Schymanski et al., 2015). A general workflow of NTA starts with sample collection followed by sample preparation procedures (filtration, extraction/cleanup, dilution/concentration) suitable for the instrument used in the study, then sample analysis to obtain

MS and MS2 using an LC/GC-HRMS, and data processing and chemometrics (Hollender et al., 2017). Data processing is the key part of NTA which involves filtering quality features from large quantities of background, such as peak picking, RT alignment, and background subtraction. (Fisher et al., 2021). At last, compounds are identified with formulas and structural annotation based on their isotope patterns, mass defect, homologous grouping, MS2, and database matching search, among others (Hollender et al., 2017).

NTA has been successful in identifying new contaminants of potential concern from environmental samples, especially transformation and degradation products, in this way, complementing target analysis in such samples (Tian et al., 2021). NTA is rapidly fitting into PFAS studies since the unknown transformation products and degradants of PFAS can make up a large proportion of total PFAS in the environment, to which no matching certified standards are available. A few studies have shown the ability of NTA to identify previously unknown PFAS, for example, a series of 37 existing and novel perfluorinated ether acid species were identified by HRMS NTA as the replacement of legacy PFAS in the Cape Fear River, North Carolina, which is known to be impacted by discharges from nearby PFAS manufacturing industry (McCord & Strynar, 2019). In another study, 41 homologous series of PFAS of which six were reported for the first time in wastewater from electronics fabrication facilities in the United States (Jacob et al., 2021). Though there is no standard workflow or criteria for PFAS NTA screening up to date, efforts are being made in improving instrumentation, analytical method, NTA software, data processing criteria, and data transparency communication (Charbonnet et al., 2022; Guelfo et al., 2021; Koelmel et al., 2020).

1.9 Research needs and Objectives of the dissertation

Most research and studies conducted on PFAS have been focused on only a few long-chain legacy species, mostly, PFOA and PFOS. However, PFAS persistence in the aquatic environment are very diverse and complicated. In addition to many other long-chain legacy PFAS, increasing emissions

from emerging short-chain PFAS substitutes that are used to replace legacy PFAS in industrial production have led to a large contribution to the amount of PFAS in the environment. What complicates the situation even more is the numerous precursors, metabolites, and transformation products that are not routinely monitored or have not been identified. Considering the aquatic environment to be the largest reservoir for PFAS, as well as the increasing concerns about the impact of these PFAS on the environment, aquatic life, and human health, the ability to be able to detect these diverse PFAS species is of the utmost importance. The high concentration of PFAS found in water in Florida suggests a potential local source of PFAS. Considering Florida supports millions of people as well as treasured, sensitive coastal and wetland ecosystems, a systematic PFAS monitoring study is needed as such information in Florida is lacking.

To address major gaps in PFAS occurrence, distribution, and fate in South Florida, this dissertation was divided in the following three major objectives:

Objective 1: Target method development for the detection of an extended list of typical PFAS groups including legacy and emerging PFAS substitutes at low parts per trillion levels

The first goal of the dissertation was to develop a target analysis method based on a semi-automated solid phase extraction (SPE) coupled to LC-MS/MS method that allows the detection of an extended list of typical PFAS groups, including long-chain legacy and emerging short-chain PFAS substitutes in water samples, with the detection limit as low as 1 ppt. The detection list includes 30 PFAS species that cover a variety of classes, such as long-chain legacy PFCA, PFSA, PFOSA, FOSAA, fluorotelomer substances, and short-chain emerging PFCA, PFSA, PFOSA, and perfluorinated ethers. The method was validated in terms of linearity, accuracy, precision, selectivity, repeatability, limits of detection, and matrix effects and applied for measurement of PFAS in environmental samples.

Objective 2: A comprehensive assessment of PFAS in drinking water and surface water from different water bodies in Florida.

The developed method in objective 1 was applied to drinking water samples from metropolitan counties and surface water samples from different water bodies in South and Central Florida, such as Biscayne Bay and canals, Tampa Bay, Everglades canals, and Key West. The occurrence, composition, spatial distribution, seasonal variation, risk assessment, and potential source analysis of PFAS were investigated. Knowledge will be gained to facilitate the development of guidelines and procedures on monitoring PFAS contamination in Florida and provide detailed information to local and regional executive agencies related to land use and water quality.

Objective 3: Development of a non-targeted approach to identify potential unknown PFAS, metabolites, and transformation products in the environment

The last objective was to develop a non-targeted approach (NTA) to evaluate potential unknown PFAS, metabolites, and transformation products in the environment, which is limited by target analysis due to the lack of certified standards. The NTA workflow based on an online solid phase extraction LC- HRMS method using an Orbitrap MS system was applied for the screening of PFAS species in drinking water and surface water in South Florida (samples collected from objective 2), complementing the previous target analysis. In addition, we proposed a semi-quantitative NTA (qNTA) method to provisionally estimate concentrations from PFAS identified without commercial standards. The qNTA method was tentatively applied to the NTA results from the environmental water samples, facilitating further understanding of the presence of these underestimated PFAS in the environment.

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Chapter 2. Assessment of Per- and Polyfluoroalkyl Substances in Biscayne Bay Surface Waters and Tap Waters from South Florida

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2.1 Introduction

Per- and poly-fluorinated alkyl substances (PFAS) are a group of synthetic chemicals, which have been widely used for over 60 years in numerous industrial and consumer products. These include water and stain proof coatings, fire retardants, aqueous film-forming foams (AFFFs), insecticides, non-stick polymers, food contact materials, surfactants, household products, medical devices, and personal care products (Baran 2001). The widespread global use of PFAS has resulted in their ubiquitous presence in the environment, such as in surface water, drinking water, in treated and untreated wastewater streams, and landfill leachates (Giesy and Kannan 2001; Lang et al. 2017; Rahman et al. 2014; Zareitalabad et al. 2013), as well as their bioaccumulation in living organisms, including humans (Kannan et al. 2004). Due to their chemical and biological recalcitrance and the fact that they are not effectively removed during conventional wastewater and drinking-water treatments, PFAS are often referred to as "forever chemicals" (Prevedouros et al. 2006). Exposure to PFAS even at low parts-per-billion (ppb) is a serious human health concern as they belong to an emerging class of endocrine-disrupting halogenated pollutants linked to cancer, developmental delays, thyroid disease, and infertility (Lopez-Espinosa et al. 2012; Vieira et al. 2013).

PFAS can be released into the aquatic environment through numerous point sources, such as industrial and municipal sewage treatment plants, PFAS production facilities, landfill sites, commercial and military airfields, and firefighting training areas, along with nonpoint sources like atmospheric deposition and surface runoffs (Müller et al. 2011). The aquatic environment, such as estuaries and coastal waters, are likely to be the ultimate sink for PFAS due to their close proximity to urban and industrial centers (Yamashita et al. 2008). Although many different classes of PFAS have been detected in environmental and biological samples, most studies and regulations have focused on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). With the emergence of short-chain PFAS, poly- and perfluoroalkyl ethers, and fluorotelomers as

replacement to the banned long-chain PFAS (Cui et al. 2020), there is a need to understand their input into the aquatic environment in order to establish preventive and management strategies and actions to reduce exposure to wildlife and humans. Recent studies have demonstrated that the environmental PFAS pollution scenario is a complicated and complex situation showing increasing emission from alternative PFAS with shorter chain, such as four carbon-based perfluoroalkyl sulfonates and carboxyl acids (PFBA, PFBS), CF_3 or a C_2F_5 based perfluorinated ethers ("GenX" and "ADONA"), among others (Wang et al. 2013). The concern increases even more with researchers showing that shorter-chain PFAS are absorbed into the liver more readily, inducing higher hepatic toxicity (Lau 2012), and are more poorly removed with the available advanced water treatments (Brendel et al. 2018; Son et al. 2020).

In Florida, few studies have reported PFOS and PFOA in drinking water with concentrations ranging from 71 to 200 ng L^{-1} (Hu et al. 2016), which exceeds the U.S. Environmental Protection Agency (EPA) health advisory level of 70 ng L^{-1} for both compounds combined (USEPA 2016). PFAS have also been found in aquatic animals including sea turtles, alligators, and dolphins from different locations in Florida (Houde et al. 2006; Kannan et al. 2001; Lynch et al. 2019; O'Connell et al. 2010; Rodriguez-Jorquera et al. 2016). While the high PFAS concentrations found in wildlife and drinking water suggests a potential local source of PFAS in Florida, critical information on the occurrence, distribution, and sources of PFAS in South Florida is lacking and is warranted to aid more stringent guidelines including other PFAS. South Florida is home to 9.3 million Floridians encompassing vital and biodiverse coastal and estuarine ecosystems, such as the Biscayne Bay, where water quality issues influenced by anthropogenic wastewater intrusions have been documented (Ng et al. 2021).

In this study, we have assessed for the first time the occurrence, composition, spatial distribution, and seasonal variation of PFAS in surface waters from Biscayne Bay and tributary canals as well

as from drinking (tap) waters from the metropolitan counties of Miami-Dade, Broward, and Palm Beach, in South Florida (with a total population of 6.2 million people). An analytical methodology based on solid-phase extraction followed by liquid-chromatography tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination of emerging and legacy PFAS (N=30) at low parts per trillion (ppt) levels in South Florida water bodies.

2.2 **Materials and Methods**

2.2.1 Chemicals and Materials

Methanol, water, hexane, acetone, methylene chloride, ammonium hydroxide, and ammonium formate, all Optima LC/MS grade, were purchased from Fisher Scientific (Waltham, MA, USA). A 30-PFAS native standard mixture (PFAC30PAR,1 μg mL^{-1} in methanol), a 19-PFAS isotopically mass-labeled standard mixture (MPFAC-24ES 1 μg mL^{-1} in methanol) and a labeled HFPO-DA standard solution (50 μg mL^{-1} in methanol) were purchased from Wellington Laboratories Inc (Guelph, Ontario, Canada). Stock solutions at a concentration of 1 μg L^{-1} were prepared using methanol and stored in the freezer (-8°C). Working solutions were prepared by diluting the stock solution to 1 ng mL^{-1} and 10 ng mL^{-1} in water and stored at 4°C. A 24-PFAS native compound standard (PFC-24, 2 μg mL^{-1} in methanol: water (80:20) was purchased from AccuStandard (New Haven, CT, USA) and used as a secondary standard for initial calibration verification (ICV). A detailed list of the PFAS included in each native standard and internal labeled standard are presented in Table 2.1, including their full name. Strata-XL-AW 100um polymeric weak anion cartridges (500mg/3mL) were purchased from Phenomenex (Torrance, CA, USA).

Table 2.1 List of 30 PFAS with their internal standards applied for quantitation and list of 24 PFAS in the secondary standard.

Abbreviation	Compound Name	Molecular Formula	Molecular Weight	I.S.	Secondary Standard
4-2 FTS	Sodium 1H,1H,2H,2H-perfluoro-1-hexanesulfonate	$C_6H_5F_9O_3S$	328.15	M2 4-2 FTS	✓
6-2FTS	Sodium 1H,1H,2H,2H-perfluoro-1-octanesulfonate	$C_8H_5F_{13}O_3S$	428.17	M2 6-2FTS	✓
8-2 FTS	Sodium 1H,1H,2H,2H-perfluoro-1-decanesulfonate	$C_{10}H_5F_{17}O_3S$	528.18	M2 8-2 FTS	✓
ADONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	$C_{10}H_{11}N_4NaO_5S$	322.27	M2 6-2FTS	
FBSA	Perfluoro-1-butanesulfonamide	$C_4H_2F_9NO_2S$	299.11	M GenX	
FHxSA	Perfluoro-1-hexanesulfonamide	$C_6H_2F_{13}NO_2S$	399.13	M8 PFOA	
FOSA	Perfluoro-1-octanesulfonamide	$C_8H_2F_{17}NO_2S$	499.14	M8 FOSA	
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	$C_6HF_{11}O_3$	330.05	M GenX	
N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	$C_{12}H_8F_{17}NO_4S$	585.23	d5 N-EtFOSAA	✓

N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	$C_{11}H_6F_{17}NO_4S$	571.21	d3 N-MeFOSAA	✓
PFBA	Perfluoro-n-butanoic acid	$C_4HF_7O_2$	214.04	MPFBA	✓
PFBS	Potassium perfluoro-1-butanesulfonate	$C_4HF_9O_3S$	300.10	M3 PFBS	✓
PFDA	Perfluoro-n-decanoic acid	$C_{10}HF_{19}O_2$	514.08	M6 PFDA	✓
PFDoA	Perfluoro-n-dodecanoic acid	$C_{12}HF_{23}O_2$	614.10	M PFDoA	✓
PFDS	Sodium perfluoro-1-decanesulfonate	$C_{10}HF_{21}O_3S$	600.14	M7 PFUdA	✓
PFHpA	Perfluoro-n-heptanoic acid	$C_7HF_{13}O_2$	364.06	M4 PFHpA	✓
PFHpS	Sodium perfluoro-1-heptanesulfonate	$C_7HF_{15}O_3S$	450.12	M3 PFHxS	✓
PFHxA	Perfluoro-n-hexanoic acid	$C_6HF_{11}O_2$	314.05	M5 PFHxA	✓
PFHxS	Potassium perfluorohexanesulfonate	$C_6HF_{13}O_3S$	400.11	M3 PFHxS	✓
PFNA	Perfluoro-n-nonanoic acid	$C_9HF_{17}O_2$	464.08	M3 PFHxS	✓

PFNS	Sodium perfluoro-1-nonanesulfonate	$C_9HF_{19}O_3S$	550.14	M8 PFOS	✓
PFOA	Perfluoro-n-octanoic acid	$C_8HF_{15}O_2$	414.07	M8 PFOA	✓
PFONS	Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	$C_8ClF_{16}KO_4S$	570.67	M8 PFOS	
PFOS	Potassium perfluorooctanesulfonate	$C_8HF_{17}O_3S$	500.13	M8 PFOS	✓
PFOUdS	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	$C_{10}ClF_{20}KO_4S$	670.69	M PFDoA	
PFPeA	Perfluoro-n-pentanoic acid	$C_5HF_9O_2$	264.05	M5 PFPeA	✓
PFPeS	Sodium perfluoro-1-pentanesulfonate Potassium	$C_5HF_{11}O_3S$	350.11	M GenX	✓
PFTeDA	Perfluoro-n-tetradecanoic acid	$C_{14}HF_{27}O_2$	714.11	M2 PFTeDA	✓
PFTrDA	Perfluoro-n-tridecanoic acid	$C_{13}HF_{25}O_2$	664.11	M2 PFTeDA	✓
PFUdA	Perfluoro-n-undecanoic acid	$C_{11}HF_{21}O_2$	564.09	M7 PFUdA	✓

2.2.2 Sampling design

Samples were collected in 500 mL pre-cleaned high density polypropylene bottles (HDPE) bottles attached to a swing arm sampler (Wooster, OH, USA) and stored on ice during transportation and then refrigerated at 4°C until the analysis. The samples were refrigerated for less than a week prior to analysis. All the samples were taken in two sampling events during the wet (October 2020) and dry (January and February 2021) seasons, respectively.

Higher environmental PFAS concentrations are normally seen in regions with dense population, high industrialization, landfill leachates, wastewater treatment plants and military bases (Cui et al. 2020). Therefore, tap water samples were collected from highly urbanized areas in Miami-Dade (11 sites), Broward (6 sites), and Palm Beach counties (4 sites), from household taps (without an additional filter system) which provides drinking water supply to about 6 million people. A total of 33 samples were collected from 21 sites.

Surface water samples were collected from water bodies where water quality issues have been documented with influences of anthropogenic wastewater intrusions such as the Biscayne Bay and tributaries/canals, including the Miami River, Little River and Biscayne C8 canal (Ng et al. 2021). A total of 15 sampling sites were assessed which encompassed a total of 27 surface water samples. Coastal Biscayne Bay supports the adjacent coral reef and mangrove ecosystem and provides critical nursery habitats and food web support for many important commercial and recreational fishery resources (Ault et al. 2001). Therefore, the presence of PFAS in these water bodies is of great concern since it could potentially have negative impacts to coral reefs, mangroves, early life stages of aquatic organisms, and other sensitive species. A detailed map outlining the sampling sites for both surface and tap waters is shown in Figure 2.1. The coordinates and descriptions of the sampling sites are listed in the supplementary material (Table 2.2)

Table 2.2 Geographical coordinates of surface water sampling locations along Biscayne Bay and adjacent canals and tap water from Miami-Dade, Broward, and Palm Beach counties

Water Type	Sampling sites	Abbv.	County	Collection Dates	Latitude	Longitude
Surface Water	Maule Lake	ML	Miami-Dade	10/26/2020, 05/01/21	25.93667	-80.14425
	Royal Galdes Canal	RGC	Miami-Dade	10/26/2020, 05/01/21	25.92927	-80.15113
	Biscayne Bay @ FIU	BB	Miami-Dade	10/26/2020, 05/08/21	25.90917	-80.13751
	Little Arch Creek	LAC	Miami-Dade	10/26/2020, 05/01/21	25.89471	-80.15435
	Biscayne Canal 8	BC-8	Miami-Dade	10/26/2020, 05/01/21	25.8712	-80.17615
	Little River Site 1	LR1	Miami-Dade	10/26/2020, 05/01/21	25.84641	-80.18605
	Little River Site 2	LR2	Miami-Dade	10/26/2020, 05/01/21	25.84602	-80.17655
	Legion Park	LP	Miami-Dade	05/01/21	25.83612	-80.18218
	Morningside Park	MP	Miami-Dade	05/01/21	25.82316	-80.17835
	Seybold Canal	SC	Miami-Dade	10/26/2020	25.7869	-80.21401
	Miami River Site 1	MR1	Miami-Dade	10/26/2020, 05/01/21	25.76997	-80.19891
	Miami River Site 2	MR2	Miami-Dade	10/26/2020, 05/01/21	25.7775	-80.20552
	Miami Beach 17th Street	MB17	Miami-Dade	10/26/2020, 05/01/21	25.79227	-80.14288
	Miami Beach 14th Street	MB14	Miami-Dade	10/26/2020, 05/01/21	25.78511	-80.14444
	Miami Beach 10th Street	MB10	Miami-Dade	10/26/2020, 05/01/21	25.78041	-80.14313
Tap Water	West Palm Beach		Palm Beach	10/2020	26.73073	-80.11627
				01/2021	26.68676	-80.19918
	Lake Worth		Palm Beach	02/2021	26.56887	-80.18442

Boynton Beach	Palm Beach	02/2021	26.53178	-80.09054
Boca Raton	Palm Beach	10/2020	26.3679	-80.12844
		02/2021	26.41273	-80.10044
Sunrise	Broward	02/2021	26.12165	-80.3321
Fort Lauderdale	Broward	02/2021	26.19332	-80.2428
Cooper City	Broward	10/2020	26.05731	-80.27171
		02/2021	26.04718	-80.23251
Pembrooke Pines	Broward	10/2020	26.00584	-80.31077
		02/2021	26.00566	-80.35756
Hollywood	Broward	10/2020	25.98669	-80.12585
		10/2020	26.03986	-80.1825
Hollywood Lakes	Broward	02/2021	26.00011	-80.12377
Dania Beach	Broward	10/2020	26.05343	-80.17407
Ives Estates	Miami-Dade	10/2020, 01/2021	25.958	-80.18611
North Miami Beach	Miami-Dade	10/2020, 01/2021	25.9272	-80.1415
North Miami	Miami-Dade	10/2020, 01/2021	25.91356	-80.15166
		10/2020, 01/2021	25.91178	-80.13997
Miami Beach	Miami-Dade	10/2020, 01/2021	25.79336	-80.1422
Grapeland Heights	Miami-Dade	10/2020, 01/2021	25.79297	-80.24552
Sweetwater	Miami-Dade	02/2021	25.75389	-80.37704

	Coral Gables	Miami-Dade	02/2021	25.70812	-80.24801
	Key Biscayne	Miami-Dade	02/2021	25.69996	-80.15672
	Kendall	Miami-Dade	02/2021	25.67176	-80.37238
	Cutler Bay	Miami-Dade	10/2020, 01/2021	25.61628	-80.32492
	Princeton	Miami-Dade	02/2021	25.53924	-80.37902

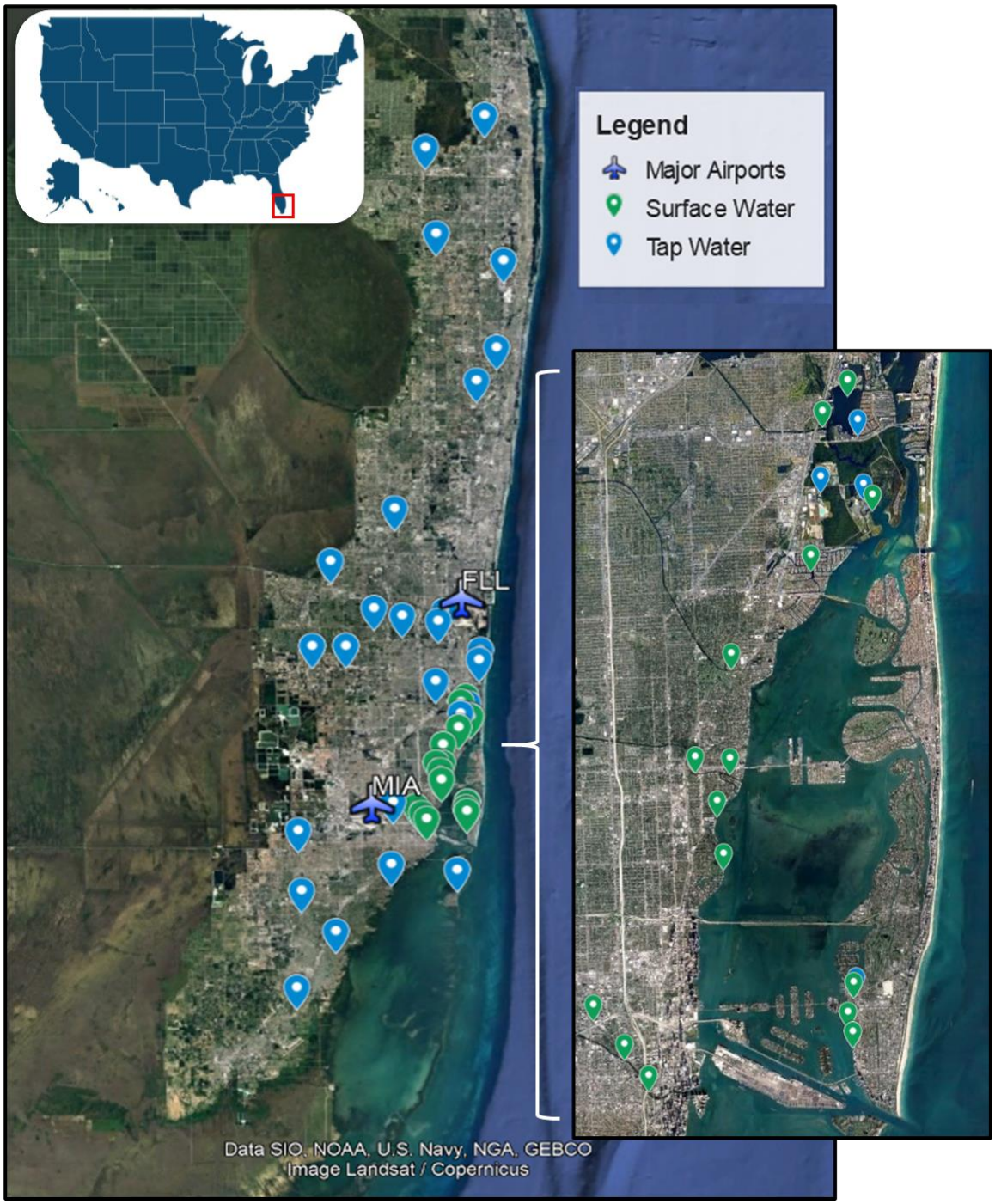


Figure 2.1 PFAS surface and tap water sampling locations in South Florida

2.2.3 Sample preparation

All containers, bottles and tubing used during extraction and sample preparation were rinsed with methylene chloride, hexane, acetone, methanol, and water at least two times and residual was left to evaporate. All the solvent used in this analysis are HPLC grade and examined for potential PFAS contamination. A 250-mL water sample spiked with 100 µL of the internal standard (IS) mixture (2.5 ng/mL) was processed through solid phase extraction (SPE) using Strata-XL AW (500 mg/ 3mL) cartridges on a semi-automated SPE equipment for extraction and preconcentration of PFAS following previous procedures published elsewhere (Patterson et al, 2020). Strata XL-AW is a polymeric weak anion exchange sorbent that couples a hydrophobic backbone with a diamino ligand, which has previously shown to enable the extraction of both short and long-chain acidic PFAS. Briefly, the cartridges were pre-conditioned with 6 mL of 0.3% ammonia in methanol, 6 mL of methanol and equilibrated with 6 mL of water according to the manufacturer protocol. After the entirety of the sample was passed through the SPE, 10 mL of ultrapure water was added to the sample bottle and agitated to rinse any sample residues. The cartridges were left to dry under vacuum for 40 min followed by elution with 10 mL of 0.3% ammonium hydroxide in methanol. The eluent was evaporated to dryness under a gentle nitrogen flow in a heated water bath at 60°C, then reconstituted to a 1 mL volume with 90:10% (vol/vol) 5 mM ammonium formate/ methanol. Also, tap water samples were subjected to a direct injection method, where 900 µL of tap water samples were spiked with 100 µL of the IS and injected to the LC-MS/MS directly (without preconcentration) for PFAS analysis. When concentrations were above the calibration curve, 100 µL of surface water samples were spiked with 90 µL of IS and combined with 810 µL of 5 mM ammonium formate to dilute the sample by ten times and then injected into the LC-MS/MS for PFAS analysis.

2.2.4 Instrumental analysis

After SPE, 100 µL of the pre-concentrated samples were analyzed by an Agilent 1290 Infinity II LC interfaced to an Agilent 6470 triple-quadrupole LC-MS/MS system equipped with Agilent Jet Stream electrospray ionization source (AJS ESI source). The LC was modified with PFAS free tubing to avoid potential contamination. A delay column (Hypersil GOLD aQ C18, 20 x 2.1 mm, 12 µm) was placed between the mobile phase mixer and the sample injector. PFAS congeners were separated on a Hypersil GOLD perfluorinated phenyl (PFP) column (150 mm x 2.1 mm, 3 µm) with a PFP guard column (Hypersil Gold PFP 5 µm drop-in guards) at a temperature of 50°C using 5 mM ammonium formate and methanol as mobile phases in a flow rate of 0.4 mL/min. The LC gradient conditions, and MS parameters of the LC-MS/MS instrument are listed in Table 2.3 and 2.4, respectively. Sample acquisition was performed using a multiple-reaction monitoring (MRM) method in negative mode for the simultaneous quantification of multiple PFAS. A summary of the MRM method including information on precursor and product ions monitored, retention time, fragmentor voltage and collision energy are presented in Table 2.5.

Table 2.3 The LC gradient conditions.

Time (min)	**A [%]**	**B [%]**	**Flow (ml/min)**
0.00	90	10	0.4
8.00	5	95	0.4
11.00	5	95	0.4
12.00	90	10	0.4

A=5mM Ammonium formate and B=Methanol

Table 2.4 MS parameters for LC-MS/MS analysis

Parameter	**Setting**
MS Acquisition	Dynamic MRM
Cycle Time	500 ms
Ion Source	ESI negative
Drying Gas Temperature & Flow	150 ℃ & 10 L/min
Nebulizer	15 psi
Sheath GasTemperature & Flow	300 ℃ & 10 L/min
Capillary	2000 V
Nozzle Voltage	0 V

Table 2.5 Summary of the MRM method for the analysis of PFAS

Compound Name	IST D	Precursor Ion	Product Ions	Retention Time (min)	Delta Retention Time	Fragmentor	Collision Energy	Cell Accelerator Voltage
4-2 FTS		327	80.9	5.69	3	125	32	5
6-2FTS		427	406.8; 79.9	6.95	3	125	24	5
8-2 FTS		527	506.8; 80.9	7.65	3	170	28	5
Adona		377	250.9	6.54	3	70	10	5
d3 N-MeFOSAA	✓	573	419	8.1	3	115	20	5
d5 N-EtFOSAA	✓	589	419	8.18	3	115	20	5
FBSA		298	77.9	6.83	3	100	28	5
FHxSA		398	77.9	7.95	3	100	28	5
FOSA		497.9	77.9	8.63	3	125	36	5
GenX		285	169	5.88	3	108	12	5
M GenX	✓	287	185	5.88	3	108	20	5
M PFDoA	✓	615	570	8.21	3	79	8	5
M2 4-2 FTS	✓	329	81	5.66	3	125	28	5
M2 6-2FTS	✓	429	81	6.94	3	125	32	5
M2 8-2 FTS	✓	529	81	7.66	3	170	36	5
M2 PFTeDA	✓	715	670	8.72	5	100	13	5
M3 PFBS	✓	302	99	4.99	3	100	33	5
M3 PFHxS	✓	402	99	6.57	3	100	41	5
M4 PFHpA	✓	367	322	6.52	3	72	8	5
M5 PFHxA	✓	318	273	5.79	3	70	8	5
M5 PFPeA	✓	268	223	4.35	3	60	4	5
M6 PFDA	✓	519	474	7.7	3	81	8	5

M7 PFUdA	✓	570	525	7.98	3	73	8	5
M8 FOSA	✓	506	78	8.63	3	125	36	5
M8 PFOA	✓	421	376	7.01	3	69	8	5
M8 PFOS	✓	507	99	7.37	3	100	46	5
M9 PFNA	✓	472	427	7.39	3	66	8	5
MPFBA	✓	217	172	2.18	3	60	8	5
N-EtFOSAA		584	525.9; 418.9	8.18	3	115	20	5
N-MeFOSAA		570	482.9;418.9	8.09	3	115	16	5
PFBA		213	168.9	2.18	3	60	8	5
PFBS		298.9	98.9;80	4.98	3	100	33	5
PFDA		513	469	7.7	3	81	8	5
PFDoA		613	569; 268.7	8.21	3	79	8	5
PFDS		598.9	99	7.93	3	100	56	5
PFDS		598.9	80	7.93	3	100	88	5
PFHpA		362.9	319; 169	6.52	3	72	5	5
PFHpS		448.9	98.7; 79.7	7.01	3	100	44	5
PFHxA		313	268.9	5.79	3	70	4	5
PFHxS		398.9	99;80	6.56	3	100	41	5
PFNA		463	419;169	7.39	3	66	8	5
PFNS		548.9	98.9; 79.9	7.67	3	165	48	5
PFOA		413	369;169	7.01	3	69	8	5
PFONS		530.9	350.9	7.44	3	100	17	5
PFOS		498.9	99; 80	7.37	3	100	46	5
PFOUDS		630.9	450.9	7.97	3	100	17	5
PFPeA		263	218.9	4.3	3	60	4	5

PFPeS		348.9	98.9;79.9	5.97	3	135	36	5
PFTeDA		713	669;169	8.6	3	100	13	5
PFTrDA		663	619;169	8.41	3	91	9	5
PFUdA		563	519	7.97	3	73	8	5
4-2 FTS		327	306.9	5.69	3	125	20	5

2.2.5 Method Validation and Quality Control/Quality Assurance (QA/QC)

Method validation was performed to assess sensitivity, linearity, matrix effects, intra- and inter-day precision and accuracy. Field blanks consisting of LC-MS grade water were carried along each sampling trip and treated the same as other samples collected. SPE method blanks were also prepared with LC-MS grade water spiked with the IS mixture and processed the same way as the environmental samples. An 11-point calibration curve was prepared in the concentration range of 2 to 1000 ng L^{-1} using diluted solutions from the 30-PFAS native standard working solution mixture. To determine the method detection limit (MDL), eight replicates of LC-MS grade water were spiked with the 30-PFAS mix to final concentration of 10, 100, 500 ng L^{-1} together with the IS, and were also analyzed through the complete analytical procedure. Precision and accuracy experiments were performed using LC-MS grade water spiked with the PFAS mixture to final concentrations of 100 and 500 ng L^{-1}, with intra-day precision evaluated by analyzing 5 replicates of each sample on the same day and inter-day precision being assessed over 3 different days with 3 replicates of each sample. Matrix effects experiments were conducted by the analysis of environmental samples (tap and surface waters) spiked with the 30-PFAS mixture at the concentration of 500 ng L^{-1}.

To ensure the quality of the analytical data being generated, control samples along each batch included procedure blanks to monitor potential carryover; method, and field/trip blanks to monitor contaminations, spiked blanks, matrix spikes, a continuing calibration verification (CCV) after 7-10 samples, and an ICV (from a secondary standard solution) at a concentration of 10 ng L^{-1} and 100 ng L^{-1} prepared with 5 mM ammonium formate and methanol (90:10 v/v). Analytical curves were run in the beginning and end of every set of 12-20 samples. The CCV and ICV measured concentration were monitored to not deviate more than 30% from the assigned value. In case of deviation, troubleshooting was conducted, and instrument was recalibrated before proceeding with the following injections.

2.2.6 PFAS Data analysis and Statistics

Peak integration and quantitation were performed using the MassHunter QQQ Quantitation analysis software. PFAS were considered present and quantifiable if the peaks meet the following criteria: 1) within 0.2 min of the same RT of corresponding isotopically labeled IS (when available), 2) presence in the confirmation peak (when 2 transitions were monitored), 3) signal to noise ratio (S/N) >3, and 4) above the detection limits established for the method, otherwise reported as below method detection limit (<MDL). For the native standards that did not have an available mass-labeled analog, the alternative mass-labeled standards were selected based on similar chain-length and functional groups, and retention time (Table 2.1). Results from the SPE method and direction injection method for the same sample were analyzed and compared, the average was taken if both meet the above-mentioned criteria.

For the compounds which showed concentrations above the calibration range from the SPE method, samples were diluted and re-run or the results from the direct injection method were reported. The software IBM SPSS Statistics version 26 was used for bar and box-plot graphs and statistical comparison of PFAS concentrations in tap water and surface water in the wet and dry seasons.

2.3 **Results and Validation**

2.3.1 Method Validation

Although not significantly different from previous methods in the literature (Coggan et al. 2019), the proposed method was validated to ensure the quality of the results reported. To assess the linearity of the method, 11-point calibration curves were plotted using the area ratio (represented by the peak area of each individual PFAS divided by the peak area of their respective isotopically labeled internal standard) as a function of the PFAS concentration. All PFAS abbreviations used are defined in Table 1.1. At the concentration range of 2 to 1000 ng L^{-1}, the curves showed to be

linear with R^2 coefficients higher than 0.99 for all the compounds. With some exceptions, higher molecular weight PFAS (N-MeFOSAA, N-EtFOSAA, PFTrDA, PFTeDA) sometimes exhibited R^2 between 0.97-0.98. The SPE MDLs were statistically calculated based on the standard deviation derived from the eight replicate spiked samples concentrations multiplied by the one-side student's t value at 99% confidence level and considering a 250-fold concentration factor of the SPE procedure. MDLs for the SPE procedure ranged from 0.01 (for PFBS) to 1.99 ng L^{-1} (for PFTeDA) as shown in Table 2.6. Since some compounds, especially short-chain PFAS, exceeded the calibration curve, tap water samples were also injected directly (without preconcentration by SPE). The instrument detection limit (IDL), determined by direct LC-MS injection, showed IDLs ranging from 0.26 (for PFBA) to 205 ng L^{-1} (for PFTeDA), which is presented in Table 2.7.

Average recoveries (%R) (Table 2.6 and 2.7) were calculated by subtracting the measured concentration of unspiked samples from the measured concentrations of spiked samples with the PFAS mixture at different concentrations (100 and 500 ng L^{-1}), then dividing by the expected spiked concentration for each PFAS, to assess the method effectiveness from extraction to final measurement. The results were above 90 % for the majority of the PFAS, ranging from 90- 128%, except for PFNS (65%). To assess repeatability, replicate spiked samples were analyzed on the same day (intra-day analysis) and the results expressed in terms of relative standard deviation (RSD) were below 10%, whereas reproducibility studies carried out in different days (inter-day analysis) showed RDS ≤ 20% for all compounds. Matrix effects (MEs), especially for complex samples having higher organic matter and salinity, can have a negative effect on the analytical method, resulting in either signal enhancement or suppression of the analyte, thus compromising the quantitative analysis of environmental samples (Kloepfer et al. 2005). MEs were estimated based on the equation $MEs\ (\%) = ([\frac{C_s - C_{us}}{C_0}] - 1) \cdot 100$, where C_s is the concentration found in the spiked sample matrix, C_{us} is the concentration measured in unspiked sample matrix, and C_0 is the

concentration found in spiked LC-MS water. For tap water assessments, some PFAS congeners showed suppression with ME ranging from -54 (PFOUDS) to -6.6 (PFTrDA) and others showed enhancement from 0.81 (PFDS) to 26 (PFUdA). Surface water samples with varied salinity (2- 40 ‰) showed ME ranging from -54 (FHxSA) to 26 (8-2 FTS). Although some high matrix effects were observed for PFOUDS and Adona (-49) for tap water samples and FHxSA in surface waters, they were not detected or reported in the analyzed environmental water samples. The method was successfully validated following the U.S. EPA guidelines delineated at EPA Method 537.1 and Method 533 (Shoemaker and Tettenhorst 2020; USEPA 2019) and MDL values obtained are consistent and similar to those previously reported in the literature for PFAS determination by LC-MS/MS methods (Coggan et al. 2019; East et al. 2021; Marchiandi et al. 2021; Munoz et al. 2017). Observed MDLs are considered adequate for the accurate determination of PFAS in tap and surface water, where they are generally present at low ppt levels.

Table 2.6 Method Validation results for PFAS SPE method

Compounds	**MDL (ng/L)**	**Intraday RSD**	**Interday RSD**	**Recovery (%)**	**Matrix Effect (tap water)**	**Matrix Effect (Surface water)**
PFBA	0.05	4.10	5.36	102	-10.8	-6.24
FBSA	0.05	2.28	5.93	113	-12.7	3.43
PFBS	0.01	0.80	1.27	101	-23.6	4.18
PFPeA	0.13	6.10	10.10	103	6.45	13.02
PFPeS	0.02	1.38	1.38	102	2.33	15.8
PFHxA	0.01	3.59	3.73	98.9	10.8	3.44
FHxSA	0.03	3.77	6.23	91.0	-25.6	-53.8
PFHxS	0.04	4.67	3.55	102	-28.7	3.43
4-2 FTS	0.03	2.68	1.96	103	2.97	4.53
Adona	0.02	4.67	4.66	100	-49.3	-21.3
GenX	0.02	1.43	2.99	100	-9.60	-7.39
PFHpA	0.05	3.62	10.00	121	7.83	-45.3
PFHpS	0.04	3.95	4.04	102	7.19	11.0
PFOA	0.04	6.18	9.29	98.9	-17.3	-10.2

FOSA	0.04	4.34	2.99	106	-14.6	0.61
PFOS	0.04	4.23	13	103	-18.4	-16.6
6-2 FTS	0.35	3.47	7.04	128	15.3	-21.5
PFONS	0.02	2.9	4.68	92.5	10.6	13.8
PFNA	0.03	4.1	5.79	91.6	-2.05	14.5
N-MeFOSAA	0.29	7.55	8.29	90.3	-17.0	2.75
N-EtFOSAA	0.45	9.61	4.45	94.5	12.9	9.80
PFNS	0.02	9.68	12.1	64.7	7.82	3.04
PFDA	0.02	2.76	5.08	98.9	11.8	15.8
PFDS	0.33	9.74	14.5	98.6	0.81	-6.91
8-2 FTS	0.34	3.25	7.15	94	1.36	26.0
PFUdA	0.20	3.69	5.22	105	25.5	17.6
PFDoA	0.22	3.42	6.83	113	-14.9	-7.49
PFTrDA	1.37	4.82	7.25	99.8	-6.59	-8.35
PFTeDA	1.99	6.55	20.2	128	10.2	-4.1
PFOUDS	0.36	9.71	13.3	103.8	-54.1	0.94

MDL: method detection limit, RSD: relative standard deviation

Table 2.7 Method Validation results for PFAS direct injection method

Compounds	**IDL (ng/L)**	**Intraday RSD**	**Interday RSD**	**Recovery (%)**	**Matrix Effect**
PFBA	0.26	6.01	5.19	98.7	-1.25
FBSA	2.14	9.83	10.7	105	4.00
PFBS	0.99	8.83	6.45	106	7.96
PFPeA	9.00	7.73	9.21	103	4.31
PFPeS	0.51	6.69	7.13	97.7	4.15
PFHxA	1.93	4.05	4.08	102	3.48
FHxSA	3.47	6.08	15.3	71.9	15.6
PFHxS	2.31	11.4	11.6	98.8	1.28
4-2 FTS	5.93	8.27	9.47	103	3.19
Adona	0.98	13.3	6.56	93.9	11.1
GenX	5.90	7.09	5.45	100	1.64
PFHpA	1.76	5.97	5.76	105	7.19
PFHpS	8.55	10.1	7.44	103	3.57
PFOA	2.04	5.11	7.23	102	5.57

FOSA	5.16	14.7	19.7	67.0	3.18
PFOS	1.35	8.85	11.9	96.8	6.32
6-2 FTS	44.6	14.45	23.1	98.3	-10.9
PFONS	1.97	17.5	21.9	93.8	3.85
PFNA	4.66	6.75	6.08	97.5	4.27
N-MeFOSAA	97.3	24.3	25.9	88.1	1.22
N-EtFOSAA	54.2	19.2	19.0	83.8	-2.73
PFNS	2.77	16.7	19.2	80.2	-9.27
PFDA	3.58	14.3	14.9	90.1	4.08
PFDS	18.3	24.0	21.0	101	-5.64
8-2 FTS	41.6	14.9	15.4	82.2	10.1
PFUdA	7.98	12.1	15.0	98.1	28.9
PFDoA	32.7	11.9	10.8	87.6	5.88
PFTrDA	107	25.3	27.8	91.8	-57.0
PFTeDA	205	19.6	38.1	87.1	-13.2
PFONDS	66.0	16.7	11.7	117	-3.68

IDL: instrument detection limit, RSD: relative standard deviation.

2.3.2 Occurrence and concentrations of PFAS in tap and surface waters from South Florida

Among 30 analyzed PFAS, 28 congeners were detected in one or more locations from the tap water samples with Adona and PFONDS below MDL in all locations, as shown in Table 2.8. The average (Min, Max) concentrations of each congener were reported for each location when two samples were taken from both the wet and dry season for the same location. Total PFAS concentration in tap water samples ranged from 1.44 to 242 ng L^{-1} from samples taken from Cooper City (dry season) and Grapeland Heights (dry season), respectively.

Table 2.8 Average and range concentrations of PFAS in Tap water samples collected in Miami-Dade, Broward and Palm Beach Counties, South Florida.

	PFAS Concentrations (ng/L)									
Locations	PFBA	PFBS	4-2 FTS	PFHxA	PFPeS	FBSA	GenX	PFHpA	PFHxS	PFOA
West Palm Beach	28.76(20.27,37.25)	4.44(4.38,4.49)	0.02(<0.03,0.05)	4.07(3.45,4.68)	0.38(0.33,0.44)	0.06(<0.05,0.12)	0.03(<0.02,0.06)	2.56(2.52,2.6)	4.1(3.03,5.16)	1.83(<0.04,3.66)
Lake Worth	1.34	0.30	<0.03	<0.01	<0.02	<0.05	<0.02	0.26	0.78	1.38
Boynton Beach	15.46	0.31	<0.03	0.33	0.08	<0.05	<0.02	0.28	0.46	1.02
Boca Raton	15.79(10.3,21.28)	2.28(0.32,4.24)	<0.03	3.74(0.33,7.15)	0.15(0.08,0.21)	<0.05	0.03(<0.02,0.05)	1.8(0.31,3.29)	1.36(0.51,2.2)	2.3(0.72,3.88)
Sunrise	1.09	0.05	7.59	0.21	<0.02	<0.05	<0.02	0.13	<0.04	3.64
Fort Lauderdale	37.09	6.52	6.37	6.31	0.82	0.11	<0.02	5.19	8.03	<0.04
Cooper City	5.7(0.65,10.75)	2.99(<0.01,5.98)	<0.03	7.18(0.02,14.34)	0.25(<0.02,0.5)	<0.05	<0.02	2.25(<0.05,4.5)	1.32(<0.04,2.64)	1.44(0.09,2.79)
Pembroke Pines	37.51(26.28,48.75)	9.53(9.38,9.69)	0.24(0.24,0.24)	16.77(14.53,19)	0.82(0.67,0.96)	1.83(1.73,1.93)	<0.02	9.66(7.61,11.72)	6.79(6.08,7.51)	7.33(4.98,9.68)
Hollywood	42.61(39.16,46.07)	6.83(5.02,8.64)	4.77(<0.03,9.54)	4.56(4.11,5.02)	0.74(0.51,0.98)	0.77(0.12,1.42)	<0.02	2.97(2.82,3.11)	4.81(4.01,5.61)	7.1(5.9,8.3)
Hollywood Lakes	7.28	4.28	<0.03	3.00	0.26	<0.05	<0.02	2.12	3.47	4.15
Dania Beach	51.80	5.12	<0.03	11.99	0.58	<0.05	<0.02	3.58	4.42	5.29
Ives Estates	23.86(11.23,36.48)	4.86(4.34,5.37)	4.34(0.06,8.62)	9.4(6.07,12.73)	0.77(0.56,0.97)	0.06(<0.05,0.12)	0.01(<0.02,0.02)	4.63(3.35,5.92)	3.95(3.47,4.43)	6.16(4.9,7.42)
North Miami Beach	13.23(0.81,25.65)	2.91(0.79,5.03)	5.5(<0.03,11)	7.68(0.61,14.74)	0.15(0.05,0.25)	0.04(<0.05,0.09)	0.02(<0.02,0.05)	2.41(0.38,4.44)	2.19(0.48,3.9)	4.18(3.72,4.65)
North Miami	38.75(27.18,50.32)	4.48(4.26,4.7)	3.42(0.08,6.76)	5.49(3.69,7.29)	0.92(0.52,1.32)	0.08(0.07,0.09)	0.05(0.04,0.06)	3.23(3.16,3.3)	4.08(3.61,4.55)	8.96(4.67,13.24)
Miami beach	20.18(17.1,23.25)	5.24(4.42,6.06)	0.02(<0.03,0.04)	9.32(7.55,11.09)	0.48(0.45,0.51)	3.39(0.13,6.65)	0.05(<0.02,0.1)	6.35(4.99,7.72)	5.01(3.54,6.49)	5.16(5.12,5.19)
Grapeland Heights	47.47(6.42,88.51)	3.16(2.12,4.19)	0.33(<0.03,0.67)	19.54(2.93,36.15)	0.51(0.21,0.81)	0.13(0.07,0.19)	<0.02	6.38(4.46,8.3)	4.06(3.68,4.45)	2.51(1.16,3.86)
Sweet water	10.05	4.06	0.18	5.47	0.82	0.21	<0.02	4.92	7.30	10.46
Coral Gables	32.20	4.08	0.15	31.62	0.82	0.08	<0.02	12.73	8.27	9.85
Key Biscayne	32.25	4.20	<0.03	6.53	0.27	0.08	<0.02	3.55	2.35	3.60
Kendall	14.85	3.43	<0.03	4.57	0.24	0.06	<0.02	3.82	2.23	4.18
Cutler Bay	16.24(9.34,23.13)	4.2(3.34,5.05)	0.04(<0.03,0.09)	5.43(4.79,6.08)	0.25(0.2,0.29)	<0.05	0.04(<0.02,0.07)	3.76(3.67,3.84)	2.04(1.93,2.15)	4.13(3.73,4.52)
Princeton	10.35	3.86	<0.03	6.04	0.26	0.24	<0.02	2.85	2.23	3.23

	PFAS Concentrations (ng/L)									
Locations	PFHpS	6-2 FTS	PFOS	PFPeA	Adona	PFNA	PFONS	PFNS	8-2 FTS	PFDA
West Palm Beach	0.32(0.26,0.38)	<0.35	10.05(7.5,12.59)	4.66(4.44,4.89)	<0.02	1.14(1.06,1.22)	1.27(<0.02,2.53)	0.04(<0.02,0.08)	<0.34	0.3(0.24,0.37)
Lake Worth	<0.04	<0.35	2.59	18.71	<0.02	0.90	<0.02	<0.02	<0.34	<0.02
Boynton Beach	0.08	<0.35	2.60	<0.13	<0.02	<0.034	<0.02	<0.02	<0.34	<0.02
Boca Raton	0.17(0.07,0.28)	1.75(<0.35,3.49)	7.74(3.09,12.39)	1.33(<0.13,2.65)	<0.02	0.41(<0.034,0.81)	<0.02	0.03(<0.02,0.06)	<0.34	0.15(<0.02,0.3)
Sunrise	<0.04	<0.35	0.53	0.19	<0.02	<0.034	<0.02	<0.02	<0.34	0.04
Fort Lauderdale	0.78	<0.35	31.52	12.00	<0.02	1.50	<0.02	<0.02	<0.34	0.84
Cooper City	<0.04	<0.35	13.37(<0.04,26.74)	<0.13	<0.02	<0.034	<0.02	0.02(<0.02,0.04)	<0.34	<0.02
Pembroke Pines	0.42(0.35,0.49)	11.24(<0.35,22.48)	20.46(14.38,26.53)	8.63(<0.13,17.26)	<0.02	2.13(1.96,2.3)	<0.02	0.02(<0.02,0.04)	3.02(2.82,3.22)	0.95(0.9,1)
Hollywood	0.52(0.46,0.59)	0.72(<0.35,1.43)	19.18(18.07,20.29)	4.49(3.67,5.3)	<0.02	2.08(0.92,3.25)	<0.02	9.84(0.06,19.61)	<0.34	0.48(0.45,0.52)
Hollywood Lakes	0.29	0.65	13.64	4.89	<0.02	0.60	<0.02	<0.02	<0.34	0.32
Dania Beach	0.44	1.77	18.06	17.45	<0.02	1.28	<0.02	0.04	<0.34	0.50
Ives Estates	0.38(0.35,0.41)	<0.35	21.33(15.32,27.35)	9.87(6.57,13.16)	<0.02	1.04(0.92,1.16)	0.66(<0.02,1.32)	9.92(1.56,18.29)	<0.34	0.51(0.43,0.6)
North Miami Beach	0.19(0.06,0.32)	<0.35	15.8(2.87,28.73)	1.16(0.76,1.55)	<0.02	1.81(0.28,3.33)	<0.02	0.02(<0.02,0.03)	<0.34	0.23(0.09,0.38)
North Miami	0.43(0.42,0.44)	<0.35	15.04(12.85,17.23)	9.72(7.54,11.91)	<0.02	1.41(1.05,1.76)	<0.02	13.29(1.36,25.21)	<0.34	0.49(0.44,0.54)
Miami beach	0.21(<0.04,0.41)	16.75(16.56,16.94)	20.07(18.37,21.77)	29.36(16.13,42.6)	<0.02	1.07(0.96,1.18)	0.06(<0.02,0.12)	0.94(<0.02,1.89)	0.49(<0.34,0.98)	0.23(<0.02,0.47)
Grapeland Heights	0.5(0.15,0.85)	48.57(28.46,68.67)	15.11(11.77,18.45)	13.11(6.87,19.35)	<0.02	4.07(0.62,7.51)	<0.02	<0.02	<0.34	0.67(0.3,1.04)
Sweet water	0.56	<0.35	8.27	20.65	<0.02	2.26	<0.02	<0.02	4.70	0.76
Coral Gables	0.58	<0.35	19.99	18.33	<0.02	2.87	<0.02	<0.02	2.96	0.79
Key Biscayne	0.22	<0.35	8.37	11.15	<0.02	0.74	<0.02	<0.02	<0.34	0.29
Kendall	0.13	<0.35	10.46	7.24	<0.02	0.66	<0.02	<0.02	<0.34	0.43
Cutler Bay	0.11(<0.04,0.22)	<0.35	10.73(9.29,12.17)	3.74(<0.13,7.49)	<0.02	0.36(<0.034,0.71)	0.04(<0.022,0.08)	0.06(<0.02,0.12)	<0.34	0.11(<0.02,0.23)
Princeton	0.20	<0.35	8.88	11.10	<0.02	0.61	<0.02	<0.02	<0.34	0.23

	PFAS Concentrations (ng/L)										
Locations	PFDS	PFONDS	PFUdA	FHxSA	N-MeFOSAA	N-EtFOSAA	PFDoA	PFTrDA	PFTeDA	FOSA	∑PFAS
West Palm Beach	<0.33	<0.36	<0.2	<0.03	1.33(<0.29,2.66)	<0.45	<0.22	<1.37	<1.99	4.75(<0.04,9.49)	70.10 (67.67,72.52)
Lake Worth	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	1.90	2.97	<0.04	31.15
Boynton Beach	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	20.62
Boca Raton	0.22(<0.33,0.43)	<0.36	<0.2	<0.03	0.09(<0.29,0.19)	<0.45	<0.22	<1.37	<1.99	<0.04	41.08(15.74,62.93)
Sunrise	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	13.48
Fort Lauderdale	1.01	<0.36	<0.2	<0.03	0.39	<0.45	<0.22	<1.37	<1.99	<0.04	118.48
Cooper City	<0.33	<0.36	<0.2	<0.03	0.2(<0.29,0.41)	0.59(0.5,0.67)	0.34(<0.22,0.68)	<1.37	<1.99	13.81(<0.04,27.62)	49.71(1.44,97.48)
Pembroke Pines	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	0.82(<1.37,1.65)	4.51(<1.99,9.01)	<0.04	153.93(94.40,190.98)
Hollywood	0.32(<0.33,0.63)	<0.36	<0.2	<0.03	<0.29	<0.45	0.18(<0.22,0.36)	<1.37	<1.99	<0.04	114.25(107.15, 118.86)
Hollywood Lakes	0.33	<0.36	2.26	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	47.54
Dania Beach	1.78	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	124.39
Ives Estates	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	5.1(<0.04,10.2)	106.89(101.01,112.77)
North Miami Beach	<0.33	<0.36	<0.2	<0.03	0.42(<0.29,0.85)	0.69(<0.45,1.38)	1.18(<0.22,2.36)	<1.37	<1.99	5.26(<0.04,10.51)	72.71(22.82,107.31)
North Miami	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	4.76(<0.04,9.52)	119.35(87.18,142.00)
Miami beach	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	0.82(<0.22,1.64)	1.1(<1.37,2.21)	<1.99	<0.04	126.31(119.26,133.36)
Grapeland Heights	0.44(<0.33,0.89)	<0.36	<0.2	0.74(<0.03,1.47)	0.19(<0.29,0.38)	0.25(<0.45,0.5)	0.33(<0.22,0.65)	<1.37	<1.99	<0.04	168.15(94.71, 241.58)
Sweet water	<0.33	<0.36	0.85	<0.03	<0.29	<0.45	<0.22	<1.37	2.03	<0.04	83.55
Coral Gables	<0.33	<0.36	1.75	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	147.07
Key Biscayne	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	73.61
Kendall	<0.33	<0.36	2.32	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	54.60
Cutler Bay	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	1.02(0.65,1.38)	<1.37	<1.99	<0.04	52.32(44.18,60.45)
Princeton	<0.33	<0.36	<0.2	<0.03	0.34	<0.45	<0.22	<1.37	<1.99	<0.04	50.42

The spatial distribution of PFAS detected in tap water is shown in Figure 2.2. Total PFAS was defined as the sum of all 30 PFAS concentrations found for each location. In total, 33 tap water samples were collected from 21 heavily populated locations. When the total concentration of PFAS for both seasons is averaged, total PFAS ranges from 70-168 ng L^{-1} for 13 locations and below 70 ng L^{-1} for 8 locations. No clear spatial distribution pattern was observed at the sites moving from north to south (as seen in Fig. 2.2). The maximum concentration of total PFAS (242 ng L^{-1}) was observed in the tap water collected in the Grapeland Heights area, where the Miami International Airport (MIA) is located 1 mile from the site. Additionally, the sample collected in the Dania Beach site, which is located only 2.5 miles from the Fort Lauderdale-Hollywood International airport (FLL), also showed to be one of the highest PFAS concentrations (124 ng L^{-1}) in this study. Airports and military bases are generally known as major PFAS sources due to the continued usage of AFFFs containing PFAS. Relatively high PFAS concentrations were therefore observed particularly in these two locations near two major airports in South Florida. PFAS levels were relatively lower (average: 57.7 ng L^{-1}) in the tap water samples collected in Key Biscayne, Kendall, Cutler Bay and Princeton area, which are farthest south from Miami. Similarly, PFAS levels from the sites farthest north from Miami were also relatively lower (average: 40.3 ng L^{-1}) in tap water samples collected in West Palm Beach, Lake Worth, Boynton Beach, and Boca Raton. This parallel can be explained by the distribution of anthropogenic activities centering in the populated area of Miami and slowly decreasing with the distance.

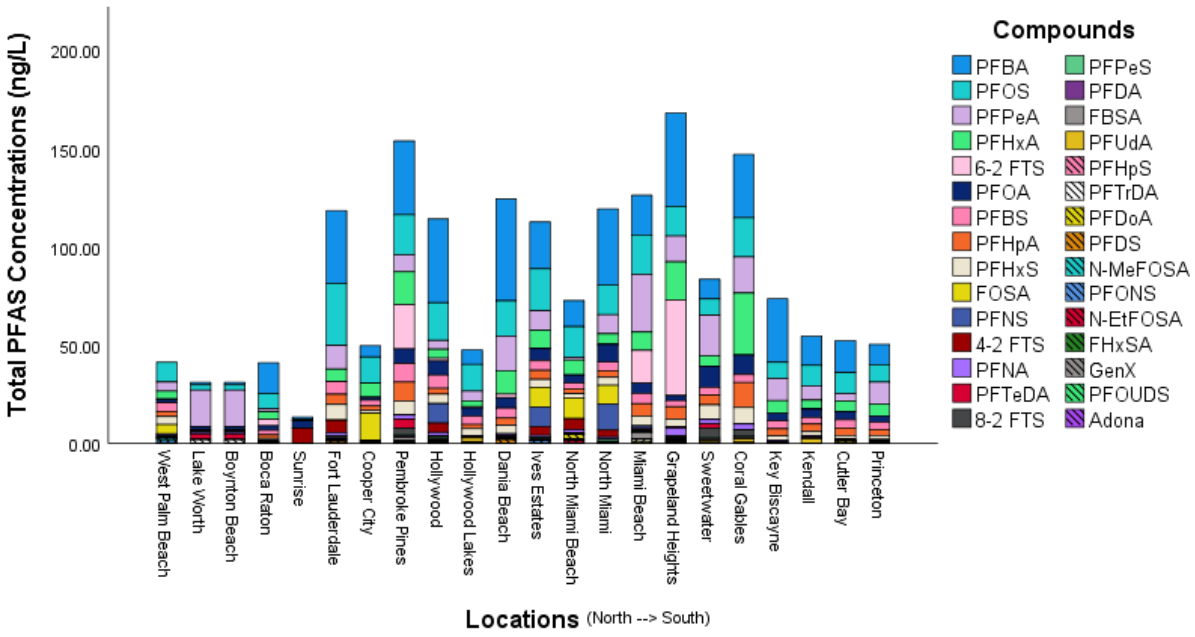


Figure 2.2 Total PFAS (average of wet and dry seasons) spatial distribution in tap water samples collected in Miami-Dade, Broward and Palm Beach Counties, South Florida.

In the surface water samples, 25 PFAS compounds were detected in one or more locations, whereas Adona, PFONS, PFOUDS, FHxSA, PFTeDA were not detected in any location. Total PFAS concentration in surface water samples ranged from 4.4 to 115.98 ng L^{-1} from samples taken from Miami Beach (dry season) and Biscayne Canal Number C-8 (wet season), respectively, as shown in Table 2.9. In total 27 surface water samples were collected from 15 sites from Biscayne Bay and adjacent river and canals from two sampling trips (wet and dry seasons), where 4 sites had total PFAS ranging from 70-106 ng L^{-1} and 11 sites below 70 ng L^{-1} based on average total concentration of PFAS for both seasons. The highest concentrations of total PFAS in surface water (average: 106 ng L^{-1}) was observed at the Biscayne Canal C-8. Relatively lower levels (average: 14.9 ng L^{-1}) were observed in 3 sites collected by Miami Beach. PFAS spatial distribution in Biscayne Bay and adjacent canals is presented in Figure 3, where no clear pattern was observed at the sites moving

from north to south, but with the highest levels concentrated in BC8, Little River (LR1 & LR2) and Seybold Canal (SC, which is close to the Miami Dade Water and Sewer distribution).

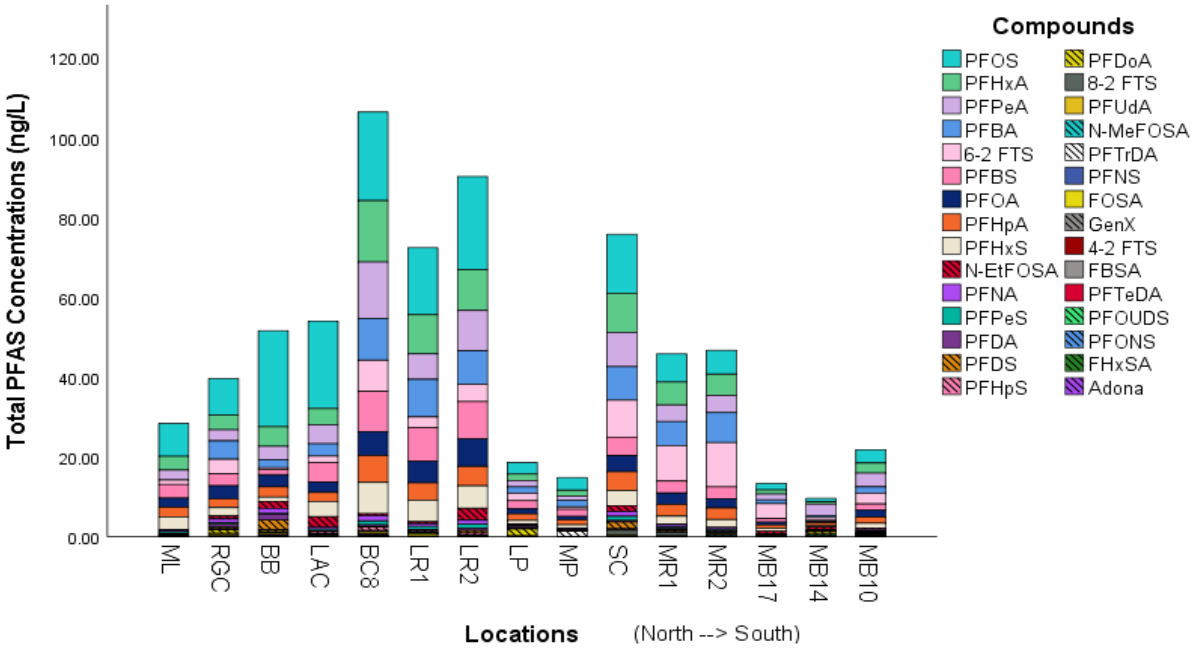


Figure 2.3 Total PFAS (average of wet and dry seasons) spatial distribution in surface water samples collected in Biscayne Bay and adjacent canals, South Florida. Sampling site descriptions, coordinates, and collection dates are presented in Table 2.2

The average of each PFAS detected in tap water and surface water samples are presented in Figure 4. As noted, PFAS levels for tap waters were higher than in the surface waters of Biscayne Bay and adjacent canals, which emphasizes the ineffective process of conventional drinking water treatment plants in the removal of this class of recalcitrant compounds (Rahman et al, 2014), especially for short-chain PFAS. Additionally, higher levels of short-chain PFAS could be due to the breakdown of precursors of longer chain PFAS and contamination coming from polyvinyl chloride (PVC) water pipes and containers (or other PFAS-containing materials) during the treatment and distribution processes (Shivakoti et al. 2010). It's important also to note that the drinking water supply from South Florida is from groundwater, including Everglades and Biscayne Bay aquifers, where PFAS sources and concentrations were not evaluated. Similarly, in a previous state-wide investigation of PFAS contamination level near fire training facilities in Florida, the concentration of PFOA and PFOS were higher in most of the monitoring wells (groundwater) than surface water collected in the same area (Hu et al. 2016, Cui et al. 2020), which suggest that the groundwater is also more readily influenced by point source of PFAS pollution than surface water.

As evidenced in Figure 2.4, PFBA, PFBS, PFHpA, and PFOS were the predominant compounds detected in all tap water sampling locations based on median concentrations throughout all the samples. Though, PFBA (detected in 100% of the samples), PFBS (97%), PFHxA (97%), PFPeS (94%), PFHxS (94%), PFOA (97%), PFHpA (97%), PFHpS (82%), PFPeA (82%), PFNA (82%), PFDA (79%) and PFOS (97%) were all frequently detected in the tap waters assessed. Highest concentrations among PFCAs were measured for PFBA (88 ng L^{-1}), followed by PFPeA (43 ng L^{-1}), PFHxA (36 ng L^{-1}), and PFOA (13 ng L^{-1}) in tap waters. Highest tap water concentrations among PFSAs were measured for PFOS (32 ng L^{-1}), followed by PFBS (9.7 ng L^{-1}), and PFHxS (8.3 ng L^{-1}). The shorter chain PFAS (PFBA, PFPeA and PFHxA) were exceeding levels of PFOA in tap water, which could be either a result of emerging PFAS substitution strategy going on in the industry or the breakdown product of related PFAS (Cao et al., 2010; Wilhelm et al., 2010). Within

the fluorinated telomers, 4-2 FTS (48% detection) was the dominant compounds, however 6-2 FTS (27% detection) showed the highest levels up to 69 ng L^{-1} (average of 5.6 ng L^{-1}), followed by 4-2 FTS (maximum of 11 ng L^{-1}, average: 1.5 ng L^{-1}), and 8-2 FTS (average: 0.56 ng L^{-1}, 15% detection). PFOSA (FBSA, FHxSA, FOSA), FOSAA (N-MeFOSAA, N-EtFOSAA), and fluorinated ether PFAS such as GenX and Adona were rarely detected (<25%) above the MDL through all the sampling sites (<MDL-28 ng L^{-1}), except for FBSA (58% detection). GenX and ADONA are new substitutes of the banned PFOA, which have been recently detected at high concentrations especially near fluorochemical production plants (Gebbink et al. 2020). Since there are no industrial point sources of PFAS in Florida, the rare presence of GenX and ADONA at very low levels might be related to air emissions and/or water currents from areas where they are used to produce polytetrafluoroethylene (Hopkins et al, 2018), as no information is available about their presence in specific consumer products.

In surface water sample, PFBA, PFBS, PFHxA, PFPeS, PFHpA, PFHxS, PFOA, PFOS, and PFNA were detected in all surface water sample as shown in Table 2.9 and Figure 2.4. Moreover, 4-2 FTS, PFHpS, 6-2 FTS, PFPeA, and PFDA were detected in more than 80% of the samples. Highest concentration among all the PFAS compounds were PFOS (up to 46 ng L^{-1}, average: 11 ng L^{-1}), followed by PFHxA (average:5.3 ng L^{-1}), PFPeA (average: 4.8 ng L^{-1}), and PFBA (average: 4.7 ng L^{-1}). Differently from the results from the tap waters, 6-2 FTS was the dominant compound among fluorinated telomers (93% detection and average concentration of 4 ng L^{-1}), although 4-2 FTS was also frequently detected (81%). Additionally, similarly to the tap waters, PFOSA, FOSAA, and fluorinated ethers showed to be detected few times above the MDL (≤30% detection) in all sampling sites and at lower concentrations (<MDL-2.97 ng L^{-1}), except for FBSA and N-EtFOSAA, both detected at 44% of the samples. Generally, in this study, the longer-chain PFAS (especially PFUdA, PFDoA, PFTrDA, PFTeDA) were detected less frequently than the shorter-

chain PFAS with C<8, which could be also due to the higher MDLs of the former compared to the latter.

Table 2.9 Average and range concentrations of PFAS in surface water samples collected in Biscayne Bay and adjacent canals, South Florida.

	PFAS Concentrations (ng/L)									
Locations	**PFBA**	**PFBS**	**4-2 FTS**	**PFHxA**	**PFPeS**	**FBSA**	**GenX**	**PFHpA**	**PFHxS**	**PFOA**
Maule Lake	4.34(1.92,6.76)	3.44(1.41,5.47)	0.04(<0.03,0.09)	3.47(1.69,5.24)	0.45(0.14,0.77)	0.03(<0.05,0.06)	0.17(<0.02,0.33)	2.4(1.26,3.54)	3.3(1.26,5.34)	2.32(1.12,3.51)
Royal Galdes Canal	4.59(2.42,6.76)	2.99(1.33,4.65)	0.04(0.04,0.04)	3.63(1.72,5.53)	0.38(0.14,0.62)	<0.05	0.03(<0.02,0.05)	2.2(1.33,3.06)	2.1(1.26,2.95)	3.3(1.72,4.88)
FIU BBC	1.92(0.68,3.16)	1.3(0.4,2.2)	0.04(0.03,0.04)	4.92(0.54,9.3)	0.27(0.04,0.5)	0.03(<0.05,0.06)	0.03(<0.02,0.06)	2.54(0.48,4.6)	1.09(0.34,1.85)	3.05(0.29,5.81)
Little Arch Creek	3.09(2.12,4.06)	4.84(2.56,7.11)	0.02(<0.03,0.04)	4.13(2.62,5.64)	0.49(0.24,0.74)	0.03(<0.05,0.07)	<0.02	2.26(1.53,3)	3.8(2.5,5.1)	2.64(1.56,3.73)
Biscayne Canal C-8	10.45(10.23,10.67)	10.06(6.41,13.7)	0.08(0.04,0.13)	15.38(15.35,15.41)	1.01(0.74,1.28)	0.07(0.05,0.1)	0.11(<0.02,0.23)	6.73(6.6,6.85)	7.81(6.29,9.33)	6.04(4.65,7.43)
Little River site 1	9.4(9.35,9.45)	8.41(5.93,10.88)	0.06(0.05,0.07)	9.8(9.44,10.16)	0.76(0.5,1.02)	0.08(0.07,0.1)	<0.02	4.37(4.22,4.53)	5.37(4.14,6.61)	5.38(4.8,5.95)
Little River site 2	8.44(7.24,9.65)	9.37(5.82,12.91)	0.04(0.04,0.04)	10.18(8.52,11.83)	1(0.44,1.55)	0.04(<0.05,0.09)	0.03(<0.02,0.06)	4.85(3.87,5.83)	5.66(3.76,7.56)	6.87(5.59,8.14)
Legion Park	1.58	2.08	<0.03	1.62	0.09	<0.05	<0.02	1.65	1.02	1.22
Morningside Park	1.64	1.75	<0.03	1.54	0.09	<0.05	<0.02	1.19	0.93	0.92
Seybold Canal	8.39	4.58	0.06	9.85	1.03	0.06	<0.02	4.76	3.73	4.10
Miami River site1	6.02(4.64,7.4)	2.98(2.47,3.5)	0.05(0.04,0.06)	5.73(4.42,7.03)	0.36(0.2,0.53)	<0.05	0.01(<0.02,0.02)	2.91(2.32,3.51)	2.15(1.72,2.57)	2.91(2.22,3.6)
Miami River site2	7.48(5.37,9.59)	2.94(2.43,3.45)	0.07(0.06,0.08)	5.35(4.1,6.6)	0.26(0.24,0.28)	0.03(<0.05,0.06)	<0.02	2.89(2.13,3.66)	2.06(1.63,2.5)	2.34(2.12,2.56)
Miami Beach 17th ST	0.98(0.74,1.22)	0.89(0.89,0.9)	0.04(0.03,0.05)	1.06(0.74,1.38)	0.1(0.08,0.11)	0.03(<0.05,0.06)	<0.02	0.84(0.78,0.91)	0.74(0.73,0.75)	0.74(0.64,0.84)
Miami Beach 14th ST	0.5(0.38,0.63)	0.28(0.16,0.4)	0.03(0.03,0.03)	0.56(0.21,0.9)	0.06(0.02,0.1)	<0.05	0.01(<0.02,0.03)	0.72(0.29,1.14)	0.25(0.14,0.35)	0.34(0.11,0.57)
Miami Beach 10th ST	1.59(0.45,2.72)	1.56(0.68,2.44)	0.02(<0.03,0.05)	2.51(0.45,4.56)	0.13(0.07,0.19)	0.03(<0.05,0.06)	0.25(<0.02,0.51)	1.61(0.69,2.54)	1.23(0.75,1.72)	1.68(0.68,2.67)

	PFAS Concentrations (ng/L)									
Locations	**PFHpS**	**6-2 FTS**	**PFOS**	**PFPeA**	**Adona**	**PFNA**	**PFONS**	**PFNS**	**8-2 FTS**	**PFDA**
Maule Lake	0.37(0.11,0.62)	1.11(0.39,1.83)	8.21(3.4,13.03)	2.46(1.21,3.72)	<0.02	0.38(0.3,0.45)	<0.022	0.08(<0.02,0.15)	<0.34	0.16(0.14,0.18)
Royal Galdes Canal	0.21(0.13,0.29)	3.65(2.18,5.12)	9.24(5.79,12.68)	2.78(2.53,3.04)	<0.02	0.98(0.91,1.05)	<0.022	0.03(<0.02,0.07)	<0.34	0.72(0.42,1.02)
FIU BBC	0.17(<0.04,0.33)	0.49(<0.35,0.98)	24.07(1.16,46.98)	3.41(0.63,6.18)	<0.02	1.26(0.08,2.44)	<0.022	0.2(<0.02,0.41)	<0.34	1.36(0.21,2.51)
Little Arch Creek	0.49(0.22,0.75)	1.64(0.72,2.56)	21.93(8.78,35.08)	4.69(4.6,4.79)	<0.02	0.46(0.22,0.7)	<0.022	0.1(<0.02,0.2)	<0.34	0.18(0.15,0.21)
Biscayne Canal C-8	0.93(0.53,1.33)	7.82(6.47,9.18)	22.19(18.49,25.89)	14.16(12.14,16.18)	<0.02	1.28(1.03,1.54)	<0.022	0.08(<0.02,0.15)	0.33(<0.34,0.63)	0.48(0.21,0.76)
Little River site 1	0.48(0.4,0.55)	2.76(1.7,3.81)	16.81(12.1,21.51)	6.38(6.1,6.65)	<0.02	0.69(0.63,0.74)	<0.022	0.08(<0.02,0.15)	<0.34	0.3(0.06,0.55)
Little River site 2	0.45(0.33,0.58)	4.32(3.72,4.93)	23.4(12.98,33.82)	10.06(9.81,10.31)	<0.02	0.99(0.72,1.26)	<0.022	0.05(<0.02,0.1)	<0.34	0.59(0.56,0.63)
Legion Park	0.07	1.87	2.89	1.59	<0.02	0.36	<0.022	<0.02	<0.34	0.60
Morningside Park	0.07	0.63	3.18	1.02	<0.02	0.22	<0.022	<0.02	<0.34	0.25
Seybold Canal	0.39	9.24	14.76	8.60	<0.02	1.19	<0.022	0.05	1.15	0.39
Miami River site1	0.16(0.1,0.21)	8.87(5.73,12.01)	7.04(5.02,9.06)	4.19(4.08,4.3)	<0.02	0.62(0.38,0.86)	<0.022	0.02(<0.02,0.04)	0.94(0.47,1.4)	0.29(0.27,0.31)
Miami River site2	0.38(0.17,0.59)	11.11(10.74,11.49)	5.98(5.66,6.3)	4.31(4.01,4.6)	<0.02	0.49(0.45,0.53)	<0.022	0.06(<0.02,0.12)	0.22(<0.34,0.44)	0.08(<0.02,0.15)
Miami Beach 17th ST	0.05(<0.04,0.09)	3.7(<0.35,7.39)	1.61(1.24,1.97)	1.44(0.78,2.11)	<0.02	0.19(0.13,0.26)	<0.022	0.06(<0.02,0.13)	<0.34	0.11(0.04,0.18)
Miami Beach 14th ST	<0.04	0.74(0.52,0.95)	0.89(0.38,1.39)	2.89(<0.13,5.68)	<0.02	0.19(0.1,0.28)	<0.022	<0.02	<0.34	0.16(0.08,0.24)
Miami Beach 10th ST	0.1(0.07,0.13)	2.69(2.05,3.33)	3.37(1.74,4.99)	3.45(2.18,4.72)	<0.02	0.37(0.16,0.59)	<0.022	0.06(<0.02,0.12)	<0.34	0.28(0.04,0.53)

	PFAS Concentrations (ng/L)										
Locations	**PFDS**	**PFONDS**	**PFUdA**	**FHxSA**	**N-MeFOSAA**	**N-EtFOSAA**	**PFDoA**	**PFTrDA**	**PFTeDA**	**FOSA**	**∑PFAS**
Maule Lake	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	32.72(15.84,49.61)
Royal Galdes Canal	0.37(<0.33,0.75)	<0.36	0.55(0.13,0.96)	<0.03	0.18(<0.29,0.37)	0.75(<0.45,1.51)	0.94(<0.22,1.88)	<1.37	<1.99	<0.04	39.69(28.81,50.57)
FIU BBC	2.51(<0.33,5.03)	<0.36	0.42(<0.2,0.84)	<0.03	0.25(<0.29,0.49)	1.74(1.28,2.21)	0.54(<0.22,1.09)	<1.37	<1.99	<0.04	51.65(8.2,95.1)
Little Arch Creek	<0.33	<0.36	<0.2	<0.03	0.28(<0.29,0.55)	2.67(<0.45,5.34)	0.18(<0.22,0.36)	<1.37	<1.99	<0.04	54.07(33.53,74.6)
Biscayne Canal C-8	<0.33	<0.36	0.58(0.33,0.83)	<0.03	0.31(<0.29,0.61)	0.53(<0.45,1.07)	<0.22	<1.37	<1.99	<0.04	106.44(96.9,115.98)
Little River site 1	<0.33	<0.36	<0.2	<0.03	<0.29	0.48(<0.45,0.96)	<0.22	<1.37	<1.99	0.69(<0.04,1.37)	72.44(63.82,81.05)
Little River site 2	0.66(<0.33,1.32)	<0.36	0.13(<0.2,0.25)	<0.03	<0.29	2.97(1.45,4.5)	<0.22	<1.37	<1.99	<0.04	90.27(65.46,115.09)
Legion Park	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	1.74	<1.37	<1.99	<0.04	18.61
Morningside Park	<0.33	<0.36	<0.2	<0.03	<0.29	<0.45	<0.22	1.42	<1.99	<0.04	14.86
Seybold Canal	1.67	<0.36	0.35	<0.03	<0.29	1.51	<0.22	<1.37	<1.99	<0.04	75.82
Miami River site1	0.45(<0.33,0.81)	<0.36	0.13(<0.2,0.26)	<0.03	<0.29	<0.45	<0.22	<1.37	<1.99	<0.04	45.83(34.17,57.49)
Miami River site2	<0.33	<0.36	0.23(<0.2,0.45)	<0.03	0.35(<0.29,0.69)	<0.45	<0.22	<1.37	<1.99	<0.04	46.75(39.7,53.8)
Miami Beach 17th ST	<0.33	<0.36	0.15(<0.2,0.29)	<0.03	<0.29	0.57(<0.45,1.14)	<0.22	<1.37	<1.99	<0.04	13.38(8.84,17.91)
Miami Beach 14th ST	<0.33	<0.36	0.12(<0.2,0.23)	<0.03	0.38(<0.29,0.77)	0.67(<0.45,1.34)	0.69(0.52,0.87)	<1.37	<1.99	<0.04	9.65(4.4,14.9)
Miami Beach 10th ST	<0.33	<0.36	<0.2	<0.03	0.34(<0.29,0.68)	0.35(<0.45,0.69)	0.17(<0.22,0.35)	<1.37	<1.99	<0.04	21.84(11.43,32.26)

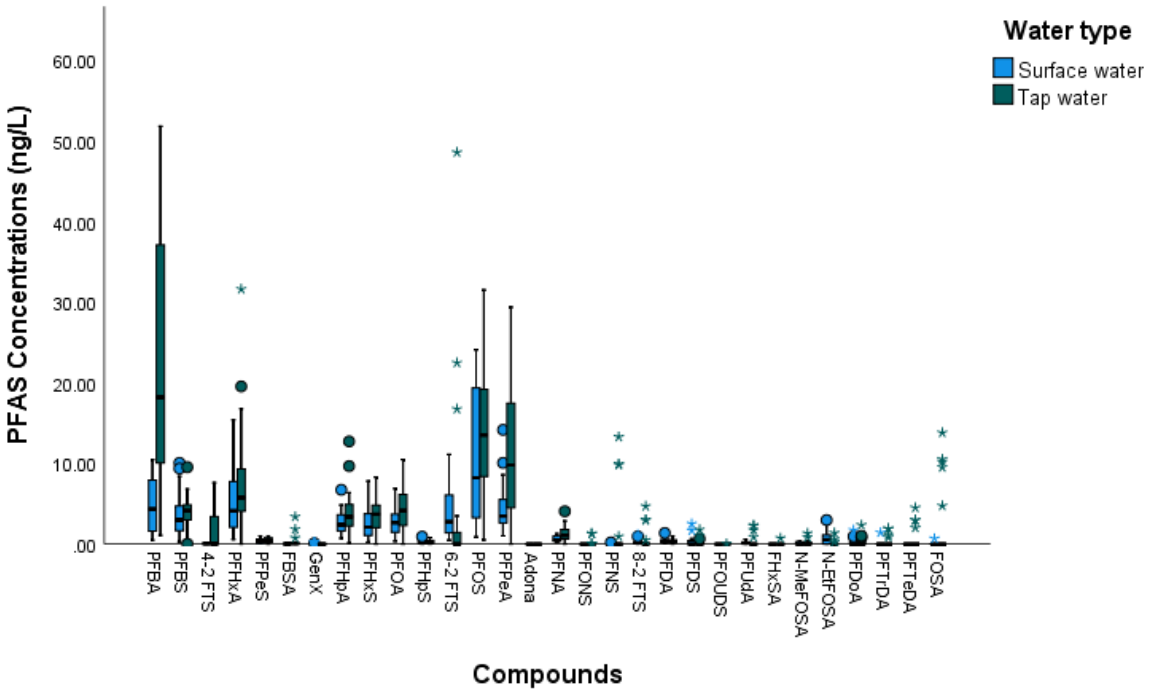
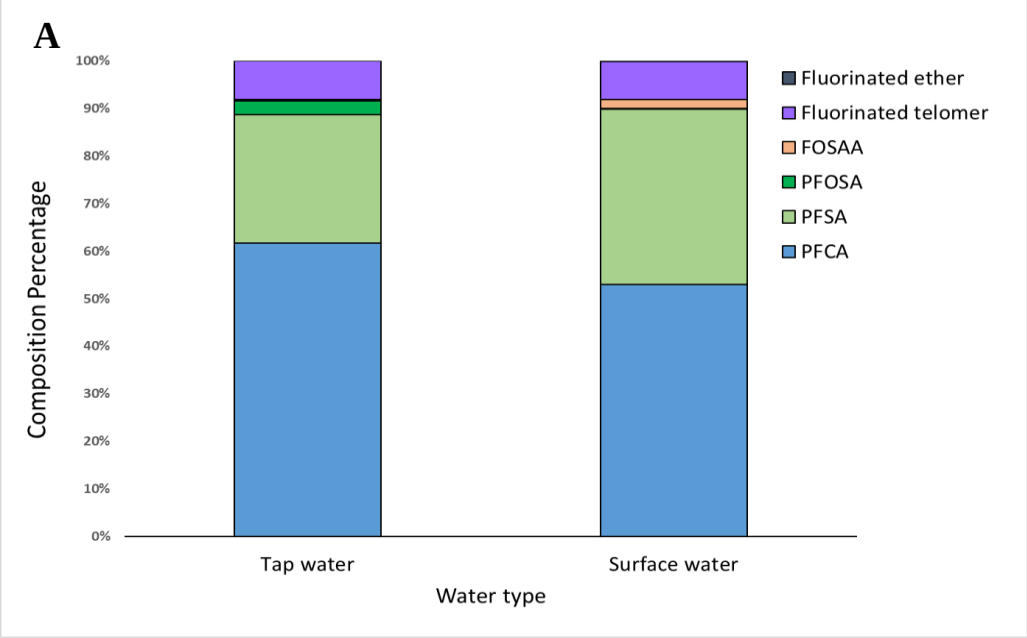


Figure 2.4 PFAS concentrations in tap water and surface water from the wet and dry seasons combined. Median (the middle line), minimum and maximum values excluding outliers (upper and lower whiskers) are shown in the boxplot. The circle and asterisks represent mild outliers and extreme outliers.

Though the composition of PFAS varies from sample to sample, when PFAS were categorized by class (PFCA, PFSA PFOSA FOSAA, fluorinated telomer and fluorinated ether) throughout all the samples by summing the concentrations from all belonging to the same category, it shows that PFCA and PFSA represent 80-90% of total PFAS being detected (they were also the majority of the compounds being analyzed). A very similar PFCA pattern was observed between the tap (88%) and surface water (89%), as seen in Figure 5A. When PFAS were categorized by chain length, with short-chain PFAS defined as the ones with carbon chain less than 8 and long-chain with carbon chain greater than or equal to 8, the results shows that the short chain PFAS represent 65% and 59% of the total PFAS in tap and surface water, respectively, as shown in Figure 5B. When comparing the total PFAS detected in the dry and wet season for surface and tap water, respectively,

the surface water results were found to be significantly different (p=0.05), which indicates the concentration of PFAS detected in the wet season had higher levels than the dry season (N=27), as shown in Figure 6. However, the difference in tap water between seasons was not significant (N=33, p=0.144), which could be attributed to the high variability of the concentrations found and that samples were not always sampled from the same exact location. A more comprehensive study of the seasonal variations in different years will be subject of a future investigation.



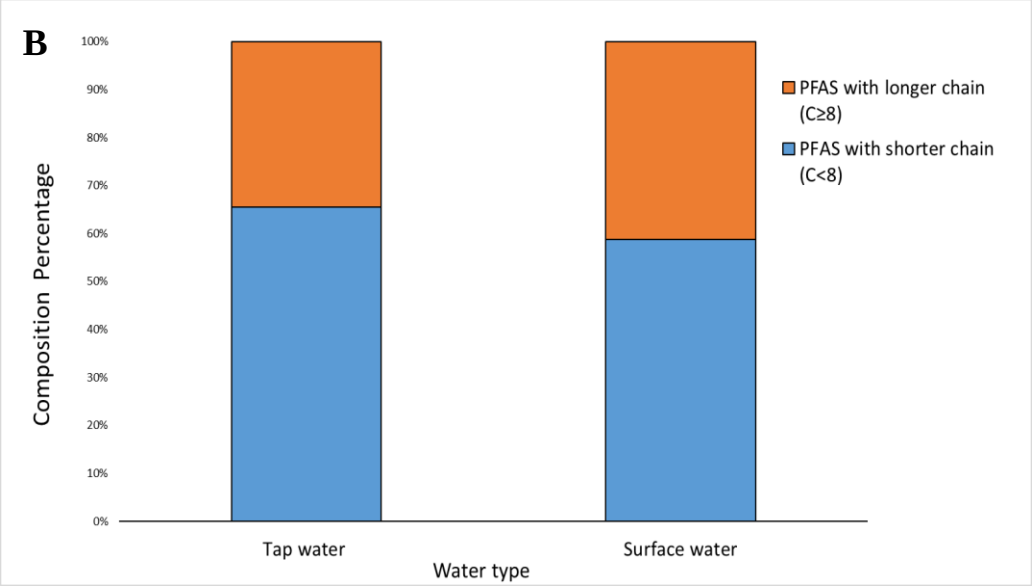


Figure 2.5 PFAS composition percentage categorized by class (A) and by chain length (B). Long chain PFAS are defined by a carbon chain greater than or equal to 8 and short-chain PFAS are defined as the ones with a carbon chain less than 8. Classes are defined in Table 1.1.

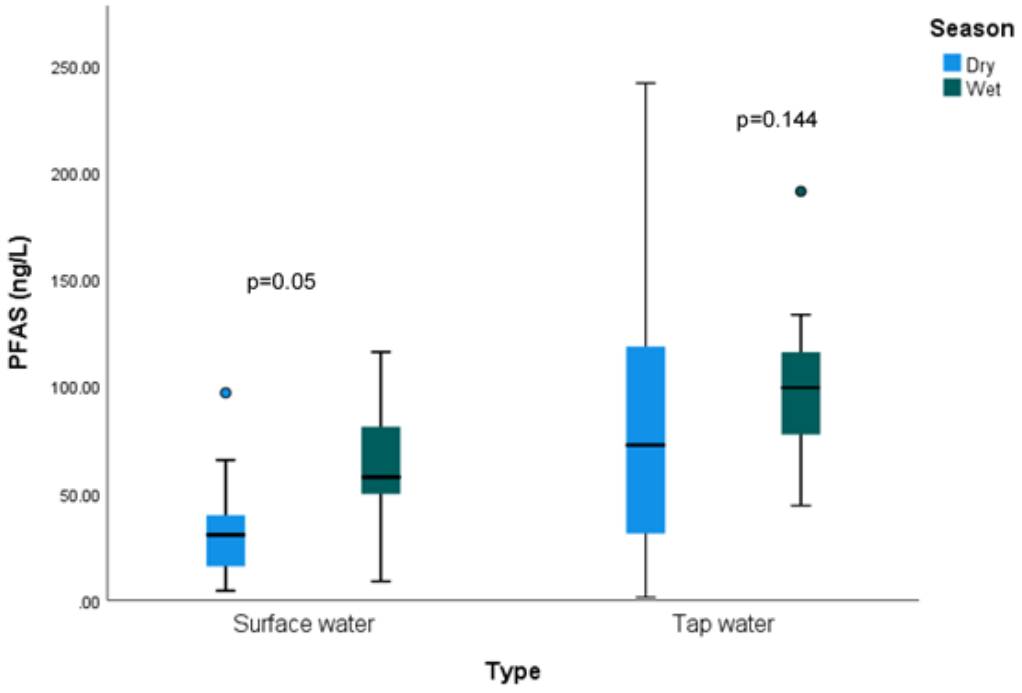


Figure 2.6 Seasonal Variation of PFAS in surface and tap water samples

2.3.3 Comparison to PFAS levels worldwide in surface and tap waters

In the past decades, PFAS have been detected in surface water and drinking water all over the world. Although PFAS exposure was different, comparison of PFAS levels to previous studies is helpful to understand and provide more information about PFAS distribution and potential exposure sources.

The U.S. EPA published the third Unregulated Contaminated Monitoring Rule (UCMR3) in 2017 (USEPA 2017), where PFOS, PFOA, PFNA, PFHxS, PFHpA and PFBS were identified for monitoring efforts at national level. In this context, nearly 1200 samples were collected from small surface water systems (≤ 10,000 population served) and approximately 13,300 samples were collected from large surface water systems (≥ 10,000 population served). In this previous study, only PFOS was detected above the minimum reporting level (MRL) in small surface water systems with an average concentration of 54 ng L^{-1}, though in large surface water systems all six monitored PFAS were detected (PFOA with a mean concentration of 31 ng L^{-1}, PFBS: 212 ng L^{-1}, PFOS: 77 ng L^{-1}, PFHxS: 69 ng L^{-1}, PFNA: 54 ng L^{-1}, and PFHpA: 19 ng L^{-1}), showing concentrations at least 2 times higher than the ones in our study.

A more recent study reported the measurement of 17 PFAS in surface water samples collected in Nevada, U.S., along Truckee River, Lake Tahoe, and Pyramid Lake (Bai and Son 2021). PFHxA, PFBS and PFOA were detected in all samples from Truckee River while PFPeA and PFHxA were found in all Las Vegas Wash samples. A total of 14 PFAS were detected in Truckee River with the highest total PFAS concentration of 203 ng L^{-1} while only 10 PFAS were detected in Las Vegas Wash with the highest concentration of 592 ng L^{-1}. In our study, 25 compounds were detected above MDL, with the highest total PFAS concentration of 106.44 ng L^{-1} in surface water, which was up to 4 times lower than in Nevada.

A tap water test conducted by the Environmental Working Group (EWG) across 44 locations in the U.S., including some sites in South Florida, reported that more than 40 samples contained PFAS levels that could pose health risks to humans. (Evans et al., 2020; Stoiber et al., 2020). Total PFAS levels ranged from below 1 ng L^{-1} to 186 ng L^{-1}, which is in line with what we have observed in this study. Specifically for the samples collected in Miami, Florida, levels reported in the previous study (PFOS and PFBA at 12 ng L^{-1}, PFPeA at 10 ng L^{-1}, PFHxA at 7 ng L^{-1}, PFOA at 5 ng L^{-1}, PFBS at 4 ng L^{-1} and PFHpA at 3 ng L^{-1}) are very close to the average concentrations found here (PFOS: 14 ng L^{-1}, PFPeA:9 ng L^{-1}, PFHxA: 8 ng L^{-1}, PFOA at 5 ng L^{-1}, PFHxA at 8 ng L^{-1}, PFBS at 4 ng L^{-1} and PFHpA at 4 ng L^{-1}). In fact, the highest PFAS levels detected by EWG were in samples from major metropolitan areas, including Miami, Philadelphia, and New Orleans (Evans et al., 2020).

PFAS contamination has also drawn a lot of attention worldwide (Domingo and Nadal 2019). Recently, mean concentration of 26 PFAS in several water sources collected in 2015 in Sweden (Gobelius et al. 2018) was 94 ng L^{-1} in surface water samples, which was only slightly higher than in Biscayne Bay area (46 ng L^{-1}). Similarly, to what was observed in our study in Biscayne Bay, among all the detected PFAS, more than 60% belonged to PFCAs, in which the predominant congeners were PFBA, PFHpA, PFHxA, PFPeA and PFOA. On the other hand, among PFSAs, PFOS was the predominant compound in our research at the Biscayne Bay area with a mean concentration of 10.47 ng L^{-1}, showing to be 16 times higher than the reported PFOS levels from Sweden inland surface water. Damià Barceló's group reported that PFOS was detected in all tap water samples from France, Spain and Brazil with mean concentrations of 7.73, 15.33 and 15.83 ng L^{-1} (Schwanz et al. 2016), which was similar to the average PFOS level found at South Florida in our study (13.35 ng L^{-1}). Similar research has also been done in China, where surface water and tap water samples had been collected across the country in 2015 (Tan et al. 2017). Yang's group reported a range of 1.4 to 175 ng L^{-1} of total PFAS in tap water samples from 17 cities in the eastern

region of China with an average of 35 ng L^{-1}, which were about 2.4 times lower than our results (84 ng L^{-1}) (Tan et al. 2017). Another tap water research reported that nationwide mean concentration of 17 PFAS in drinking water from China was 35.13 ng L^{-1}, which is approximately 50% lower than our detected level of 15 overlapped compounds (Li et al. 2019). Similar to our results, PFAS profile showed high PFAS contributions of PFBA and PFBS, but with concentrations in China up to 50 times lower than in our study (Li et al. 2019).

2.3.4 Ecological Risk Assessment

The aquatic ecosystem is under constant pressure of anthropogenic contaminants, such as the persistent PFAS, and the coastal areas are known for concentrating these pollutants (Xiao, 2017), which can lead to potential impacts on coral reefs, early life stages of aquatic organisms, and other sensitive species inhabiting Biscayne Bay area. Although there are no established guidelines for ecological risk-based assessment in surface water or screening thresholds for PFAS that are recommended and/or reinforced by the U.S. EPA, other countries, and some states within the U.S, including Florida, have defined provisional surface water screening levels for certain PFAS, especially PFOS and PFOA (FL DEP, 2021). The Florida Department of Environmental Protection (FL DEP) has developed surface water screening values of 1,300 µg L^{-1} PFOA and 37 µg L^{-1} PFOS for freshwater systems, and 13 µg L^{-1} PFOS in saltwater systems, considering the protection of aquatic biota (fish and shellfish) (FL DEP, 2021). Our PFOS and PFOA values are orders of magnitude below this threshold. However, more restrictive regulatory threshold/screening values of 0.00023 µg L^{-1} PFOS was recommended by the National Chemicals Working Group in Australia and New Zealand for 99% species protection value (draft default guideline value), 0.035 µg L^{-1} PFOS by the Michigan Department of Community Health for surface water value protective of avian wildlife, 0.023 µg L^{-1} PFOS by the European Commission as quality standard for saltwater pelagic community, with 0.00013 µg L^{-1} PFOS as annual average environmental quality

standard.(Ankley et al., 2004). In our study, we have observed PFOS levels up to 29 ng L^{-1} which is above most of these lower thresholds recommended in Europe, Australia and New Zealand, which could potentially affect marine organisms within Biscayne Bay and adjacent canals.

2.3.5 Human Health Risk Assessment

Humans are exposed to PFAS through food and water ingestion, dust inhalation, and hand- to-mouth transfer from contaminated areas. Since PFAS have been linked to severe health issues such as kidney and testicular cancer, elevated cholesterol, decreased fertility, decreased immune response to vaccinations in children, and obesity (Kato et al., 2018), it is critical to assess the potential human health risks based on PFAS exposure through tap water consumption. For that, estimated daily intakes (EDI) of PFAS exposure via drinking water consumption were calculated as:

$$EDI = \frac{C_{dw} \times q_{dw}}{m_{bw}} f_{uptake}$$

Where, C_{dw} is the median (or maximum) concentration of PFAS in drinking/tap water ($ng \cdot L^{-1}$), q_{dw} is the daily amount of drinking water consumed per person ($L \cdot day^{-1}$), m_{bw} is the body weight (kg) and $F_{uptake}$is the gastrointestinal uptake fraction (no unit) (Chen et al. 2018). F_{uptake} values used here were based on previous rodent studies and estimated as 0.66, 0.80 and 0.91 for low-, intermediate-, and high-exposure scenarios for PFOA and PFOS (Trudel et al., 2008). For this study, in the absence of available literature values, we have adopted the F_{uptake} values for all PFAS considering the highest-exposure scenario (0.91), knowing of the limitations inherent to the broad range of PFAS physical-chemical properties. We have assumed a daily water consumption of 2.5 L day^{-1} per person, and 78.0 kg was taken as the average human body weight (Chen et al., 2018; Fryar et al., 2018).

Human health risks from PFAS exposure were evaluated by comparing the estimated PFAS daily intakes (EDI) through tap water consumption previously calculated with the established reference dose (Rf) levels. Expressed as Hazard index (HI), health risks were calculated as:

$$HI = EDI / Rf$$

Based on the derived oral minimal risk levels (MRLs) recommended by the Agency for Toxic Substances and Disease Registry for PFOA, PFOS, PFHxS and PFNA, reference dose values adopted were 3, 2, 20 and 3 ng/kg * day, respectively (ATSDR, 2021). In the absence of Rf values for other PFAS, Rf values for PFCAs were assumed as a first approximation to be similar to that of PFOA. Similarly, for other PFSAs, the Rf value of PFOS was used. The highest PFAS daily intake was observed for PFBA at 2.58 ng/kg*day, while PFOA exhibited a maximum daily intake of 0.39 ng/kg*day and PFOS presented an EDI of 0.92 ng/kg*day, as shown in Table 2.10. Total maximum daily intake of PFAS by tap water consumption was estimated as 12 ng/kg*day. Furthermore, the HI of individual PFAS ranged from 0.01 to 0.86 when calculated based on maximum EDI, while the total HI considering the sum of all PFAS exposure was calculated to be 0.62 (based on median exposure) or 4.1 (based on highest exposure) (Table 2.10). Although the calculated HI represent a low human health risk (HI<1) when considering median concentrations observed in tap waters, the HI was above 4 in the worst-case scenario (considering highest PFAS levels observed), which raises great human health concern, even though reference doses for most of the PFAS are not available and values used based on PFOA and PFOS could be underestimating or overestimating the actual HI.

Currently, more stringent PFAS regulations in drinking water have been developed by different US states, including not only PFOA and PFOS, but a greater number of long chain and short chain PFAS (Post et al., 2021), suggesting the lack of protection offered by the U.S. EPA advisory guideline level of 70 ng L^{-1} for PFOA and PFOS combined. Although concentrations of PFOA and

PFOS found in this study were below the lifetime health advisory levels established by the U.S. EPA, when considering established guidelines in place in Vermont (20 ng L^{-1} for PFOS), Massachusetts (20 ng L^{-1} for PFOS), Michigan (16 ng L^{-1} for PFOS, 8 ng L^{-1} for PFOA and 6 ng L^{-1} for PFNA), New York (10 ng L^{-1} for PFOS), New Jersey (13 ng L^{-1} for PFOS), New Hampshire (15 ng L^{-1} for PFOS and 12 ng L^{-1} for PFOA), Minnesota (15 ng L^{-1} for PFOS), Washington (15 ng L^{-1} for PFOS), and California (10 ng L^{-1} for PFOA), levels found in tap water for PFOS in several locations from 11.8 to 29 ng L^{-1}, PFOA levels of 9.7 and 13 ng L^{-1}, and PFNA concentration of 7.5 ng L^{-1} raises health concerns and requires further monitoring. The most frequent and predominant PFAS in tap water from South Florida was PFBA, which while being currently regulated only in Minnesota (at a 7,000 ng L^{-1} level which is extremely higher than the levels observed in this study), it has been recently found to accumulate in the lungs and to adversely affect the body's immune response (Grandjean et al., 2020). Therefore, there is a need for further studies on short-chain PFAS to better understand the risks posed specially by PFBA exposure through diet (water and food).

Table 2.10 Estimated daily intake (EDI) and Hazard index (HI) for PFAS exposure through tap water consumption.

PFAS	Median (Min, Max) concentration of PFAS (ng/L)	Estimated daily intakes (EDI) (ng/kg*day) Based on Median, Max	Established reference dose (Rf) (ng/kg * day)	Hazard index (HI) Based on Median, Max
PFBA	18.2 (0.65,88.5)	0.531, **2.581**	3	0.18, **0.860**
PFBS	4.14(0,9.69)	0.121, **0.283**	2	0.06, **0.141**
4-2 FTS	0.03(0,11.0)	0.001, **0.321**	-	-
PFHxA	5.77(0,36.2)	0.168, **1.054**	3	0.06, **0.351**
PFPeS	0.32(0,1.32)	0.009, **0.039**	2	0.00, **0.019**
FBSA	0.06(0,6.65)	0.002, **0.194**	-	-
GenX	0(0,0.1)	0.000, **0.003**	-	-
PFHpA	3.39(0,12.7)	0.099, **0.371**	3	0.03, **0.124**
PFHxS	3.71(0,8.27)	0.108, **0.241**	20	0.01, **0.012**
PFOA	4.14(0,13.2)	0.121, **0.386**	3	0.04, **0.129**
PFHpS	0.26(0,0.85)	0.007, **0.025**	2	0.00, **0.012**
6-2 FTS	0(0,68.7)	0.000, **2.003**	-	-
PFOS	13.5(0,31.5)	0.394, **0.919**	2	0.20, **0.460**
PFPeA	9.18(0,42.6)	0.268, **1.242**	3	0.09, **0.414**
Adona	0(0,0)	0.000, **0.000**	-	-
PFNA	1.05(0,7.51)	0.031, **0.219**	3	0.01, **0.073**
PFONS	0(0,2.53)	0.000, **0.074**	-	-
PFNS	0.01(0,25.2)	0.000, **0.735**	2	0.00, **0.368**
8-2 FTS	0.01(0,4.70)	0.000, **0.137**	-	-
PFDA	0.31(0,1.04)	0.009, **0.030**	3	0.00,**0.010**
PFDS	0(0,1.78)	0.000, **0.052**	2	0.00, **0.026**
PFOUDS	0(0,0.03)	0.000, **0.001**	-	-
PFUdA	0(0,2.32)	0.000, **0.068**	3	0.00, **0.023**
FHxSA	0(0,1.47)	0.000, **0.043**	-	-
N-MeFOSAA	0(0,2.66)	0.000, **0.078**	-	-
N-EtFOSAA	0(0,1.38)	0.000, **0.040**	-	-
PFDoA	0(0,2.36)	0.000, **0.069**	3	0.00, **0.023**
PFTrDA	0(0,2.21)	0.000, **0.064**	3	0.00, **0.021**
PFTeDA	0(0,9.01)	0.000, **0.263**	3	0.00, **0.088**
FOSA	0(0,27.6)	0.000, **0.806**	-	-
Total PFAS	64.1(0.63, **423)**	1.870, **12.30**		0.62, **4.11**

[1] ATSDR, 2021. Rf of PFOA was used for other PFCAs and Rf value of PFOS was used for PFSAs when values were not available. Maximum values are in bold. MDL: method detection limit. Values

2.4 **Acknowledgements**

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Chapter 3. Spatial Distribution of Per- and Polyfluoroalkyl Substances (PFAS) in Waters from Central and South Florida.

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3.1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of notoriously persistent pollutants found worldwide. They have been manufactured for over 60 years and have wide applications in industrial and consumer products, such as non-stick cookware, waterproof fabrics, stain-resistant products, surfactants, protective coatings, cosmetics, firefighting foams, food packaging, wire manufacturing, paints, and more (Baran, 2001). PFAS possess incredible chemical and thermal stability due to their strong C-F bonds (Buck et al., 2011; Podder et al., 2021), and are consequently extremely persistent in the environment and can bioaccumulate through the food chain, which is often referred to as "forever chemicals" (Skutlarek et al., 2006). Even at low parts-per-billion level exposure, PFAS have proven to be associated with reproductive, developmental, hepatic, neurological, immunosuppressive, and endocrine disruptive toxicity (Grandjean & Clapp, 2015; Kato et al., 2018; Lopez-Espinosa et al., 2012; Vieira et al., 2013).

Generally, PFAS can be released into the environment through specific point sources areas: production and manufacturing facilities, facilities using Aqueous Fire Fighting Foams (AFFF) (Airport, military bases, firefighting training areas), landfills and wastewater treatment plants (WWTPs) (Cui et al., 2020). Nonpoint sources like atmospheric deposition and water runoffs (from industrial, wastewater, agriculture) can also introduce PFAS into the environment (Ahrens & Bundschuh, 2014). For example, PFAS-containing materials disposed into landfills will generate leachate, contributing to the discharge of PFAS to surface water, groundwater, and wastewater treatment plants (WWTPs) which are ineffective in removing these compounds (Rahman et al., 2014). Using PFAS contaminated water as a source of tap water can recirculate PFAS in the water cycle (Winchell et al., 2021) and catalyze bioaccumulation in a plethora of biological organisms, which pose a huge concern to human health. The prevalence of PFAS in surface water also threatens the health condition of wildlife, such as aquatic animals, birds, especially the predators at the top of the food chain through bioaccumulation and biomagnification from contaminated waters

and preys (Grønnestad et al., 2019; Quinete et al., 2011). PFAS have been observed in several aquatic animals in Florida, including alligators which hold a significant role in the food chain in the Florida Everglades (Bangma et al., 2017; Wood et al., 2021).

Higher PFAS levels are often associated with highly urbanized and industrialized areas having commercial and industrial complexes, ports and marinas, military bases, human waste facilities, etc. (Sardiña et al., 2019). Coastal estuarine environments make up the leading environment for PFAS pollution due to their proximity to urban and industrial centers (Yamashita et al., 2008). Recently, we have assessed a total of 30 PFAS for the first time in surface waters from Biscayne Bay and adjacent canals, which showed concentrations up to 106 ng L^{-1} of total PFAS, and in tap waters from populated counties in South Florida with levels up to 242 ng L^{-1} of total PFAS (Li et al., 2022). The PFAS levels found in these areas represent significant human health and ecological risks. Considering that Florida supports over 20 million people, as well as treasured, sensitive coastal and wetland ecosystems, it is vital to expand the PFAS monitoring study encompassing a broader area to provide needed information to the public and regional authorities addressing the issue.

In this study, we have expanded the study area to include tap waters from other major counties that are highly populated in Florida, and surface waters from important coastal and wetland ecosystems subject to anthropogenic activities that might be impacting the water quality. These areas include Tampa Bay and its adjacent area on the West coast of South Florida, Biscayne Bay and its adjacent area on the East coast of South Florida, the canals coming from preserved Everglades area-Everglades National Park (ENP), and coastal Key West. Therefore, our main goal was to evaluate the occurrence, levels, composition, and spatial distribution of PFAS along surface and tap waters in Central and South Florida. A total of 30 PFAS, including legacy long-chain and especially, emerging short-chain PFAS, were analyzed in this study to allow better coverage and understanding of the input and fate of PFAS in the environment.

3.2 **Materials and Methodology**

3.2.1 Chemicals and standards.

All the chemicals and solvents used in this study were Optima LC/MS grade and purchased from Fisher Scientific (Waltham, MA, USA), including methanol, water, hexane, acetone, methylene chloride, ammonium hydroxide, and ammonium formate. Strata-XL-AW 100 μm polymeric weak anion cartridges (500 mg/3 mL) were purchased from Phenomenex (Torrance, CA, USA), and used for the solid phase extraction (SPE) process.

30 PFAS native standards (PFAC30PAR), isotopically mass-labeled 19 PFAS standards (PFAC-24ES), and isotopically mass-labeled HFPO-DA (M3HFPO-DA), were purchased from Wellington Laboratories Inc (Guelph, Ontario, Canada). PFAC30PAR and PFAC-24ES were purchased as solutions of 1 mg L^{-1} in MeOH, and M3HFPO-DA was of 50 mg L^{-1} in MeOH. In addition, 24 PFAS native standard (PFC-24) was purchased from AccuStandard (New Haven, CT, USA) as solution of 2 mg L^{-1} in methanol: water (80:20) and used as a secondary standard for initial calibration verification (ICV) purpose. Working solutions of native standards (PFAC30PAR) were prepared at concentrations of 10 μg L^{-1} and 1 μg L^{-1} in water, while for the secondary standards PFC-24, a concentration of 1 μg L^{-1} was prepared in water. For internal standards (IS), the working solution was prepared as a mixture of PFAC-24 ES and M3HFPO-DA at a concentration of 2.5 μg L^{-1} in methanol. All the standards and working solutions were stored refrigerated at 4 °C. The list of all the compounds presented in the native and internal standards used is shown in Table 2.1.

3.2.2 Study area and sample collection

Our study areas covered Biscayne Bay, Tampa Bay and their adjacent canals, canals coming from the ENP, and Key West. Surface water samples were collected from the nearshore and their adjacent rivers and canals. Biscayne Bay is a rectangular-shaped estuary located along the southeast

coast of South Florida, which provides important habitat for a variety of wildlife as well as is a key part of the recreational, social, economic, and cultural life of South Florida. This study focused on the North region of the Biscayne Bay (North Bay), which is heavily populated (population: 2.5 million in Miami Dade), urbanized, and influenced by freshwater releases from rivers and canals such as Arch Creek, Biscayne Canal, Little River, Miami River (Caccia & Boyer, 2005). Similarly, Tampa Bay on the West coast of Florida also has its environmental significance as well as regional economic significance. It adjoins the highly urbanized land area with many industrial and agricultural cities surrounding Tampa Bay, such as Tampa city and St. Petersburg, with approximately 1.3 million population (Hillsborough and Pinellas County) (Yates et al., 2011). Key West is the southernmost tip of the Florida Keys and a popular vacation destination. It is occupied mostly by hotel businesses and single-family houses with a population of 24,649. In Key West, despite recent changes related to the wastewater collection and transmission system with some progress towards connecting some areas to municipal sewages sources, most of the single-family houses (especially trailer camps) and small businesses (e.g., hotels) are not served by modern sewage treatment plants. Instead, they rely on the use of onsite treatment systems, including septic tanks and shallow (90-120 feet) injection wells, which could lead to micropollutants leaching to groundwater and adjacent surface water (Yang et al., 2017). The ENP is the largest tropical wilderness in the United States and constitutes a preserved area due to its biodiversity and ecological importance. The samples were not collected inside the ENP but from its adjacent freshwater canals on the eastern boundaries, which are under the influence of subtropical agricultural lands and urban development areas (Quinete et al., 2013).

Surface water samples were collected using a swing arm sampler (Wooster, OH, US) with 500 mL pre-cleaned high-density polyethylene bottles (HDPE). Samples (N=13) from Biscayne Bay (North Bay) were collected in Aug 2021. Samples (N=12) from ENP were collected in Feb 2020 and July 2021. Samples (N=7) from Tampa Bay area were collected in May 2021. Samples (N=6) from Key

West were collected in July and Sep 2021. The map of collection sites is shown in Figure 3.1. The corresponding coordinates, abbreviations, date of the sampling event, and salinity of the samples can be found in Table 3.1. Tap water samples were also collected in 500 mL pre-cleaned HDPE bottles from major municipalities in 9 counties in Florida (N=10), including St. Lucie, Orange, Hillsborough, Pinellas, Manatee, Sarasota, Charlotte, Lee, and Collier, which covers major cities on the West coast of South Florida and Central Florida. The sampling event for tap water was conducted in May 2021.

Table 3.1 Geographical coordinates of surface water sampling locations along Biscayne Bay and adjacent canals, ENP and adjacent Canals, Key West, and Tampa Bay; and tap water from Collier, Charlotte, St. Lucie, Okeechobee, Orange, Hillsbourough, Pinellas, Lee, Osceola, and Sarasota counties.

Water Type	Sampling Sites	Abbv.	County	Collection Dates	Latitude	Longitude	Salinity (ppt or PSU)
Surface Water	Maule Lake	ML	Miami-Dade	08/2021	25.93667	-80.14425	27
	Royal Glades Canal	RGC	Miami-Dade	08/2021	25.92927	-80.15113	22
	Biscayne Bay @ FIU	BBC	Miami-Dade	08/2021	25.90917	-80.13751	
	Little Arch Creek	LAC	Miami-Dade	08/2021	25.89471	-80.15435	29
	Biscayne Canal 8	BC8	Miami-Dade	08/2021	25.8712	-80.17615	25
	Little River site 1	LR1	Miami-Dade	08/2021	25.84641	-80.18605	2

	Little River site 2	LR2	Miami-Dade	08/2021	25.84602	-80.17655	4
	Legion Park	LP	Miami-Dade	08/2021	25.83584	-80.18008	39
	Morningside Park	MP	Miami-Dade	08/2021	25.82316	-80.17834	30
	Miami River site 1	MR1	Miami-Dade	08/2021	25.76997	-80.19891	17
	Miami River site 2	MR2	Miami-Dade	08/2021	25.7775	-80.20552	14
	Miami Beach 17th street	MB17th	Miami-Dade	08/2021	25.79227	-80.14288	36
	Miami Beach 14th street	MB14th	Miami-Dade	08/2021	25.78511	-80.14444	36
	Miami Beach 10th street	MB10th	Miami-Dade	08/2021	25.78041	-80.14313	37

	Homestead Air Reserve Base	HB	Miami-Dade	02/2021; 07/2021	25.47332	-80.39608	2
	C103-217 Mowry Canal	C103	Miami-Dade	02/2021; 07/2021	25.51721	-80.54271	1
	C113-217Biscayne-Evergalades trail	C113	Miami-Dade	02/2021; 07/2021	25.48138	-80.54317	1
	L31W	L31W	Miami-Dade	02/2021; 07/2021	25.39836	-80.57248	1
	S176 C111 Biscayne-Evergalades trail	S176	Miami-Dade	02/2021; 07/2021	25.4679	-80.5625	1
	S178 Southern Glades Trail	S178	Miami-Dade	02/2021; 07/2021	25.4084	-80.5237	0
	Truman Waterfront Park	TWP	Monroe	08/2021	24.5515	-81.80725	38
	Garrison Bright	GB	Monroe	08/2021	24.56091	-81.78486	38

	Smathers Beach	SB	Monroe	07/2021	24.55038	-81.76815	38
	Seaport Bright	SeB	Monroe	08/2021	24.56237	-81.80073	38
	Edward B. Knight Pier	EKP	Monroe	07/2021	24.54544	-81.78353	38
	Fort Zachary Taylor Historic Park Beach	FZB	Monroe	08/2021	24.54561	-81.8123	38
	Central Tampa Bay	CTB	Hillsborough	05/2021	27.65377	-82.67778	34
	Causeway Boulevard	CB	Hillsborough	05/2021	27.96067	-82.69837	27
	Port Charlotte	PC	Charlotte	05/2021	26.96166	-82.11120	29
	Terra Ceia	TC	Manatee	05/2021	27.55414	-82.57290	35

	Ballast Point Park	BPP	Hillsborough	05/2021	27.88903	-82.48083	29
	Vinoy Park	VP	Pinellas	05/2021	27.78213	-82.62500	31
	North Tampa Bay	TB	Hillsborough	05/2021	27.86755	-82.53658	30
	Marco Island	MI	Collier	05/2021	25.9331	-81.71558	
Tap Water	Naples		Collier	05/2021	26.15825	-81.68380	
	Port Charlotte		Charlotte	05/2021	26.96192	-82.11080	
	Port St. Lucie		St. Lucie	05/2021	27.12822	-80.33308	
	Fort Drum		Okeechobee	05/2021	27.60028	-80.82206	
	Orlando		Orange	05/2021	28.41808	-81.18399	
	Tampa		Hillsborough	05/2021	27.88960	-82.50750	
	St. Petersburg		Pinellas	05/2021	27.81912	-82.65272	

	Fort Myers		Lee	05/2021	26.68593	-81.79483	
	St. Cloud		Osceola	05/2021	28.09429	-81.27405	
	Sarasota		Sarasota	05/2021	27.35354	-82.54764	

3.2.3 SPE LC-MS method

A SPE method followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was used for PFAS extraction, preconcentration, and determination following the previously published procedure (Li et al., 2022). Briefly, 250 mL of the water sample was spiked with 100 µL of a 2.5 µg L^{-1} IS and were passed through preconditioned Strata-XL AW cartridges on a semi-automated SPE system (12 samples per batch). Samples were eluted with 10 mL of 0.3% ammonium hydroxide in methanol, then evaporated to dryness using a nitrogen evaporator, and reconstituted to 1 mL with 90:10 (vol/vol) 5 mM ammonium formate /methanol for LC-MS/MS analysis. A volume of 100 µL was injected into the Agilent 1290 Infinity II LC interfaced to an Agilent 6470 triple-quadrupole LC-MS/MS system for PFAS identification and quantification. A Hypersil GOLD pentafluorophenyl (PFP) column (150 mm × 2.1 mm, 3µm) with a PFP guard column (Hypersil Gold PFP 5µm drop-in guards) was used as the analytical column. The LC conditions, MS parameters, and MRM method can be found in Table 2.3, 2.4, and 2.5.

3.2.4 Quality control/quality assurance (QA/QC)

The methodology has been validated as described in our previous publication (Li et al., 2022). To ensure the quality of the obtained data, LC-MS Blanks (LC-MS water), procedural blanks (LC-MS water spiked with IS and processed through SPE), and field/trip blanks (LC-MS water carried through sampling events and processed through SPE) were also run with every batch of samples. Spiked blanks, which consisted of LC-MS water (250 mL) spiked with native standards at a final concentration of 10 ng L^{-1} and 100 ng L^{-1} and IS at a final concentration of 250 ng L^{-1}, were prepared and analyzed every 8-10 samples. An initial calibration verification (ICV) solution was prepared as secondary standards and run with every batch of samples after the calibration curve. An 11-point

calibration curve was prepared in the concentration range of 2 to1000 ng L^{-1} for quantitation purposes. The LC-MS system was modified with PFAS free tubing, and a delay column (Hypersil GOLD aQ C18, 20 × 2.1 mm, 12μm) to avoid potential contamination coming from the solvents. All bottles, vials, and tubing used in this study were cleaned with solvents, including methylene chloride, hexane, acetone, methanol, and water at least two times and airdried. The solvents used in this analysis were examined for potential PFAS contamination. Concentrations found in blanks ranged from <MDL to 0.2 ng L^{-1} (for PFTrDA) and were subtracted from results in the environmental waters.

3.2.5 Data analysis and statistics

PFAS quantitation was performed using Mass Hunter QQQ quantitation analysis software and the criteria of peak integration and quantitation follows the one reported in Li et al., 2022 for retention time matching (<0.1 min of IS or native standards in the calibration curve), presence in the confirmation peak when available, signal to noise ratio (>3), and above the method detected limits, otherwise reported as <MDL. Statistical tests and plots were conducted in the R software (version 3.5.0; R Team, 2019), with an alpha set at 0.05.

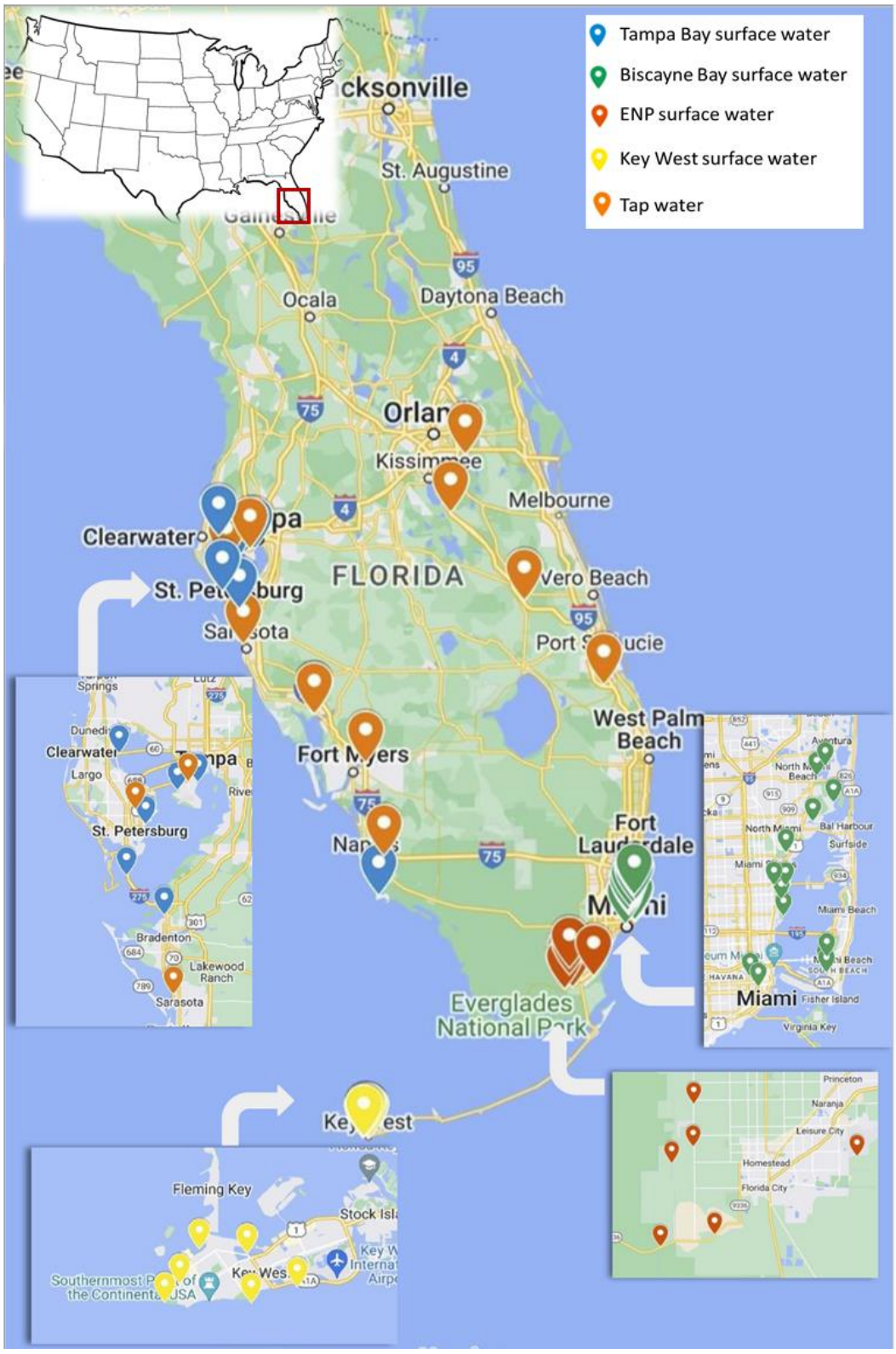


Figure 3.1 PFAS surface water and tap water sampling sites across Central and South Florida

3.3 **Results and Discussion**

3.3.1 Occurrence, concentrations, and composition of PFAS in surface waters from Biscayne Bay, Tampa Bay, ENP, and Key West in Central and South Florida

PFAS were detected in samples from 33 sampling sites that cover 13 sites surrounding the Biscayne Bay area, 8 sites from the Tampa Bay area, 6 sites from the ENP adjacent canals, and 6 sites from Key West. The concentration of individual PFAS congeners and total PFAS concentrations (the sum of all the PFAS congeners detected for the same sites) are shown in Table 3.2. For the sampling sites collected multiple times (≥2), the average (Min, Max) concentrations were reported. The spatial distribution of total PFAS in the sampling sites along Central and South Florida is displayed in Figure 3.2. Overall, total PFAS concentrations ranged from 6.50 ng L^{-1} (TWP, Key West) to 169 ng L^{-1} (HB, Miami) with 6 locations above 60 ng L^{-1} and 28 locations below 60 ng L^{-1}.

To better understand spatial variations in Biscayne Bay and canals, we have combined data from samples collected in Oct 2020 (N=13), Jan 2021 (N=14), which were previously analyzed and presented (Li et al., 2022), with recently collected samples in Aug 2021 (N=13). As a result, a total of 40 samples were averaged from 3 sampling trips during 2020-2021, with the average of total PFAS concentrations ranging from 11.4 ng L^{-1} (MB17th) to 91.0 ng L^{-1} (LR2). Relatively higher concentrations were identified at two sites from Little River (81.0, 91.0 ng L^{-1}), two sites from Miami River (72.4, 72.1 ng L^{-1}), Little Arch Creek (54.5 ng L^{-1}), and Biscayne Bay canal C-8 (53.9 ng L^{-1}), whereas sites from Miami Beach (MB10th, MB14th, and MB17th) were all below 20 ng L^{-1}. The variability of the three sets of data is presented as error bars in Figure 3.2. Results from multiple sampling events conducted during rainy and dry seasons suggested that PFAS concentration from the same location remains relatively stable with small variations throughout the whole period of study. Little River, Miami River, and Biscayne Bay canal BC-8 are identified as

"hot spots", where the highest levels were observed in this study and the previous one (Li et al., 2022).

From 10 samples collected at ENP adjacent canals in two sampling trips, the average of total PFAS concentrations ranged from 35.0 ng L^{-1} (C103) to 52.1 ng L^{-1} (C113), as shown in Figure 3.2, Table 3.2. The surface water at site HB was also from canal C-103 closer to its east opening to Biscayne Bay passing Homestead Air Reserve Base. The sampling site HB is around 16 km away from site C103 adjacent to ENP and showed a total concentration of 169 ng L^{-1}, which was the highest total PFAS concentration detected throughout this study. The 6 samples spreading along the coast of Key West presented total PFAS concentrations in the range of 6.50 ng L^{-1} (TWP) to 19.1 ng L^{-1} (EKP).

Total PFAS concentrations ranged from 17.4 (TC) to 60.6 ng L^{-1} (TB) from the 7 samples collected from Tampa Bay and its adjacent area. Three sites from the North region of Tampa Bay (TB, CB, BPP) and two sites from central Tampa Bay (VP, CTB) falls into a range of 37.8-60.6 ng L^{-1}, whereas the surface water sample from Terra Ceia aquatic preserve located in South Tampa Bay (TC) had the lowest total PFAS concentration of 17.4 ng L^{-1}. Two samples collected along the west coast in Port Charlotte (PC) and Marco Island (MI) showed concentrations of 26.11, and 38.23 ng L^{-1}, respectively.

Table 3.2 The concentrations of PFAS in surface water samples collected in Biscayne Bay, Everglades, Key West, and Tampa Bay, Florida. Average (Min, Max) is shown if the sampling site was collected ≥2.

		Concentrations (ng L-1)									
	Abbr	PFBA	PFBS	4-2 FTS	PFHxA	PFPeS	FBSA	GenX	PFHpA	PFHxS	PFOA
Biscayne Bay	ML	3.3(1.92,4.88)	2.87(1.41,5.47)	0.02(<MDL,0.09)	3.19(1.69,4.93)	0.37(0.23,0.77)	0.06(<MDL,0.09)	<MDL	2.00(1.26,2.55)	2.61(1.26,5.32)	2.18(1.12,3.63)
	RGC	3.71(2.42,6.76)	2.77(1.33,4.65)	0.02(0.04,0.04)	3.57(1.72,5.53)	0.34(0.23,0.62)	0.05(<MDL,0.1)	0.01(<MDL,0.05)	2.83(1.33,3.46)	2.01(1.26,2.95)	3.02(1.72,4.88)
	BBC	1.92(<MDL,3.16)	1.3(0.4,4.62)	0.04(0.03,0.04)	4.92(0.54,9.3)	0.29(0.08,0.57)	0.03(<MDL,0.08)	0.03(<MDL,0.08)	2.54(0.48,4.6)	1.09(0.34,4.5)	3.05(0.29,5.81)
	LAC	2.95(2.12,3.51)	4.73(2.56,7.11)	0.01(<MDL,0.04)	4.04(2.62,4.73)	0.65(0.57,0.74)	0.06(<MDL,0.08)	0.04(<MDL,0.08)	2.57(1.53,3.26)	4.2(2.5,5.32)	3.35(1.56,4.05)
	BC8	4.83(4.83,10.67)	5.88(5.88,13.7)	<MDL	4.17(4.17,15.41)	0.62(0.62,1.56)	<MDL	<MDL	3.53(3.53,6.6)	3.74(3.74,8.5)	2.79(2.79,7.86)
	LR1	10.21(7.27,12.07)	8.4(5.93,10.88)	0.03(0.05,0.07)	10.2(7.9,11.72)	0.86(0.59,1.24)	0.14(0.07,0.2)	<MDL	4.66(3.78,5.33)	5.17(4.14,6.35)	6.26(4.39,7.35)
	LR2	9.88(7.24,11.32)	8.58(5.82,12.91)	0.04(0.03,0.04)	10.94(8.52,11.83)	1.03(0.78,1.55)	0.06(<MDL,0.09)	0.01(<MDL,0.06)	5.01(3.87,5.83)	5.58(3.76,7.56)	6.9(5.59,8.14)
	LP	2.2(1.58,2.82)	2.09(2.08,2.1)	<MDL	2.45(1.62,3.29)	0.19(0.17,0.21)	0.05(<MDL,0.09)	<MDL	1.61(1.57,1.65)	1.28(1.02,1.54)	1.89(1.22,2.56)
	MP	2.36(1.64,3.08)	2.4(1.75,3.05)	<MDL	2.32(1.54,3.1)	0.21(0.18,0.23)	<MDL	<MDL	1.86(1.19,2.54)	1.62(0.93,2.3)	2.05(0.92,3.18)
	MR1	7.05(4.64,8.09)	3.44(2.47,3.9)	0.1(0.04,0.14)	8.13(4.42,10.54)	0.43(0.32,0.56)	0.05(<MDL,0.1)	0.07(<MDL,0.13)	3.9(2.32,4.89)	2.45(1.72,2.75)	3.25(2.22,3.6)
	MR2	7.82(5.37,9.42)	3.44(2.43,3.95)	0.11(0.06,0.15)	8.18(4.1,11.22)	0.38(0.28,0.42)	0.01(<MDL,0.06)	<MDL	3.95(2.13,5.17)	2.34(1.63,2.72)	2.69(2.12,2.88)
	MB17th	1.15(0.74,1.32)	0.83(0.76,0.9)	0.02(0.03,0.05)	1.15(1.05,1.38)	0.1(0.06,0.16)	0.02(<MDL,0.06)	0.02(<MDL,0.03)	0.77(0.64,0.91)	0.59(0.44,0.77)	0.72(0.67,0.84)
	MB14th	1.24(0.38,1.97)	0.67(0.16,1.06)	0.02(0.03,0.03)	1.24(0.21,1.92)	0.08(0.05,0.1)	<MDL	0.01(<MDL,0.03)	1.23(0.29,1.74)	0.52(0.14,0.79)	0.66(0.11,0.98)
	MB10th	1.15(0.45,2.72)	1.01(0.45,2.44)	0.01(<MDL,0.05)	1.58(0.54,4.56)	0.12(0.02,0.37)	0.01(<MDL,0.06)	<MDL	1.16(0.31,2.54)	0.77(0.3,1.72)	0.9(0.29,2.67)
		4.27	3.46	0.03	4.72	0.40	0.04	0.01	2.69	2.43	2.84
Everglades	C103	11.98(9.56,14.4)	2.01(1.91,2.11)	0.03(<MDL,0.05)	2.62(2.11,3.12)	0.26(0.13,0.38)	0.03(<MDL,0.06)	0.03(<MDL,0.05)	1.53(1.32,1.73)	0.52(0.41,0.64)	1.69(1.46,1.91)
	C113	8.97(7.13,10.8)	4.41(2.98,5.83)	0.02(<MDL,0.05)	1.96(1.45,2.48)	0.37(0.32,0.41)	0.03(<MDL,0.06)	0.03(<MDL,0.07)	0.99(0.77,1.2)	0.83(0.8,0.85)	1.56(0.95,2.17)
	S176	11.46(9.91,13.01)	2.56(2.1,3.02)	<MDL	2.42(1.83,3)	0.21(0.16,0.27)	<MDL	0.1(<MDL,0.2)	1.49(1.11,1.87)	0.84(0.59,1.1)	2.4(1.93,2.87)
	S178	5.33(3.96,6.71)	5.54(2.94,8.14)	<MDL	1.14(0.82,1.45)	0.45(0.42,0.48)	0.06(0.06,0.06)	0.01(<MDL,0.02)	0.36(<MDL,0.73)	1.36(0.8,1.91)	0.98(0.52,1.45)
	L31W	12.16(7.01,17.32)	2.72(2.31,3.12)	<MDL	2.19(0.87,3.51)	0.41(0.28,0.54)	<MDL	0.06(0.06,0.06)	1.44(0.59,2.29)	0.61(0.55,0.67)	2.07(0.99,3.14)
	HB	15.38(11.23,19.54)	21.46(15.92,27)	0.05(0.04,0.07)	19.1(13.82,24.38)	3.74(2.43,5.06)	0.64(0.23,1.05)	0.02(<MDL,0.04)	9.12(6.06,12.17)	22.92(17.16,28.68)	10.23(8.62,11.84)
		10.88	6.45	0.03	4.90	0.91	0.13	0.04	2.55	4.51	3.16
Key West	SeB	1.20	0.48	<MDL	0.37	0.03	<MDL	<MDL	1.18	0.29	0.27
	GB	1.97	1.41	<MDL	1.21	0.13	<MDL	<MDL	1.16	1.27	0.98
	TWP	1.18	0.46	<MDL	0.40	0.06	<MDL	<MDL	0.57	0.50	0.28
	SB	2.15	0.70	<MDL	1.12	0.09	<MDL	0.03	2.62	0.36	1.90
	FZB	1.24	0.45	<MDL	0.53	0.04	<MDL	<MDL	1.15	0.43	0.43
	EKP	2.09	0.71	<MDL	1.29	0.15	0.10	0.16	2.19	0.90	0.94
		1.64	0.70	<MDL	0.82	0.08	0.02	0.03	1.48	0.62	0.80
Tampa Bay	TB	3.40	4.22	<MDL	3.60	0.68	<MDL	<MDL	6.47	4.66	2.72
	CB	3.76	4.49	<MDL	4.38	0.65	0.13	<MDL	4.25	5.48	2.81
	BPP	3.01	4.35	<MDL	3.65	0.51	0.21	<MDL	3.74	4.08	2.75
	VP	2.93	3.49	<MDL	3.42	0.48	0.22	<MDL	8.39	3.59	2.52
	CTB	1.53	2.00	<MDL	1.36	0.23	0.20	<MDL	23.11	1.41	1.21
	TC	2.05	1.86	<MDL	1.53	0.23	<MDL	<MDL	3.76	1.61	1.16
	PC	3.50	3.06	<MDL	1.64	0.22	0.21	<MDL	1.01	1.28	2.13
	MI	4.49	1.19	<MDL	1.33	0.07	<MDL	0.08	16.17	0.56	1.52
		3.08	3.08	<MDL	2.61	0.38	0.12	0.01	8.36	2.83	2.10

		Concentrations (ng L-1)									
	Abbr	PFHpS	6-2 FTS	PFOS	PFPeA	Adona	PFNA	PFONS	8-2 FTS	PFNS	PFDA
Biscayne Bay	ML	0.17(0.1,0.38)	0.56(<MDL,1.83)	6.17(3.4,13.03)	3.06(1.21,3.72)	<MDL	0.56(0.3,0.75)	<MDL	<MDL	0.04(<MDL0,0.15)	0.26(0.14,0.36)
	RGC	0.19(0.13,0.29)	1.82(<MDL,5.12)	11.91(5.79,14.58)	3.35(2.53,3.92)	<MDL	1.08(0.91,1.17)	<MDL	0.01(<MDL,0.06)	0.02(<MDL,0.07)	0.7(0.42,1.02)
	BBC	0.17(0.01,0.33)	0.49(<MDL,0.98)	24.07(1.16,46.98)	3.41(0.63,6.18)	<MDL	1.26(0.08,2.44)	<MDL	0.04(<MDL,0.07)	0.2(<MDL,0.41)	1.36(0.21,2.51)
	LAC	0.4(0.22,0.72)	1.19(0.72,2.56)	20.29(8.78,35.08)	5.39(4.6,6.1)	<MDL	0.61(0.22,0.75)	<MDL	0.07(<MDL,0.28)	0.05(<MDL,0.2)	0.21(0.15,0.24)
	BC8	0.52(0.52,0.9)	0.79(0.79,9.18)	10.88(10.88,25.89)	11.25(11.25,16.18)	<MDL	1.31(1.03,1.54)	<MDL	<MDL	0.04(<MDL,0.15)	0.78(0.21,0.78)
	LR1	0.43(0.4,0.51)	3.42(1.7,4.08)	17.5(12.1,21.51)	10.62(6.1,14.87)	<MDL	1.07(0.63,1.46)	<MDL	0.08(<MDL,0.2)	0.04(<MDL,0.15)	0.43(0.06,0.55)
	LR2	0.56(0.33,0.66)	4.49(3.72,4.93)	19.46(12.98,33.82)	12.49(9.81,14.93)	<MDL	1.62(0.72,2.24)	<MDL	0.29(0.13,0.41)	0.02(<MDL,0.1)	0.91(0.56,1.22)
	LP	0.11(0.07,0.15)	1.34(0.81,1.87)	5.8(2.89,8.71)	2.25(1.59,2.91)	<MDL	0.86(0.36,1.36)	<MDL	0.11(<MDL,0.22)	<MDL	0.79(0.6,0.98)
	MP	0.18(0.07,0.29)	0.31(<MDL,0.63)	5.74(3.18,8.3)	2.48(1.02,3.94)	<MDL	0.81(0.22,1.4)	<MDL	<MDL	<MDL	0.72(0.25,1.2)
	MR1	0.17(0.1,0.21)	21.79(5.73,34.72)	7.67(5.02,9.06)	9.39(4.08,14.59)	<MDL	0.89(0.38,1.17)	<MDL	1.14(0.47,1.4)	0.01(<MDL,0.04)	0.34(0.27,0.4)
	MR2	0.26(0.17,0.33)	20.15(10.74,29.18)	6.03(5.66,6.3)	10.42(4.01,16.54)	<MDL	1.27(0.45,2.04)	<MDL	1.77(0.25,3.19)	0.06(<MDL,0.12)	0.63(0,1.19)
	MB17th	0.02(<MDL,0.09)	2.08(0,7.39)	1.58(1.24,1.97)	1.5(0.78,2.11)	<MDL	0.23(0.13,0.27)	<MDL	0.04(<MDL,0.15)	0.03(<MDL,0.13)	0.12(0.04,0.18)
	MB14th	0.04(<MDL,0.08)	0.59(0.44,0.95)	1.16(0.38,1.44)	2.71(0.1,5.68)	<MDL	0.41(0.1,0.63)	<MDL	0.01(<MDL,0.02)	<MDL	0.48(0.08,0.81)
	MB10th	0.03(<MDL,0.13)	1.45(0.21,3.33)	2.63(1.74,4.99)	2.17(0.89,4.72)	<MDL	0.32(0.16,0.59)	<MDL	0.02(<MDL,0.09)	0.03(<MDL,0.12)	0.28(0.04,0.53)
		0.23	4.32	10.06	5.75	<MDL	0.88	<MDL	0.26	0.04	0.57
Everglades	C103	0.06(0.06,0.06)	<MDL	3.43(3.17,3.69)	3.24(1.9,4.58)	<MDL	0.34(0.16,0.51)	<MDL	0.02(<MDL,0.04)	<MDL	0.48(<MDL,0.95)
	C113	0.15(0.13,0.17)	<MDL	10.86(8.14,13.58)	3.04(1.34,4.73)	<MDL	0.75(0.19,1.32)	<MDL	<MDL	0.01(<MDL,0.01)	1.64(0.12,3.16)
	S176	0.09(0.05,0.12)	<MDL	5.02(3.13,6.9)	3.27(2.44,4.1)	<MDL	1.82(0.77,2.87)	<MDL	0.08(<MDL,0.17)	0.02(0,0.04)	0.65(0.61,0.7)
	S178	0.31(0.18,0.44)	<MDL	13(12.46,13.54)	1.85(1.83,1.88)	<MDL	0.25(0.25,0.26)	<MDL	<MDL	0.01(0,0.02)	0.51(0.06,0.97)
	L31W	0.05(0.05,0.05)	<MDL	5.11(3.65,6.57)	3.57(1.21,5.92)	<MDL	1.02(0.12,1.92)	0.01(<MDL,0.02)	0.04(<MDL,0.07)	<MDL	1.17(0.27,2.07)
	HB	1.75(1.48,2.01)	<MDL	24.21(0.05,48.37)	4.88(<MDL,9.75)	<MDL	3.14(1.62,4.66)	<MDL	0.74(0.71,0.78)	0.13(0.1,0.16)	1.62(0.46,2.78)
		0.40	<MDL	10.27	4.12	<MDL	1.22	<MDL	0.17	0.03	1.01
Key West	SeB	<MDL	<MDL	0.68	0.50	<MDL	0.16	<MDL	<MDL	<MDL	0.19
	GB	0.06	<MDL	0.93	1.89	<MDL	0.39	<MDL	<MDL	<MDL	0.33
	TWP	<MDL	<MDL	1.28	0.49	<MDL	0.18	<MDL	<MDL	<MDL	0.04
	SB	0.05	0.66	2.49	1.81	<MDL	1.13	0.12	<MDL	<MDL	0.32
	FZB	<MDL	<MDL	2.83	0.59	<MDL	0.22	<MDL	<MDL	<MDL	0.09
	EKP	<MDL	0.52	3.70	2.09	<MDL	0.43	0.04	<MDL	<MDL	2.00
		0.02	0.20	1.98	1.23	<MDL	0.42	0.03	<MDL	<MDL	0.49
Tampa Bay	TB	0.41	<MDL	25.74	4.91	<MDL	1.43	<MDL	<MDL	0.05	0.99
	CB	0.43	<MDL	14.84	6.10	<MDL	0.86	<MDL	<MDL	0.06	0.31
	BPP	0.34	<MDL	11.76	4.96	<MDL	0.84	<MDL	<MDL	<MDL	0.45
	VP	0.29	<MDL	9.58	4.88	<MDL	0.78	<MDL	<MDL	<MDL	0.30
	CTB	0.14	0.64	3.46	1.92	<MDL	0.37	<MDL	<MDL	<MDL	0.20
	TC	0.07	<MDL	2.40	2.42	<MDL	0.20	<MDL	<MDL	<MDL	0.09
	PC	0.27	<MDL	9.19	2.41	<MDL	0.55	<MDL	0.22	<MDL	0.43
	MI	0.04	<MDL	3.32	2.18	0.02	0.94	<MDL	<MDL	<MDL	1.04
		0.25	0.08	10.04	3.72	<MDL	0.75	<MDL	0.03	0.01	0.48

		Concentrations (ng L-1)										
	Abbr	PFDS	FHxSA	PFONDS	PFUdA	N-MeFOSAA	N-EtFOSAA	PFDoA	PFTrDA	PFTeDA	FOSA	∑PFAS
Biscayne Bay	ML	<MDL	<MDL	0.06(<MDL,0.13)	<MDL	<MDL	<MDL	1.15(<MDL,2.3)	<MDL	<MDL	0.05(<MDL,0.1)	28.69
	RGC	0.19(<MDL,0.75)	<MDL	<MDL	0.27(<MDL,0.96)	0.09(<MDL,0.37)	0.38(<MDL,1.51)	1.04(<MDL,1.88)	<MDL	<MDL	<MDL	39.39
	BBC	2.51(<MDL,5.03)	<MDL	<MDL	0.42(<MDL,0.84)	0.25(<MDL,0.49)	1.74(<MDL,2.21)	0.54(<MDL,1.09)	<MDL	<MDL	<MDL	51.67
	LAC	<MDL	0.03(<MDL,0.07)	<MDL	<MDL	0.14(<MDL,0.55)	1.34(<MDL,5.34)	0.54(<MDL,0.9)	<MDL	1.09(<MDL,2.18)	0.51(<MDL,1.03)	54.47
	BC8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.51(<MDL,2.51)	0.22(<MDL,0.22)	53.85
	LR1	<MDL	0.03(<MDL,0.07)	<MDL	<MDL	0.24(<MDL,0.47)	0.47(<MDL,0.96)	0.16(<MDL,0.32)	<MDL	<MDL	0.53(<MDL,1.37)	80.97
	LR2	0.33(<MDL,1.32)	<MDL	<MDL	0.06(<MDL,0.25)	1.27(<MDL,2.54)	1.49(<MDL,4.5)	<MDL	<MDL	<MDL	<MDL	91.01
	LP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.87(<MDL,1.74)	<MDL	<MDL	0.59(<MDL,1.17)	24.48
	MP	<MDL	<MDL	<MDL	0.57(<MDL,1.14)	<MDL	<MDL	1.28(<MDL,2.56)	0.71(<MDL,1.42)	<MDL	<MDL	25.63
	MR1	0.22(<MDL,0.81)	<MDL	<MDL	0.07(<MDL,0.26)	0.47(<MDL,0.95)	0.47(<MDL,0.95)	0.46(<MDL,0.92)	<MDL	<MDL	0.16(<MDL,0.32)	72.14
	MR2	<MDL	<MDL	<MDL	0.11(<MDL,0.45)	0.88(0,1.41)	0.43(<MDL,0.87)	<MDL	<MDL	1.33(<MDL,2.66)	0.1(<MDL,0.2)	72.38
	MB17th	<MDL	0.02(<MDL,0.04)	<MDL	0.07(<MDL,0.29)	<MDL	0.28(0,1.14)	<MDL	<MDL	<MDL	0.05(<MDL,0.1)	11.39
	MB14th	0.08(<MDL,0.31)	<MDL	<MDL	0.12(<MDL,0.23)	0.36(<MDL,0.77)	0.79(<MDL,1.34)	0.35(<MDL,0.87)	<MDL	<MDL	0.59(<MDL,1.17)	13.34
	MB10th	<MDL	<MDL	<MDL	<MDL	0.17(<MDL,0.68)	0.17(<MDL,0.69)	2.03(<MDL,3.89)	<MDL	<MDL	<MDL	16.02
		0.24	0.01	<MDL	0.12	0.28	0.54	0.60	0.05	0.35	0.20	
Everglades	C103	<MDL	<MDL	<MDL	<MDL	0.25(<MDL,0.51)	0.42(<MDL,0.85)	<MDL	2.89(<MDL,5.79)	<MDL	<MDL	35.02
	C113	0.56(<MDL,1.12)	<MDL	4.95(<MDL,9.89)	<MDL	0.33(<MDL,0.66)	1.66(<MDL,3.33)	7.85(1.43,14.27)	2.02(<MDL,4.03)	<MDL	<MDL	52.06
	S176	<MDL	<MDL	0.64(<MDL,1.27)	3.59(<MDL,7.18)	0.64(0,1.28)	0.87(<MDL,1.74)	3.51(2.46,4.56)	<MDL	<MDL	<MDL	43.93
	S178	<MDL	<MDL	1.38(<MDL,2.76)	<MDL	<MDL	0.48(<MDL,0.97)	3.23(<MDL,6.47)	3.87(<MDL,7.74)	<MDL	<MDL	49.48
	L31W	<MDL	<MDL	5.26(<MDL,10.52)	1.78(<MDL,3.57)	0.15(<MDL,0.3)	1.78(<MDL,3.56)	<MDL	1.19(0,2.38)	<MDL	<MDL	40.70
	HB	0.82(0.8,0.84)	0.12(0.08,0.16)	2.51(<MDL,5.01)	0.97(<MDL,1.94)	0.69(<MDL,1.38)	0.97(<MDL,1.93)	3.63(<MDL,7.27)	<MDL	<MDL	3.77(<MDL,7.54)	169.05
		0.23	0.02	1.51	1.06	0.69	1.99	4.18	3.32	0.00	1.26	
Key West	SeB	<MDL	<MDL	0.81	<MDL	<MDL	<MDL	2.75	<MDL	<MDL	<MDL	8.89
	GB	<MDL	<MDL	<MDL	<MDL	1.46	<MDL	<MDL	<MDL	<MDL	<MDL	13.18
	TWP	<MDL	<MDL	<MDL	<MDL	0.93	<MDL	<MDL	<MDL	<MDL	0.14	6.50
	SB	<MDL	<MDL	<MDL	<MDL	<MDL	0.79	1.93	<MDL	<MDL	<MDL	18.27
	FZB	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	8.00
	EKP	<MDL	<MDL	<MDL	<MDL	1.23	0.57	<MDL	<MDL	<MDL	<MDL	19.11
		<MDL	<MDL	0.13	<MDL	0.60	0.23	0.78	<MDL	<MDL	0.02	
Tampa Bay	TB	0.55	<MDL	<MDL	0.59	<MDL	<MDL	<MDL	<MDL	<MDL	0.17	60.60
	CB	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.37	48.92
	BPP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	40.66
	VP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.11	40.97
	CTB	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	37.78
	TC	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	17.38
	PC	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	26.11
	MI	<MDL	<MDL	1.74	<MDL	<MDL	<MDL	3.52	<MDL	<MDL	<MDL	38.23
		0.07	<MDL	0.22	0.07	<MDL	<MDL	0.44	<MDL	<MDL	<MDL	

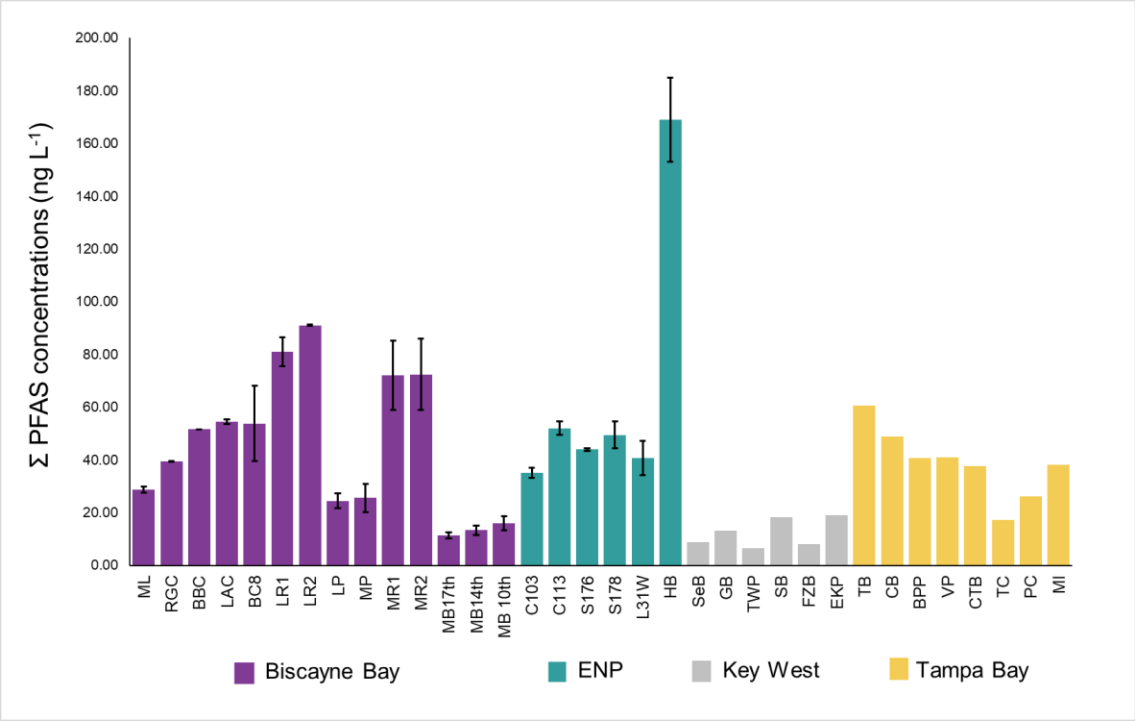


Figure 3.2 Average of total PFAS concentrations in samples collected from Biscayne Bay, ENP, Key West, and Tampa Bay. The variability of the data from the same sampling sites collected ≥2 times is presented as error bar.

Overall, among 30 PFAS congeners covered in this study, all were detected in one or more sites. The PFAS composition in each location is presented in Figure 3.3. It can be observed that compositions vary from location to location, however, predominant congeners of each defined area can be identified based on detection rates and mean concentrations.

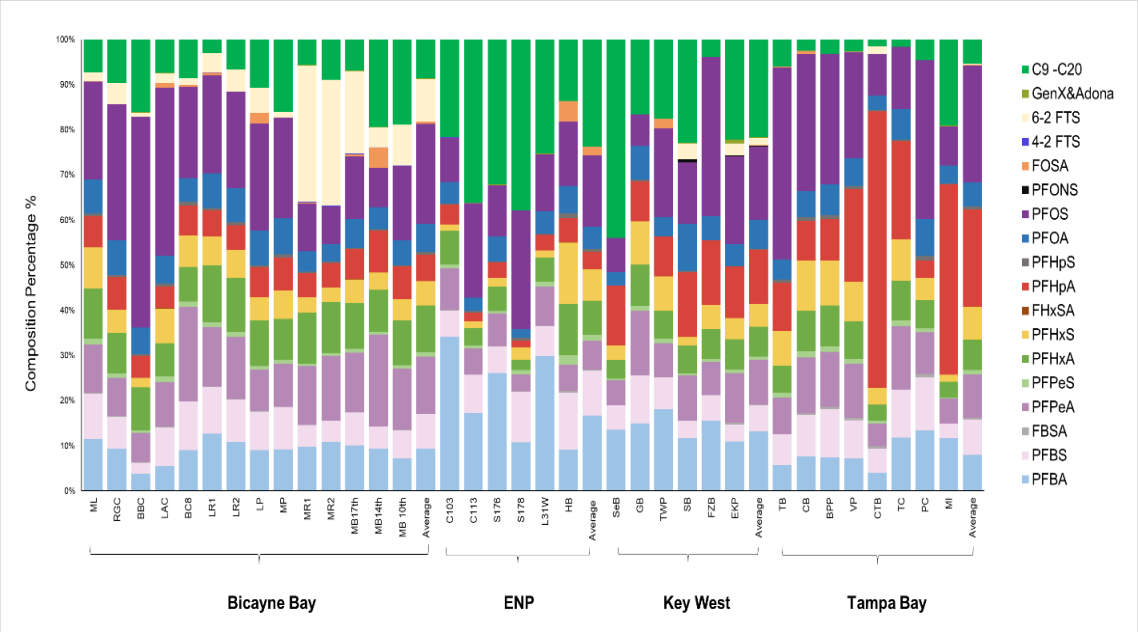


Figure 3.3 PFAS composition percentage in surface water sample along Biscayne Bay, ENP, Key West, and Tampa Bay. Group C9-C20 combined PFAS congeners with carbon chain ≥9 in each sample. PFAS classes and carbon chain length groups can be found in Table 2.1.

In Biscayne Bay samples, among 28 PFAS congeners detected (Adona and PFONS were not detected), PFOS was the predominant PFAS, with concentrations ranging from 1.16 ng L^{-1} to 24.1 ng L^{-1} (mean: 10.1 ng L^{-1}), followed by PFPeA (1.50-12.5 ng L^{-1}; mean: 5.75 ng L^{-1}), PFHxA (1.15-10.9 ng L^{-1}; mean: 4.72 ng L^{-1}), 6-2 FTS (0.313-21.8 ng L^{-1}; mean: 4.32 ng L^{-1}), PFBA (1.15-10.2 ng L^{-1}; mean: 4.26 ng L^{-1}), PFBS (0.671-8.58 ng L^{-1}; mean: 3.46 ng L^{-1}), PFOA (0.660-6.90 ng L^{-1}; mean: 2.84 ng L^{-1}), PFHpA (0.766-5.01 ng L^{-1}; mean: 2.69 ng L^{-1}), and PFHxS (0.520-5.58 ng L^{-1}; mean: 2.43 ng L^{-1}). PFOS, PFPeA, PFHxA, PFBA, PFBS, PFOA, PFHpA PFHxS had detection rates of 100%, and 6-2 FTS had a detection rate of 92.5%.

In the ENP canal samples, 25 PFAS congeners were detected (6-2 FTS, Adona, FHxSA, PFTeDA, FOSA were not detected). Since HB is apart from the ENP adjacent canals, thus it was not included in the following composition analysis represented by ENP samples. PFBA (5.33-12.2 ng L^{-1}; mean: 9.98 ng L^{-1}; detection frequency- DF:100%), PFOS (3.42-13.0 ng L^{-1}; mean: 7.48 ng L^{-1}; DF: 100%), PFBS (2.01-5.54 ng L^{-1}; mean: 3.45 ng L^{-1}; DF:100%), PFPeA (1.85-3.56 ng L^{-1}; mean:

2.99 ng L^{-1}; DF:100%), PFHxA (1.14-2.62 ng L^{-1}; mean: 2.06 ng L^{-1}; DF:100%), PFOA (0.985-2.40 ng L^{-1}; mean: 1.74 ng L^{-1}; DF: 100%), and PFHpA (0.73-1.53 ng L^{-1}; mean: 1.23 ng L^{-1}; DF: 90%), were identified as the predominant congeners in the ENP canals. Different from Biscayne Bay samples, some long-chain PFAS such as PFOUDS, PFUdA, N-EtFOSAA, PFDOA, PFTrDA were also predominantly present in the samples.

In Key West samples, 23 PFAS congeners were detected (8-2 FTS, PFNS, PFDS, FHxSA, PFUdA, PFTrD, PFTeDA were not detected), whereas PFOS (0.675-3.70 ng L^{-1}; mean:1.98 ng L^{-1}), PFBA (1.18-2.15 ng L^{-1}; mean: 1.64 ng L^{-1}), PFHpA (0.568-2.62 ng L^{-1} mean: 1.48 ng L^{-1}), and PFPeA (0.494-2.09 ng L^{-1}; mean: 1.23 ng L^{-1}) were identified as the predominant congeners at 100% detection rates.

In samples from Tampa Bay area, 19 PFAS were detected (4-2 FTS, GenX, Adona, PFONS, PFOUDS, FHxSA, N-MeFOSAA, N-EtFOSAA, PFDoA, PFTrDA, and PFTeDA were not detected), and PFOS was the predominant PFAS, with a concentration ranging from 2.40 ng L^{-1} to 25.7 ng L^{-1} (mean: 10.0 ng L^{-1}), followed by PFHpA (1.01-23.1 ng L^{-1}; mean: 8.36 ng L^{-1}), PFHxS (0.564-5.48 ng L^{-1}; mean: 2.83 ng L^{-1}), PFPeA (1.92-6.10 ng L^{-1}; mean: 3.72 ng L^{-1}), PFBS (1.19-4.49 ng L^{-1}; mean: 3.08 ng L^{-1}), PFBA (1.53-4.49 ng L^{-1}; mean: 3.08 ng L^{-1}), and PFHxA (1.33-4.38 ng L^{-1}; mean: 2.6 ng L^{-1}), with detection rates of 100% for all the congeners.

3.3.2 Occurrence, concentrations, and composition of PFAS in tap waters from West Coast and Central Florida

Tap water samples were collected along the West coast of Florida (Sarasota, Tampa City, St. Petersburg, Port Charlotte, Ft. Meyers, and Naples), as well as from cities in Central Florida, (Port St. Lucie, Fort Drum, St. Cloud, and Orlando). Total PFAS concentrations ranged from 1.61 ng L^{-1} (Naples) to 45.2 ng L^{-1} (Tampa City) in tap water as shown in Figure 3.4, and Table 3.3. Most of the samples fell below 20 ng L^{-1} with the exception of Tampa City, where was found a concentration

of 45.2 ng L^{-1}. Tap waters from Naples, Ft Meyers, Sarasota, Orlando, St. Cloud showed total PFAS concentrations below 6 ng L^{-1}.

Table 3.3 The concentrations of PFAS in tap water samples from major cities on the West coast and Central Florida

Concentrations (ng L-1)															
Location	**PFBA**	**PFBS**	**FBSA**	**PFPeA**	**PFPeS**	**PFHxA**	**PFHxS**	**FHxSA**	**PFHpA**	**PFHpS**	**PFOA**	**PFOS**	**PFONS**	**FOSA**	**PFNA**
Orlando	0.21	0.17	<MDL	0.34	0.07	0.27	0.45	<MDL	0.67	<MDL	0.53	0.17	<MDL	<MDL	0.03
St. Cloud	<MDL	<MDL	<MDL	0.22	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Fort Drum	<MDL	0.45	<MDL	0.87	0.03	0.76	0.52	<MDL	8.57	<MDL	1.02	0.54	<MDL	<MDL	<MDL
Port St. Lucie	<MDL	0.58	<MDL	1.89	0.10	2.80	0.60	<MDL	3.68	0.07	1.94	1.37	<MDL	<MDL	0.23
Tampa	4.02	4.12	2.69	8.76	1.77	3.70	4.69	<MDL	2.96	0.23	2.97	6.50	<MDL	<MDL	0.37
St. Petersberg	1.14	1.24	<MDL	1.59	0.10	1.29	0.75	<MDL	0.76	0.06	1.15	1.68	<MDL	<MDL	0.18
Sarasota	0.10	<MDL	<MDL	0.13	<MDL	<MDL	<MDL	<MDL	1.10	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Port Charlotte	<MDL	3.27	5.40	3.53	0.09	2.33	0.42	<MDL	1.30	<MDL	1.76	0.82	<MDL	<MDL	0.07
Ft. Meyers	3.73	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Naples	<MDL	0.10	<MDL	0.21	0.03	0.11	0.11	<MDL	0.57	<MDL	0.13	0.36	<MDL	<MDL	<MDL
Average of all sites	1.84	1.42	4.04	1.95	0.31	1.61	1.08	<MDL	2.45	0.12	1.36	1.63	<MDL	<MDL	0.18
Sum	9.20	9.93	8.09	17.53	2.19	11.26	7.54	0.00	19.59	0.36	9.51	11.44	0.00	0.00	0.88
Detection rates	50%	70%	20%	90%	70%	70%	70%	0%	80%	30%	70%	70%	0%	0%	50%

Concentrations (ng L-1)																
Location	**PFNS**	**PFDA**	**PFDS**	**PFUdA**	**PFDoA**	**PFTrDA**	**PFTeDA**	**4-2 FTS**	**6-2 FTS**	**8-2 FTS**	**N-MeFOSA**	**N-EtFOSA**	**PFONDS**	**GenX**	**Adona**	**∑PFAS**
Orlando	<MDL	0.03	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.94
St. Cloud	<MDL	<MDL	<MDL	<MDL	<MDL	2.68	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.89
Fort Drum	<MDL	0.03	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	12.77
Port St. Lucie	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	13.25
Tampa	<MDL	0.13	<MDL	<MDL	1.83	<MDL	<MDL	<MDL	<MDL	0.05	0.37	<MDL	<MDL	<MDL	<MDL	45.17
St. Petersberg	<MDL	0.06	<MDL	<MDL	0.33	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	10.33
Sarasota	<MDL	4.63	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	5.96
Port Charlotte	<MDL	<MDL	<MDL	<MDL	0.59	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	19.59
Ft. Meyers	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.73
Naples	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.61
Average of all sites	<MDL	0.98	<MDL	<MDL	0.92	2.68	<MDL	<MDL	<MDL	0.05	0.37	<MDL	<MDL	<MDL	<MDL	22.98
Sum	0.00	4.88	0.00	0.00	2.75	2.68	0.00	0.00	0.00	0.05	0.37	0.00	0.00	0.00	0.00	118.24
Detection rates	0%	50%	0%	0%	30%	10%	0%	0%	0%	10%	10%	0%	0%	0%	0%	

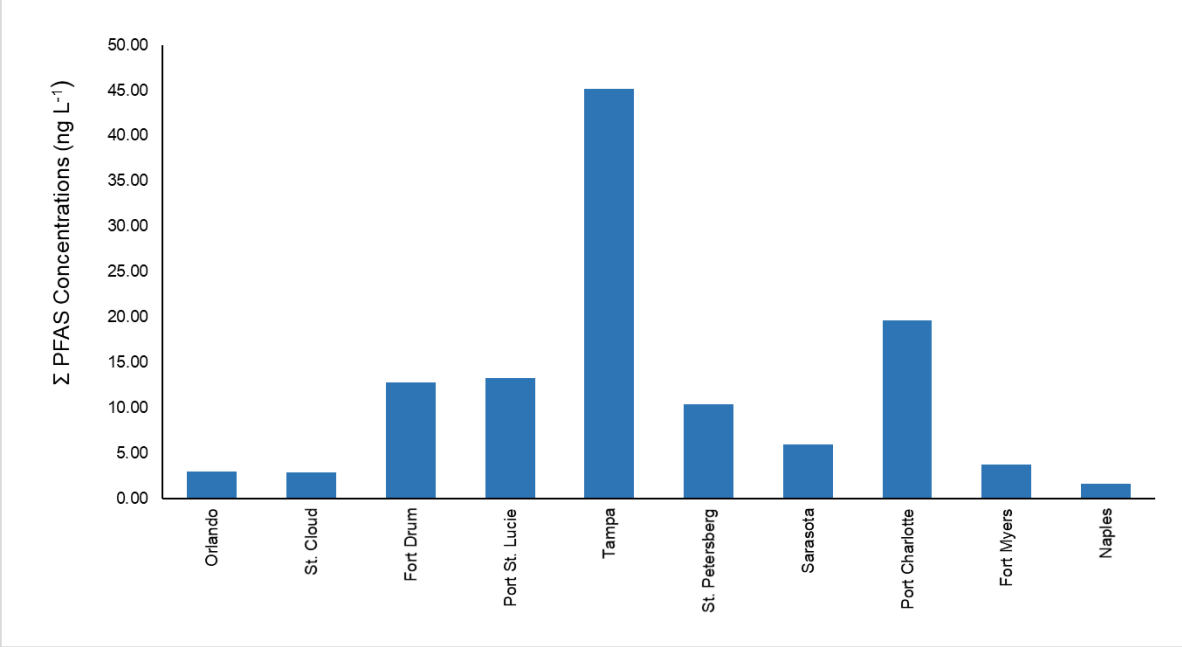


Figure 3.4 Total PFAS concentration of tap water samples from major cities on the West coast and Central Florida.

Among 30 PFAS, 17 compounds were detected in one or more samples, with FHxSA, PFONS, FOSA, PFNS, PFDS, PFUdA, PFTeDA, 4-2 FTS, 6-2 FTS, N-EtFOSAA, PFONDS, GenX, and Adona not being detected. The predominant congeners were PFHpA (<MDL-8.57 ng L^{-1}; mean: 2.45 ng L^{-1}), PFPeA (<MDL-8.76 ng L^{-1}; mean: 1.95 ng L^{-1}), PFOS (<MDL-6.50 ng L^{-1}; mean: 1.63 ng L^{-1}), PFHxA (<MDL-3.70 ng L^{-1}; mean: 1.61 ng L^{-1}), PFBS (<MDL-4.12ng L^{-1}; mean: 1.42 ng L^{-1}), PFOA (<MDL-2.94 ng L^{-1}, mean: 1.36 ng L^{-1}), and PFBA (<MDL-4.02 ng L^{-1}; mean: 1.84 ng L^{-1}). The detection rates of PFBS, PFPeA, PFPeS, PFHxA, PFHxS, PFHpA, PFOA, and PFOS, ranged from 70 to 90 %, while the detection rates of PFBA, FBSA, PFHpS, PFNA, PFDA, PFDoA, and PFTrDA ranged from 10-50%. The composition of PFAS congeners of each sample is shown in Figure 3.5. Overall, PFHpA accounts for 16.6% of total PFAS detected, followed by PFPeA

(14.8%), PFBA, PFBS, FBSA, PFHxA, PFHxS, PFOA, and PFOS ranged from 6.38% to 9.67%, while other PFAS contributed with less than 4.12%.

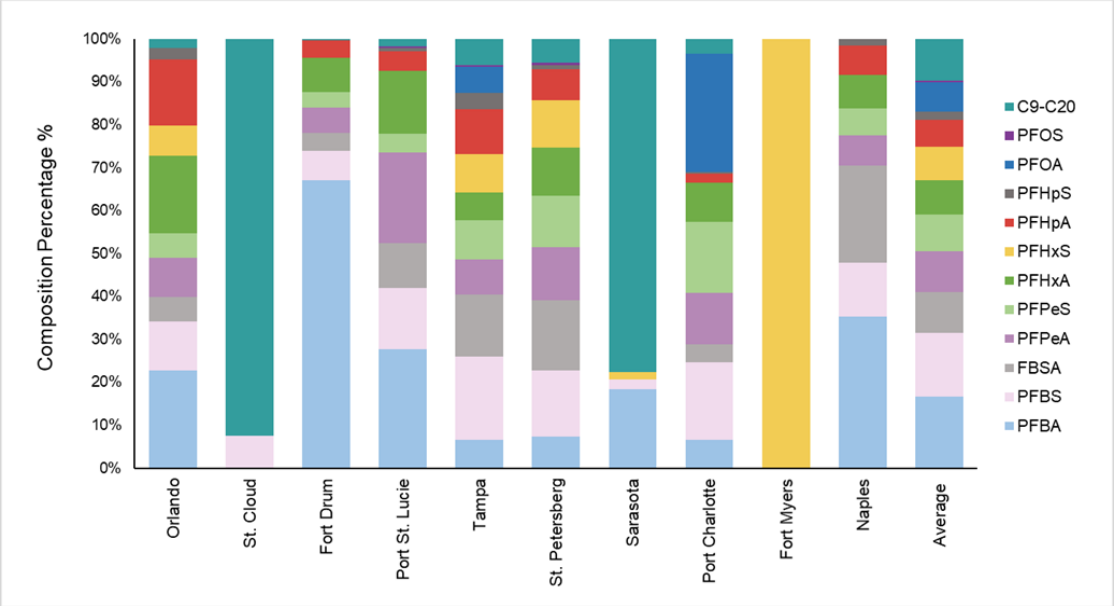


Figure 3.5 PFAS composition percentage in tap water sample from Central Florida. Group C9-C20 combined PFAS congeners with carbon chain ≥9 in each sample.

3.3.3 Spatial distribution and potential sources of PFAS in surface waters

Higher concentrations of PFAS are mostly observed in samples from polluted rivers, or samples near coastal estuaries, and point sources, such as military airbases, WWTPs, airports, etc. These water bodies pass through highly urbanized areas with large populations, businesses, in which various chemicals and waste discharges, drainage and runoffs might all end up to. The highest concentration in surface water was observed in the C103 canal (HB: 169 ng L^{-1}) at 2 km from the Homestead Air Reserve Base, where the historical use of AFFF containing PFAS is well known, and whereas the sample from the same canal on the west side close to ENP is nearly 5-fold lower, suggesting the potential impact of this point source on PFAS levels. The highest levels in Biscayne

Bay surface water were found in Miami River (98.9 ng L^{-1}; Sep 2021), Little River (91.7 ng L^{-1}; Sep 2021), and BC-8 canal (103 ng L^{-1}; Aug 2020), which were previously also reported as polluted waterways in South Florida with high levels of wastewater tracers, pharmaceutical and personal care products (PPCPs), and steroid hormones (Blair & Kemp, 2004; Ng et al., 2021).

The ENP adjacent canals presented PFAS levels ranging from 30 to 60 ng L^{-1}. These freshwater canals on the eastern boundaries serve as a buffer zone that separates the wetlands of ENP from highly productive subtropical agricultural lands and urban development areas (Quinete et al., 2013). Therefore, potential sources are likely to be a mixture of rainfall and runoffs from urban and agricultural areas of southeast Florida, such as from the usage of PFAS containing insecticides and fertilizers (Savvaides et al., n.d.) (Borthakur et al., 2022). Since there are no studies to date performed inside the ENP preserved area, the impact of PFAS in the Everglades water quality is still uncertain.

Key West and Miami beach surface waters showed concentrations below 20 ng L^{-1}. Though the sample locations include tourist beaches, marinas, drainage openings from apartment buildings, which could have contributed with PFAS input, it still presented a relatively low PFAS pollution level. These samples are associated with the highest salinities observed in this study, one possible reason is that PFAS levels can be substantially lowered by the dilution effect in seawater (Wang et al., 2019).

North Tampa Bay (where Tampa city is located) surface water showed slightly higher concentration than samples from central Tampa Bay followed by samples from South Tampa Bay, which coincides with the population in this area decreasing from North to South, for example, North Tampa Bay (Tampa city) has a population of 383,959, followed by Central Tampa Bay (St. Peterburg) with a population of 258,308, and South Tampa Bay (Palmetto City) with a population of 13,323. (census.gov, April 1, 2020). In addition, airports (Tampa International Airport, Clearwater International Airport, and Peter O. Knight Airports), military bases (MacDill Air Force

Base), landfills (Pinellas County Solid Waste Disposal), wastewater treatment plants (St Petersburg Wastewater Treatment and Howard F. Curren Advanced Wastewater Treatment Plant) are all found concentrated in North Tampa Bay, which could have contributed to PFAS input in this area. In April 2021, 814 million liters of legacy phosphate mining wastewater and marine dredge water from Piney Point Mining Phosphate Facility were discharged into South Tampa Bay and Port Manatee. Our sampling trip was conducted about one month after the incident, whereas the wastewater input and runoff it carried over might have contribute to the water quality deterioration in this area, potentially affecting the PFAS levels as well.

PFAS concentrations identified in each sampling area are compared and presented in Figure 3.6. The identified predominant PFAS included PFOS, PFPeA, PFHxA, PFBA, PFOA, PFHxA, PFHpA, PFHxS, PFNA, PFHpS, PFPes, and 6-2 FTS. As seen in Figure 3.6, in general, highest concentration of PFAS were found in Biscayne Bay and canals waters followed by Tampa Bay, ENP canals, and Key West for most of the congeners, except for PFBA, which was higher in ENP canals, and PFHpA, which showed the highest level in Tampa Bay.

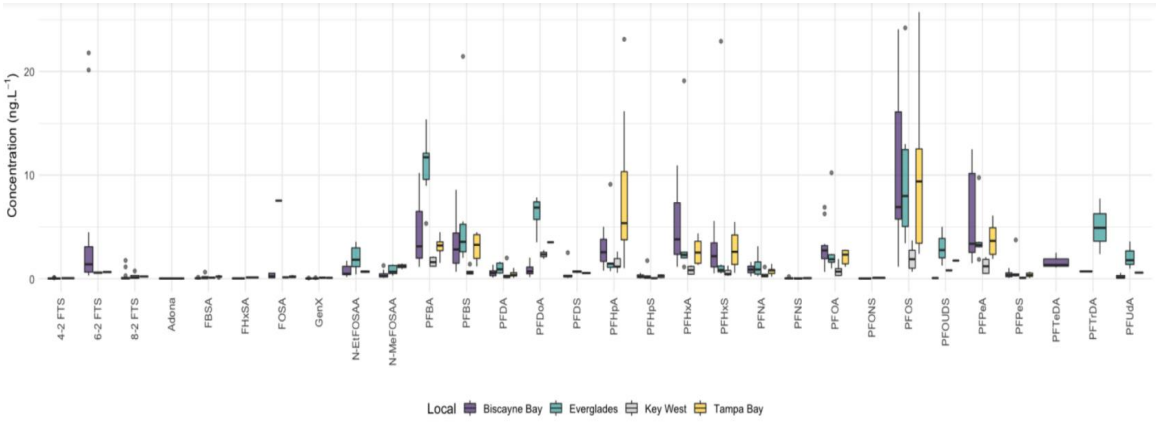


Figure 3.6 Boxplot of PFAS concentration in surface water from Biscayne Bay, ENP canals, Tampa Bay, and Key West. Median (the middle line), minimum and maximum values excluding outliers (upper and lower whiskers) are shown in the boxplot. The circles represent outliers.

3.3.4 Spatial distribution and potential sources of PFAS in tap water from Florida

To better address PFAS spatial distribution, tap water samples collected in this study from Central Florida and the West coast of South Florida are compared to samples collected from the metropolitan area on the East coast of South Florida previously published in Li et al., 2022 using the same method. The average PFAS concentration of the samples from the same region were calculated for comparison and displayed in Figure 3.7. The regions in Florida were divided into three groups: Central Florida (St. Lucie, Okeechobee, Osceola, Orange counties; N=4); West coast of South Florida (Sarasota, Hillsborough, Pinellas, Charlotte, Lee, Collier counties; N=6), and East coast of South Florida (Palm Beach, Broward, Miami Dade counties; N=22, data from Li et al. 2022). The total PFAS concentrations were the highest in the East coast of South Florida (mean: 83.0 ng L^{-1}), followed by the West coast of South Florida (mean: 14.4 ng L^{-1}), and Central Florida (mean: 8.00 ng L^{-1}) as shown in Figure 3.7. This trend coincides with the increased population in the defined groups: East coast of South Florida with a population of 5.6 million followed by West coast of South Florida with 3.6 million, and Central Florida with 1.7 million. Though PFAS levels could be associated with demographics factors (higher population number) and related human activities (higher production and discharge of industrial and domestic wastewater, landfills disposals, among others), the number of samples assessed in other regions and counties were low and further studies including a larger number of samples are needed to allow a more comprehensive and better understanding on the occurrence, distribution, and fate of PFAS in Florida.

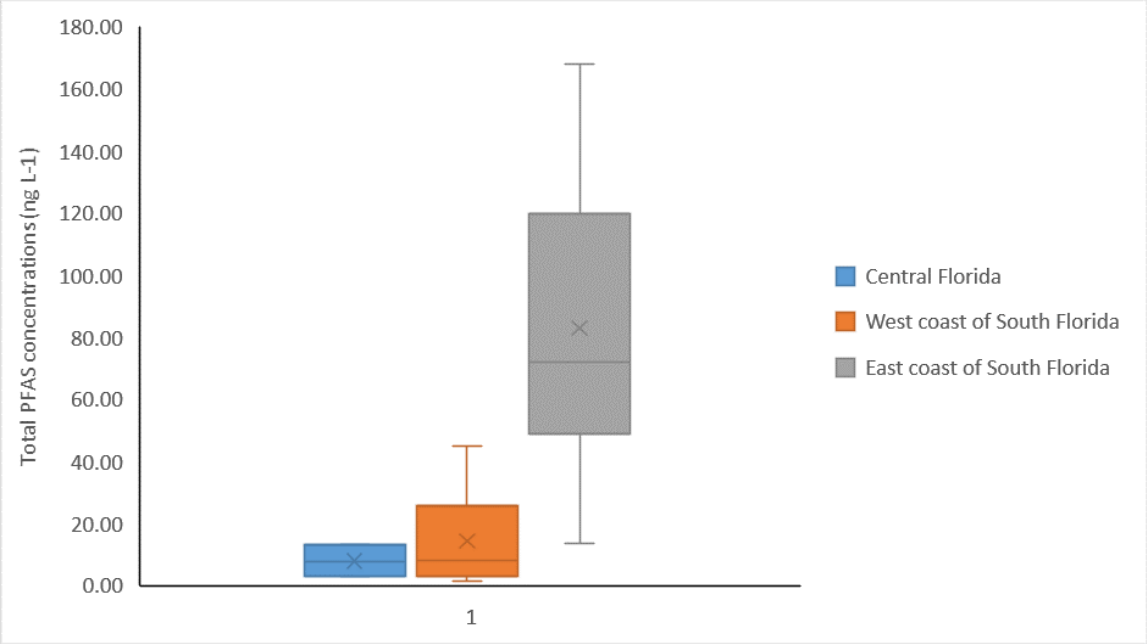


Figure 3.7 Boxplot of PFAS concentration of tap water in Central Florida, West coast of South Florida, and East coast of South Florida. Central Florida included St. Lucie, Okeechobee, Osceola, and Orange counties; N=4; West coast of South Florida included Sarasota, Hillsborough, Pinellas, Charlotte, Lee, and Collier counties; N=6; and East coast of South Florida included Palm Beach, Broward, and Miami Dade counties.

The concentrations of most PFAS congeners were higher on the East coast of South Florida, followed by West coast of South Florida, and Central Florida as shown in Figure 3.8, except for PFPeS, GenX, PFHpA, and Adona. Adona is an emerging PFAS substitute of PFOA and PFOS which was not detected in any East coast samples but showed concentration up to 6.50 ng L^{-1} in the West Coast and Central Florida tap waters. PFBA, PFOS, PFPeA, and PFHxA levels on the East coast showed higher concentration trend compared to the other regions in Florida.

The source of tap water in most of the cities in South and Central Florida such as Miami, Orlando, Fort Mayers, Naples, and Port St. Lucie is primarily from the Floridian aquifer, whereas the sources of tap water in Tampa region which include Tampa city, St. Peterburg, Sarasota, Port Charlotte in this study, are diverse coming from surface water from rivers and canals, groundwater from Floridian aquifer, and desalinated seawater (Tampa.gov, April 1, 2020). However, studies on PFAS

occurrence and levels in Floridan aquifers and surface water sources used for drinking purposes are still lacking to be able to draw any conclusion on the contamination source of PFAS in drinking water. It was found that the PFAS level in drinking water is higher than that of surface water in the east coast samples, which could arise from precursors breakdown processes during the water treatment and contamination during distribution processes (Li et al., 2022), but in the West coast and Central Florida water, more samples are needed to evaluate the difference on PFAS levels in surface water and tap waters.

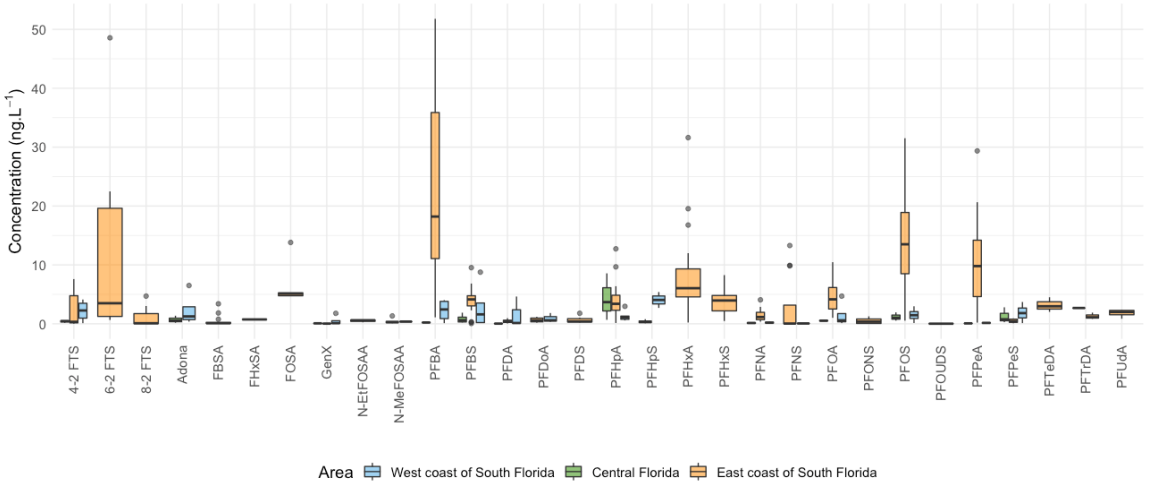


Figure 3.8 Boxplot of PFAS concentration of tap water samples in defined Central Florida, West coast of South Florida, and East coast of South Florida. Median (the middle line), minimum and maximum values excluding outliers (upper and lower whiskers) are shown in the boxplot. The circles represent outliers.

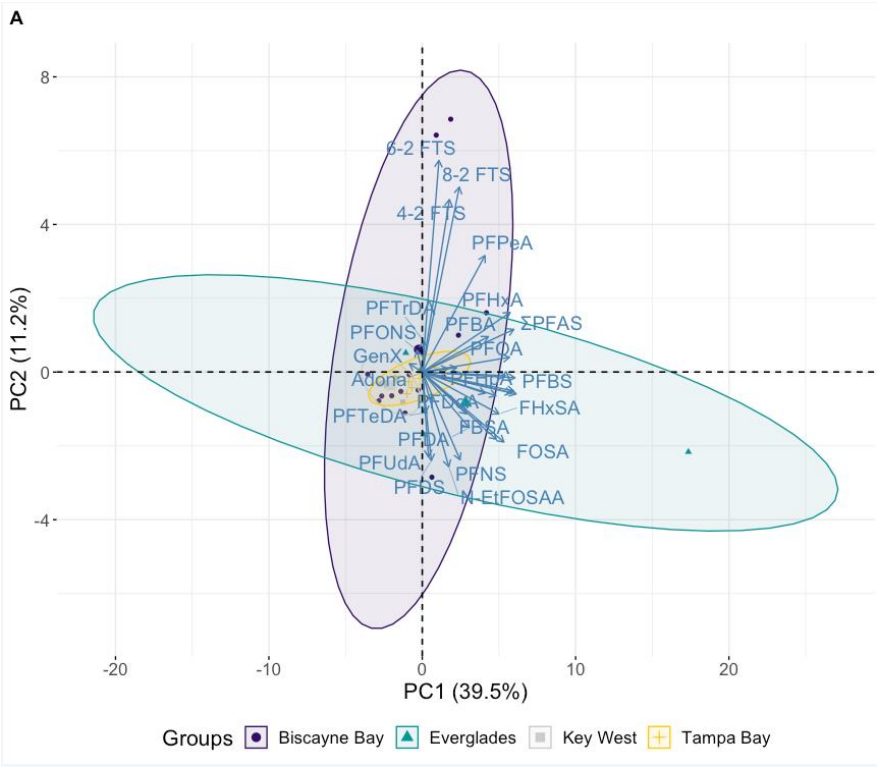
3.3.5 Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) determined the total variance of the dataset explained by the principal components (PCs) and their eigenvalues in both surface and tap waters. The number of PCs was determined using the Kaiser criterion (eigenvalues > 1; (Mooi & Sarstedt, 2011). Eight PCs were extracted from the surface waters' dataset, displaying a cumulative variance

of 84.71%. As for the tap waters' dataset, eleven PCs were extracted, exhibiting a cumulative variance of 83.55%.

A representation of the results of the PCA is given by the PCA biplots in Figure 3.9. These plots display loadings of the variables (i.e., vectors), determining how strongly each of the variables influence a PC. The further away these vectors are from a PC origin, the higher the influence they have on that PC. Small angles in these loadings indicate positive correlations, while large angles indicate negative correlations, and a 90° angle indicates no correlation. In Figure. 3.9A, which represents the PCA results for surface waters, a higher loading of the congeners 4-2 FTS, 6-2 FTS, and 8-2 FTS was noted, showing a high influence of these congeners, especially in the Biscayne Bay area. These congeners displayed a strong positive correlation, suggesting they have similar sources. The PCA biplot also showed clusters of samples based on their similarities. Samples from the same areas clustered in groups suggest shared similarities in compounds' composition, which was especially true in Key West.

In Fig. 3.9B, which represents the PCA results for tap waters, multiple high loadings were noted, including total PFAS (ΣPFAS), and the congeners PFOA, PFHpA, PFHxA, PFBA, PFNA, PFHxS, and PFOS. This shows a higher influence of these congeners in the data variability, especially in the East coast of South Florida region. Interestingly, all of these congeners are from the same two categories: perfluoroalkyl carboxylic acid (PFCA, including PFOA, PFHpA, PFHxA, PFBA, and PFNA) and perfluoroalkyl sulfonic acid (PFSA; including PFHxS, and PFOS), indicating similar composition and potential sources.


A
8
4
0
-4
PC2 (11.2%)
6-2 FTS
8-2 FTS
4-2 FTS
PFPeA
PFTrDA
PFHxA
PFBA
ΣPFAS
PFONS
GenX
PFOA
Adona
PFHpA
PFBS
PFDoA
FHxSA
PFTeDA
PFBSA
PFDA
FOSA
PFUdA
PFNS
PFDS
N-EtFOSAA
-20
-10
0
10
20
PC1 (39.5%)
Groups
Biscayne Bay
Everglades
Key West
Tampa Bay

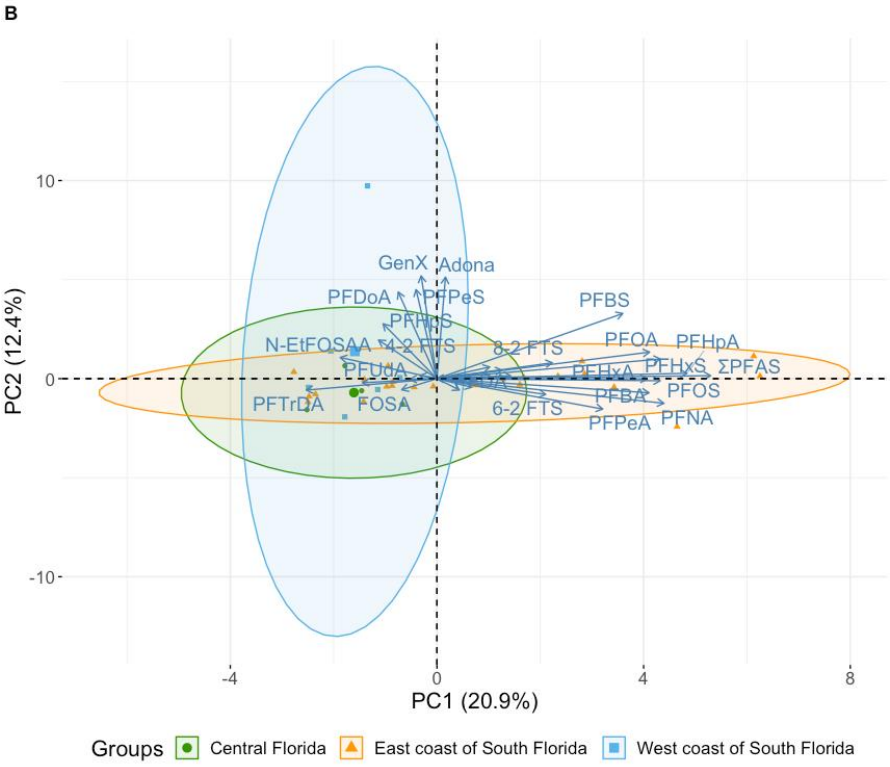

B
10
0
-10
PC2 (12.4%)
GenX
Adona
PFDoA
PFPeS
PFBS
PFHxS
N-EtFOSAA
4-2 FTS
8-2 FTS
PFOA
PFHpA
PFUdA
PFHxA
PFHxS
ΣPFAS
PFTrDA
FOSA
PFBA
PFOS
6-2 FTS
PFPeA
PFNA
-4
0
4
8
PC1 (20.9%)
Groups
Central Florida
East coast of South Florida
West coast of South Florida

Figure 3.9 PCA biplots of PFAS loadings relative to location in (A) surface waters, and (B) tap waters

.

3.3.6 Ecological and human health risk assessment

PFAS have been identified as Contaminants of Emerging Concern (CEC), which their ubiquitous presence may pose ecological and public health risks. Levels of PFOA and PFOS found here in tap water are below the health advisory guideline from the U.S EPA, which the sum of PFOA and PFOS should not be above 70 ng L^{-1}. Nevertheless, this level is being currently re-evaluated considering more recent scientific data and new analyses which indicate that negative effects might occur in levels lower than the established advisory value (Mccarthy et al., 2017; Post, 2021). Seafood consumption is another important route of dietary exposure to humans, as PFAS were found in fish tissues including Striped Mullets (Mugil Cephalus) which is a native Floridian fish (Bangma et al., 2018; Denys et al., 2014). Though there are no federal established guidelines that monitor surface water contamination for PFAS in the U.S., currently, the Florida Department of Environmental Protection (FDEP) has developed provisional surface water screening values of 1300 μg L^{-1} of PFOA and 37 μg L^{-1} of PFOS for fresh-water systems, and 13 μg L^{-1} of PFOS in saltwater systems, considering the protection of human health for the consumption of freshwater and estuarine finfish and shellfish (FDEP, 2021). The levels of PFOA ranged from 0.265 ng L^{-1} to 10.2 ng L^{-1}, and PFOS ranged from 0.68 ng L^{-1} to 25.7 ng L^{-1} in surface water samples from Biscayne Bay, Tampa Bay, ENP, and Key West covered in this study, which are all below these screening levels. However, considering that coastal Florida supports heavy seafood production and consumption, PFAS monitoring on these areas is needed for further human health risk assessment.

In addition, these aquatic ecosystems that support indispensable biomes are incessantly stressed due to these anthropogenic pollutants. As PFAS were identified in Florida coastal water samples,

previous study evaluated West Indian manatees inhabiting three coastal sites in Florida (Brevard County, Crystal River, and ENP), where PFOS was detected in the plasma of every Manatee (N= 69) with concentrations up to 166 ng/g ww. Coastal area covered in this study are natural habitats for manatees (Deutsch et al., 2003), and PFOS was also found to be the most predominant PFAS determined in our surface water samples, which suggest the potential environmental impact on these vulnerable and endangered species. Moreover, another study showed that corals, a crucial component to wildlife, tourism, and storm control, can rapidly bioconcentrate and eliminate PFOS, and exposure to PFOS (100 ng L^{-1}) was associated with increased oxidative stress (Bednarz et al., 2022). When combined with elevated temperature, PFOS can exacerbate the oxidative stress response leading to impaired photosynthesis in corals, which indicates that interactive effects of PFOS exposure with other environmental stressors can induce additional biological effects (Bednarz et al., 2022).

The levels of PFOS found in this study are above most of strict thresholds recommended in Europe, Australia, and New Zealand (0.23 to 23 ng L^{-1}) for the purpose of protecting aquatic biota. Though PFOS is the most prevalent PFAS detected in our study, PFBA, PFBS, and PFPeA were also predominant in the aquatic environment, therefore, it is important to take into consideration the potential PFAS synergetic effects on aquatic wildlife, which are currently not well understood and demands further assessments.

3.4 **Acknowledgment**

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Chapter 4. Non-targeted analysis for the screening and semi-quantitative estimates of Per-and Polyfluoroalkyl substances in water samples from South Florida environments

Xuerong Li, Danni Cui, Brian Ng, Piero Gardinali, and Natalia Soares Quinete

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of persistent contaminants that are found in aquatic environments ubiquitously worldwide (Ahrens, 2011; Domingo & Nadal, 2019). PFAS molecules usually consist of C-F bonds which make them thermally and chemically stable (Buck et al., 2011). Combined with their amphiphilic chemical properties, they are synthesized and valued in the production of a vast variety of commercial and consumer products, such as textiles, water/grease repellent, firefight foam, paints, and non-stick coating (Buck et al., 2021). However, recent in vivo and in vitro studies have shown that exposure to PFAS is associated with reproductive, developmental, hepatic, immunosuppressive, and endocrine disruptive toxicity, as well as bioaccumulating through the food chain (Fenton et al., 2021; Xu et al., 2014).

Though the production of some PFAS compounds has been banned in the U.S. due to health concerns, alternative PFAS molecules are brought into the market as replacements (Dhore & Murthy, 2021),which the impact on human health and the environment is still unclear. However, a few studies have reported that the emerging PFAS substitutes have shown similar toxicity and bioaccumulation just as the banned legacy PFAS (F. Li et al., 2020; Pérez et al., 2013). In addition to the continuous production of these emerging PFAS substitutes, the highly persistent legacy PFAS such as perfluorooctanoic acid (PFOS) and perfluorooctane sulfonic acid (PFOA) are still found to be present in the environment worldwide, along with their degradation and transformation products (Guelfo et al., 2021). As a result, the PFAS species in the environment are extremely diverse, with potentially over 100,000 compounds in existence that are covered in databases up to this date (EPA, 2021). Current target analytical methods based on liquid chromatography (LC) - mass spectrometry (MS) applied to environmental water studies are able to detect up to 40 PFAS species (USEPA, 2021). Although targeted methods can achieve high sensitivity and accuracy, the number of PFAS molecules detected and quantified is restricted since it requires certified standards,

many of which are not available at present for most PFAS. Thus, the environmental and public health risk associated with the "undetected" PFAS chemical space remains unknown.

There has been an increasing number of non-targeted analysis (NTA) approaches based on high-resolution mass spectrometry (HRMS) that allow for more comprehensive detection of total PFAS potentially present in environmental samples without the need for certified standards (Jacob et al., 2021; McCord & Strynar, 2019; Y. Q. Wang et al., 2022). The NTA workflow typically starts with obtaining HRMS full scan spectra and MS^2 spectra of a sample with no prior information needed, followed by data processing that involves obtaining quality features from large quantities of background, as well as PFAS structural annotation using NTA software(s) based on different algorithms (Hollender et al., 2017). Since there is no established workflow or criteria for PFAS NTA screening, variability and uncertainty exist when it comes to differences in instrumentation, analytical method, NTA software used, and data processing criteria (Guelfo et al., 2021).

Previously, we developed a target analysis based on LC/MS and analyzed 30 PFAS in drinking and surface water from Florida (Li et al., 2022). A full assessment of both target and non-targeted approaches will be complementing the coverage of PFAS species identified in environmental samples, which plays a crucial role in further understanding their toxicological and environmental impact. Therefore, in this study, we have developed a workflow for PFAS NTA screening based on an online solid phase extraction (SPE) coupled to LC-HRMS method using a Q-Exactive Orbitrap system for the screening of PFAS species in drinking water from populated counties in South Florida, as well as in surface water from Biscayne Bay, Key West, and Everglades canals. Data post-processing was conducted using the small molecules identification software Compound Discoverer 3.3 and FluoroMatch, a software specific for PFAS NTA, with the goal to compare the variability in processing NTA data for PFAS analysis in water samples. In addition, we propose a semi-quantitative NTA (qNTA) method to provisionally estimate concentrations from PFAS identified without commercial standards. The method was validated with spiked samples of 30

native standards (NS) and corresponding 19 labeled standards, and the variability was discussed for each compound. The semi-quantitative method was tentatively applied to the NTA results from the environmental water samples and concentrations were compared to the results from target analysis.

4.1 Materials and methods

4.1.1 Preparation of PFAS standards

A 30 native PFAS solution and 19 isotopically labeled PFAS mix were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada) as native standards (NS) and internal standards (IS), respectively. Both stock solutions (1 mg/L in methanol) were further diluted to 10 µg/L in LC-MS grade water and stored at 4°C for sample spiking. The list of PFAS compounds in the standard solutions is presented in Table 4.1, the list of IS can be found in Table 2.1. Each water sample was spiked with 100µL of 10 µg/L of labeled 19 PFAS mix as the IS to a final volume of 10.5 mL.

Table 4.1 List of 30 PFAS native standards and their exact mass.

Compound Class	Compound Name	Abbreviation	Chemical formula	Exact Mass
Perfluoroalkyl carboxylic acid (PFCA)	Perfluorobutanoic acid	PFBA	C_3F_7COOH	212.9792
	Perfluoropentanoic acid	PFPeA	C_4F_9COOH	262.9760
	Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	312.9728
	Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	362.9696
	Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	412.9664
	Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	462.9632
	Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	512.9600
	Perfluoroundecanoic acid	PFUdA	$C_{10}F_{21}COOH$	562.9568
	Perfluorododecanoic acid	PFDoA	$C_{11}F_{23}COOH$	612.9537
	Perfluorotridecanoic acid	PFTrDA	$C_{12}F_{25}COOH$	662.9505
	Perfluorotetradecanoic acid	PFTeDA	$C_{13}F_{27}COOH$	712.9473
Perfluoroalkyl sulfonic acid (PFSA)	Perfluorobutane sulfonic acid	PFBS	$C_4F_9SO_3H$	298.9429
	Perfluoropentane sulfonic acid	PFPeS	$C_5F_{11}SO_3H$	348.9398

	Perfluorohexane sulfonic acid	PFHxS	$C_6F_{13}SO_3H$	398.9366
	Perfluoroheptane sulfonic acid	PFHpS	$C_7F_{15}SO_3H$	448.9334
	Perfluorooctane sulfonic acid	PFOS	$C_8F_{17}SO_3H$	498.9302
	Perfluorononane sulfonic acid	PFNS	$C_9F_{19}SO_3H$	548.9270
	Perfluorodecane sulfonic acid	PFDS	$C_{10}F_{21}SO_3H$	598.9238
Fluorotelomer sulfonic acid (FTS)	4:2 Fluorotelomer sulfonic acid	4-2 FTS	$C_4F_9C_2H_4SO_3H$	326.9743
	6:2 Fluorotelomer sulfonic acid	6-2 FTS	$C_6F_{13}C_2H_4SO_3H$	426.9679
	8:2 Fluorotelomer sulfonic acid	8-2 FTS	$C_8F_{17}C_2H_4SO_3H$	526.9615
Perfluoroether carboxylic acid (PFECA)	Perfluoro-2-methyl-3-oxahexanoic acid	GenX	$C_6F_{11}O_3H$	284.9779
	4,8-Dioxa-3H-perfluorononanoic acid	Adona	$C_7H_2F_{12}O_4$	376.9689
Perfluoroalkane sulfonamido acetic acid (FOSAA)	N-Methyl perfluorooctane sulfonamido acetic acid	N-MeFOSAA	$C_8F_{17}SO_2N[CH_3]CH_2COOH$	569.9673
	N-Ethyl perfluorooctane sulfonamido acetic acid	N-EtFOSAA	$C_8F_{17}SO_2N[C_2H_5]CH_2COOH$	583.9830
Perfluoroalkane sulfonamide (PFOSA)	Perfluorobutanesulfonamide	FBSA	$C_4F_9SO_2NH_2$	297.9589
	Perfluorohexanesulfonamide	FHxSA	$C_6F_{13}SO_2NH_2$	397.9526
	Perfluorooctanesulfonamide	FOSA	$C_8F_{17}SO_2NH_2$	497.9462

4.1.2 Environmental samples

Water samples were collected in 500 mL pre-cleaned high-density polyethylene (HDPE) bottles with a swing arm sampler (Wooster, OH, USA), transported in a cooler with ice to the lab, and stored refrigerated at 4°C until analysis. Selected samples were from surface water samples of adjacent canals and water bodies of Biscayne Bay (N=5), Key West (N=3), and Everglades area (N=3), as well as tap water samples (N=6) from populated areas from South Florida (Pembrooke Pines, North Miami, Grapeland Heights, Fort Lauderdale, Miami Beach, and Davie.). Details about

the sampling sites and the concentration of 30 PFAS congeners that have been previously reported in our studies using a target LC-MS method are summarized in Table 4.2.

Table 4.2 Geographical coordinates and the results of the concentration of 30 PFAS previously reported in surface water sampling locations along Biscayne Bay, Key West, Everglades adjacent canals, and tap water from populated counties in South Florida.

Water Type	**Sampling Site**	**Sample Number**	**Total Conc PFAS* (ng/L)**	**Latitude**	**Longitude**	**Salinity (ppt or PSU)**	**Collection Dates**
Tap water	Pembroke Pines	Tap 1	94.40	26.00566	-80.35756	-	02/2021
	North Miami	Tap 2	142.0	25.91356	-80.15166	-	02/2021
	Dave	Tap 3	191.0	26.00584	-80.31077	-	10/2020
	Grapeland Heights	Tap 4	241.6	25.79297	-80.24552	-	02/2021
	Miami Beach	Tap 5	119.3	25.79336	-80.1422	-	01/2021
	Fort Lauderdale	Tap 6	118.5	26.19332	-80.2428	-	02/2021
Biscayne Bay Surface water	Royal Glade Canal	BB 1	28.8	25.92927	-80.15113	22	01/2021
	Biscayne Canal C-8	BB 2	53.8	25.8712	-80.17615	25	08/2021
	Miami River site 1	BB 3	98.3	25.76997	-80.19891	17	09/2021
	Little River site2	BB 4	92.0	25.84602	-80.17655	4	09/2021
	Miami River site 2	BB 5	99.5	25.7775	-80.20552	14	08/2021
Everglades Surface water	Homestead Air Reserve Base	EV 1	130.0	25.47332	-80.39608	2	07/2021
	C103-217 Mowry Canal	EV 2	29.1	25.51721	-80.54271	1	07/2021
	S178 Southern	EV 3	47.3	25.4084	-80.5237	0	07/2021

	Glades Trail						
Key West Surface water	Edward B. Knight Pier	KW 1	19.1	24.54544	-81.78353	38	07/2021
	Smathers Beach	KW 2	18.3	24.55038	-81.76815	38	07/2021
	Garrison Bright	KW 3	13.2	24.56091	-81.78486	38	08/2021

*Total PFAS concentration is the sum of 30 PFAS congeners using target analysis

4.1.3 QA/QC

The Q-Exactive Orbitrap (Thermo Scientific) was calibrated in positive and negative mode with mass tolerance < 5ppm with Pierce LTQ ESI ion calibration solutions (Thermo Scientific). Quality control (QC) samples consisted of LC-MS grade water spiked with NS at final concentration of 50 and 100 µg/L and IS at final concentration of 100 µg/L to a final volume of 10.5 ml. QC samples were analyzed at the beginning of the sequence and every five environmental samples to ensure the instrument's performance. Blank samples (LC/MS grade water) were analyzed at the beginning and between every environmental sample. The chromograms of PFAS QC samples (native standards) showing the peak separation and intensity obtained by online SPE-LC-HRMS extracted by X-Calibur software through exact mass search is presented in Figure 4.1.

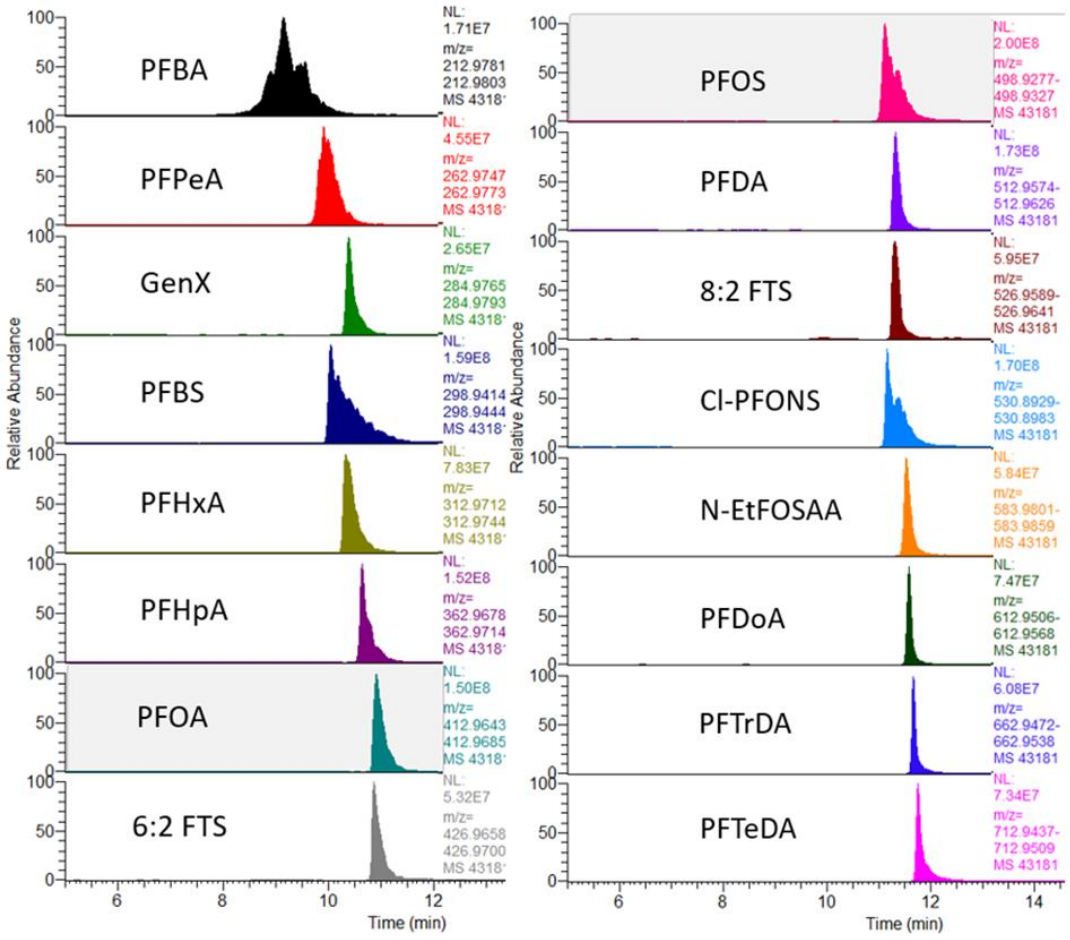

Figure 4.1 The chromograms of 30 PFAS standards showing peak separation in the QC samples

4.1.4 LC-MS/MS data acquisition

Data acquisition is based on an online SPE- liquid chromatography (LC)- HRMS method using a Q-Exactive Orbitrap system equipped with heated electrospray ionization (HESI) interface. Hypersil GOLD PFP HPLC Column (100 mm x 2.1 mm, 3 μm) was used for chromatogram separation. Waters Oasis WAX Online Column (20 mm × 2.1 mm, 30 μm) was used for online SPE column. The mobile phase gradient program for the online SPE and analytical pumps are presented in Table 4.3. Water samples were run in full scan negative mode with a scan range from 100.0 to 800.0 m/z at a resolution of 140,000, followed by data-dependent MS/MS with a

normalized collision energy of 30 and at a resolution of 35,000. Detailed MS parameters can be found in Table 4.4.

Table 4.3 The gradient program of on-line SPE and analytical column

Time (min)	A [%]	B [%]	C [%]	D [%]	Flow (ul/min)
0.00	90.0	5.0	0.0	5.0	400.0
5.00	95.0	5.0	0.0	0.0	400.0
5.50	95.0	5.0	0.0	0.0	400.0
6.50	0.0	10.0	0.0	90.0	400.0
10.50	0.0	95.0	0.0	5.0	400.0
20.00	0.0	95.0	0.0	5.0	400.0

Table 4.4 MS parameters for Full MS and data-dependent MS (ddMS2)

Time (min)	A [%]	B [%]	C [%]	D [%]	Flow (ul/min)
0.00	99.0	1.0	0.0	0.0	2000.0
5.00	99.0	1.0	0.0	0.0	2000.0
5.50	99.0	1.0	0.0	0.0	100.0
7.00	0.0	0.0	100.0	0.0	100.0
9.00	0.0	0.0	100.0	0.0	100.0
10.00	0.0	100.0	0.0	0.0	100.0
13.00	0.0	100.0	0.0	0.0	500.0
14.50	0.0	100.0	0.0	0.0	500.0
18.50	0.0	100.0	0.0	0.0	500.0
19.00	99.0	1.0	0.0	0.0	1500.0
20.00	99.0	1.0	0.0	0.0	1500.0

Solvent A: water; Solvent B: Methanol; C: Acetonitrile; D: Ammonia Formate (2mM) in water

4.1.5 Data Post-Processing

Two non-target workflows were established and optimized using Compound Discoverer 3.0 and FluoroMatch 2.0 from sample collection to data processing. Samples were post-processed with both software to screen for potential PFAS compounds.

Compound Discoverer 3.0

The data processing and annotation are based on the CD workflow "Environmental w Stats Unknown ID w Online and Local Database Searches" embedded in the software, as shown in Figure 4.2. The samples from the same source (Biscayne Bay, Key West, Everglades, and tap water) were processed together in one batch, as well as Blanks and QC samples. The workflow The list of detected PFAS features generated by CD as further reduced by additional filtering criteria to improve confidence level, whereas only features meeting the criteria below were kept for further analysis: 1) mass defect ≥ 0.75 or ≤ 0.1, (Nason et al., 2020); 2) Molecular Formula is proposed in the data file; 3) Mass list match was found in the EPA Master list (Citation); 4) MS2 were found for preferred ion in Data Dependent Analysis (DDA); 5) class scoring >6.25 for common fragments match (CF_3-, C2F5O-, etc.); 6) the feature was found ≥2 samples from the same area. A feature is defined as a detected m/z, its retention time, and its intensity (Pastore et al., 2018). The list of features with highest peak intensities detected in each location and type of water can be found in Table 4.6.

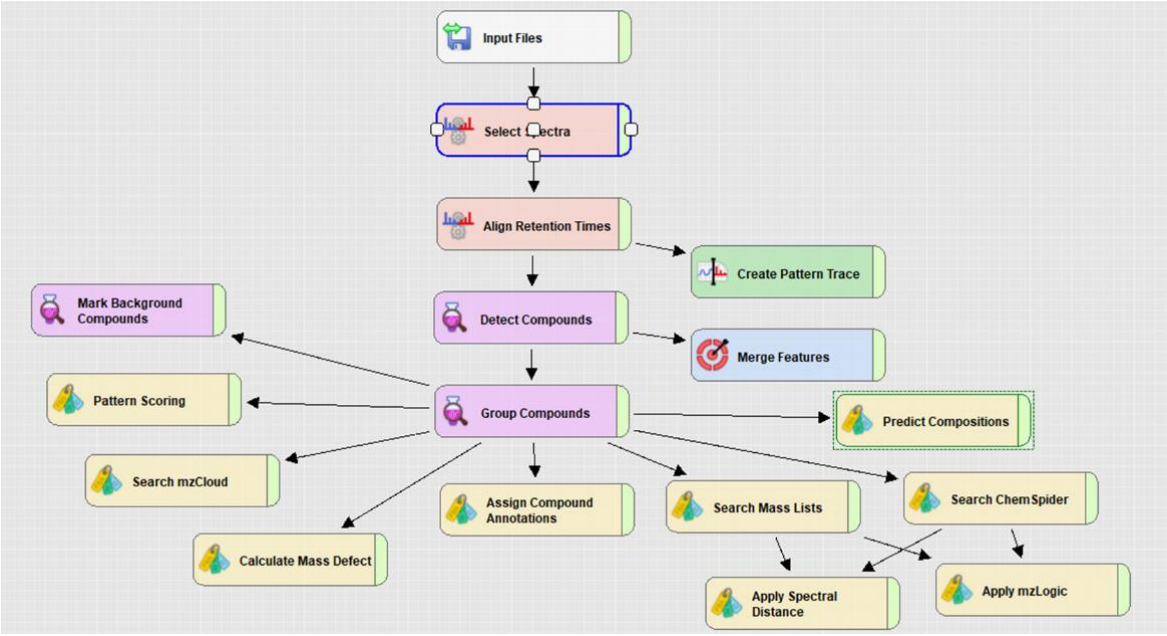

Figure 4.2 The workflow established with Compound Discoverer for the screening of PFAS

FluoroMatch 2.0

Data were processed on FluoroMatch software version 2.0 using the default setting parameters. The same strategies were employed as in compound Discoverer 3.0, the samples from the same source (Biscayne Bay, Key West, Everglades, and tap water) were processed in one batch. In the data output file, data were further reduced through manual curation based on the following criteria: 1) Exact Mass and MS2 Match in class-based standards and in-silico library; 2) Chemical Formula is proposed; 3) Score annotation in A (confident identification) (Koelmel et al., 2020); and 4) Score annotation in B (highly likely PFAS identification). The final list of features detected from different sources by FluoroMatch can be found in Table 4.7.

4.1.6 Semi-quantification

The 30 native PFAS standards mix was used to prepare a five-point calibration curve at concentrations of 0, 10, 50, 100, and 500 ng/L. The labeled 19 PFAS IS mix was spiked to the standard calibration samples as well as water samples at a concentration of 100 ng/L for standardization, accounting for variabilities in sample preparation and LC-HRMS measurements. The average response (peak intensity) from all labeled 19 IS was used to normalize the average response of all 30 PFAS NS. A global calibration curve was created by plotting a quadratic regression fit curve of the concentration (X) versus the response factor between the average response of NS and IS (peak intensity of the native standard/peak intensity of the internal standard), as shown in Figure 4.3 The total PFAS concentration in each sample was estimated based on the sum of peak intensity and response factor using this global calibration curve.

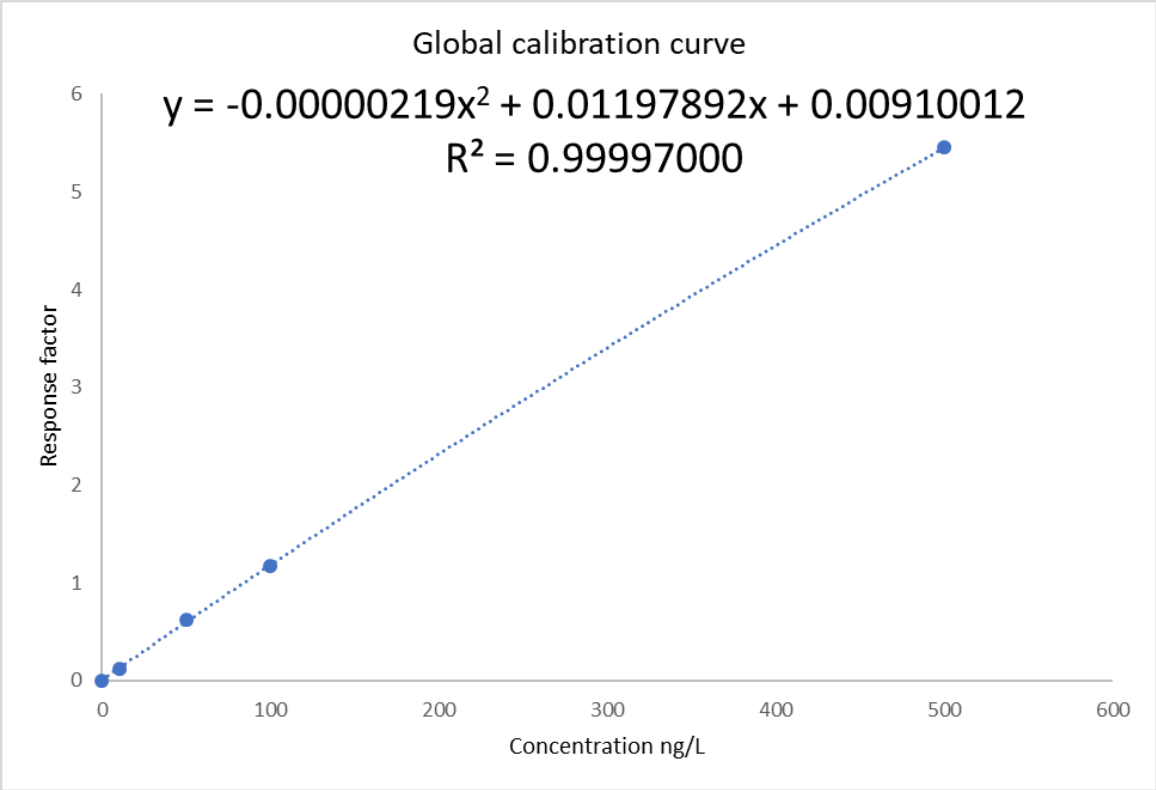

Figure 4.3 The global calibration curve established for qNTA.

4.2 **Results and discussion**

4.2.1 QA/QC

QC samples at concentrations of 50 ng/L and 100ng/L were run in the same condition as the environmental samples. The peak integration of 30 NS was processed with Xcalibur software and based on exact mass search. All 30 NS were able to be separated by chromatography and detected by MS, as shown in Figure 4.1. The QC samples were analyzed through the NTA workflow by Compound Discoverer. The detected and identified features generated directly from the compound discoverer were manually curated following the same criteria as for the environmental samples, which aimed at removing noise and falsely identified (false positive) compounds. The number of features was reduced from 2036 to 78 after applying filtering criteria, in which all 30 PFAS

compounds were correctly identified. True Positives (TPs) are the PFAS NS correctly identified, which in this case TPs=30. False Positives (FPs) are the compounds incorrectly identified as true positives, in which FPs=48. True negatives (TNs) are the PFAS compounds correctly identified as not in the samples, TNs=1958. False negatives (FNs) are the NS incorrectly identified, in this case FNs=0. The precision and accuracy are used to access the performance of the NTA method as presented in Table 4.5 (Fisher et al., 2022). Overall, the optimized NTA workflow with additional manual data post-processing was able to reduce falsely identified compounds successfully with acceptable precision (0.38) and accuracy (0.97) or a mixture of 30 PFAS compounds in the QC samples.

Table 4.5 The performance metrics used to assess the NTA performance

True Positive (TP) TP=30	False Positive (FP) FP=48	$precision = \frac{TP}{TP + FP}$ Precision=0.38
False Negative (FN) FN=0	True Negative (TN) TN=1958	$Accuracy = \frac{TP + TN}{TP + FP + FN + TN}$ Accuracy=0.97

4.2.2 Screening of PFAS in water samples from South Florida using Compound discoverer 3.0 and FluoroMatch

Based on the criteria outlined in section 4.2.5, 409 features in total were filtered out by Compound Discoverer in the water samples from different sources, annotated with compound name, chemical formula, m/z, RT, class score, and MS^2 match in the EPA PFAS master list. In Biscayne Bay samples, 53 tentatively identified PFAS were filtered out from 689 features (reported directly from the software). 7-(Heptafluoropropyl)-4,9-dimethoxy-5H-furo[3,2-g][1]benzopyran-5-one ($C_{16}H_9F_7O_5$), 1,1,2,2,3,3,5,5,6,6,6-undecafluoro-4,4-bis(trifluoromethyl)hexane-1-sulfonic acid

($C_8HF_{17}O_3S$), N-Carboxymethyl-N,N-dimethyl-2-(perfluoroethyl)-2-fluoroethan-1-aminium ($C_8H_{12}F_6NO_2$) were found to be the most prevalent PFAS (highest peak intensity) in Biscayne Bay water samples. In tap water samples, 271 features were filtered out of 2216 features, whereas perfluoro-2-(trifluoromethyl)propanesulfonic acid ($C_4HF_9O_3S$), 2,2,3,4,4,5,5,5-octafluoro-3-(trifluoromethyl)pentanoic acid ($C_6HF_{11}O_2$), and perfluoro-p-ethylcyclohexylsulfonic acid ($C_8HF_{15}O_3S$) were the most prevalent species. In samples from Everglades adjacent canals, 151 were filtered out 1155 features; the prevalent PFAS included 6:2 fluorinated telomer sulfonate ($C_8H_5F_{13}O_3S$), 1-chloro-2,2,3,3-tetrafluorocyclobutane ($C_4H_3ClF_4$), perfluoro(2,7-dimethyl-3,6-dioxasuberoyl) fluoride ($C_8F_{14}O_4$), followed by PFOS, PFBS, PFHxS. In Key West samples, 14 were filtered out of 196 features. The compounds 3,3,3',3'-tetrakis(trifluoromethyl)-1,1'-spirobi[2,1-benzoxaphosphol-1-ium] ($C_{18}H_8F_{12}O_2P$), Dichloro(1,1,1,2,3,3-hexafluoropropan-2-yl)methylsilane ($C_4H_4Cl_2F_6Si$), and 5-(Perfluoropropyl)-2,2-dimethyl-3,5-pentanedione barium(2+) ($C_{20}H_{22}BaF_{14}O_4$) were the prevalent species.

The Venn diagram in Figure 4.4 shows the features that are unique or overlapping from different water sources. Tap water has the most detected features and unique features. This can be attributed to higher PFAS concentrations being prevalent in highly urbanized areas and the ineffective removal of these recalcitrant compounds by drinking water treatment plants (Cui et al., 2020; Li et al., 2022; Li et al., 2022). Tap water also has the largest overlap (N=50) with Everglades samples. This large overlap of features between tap water and water from the Everglades can be attributed to the fact that the major source of drinking water in South Florida is groundwater, coming especially from the Everglades aquifers. Everglades samples also shared 15 features with Biscayne Bay. These overlapping features can be a result of the adjacent canals near the Everglades flowing towards Biscayne Bay. The lower number of detected features in Biscayne Bay compared to the Everglades can be because as go downstream, PFAS compounds can be lost due to potential bioaccumulation into aquatic plants and organisms as well as susceptible to dilution effect (Martin

et al., 2013; Q. Wang et al., 2019). However, no feature was found in common among all four sources. The number of features in each different water source has been corroborated by previous studies that showed that the concentration of detected PFAS compounds in tap water are higher than that of surface water, with surface water samples from Key West having very low concentrations of PFAS, leading to its low number of features detected by NTA (Li et al, 2022, Li et al, 2022).

Table 4.6 The list of features with highest peak intensities detected from different sources by Compound Discoverer.

Tapwater								
Name	Formula	Calc. MW	m/z	RT [min]	Area (Max.)	Class Coverage	Mass List Match: Chemical	MS2
Perfluoro-2-(trifluoromethyl)propanesulfonic acid	C4 H F9 O3 S	299.9514	298.9441	10.429	69920772.2	6.25	Multiple matches found	DDA for preferred ion
2,2,3,4,4,5,5,5-Octafluoro-3-(trifluoromethyl)pentanoic acid	C6 H F11 O2	313.9812	312.9739	10.405	46561683.9	6.25	Multiple matches found	DDA for preferred ion
Perfluoro-p-ethylcyclohexylsulfonic acid	C8 H F15 O3 S	461.9425	460.9352	10.85	36936092.6	6.25	Multiple matches found	DDA for preferred ion
2,2,3,3,3-Pentafluoro-1-(pyrrolidin-2-yl)propan-1-one	C7 H8 F5 N O	217.0511	216.0439	9.494	21075618.8	6.25	Multiple matches found	DDA for preferred ion
Octafluorotetrahydrothiophene 1,1-dioxide	C4 F8 O2 S	263.9472	262.9399	8.096	18306175.1	6.25	Multiple matches found	DDA for preferred ion
Perfluorodecane	C10 F22	537.9635	536.9562	11.474	16602617.4	6.25	Multiple matches found	DDA for preferred ion
2-(Pentafluoroethyl)-4H-pyran-4-one	C7 H3 F5 O2	214.0044	212.9972	9.885	13814616.1	6.25	Multiple matches found	DDA for preferred ion
Chloroheptafluorocyclobutane	C4 Cl F7	215.9572	214.9499	8.346	10676511	6.25	Multiple matches found	DDA for preferred ion
1,1,1,2,2-pentafluoro-6-iodohexane	C6 H8 F5 I	301.9582	300.9509	10.006	9819022.19	6.25	Multiple matches found	DDA for preferred ion
2-(Trifluoromethyl)-1,1,1,3,3,3-hexafluoropropane	C4 H F9	219.9944	218.9871	10.263	9678015	6.25	Multiple matches found	DDA for preferred ion

Biscayne Bay surface water								
Name	Formula	Calc. MW	m/z	RT [min]	Area (Max.)	Class Covera	Mass List Match: Chemical I	MS2
7-(Heptafluoropropyl)-4,9-dimethoxy-5H-furo[3,2-g][1]benzopyran-5-one	C16 H9 F7 O5	414.0334	413.0261	11.725	129035578.3	6.25	Single match found	DDA for preferred ion
1,1,2,2,3,3,5,5,6,6,6-undecafluoro-4,4-bis(trifluoromethyl)hexane-1-sulfonic acid	C8 H F17 O3 S	499.9386	498.9313	11.731	51732911.34	6.25	Multiple matches found	DDA for preferred ion
N-Carboxymethyl-N,N-dimethyl-2-(perfluoroethyl)-2-fluoroethan-1-aminium	C8 H12 F6 N O2	268.0777	267.0704	10.416	39188104.41	6.25	Single match found	DDA for preferred ion
Bis(2,2,3,3,3-pentafluoropropyl)sulfoxide	C6 H4 F10 O S	313.9813	312.9741	11.5	29044253.16	12.5	Multiple matches found	DDA for preferred ion
Perfluoro-4,5-dimethylhexanoic acid	C8 H F15 O2	413.9748	412.9675	11.604	27494033.46	6.25	Multiple matches found	DDA for preferred ion
Methyl 1-(4-ethoxyphenyl)-2,2,3,3-tetrafluorocyclobutane-1-carboxylate	C14 H14 F4 O3	306.0868	305.0796	13.312	26359759.96	6.25	Single match found	DDA for preferred ion
2,3-Bis(1,1,1,2,3,3-hexafluoropropan-2-yl)but-2-enedioate	C10 H2 F12 O4	413.9755	412.9682	11.223	26228058.99	6.25	Multiple matches found	DDA for preferred ion
1,1,1,2,2,3,3,4,5,5,5-Undecafluoropentane	C5 H F11	269.9913	268.9841	11.467	20125692.5	6.25	Multiple matches found	DDA for preferred ion
2,2,3,3,4,4,4-Heptafluoro-N-pentylbutanamide	C9 H12 F7 N O	283.078	282.0707	11.242	16318865.83	6.25	Single match found	DDA for preferred ion
2'-Deoxy-5-(nonafluorobutyl)uridine	C13 H11 F9 N2 O5	446.0522	445.0449	11.105	14514092.87	6.25	Multiple matches found	DDA for preferred ion

Everglades surface water								
Name	Formula	Calc. M	m/z	RT [mir	Area (N	Class C	Mass List Match: Chem	MS2
6:2 Fluorinated telomer sulfonate	C8 H5 F13 O3 S	427.976	426.9687	10.745	4.08E+08	18.75	Multiple matches found	DDA for preferred ion
1-Chloro-2,2,3,3-tetrafluorocyclobutane	C4 H3 Cl F4	161.9852	160.9779	10.382	2.96E+08	6.25	Single match found	DDA for preferred ion
Perfluoro(2,7-dimethyl-3,6-dioxasuberoyl) fluoride	C8 F14 O4	425.9611	424.9538	10.771	2.88E+08	12.5	Multiple matches found	DDA for preferred ion
Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	499.9388	498.9316	11.084	2.67E+08	12.5	Multiple matches found	DDA for preferred ion
Perfluoro-1-butanesulfonic acid (PFBS)	C4 H F9 O3 S	299.9506	298.9434	10.255	1.24E+08	6.25	Multiple matches found	DDA for preferred ion
Perfluoro-1-hexanesulfonic acid (PFHxS)	C6 H F13 O3 S	399.9448	398.9375	11.149	1.11E+08	6.25	Single match found	DDA for preferred ion
Carboxydimethyl(4,4,5,5,5-pentafluoro-2-hydroxypentyl)azanium	C8 H13 F5 N O3	266.0795	265.0723	11.887	1.07E+08	6.25	Single match found	DDA for preferred ion
4-Chloro-5-fluoro-2,2-bis(trifluoromethyl)-1,3-dioxole	C5 Cl F7 O2	259.9499	258.9427	7.9	42129956	6.25	Single match found	DDA for preferred ion
4-Hydroxy-3-(hydroxyimino)-4-(nonafluorobutyl)-1,3,4,5-tetrahydro-	C13 H8 F9 N3 O3	425.0401	424.0328	10.788	36965609	12.5	Single match found	DDA for preferred ion
Sodium perfluoropentanesulfinate	C5 F11 Na O2 S	355.9326	354.9253	7.916	26843578	6.25	Single match found	DDA for preferred ion

Key west surface water								
Name	Formula	Calc. M	m/z	RT [mir	Area (M	Class C	Mass List Match: Chem	MS2
3,3,3',3'-tetrakis(trifluoromethyl)-1,1'-spirobi[2,1-benzoxaphosphol-1-ium]	C18 H8 F12 O2 P	515.0042	513.997	8.845	9224064	6.25	Single match found	DDA for preferred ion
Dichloro(1,1,1,2,3,3-hexafluoropropan-2-yl)methylsilane	C4 H4 Cl2 F6 Si	263.9383	262.931	17.025	7239698	6.25	Multiple matches found	DDA for preferred ion
5-(Perfluoropropyl)-2,2-dimethyl-3,5-pentanedione barium(2+) (2:1)	C20 H22 Ba F14 O4	730.035	729.0278	11.783	5932107	6.25	Single match found	DDA for preferred ion
1,6-Dibromododecafluorohexane	C6 Br2 F12	457.8158	456.8086	5.773	4032653	6.25	Single match found	DDA for preferred ion
1,1,1,2,2,3,3,4,4,5,5,6,6,9,9,10,10,11,11,11-Icosafluoroundecane	C11 H4 F20	516.0023	514.9951	8.734	3404431	6.25	Multiple matches found	DDA for preferred ion
1-Bromo-1,1,2,2-tetrafluoro-4-iodobutane	C4 H4 Br F4 I	333.8451	332.8378	5.765	2992976	6.25	Single match found	DDA for preferred ion
1-Chloro-1,2,2,2-tetrafluoroethane-1-sulfinyl chloride	C2 Cl2 F4 O S	217.8968	216.8895	6.118	1343217	6.25	Single match found	DDA for preferred ion
1-Bromo-1,1,2,2-tetrafluoroethane	C2 H Br F4	179.9206	178.9133	5.648	400723.6	6.25	Multiple matches found	DDA for preferred ion
5-Chloro-1,1,1,2,2-pentafluoropentane	C5 H6 Cl F5	196.0084	195.0011	5.545	278163.2	6.25	Single match found	DDA for preferred ion
(Pentafluoroethyl)phosphonous dichloride	C2 Cl2 F5 P	219.902	218.8947	5.801	241331.3	6.25	Single match found	DDA for preferred ion

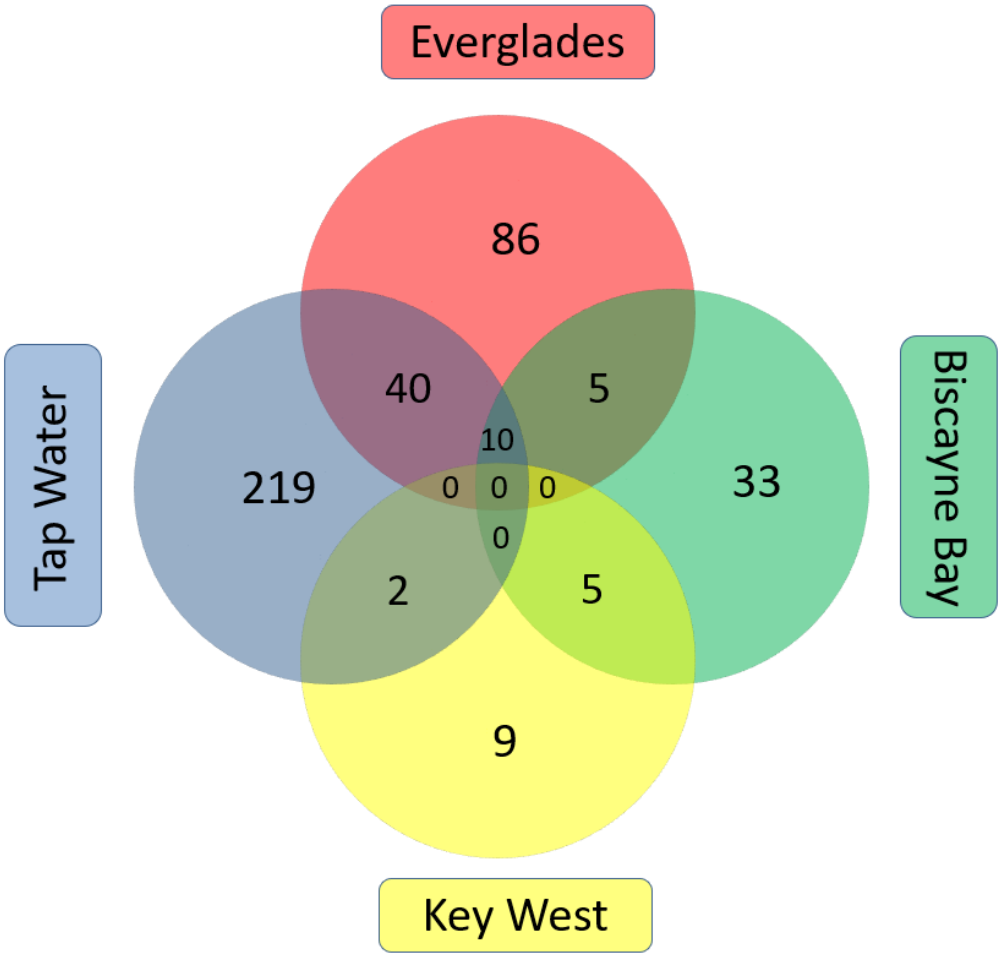

Figure 4.4 Venn diagram of PFAS screened in water samples from different sources (Tap water, surface water from Biscayne Bay, Key West, and Everglades adjacent canal) using Compound Discoverer 3.0.

The identified features from different sources are visualized in the Kendrick mass defect (KMD) against the Nominal Kendrick Mass (NKM) plot, as shown in Figure 4.5. The Kendrick mass (KM) of an observed mass (OM) is normalized by the ratio of nominal mass to the exact mass of the repeating unit of CF_2. The KMD is calculated as the difference between the NKM and exact KM. PFAS homologues series with only varying numbers of repeating units of CF_2 (m/z 49.9968) share the same KMD and thus align horizontally on the KMD plot. The KMD can be plotted based on

different repeating units that are common to PFAS, such as CF_2O (m/z 65.9917) and C_2F_4O (m/z 115.9885) (Dimzon et al., 2016). Due to fluorine atoms having a negative mass defect of $\Delta m/z = -0.0016$, PFAS have low and often increasingly negative mass defects as the number of fluorine atoms increases (Liu et al., 2019). The plot presented here is based on the most common repeating unit of CF_2. Samples from different water sources are presented in Figure 4.5 to visualize the difference between sample types. The tap water has the most detected features with the majority spanning the NKM range of 250-500 and mostly occupying the negative region of the KMD (-0.025 to -0.125). It was previously reported that the majority (> 90%) of PFAS from a curated PFAS list containing 3213 PFAS compounds had a mass defect between -0.25 and +0.1 which was also observed in this study (Bugsel & Zwiener, 2020). Water samples from Everglades adjacent canals show a similar pattern as tap water. In contrast, the samples from Biscayne Bay and Everglades are more evenly distributed on the positive and negative KMD, although there were very few features detected. The common overlapping area in which the majority of the features among all the sample types is observed is in the negative region of the KMD between NKM of 200-500.

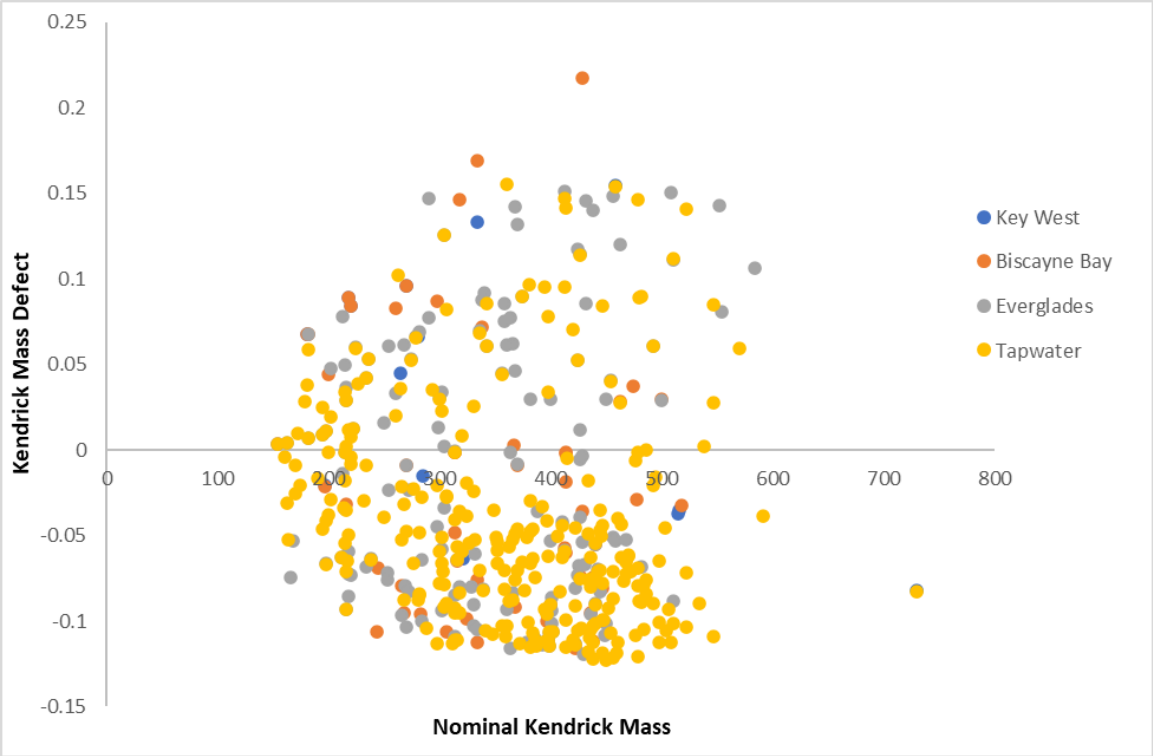

Figure 4.5 Kendrick mass defect plot of PFAS screened from different water sources using Compound Discoverer 3.0.

A Van Krevelen diagram as shown in Figure 4.6 is also presented to visualize the detected PFAS features of the water samples from different sources, in which the ratio of Fluorine and Carbon (F:C) is plotted against the ratio of oxygen to carbon (O:C) for each feature. Based on the degree of saturation and oxygen content, the features are localized in different regions on the Van Krevelen diagrams. As described in (Ng et al., 2022), for example, the compounds containing no oxygen (aromatic hydrocarbons) appear at the y-axis, and PFAS with high content of fluorine would appear in the upper region given the F/C ratio is relatively high, especially for a lot of the legacy PFAS where all the hydrogens on the carbon chain are substituted by F. In our data, the clutter of more densely populated area appears to be in the region with F:C from 0.25-2.5, and O:C from 0-0.4, which suggest the majority of the PFAS compounds has a low content of oxygen and are polyfluoroalkyl where H are only partially substituted by the fluorine in the carbon chain.

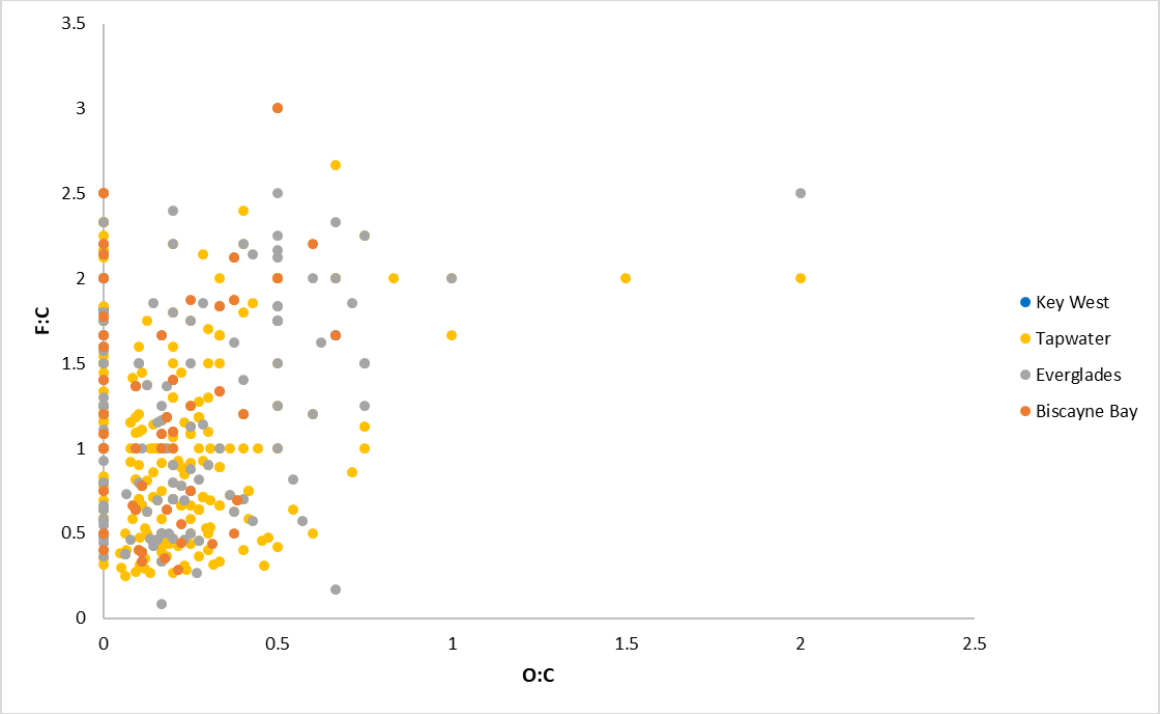

Figure 4.6 Van Krevelen diagram of PFAS screened from different water sources using Compound Discoverer 3.0.

These water samples raw data were also post-processed using FluoroMatch, an open-source software tailored toward PFAS annotation (Koelmel et al., 2020). This data post-processing yielded 61 features in total from the water samples from different sources, annotated with compound name, chemical formula, m/z, RT, matching fragments in-house library, and confidence score. In Tap water samples, 56 PFAS were filtered out from 8227 features (reported directly from the output), whereas perfluorobutanesulfonic acid ($C_4HF_9O_3S$), perfluorooctanesulfonic acid ($C_8HF_{17}O_3S$), and PFOA ($C_8HF_{15}O_2$) were found to be the most prevalent PFAS (highest peak intensity). In Biscayne Bay (BB) samples, 33 features were filtered out of 10416 features. 2-[Chloro(difluoro)methoxy]-1,1,2,2-tetrafluoroethanesulfonic acid ($C_3HClF_6O_4S$), Perfluorobutanesulfonic acid ($C_4HF_9O_3S$), and PFPeS ($C_5HF_{11}O_3S$) were the most prevalent species in BB. In samples from Everglades adjacent canals, 23 were filtered out of 3249 features. The prevalent PFAS included 5-

(1,1,2,2,3,3,4,4,4-nonafluorobutyl)-1H-pyrimidine-2,4-dione ($C_8H_3F_9N_2O_2$), Chloro-perfluoropropane sulfonate ($C_3HClF_6O_3S$), Perfluoro-6-methylheptanecarboxylic acid ($C_9HF_{17}O_2$) in the Everglades canals. In Key West samples, 21 were filtered out of 3249 features, with 6:2 FTS ($C_8H_5F_{13}O_3S$) and 2,2,3,3,4,4,5,5,5-Nonafluoropentanal (C_5HF_9O) being the prevalent species.

As shown in the Venn diagram in Figure 4.7, the tap water has the most detected features followed by Biscayne Bay samples. Three features were in common by all four sources, which were tentatively identified as $C_3HF_5O_3S$ (structure not determined), PFBS ($C_4HF_9O_3S$), and PFPeS ($C_5HF_{11}O_3S$).

Table 4.7 The list of features with highest peak intensities detected from different sources by FluoroMatch.

Score	SeriesType_Identifier	Name_or_Class	Formula	m/z	Retention Time	Area Max
Tap water						
B+	[CF2]n_138	PFSA-perfluoroalkyl	C4HF9O3S	298.9436	10.358617	2.86E+07
B	[CF2]n_138	Perfluorooctanesulfonic acid	C8HF17O3S	498.9314	11.328822	2.83E+07
B+	[CF2]n_162	PFCA-perfluoroalkyl_branched	C8HF15O2	412.9673	11.003244	2.74E+07
B+	PVDF_[CH2CF2]n_92	PFSA-pentafluorosulfide	CHF7O3S2	256.9203	5.913812	2.70E+07
B	PVDF_[CH2CF2]n_133	NA	C10H7F15O2	443.0111	9.646277	1.57E+07
B	[CF2]n_184	NA	C7H3F9O	272.997	10.892528	1.39E+07
A	[CF2]n_162	PFCA-perfluoroalkyl_branched	C7HF13O2	362.9708	10.702467	1.22E+07
B	[CF2]n_96	NA	C7H6ClF8IO	418.8957	8.323713	1.03E+07
B	[CF2]n_137	NA	C2HF6O4P	232.9466	8.186213	6.31E+06
B	PVF_[CH2CHF]n_83	NA	C4H3F6IN2O	334.9099	8.336257	4.61E+06
Bisayne Bay						
B-	[CF2]n_0	NA	C3HClF6O4S	280.9129	8.601513	4.39E+06
B	[CF2]n_154	Perfluorobutanesulfonic acid	C4HF9O3S	298.9437	10.283531	4.04E+06
B+	[CF2]n_161	FT-PFSA	C3H5F3O3S	176.9855	14.406217	3.30E+06
B	[CF2]n_154	Perfluoropentanesulfonic acid	C5HF11O3S	348.9404	10.705771	1.27E+06
B	PVF_[CH2CHF]n_248	NA	C12H22F6O4Si	371.112	10.579448	1.33E+05
B	PVDF_[CH2CF2]n_148	NA	C12H9F7N2	313.0574	7.614063	1.18E+05
B-	[CF2]n_219	NA	C14H11F7N2O	355.0681	7.677517	1.07E+05
B	PVF_[CH2CHF]n_174	NA	C18H17F7N2O2	425.1104	8.340733	8.90E+04
B	PVDF_[CH2CF2]n_141	NA	C14H11F7N2O2	371.0629	7.737304	8.07E+04
B+	PVDF_[CH2CF2]n_83	PFSA-unsaturated	C8HF15O3S	460.9351	11.124688	7.80E+04
Everglades						
B	[CF2]n_245	NA	C7H3F9O	272.9972	10.401962	1.07E+08
B	[CF2]n_245	SCHEMBL1270621	C8H3F9N2O2	272.9972	10.527126	6.03E+07
B-	[CF2]n_0	PFSA-Cl	C3HClF6O3S	264.9166	10.247996	5.61E+06
B	[CF2]n_235	Perfluoro-6-methylheptanecarboxylic acid	C9HF17O2	462.9645	11.323643	5.52E+06
A	[CF2]n_235	PFCA-perfluoroalkyl	C5HF9O2	262.9765	10.560521	5.10E+06
B	[CF2]n_227	Perfluoropentanesulfonic acid	C5HF11O3S	348.9401	10.652439	2.41E+06
A	[CF2]n_235	PFCA-perfluoroalkyl	C6HF11O2	312.9736	10.862431	2.22E+06
B+	[CF2]n_227	PFSA-perfluoroalkyl_branched_C3	C6HF13O3S	398.9375	12.574217	2.00E+06
B	[CF2]n_227	Perfluorohexanesulfonic acid	C6HF13O3S	398.9377	12.806224	2.00E+06
A	[CF2]n_235	PFCA-perfluoroalkyl	C4HF7O2	212.9802	9.308112	1.82E+06
Key West						
B	PVDF_[CH2CF2]n_166	6:2 Fluorotelomer sulfonic acid	C8H5F13O3S	426.9685	11.672161	1.98E+07
B+	PVDF_[CH2CF2]n_166	FT-PFSA	C8H5F13O3S	426.9689	11.271224	1.02E+07
B	[CF2]n_239	NA	C5HF9O	246.9827	11.984433	5.42E+06
B	[CF2]n_245	NA	C7H3F9O	272.9972	10.401962	4.30E+06
B	[CF2]n_245	SCHEMBL1270621	C8H3F9N2O2	272.9972	10.527126	3.42E+06
B	[CF2]n_239	1H,1H,5H-Perfluoropentane-1-thiol	C5H4F8S	246.9821	10.723788	2.00E+06
B	[CF2]n_227	Perfluorohexanesulfonic acid	C6HF13O3S	398.9377	12.806224	2.54E+05
B+	[CF2]n_227	PFSA-perfluoroalkyl_branched_C3	C6HF13O3S	398.9375	12.574217	2.26E+05
A	[CF2]n_235	PFCA-perfluoroalkyl	C5HF9O2	262.9765	10.560521	1.93E+05
B	[CF2]n_235	Perfluoro-6-methylheptanecarboxylic acid	C9HF17O2	462.9645	11.323643	1.07E+05

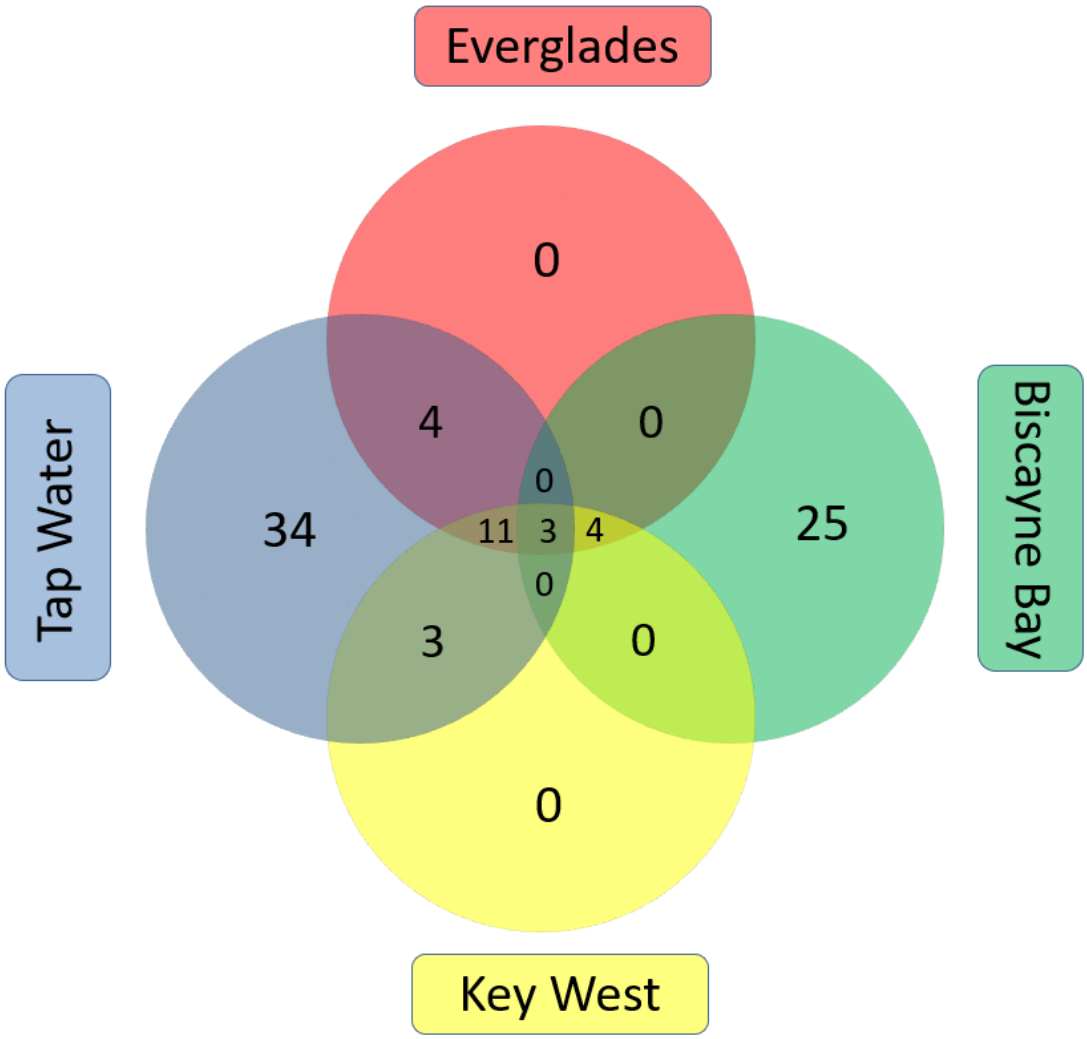

Figure 4.7 Venn diagram of PFAS screened in water samples from different sources (Tap water, surface water from Biscayne Bay, Key West, and Everglades adjacent canal) using FluoroMatch.

The identified features from different sources are visualized in the plot of KMD against NKM as shown in Figure 4.8. The tap water has the most detected features and is spread out on the nominal mass range between 200 to 500 and has both positive and negative KMD. However, water samples from Everglades adjacent canals clustered more on the negative region of KMD (-0.07 to 0.17). Features from different sources are also visualized as a Van Krevelen diagram in Figure 4.9. The

cluster of the more densely populated area appears to be in the region with F:C from 0.25-2.5, and O:C from 0-0.5, which suggest the majority of the PFAS compound has a slightly higher content of oxygen, and PFAS with the H completely or partially substituted by fluorine in the carbon chain both present in the features. Although the number of features detected by FluoroMatch is significantly less (79% less) than that of Compound Discoverer, the observed results in both Van Krevelen diagrams are very similar. One data point that falls out of the cluster is PFSA-pentafluorosulfide ($CHF_7O_3S_2$) found in tap water, which has an extremely high ratio of F due to the bonding to sulfur.

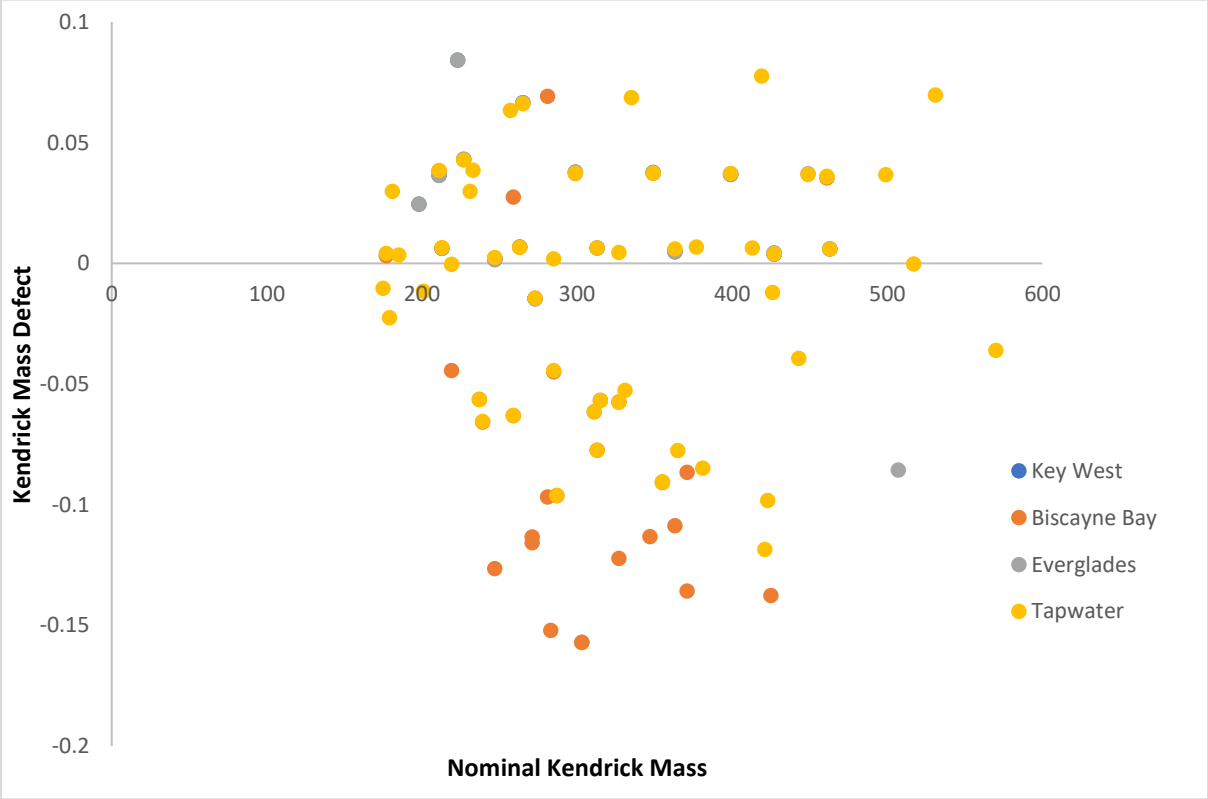

Figure 4.8 Kendrick mass defect plot of PFAS screened from different water sources using FluoroMatch.

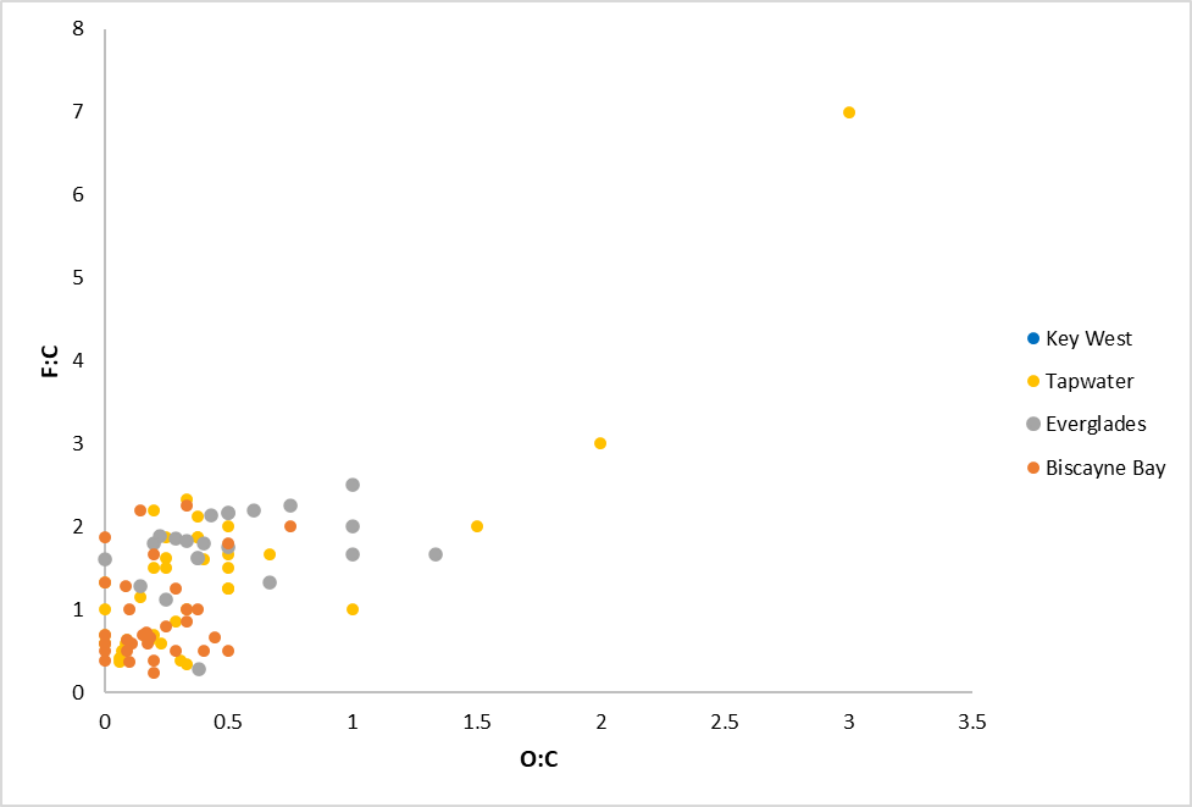

Figure 4.9 Van Krevelen diagram of PFAS screened from different water sources using FluoroMatch.

4.2.3 PFAS Semi-quantitative assessments

qNTA was performed for PFAS tentatively identified with Compound Discoverer 3.0 using a global calibration curve created based on the available 30 PFAS NS. First, to estimate the accuracy of using the global calibration curve for concentration estimates, the concentration of a QC sample containing NS and IS spiked in LC-MS grade water (50 ng/L) was calculated based on the global calibration curve and compared to their added concentrations. The percentage error (PE) for each compound is presented in table 4.8.

Table 4.8 The calculated concentration of 50 ng/L QC sample based on the global calibration curve. The percentage error (PE) for each NS is calculated compared to the actual concentration.

QC 50ng/L	Calculated concentration in ng/L	Percentage error (%)
PFBA	0.506996595	-98.98600681
PFPeA	42.93002507	-14.13994985
PFHxA	-0.608204684	-101.2164094
PFHpA	59.059716	18.11943199
PFOA	63.11276017	26.22552034
PFNA	66.66902078	33.33804156
PFDA	60.11956448	20.23912897
PFUdA	49.04189939	-1.916201215
PFDoA	19.29153945	-61.41692111
PFTrDA	-0.71	-101.42
PFTeDA	3.625140997	-92.74971801
FBSA	73.45140391	46.90280782
FHXSA	102.6161339	105.2322678
FOSA	29.66997375	-40.66005251
GenX	15.49703581	-69.00592838
MeFOSAA	27.55004626	-44.89990748
Et-FOSAA	23.08177075	-53.8364585
PFBS	162.9362302	225.8724604
PFPeS	121.2978579	142.5957158
PFHxS	119.9368488	139.8736975

PFHpS	90.5754954	81.15099079
PFOS	109.4796763	118.9593526
PFNS	105.4510513	110.9021026
PFDS	64.38207872	28.76415744
4:2 FTS	15.80447639	-68.39104723
6:2 FTS	16.73316473	-66.53367053
8:2 FTS	21.62121858	-56.75756284
NaDoNA	2.238216288	-95.52356742
PFONS	72.96458295	45.9291659
PFOUds	24.61009387	-50.77981225

The PE of PFHpA, PFOA, PFNA, PFDA, FBSA, PFDS, and PFONS was overestimated by up to 50% of the actual concentration, and of PFPeA, PFUdA, FOSA, MeFOSAA was underestimated by up to 50%. The PE of PFHpS was overestimated by 51-100% of the actual concentration, whereas the compounds PFBA, PFDoA, PFTeDA, GenX, EtFOSAA, 4:2 FTS, 6:2 FTS, 8:2 FTS, NaDoNA, and PFOUds showed to be underestimated by 51-100%. The PE of FHxSA, PFBS, PFPeS, PFHxS, PFOS, PFNS was overestimated by over 100% of the actual concentration, and PFHxA and PFTrDA were underestimated by over 100%. Variations of systemic overestimation and underestimation are generally caused by using a global calibration curve, especially with very diverse chemical structures of PFAS resulting in differences in ionization efficiency and chromatography (McCord et al., 2018). With target analysis, these errors can be corrected when using isotopically labeled matching standards which leads to more accurate measurement. However, the purpose of the qNTA shown here is to provide a provisional estimate of the total

concentration of PFAS in a sample, which can provide complementary information for further studies on the toxicological and environmental impacts of PFAS.

The above qNTA method was applied to tap water samples from South Florida and environmental surface water samples collected from Biscayne Bay, Key West, and Everglades adjacent canals. The total concentrations of all the PFAS tentatively identified in the sample were calculated based on the global calibration curve, which was compared to the total concentration of 30 PFAS from target analysis for each sample, as shown in Figure 4.10.

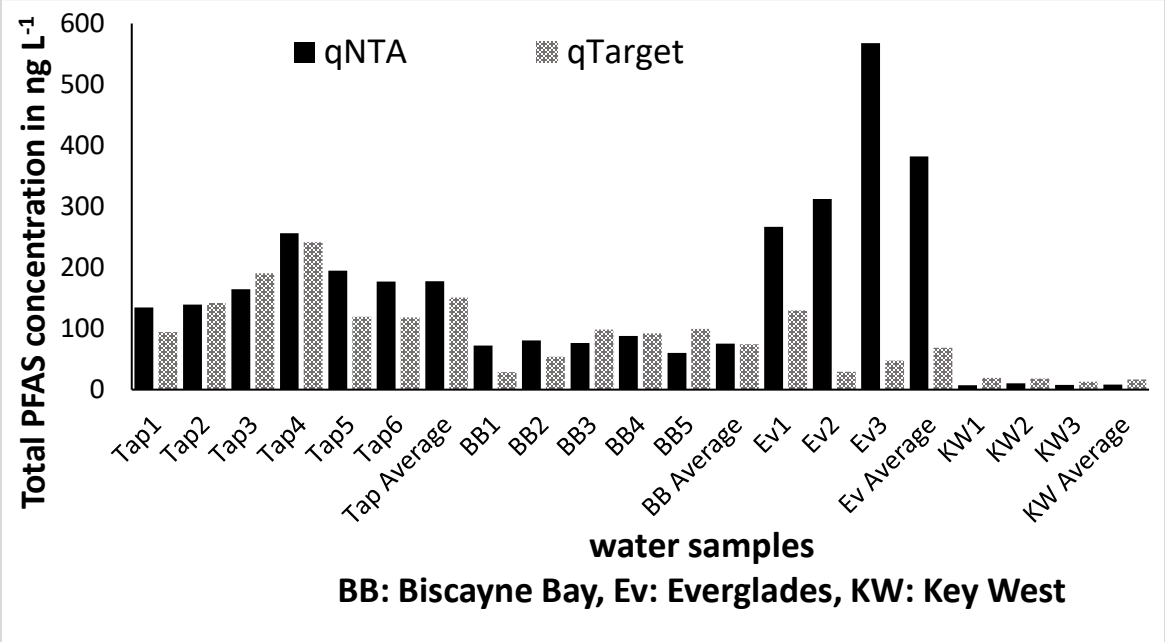

Figure 4.10 Estimated total PFAS concentrations of water samples using a global calibration of 30 NS (qNTA) and concentrations of the samples using target analysis developed previously in Li et.al.

The results from qNTA and target analysis are in the same order of magnitude or most samples from Tap water, Biscayne Bay, and Key west samples, with the difference within the range of -39.52 to 75.50 ng/L. In general, the total PFAS concentration obtained from qNTA is higher than that calculated by target analysis, which represents only the sum of 30 PFAS. This was expected

since NTA incorporates much more species that were not quantifiable by the latter. However, it has to be taken into consideration the limitations of this approach which introduce errors (in some cases higher than 100%) coming from using the global calibration curve, therefore contributing to one to two orders of magnitude higher or lower than the actual value. Another limitation of this NTA approach that could have contributed to errors in the estimates is that many PFAS that were able to be detected in target analysis may not be detected in NTA due to their low concentrations in the environmental samples and the higher detection limits of the NTA (whereas detection limits of the target analysis were as low as 0.01-0.35 ng/L. It was surprising that in the samples from the Everglades adjacent canals, PFAS total concentration estimated by qNTA is 2-12 fold higher than what was previously reported by target analysis, which could suggest a more complex composition of PFAS in higher concentration, which was neglected by the target analysis. Further investigation should focus on the potential sources of PFAS that could have contributed to this substantially elevated PFAS concentration revealed by NTA in the Everglades area.

4.3 **Acknowledgment**

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Chapter 5. Conclusions

Numerous scientific gaps have been noted in PFAS research, especially in the occurrence, distribution, and sources of these pollutants in Florida environments. A sensitive and reliable method based on SPE followed by LC-MS/MS analysis has been successfully developed and validated, then further applied for the assessment of legacy and emerging PFAS in surface waters from Biscayne Bay and adjacent canals and in tap waters from the most populated counties in South Florida (Miami-Dade, Broward, and Palm Beach Counties). The most frequent and predominant PFAS detected in surface water and tap water were PFBA, PFBS, PFPeA, PFHxA, PFHxS, PFOA, and PFOS, with tap water having higher average PFAS concentrations (86 ng L-1) than in surface waters (46 ng L-1), which raise questions on PFAS sources and occurrence in the Everglades ecosystem, one of the major sources of Florida aquifers that supply drinking water for eight million Floridians, highlighting the need for future investigations.

Concentrations of PFOA and PFOS in more than 50% of the tap water found in this study were above the new health advisory levels revised by the US EPA in 2022 (4 parts per quadrillion for PFOA and 20 parts per quadrillion for PFOS). The levels found in tap water for PFOS, PFOA, in some locations in this study could represent potential human health effects and requires further monitoring. The highest PFAS concentrations in tap water were found in the closest location to MIA airport, suggesting a potential pollution source. In surface water, the highest PFAS level was found in the Biscayne Canal C-8 (average of 106 ng L^{-1}), which has shown also in previous studies the highest levels of wastewater tracers and other endocrine disruptor chemicals, corroborating anthropogenic sources of wastewater intrusion pollution. Even though PFAS composition and concentrations varied in different countries and within the US, PFAS levels in Biscayne Bay and adjacent canals waters and South Florida tap waters are similar or higher than concentrations found in Europe, Brazil, and China, while in the same order of magnitude than the PFAS levels

detected in other states. PFAS levels found in tap water indicated a maximum HI of 4, suggesting a high human health risk in the worst-case scenario. Nevertheless, these results should be carefully interpreted due to the lack of available data in the literature on the reference dose for most PFAS, while assumptions based on existing reference doses (e.g., PFOA reference dose was used for all PFCA compounds) were made to estimate HI in this study.

Overall, this study brings new knowledge on PFAS occurrence and distribution in selected South Florida environments, offering an insight into the current state of our drinking water and waterways, and providing needed information to the public and regional government agencies on water quality, which hopefully can facilitate the development of guidelines and innovative water treatment procedures for removal or reduction of PFAS, especially in areas where levels could potentially have a greater environmental concern.

This study has investigated PFAS occurrence, concentration, composition, spatial and seasonal distribution, and potential sources in tap water and surface water in Central and South Florida environments. For tap waters, PFAS concentrations showed the highest on the East coast of South Florida (mean: 83.0 ng L^{-1}), followed by the West Coast of South Florida (mean: 14.4 ng L^{-1}), and Central Florida (mean: 8.0 ng L^{-1}). It could be associated with population and related human activities that have a potential impact on the quality of groundwater as drinking water source through the water cycle. PFOS, PFPeA, PFHpA, PFHxA, PFHxS, PFBA, and PFBS are found predominant in tap water samples. Studies on PFAS occurrence and levels in Floridian aquifers (such as Biscayne Bay aquifers), groundwater, and surface water sources used for drinking purposes are still needed to further elucidate the source of PFAS in the drinking water supply. In surface water, higher

PFAS concentrations (> 60 ng/L) are mostly observed from polluted rivers or coastal estuaries in Biscayne Bay, and sites collected nearby point sources (military airbases, WWTPs, airports, etc.). Predominant PFAS in surface waters were very similar to the ones observed in tap waters and included PFOS, PFPeA, PFHxA, PFBA, PFOA, PFHpA, and PFHxS, which levels followed the trend Biscayne Bay > Tampa Bay > ENP canals > Key West. Though the levels of PFOA (up to 22.9 ng L^{-1}) and PFOS, (up to 25.7 ng L^{-1}) found in this study are below the provisional surface water screening level for both fresh water and saltwater systems set up by FL DEP for the protection of human health from consumption of freshwater and estuarine finfish and shellfish, they are above most of strict thresholds recommended in Europe, Australia and New Zealand (0.23 to 23 ng L^{-1} for PFOS) for the purpose of protecting aquatic biota. The levels reported here constitute a potential ecological risk to coral reefs, early life stages of aquatic organisms, and other sensitive species inhabiting Florida. Other PFAS frequently found in the aquatic environment should be taken into consideration in further toxicology studies to better understand PFAS synergetic effects on aquatic wildlife, allowing a more comprehensive and sensitive assessment of ecological risks. Principal component analysis (PCA) was able to identify similarities in potential sources of PFAS that have strong correlations or show geographic clustering, which can be a powerful tool for investigating the fate and source of PFAS.

A NTA workflow for the screening of potential emerging PFAS, degradants, and transformation products was developed and optimized. The method was validated using a mixture of 30 PFAS native standards as QC samples. The optimized data post-processing was able to reduce falsely identified compounds (false positive) with precision (0.38) and

accuracy (0.97). When the workflow was applied for the screening of PFAS species in environmental samples, a total of over 500 PFAS were tentatively identified in selected drinking water samples (N=6) from populated counties in South Florida, as well as in surface waters (N=9) from Biscayne Bay, Key west, and Everglades canals. Major species (highest peak intensity and present in more than 2 samples from the same source) were identified for each source: chlorinated PFAS and PFBS in tap water, 7-(heptafluoropropyl)-4,9-dimethoxy-5H-furo[3,2-g][1]benzopyran-5-one and 2-[Chloro(difluoro)methoxy]-1,1,2,2-tetrafluoroethanesulfonic acid in Biscayne Bay, 6:2 FTS and 5-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)-1H-pyrimidine-2,4-dione in Everglades, and 3,3,3',3'-tetrakis(trifluoromethyl)-1,1'-spirobi[2,1-benzoxaphosphol-1-ium] and 6:2 FTS in Key West.

Data post-processing using Compound Discoverer (CD, a total of 481 feature detected) for the NTA screening of PFAS species yielded a greater number of PFAS species than FluoroMatch (FM, 130 features), which could be an indication that Compound Discoverer may be better suited for the detection of a wider range of PFAS. The results showed tap water containing the greatest number of PFAS species (CD=271 features from Compound Discoverer-CD; and FM= 55 features from FluoroMatch), followed by Everglades (CD=141; FM=22) and Biscayne Bay (CD=53; FM=32), with Key West (CD=16; FM=21) having the least amount. It was found that tap water and the surface water from the Everglades canal have the greatest overlap (CD=50; FM=18), possibly due to drinking water in South Florida being supplied by groundwater sources including the Everglades aquifers. A semi-quantitation method for NTA (qNTA) utilizing a global calibration curve on existing native and labeled internal standards was explored and applied to

environmental samples to estimate the total PFAS concentration. Variations of systemic overestimation and underestimation were evaluated with a mixture of 30 PFAS native standards. The high variability (% error up to 150%) is caused by using a global calibration curve for PFAS with very diverse chemical structures in which the ionization efficiency and chromatography are quite different from one to another. For most samples, the concentration from qNTA is substantially higher than in target analysis since NTA incorporates much more species that were not measured and quantified by the latter. Especially it was found that total PFAS concentration from qNTA in Everglades adjacent canals samples is 2-12 fold higher than from the previously reported target analysis, suggesting potential contamination sources coming from the Everglades area that needs to be further explored. Overall, NTA can provide complementary information on PFAS species in environmental samples, which is needed to better evaluate their toxicological and potential impacts.

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