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Determination of best analytical methods for per and polyfluoroalkyl substances (PFASs) in non-drinking water Matrices

by

Nazifa Nusrat Ahmed

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The members of the committee appointed to examine the thesis of NAZIFA NUSRAT AHMED find it satisfactory and recommend that it be accepted.

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List of Abbreviations

AFFF	Aqueous film-forming foams
CE	Collision cell
Cl-PFESA	Clorinated perfluoroether sulphonate
CTD	Conductivity-temperature-depth
ECF	Electrochemical fluorination
EI	Electron ionization
DIW	De ionized water
diPAP	Fluorotelomer phosphate diester
DNA	Deoxyribo Nucleic Acid
EIC	Extracted ion chromatogram
ENVI	Environment for Visualizing Images
EtFOSAA	Ethyl perfluorooctane sulphonamidoacetate
FETAX	Frog Embryo Teratogenicity Assay-Xenopus
FOSAA	Perfluorooctane sulfonamidoacetate
FRB	Field Reagent Blanks
FTs	Fluorotelomers
FTAB	Fluorotelomer sulphonamide alkylbetaine
FTCA	Fluorotelomer (saturated) Carboxylate
FTUCA	Fluorotelomer (unsaturated) Carboxylate
FTOH	Flurotelomer alcohol
FTSAs	Fluorotelomer Sulphonate
FtSaAM	Fluorotelomer sulphonamide propyl N.N dimethylamine

FtToAS	Fluorotelomer Thio Ether Amido Sulfonate
GC–MS	Gas chromatography mass spectrometry
HDPE	High density poly ethylene
HPLC	High Performance Liquid Chromatography
IS	Internal standard
LOD	Limit of detection
LC-MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
LC-QTOF	Liquid chromatography-quadrupole time of flight
LOQ	Limit of quantification
L-PFOS	Sodium perfluoro-1-octanesulfonate
L-PFDS	Sodium perfluoro-1-decanesulfonate
MeFOSAA	Methyl perfluorooctane sulphonamidoacetate
MPFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid
MTBE	Methyl-t-butyl ether
N-EtFOSE	N-ethyl perfluorooctane sulfonamido ethanol
N-MeFOSE	N-methyl perfluorooctane sulfonamido ethanol
NSAID	Non-Steroidal Anti Inflammatory Drug
NASF	North Atlantic Seafood Forum
PFAAs	Perfluoroalkyl acids
PFASs	Per- and polyfluoroalkyl substances
PFCAs	Perfluorocarboxylic acids
PFDA	Perfluorodecanoic acid
PFOA	Perfluorooctanoic acid

PFOS	Perfluorooctane sulphonate
PFPiA	Bis (perfluorohexyl) phosphinate
PFODA	Perfluoro-n-dodecanoate
PFSAs	Perfluoroalkyl sulphonic acids
PFTrDA	Perfluorotridecanoate
PFUnDA	Perfluoroundecanoate
PFTeDA	Perfluorotetradecanoate
PP	Polypropylene
PUF	Polyurethane Foam
QC	Quality Control
RSD	Relative Standard Deviation
RT	Retention Time
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
%R	Recovery Efficiency or Percent Recovery
SPE	Solid Phase Extraction
TBAHS	Tetra butyl ammonium hydrogen sulfate
TFA	Trifluoroacetic Acid
TOPAM	Total Oxidizable Precursor Assay Method
TQ-MS	Triple quadruple mass spectrometer
UNEP	United Nation Environment Programme
UPW	Ultra pure water
USEPA	United State Environmental Protection Agency

Determination of best analytical methods for per and polyfluoroalkyl substances (PFASs) in non-drinking water Matrices

Thesis Abstract-Idaho State University (2021)

Per- and polyfluoroalkyl substances (PFASs) are a group of anthropogenic organic fluorinated compounds that have been widely used in various industrial, commercial, and consumer products, such as carpet protector sprays, food containers, cosmetics, and firefighting foam. Many studies exsit ondifferent methods of detection and quantification of PFAS in drinking water.U.S. EPA has approved a method for detecting and quantifying of PFAS in drinking water matrices (Method 537.1). However there is no approved method for analyzing non-drinking water matrices.(e.g., surface water, subsurface water, wastewater, sludge, air, sediment, biosolids, soil and biota). These non-drinking water matrices are important they are related to food chain affecting wildlife and humans. Surface, subsurface water, and treated wastewater are used as the sources of drinking water. Air is directly inhaled by all animals, human and plants. Fish or biota is directly consumed by human and soil, sediment, sludge and biosolids are related to plants which ultimately related to food chain and consumer product of human. For these reasons, analysis of non-drinking water matrices is needed. So the present study has performed a review of papers on Web Science and Google Scholar published from January 2000 to April 2020 regarding detection and quantification method of PFAS on non-drinking water matrices. After the evaluation of the analytical methods, the best methods currently available for non-drinking water matrices were selected.

Key words: Per- and polyfluoroalkyl substances, Environmental Protection Agency (EPA).

Chapter 1: Introduction

1.1 Background

Poly- and perfluoroalkyl substances (PFASs) have been produced in the past 60 years, and used in many consumer products, such as surface protectors in fast food containers, surface tension lowering agents in fire-fighting foams, water-proof fabric, carpets, greaseproof paper, stain-resistant coatings, metal plating paints, pesticides, fluoropolymers in the semi-conductor and aviation industries [Coggan et al. 2019; Boiteux et al. 2016]. Because most PFAS are resistant to degradation in the environment, they are omnipresent in water, soil, air, food, wildlife, and humans, and have the potential to cause adverse impacts on the exposed organisms. PFAS are a group of emerging man-made pollutants challenging water sectors for their practices such as recycling/reuse and discharges to the environment [Coggan et al. 2019].

It has been shown that short-chain PFAS can be absorbed into the livers more readily with longer chain PFAS, and concentrated in blood proteins [Banzhaf et al. 2016]. Some of the long-chain PFAS ($C \geq 8$) are bio-accumulative and toxic, thus they are included in the national and international regulations [Zhao et al. 2017]. Perfluorooctane sulfonic acid (PFOS) and its related compounds were added to the Stockholm Convention's Annex B in 2009. Since then, their production and use decreased significantly [UNEP 2009]. Major global manufacturers of perfluorooctanoic acid (PFOA) and its precursors promised to voluntarily cease their productions by 2015 [USEPA 2006]. However, the release of PFAS has been continuing in other means, for example, the productions of irreplaceable homologues, the consumption of stockpiles, and emissions from previously-sold commercial products [Zhao et al. 2017]. These practices have resulted in unacceptably high PFAS levels in some areas. In the evaluation of PFAS such as their

toxicity and health effects, sources and exposure pathways, environmental impacts, treatment technologies and remediation of contaminated sites, scientists and engineers rely heavily on the detection and quantification levels of the analytical methods. One of the challenges related to the detection and quantification is the diversity of PFAS including numerous degradation byproducts.

Over twentyone years (2000 to 2020), many researchers have been developed analytical methods to identify and quantify or measure concentration of PFAS in drinking water and non-water matrices. Now one of the important questions comes forward, how accurate these concentrations or their methods performance are. Answering this question is the objectives of this study. Evaluation of the analytical methods can be done by analyzing the method performance in terms of limit of detection (LOD), limit of quantification (LOQ), and recovery efficiency (%R) [Karnes and March 1993]. LOD and LOQ indicate quality control and quality assurance measure of the method. The percent recovery (%R) close to 100% indicates nearly complete extraction, minimal losses, and good alignment between spiking and calibration solution [Karnes and March 1993]. Currently, there is an analytical method for PFAS (Method 537.1) approved by EPA. However, this method is approved only for the analysis of PFAS in drinking water. Consequently, researchers have developed their own methods to measure PFAS concentrations in non-drinking matrices (surface, subsurface, wastewater, sludge, soil, sediment, air, biota, and biosolids) for the purposes of screening and studying. Detection and identification of PFAS is critical for successful investigations. This study has attempted to identify the best methods currently available for the analysis of PFAS and their byproducts in non-drinking matrices.

1.2 Objectives

The main objectives of the present study are as follows:

1. By reviewing previous work published for the last 20 years, summarize and compile the analytical methods for PFASs in non-drinking water matrices including surface water, subsurface water, wastewater, sludge, biosolids, sediment, soil, air, and biota.
2. Identify the best analytical methods currently available for PFAS in non-drinking water matrices by comparing their performance evaluation parameters such as limit of detection (LOD), limit of quantification (LOQ), and percent recovery (%R).
3. Propose recommended methods for the analysis of PFAS in surface water, subsurface water, wastewater, sludge, biosolids, sediment, soil, air, and biota.

Chapter 2: Literature review

The focus of this study is to conduct a literature review to compile and compare methods that have been developed and used by previous researchers.

An extensive literature review on analytical methods, focusing on detection and quantification, was performed for PFAS in non-drinking water matrices. The papers reviewed in this study are those published from January 2000 to April 2020, and identified by literature search using Web of Science and Google scholar. The non-drinking water matrices include surface water, subsurface water, wastewater, sludge, biosolids, sediment, soil, air and biota. This effort found the following literatures:

1) Twelve journal articles on surface water analysis; i.e., Moody et al. [2001], Hansen et al. [2002], Yamashita et al. [2004], Cai et al. [2012], Boiteux et al. [2016], Zhao et al. [2016], Yeung et al. [2017], Zhao et al. [2017], Pan et al. [2019],Wang et al. [2019],Lee et al. [2020], and Hung et al. [2020].
2) Eight journal articles on the analytical methods for subsurface water; i.e., Schultz et al. [2004], Houtz et al. [2013], Backe et al. [2013], Anderson et al. [2016], Boiteux et al. [2016], Weber et al. [2017], Szabo et al. [2018], and Hepburn et al. [2019].
3) Eleven articles on the analytical methods for waste water; i.e., Sinclair and Kannan [2006], Schultz et al. [2006], Guo et al. [2010], Chen et al. [2012], Zhang et al. [2013], Houtz et al. [2016], Dimzon et al. [2017], Eriksson et al. [2017], Houtz et al. [2018], Dauchy et al. [2018], and Coggan et al. [2019].

4) Seven journal articles on the analytical methods for sludge; i.e., Higgins et al. [2005], Li et al. [2009], Sindiku et al. [2013], Chen et al [2012], Boiteux et al. [2016], Eriksson et al. [2017], and Coggan et al. [2019].
5) Eight journal publications on the analytical methods for soil; i.e., Li et al. [2009], Washington et al. [2010], Houtz et al. [2013], Xiao et al. [2015], Meng et al. [2015], Anderson et al. [2016], Boiteux et al. [2016], and Lee et al. [2019].
6) Nine journal articles on the analytical methods for sediment; i.e., Higgins et al. [2005], Li et al. [2009], Zhao et al. [2016], Anderson et al. [2016], Boiteux et al. [2016],Munoz et al. [2017], Wang et al. [2019], Lee et al. [2019], and Hung et al. [2020].
7) Four articles on the analytical methods for air; i.e., Vento et al. [2012], Zhao et al. [2017], Dimzon et al. [2017], and Lee et al. [2019].
8) Three journal papers on the analytical methods for biota; i.e., Munoz et al. [2017], Lee et al. [2019], and Hung et al. [2020].
9) Two journal articles on the analytical methods for biosolids; i.e., Chen et al. [2012], Venkatesan, and Halden [2013].

In the early years, scientists have had major challenges with the analysis of PFAS in environmental samples [Benzhaf et al. 2016]. The analytical methods were less technical, stable isotopically labeled internal standards were lacking, and the techniques such as liquid chromatography couple with tandem mass spectrometry (first generation LC-MS/MS) were not very sensitive [Benzhaf et al. 2016]. For those reasons, the studies in the early 2000s reported mainly two types of PFAS; that is, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) [Benzhaf et al. 2016]. Over the last 21 years, the PFAS detection technology has made huge advances [Benzhaf

et al. 2016]. Today, numerous stable isotopically labeled internal standards are commercially available, and second generation mass spectrometry have lowered instrument's detection limits from µg/L to ng/L [Benzhaf et al. 2016]. The third generation instruments can reach the detection limits to pg/L, more than three orders of magnitude sensitive in response [Benzhaf et al. 2016]. Furthermore, the recovery efficiency has increased from a first generation instrument with a range of 10 - 12% to the range of 90 - 100% by the third generation liquid chromatography couple with tandem mass spectrometry (LC-MS/MS) instrument. The introduction of liquid chromatography/ion mobility - quadrupole time-of-flight mass spectrometry (LC/IM-QTOF-MS) has made the PFAS analysis more practical. The current LC/IM-QTOF-MS instrument is capable of detecting non-targeted PFAS and their precursors [Yukioka et al. 2020].

As of today, little attempt has been made to review and comprehensively compare the analytical methods for PFAS and their byproducts in non-drinking water matrices. In an attempt to identify best analytical method currently available for PFAS, the present study evaluated the published analytical methods in terms of the detection limits such as limit of detection (LOD) and limit of quantification (LOQ), recovery efficiency (%R), detectability (detection of degradation byproducts and related species such as homologs), if such data were reported.

In addition to the analytical methods, this study reviewed the PFAS characteristics, toxicity and health effects, sources and pathways in the environment.

2.1 Characteristics of PFAS

PFAS are characterized by a hydrophobic alkyl chain with varying length (typically C4–C16), in which all or most of the carbon–hydrogen (C-H) bonds are replaced by carbon–fluorine (C-F) bonds [Boiteux et. al. 2016]. The C-F part forms a hydrophilic end group and the hydrophilic part defines the type of PFAS. Perfluoroalkyl acids (PFAAs), including perfluorocarboxylic acids (PFCAs) and perfluoroalkyl sulphonic acids (PFSAs), are the most widely studied PFAS due to their unique physicochemical properties including thermal and chemical stability [Boiteux et. al. 2016]. The properties and fate of PFAS are closely related to their chain length (C_nF_{2n+1}). The short-chain PFAS are defined as $n < 6$ for perfluorosulfonic acids (PFSAs) and $n < 7$ for perfluorocarboxylic acids (PFCAs) [Zhao et al. 2016]. The short-chain PFAS have been detected and even spread more widely than long-chain PFAS in some areas [Zhao et al. 2016].

Fluorotelomers (FTs) are a subgroup of PFAS and are partially fluorinated. In FTs such as fluorotelomer sulfonic acid (FTSA), fluorotelomer saturated carboxylic acid (FTCA), or fluorotelomer unsaturated carboxylic acid (FTUCA), a small C-H chain (generally two carbons) is linking the perfluorinated carbon chain to a functional group [Boiteux et al. 2016]. FTs are used in many industrial applications such as surfactants or surface protection products [Boiteux et al. 2016]. In 4:2 fluorotelomer sulfonic acid (4:2 FTSA), 6:2 fluorotelomer sulfonic acid (6:2 FTSA) and 8:2 fluorotelomer sulfonic acid (8:2 FTSA), two of the carbons in the tail are not fully fluorinated, while the remaining carbons are fully fluorinated [Mueller and Yingying 2017]. Note that the “n:x” is a naming convention where “n” is the number of fully fluorinated carbons (in the above case, 4, 6 and 8) and “x” is the number of carbons that are not fully fluorinated.

The characteristics of PFAS can be found in the Pub Chem (https://pubchem.ncbi.nlm.nib.gov). The Pub Chem is a database of chemical molecules and chemical activities in biological assays. The system is maintained by the National Center for Biotechnology Information, the National Library of Medicine, USA National Institutes of Health. The characteristics of PFAS investigated in this study are presented in Table 1.

Table 1: Characteristics of per-polyfluoroalkyl substances investigated in this study.

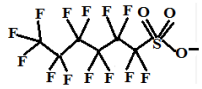
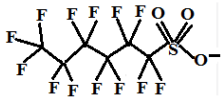
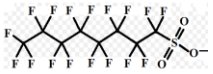
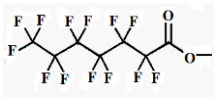
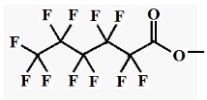
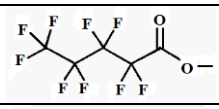
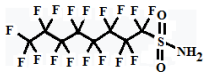
Name	Acronyms	Structure	Characteristics
Perfluorobutane sulphonate	PFBS		A chemical compound with a four carbon fluorocarbon chain and a sulfonic acid functional group (Pub Chem). As an anion it characterizes as a stable fluorosurfactant because of the strength of carbon–fluorine bonds (Pub Chem).
Perfluorohexane sulphonate	PFHxS		A perfluoroalkane sulphonic acid conjugate base that is hexane-1-sulfonic acid in which all thirteen of the hydrogens that are attached to carbons have been replaced by fluorines which are characterized as firefighting foam surfactants and metal plating chemical (Pub Chem).
Perfluorooctane sulphonate	PFOS		Characterized as an anthropogenic fluorosurfactant and global pollutant (Pub Chem).
Perfluorodecane sulphonate	PFDS		Characterized as cleaning-washing product for industrial and commercial purpose (Pub Chem).
Perfluoro heptanoate	PFHpA		A fluoroalkanoic acid conjugate base and is characterized as a xenobiotic (a chemical substance found within an organism that is not naturally produced or expected to be present within the organism) and an environmental contaminant (Pub Chem).
Perfluoro hexanoate	PFHxA		A monocarboxylic acid conjugate base and is characterized as an environmental contaminant and a xenobiotic compound (Pub Chem).
Perfluoro pentanoate	PFPeA		A short-chain perfluorocarboxylic acid (PFCA) conjugate base generally characterizes as an industrial surfactant and surface protector (Pub Chem).
Perfluoro octanoate	PFOA		A conjugate base which characterizes as an environmental contaminant, a xenobiotic, a carcinogenic agent, a surfactant and an endocrine disruptor (Pub Chem).
Perfluoro nonanoate	PFNA		Characterized as surfactant for the production of the fluoropolymers, polyvinylidene fluoride (Pub Chem). It is produced mainly in Japan by the oxidation of a linear fluorotelomer olefin mixture (Pub Chem).
Perfluoro decanoate	PFDA		A conjugate base that characterizes as a xenobiotic and an environmental contaminant (Pub Chem).
Perfluoro octane sulfonamide	PFOSA		Characterized as a persistent organic pollutant, and the compound used to repel grease and water in food packaging along with other consumer applications (Pub Chem).

Table 1: Continued

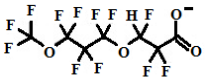
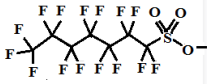
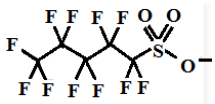
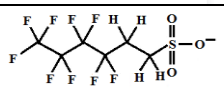
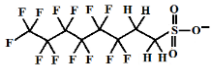
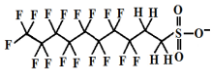
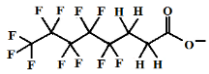
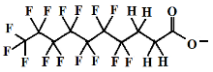
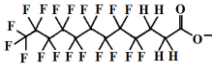
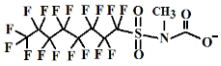
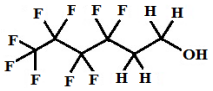
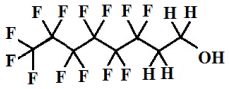
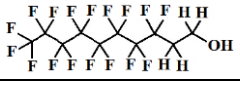
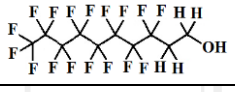
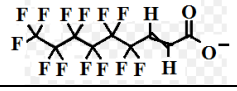
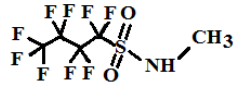
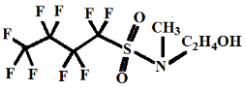
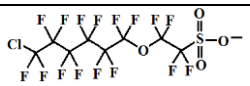
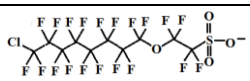
Name	Acronyms	Structure	Characteristics
Perfluoro dodecanoate	PFDoDA		It characterizes as a highly persistent, bioaccumulative breakdown product of stain- and grease-proof coatings on food packaging, soft furnishings, and carpets (Pub Chem).
Carbazochrome sodium sulphonate	ADONA		Characterized as a hemostatic) (a hemostat is used to bluntly tunnel through the abdominal musculature and subcutaneous tissue, exiting the skin lateral to the laparotomy) agent that promotes clotting, preventing blood loss from open wounds (Pub Chem).
Perfluoro heptane sulphonate	PFHpS		This compound characterizes as involving in ionic interaction, that is ion-exchange, ion-pair, ion-suppression or ion-exclusion reaction (Pub Chem).
Perfluoro pentane sulphonate	PFPeS		It is characterized as non-central analgesic, antipyretic or antiinflammatory agents, e.g antirheumatic agents; Non-steroidal antiinflammatory drugs (NSAIDs) (Pub Chem).
4:2 fluorotelomer sulphonate	4:2 FTSA		Characterized as surfactant and environmental transformation products (Mueller and Yingling 2017).
6:2 fluorotelomer sulphonate	6:2 FTSA		Characterized as surfactant and environmental transformation products (Mueller and Yingling 2017).
8:2 fluorotelomer sulphonate	8:2 FTSA		Characterized as surfactant and environmental transformation products (Mueller and Yingling 2017).
6:2 fluorotelomer carboxylate	6:2 FTCA		Characterized as thermally and chemically stable with low reactivity (Mueller and Yingling 2017).
8:2 fluorotelomer carboxylate	8:2 FTCA		Characterized as thermally and chemically stable with low reactivity (Mueller and Yingling 2017).
10:2 fluorotelomer carboxylate	10:2 FTCA		Characterized as thermally and chemically stable with low reactivity (Mueller and Yingling 2017).
N-Methyl perfluorooctane sulphonamido acetate	N-MeFOSAA		Characterized as intermediate environmental transformation product (Mueller and Yingling 2017).
4:2 fluorotelomer alcohol	4:2 FTOH		Characterized as major raw material for surfactant and surface protection products (Mueller and Yingling 2017).
6:2 fluorotelomer alcohol	6:2 FTOH		Characterized as major raw material for surfactant and surface protection products (Mueller and Yingling 2017).
8:2 fluorotelomer alcohol	8:2 FTOH		Characterized as major raw material for surfactant and surface protection products (Mueller and Yingling 2017).
10:2 Fluorotelomer alcohol	10:2 FTOH		Characterized as major raw material for surfactant and surface protection products (Mueller and Yingling 2017).
6:2 fluorotelomer unsaturated carboxylate	6:2 FTUCA		Characterized as metabolites or degradation products from FTOH exposure (Butt et al. 2013).

Table 1: Continued

Name	Acronyms	Structure	Characteristics
8:2 fluorotelomer unsaturated carboxylate	8:2 FTUCA		Characterized as metabolites or degradation products from FTOH exposure (Butt et al. 2013).
19:2 fluorotelomer unsaturated carboxylate	19:2 FTUCA		Characterized as metabolites or degradation products from FTOH exposure (Butt et al. 2013).
N-Methyl perfluorobutane sulphonamide	*N*-MeFBSA		It is a byproduct of N-MeFBSE and the production of this compound exposes a mechanism by which N-MeFBSE contribute to the burden of perfluorinated contamination in remote location (D'eon et al. 2006)
N-Methyl perfluorobutane sulphonamidoethanol	*N*-MeFBSE		It is a parent compound of N-MeFBSA, PFBA, PFPrA, PFBS and trifluoroacetic acid. Anthropogenic production of this compound contributed to the ubiquity of perfluoroalkyl sulfonate and carboxylate compound in the environment (D'eon et al. 2006)
6:2 Clorinated perfluorinated ether sulphonate	6:2 Cl-PFESA		Characterized as it exhibits higher activity towards peroxisome (a small organelle present in the cytoplasm of many cells, which contains the reducing enzyme catalase and usually some oxidases) proliferator-activated receptors (are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes) signaling pathways than perfluorooctane sulfonates (Li et al. 2018).
8:2 Clorinated perfluorinated ether sulfonate	8:2 Cl-PFESA		Characterized as it exhibits higher activity towards peroxisome (a small organelle present in the cytoplasm of many cells, which contains the reducing enzyme catalase and usually some oxidases) proliferator-activated receptors (are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes) signaling pathways than perfluorooctane sulfonates (Li et al. 2018).
N-Methyl perfluorooctane sulfonamide ethanol	*N*-MeFOSE		Characterized as it is a fluorocarbon derivative and a perfluorinated compound ,having an eight-carbon chain and a terminal sulfonamide functional group, which is used as a building material, wood preservatives (Pub Chem).
N-ethyl perfluorooctane sulfonamide ethanol	*N*-EtFOSE		Characterized as it is a monomer used in the aqueous treatment mass for building walls, ceiling or floor and is a typical precursor of perfluorooctane sulfonate (PFOS) (Pub Chem).

In this present study, literature review is performed for the paper published from January 2000 through April 2020 and found using Web of Science and Google Scholar. The characteristics of the following compounds were not available at the time when this literature review was conducted: PFUnDA, PFTrDA, PFTeDA, FOSA, PFPeS, PFHxDA, PFODA, 5:3 ACID, 4:2 diPAP, 6:2 diPAP, 8:2 diPAP, C6/C6 PFPiA,C6/C8 PFPiA, C8/C8 PFPiA,6:2 FTAB, 6:2 FtSaAM, L-PFBS, L-PFHxS, L-PFHpS, L-PFOS, and L-PFDS.

2.2 Toxicity and Health effects of PFAS

PFAS are highly recalcitrant and could persist for a long period of time in the environment and a human body (e.g., human serum, milk, and tissues) [Li et al. 2009]. Certain type of PFAS, such as perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids (PFSA) are likely toxic, bioaccumulate, and pose adverse effects on human health [Li et al. 2009]. Survey on mortality in USA caused by PFAS contamination present that, 5.7% mortality is due to heart/cerebro vascular diseases and 4.5% mortality is due to all types of cancer and 12% mortality by renal, hepatic and pulmonary failure [Fry and Power 2017]. Another survey on mortality in Italy found that 6.9% mortality cause by hepatic cancer, 5.6% mortality by renal cancer, 5.7% mortality by pancreatic cancer [Mastrantonio et al. 2017].

Epidemiological studies have revealed associations between exposure to specific PFAS and a variety of health effects, including altered immune and thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, adverse reproductive and developmental outcomes, and cancer [Fenton et al, 2020]. In a single study, modest down-regulation of C-reactive protein response, a marker of human systemic inflammation, was also reported to be associated with perfluorooctanoic acid (PFOA) blood levels [Fenton et al, 2020]. A pregnancy cohort study prospectively detected increased risk of airway and throat infections and diarrhea in children through age 10 yr, correlated with cord-blood PFAS measurements [Fenton et al, 2020]. Immunological study on human body provides strong evidence that PFAS exposure can suppress the human immune response [Fenton et al, 2020].

Selected PFAS

Various human studies have examined possible relationships between the perfluorobutane sulphonate (PFBS) exposure and the potential health outcomes such as alteration of menstruation, reproductive hormones or semen parameters, kidney function (uric acid production), lung function (induction of asthma), and lipid profile [EPA-823-R-18-307 Public Comment Draft in 2018]. In short-term exposures, perfluorohexane sulphonate (PFHxS) has the potential to stay in a human body for an extended period of time [Persistent Organic Pollutants Review Committee, 2011]. PFHxS is more potent for the endpoints (gosner stage development) and (snout-vent length) at 40 days [Persistent Organic Pollutants Review Committee, 2011]. The sublethal effects have been shown by PFHxS on amphibians embryo at present environmental levels [Persistent Organic Pollutants Review Committee, 2011]. A study with the African clawed frog tadpoles (*Xenopus laevis*) indicated possible endocrine disruption [Persistent Organic Pollutants Review Committee, 2011]. The exposure of perfluorooctane sulfonate (PFOS) to animal and human bodies caused hepatotoxicity, neurotoxicity, reproductive toxicity, immune toxicity, thyroid disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity in laboratory animals and many in vitro human systems [Zeng et al. 2019]. In a 90-day rat study, perfluorodecane sulfonate (PFDS) produced hyperplasia of the medullary and papillary tubular and ductal epithelial cells in the inner medullary region at 600 mg/kg/day, but it did not produce the adverse effects at 200 mg/kg/day [Appendix I – PFAS Toxicity Profiles – Department of Defense, 2019]. An initial frog embryo teratogenicity assay-Xenopus (FETAX) assay identified perfluorohexanoate (PFHxA) and perfluoroheptanoate (PFHpA) are potential teratogens (an agent that causes embryo malformation) and developmental toxicants [Kim et al. 2015]. Following the exposure to PFHxA or PFHpA, severe defects in the liver and heart of amphibian embryo have been found by the whole mount

in situ hybridization, reverse transcriptase polymerase chain reaction (RT-PCR), and histologic analyses [Kim et al. 2015]. Perfluorooctanoate (PFOA) is not lipophilic and not metabolized in an animal body. Although PFOA is not directly genotoxic, animal data indicate that it can cause several types of tumors and neonatal death and may have toxic effects on the immune, liver, and endocrine systems [Steenland et al. 2010]. Note that, in genetics, genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer [Steenland et al. 2010].

Perfluorononanoate (PFNA) and perfluorodecanoate (PFDA)

The toxicity of perfluorononanoate (PFNA) was evaluated using the 48-h acute toxicity test with *Daphnia magna* (water fleas) [Lu et al. 2015]. In their study, PFNA inhibited both growth and reproduction of *Daphnia magna*. Perfluorodecanoate (PFDA) is a non-carcinogenic compound (not listed by International Agency for Research on Cancer) but a potentially toxic compound [Pub Chem]. PFDA are thought to be endocrine disruptors [Pub Chem].

Perfluorooctane sulphonamide (PFOSA), perfluorododecanoate (PFDoDA), and carbazochrome sodium sulphate (ADONA)

Perfluorooctane sulphonamide (PFOSA) is a potential mitochondrial toxicant that causes significant inhibition of DNA synthesis [Slotkin et al. 2008]. Mitochondria are a semiautonomous double-membrane-bound organelle found in most eukaryotic organisms. Vaccine containing perfluorododecanoate (PFDoDA) decrease antibody and increased risk of early menopause

[Agency for Toxic Substances and Disease Registry (ATSDR), 2014]. Carbazochrome sodium sulphate (ADONA) causes ptosis of eyes, somnolence (sleepiness) due to depression, gastrointestinal hypermotility and diarrhea [Pub Chem]. Ptosis is the drooping of the upper eyelid due to paralysis or disease, or as a congenital condition.

Perfluoroheptane sulphonate (PFHpS) and perfluoropentane sulphonate (PFPeS)

Perfluoroheptane sulphonate (PFHpS) has acute oral toxicity if swallowed and causes acute dermal toxicity in contact with skin, severe skin burns and eye damage and acute inhalation toxicity if inhaled [Pub Chem]. Perfluoropentane sulphonate (PFPeS) is not a highly toxic compound like PFOS, PFOA, and PFHxS. According to Pub Chem, PFPeS is a non-central analgesic, anti-pyretic, or anti-inflammatory agent; e.g., anti-rheumatic agents, non-steroidal anti-inflammatory drugs (NSAIDs).

Short-chain fluorotelomers

6:2 fluorotelomer sulphonate (6:2 FTSA) can cause kidney and liver damages in rodent models, however they are less toxic than PFOS in the studies with fish, algae, water fleas (*Daphnia magna*), and earthworms [Hoke et al. 2015]. These compounds do not bioaccumulate in fish and present little risk to aquatic organisms [Hoke et al. 2015]. In a study with adult male mice, 6:2 fluorotelomer carboxylate (6:2 FTCA), 8:2 fluorotelomer carboxylate (8:2 FTCA) and 10:2 fluorotelomer carboxylate (10:2 FTCA) exhibited weak and moderate hepatotoxicity compared with those reported for PFOA and PFOS [Sheng et al. 2017]. In Daphnia magna tests the FTCA

was consistently more toxic than the FTUCA and PFCAs [Phillips et al. 2010]. N-methyl perfluorooctane sulfonamido acetic acid (MeFOSAA) and n-ethyl perfluorooctane sulfonamido acetic acid (EtFOSAA) increased the hypertension in pre-diabetic adults (male) at high concentrations [Lin et al. 2020]. In 90-day sub chronic study in rat, 6:2 fluorotelomer alcohol (6:2 FTOH), was administered to rat by oral gavages and mortality was observed at 125 mg/kg/day, deaths occurred after three weeks of dosing and continued sporadically [Serex et al. 2014]. A chronic toxicity assessment showed that 6:2 fluorotelomer unsaturated carboxylate (6:2 FTUCA), 8:2 fluorotelomer unsaturated carboxylate (8:2 FTUCA) and 19:2 fluorotelomer unsaturated carboxylate (19:2 FTUCA) are less toxic to *Daphnia magna* and *Chironomus dilutus* in separate life-cycle tests than fluorotelomer saturated carboxylic acid (FTCA) [Pillips et al. 2010]. The reproduction of *Daphnia magna* and *Chironomus dilutes* was significantly reduced as compared to the controls, with respective to median effective concentration of 287 µg/L for offspring, and the mean number of female of 214 for fluorotelomer unsaturated carboxylate (FTUCA) [Pillips et. al. 2010]. 6:2 and 8:2 chlorinated perfluoroether sulphonate (6:2 Cl-PFESA and 8:2 Cl-PFESA) cause thyroid hormone disruption effects through competitive binding to transport proteins and activation of thyroid hormone receptors. These compounds have also toxic effects to freshwater algae [Xin et al. 2018]. N-MeFOSE is harmful if swallowed, if inhaled, in contact with skin and may cause serious eye and respiratory track irritation [Pub Chem]. N-EtFOSE treatment significantly decreased the growth rate, increased relative liver weight and activity of superoxide dismutases (SOD) in liver and uterus of female rat (Xie et al. 2009). The toxicity and health effects of n-methyl perfluorobutane sulfonamide (MeFBSA) and n-methyl perfluorobutane sulfonamide ethanol (MeFBSE) have not been reported at the time this study was conducted.

2.3 Sources and exposure pathways

Ingestion of food and drinking water contaminated with PFAS are the major human exposure pathways [Fry and Power 2017]. Inhalation of dust contaminated with PFAS and dermal (skin) contact with PFAS are considered to be minor exposure pathways [Fry and Power 2017]. As mentioned previously, surface water, subsurface water and wastewater are the major sources of drinking water contamination. Soil, sediment, sludge and biosolids responsible for plant growth eventually become the sources of food and consumer products of human. Air with PFAS contaminated dust is another source of PFAS. Proper investigation of sources and exposure pathways of PFAS in environment needs reliable analytical methods to identify and quantify PFASs accurately. Thus, it is important to identify reliable analytical methods in non-drinking water matrices.

The sources and exposure pathways of PFAS in an aquatic environment have been studied in many countries [Boiteux et al. 2016]. In the United States, the Environmental Protection Agency (EPA) together with manufacturers and users of perfluoroalkyl chemicals has investigated their sources and exposure pathways [Sinclair and Kannan 2005]. Industrial and commercial wastewaters, food, consumer products and dust have been implicated as likely sources of PFAS in human bodies and the environment [Sinclair and Kannan 2005]. Figure 1 shows the pathways of human exposure to PFAS.

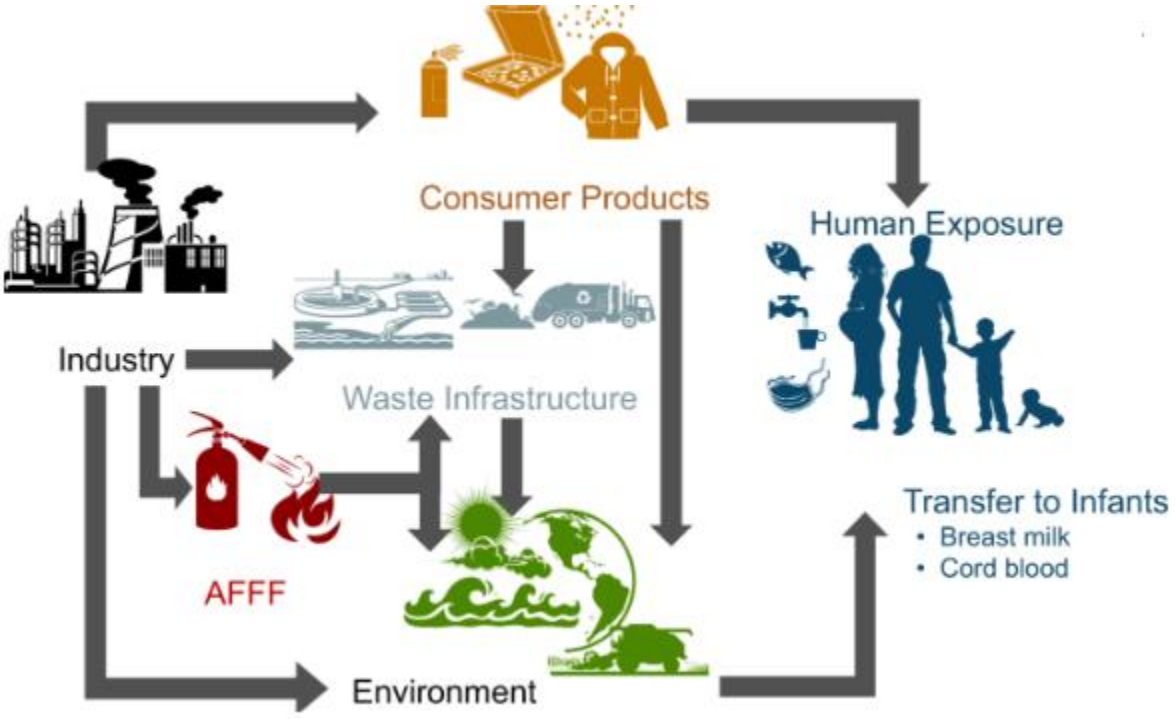


Figure 1: The pathway of human exposure to PFASs [Sunderland et al. 2018]

In recent years, the effluents of domestic and/or industrial wastewater treatment plants (WWTPs) have been found as one of the major contributors of PFAS in natural waters [Boiteux et al., 2016]. Conventional domestic WWTPs are not capable of removing PFAS or any other bio-refractory chemicals. Since 2002, many industries in the U.S. are voluntarily phasing out using PFAS [Houtz et al. 2013]. However, PFAS have been found in the WWTPs, which are major PFAS contributors in a natural water system. There are multiple routes or pathways through which PFOS and PFOA enter a human body. The ingestion of contaminated groundwater has shown to be an important route of PFAS exposure to humans [Xiao et al. 2015]. Soils and ground water adjacent to unlined disposal dumps or landfills (which accepting industrial waste) act as a major source and exposure pathway of PFOS and PFOA for residents living near the sites [Xiao et al. 2015]. The fate and behavior of PFASs in WWTPs are not well understood because the number of PFAS analyzed has

been very limited and precursors of perfluoroalkyl acids (PFAAs), such as fluorotelomers (FTs) have not been included. Another source of PFAS for the significant environmental contamination may be the use of aqueous film-forming foams (AFFFs). The firefighting training sites where aqueous film-forming foams (AFFFs) are frequently used have been significant sources of PFAS [Coggan et al. 2019].

Ionic PFAS are resistant to photolysis, pyrolysis, and biotransformation; thus they are highly persistent in the environment [Zhao et al. 2017]. These compounds have high water solubility owing to their carboxylic or sulfonic acid groups, and can migrate significant distances in the water environment [Zhao et al. 2017]. An open ocean is presumed to be an important sink of ionic PFAS homologues [Zhao et al. 2017]. Neutrally occurring PFAS such as fluorotelomer alcohols (FTOHs) are volatile and distributed mainly in air rather than in water [Zhao et al. 2017]. Following oxidation by radicals and oxidants in atmosphere, neutral PFAS transform to ionic forms and then reach the earth's surface by dry or wet deposition. The dry and wet depositions are considered indirect sources of PFAS [Zhao et al. 2017].

PFAS are ubiquitous, non-biodegradable, bio-accumulative, and toxic in the environment; thus they have the potential to cause health hazard to human and wildlife globally. Evaluation methods for the remediation of PFAS contaminated sites are highly dependent on the detection and quantification levels of the analytical methods. Therefore, the reliable analytical method for quantifying PFAS in the environmental samples is critically important.

Chapter 3: Methods

In this study, the analytical methods for poly- and perfluoroalkyl substances (PFAS)in non-drinking water matrices were investigated through literature review. First, literature search was carried out to identify peer-reviewed papers on PFAS (including their homologs and degradation byproducts) using Web of Science and Google Scholar. This literature search is limited to the papers published between January 2000 and April 2020.To identify the reliable analytical methods for PFAS in non-drinking water matrices, the available analytical methods were evaluated based on the following parameters:

(1) Limit of detection (LOD), (2) limit of quantification (LOQ), (3) percent recovery (%R) of the analytes, (4) detectability (detection of unknown byproducts, homologs), and (5) precisions as relative standard deviation (RSD) for the data obtained. LOD is defined as the lowest analyte concentration that can be quantitatively detected with an acceptable range of precision or as the analyte peak required to yield a background signal-to-noise ratio of 3:1 [Chen et al. 2012]. LOQ is the lowest analyte concentration that can be quantitatively detected with an acceptable range of accuracy and precision or is the analyte peak required to yield a background signal-to-noise ratio larger than10:1 [Chen et al. 2012]. LOD and LOQ values may also be calculated based on the standard deviation (Std) of the LC-MS/MS instrumental responses or signal of the calibration curve between instrumental responses and concentrations of a analyte and the slope of the calibration curve (S) according to the formula: LOD = 3.3*(Std/S) and LOQ = 10*(Std/S) [Armbruster and Pry 2008]. The coefficients 3.3 and 10 are called expansion factors and are obtained assuming a 95% confidence level [Armbruster and Pry 2008]. 95% confidence level is a range of values that anybody can be 95% certain that this range contains the true mean of the

population [Morey et al. 2016]. This is not the same as a range that contains 95% of the values [Morey et al. 2016]. There is no specific range or value for LOD and LOQ; however, the LOD and LOQ values at a level of pg/L, pg/g or pg/m^3 indicate highest sensitivity of the state-of-the-art analytical technology; i.e. liquid chromatography interface with tandem mass spectrometry (LC-MS/MS) [Banzhaf et. al. 2017]. Recovery efficiency or percent recovery (%R) for an analyte is computed by the equation: %R= [(A-B)/C]*100, where A is the measured concentration of the fortified sample, B is the measured concentration of the non-fortified sample, and C is the fortification concentration [Shoemaker and Tettenhorst, 2018]. The accepted range of the %R value is 80–120% according to the Food and Agriculture Organization of the United Nation [2018]. When a sample is analyzed several times, the individual results vary from trial-to-trial. Precision is a measure of this variability [Karnes and March 1993]. The closer the agreement between individual analyses, the more precise the results [Karnes and March 1993]. From the measured standard deviation and mean values, the precision value (as relative standard deviation) is calculated using the formula: RSD (%) = (Standard deviation/Mean)*100 [Karnes and March 1993].The acceptable range of precision in terms of %RSD is ±20% [Karnes and March 1993]. Accuracy is how closely the result of an experiment agrees with the "true" or expected result. Accuracy value is express as an absolute error (e), e = (obtained result−expected result) or as a percentage relative error (%e_r), %e_r = (obtained result−expected result/expected result)*100 [Karnes and March 1993]. There is no accepted limit for absolute error (e) or percent relative error (%e_r) but it need to be remember that the lower the absolute error or percent relative error the higher the accuracy of measurement or prediction [Karnes and March 1993].

This research work started with the collection of and review of the papers that describe the analytical methods for PFAS in non- drinking water matrices, including surface water, subsurface water, waste water, sludge, biosolids, soil, sediment, air, and biota. The findings are tabulated in terms of:

- authors (year);
- analytical instrument used;
- analytical approach;
- PFAS investigated;
- media, sampling sites, and application;
- performance evaluation parameters such as LOQ, LOD, percent recovery (%R), detectability, and precision;
- relevant comments.

The analytical methods for sludge and biosolids are combined, as their methods are identical [Chen et al. 2012]. The selection of the analytical methods is based on two conditions: a) the LOD and LOQ values or their range must be available; and b) the recovery efficiency (%R) must be within the acceptable range suggested by the Food and Agricultural Organization of United Nation. Acceptable range of percent recovery is not related to water quality standard. If two analytical methods produced the same performance values, one method is selected based on the number of analytes detected. The analytical methods selected as the best methods currently available are given in the Conclusions chapter, and the method protocols are presented in Appendices.

Chapter 4: Results and Discussion

The assessment of each method was conducted based on rating of very good, good, fair, and poor. Rating “very good” is indicating that both LOD and LOQ range must be available in the method and percent recovery (%R) range is within the acceptable range (80-120%) provided by the Food and Agriculture Organization of the United Nation [2018]. Rating “good” is that both LOD and LOQ range are available in the method but percent recovery (%R) range is not within the acceptable range (80-120%). Rating “fair” indicates that at least one range of LOD or LOQ is available and the %R range can be inside or outside the acceptable range. Rating “poor” indicates that both LOD and LOQ range are not available and %R range is outside the acceptable range. The analytical method with the rating of “very good” is considered as the best analytical methods. An additional condition is that analytical methods must measure at least ten analytes (PFAS) to be considered as the best analytical methods.

In this study, it was found that three types of liquid chromatography interface with tandem mass spectrometry (LC-MS/MS) instruments have been used for analysis. These are the first generation, second generation, and third generation instrument. First generation instrument are those who provides their detection limits in microgram/liter, microgram /gram, and microgram /meter^3 [Banzhaf et al. 2016]. The second generation instruments are those provide the detection limits are in nanogram/liter, nanogram /gram, and nanogram /meter^3 and the third generation instruments with the detection limits in picogram/liter, picogram/gram, and picogram /meter^3 [Banzhaf et al. 2016]. Besides these three generations of instruments, another type of instrument known as Liquid chromatography interfaced with triple quadrupole mass spectrometry is available for the PFAS analysis. In this instrument, chemical compounds are separated using traditional liquid

chromatography, then the analytes are directed into a series of quadrupole where they are ionized as the molecular ion then fragmented and selected fragments are detected and quantified. The determination of both molecular ion and fragment ions (due to thermal decomposition, spontaneous fragmentation at ionization or secondary fragmentation of the energized radical molecular ion) permits more accurate identification analysis than identification using molecular ion alone in case of liquid chromatography interface with tandem mass spectrometry instrument. For this reason the performance of this instrument is higher than LC-MS/MS instrument. All four types of instruments are related to targeted analysis, and most of the methods considered in this study are these types of instruments [Peng et al. 2014].

Result of the literature review on PHAS is presented in the order of: (1) surface water,(2) subsurface water,(3) wastewater,(4) sludge and biosolids,(5) soil,(6) sediment,(7) air, and (8) biota (fish), and summarized in Tables 2 to 9, respectively.

4.1 Surface water

For the analytical methods for PFAS in surface water, review results are summarized in Table 2, reported ranges of the LOD and LOQ values are presented in Figure 2, and ranges of the percent recovery (%R) values are shown in Figure 3.

Table 2: Analytical methods for PFAS in surface water.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Hung et al. (2020)	LC-MS/MS	Targeted analysis	13 PFAS from three classes (PFCAs, PFSAs and FTs)	Surface water from three special management sea area in Korea (Gwangyang Bay, Mansan Bay and Busan Harbor)	LOD range 1-6 pg/L; %R for $^{13}C_4$ PFOA and $^{13}C_4$ PFOS are 85.35% and 103.23%.	All targeted PFASs except PFDS and PFDA are observed in the sea water. Rating: Fair.

Table 2: Continued.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Lee et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from three classes (PFCAs, PFSAs and FOSA)	Surface water collected from Asan Lake area of South Korea	LOD range 1-8 pg/L; LOQ range 3-17 pg/L; %R range 89%- 117%.	16 PFASs were measured and 9 were detected. PFCAs in water samples accounted for 77.6% of PFASs. Rating: Very good.
Wang et al. (2019)	LC-MS/MS	Targeted analysis	20 PFAS from three classes (PFCAs, and PFSAs and four compounds-HFPO-DA, ADONA, 6:2 Cl-PFESA, 8:2 Cl-PFESA of unknown class	Surface and bottom water have been collected from South China Sea (SCS) region.	%R range 73%-98%; LOQ range from 5-20 ng/L.	6:2 and 8:2 Cl-PFESA and HFPO-DA were detected in the SCS, while ADONA was below the LOD. Rating: Fair.
Pan et al. (2019)	Liquid chromatography interfaced with triple quadrupole mass spectrometry	Targeted analysis	12 PFAS from three classes (PFCAs, PFSAs and FOSA)	The study area comprised rivers and nearshore waters along the coast of the Beibu Gulf in South China.	LOD range 4-25 ng/L; LOQ range 13- 83 ng/L; %R are 69.7%- 112.5%.	There were 11 and 12 out of the 18 targeted PFASs found in riverine water samples from the Beibu Golf in the summer and winter. Rating: Good.
Zhao et al. (2017)	LC-MS/MS	Targeted analysis	12 PFAS from three classes (PFSAs, PFCAs and FOSA)	The study area covered the Bohai Sea, Yellow Sea and Yangtze River estuary in China.	LOD range 4-15 ng/L; LOQ range 13- 23 ng/L; %R are 38%- 119%.	In the water dissolve phase, 10 out of 12 PFASs were detected. Rating: Good.
Yeung et al. (2017)	LC-MS/MS	Targeted analysis	31 PFAS from three classes (PFSAs, PFCAs and FTs)	Surface water were collected on two separate cruises in the Central Artic Ocean from surface to bottom.	LOQ range 5-20 ng/L; %R are 85% -109% except 60% for FOSA.	69 samples were analyzed for 31 PFASs in seawater, snow and melt pond water. Rating: Fair.
Zhao et al. (2016)	LC-MS/MS	Targeted analysis	11 PFAS from two classes (PFCAs- PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA) and (PFSAs-PFOS, PFBS)	Surface water samples were collected from 20 sites along the middle and lower reaches of the Yellow River.	LOD range 6-18 µg/L; LOQ range 9-23 µg/L; %R are 81%- 124%.	All of the 11 studied PFASs showed very high frequencies in the water phase, only PFBS, PFPeA, PFHpA and PFUdA were not detected in the Yellow River. Rating: Good.
Boiteux et al. (2016)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Surface water samples were collected in the river which receives wastewater from a training site using AFFF.	LOD range 2-13 ng/L; LOQ range 4-20 ng/L; %R are 70%-145% except for 5:3 ACID (36%).	The aim of this study was to optimise several analytical methods (oxidative conversion to PFAAs) for the determination of PFASs in water. Rating: Good.
Cai et al. (2011)	LC-MS/MS	Targeted analysis	24 PFAS from three classes (PFSAs, PFCAs and FOSA)	Surface water samples were collected at 22 stations from the North Pacific to Arctic Ocean.	LOD range 4-22 ng/L; LOQ range 6-23 ng/L; %R are 70%-145% except for PFHxA (57%).	Overall 14 of 24 PFCAs were quantified in surface water samples at 22 sampling sites. Rating: Good.
Yamashita et al. (2004)	LC-MS/MS	Targeted analysis	7 PFAS from two classes (PFSAs and PFCAs)	Surface water samples were collected in the central and eastern Pacific Ocean, South China and SULU Seas, mid Atlantic Ocean and Tokyo Bay.	LOD range 4-23 ng/L; LOQ range 9-17 ng/L; %R are 61%-147% except for PFBS which is too low.	PFOA is the predominant fluorochemical followed by PFOS. Rating: Good.
Hansen et al. (2002)	LC-MS/MS	Targeted analysis	2 PFAS from two classes (PFOS and PFOA)	Surface water samples were collected at approximately 2-mi intervals along the Tennessee River.	LOD range 5-18 ng/L; LOQ range 10-21 ng/L; %R is 96% for PFOS and is 83% for PFOA; Reproducibility for all analytes was within 6%.	The purpose of this study was to developed a method for analysis of PFASs if manufacturing facilities may be a source of PHOS and PFOA in the enviroment. Rating: Very good.
Moody et al. (2001)	LC-MS/MS	Targeted analysis	8 PFAS from two classes (PFCAs and PFSAs)	Surface water samples were collected from Etobicoke Creek over a period of 153 days after the AFFF spill. Sample site is upstream of the airport and the AFFF spill.	LOD is 4 ng/L for PFOS and 12 ng/L for PFOA; LOQ is 9 ng/L for PFOS and 19 ng/L for PFOA; %R is 68% for PFOS and is 93% for PFOA; The RSD was 6% for PFOS and 7.5% for PFOA.	PFOS was the predominant anionic perfluorinated surfactant detected in surface water samples by this method and accounting for >90% of the total PFSAs. Rating: Good.

In their research work with surface water collected from Etobicoke Creek, Moody et al. [2001] analyzed eight PFAS of two classes (PFCA and PFSA). Of which, only PFOA and PFOSwere evaluated for the analytical performance. The reported LOD values are 4 ng/L for PFOS and

12ng/L for PFOA. The LOQ values are9 ng/L and 19 ng/L for PFOS and PFOA, respectively. The recovery efficiencies (%R) are 68% and 93% for PFOS and PFOA, respectively. The values of the relative standard deviation (RSD) are 6% and 7.5% for PFOS and PFOA, respectively. Overall, the performance of the analytical method employed by Moody et al. [2001] is good. Figure 2 shows the availability and non availability of LOD and LOQ of the methods.

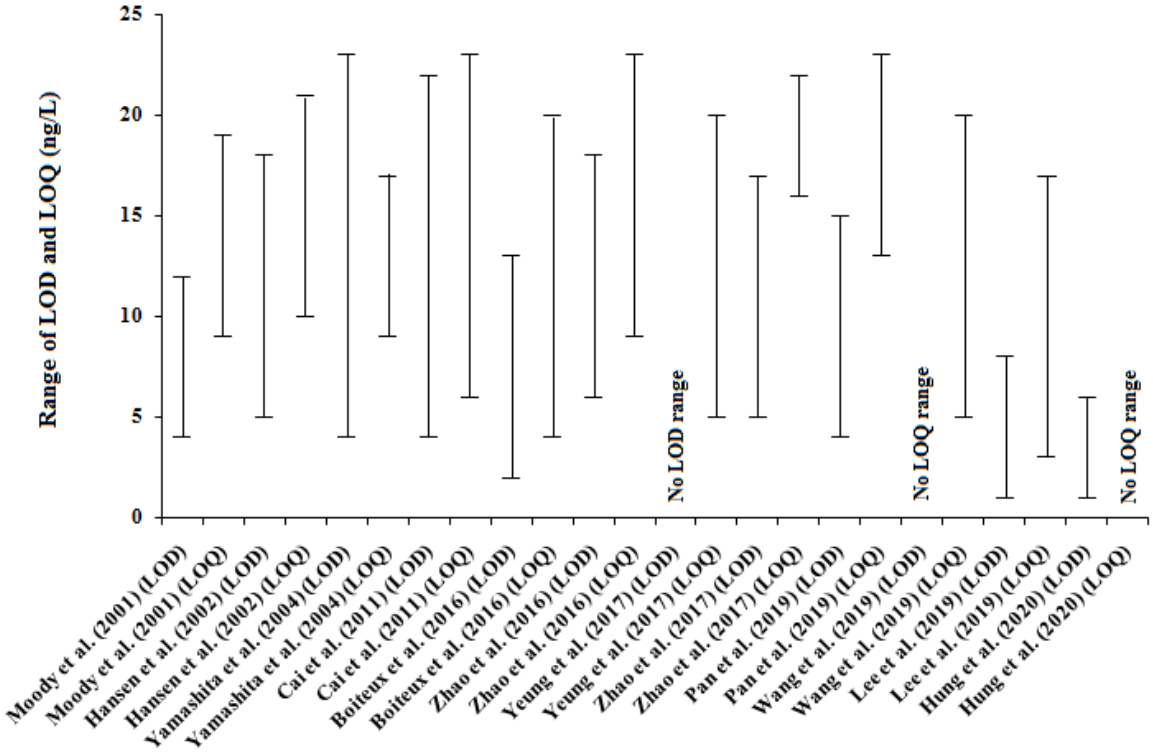


Figure 2: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in surface water.

In a study with surface water of the Tennessee River in the U.S., Hansen et al. [2002] analyzed the water samples for PFOA and PFOS. The LOD values reported for PFOA and PFOS are 5 ng/L and 18 ng/L, respectively. The LOQ values for PFOA and PFOS are 10 ng/L and 21 ng/L, respectively. The values of the percent recovery (%R) for PFOA and PFOS are 83% and 96%, respectively. Reproducibility or precision for all the analytes is within 6%. Overall, the analytical

performance for the method employed by Hansen et al. [2002] is evaluated asvery good.Although the rating of this method is very good, this method is not the best analytical method for surface water, as the number of analytes measured are two, less than ten. Figure 3 shows the range %R for the method and acceptable %R range suggested by the Food and Agricultural Organization of United Nation [FAO Animal production and Health 2018].

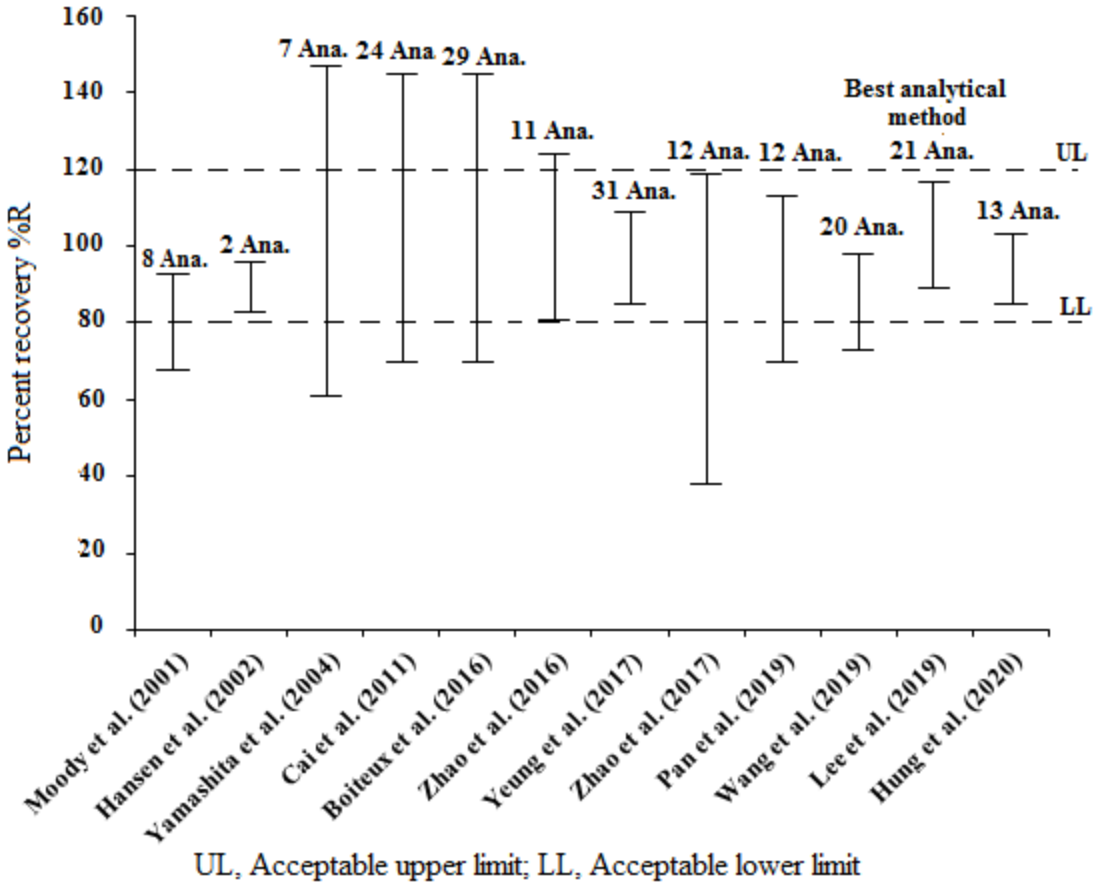


Figure 3: Percent recovery by the analytical methods for PFAS in surface water.

Yamashita et al. [2004] analyzed surface water samples collected from the central and the eastern Pacific Ocean, South China, and identified seven PFAS of two classes (PFSAs and PFCAs). The reported LOD values are in a range from 4 to 23ng/L, and the LOQ values from 9 to17 ng/L. The percent recovery efficiency (%R) ranges from 61to147%, except for PFBS which exhibited a

considerably low %R value. The lower limit of the %R is below 80% and the upper limit is above 120%. These ranges are outside the range of 80 to 120% recommended by the Food and Agricultural Organization of United Nation [FAO Animal production and Health 2018]. Overall, the performance of the analytical method employed by Yamashita et al. [2004] is evaluated to be good.

In analyzing surface water samples collected from the North Pacific Ocean to the Arctic Ocean, Cai et al. [2011] detected 24 PFAS of three classes (PFSAs, PFCAs, and FOSA) with the LOD and LOQ values rangingfrom4to 22ng/L and 6to 23ng/L, respectively. The reported %R values are in a range between 70% and 145%, except for PFHxA (57%). The lower limit of the %R value is below 80% and the upper limit is above 120%.Overall, the performance of the analytical method used by Cai et al. [2011] is considered good.

In the analysis of surface water collected from the river that receives wastewater from a fire-fighting training site (where AFFF was used) in France, Boiteux et al. [2016] detected 29 PFAS of three classes (PFSAs, PFCAs, and FTs). The reported LOD and LOQ values are in a range from 2 to13 ng/L and 4 to 20 ng/L, respectively. The reported %R values range from 70 to 145%, except for 5:3ACID (36%). The lower and upper limit of the %R values are outside of the acceptable range (80 - 120%) provided by the Food and Agriculture Organization of the United Nation [FAO Animal production and Health 2018]. Overall, the performance of the analytical method used by Boiteux et al. [2016] is rated good.

In their work with surface water collected from the middle and lower reaches of the Yellow River in China, Zhao et al. [2016] detected 11 PFAS of two classes (PFCAs and PFSAs) with the LOD values ranging from 6 to18μg/L, the LOQ values from 9 to 23μg/L, and the %R values from 81 to 124%. The reported LOD and LOQ values are in a μg/L range. The lower limit of the percent recovery (%R) is within the acceptable limit of 80%; however, the upper limit is above the acceptable 120% limit. Overall, the analytical method employed by Zhao et al. [2016] is rated good.

Yeung et al. [2017] analyzed surface water collected from Central Artic Ocean seawater, snow and melt pond water, and detected 31 PFAS of three classes (PFSAs, PFCAs, and FTs) with the LOQ values ranging from 5 to 20 ng/L. The reported %R values range from 85 to 109%, with an exception of FOSA (60%). No LOD values were reported. Overall, the analytical method used by Yeung et al. [2017] is considered fair.

In the study with surface water of the Bohai Sea, Yellow Sea, and Yangtze River estuary in China, Zhao et al. [2017] detected 12 PFAS of three classes (PFSAs, PFCAs and FOSA)with the LOD values ranging from 4 to15ng/L, the LOQ values from 13 to 23ng/L. The reported %R values are in a range from 38% to119%. The lower limit of %R value is below the lower limit of the preferred 80% recommended by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the analytical method employed by Zhao et al. [2017] is evaluated to be good.

Pan et al. [2019] analyzed surface water collected from the rivers and near shore along the coast of the Beibu Gulf in South China. In their work, 12 PFAS of three classes (PFCAs, PFSAs and FOSA) were detected with the LOD and LOQ values ranging from 4 to 25 ng/L and 13 to 83 ng/L, respectively. The reported %R values are in a range between 69.7% and 112.5%. The lower limit of the %R values is below the acceptable limit (80%). Overall, the analytical performance is ranked good.

In analyzing surface water collected from South China Sea, Wang et al. [2019] detected 20 PFAS of three classes (PFCAs, and PFSAs, and four compounds-HFPO-DA, ADONA, 6:2 Cl-PFESA, and 8:2 Cl-PFESA from unknown class. The reported LOQ values are in a range from5 to20 ng/L and the %R values from 73to 98%. No LOD values were reported. The lower limit of the %R values is below the accepted limit of 80%. Overall, the analytical method employed by Wang et al. [2019] is evaluated as fair.

Lee et al. [2019] investigated surface water in Asan Lake in South Korea, and detected 21 PFAS of three classes (PFCAs, PFSAs and FOSA) with the LOD values ranging from 1 to 8pg/Land the LOQ values from 3 to17 pg/L (Fig. 2). The %R values are in a range between 89%and 117%(Fig. 3). Overall, the analytical method used by Lee et al. [2019] is evaluated as very good, and selected as the best analytical method currently available for surface water.

In the study with surface water from three special management sea areas (i.e., Gwangyang Bay, Mansan Bay, and Busan Harbor) in Korea, Hung et al. [2020] detected 13 PFAS of three classes (PFCAs, PFSAs and FTs). The values of LOD range from 1 to 6 pg/L. The %R values for

$^{13}C_4$PFOA and $^{13}C_4$ PFOS are 85.35% and 103.23%, respectively. No LOQ values were reported. The %R values are given for only two analytes, which implies that most of the analytes were not measured. Overall, the analytical method used by Hung et al. [2020] is evaluated as fair.

4.2 Subsurface Water

For the analytical methods for PFAS in subsurface water, review results are summarized in Table 3, the reported ranges of the LOD and LOQ values are presented in Figure 4, and the ranges of the percent recovery (%R) values are shown in Figure 5.

Table 3: Analytical methods for PFAS in Subsurface Water.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Hepburn et al. (2019)	LC-MS/MS	Targeted analysis	17 PFAS from three classes (PFCAs, PFSAs and FTs)	Ground water samples were collected from thirteen shallow monitoring bores near the Port Melbourne area.	LOD is 10-29 pg/L; LOQ range 18-36 ng/L; %R are 70%-130% except for PFDS (53%)	PFAS were detected in all groundwater samples and PFOS, PFHxS, PFOA and PFBS were detected at all locations. Rating: Good.
Szabo et al. (2018)	LC-MS/MS	Targeted analysis	20 PFAS from three classes (PFCAs, PFSAs and FTs)	Ground water samples were abstracted from twenty monitoring wells within the Werribee Irrigation District in Victoria.	LOD range 13-35 pg/L; LOQ range 21-47 pg/L; %R are 73%-145% except for PFPeS is 180%.	PFAS were detected in all groundwater samples and the four most detectable compounds were PFOS, PFBS, PFOA and PFBA. Rating: Good.
Weber et al. (2017)	Liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	16 PFAS from four classes (PFCAs, PFSAs, FOSA and FTs)	Ground water samples were collected from a netwowork of monitorinr-well cluster at Western Cape Cod, Massachusetts.	LOD ranged from 22-48 ng/L; %R ranged from 98%-130% for PFBS, PFHxS and PFOS after oxidation.	Total Oxidizable Precursor (TOP) Assay analytical method was employed in this study. Rating: Fair.
Boiteux et al. (2016)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Ground water samples were collected in the vicinity of the training site using AFFF.	LOD range 12-34 ng/L; LOQ range 20-42 ng/L; %R are 70%-145% except for 5:3 ACID (36%).	The aim of this study was to optimise several analytical methods (oxidative conversion to PFAAs) for the determination of PFASs in water. Rating: Good.
Anderson et al. (2016)	LC-MS/MS	Targeted analysis	16 PFAS from three classes (PFSAs, PFCAs and FOSA)	A total of ten active US Air Force installations were selected for sampling throughout the continental United States.	No method performance evaluation criteria (LOD, LOQ and %R) values are mentioned	Statistical analysis (linear discriminant analysis) was used to evaluate inter-media variability as a function of all 16 PFASs being analyzed. No rating.
Backe et al. (2013)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Ground water were collected from two different U.S. military bases (sites A and B) at Oregon State.	LOD range 7-36 ng/L; LOQ range 16-47 ng/L; %R range 78% and 144%; Accuracy ranged 96%-106% for quantitative and 87%-155% for semi-quantitative analytes.	At three out of five military sites fluorotelomer thioamidosulfonates were found in groundwater at concentrations lower than those of fluorotelomer sulfonates and other legacy PFAS. Rating: Good.

Table 3: Continued.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Houtz et al. (2013)	LC-MS/MS	Targeted analysis	42 PFAS from three classes (PFSAs, PFCAs and FTs)	Groundwater samples were collected from a location at Ellsworth Air Force Base in Piedmont firefighting training center, South Dakota.	LOD range 10-25 ng/L; %R range 56%- 130% ; Precision range 0.0%-11%.	AFFF samples manufactured by 3M, National Foam, Ansul, Chemgurd and Buckeye were directly analyzed for AFFF-related PFAA precursors, PFSAs, PFCAs generated upon oxidation. Rating: Fair.
Schultz et al. (2004)	High perfomance liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	14 PFAS from two classes (PFSAs-2-PFBS, 2-PFHxS, 2-PFOS and FTs- 5-6:2 FtS)	Samples were collected from sites associated with fire-training activities at Wurtsmith Air Force Base, Michigan, Tyndall Air Force Base,Florida and Naval Air Station Fallon,	LOD range 15-36 µg/L; LOQ range 27-43 µg/L ; %R range 82%- 120%.	The fluorotelomer sulfonates were the most abundant (80%) fluorosurfectant observed at these three sites. Rating: Very good.

Schultz et al. [2004] analyzed subsurface samples collected from fire-training sites at the Wurt Smith Air Force Base in Michigan, the Tyndall Air Force Base in Florida, and the Naval Air Station Fallon in Nevada. In their study, 14 PFAS of two classes (PFSAs-2-PFBS, 2-PFHxS, 2-PFOS and FTs-5 to 6:2 FtS) were detected with the LOD and LOQ values ranging from 15 to 36 µg/L and 27-43 µg/L, respectively (Fig. 4). The recovery efficiency (%R) values reportedare in a range from 82 to 120% (Fig. 5). The LOD and LOQ values are in the µg/L level. The detection levels can be improved to the level of ng/L or pg/L by using a second or third generation LC-MS/MS instrument. This method, however, is emerged as the best analytical method currently available for subsurface water with a rating of very good.

Houtz et al. [2013] investigated groundwater at the site of the Piedmont firefighting training center, the Ellsworth Air Force Base in South Dakota, USA. They detected 42 PFAS of AFFF-related PFAS precursors from three classes (PFSAs, PFCAs, and FTs) with the LOD values ranging from 10 to 25 ng/L. The reported %R values are in a range between 56 and 130%. The range of precisions is between 0.0 and 11%. No LOQ values were reported. The lower and upper limits of the %R values are outside the range recommended by the Food and Agriculture Organization of

the United Nation [FAO Animal Production and Health 2018]. The reported range of the precision values is acceptable. Overall, the analytical method employed by Houtz et al. [2013] is considered fair.

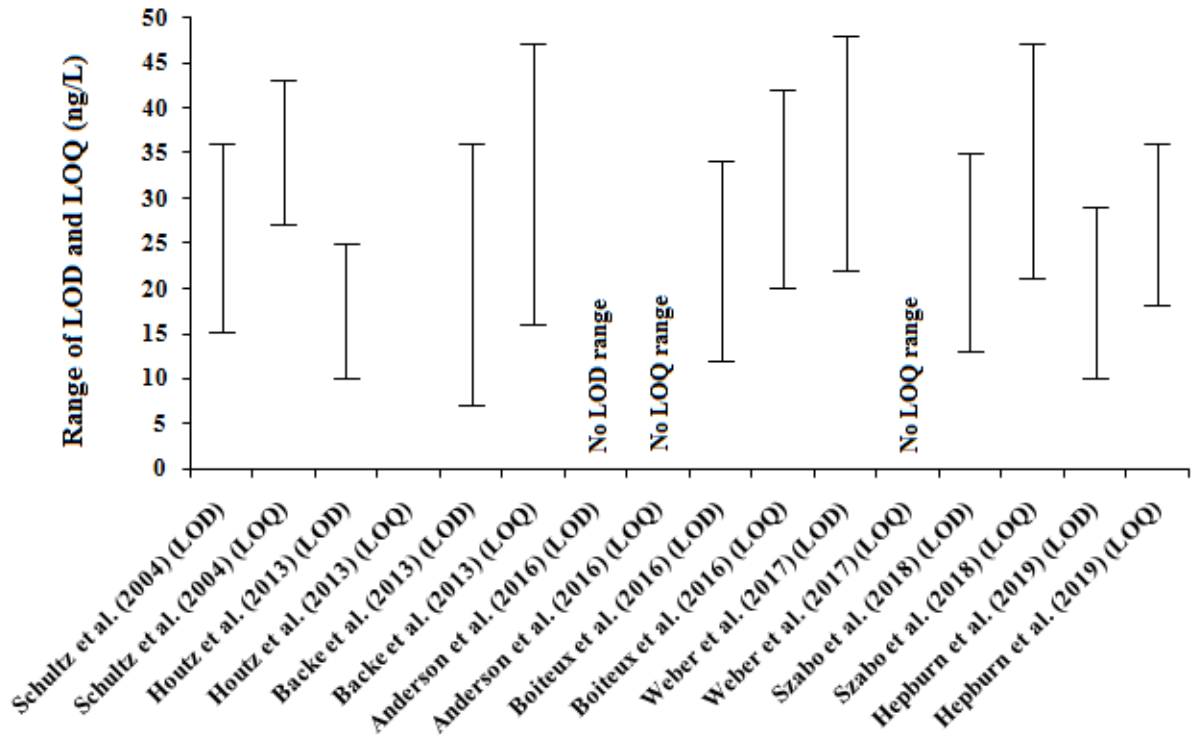


Figure 4: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in subsurface water.

In their study with subsurface water at the two different U.S. military bases in Oregon, USA. Backe et al. [2013] detected 29 PFAS of three classes (PFSAs, PFCAs and FTs including fluorotelomer thioamido sulfonates) with the LOD and LOQ values ranging from 7 to 36 ng/L and 16 to 47 ng/L, respectively. The range of %R is between 78% to 144%. The accuracy ranges are between 96% and 106% in the quantitative analysis, and 87% and 155% in the semi-quantitative analysis. Overall, the analytical method employed by Backe et al. [2013] is rated good.

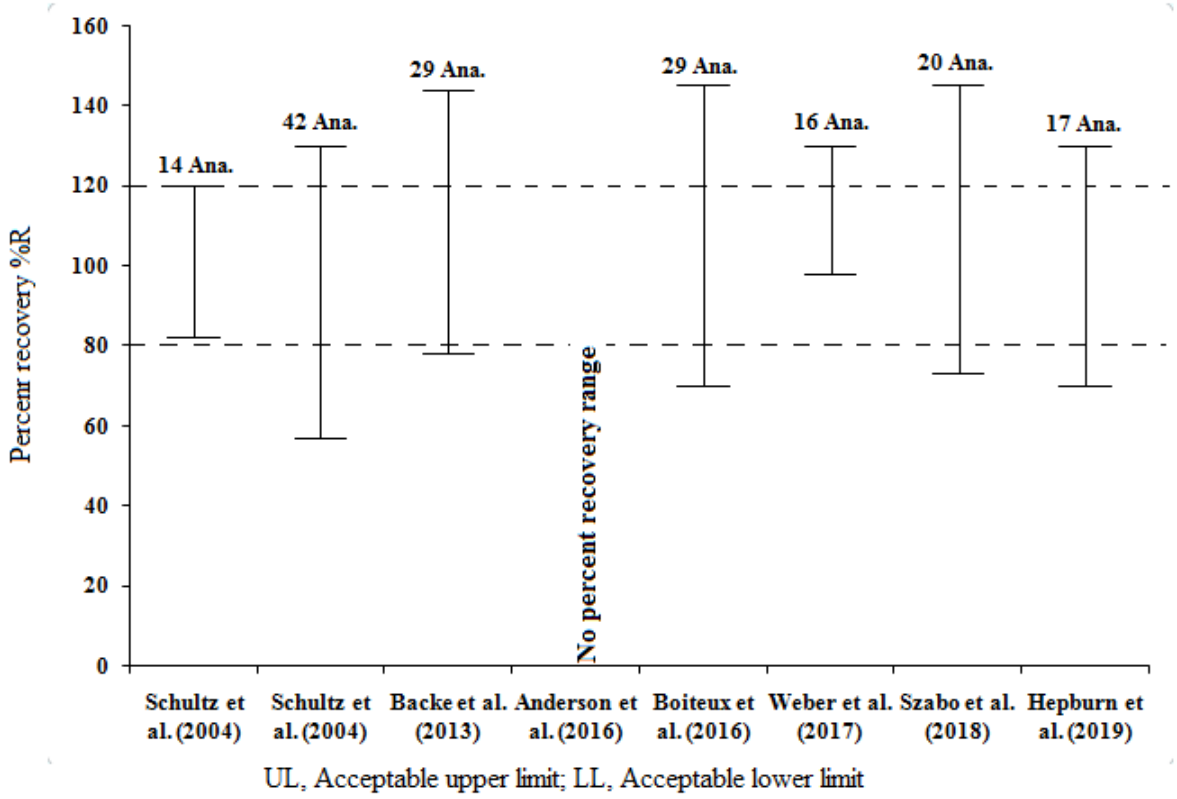


Figure 5: Percent recovery by the analytical methods for PFAS in subsurface water.

Anderson et al. [2016] analyzed subsurface water at ten active US Air Force installations throughout the United States. In their study, 16 PFAS of three classes (PFSAs, PFCAs and FOSA) were detected. They carried out statistical analysis (linear discriminant analysis) to obtain the inter-media variability for the 16 PFASs analyzed. No values of the LOD, LOQ, and %R were reported.

Analyzing subsurface water in the vicinity of the fire-fighter training site (where AFFF had been used) in France, Boiteux et al. [2016] detected 29 PFAS of three classes (PFSAs, PFCAs, and FTs).The reported LOD and LOQ values are ranging from 12 to 34 ng/L and 20 to 42 ng/L, respectively. The reported %R values are in a range from 70% to 145% with an exception of 5:3

ACID (36%). The lower and upper limits of the %R values are outside the acceptable range (80%-120%). Overall, the analytical method employed by Boiteux et al. [2016] is rated good.

Using the Total Oxidizable Precursor Assay method, Weber et al. [2017] analyzed subsurface water collected from a network of monitoring-well cluster at Western Cape Cod, Massachusetts, U.S.A. They measured 16 PFAS from four classes (PFCAs, PFSAs, FOSA, and FTs). The LOD values reported for PFBS, PFHxS, and PFOS are in a range from 22 to 48 ng/L, and the %R values from 98 to 130% after oxidation. No LOQ values were reported. The upper limit of the %R values for these three PFAS is above the acceptable limit 120% provided by the Food and Agriculture Organization of the United Nation[FAO Animal Production and Health 2018].Overall, the analytical method employed by Weber et al. [2017]is rated fair.

Szabo et al. [2018] analyzed subsurface water samples collected from twenty monitoring wells in the Werribee Irrigation District in Victoria, and detected 20 PFAS from three classes (PFCAs, PFSAs and FTs). The reported LOD values range from 13 to 35pg/L, the LOQ values from 21 to 47 pg/L, and the %R from 73 to 145% with an exception of PFPeS (180%).The lower and upper limits of the %R values are outside the accepted range provided by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018].Overall, the analytical method employed by Szabo et al. [2018] is considered good.

In analyzing subsurface water samples collected from thirteen shallow monitoring bores near the Port Melbourne area in Australia, Hepburn et al. [2019] detected17 PFAS of three classes (PFCAs,

PFSAs, and FTs) with the LOD in a range of 10 to 29pg/Land the LOQ in a range from18 to 36pg/L. The range of the %R values is between 70 and 130%, except for PFDS (53%). The lower and upper limit of the %R values is outside the acceptable range (80-120%). Overall, the analytical method used by Hepburn et al. [2019] is evaluated to be good.

4.3 Waste water

For the analytical methods for PFAS in wastewater, review results are summarized in Table 4, the reported ranges of the LOD and LOQ values are presented in Figure 6, and the ranges of the percent recovery (%R) values are shown in Figure 7.

Table 4: Analytical method for PFAS in wastewater (Domestic, industial)

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Coggan et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from four classes (PFCAs, PFSAs, 6:2 FTs and Cl-PFESA)	Domestic wastewater	LOQ range 4-20 pg/L; LOD range 1-11 pg/L; %R in LCS samples range 80-120%; %R for PFDS, 8:2 Cl-PFESA, PFTrA, & PFTeA range 70-76%.	21 PFAS from four classes were measured in WWTP solids and aqueous samples from 19 Australian WWTPs. Rating: Very good.
Dauchy et al. (2018)	LC-MS/MS	Targeted analysis	34 PFAS from four classes (PFCAs, PFSAs, FOSAs and FTs)	Wastewater drained from the Fire Fighting Training Area	LOD range 4-29 pg/L; %R in LCS samples range 70-120%.	The fluorotelomer contribution in waste water to the total PFAS concentration was overwhelming (86.6-98.4%) and mainly accounted for by FTAB. Rating: Fair.
Houtz et al. (2018)	LC-MS/MS	Targeted analysis, TOP Assay; Nontargeted analysis with QTOF	15 PFAS from four classes (FtTAoS, UPFSA, FTSHC and PFASAC)	Wastewater at Trickling Filter plant	%R ranged 80%-150%.	PFASs from AFFF was investigated in a WWTP. 6:2 FtS, PFOS, PFPeA, PFHxA, PFHpA, PFHxS, 8:2 FtS, and PFBA had the highest amount of mass discharge. Rating: Poor.
Eriksson et al. (2017)	Liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	54 PFAS six classes (PFCAs, PFSAs, FOSA, FTs, PFPA and PAPs)	Wastewater sample were taken from three WWTPs which receives water from industries and hospitals.	%R range 52%-90% for PFCAs, 74%-83% PFSAs, 49%-78% for FTCA/FTUCAs, 74%-81% for FTSAs, 32%-40% for monoPAPs and 22%-32% for diPAPs.	A broad range of compound classes were detected in the filtered effluent water. Among the precursor compound classes only FTSA was detected; 6:2 FTSA was found in all three WWTPS. Rating: Poor.
Dimzon et al. (2017)	GC-MS/MS	Targeted analysis	13 volatile PFAS of two classes (PFAIs and FTs)	Wastewater sample were collected from different industrial and municipal WWTPs in Netherlands and Germany.	LOD range 3-13 ng/L; %R range for the analytes and control standards are between 50% to 75% with high variation.	Due to the highly volatile and hydrophobic nature, the PFAS partitioned more into the headspace than in water. This reduces the percent recoveries of the PFASs. Rating: Fair.
Houtz et al. (2016)	LC-MS/MS	Targeted analysis	20 PFAS of five classes (Fts, FOSAAs, PFOPAs, PFSAs and PFCAs)	Single grab samples of treated final effluent were collectes from eight WWTPS that discharge to San Francisco Bay.	LOD range 16-39 ng/L; LOQ range 14-33 ng/L; %R range 80%-131%; Precision as RSD range 4%-16%.	With direct measurement of twenty specific PFAS analytes, the total concentration of perfluoroalkyl acid (PFAA) precursors was also indirectly measured by total oxidation precursor (TOP) assey. Rating: Good.

Table 4: Continued.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Zhang et al. (2013)	LC-MS/MS	Targeted analysis	16 volatile PFAS of three classes (PFSAs, PFCAs and FOSAs)	Wastewater sample (influent and effluent) were collectd from 28 WWTPs in 11 cities in China.	LOQ range 10-36 ng/L; %R range 75%-109%.	PFOA was in greatest concentrations in both influents and effluents, the highest PFOA levels were found at Dairy industry WWTP in Shanghai. Rating: Fair.
Chen et al. (2012)	Liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	2 compounds from two classes (PFOA and PFOS)	Wastewater sample were collected from the sea water which receiving wastewater discharge from WWTPs of three coastal cities of China.	LOD range 6-18 ng/Land LOQ range 9-31 ng/L; %R range 87%-104%.	Concentration of PFOS and PFOA were analyzed in wastewater samples from ten municipal WWTPs and two industrial WWTPs. Rating: Very good.
Guo et al. (2010)	LC-MS/MS	Targeted analysis	10 PFAS from two classes (PFCAs and PFSAs)	Influent and effluent of municipal, livestock and industrial WWTPs in Korea.	%R of real sample ranged 69-119% (except PFDS); RSD <20%; LOQ ranged 3-13 ng/mL.	10 perfluoroalkyl compounds (PFCs) were analyzed in influent and effluent wastewater and sludge samples in 15 municipal, 4 livestock and 3 industrial WWTPs in Korea. Rating: Fair.
Schultz et al. (2006)	Large-Volume-Injection LC-MS/MS	Targeted analysis	15 PFAS from four classes (PFCAs, PFSAs, FOSA and FTs)	WWTPs (Raw influents, Final effluent)	%R (travel spikes) ranged 87%-98%; R% (Raw influent filed matrix spikes) ranged 77-96%; R% (Final effluent filed matrix spikes) ranged 80-99%. Lower LOQs range 0.5-3.0 ng/L. RSD (single influent) ranged 2-18%; RSD (single effluent) ranged 4-22%.	A quantitative method was developed, which consisted of centrifugation followed by large-volume injection (500 µL) of the supernatant onto an LC with a reverse-phase column and detection by electrospray ionization and MS/MS. Rating: Fair.
Sinclair and Kannan (2005)	LC-MS/MS	Targeted analysis	15 PFAS from three classes (PFCAs, PFSAs and FTs)	Influent, primary-treated and effluent waters were collected from six WWTPs receiving domestic and commercial wastewaters in New York State.	Mean percent recoveries of PFUnDA, PFDoDA, PFTDA, PFHxDA and PFOcDA range from 25% to 75%. PFNA recoveries range from 95% to 179%.	PFOA was the dominant PFAS and was measured in all six WWTPs, the concentratios of PFOA determined here are comparable to those measured in a WWTP on Cleveland, Ohio state. Rating: Poor.

Sinclair and Kannan [2005] analyzed domestic and commercial wastewaters, including influent, primary-treated, and effluent wastewater samples collected from six WWTPs in the State of New York. In their work, they detected 15 PFAS from three classes (PFCAs, PFSAs, and FTs. The values of the mean percent recovery (%R) range from 25 to 75% for PFUnDA, PFDoDA, PFTDA, PFHxDA, and PFOcDA, and 95 to 179% for PFNA. No LOD and LOQ values were reported. The %R values for PFNA is fair. The lower limit of the %R values for PFNA is within the accepted range (80-120%) recommended by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018], whereas the upper limit is outside the 120% limit. The lower and upper limits of the %R for PFUnDA, PFDoDA, PFTDA, PFHxDA, and PFOcDA, are outside the acceptable range. It appears that out of 15 analytes 6 analytes were measured and

9 analytes remain unmeasured. Overall, the analytical method employed by Sinclair and Kannan [2005] is considered poor.

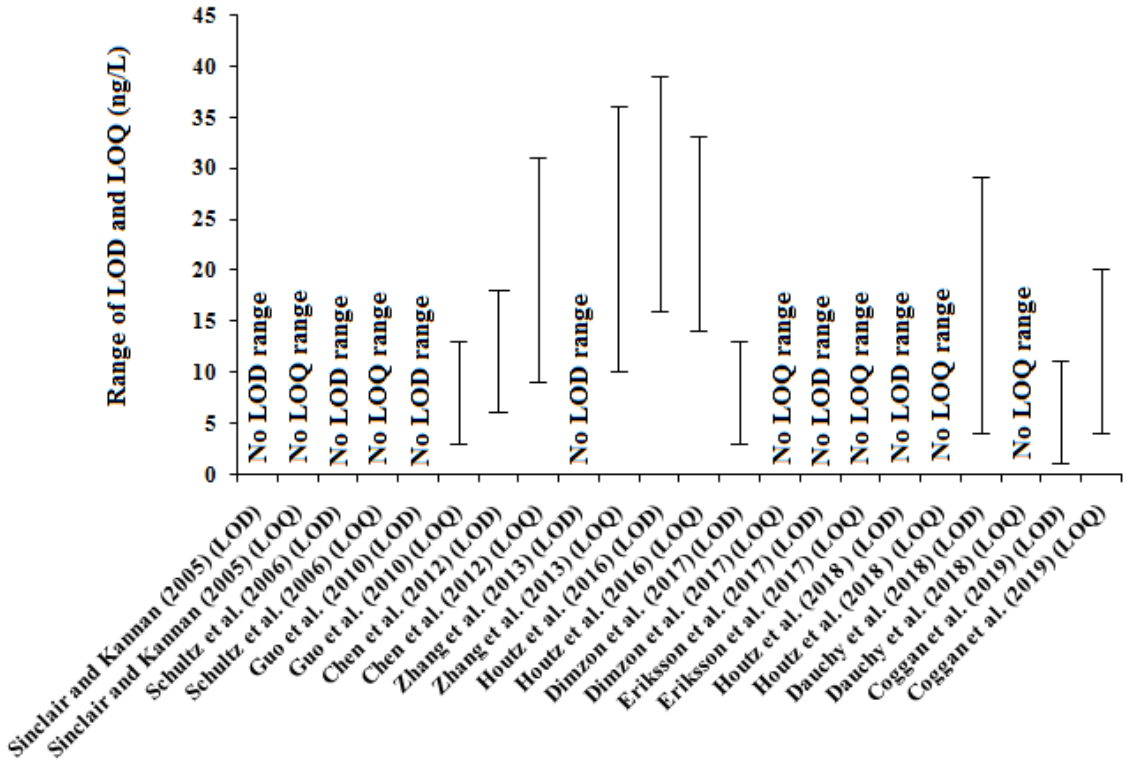


Figure 6: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in wastewater.

Schultz et al. [2006] analyzed wastewater samples collected from influents (raw wastewater) and final effluent of WWTPs in the State of Oregon, USA, and detected 15 PFAS from four classes (PFCAs, PFSAs, FOSA and FTs). The values of the %R for travel spikes are in a range from 87 to 98%, the R% for raw influent filed matrix spikes are from 77 to 96%, and the R% for final effluent filed matrix spikes are from 80 to 99%. The reported LOQ values are ranging from 0.5 to 3.0 ng/L and the RSD values (for single influent) are from 2 to18%, the RSD (for single effluent) are from 4 to 22%. In their paper, LOD values are not reported, and three types of the %R values are given, but all values are not acceptable. The lower limit of the %R for second type of spikes is

below the acceptable range. The precision (as RSD) for the method are good. These values indicate that the method is fair.

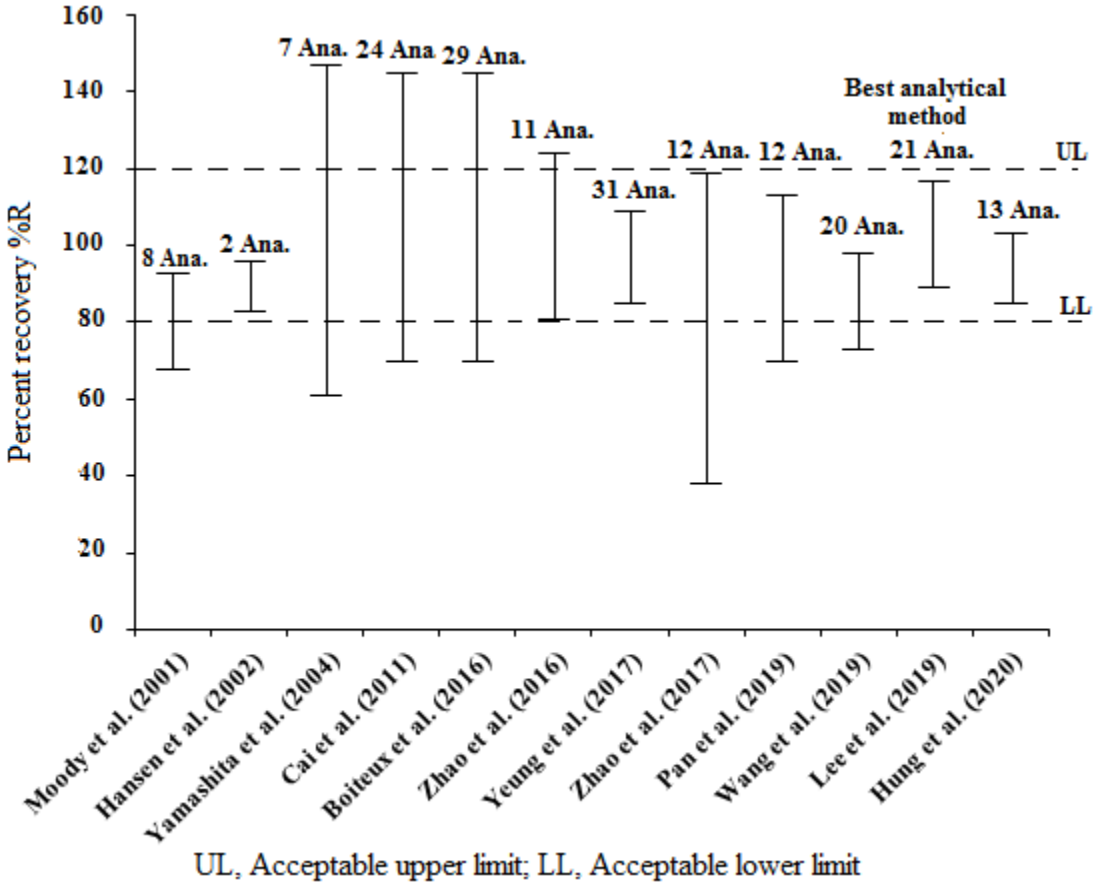


Figure 7: Percent recovery by the analytical methods for PFAS in wastewater.

In the study by Guo et al. [2010] wastewater samples were collected from influent and effluent of municipal, livestock and industrial WWTPs in Korea. In their study, 10 PFAS of two classes (PFCAs and PFSAs) were detected. The reported %R values are in a range from 69 to 119% (except for PFDS), the LOQ from 3 to 13 ng/mL, and the RSD is <20%.The %R values are below the lower limit provided by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. The RSD value is acceptable. No LOD values were provided. Overall, the analytical performance is rated fair.

Inanalyzing water collected from the sea that receives wastewater discharges from WWTPs in three coastal cities in China, Chen et al. [2012] detected 2 compounds from two classes (PFOA and PFOS) with the LOD values ranging from 6 to 18 ng/L, the LOQ from 9 to 31 ng/L, and the %R value from 87 to104%. The reported range of %R is acceptable. Overall, the performance is very good. The number of analytes is only two less than ten, which is the basic number of analytes considered for best analytical method.

In the research work by Zhang et al. [2013], wastewater samples were collected from the influent and effluent of 28 WWTPs in 11 cities in China including Dairy industry WWTP in Shanghai. In their efforts, 16 volatile PFAS of three classes (PFSAs, PFCAs and FOSAs) were detected. The reported LOQ values are in a range from 10 to 36 ng/L andthe %R values from75 to 109%. No LOD values were reported. The lower limit of the %R values is below the accepted range (80-120%) given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the analytical method used by Zhang et al. [2013] is ranked fair.

Houtz et al. [2016] analyzed treated final effluent of eight WWTPs that discharge to San Francisco Bay in California, U.S.A. In their study, 20 PFAS of five classes (Fts, FOSAAs, PFOPAs, PFSAs and PFCAs) were detected with the LOD values in a range from 16 to 39 ng/L, the LOQ values from 14 to 33 ng/L. The reported %R values are in a range between 80% and 131%. The reported precision (as RSD) is in a range between 4% and 16%. The upper limit of the %R values is above the range given by the Food and Agriculture Organization of the United Nation [FAO Animal

Production and Health 2018]. Overall, the analytical method employedby Houtz et al. [2016] is good.

In the study by Dimzon et al. [2017], wastewater samples were collected from industrial and municipal WWTPs in Netherlands and Germany. In their study, 13 volatile PFAS of two classes (PFAIs and FTs) were detected. The reported LOD values are in a range from 3 to 13ng/L. No value or range for the LOQ was reported. The %R range for the analytes and control standards are between 50% to 75% with high variation. The %R value is below the acceptable range considered in this study. Overall, the analytical method used by Dimzon et al. [2017] is fair.

In analyzing wastewater collected from three WWTPs that receives wastewater from industries and hospitals in Sweden, Eriksson et al. [2017] detected 54 PFAS of six classes (PFCAs, PFSAs, FOSA, FTs, PFPA and PAPs). Their method provided the %R values ranging from 52% to 90% for PFCAs, 74% to 83% for PFSAs, 49% to 78% for FTCA/FTUCAs, 74% to 81% for FTSAs, 32% to 40% for monoPAPs, and 22% to 32% for diPAPs. No values or ranges of LOD and LOQ were provided. The lower limit of the %R values is below the acceptable range considered in this present study. Overall, the analytical method employed by Eriksson et al. [2017] is considered poor.

In the study by Houtz et al. [2018], wastewater samples were collected from Trickling Filter plants that receive large quantities of AFFF discharges during annual Federal Aviation Authority (FAA) foam refractory testing at an airport in Berkeley California, USA. In their analysis, Houtz et al. [2018] detected 15 PFAS of four classes (FtTAoS, UPFSA, FTSHC and PFASAC). The reported

%R values are ranging from 80% to 150%. No LOD and LOQ values were reported. The upper limit of the %R value is above the accepted range provided by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the analytical method used by Houtz et al. [2018] is poor.

In analyzing wastewater discharged from a fire fighting training site in France, Dauchy et al. [2018] identified 34 PFAS of four classes (PFCAs, PFSAs, FOSAs and FTs). The reported LOD values are in a range between 4 and 29pg/L, and %R values ranging between70 and 120%. No LOQ data was reported. The lower limit of the %R values is below the acceptable range considered in the present study. Overall, the analytical method used by Dauchy et al. [2018] is considered fair.

In their research with domestic wastewater at nineteen WWTPs in Australia, Coggan et al. [2019] detected 21 PFAS off our classes (PFCAs, PFSAs, 6:2 FTs and Cl-PFESA) with the LOD values ranging from 1 to 11 pg/L and the LOQ values from 4 to 20pg/L. The %R values for the LCS samples are in a range from 80 to 120%, and the %R values for PFDS, 8:2 Cl-PFESA, PFTrA, and PFTeA are in a range from 70 to 76%. The %R values for16analytes (of 21) are within the range provided by the Food and Agriculture Organization of United Nation [FAO Animal Production and Health 2018]. These results suggest that theanalytical method employed by Coggan et al. [2019] is very good. This method is selected as the best method currently available for wastewater.

4.4 Sludge and Biosolids

For the analytical methods for PFAS in sludge and biosolids, review results are summarized in Table 5, the reported ranges of the LOD and LOQ values are presented in Figure 8, and the ranges of the percent recovery (%R) values are shown in Figure 9.

Table 5: Analytical methods for PFAS in sludge and biosolids.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Coggan et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from four classes (PFCAs, PFSAs, 6:2 FTs and Cl-PFESA)	Three replicate solid samples were collected from each of noneteen Australian WWTPs in 50 mL polypropylene centifuge tube	LOD range 15-33 pg/g; LOQ range 21-44 pg/g; %R in LCS samples range 80-120%; %R for 6:2 FTS was 61%.	PFAS were detected in all WWTP solid samples, the compounds PFOS, PFDoA, and PFTeA were detected in >90% of samples. Rating: Very good.
Eriksson et al. (2017)	LC-MS/MS	Targeted analysis	54 PFAS six classes (PFCAs, PFSAs, FOSA, FTs, PFPA and PAPs)	Sludge sample were taken from three WWTPs which receives water from industries and hospitals.	%R range 83%-92% for PFCAs, 86%-87% PFSAs, 66%-77% for FTCA/FTUCAs, 91%-132% for FTSAs, 53%-69% for monoPAPs, 25%-85% for diPAPs and 64%-86% for FOSA/FOSEs.	Several classes of precursors, intermediates and persistent PFASs were frequently detected. Rating: Poor.
Boiteux et al. (2016)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Sludge samples were collected in the river which receives wastewater from a training site using AFFF.	LOD is 11-27 ng/g; LOQ is 17-35 ng/g; %R range 77%- 114% except for PFTeDA (57%); Precision (RSD) below 20%.	The aim of this study was to optimise several analytical methods (oxidative conversion to PFAAs) for the determination of PFASs in sludge. Rating: Good.
Venkatesan and Halden (2013)	LC-MS/MS	Targeted analysis	9 PFAS from two classes (PFCAs and PFSAs)	Biosolids samples were collected by EPA from 94 WWTPs in 32 states and the District of Columbia as part of the 2001 National Sewage Sludge Survey.	LOD range 20-42 ng/g; %R range from 75%-110%; Precision as (RSD) was within 20%.	The study demonstrates both human exposure risk assessments and regulatory requirements for these recalcitrant PFAS chemicals. Rating: Fair.
Sindiku et al. (2013)	LC-MS/MS	Targeted analysis	20 PFAS from three classes (PFCAs, PFSAs and MPFAA)	Sewage Biosolids was sampled in four domestic WWTPs, five industrial effluent treatment plants and one hospital wastewater treatment plant.	LOD range 13-31 ng/g; LOQ range 18-36 ng/g; %R range 50%-104%.	PFASs were detected in all analyzed Nigerian sewage sludge samples from industrial, domestic and hospital WWTPs. PFOS is the most dominamt and detected PFASs. Rating: Good.
Chen et al. (2012)	High perfomance liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	2 PFAS from two classes (PFCAs-PFOA and PFSAs-PFOS)	Sludge sample were collected from the sea water which receiving wastewater discharge from WWTPs of three coastal cities of China.	LOD range 9-23 ng/Land LOQ range 17-40 ng/L; %R range 83%-94%.	Compared with other studies, PFOS and PFOA concentrations in sludge samples from WWTPs in China were comparable to those from Asia countries, but lower than those from Denmark and USA. Rating: Very good.
Li et al. (2009)	LC-MS/MS	Targeted analysis	15 PFAS from two classes (PFSAs and PFCAs)	Waste activated sludge, activated sludge of aeration tank and primary sludge were collected from eight WWTPs in Shanghai, China.	%R range 57% -115%; Precision of the method (RSD) range 2-18%.	The long chain PFCAs ($>C_8$) were more frequently detected in sludge samples than in soil. Rating: Poor.
Higgins et al. (2005)	LC-MS/MS	Targeted analysis	12 PFAS from three classes (PFSAs, PFCAs and FOSA)	Digested sludge samples were collected from eight WWTPs receiving 50% domestic wastewater.	LOD range 16-29 ng/g; LOQ range 21-37 ng/g; %R range 41%-91% for digested sludge and 37%-98% for primary sludge.	*N*-EtFOSAA was the dominant analyte in 6 of the 10 digested sludge samples, while PFOS was dominant in 4. Rating : Fair.

In the study by Higgins et al. [2005], digested sludge samples were collected from eight WWTPs that were receiving domestic wastewater in the San Francisco Bay Area, California, USA. In their work, 12 PFAS of three classes (PFSAs, PFCAs and FOSA) were detected. The reported LOD

values range from 16 to 29 ng/g, the LOQ values from 21 to 37 ng/g. The %R values are in a range between 41 and 91%for digested sludge and 37% and 98%for primary sludge. The lower limit of the %R values for these two types of sludge is far below the accepted range given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the analytical method used by Higgins et al. [2005] is evaluated to be fair.

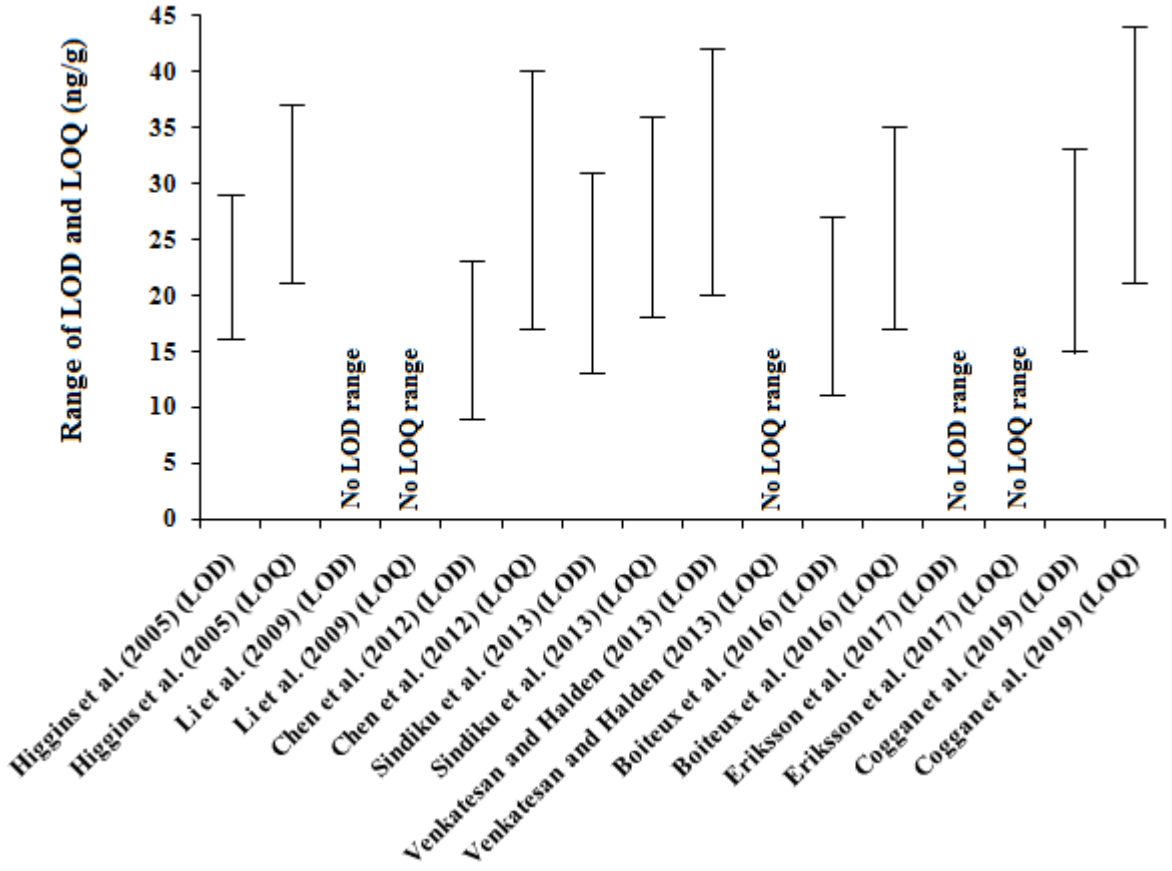


Figure 8: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in sludge and biosolids.

Li et al. [2009] analyzed waste activated sludge, mixed liquor suspended solids (activated sludge in an aeration tank), and primary sludge, collected from eight WWTPs in Shanghai, China. In their work, 15 PFAS of two classes (PFSAs and PFCAs) were detected. The reported %R values range

from 57 to 115%, the precision (RSD) values is in a range from 2 to 18%. No LOD and LOQ values were reported. The lower limit of the %R is below the acceptable limit (80%) considered in this present study. Overall, the analytical method employed by Li et al. [2009] is considered poor.

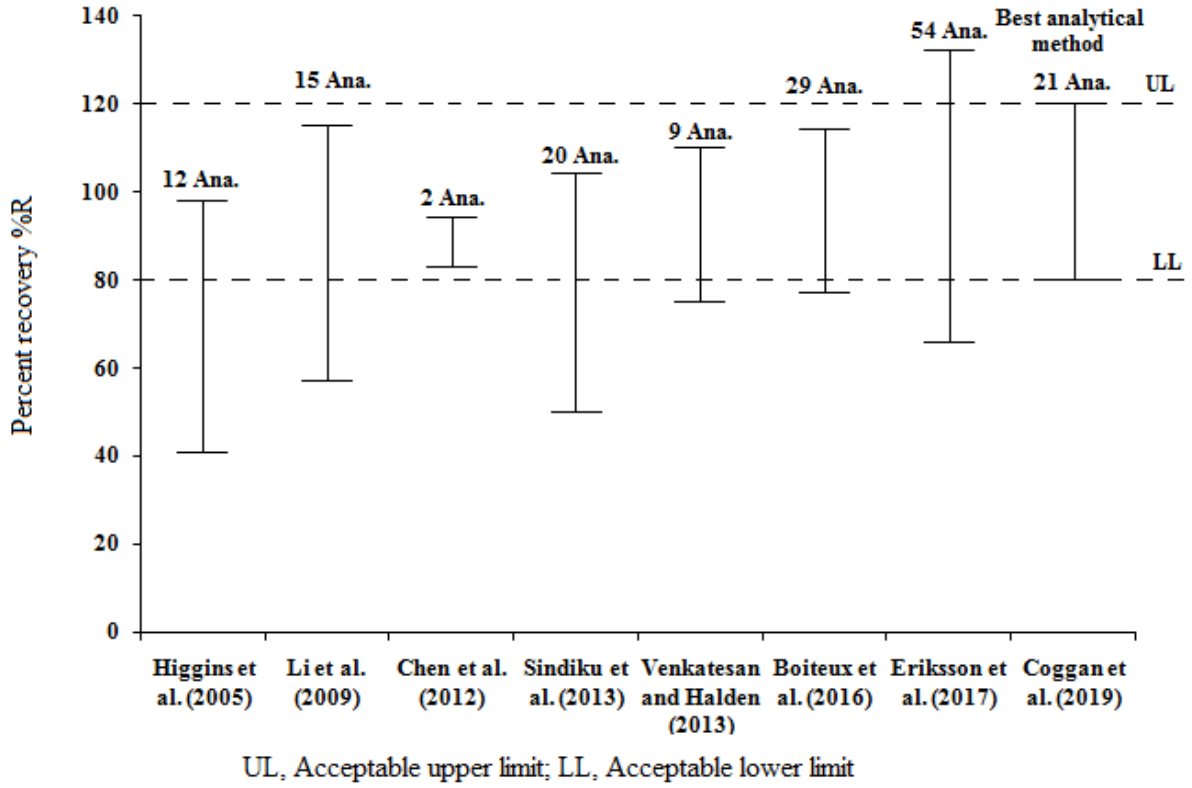


Figure 9: Percent recovery by the analytical methods for PFAS in sludge and biosolids.

In analyzing sludge samples collected from the sea that receives WWTP discharge in three coastal cities in China, Chen et al. [2012] detected 2 PFAS from two classes (PFCAs- PFOA and PFSAs-PFOS). The reported LOD and LOQ values are in a range from 9 to 23 ng/L and 17 to 40 ng/L, respectively. The reported %R values are in a range from 83 to 94%. These values indicate that the analytical method is very good; however, only two analytes were measured, less than 10 analytes considered for best analytical method.

Sindiku et al. [2013] analyzed sewage biosolids collected from four domestic WWTPs, five industrial effluent treatment plants, and one hospital wastewater treatment plant in Nigeria. They identified 20 PFAS of three classes (PFCAs, PFSAs and MPFAA) with the LOD and LOQ values rangingfrom13 to 31ng/g and 18 to 36ng/g, respectively. The reported %R values are in a range from 50 to 104%. The lower limit of %R is below the acceptable range of 80% set in the present study. Overall, the analytical method used by Sindiku et al. [2013] is considered good.

Biosolids samples were collected by the U.S. EPA from 94 WWTPs in 32 states and the District of Columbia as part of the 2001 National Sewage Sludge Survey. In analyzing these samples, Venkatesan and Halden [2013] identified 9 PFAS from two classes (PFCAs and PFSAs)with the LOD values ranging from 20 to 42 ng/g, the %R values between 75 and 110%, and the precision (RSD) within 20%. No LOQ values were reported. The lower limit of the %R values is below the acceptable range given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018].These results indicate that the method employed by Venkatesan and Halden [2013] is fair.

Boiteux et al. [2016] collected sludge from the river that receives wastewater from a fire-fighter training site (using AFFF) in France. In analyzing these samples, they identified 29 PFAS of three classes (PFSAs, PFCAs and FTs) with the LOD and LOQ values ranging from11 to 27 ng/g and 17 to 35 ng/g, respectively. The reported %R values range from 77 to114%, except for PFTeDA (57%). The precision (RSD) is below 20%. The lower limit of the %R values is below the acceptable limit of 80%. Overall, the analytical method employed by Boiteux et al. [2016] is considered good.

Analyzing sludge samples collected from three WWTPs that receive wastewater from industries and hospitals in Sweden, Eriksson et al. [2017] detected54 PFAS of six classes (PFCAs, PFSAs, FOSA, FTs, PFPA and PAPs). The reported %R values range from83 to 92% for PFCAs, 86 to 87% for PFSAs, 66 to 77% for FTCA/FTUCAs, 91 to 132% for FTSAs, 53 to 69% for monoPAPs, 25 to 85% for diPAPs, and 64 to 86% for FOSA/FOSEs. The %R rangesof PFCAs and PFSAs are good. The upper limit of the %R values for FTSAs is above the acceptable range provided by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018].The lower limit of the %R values for FTCA/FTUCAs, monoPAPs, diPAPs, and FOSA/FOSEs are below the acceptable range (80%). No LOD and LOQ values were reported. Overall, the analytical method by Eriksson et al. [2017] is ranked poor.

Coggan et al. [2019] collected three replicate sludge samples from each of nineteen WWTPs in Australia. In their analytical work, they detected 21 PFAS of four classes (PFCAs, PFSAs, 6:2 FTs and Cl-PFESA), with the LOD and LOQ values ranging from 15 to 33pg/L and 21 to 44 pg/L, respectively. The reported %R values range from 80 to120% for the laboratory control samples (LCS), and 61% for 6:2 FTS. The %R ranges for 20 analytes (except for 6:2 FTS) are within the range given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018].Overall, the analytical method employed by Coggan et al. [2019] is very good, and selected as the best method currently available for the analysis of sludge and biosolids.

4.5 Soil

For the analytical methods for PFAS in soil, review results are summarized in Table 6, the reported ranges of the LOD and LOQ values are presented in Figure 10, and the ranges of the percent recovery (%R) values are shown in Figure 11.

Table 6: Analytical methods for PFAS in soil

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Lee et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from three classes (PFCAs, PFSAs and FOSA)	Soil samples were collected from near the Asan Lake area of South Korea	LOD range 6-21 pg/g; LOQ range 11-29 pg/g; %R range 88%- 122%.	16 PFASs were measured and 12 were detected. PFCAs in soil samples accounted for 76.6% of PFAS, the pridominant PFAS in soils was PFOA followed by PFOS. Rating: Good.
Boiteux et al. (2016)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Three surface soil samples were collected at different locations on the training site, one of the soil samples was collected in an area suspected to have been used as a training area before the 1970s.	LOQ is 2 to 11 ng/g; %R are 59%- 120% with precision as RSDs over 30%.	The aim of this study was to optimise several analytical methods (different extraction techniques to optimise and couple to LC-MS/MS analysis) for the determination of PFASs in soil. Rating: Fair.
Anderson et al. (2016)	LC-MS/MS	Targeted analysis	16 PFAS from three classes (PFSAs, PFCAs and FOSA)	A total of ten active US Air Force installations were selected for sampling throughout the continental	No method performance evaluation criteria (LOD, LOQ and %R) values are mentioned	Statistical analysis (linear discriminant analysis) was used to evaluate inter-media variability as a function of all 16 PFASs are being analyzed. No rating.
Meng et al. (2015)	LC-MS/MS	Targeted analysis	14 PFAS from two classes (PFCAs and PFSAs)	A total of seventy-nine surface soils were collected from estuarine and coastal areas adjacent to the Bohai and Yellow Seas in China.	LOD range 8-21 ng/g; LOQ range 10-30 ng/g; %R range 86%- 119%.	The urbanization and industrialization in the coastal region are growing dramatically along with the rapid economic development in China. As a result PFASs produced from production and consumption becaome urgent environmental issues. Rating: Very good.
Xiao et al. (2015)	Liquid chromatography interfaced with triple quadrupole mass spectometry	Targeted analysis	2 PFAS from two classes (PFSAs and PFCAs)	Soil samples were collected at 28 sites along the U.S. Highway 10 from the City of Cottage Grove to the City of Big Lake in St. Paul metropolitan area, Minneapolis.	LOD are 13 and 27 ng/g for PFOS and PFOA; %R are 115% and 100% for PFOS and PFOA .	PFOS and PFOA compounds were found in all of the soil samples collected, the adsorption of PFOS and PFOA highly depends on the organic carbon content and available cations. Rating: Very good.
Houtz et al. (2013)	LC-MS/MS	Targeted analysis	42 PFAS from three classes (PFSAs, PFCAs and FTs)	Soil samples were collected from a location at Ellsworth Air Force Base in Piedmont firefighting training center, South Dakota.	LOD range 6-19 ng/g; %R range 56%- 131% ; Precision (RSD) range 1%- 13%.	Perfluorinated sulfonates (PFSAs) and perfluoroalkyl acid (PFAAs) precursors acxcounted for a large fraction of total PFAS conecntration in soil samples. Rating: Fair.
Washington et al. (2010)	LC-MS/MS	Targeted analysis	18 PFAS from three classes (PFSAs, PFCAs and FTs)	Soil samples were collected by USEPA regional scientists from 2 sludge-applied fields and 1 sludge-free background field at Decatur, Alabama.	%R range 59%-112%.	In the sludge-applied surface soils, PFA analytes summed to as high as 5 µg/g and short-chain concentrations generally fell with increasing time since last sludge application. Rating: Poor.
Li et al. (2009)	LC-MS/MS	Targeted analysis	15 PFAS from two classes (PFSAs and PFCAs)	Surficial soils were collected from agricultural, residential and industrial areas in Shanghai, China in triplicate.	%R range 73% -112%; Precision of the method (RSD) range 1-19%.	The dominant analyte in soil samples was TFA, which followed by PFOA and PFOS in most cases, the short-chain PFCAs were more frequently detected than the long-chain. Rating: Poor.

In their study with surface soils in agricultural, residential and industrial areas in Shanghai, China, Li et al. [2009] detected 15 PFAS of two classes (PFSAs and PFCAs) with the %R between 73%

and 112%, and the precision (RSD) ranging from 1 to 19%. No LOD and LOQ values were reported. Although the precision range is acceptable, the lower limit of the %R valuesis below the acceptable limit provided by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the analytical method used by Li et al. [2009] is considered poor.

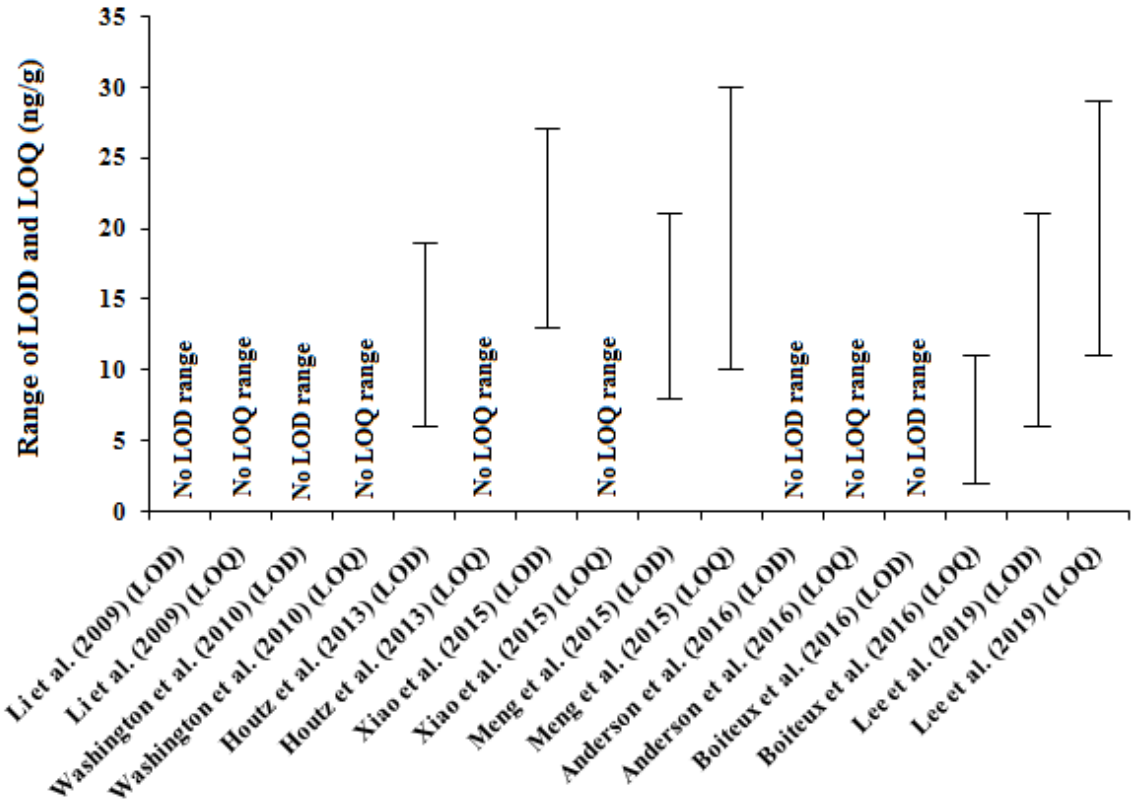


Figure 10: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in soil.

Washington et al. [2010] analyzed soil samples collected from two sludge-applied fields and one sludge-free background field at Decatur, Alabama, USA. In their work, they detected 18 PFAS of three classes (PFSAs, PFCAs and FTs) with the %R values in a range from 59% to 112%. No LOD and LOQ values were reported. The lower limit of the %R values is below the acceptable

limit (80%) considered in the present study. The statistical results indicate that the analytical method employed by Washington et al. [2010] is poor.

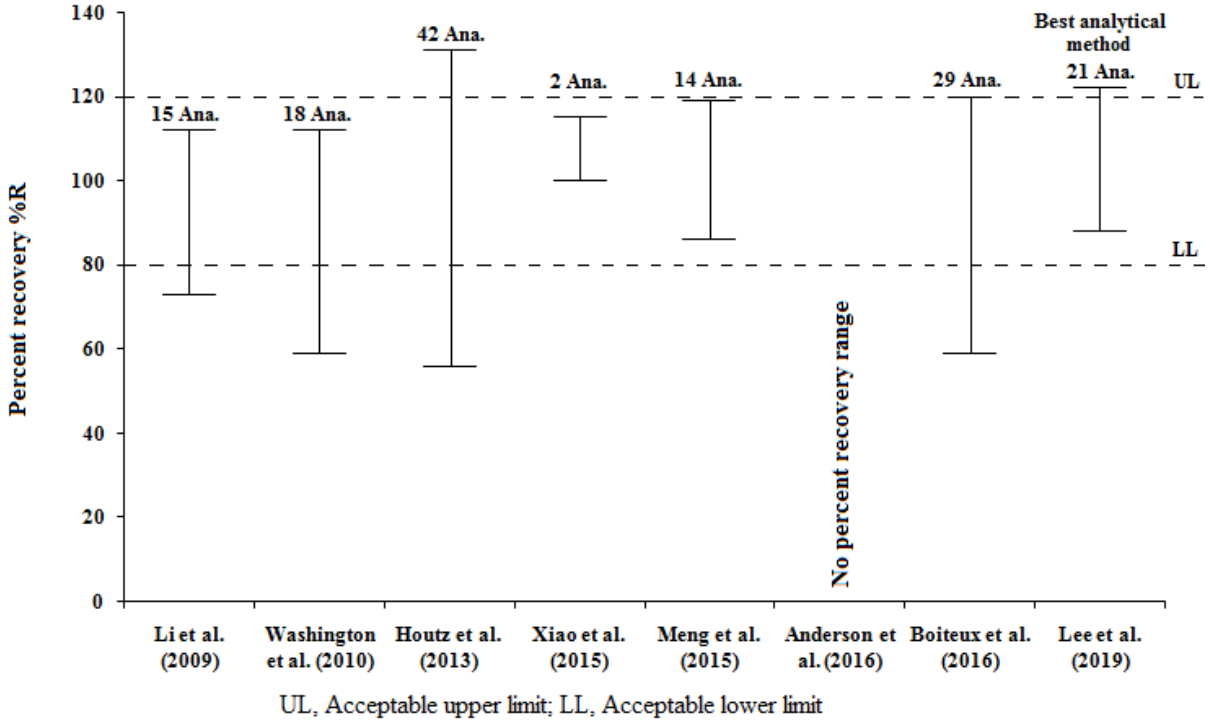


Figure 11: Percent recovery by the analytical methods for PFAS in soil.

In the study by Xiao et al. [2015], soil samples were collected along the U.S. Highway 10 from the City of Cottage Grove to the City of Big Lake in St. Paul metropolitan area, Minneapolis, USA. In analyzing these samples, Xiao et al. [2015] detected 2 PFAS of two classes (PFSAs and PFCAs) with the LOD values of 13 ng/g for PFOS and 27 ng/g for PFOA. No LOQ values were reported. The reported %R values are 115 and 100% for PFOS and PFOA, respectively. These %R values are acceptable. The results indicate that the analytical method employed by Xiao et al. [2015] is very good. But only two analytes were reported.

Meng et al. [2015] analyzed surface soils collected from the estuarine and coastal areas adjacent to the Bohai and Yellow Seas in China, and detected 14 PFAS of two classes (PFCAs and PFSAs) with the LOD and LOQ values ranging from 8 to 21 ng/g and 10 to 30 ng/g, respectively. The reported %R values are between 86% and 119%. Overall, the performance is very good, and the method employed by Meng et al. [2015] is selected as the best analytical method currently available for soil.

Anderson et al. [2016] collected soil samples from the US Air Force installations throughout the continental United States. In their work, 16 PFAS of three classes (PFSAs, PFCAs and FOSA) were detected. No values were provided for the LOD, LOQ, nor %R. They carried out statistical analysis (linear discriminant analysis) to obtain the inter-media variability for the 16 PFASs analyzed.

In analyzing soil samples collected from different locations in the fire fighter training sites in France, Boiteux et al. [2016]identified29 PFAS of three classes (PFSAs, PFCAs and FTs).The reported LOQ values are in a range from 2 to 11 ng/g, the %R is between 59% and 120%, and the precision (as RSDs) is over 30%. No LOD value or range was reported. The lower limit of the %R is outside the acceptable limit (80%) given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Precision (as RSD) value is lower than the acceptable level (RSD≤20%). Overall, the analytical method used by Boiteux et al. [2016] is considered fair.

Lee et al. [2019] collected soil samples near the Asan Lake area in South Korea. In analyzing these samples, they identified 21 PFAS of three classes (PFCAs, PFSAs and FOSA) with the LOD and LOG values ranging from 6 to 21 pg/g and 11 to 29 pg/g, respectively. The reported %R values are in a range from 88 to 122%. The upper limit of the %R is outside the acceptable limit (120%) considered in the present study. Overall, the analytical method employed by Lee et al. [2019] is good.

4.6 Sediment

For the analytical methods for PFAS in sediment, review results are summarized in Table 7, the reported ranges of the LOD and LOQ values are presented in Figure 12, and the ranges of the percent recovery (%R) values are shown in Figure 13.

Table 7: Analytical methods for PFAS in sediment.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Hung et al. (2020)	LC-MS/MS	Targeted analysis	13 PFAS from three classes (PFCAs, PFSAs, FTs)	Sediment samples were collected from three special management sea area in Korea (Gwangyang Bay, Mansan Bay and Busan Harbor)	LOD range 17-34 pg/g; %R for $^{13}C_4$ PFOA and $^{13}C_4$ PFOS are 98.88% and 87%.	The average PFAS concentration in the sediment samples was generally lower than the LOD. Rating: Fair.
Lee et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from three classes (PFCAs, PFSAs and FOSA)	Sediment samples were collected from Asan Lake area of South Korea	LOD range 10-26 pg/g; LOQ range 16-32 pg/g; %R range 89%- 109%.	16 PFASs were measured and 14 were detected. The predominant PFAS analyte in sediments was PFOS, followed by PFDA. Rating: Very good.
Wang et al. (2019)	LC-MS/MS	Targeted analysis	20 PFAS from three classes (PFCAs, and PFSAs) and HFPO-DA, ADONA, 6:2 Cl-PFESA, 8:2 Cl-PFESA	Sediment samples were collected from South China Sea (SCS) region.	LOQ range from 13-35 pg/g; %R range 68%-98%.	Of the 20 targeted compounds, 11 PFASs were detected in sediment, PFPeA, PFDS, PFDoDA, PFTeDA, PFHxDA, PFOcDA, ADONA, 8:2 Cl-PFESA and HFPO-DA were all below the LOQ. Rating: Fair.
Munoz et al. (2017)	LC-MS/MS	Targeted analysis	17 PFAS from three classes (PFCAs, PFSAs and FOSA)	Sediments were collected in the lake Megantic and along a logitudinal gradient in the Chaudiere River.	LOD range 8-19 ng/g; LOQ range 15-28 ng/g; %R range 76%-95% .	In sediments from Lake Megantic and Chaudiere river, up to 6 PFAAs were detected, a low burden compared to urban environments in Canada. Rating: Good.
Boiteux et al. (2016)	LC-MS/MS	Targeted analysis	29 PFAS from three classes (PFSAs, PFCAs and FTs)	Sedimeny samples were collected in the river which receives wastewater from a training site using AFFF.	LOQ is 13 to 29 ng/g; %R are 78%- 113% except for PFTeDA (62%) and FOSA (59%).	The aim of this study was to optimise several analytical methods (oxidative conversion to PFAAs) for the determination of PFASs in sediment. Rating: Fair.

Table 7: Continued.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Anderson et al. (2016)	LC-MS/MS	Targeted analysis	16 PFAS from three classes (PFSAs, PFCAs and FOSA)	A total of ten active US Air Force installations were selected for sampling throughout the continental United States.	No method performance evaluation criteria (LOD, LOQ and %R) values are mentioned	Statistical analysis (linear discriminant analysis) was used to evaluate inter-media variability as a function of all 16 PFASs are being analyzed. No rating.
Zhao et al. (2016)	LC-MS/MS	Targeted analysis	11 PFAS from two classes (PFCAs- PFBA, PFPeA, PFHxA, PFHpA PFOA, PFNA, PFDA, PFUdA, PFDoA and PFSAs- PFOS, PFBS)	Sediment samples were collected from 20 sites along the middle and lower reaches of the Yellow River, 14 of which were at the main stream and the other 6 sites were at major tributaries.	LOD range 7-17 µg/g; LOQ range 12-25 µg/g; %R are 51%- 96%.	PBAS was only detected at 16 sites while other PFASs were detected at all the sampling sites. For the main stream, the maximum value was observed at the Ashan station, with PFOA and PFPnA being the predominant compounds. Rating: Fair.
Li et al. (2009)	LC-MS/MS	Targeted analysis	15 PFAS from two classes (PFCAs and PFSAs)	Surficial sediment samples were collected from Huangpu River and Suzhou River in Shanghai, China.	%R are 66%- 111%; Precision of the method (RSD) range 1-15%.	All the sediments contained at least nine PFASs monitored in this study at measurable concentrations. Rating: Poor.
Higgins et al. (2005)	LC-MS/MS	Targeted analysis	12 PFAS from three classes (PFSAs, PFCAs and FOSA)	Surficial sediment samples were collected from the outlets of various rivers and creeks in the San Francisco Bay Area.	LOD range 11-24 µg/g; LOQ range 14-33 µg/g; %R range 73%- 98% for extraction spike and 56%-93% for aged spike.	No single analyte was detected in every sediment, PFOS, PFDS, *N*-MeFOSAA, *N*-EtFOSAA, PFOA and PFDA were the most commonly detected PFASs. Rating: Fair.

In the study by Higgins et al. [2005], sediment samples were collected from the outlets of various rivers and creeks in the San Francisco Bay Area in the U.S. In analyzing these samples, they detected 12 PFAS of three classes (PFSAs, PFCAs and FOSA) with the LOD and LOQ values ranging from 11 to 24µg/g and 14 and 33µg/g, respectively. The reported %R values arein a range between 73% and 98% for extraction spike and 56-93% for aged spike. The LOD and LOQ values are at a level of µg/g, which can be enhanced by using a second or third generation LC-MS/MS instrument. The lower limits of the %R for both types of spike are below the accepted value of 80% given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018].Overall, the performance is fair.

Li et al. [2009] collected sediment samples from the Huangpu River and the Suzhou River in Shanghai, China. In their work, 15 PFAS of two classes (PFCAs and PFSAs) with the %R values ranging from 66 to 111%, and the precision (RSD) ranging from 1 to 15%. No LOD and LOQ

values were reported. The lower limit of the %R is below the acceptable limit of 80% considered in the present study. The precision range for the method is acceptable. Overall, the results indicate that the analytical method used by Li et al. [2009]is poor.

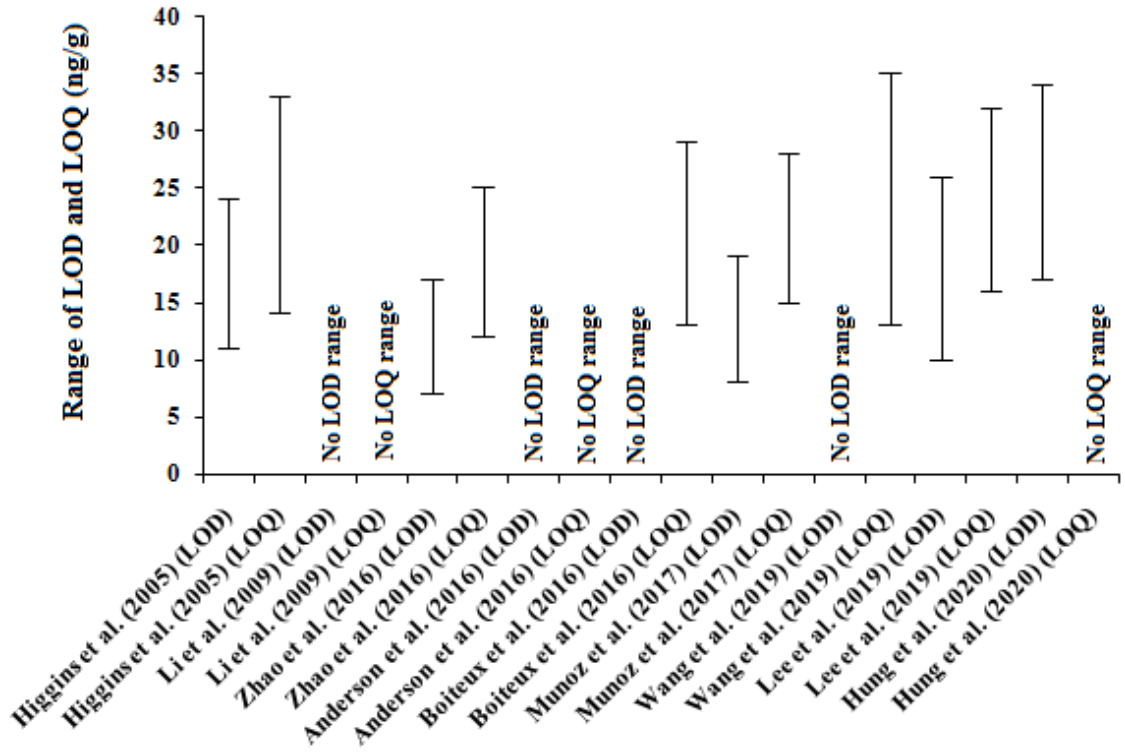


Figure 12: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in sediment.

In analyzing sediment samples collected from 20 sites along the middle and lower reaches of the Yellow River in China, Zhao et al. [2016] detected 11 PFAS of two classes (PFCAs and PFSAs) with the LOD and LOQ values ranging from 7 to 17 µg/g and from 12 to 25 µg/g, respectively. The reported %R values are in a range from 51 to 96%.The lower limit of the %R values is below the acceptable 80% limit provided by the Food and Agriculture Organization of the United Nation[FAO Animal Production and Health 2018].The LOD and LOQ values are at a level of µg/g,

which can be enhanced by using second or third generation LC-MS/MS instrument. Overall, the analytical method used by Zhao et al. [2016] is considered fair.

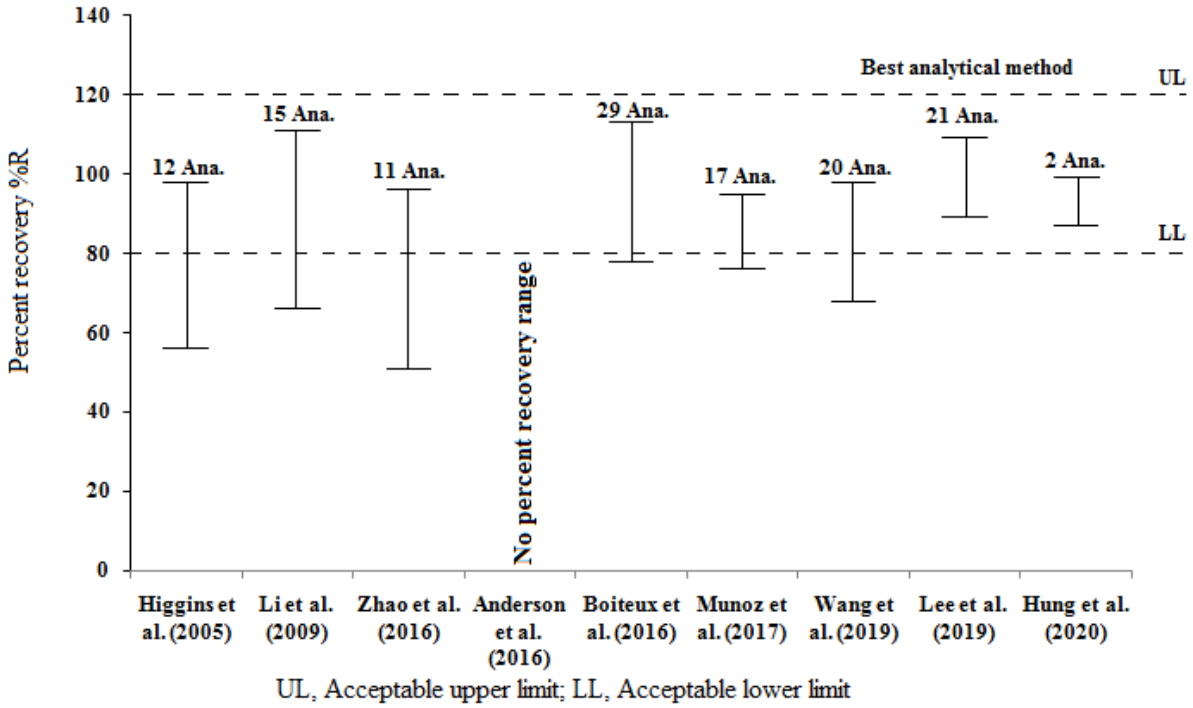


Figure 13: Percent recovery by the analytical methods for PFAS in sediment.

In a study developed by Anderson et al. [2016], sediment sample were collected from a total of ten active US Air Force installations selected throughout the continental United States.In their study, 16 PFAS of three classes (PFSAs, PFCAs and FOSA) were detected. Novalues were reported for LOD, LOQ, %R or RSD. Instead, linear discriminant analysis was carried out to obtain the inter-media variability for the 16 PFAS analyzed.

Boiteux et al. [2016] analyzed sediment samples collected from the river that receives wastewater from a fire-fighting training site using AFFF in France, and identifies 29 PFAS of three classes (PFSAs, PFCAs and FTs).They reported the LOQ values in a range from 13 to 29ng/g. The %R

values are in a range from 78 to 113%, except for PFTeDA (62%) and FOSA (59%). No values or ranges were reported for LOD. The lower limit of the %R values is slightly below the acceptable 80% limit considered in the present study. The result indicates that the analytical method used by Boiteux et al. [2016] is fair.

In the study by Munoz et al. [2017], sediments samples were collected from the Lake Megantic and along the longitudinal gradient of the Chaudiere River in Quebec, Canada. In their work, 17 PFAS of three classes (PFCAs, PFSAs and FOSA) were detected with the LOD and LOQ values are in a range from 8 to 19 ng/g and 15 to 28 ng/g, respectively. The reported %R values are in a range between 76% and 95%.The lower limit of the %R value is below the acceptable 80% limit suggested by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the results indicate that the analytical method used by Munoz et al. [2017] is good.

In analyzing sediment samples collected from the South China Sea (SCS) region, Wang et al. [2019] identified 20 PFAS of three classes (PFCAs, and PFSAs and HFPO-DA, ADONA, 6:2 Cl-PFESA, 8:2 Cl-PFESA). The reported LOQ values are in a range from 13 to 35 pg/g and the %R values from 68 to 98%.No LOD values were reported. The lower limit of the %R values is below the acceptable limit of 80%. The range of the LOQ value is in a pg/g level. The analytical method used by Wang et al. [2019] is considered fair.

Lee et al. [2019] analyzed sediment samples collected from the Asan Lake area in South Korea, and detected21 PFAS of three classes (PFCAs, PFSAs and FOSA). The LOD and LOQ values are in a range from 10 to 26 pg/g and 16 to 32pg/g, respectively. The reported %R values are in a range from 89 to 109%. Overall, the analytical method employed by Lee et al. [2019] is very good and selected as the best method currently available for the sediment analysis.

In the study by Hung et al. [2020], sediment samples were collected from three special management sea area in Korea (Gwangyang Bay, Mansan Bay and Busan Harbor).In their study, 13 PFAS of three classes (PFCAs, PFSAs, FTs)were detected with the LOD range from 17 to 34pg/g. No LOQ values were reported. The reported %R values for $^{13}C_4$ PFOA and $^{13}C_4$ PFOS are 98.88% and 87%, respectively. The %R values are acceptable for 2 analytes ($^{13}C_4$ PFOA and $^{13}C_4$ PFOS) from 13 PFAS. The results indicate that the analytical method used by Hung et al. [2020] is fair.

4.7 Air

For the analytical methods for PFAS in air, review results are summarized in Table 8, the reported ranges of the LOD and LOQ values are presented in Figure 14, and the ranges of the percent recovery (%R) values are shown in Figure 15.

Table 8: Analytical methods for PFAS in Air.

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Lee et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from three classes (PFCAs, PFSAs and FOSA)	Air samples were collected 1 m above groung level from near the Asan Lake area of South Korea	LOD range 7-23 pg/m^3; LOQ range 9-26 pg/m^3; %R range 87%- 108%.	Of the 16 PFASs species measured, 11 were detected in air samples. The pridominant PFAS species in the air was PFOA (75.9% of the total). Rating: Very good.
Dimzon et al. (2017)	GC-MS/MS	Targeted analysis	13 volatile PFAS of two classes (PFAIs and FTs)	Air sample were collected from different industrial and municipal WWTPs in Netherlands and Germany.	LOQ range 6-27 ng/m^3; %R range 60%-120% by volatilization and direct addition method for ten analytes and 80%-100% for FTOs, PFAIs and FTIs.	Substantially high amounts of 6:2-FTMAC per liter of air were detected in the air above the industrial WWTP influent. Rating: Fair.
Zhao et al. (2017)	LC-MS/MS	Targeted analysis	12 PFAS from three classes (PFSAs, PFCAs and FOSA)	The study area covered the Bohai Sea, Yellow Sea and Yangtze River estuary in China.	LOD range 10-25 ng/m^3; LOQ range 12-31 ng/m^3; %R range 38% -119%.	In the gas phase, 10 neutral PFASs were detected, i.e., 6:2, 8:2, 10:2 and 12:2 FTOH; 8:2 FTAC; MeFOSA, EtFOSA; MeFBSA; EtFOSE; and MeFBSE. Rating: Good.
Vento et al. (2012)	Gas Chromatography couple with thermo DSQ Quadrupole, Mass Spectometer	Targeted analysis	11 PFAS from three classes (FTOHs. FASAs and FASEs)	Sampling of air was undertaken over the geographical region of 54-69 °S and 60-75 °W, in places close to the Anterctica Peninsula.	%R range 45%-129% except for *N*-MeFOSE (137%) and *N*-EtFOSE (148%).	The most abundant compounds in air were in the order 8:2 FTOH >10:2 FTOH > MeFBSA ~ MeFBSE. The FTOHs were the dominant compounds. Rating: Poor.

In the research work by Vento et al. [2012], air samples were collected over the geographical region (54-69^{o}S, 60-75^{o}W) in the places close to the Antarctica Peninsula. In those samples, 11 PFAS of three classes (FTOHs. FASAs and FASEs) were detected. No LOD or LOQ values were reported. The %R values are in a range from 45 to 129%, except for *N*-MeFOSE(137%) and *N*-EtFOSE (148%). The lower and upper limit of the %R values is outside the acceptable range suggested by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. The results suggest that the analytical method used by Vento et al. [2012] is poor.

In the study by Zhao et al. [2017], the air-sampling are as covered the Bohai Sea, Yellow Sea, and Yangtze River Estuary in China. In their work, 12 PFAS of three classes (PFSAs, PFCAs and FOSA) were detected with the LOD and LOQ values ranging from 10 to 25ng/m^3 and from 12 to 31ng/m^3, respectively.The reported %R values are in a range between38% and 119%. The lower

limit of the %R value is below the acceptable limit (80%) considered in the present study. The results indicate that the analytical method used by Zhao et al. [2017] is good.

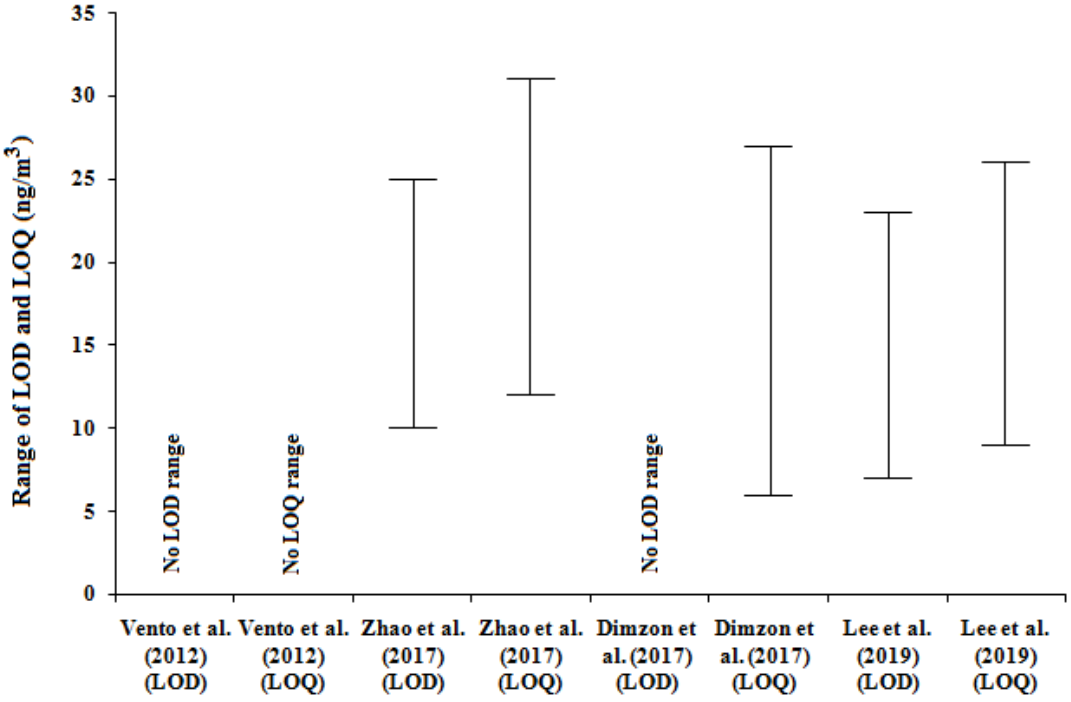


Figure 14: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in Air.

Dimzon et al. [2017] collected air samples from different industrial and municipal WWTPs in Netherlands and Germany. By applying a volatilization and direct addition method, they detected 13 volatile PFAS of two classes (PFAIs and FTs) with the LOQ values ranging from 6 to 27ng/m^3. No LOD values were reported. The reported %R values fall in between60 and 120% for ten analytes, and 80 and 100% for FTOs, PFAIs, and FTIs. The %R range is acceptable for three analytes (FTOs, PFAIs and FTIs), whereas the lower limit of the %R is below the acceptable limit for ten analytes. Overall, the analytical method employed by Dimzon et al. [2017] is considered fair.

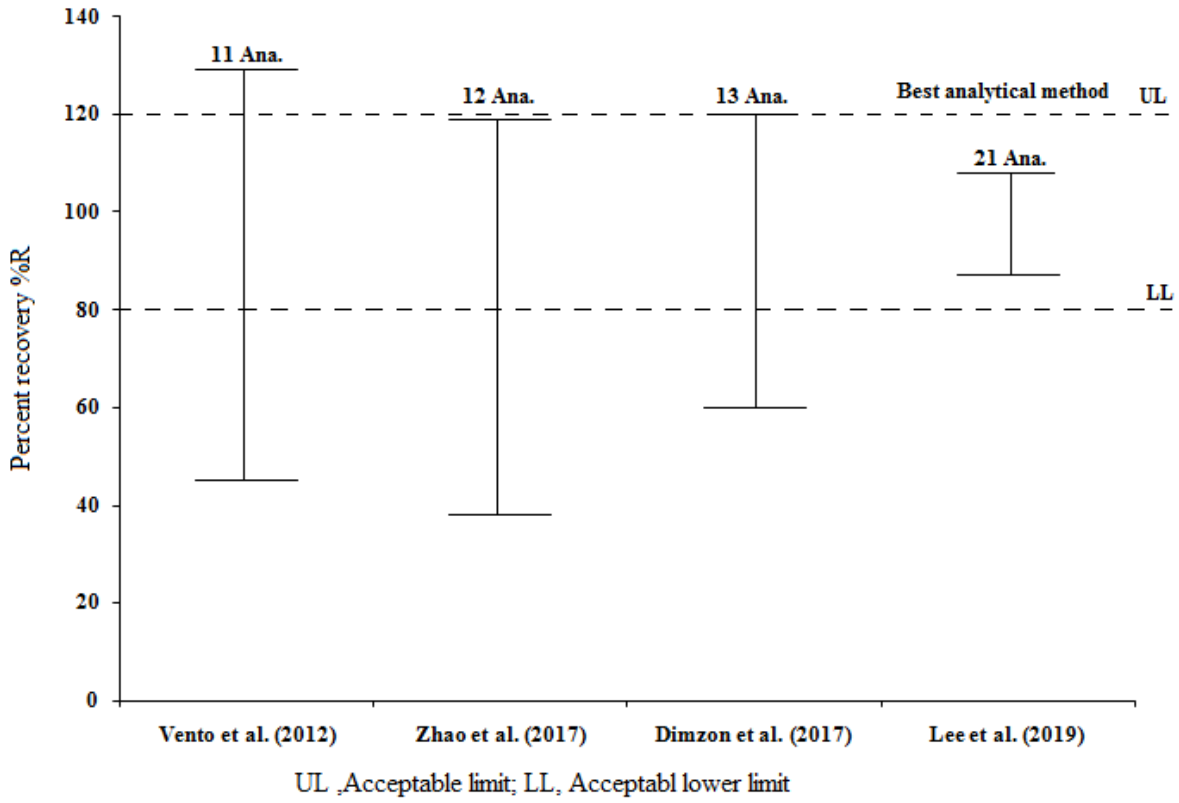


Figure 15: Percent recovery by the analytical methods for PFAS in air.

In the study by Lee et al. [2019], air samples were collected 1 m above ground level near the Asan Lake area in South Korea. In their work, 21 PFAS of three classes (PFCAs, PFSAs and FOSA) were identified with the LOD and LOQ values ranging from 7 to 23 pg/m^3 and9 to26 pg/m^3, respectively. The reported %R values are in a range between 87 and 108%.The results indicates that the analytical method used by Lee et al. [2019] is very good. This method is selected as the best method currently available for the analysis of air samples.

4.8 Biota (fish)

For the analytical methods for PFAS in biota (fish), review results are summarized in Table 9, the reported ranges of the LOD and LOQ values are presented in Figure 16, and the ranges of the percent recovery (%R) values are shown in Figure 17.

Table 9: Analytical methods for PFAS in biota (fish)

Authors (Year)	Analytical Instrument	Analytical approach	PFAS investigated	Media, sampling sites, application	Performance: Limit of Quantification (LOQ); Limit of Detection (LOD); Percent Recovery (%R)	Comments
Hung et al. (2020)	LC-MS/MS	Targeted analysis	13 PFAS from three classes (PFCAs, PFSAs, FTs)	Fish samples were collected from three special management sea area in Korea (Gwangyang Bay, Mansan Bay and Busan Harbor)	LOD range 15-27 pg/g; %R for $^{13}C_4$ PFOA and $^{13}C_4$ PFOS are 94.9% and 90.5% for blood, 105.87% and 84.62 for muscle and 99.65% and 97.77% for liver.	The highest PFAS concentrationwas found in the flathead grey mullet in Busan Bay, followed by the Japanese amberjack fish sample. Rating: Fair.
Lee et al. (2019)	LC-MS/MS	Targeted analysis	21 PFAS from three classes (PFCAs, PFSAs and FOSA)	Fish specimens of crucian carp, skygager, bluegill, bass, barbel steed and common carp, were collected from Asan Lake.	LOD range 12-25 pg/g; LOQ range 17-33 pg/g; %R range 82%- 116% for fish muscle.	Of the 19 PFAS species measured, 18 were were detected in fish samples. Rating: Very good.
Munoz et al. (2017)	High performance liquid chromatography coupled to a Q-Exactive Orbitrap mass spectrometer.	Targeted analysis	17 PFAS from three classes (PFCAs, PFSAs and FOSA)	Following the accident, white suckers were collected from Lake Megantic as well as from the Chaudiere River downsream from the AFFF-impacted site.	LOD range from 9-23 ng/g; LOQ range from 15-34 ng/g; %R range 74%-101% except for PFTeDA (41%) for fish muscle; Precision as (RSDs) remained <13%.	In adult fish muscle, PFOS and long-chain PFCAs were found systematically and high detection frequencies were also reported for PFNA and PFTeDA. Rating: Poor.

In the research work by Munoz et al. [2017], white suckers were collected from Lake Megantic and the Chaudiere River downstream from the AFFF-impacted site. In their study, 17 PFAS of three classes (PFCAs, PFSAs and FOSA) were identified with the LOD and LOQ values in a rangefrom 9 to 23 ng/g and 15 to 34 ng/g, respectively. The reported %R values fall between 74 and 101%, except for PFTeDA (41%) in white suckers muscle. The analytical precision (RSD) is <13%. The lower limit of the %R values is below the accepted range given by the Food and Agriculture Organization of the United Nation [FAO Animal Production and Health 2018]. Overall, the results suggest that the analytical method used by Munoz et al. [2017] is poor.

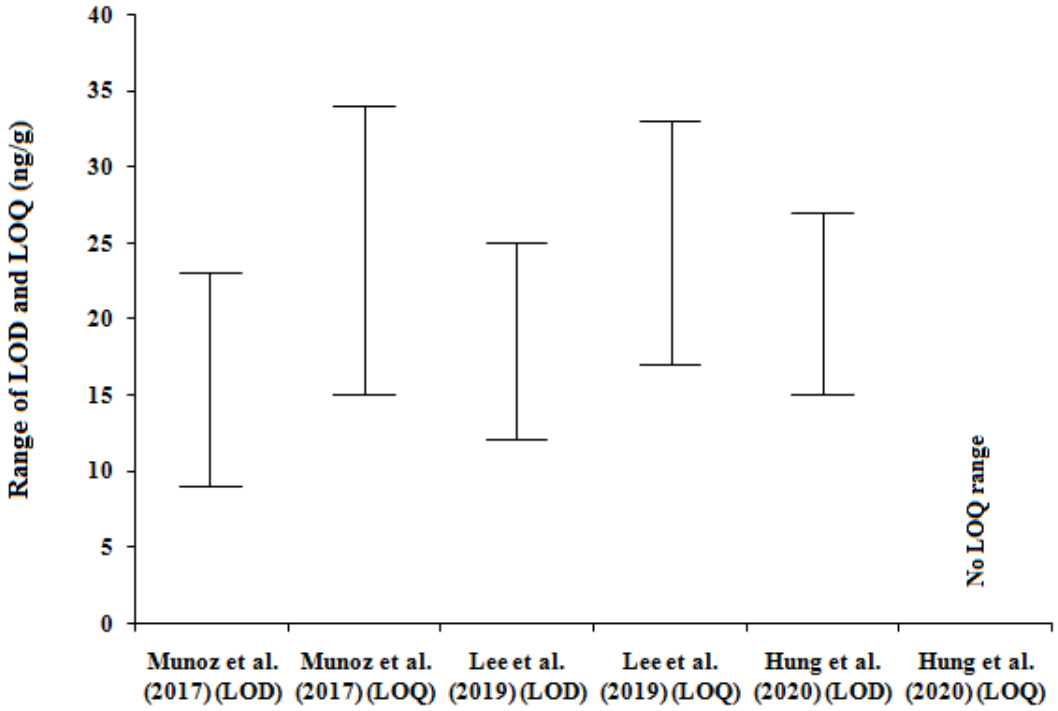


Figure 16: Limit of detection (LOD) and limit of quantification (LOQ) by the analytical methods for PFAS in biota (fish).

Lee et al. [2019] analyzed fish specimens of crucian carp, skygager, bluegill, bass, barbel steed, and common carp; all of which were collected from Asan Lake in Korea. In their work,21 PFAS from three classes (PFCAs, PFSAs and FOSA) were detected with the LOD and LOQ valuesranging from 12 to 25pg/g, and 17 to 33pg/g, respectively. The reported %R values are in a range from 82% to 116% for muscle tissues. These results indicate that the analytical method employed by Lee et al. [2019] is very good. This method is selected as the best analytical method currently available for biota, specifically for fish.

In the study by Hung et al. [2020], fish samples(Japanese amberjack and flathead grey mullet) were collected from three special management sea areas (Gwangyang Bay, Mansan Bay and Busan

Harbor) in Korea. In their analysis, 13 PFAS of three classes (PFCAs, PFSAs, FTs) were detected with the LOD values ranging from 15 to 27 pg/g. No LOQ values or ranges were reported. The %R values for $^{13}C_4$ PFOA and $^{13}C_4$ PFOS are 94.9% and 90.5%, respectively, for blood samples; 94.49% and 87.62, respectively, for muscle samples; and 99.65% and 97.77%, respectively, for liver samples. The %R values are acceptable for only two analytes ($^{13}C_4$ PFOA and $^{13}C_4$ PFOS). These results indicate that the analytical method used by Hung et al. [2020] is fair.

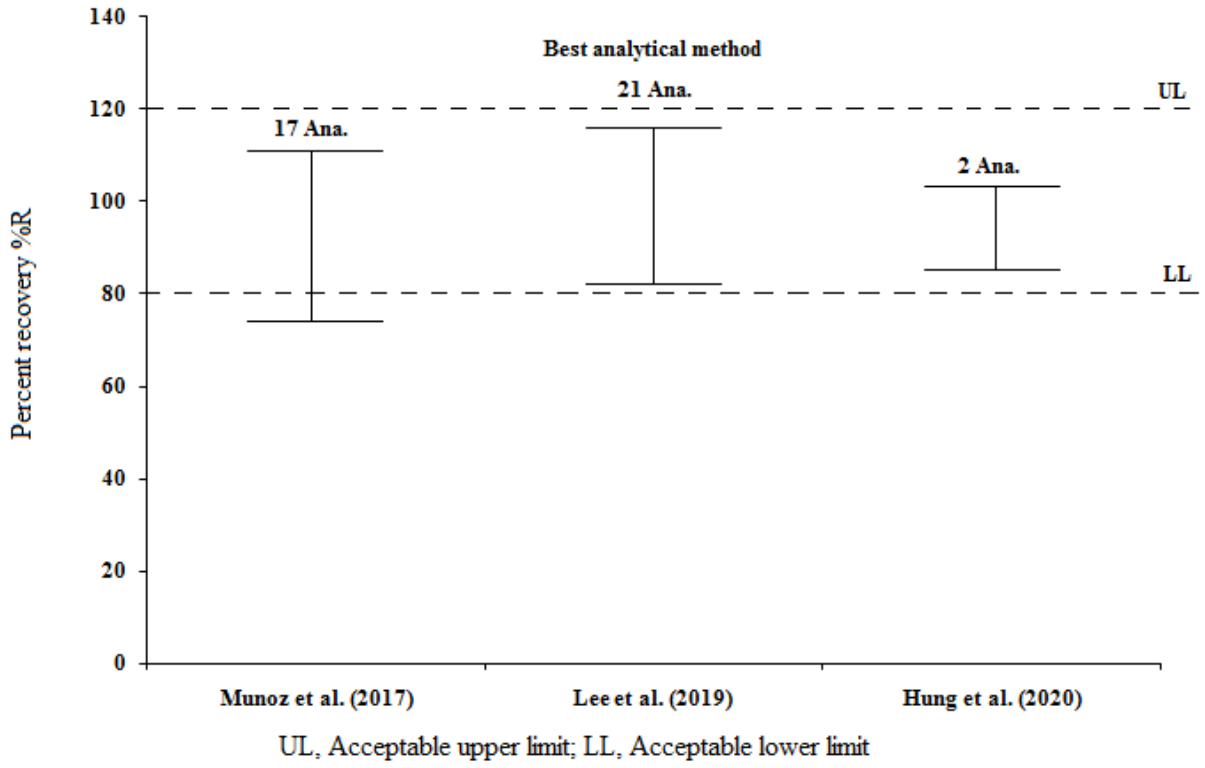


Figure 17: Percent recovery by the analytical methods for PFAS in biota (fish).

Chapter 5: Discussion

While working on this study, a question has arisen regarding the analytical approach. The question is which approach is better between the targeted and non-targeted analysis. There are advantages and disadvantages to both approaches. In the targeted analysis, specific chemicals are investigated by adding stable isotopically labeled internal standard solution to a sample and the behavior of these standard chemicals (e.g., mass spectra, retention times) are known. However, it is not possible to find other chemicals (untargeted compounds) that potentially present in the sample. A typical mass spectrometric targeted analysis method is based on selected reaction monitoring (SRM) on a triple quadrupole or tandem mass spectrometry instrument. In this system, both selectivity and sensitivity can be increased by limiting the amount of measured data (Martin Soderstrom, University of Helsinki, Finland, 2019).

In the non-targeted analysis, the aim is principally to find any chemicals present in a sample (noting that practically it is not possible to detect all the compounds present in a sample). A typical mass spectrometric instrument for this analysis would be a time-of-flight (TOF) or orbitrap instrument. In a typical non-targeted method, we search for protonated molecules at high-resolution and then measure MS/MS spectra for identification. Non-targeted methods are becoming more popular as researchers have to look for harmful chemicals widely in the environment (Martin Soderstrom, University of Helsinki, Finland, 2019). The main problem of the non-targeted analysis is the amount of work required for the data processing.

The primary concern in the present study is uncertainty associated with the quality assurance and quality control in the processes from sampling to data analysis.

Human and non-human variabilities all contribute to uncertainty in the results. Potential variables include but are not limited to:

1) Sampling sites – diverse sampling sites could result in different quality of raw samples. Some samples could be more difficult to handle (requires an extensive pretreatment) than others.
2) Analytical instruments – different makes/models of LC-MS/MS have various capabilities and could produce different results with varying selectivity and sensitivity.
3) Humans – different levels of training could result in incomparable human errors during the collection, handling, and treatment of samples, PFAS extraction, and instrumental analysis of the samples.
4) Analytes – different authors targeted different analytes, which are not comparable with other researchers' targets.

These variations can introduce potential errors and uncertainties in the results and thus conclusions of this study. Because this study totally relies on the information provided in the available papers, these variables are uncounted.

Chapter 6: Conclusions

The literature review on analytical methods for PFAS in non-drinking water matrices (i.e., surface water, ground water, wastewater, sludge and biosolids, soil, sediment, air, and biota-fish) were performed. Based on the peer-reviewed papers published from January 2000 to April 2020 and identified using Web of Science and Google Scholar, the best analytical methods available during the study period are as follows:

- for surface water, the method employed by Lee et al. [2019]
- for sub surface water, the method by Schultz et al. [2004]
- for wastewater, the method used by Coggan et al. [2019]
- for sludge and biosolids, the methods by Coggan et al. [2019]
- for soil, the methods employed by Meng et al. [2015]
- for sediment, the method used by Lee et al. [2019]
- for air, the method employed by Lee et al. [2019]
- for biota-fish, the method used by Lee et al. [2019].

For these eight analytical methods are best analytical methods, authors have provided the small limit of detection (LOD) and limit of quantification (LOQ) values for method's quality control and quality assurance measure. Again, percent recovery ranges (efficiency of the method) of these methods are within the acceptable range (80-120%) suggested by Food and Agricultural Organization of United Nation. The number of analytes measured is more than ten, for selecting the best methods.

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Appendix I – PFAS Toxicity Profiles – Department of Defense, 2019.

Martin Soderstrom, University of Helsinki, Finland, 2019.

APPENDICES

In this section, the analytical methods recommended for per-polyfluoroalkyl substances (PFAS) in non- drinking water matrices (Surface water, subsurface water, wastewater, sludge and biosolids, sediment, soil, air and biota) are presented.

The style of presentation given in the EPA approved analytical method 537.1 of per-polyfluoroalkyl substances in drinking water has been followed. The EPA method 537.1 is consisted of fifteen steps and corresponding sub-steps. Of these fifteen steps, Definitions, Interferences, Safety, Equipment and Supplies, Reagent and Standards, Quality Control, Calibration and Standardization, Data Analysis and Calculation, Method of Performance, Pollution Prevention, and Waste Management are common for drinking water and non-drinking water matrices. To avoid repetition, these steps are not presented in this section. Only Scope and Application, Summary of Method, Sample Collection, Preservation and Storage, and Procedure for these non-drinking water matrices are presented in the following order:

Appendix A: Analytical method for surface water [Lee et al. 2019]

Appendix B: Analytical method for subsurface water [Schultz et al. 2004]

Appendix C: Analytical method for wastewater [Coggan et al. 2019]

Appendix D: Analytical method for sludge and biosolids [Coggan et al. 2019]

Appendix E: Analytical method for soil [Meng et al. 2015]

Appendix F: Analytical method for sediment [Lee et al. 2019]

Appendix G: Analytical method for air [Lee et al. 2019]

Appendix H: Analytical method for fish [Lee et al. 2019]

Appendix A: Analytical method for PFAS in surface water [Lee et al. 2019]

A.1 Scope and Application

This is a solid phase extraction (SPE), centrifuged, liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in surface water. Surface water samples are, in general, waters collected from the sea, river/stream, lake/reservoir, and pond/impoundment. PFAS for which this analytical method has targeted are presented in Table A.1.

Table A.1: Name and abbreviation/acronym of the PFAS in surface water.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoro-n-undecanoate	PFUdA
Perfluoro-n-dodecanoate	PFDoA
Perfluorotetradacanoate	PFTeDA
Perfluorotridacanoate	PFTrDA
Perfluoro-n-undecanoate	PFHxDA
Perfluoro-n-dodecanoate	PFODA
Perfluorobutanesulfonate	PFBS
Perfluorohexanesulfonate	PFHxS
Perfluoroheptanesulfoate	PFHpS
Perfluorooctanesulfonate	PFOS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Perfluorooctanesulfonamide	FOSA
N-Methyl-perfluorooctane-sulfonamido	MeFOSAA
N-Ethyl-perfluorooctane-sulfonamido	EtFOSAA

A.2 Summary

Water samples (500mL) are filtered using prebaked (450 °C, 12 h) GC-50 filters (GF/F Whatman, 0.7 μm), and then spiked with 300 μL of the mixed working internal standard solution containing

(MPFHxA, M5PFHxA, M4PFHpA, MPFOA, M8PFOA, MPFNA, M9PFNA, MPFDA, M6PFDA, MPFUdA, M7PFUdA, MPFDoA, MPFHxS, MPFOS, M8PFOS, d3-N-MeFOSAA and d5-N-EtFOSAA). The concentration of these constituents is 10 ng/mL. Sixteen (16) target analytes dissolved in water are extracted using a weak anion-exchange solid phase exchange (WAX) cartridge. The cartridges are preconditioned by sequentially passing through 4 mL of 0.5% (v/v) ammonium hydroxide (NH_4OH, 30-32%) in methanol, 4 mL of methanol, and 4 mL of de-ionized water (DIW). After sample loading, the cartridges is washed with 4 mL of25 mM sodium acetate buffer solution, and then dried under vacuum for 5-10 min. The extracts are sequentially eluted with 3 mL of methanol and 3 mL of 0.5% NH_4OH in methanol. Finally, the elutes are evaporated to dryness, reconstituted in 1 mL methanol, and transferred to polypropylene (PP) injection vials prior to analysis by LC-MS/MS.

A.3 Sample Collection, Preservation and Storage

A.3.1Sample bottle preparation

Sample bottle: Samples must be collected in a polypropylene (PP) bottle (250 - 1000 mL) fitted with a PP screw-cap (Shoemaker and Tellenhorst 2018).

Sample preservation: The preservation reagent, recommended by EPA Analytical Method 537.1 for drinking water may be added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

A.3.2 Sample collection

Precaution: Sample handlers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. During sampling, PFAS contamination can occur from a

number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves aid in minimizing accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Water samples are collected continuously at the same spot in a water column, by dipping a pre-cleaned 1000mL polypropylene (PP) bottle in the water. When filling the sample bottle, be careful not to flush out sample preservation reagent. Samples do not need to be collected headspace free (Shoemaker and Tellenhorst 2018). After collecting samples, cap the bottles and agitate by hand until preservative is completely dissolved. Keep the sample bottles sealed from time of collection till initial preparation or extraction (Shoemaker and Tellenhorst 2018).

Ambient water quality parameters: During sampling, physicochemical properties of the water are measured at each site. Temperature of the sample is expected to be the same as inside the water column where the sample is taken (Shoemaker and Tellenhorst 2018).

A.3.3 Field reagent blanks (FRB)

The procedure for the preparation of Field Reagent Blanks (FRB) is the same as the EPA method (EPA Method 537.1) for drinking water (Shoemaker and Tellenhorst 2018).

A.3.4 Sample shipment and storage

Samples must be chilled during shipment and must not exceed 10°C (normally at 4^{o}C) during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C (normally at 4^{o}C) when the samples are received at the laboratory. Samples stored in the lab must

be held at or below 6°C (normally at 4ºC) until initial preparation, but must not be frozen (Shoemaker and Tellenhorst 2018).

Results of a sample storage stability study indicated that all compounds listed in this method have adequate stability for 14 days (since samples are collected, preserved, shipped and stored). It is recommended that samples should be extracted as soon as possible but must be extracted within 14 days. After extraction, extracts must be stored at room temperature and analyzed within 14 days as suggested by EPA (Shoemaker and Tellenhorst 2018).

A.4 Procedure

A.4.1 Sample preparation

Initial preparation:—Water samples (500mL) is filtered using prebaked (450 ℃, 12 h) GC-50 filters (GF/F Whatman, 0.7 μm), and then is spiked with 300 μL of amixed working internal standard solution containing target PFAS (e.g., MPFHxA, M5PFHxA, M4PFHpA, MPFOA, M8PFOA, MPFNA, M9PFNA, MPFDA, M6PFDA, MPFUdA, M7PFUdA, MPFDoA, MPFHxS, MPFOS, M8PFOS, d3-N-MeFOSAA and d5-N-EtFOSAA).The concentration of these constituents is 10 ng/mL. The target analytes in the dissolved water phase are extracted by solid-phase extraction (SPE) with a weak anion-exchange solid phase exchange (WAX)cartridge.

A.4.2 Sample extraction

The sampling bottle is amended with 5 mL of methanol to enhance desorption of PFAS from the bottle wall (Shoemaker and Tellenhorst 2018).The cartridges is preconditioned by sequentially passing through 4 mL of 0.5% (v/v) ammonium hydroxide (NH_4OH, 30-32%) in methanol, 4 mL

of methanol, and 4 mL of de-ionized water (DIW).Then, 200/400 mL of sample is loaded onto the preconditioned cartridge. Turn on the vacuum and begin adding sample to the cartridge. The entire sample is passed through the cartridge under vacuum at approximately one drop per second (Shoemaker and Tellenhorst 2018).

Sample bottle and cartridge elution: – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges (Shoemaker and Tellenhorst 2018). After sample loading, the cartridges is washed with 4 mL of 25 mM sodium acetate buffer solution, and then dried under vacuum for 5-10 min. The extracts are sequentially eluted with 3 mL of methanol and 3 mL of 0.5% NH_4OH in methanol.

Extract concentration: – Concentrate the extract to dryness under vacuum or a gentle stream of nitrogen gas in a heated water bath at 60-65℃ for 20 min to remove all the water/methanol mix (Shoemaker and Tellenhorst 2018). Finally, elute is reconstituted in 1 mL of methanol, and transferred to polypropylene (PP) injection vials prior to analysis by LC-MS/MS.

Sample volume determination: The procedure by EPA (Analytical method 537.1) approved for drinking water can be followed (Shoemaker and Tellenhorst 2018).

A.4.3 Extract Analysis

The procedure for high performance liquid chromatography interfaced with tandem mass spectrometry (HPLC-MS/MS) is found in SectionH.4.3. The extract analysis is common to all PFAS in non- drinking water matrices.

Appendix B: Analytical method for PFAS in subsurface water [Schultz et al. 2004]

B.1 Scope and Application

This is a solid phase extraction (SPE), derivatization, liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in subsurface water. PFAS for which this method has targeted are presented in Table B.1.

Table B.1: Name and abbreviation/acronym of PFAS in subsurface water

Name	Abbreviation/Acronym
4:2 Fluorotelomer sulphate	4:2 FTSA
6:2 Fluorotelomer sulphate	6:2 FTSA
8:2 Fluorotelomer sulphate	8:2 FTSA
10:2 Fluorotelomer sulphate	10:2 FTSA
Perfluorobutane sulphonate	PFBS
Perfluoropentane sulphonate	PFPeS
Perfluorohexane sulphonate	PFHxS
Perfluoroheptane sulphonate	PFHeS
Perfluorooctane sulphonate	PFOS
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA

B.2 Summary

An aliquot (55-200 mL) of subsurface water sample is extracted through a 25-mm strong anion exchange (SAX) disk. The SAX disk is pretreated prior to use to remove interfering disk impurities. Pretreatment is consisted of soaking the disk in 12mM HCl/acetonitrile for 2 days. Then the disk is soaked in pure acetonitrile for several hours. Just prior to use, the disk is rinsed with a minimum of 350 mL of de-ionized water (DIW) in order to sufficiently rinse the HCl from the disk and wet it prior to passing groundwater samples through it. The sample (55-200 mL) is passed through the disk under full vacuum, and the disk is then allowed to dry. The disk containing

exchanged analytes is placed in a 2-mL auto sampler vial together with 1 mL of acetonitrile, 51.2 µg of internal standard, 2-chlorolepidineand, and 100 µL of methyl iodide. When heated at 80 ℃ for 1 h, the acids are simultaneously eluted from the disk and derivatized to their methyl esters.

B.3 Sample Collection, Preservation and Storage

B.3.1Sample bottle preparation

Samples must be collected in a polypropylene (PP) bottle (250mL – 1000 mL) fitted with a PP screw-cap (Shoemaker and Tellenhorst 2018).

Sample preservation: The preservation reagent, recommended by EPA (Analytical Method 537.1 for drinking water) can be added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

B.3.2Sample collection

Precaution: Sample handler must wash their hands before sampling and wear nitrile gloves while filling or sealing the sample bottles. During sampling, PFAS contamination can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves aid in minimizing accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: From subsurface abstraction wells, ground water is collected in a pre rinsed wide-mouth polypropylene (PP) bottle using a stainless steel bailer with bottom check-valve. Do not flush out the sample preservation reagent. Samples do not need to be collected headspace free

(Shoemaker and Tellenhorst 2018). After collecting sample, cap the bottle and agitate by hand until preservative is dissolved. Keep the sample sealed from time of collection till initial preparation or extraction (Shoemaker and Tellenhorst 2018).

Ambient water quality parameters: During sampling, physicochemical properties of the groundwater are measured at each site. Temperature of the sample is expected to be the same as the groundwater inside the wells (Shoemaker and Tellenhorst 2018).

B.3.3Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) is the same as the EPA Method 537.1 recommended for drinking water (Shoemaker and Tellenhorst 2018).

B.3.4Sample shipment and storage

Samples must be chilled during shipment and must not exceed 10°C (normally at 4^{o}C) during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C (normally at 4^{o}C) when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6°C (normally at 4^{o}C) until initial preparation, but must not be frozen (Shoemaker and Tellenhorst 2018).

Results of a sample storage stability study indicated that all the compounds listed in this method have adequate stability for 14 days when collected, preserved, shipped and stored. Therefore, water samples should be extracted as soon as possible, and must be extracted within 14 days. Extracts

must be stored at room temperature and analyzed within 14 days after extraction (Shoemaker and Tellenhorst 2018).

B.4 Procedure

B.4.1 Sample preparation

Initial preparation: – In this extraction method, no initial preparation of sample may not be required. Sample preparation can start with the solid phase extraction.

B.4.2 Sample extraction

The sample bottles are amended with 5 mL of methanol to enhance desorption from the wall (Shoemaker and Tellenhorst 2018). An aliquot (55-200 mL) of subsurface water sample is extracted through a 25-mm strong anion exchange (SAX) disk. Prior to use, the SAX disk is pretreated to remove interfering impurities. Pretreatment is consisted of soaking the disk in a 12-mM HCl/acetonitrile solution for 2 days; after which the disk is soaked in pure acetonitrile for several hours. Just prior to use, the disk is rinsed with a minimum of 350 mL of de-ionized water in order to sufficiently rinse the HCl from the disk and wet it prior to passing subsurface water samplethroughit. After turning on the vacuum, the entire sample is passed through the SAX disk under vacuum at a rate approximately one drop per second (Shoemaker and Tellenhorst 2018).

Sample bottle and cartridge elution: – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the SAX disks (Shoemaker and Tellenhorst 2018). After sample loading, the disks are then allowed to dry. Then the Sax disks is dried under vacuum for 5-10 min. Use a low

vacuum such that the solvent exits the cartridge in a drop wise fashion (Shoemaker and Tellenhorst 2018).The disks containing the exchanged analytes is placed in a 2-mL autosampler vial together with 1 mL of acetonitrile, 51.2 µg of internal standard, 2-chlorolepidineand 100 µL of methyl iodide.

Extract concentration: – Concentrate the extract to dryness under vacuum or a gentle stream of nitrogen in a heated water bath at 60-65°C for 1 hr to remove all the water/chemicals mix (Shoemaker and Tellenhorst 2018). When heated, the acids are simultaneously eluted from the disk and derivatized to their methyl esters. The extract is analyzed by HPLC-MS/MS.

Sample volume determination: The procedure by EPA (Analytical method 537.1 for drinking water) can be followed (Shoemaker and Tellenhorst 2018).

B.4.3 Extract analysis:

The extract analysis is common to all PFAS in non- drinking water matrices. The procedure for HPLC-MS/MS analysis can be found in Section H.4.3.

Appendix C: Analytical method for PFAS in wastewater [Coggan et al. 2019]

C.1 Scope and Application

This is a filtered, solid phase extraction (SPE), liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in wastewater. PFAS for which this method targeted are presented in Table C.1

Table C.1: Name and abbreviation/acronym of the PFAS in wastewater.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBS
Perfluoropentanoate	PFPeA
Perfluorohexanonate	PFHxA
Perfluoroheptanonate	PFHpA
Perfluorooctanonate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoroundecanoate	PFUnDA
Perfluorododecanoate	PFDoDA
Perfluorotridecanoate	PFTrA
Perfluorotetradecanoate	PFTeA
Perfluorobutane sulphonate	PFBS
Perfluoropentane sulphonate	PFPeS
Perfluorohexane sulphonate	PFHxS
Perfluoroheptane sulphonate	PFHpS
Perfluorooctane sulphonate	PFOS
Perfluorodecane sulphonate	PFDS
6:2 Fluorotelomer sulphonate	6:2 FTSA
6:2 Fluorotelomer sulphonate	8:2 FTSA
6:2 Clorinated perfluorinated ether sulphonate	6:2 Cl-PFESA
8:2 Clorinated perfluorinated ether sulphonate	8:2 Cl-PFESA

C.2 Summary

Aqueous samples is filtered using 1-µm glass fiber filters and then spiked with 5 ng of isotopically labeled PFAS internal standards solution (e.g., $^{13}C_2$-PFTeA,$^{13}C_2$-PFDoA, $^{13}C_2$-PFDA, $^{13}C_8$-PFOA, $^{13}C_2$-PFHxA, $^{13}C_3$-PFPeA, $^{13}C_3$-PFBA, $^{13}C_4$-PFOS, $^{13}C_3$-PFHxS,$^{13}C_2$-PFBS, $^{13}C_2$-6:2 FTS and $^{13}C_4$-PFOS).Then, the sample is extracted by solid-phase extraction (SPE) using (weak anion

exchange, 6 mL, 150 mg) cartridges. A 15 mL polypropylene centrifuge vial is used to collect extracted sample. All cartridges are conditioned sequentially with 4 mL of 0.1% (v/v) ammonium hydroxide in methanol, 4 mL of methanol, and 4 mL of ultrapure water. The entire sample is allowed to pass through the conditioned cartridge under vacuum at approximately one drop per second, then washed with 4mL of apH4 buffer (sodium acetate/acetic acid) and then dried under vacuum for 10 min. The SPE cartridge is eluted using 2 mL of methanol that is used to rinse the sample bottle, followed by 4 mL of 0.1% (v/v) ammonium hydroxide in methanol. Extracts are evaporated to 500 μL under a gentle stream of nitrogen (at 25^{o}C) and reconstituted to 1 mL in methanol and transferred to a polypropylene chromatography vial with PP lid for analysis.

C.3 Sample Collection, Preservation and Storage

C.3.1 Sample bottle preparation

Sampling bottle: Sample must be collected in a polypropylene (PP) bottle (250– 1000 mL) fitted with a PP screw-cap (Shoemaker and Tellenhorst 2018).

Sample preservation: The preservation reagent, recommended by EPA Analytical Method 537.1 for drinking water can be added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

C.3.2 Sample collection

Precaution: Sample handlers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. PFAS contamination during sampling can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand

washing and wearing nitrile gloves aid in minimizing accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Wastewater sample are collected from wastewater treatment plants (WWTPs). Temperature of the sample is expected to be the same as wastewater in the WWTP where the sample is collected (Shoemaker and Tellenhorst 2018).

Open a WWTPs outlet tap fill sample bottles, carefully not to flush out the sample preservation reagent. Sample does not need to be collected headspace free (Shoemaker and Tellenhorst 2018).

After sample is collected, cap the bottle and agitate by hand until preservative is dissolved. Keep the sample sealed from the time of collection until initial preparation or extraction (Shoemaker and Tellenhorst 2018).

C.3.3 Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) for wastewater samples are the same method found in the EPA Method 537.1 for drinking water (Shoemaker and Tellenhorst 2018).

C.3.4 Sample shipment and storage

Samples must be chilled during shipment and must not exceed 10°C (normally at 4^{o}C) during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C (normally at 4^{o}C) when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6°C (normally at 4^{o}C) until initial preparation, but must not be frozen (Shoemaker and Tellenhorst 2018).

Sample and extract holding times:

Results of the sample storage stability study indicated that all compounds listed in this method have adequate stability for 14 days when collected, preserved, shipped and stored. Therefore, water samples should be extracted as soon as possible but must be extracted within 14 days. Extracts must be stored at room temperature and analyzed within 14 days after extraction (Shoemaker and Tellenhorst 2018).

C.4 Procedure

C.4.1 Sample preparation

Initial preparation: – Non filtered water samples (250 mL) are spiked with 5 µL of a 1 ng/µL labeled internal standards containing mPFOS, mPFBA, mPFHxA, mPFOA, mPFDA, mPFUnDA, mPFDoDA, m6:2 FTSA, m6:2 FTCA, m8:2 FTCA, m8:2FTUCA and m10:2 FTUCA and concentrated by solid phase extraction (SPE) (cartridge Strata X-AW 200 mg/6 mL).

C.4.2 Sample extraction

Sample bottles are amended with 5 mL of methanol to enhance desorption from reactor walls (Shoemaker and Tellenhorst 2018).The cartridges are preconditioned by sequentially passing through 4 mL of 0.5% (v/v) ammonium hydroxide (NH_4OH, 30-32%) in methanol, 4 mL of methanol, and 4 mL of de-ionized water (DIW).Turn on the vacuum and begin adding sample to the cartridge. The entire sample will be passed through the cartridge under vacuum at approximately one drop per second (Shoemaker and Tellenhorst 2018).

Sample bottle and cartridge elution: – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges (Shoemaker and Tellenhorst 2018). After sample loading, the cartridges are washed with 4 mL of 25 mM sodium acetate buffer solution. Use a low vacuum such that the solvent exits the cartridge in a drop wise fashion (Shoemaker and Tellenhorst 2018). Then the cartridge are dried under vacuum for 5-10 min. PFASs will be eluted from the cartridges by the successive use of 1 mL of methanol (CH_3OH), 4 mL of 0.1% (v/v)ammonium hydroxide (NH_4OH) in CH_3OH, and 2 mL of 0.1% (v/v)NH_4OH in isopropanol/dichloromethane (30/70).

Extract concentration: – Concentrate the extract to dryness under vacuum or a gentle stream of nitrogen in a heated water bath at 60-65°C for 20 min to remove all the water/methanol mix (Shoemaker and Tellenhorst 2018). Finally, the extract is reconstituted to a final volume of100 µL in methanol and transferred to polypropylene (PP) injection vials prior to analysis by LC-MS/MS.

Sample volume determination: The procedure by EPA (Analytical method 537.1 for drinking water) can be followed (Shoemaker and Tellenhorst 2018).

C.4.3 Extract analysis

The extract analysis is common to all PFAS in non-drinking water matrices. The procedure for HPLC-MS/MS analysis can be found in Section H.4.3.

Appendix D: Analytical method for Sludge and Biosolids [Coggan et al. 2019]

D.1 Scope and Application

This is a sonicated-shaken, centrifuged, filtered and liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in sludge and biosolids. The compounds for which this method targeted are presented in Table D.1.

Table D.1: Name and abbreviation/acronym of the PFASs in sludge and biosolids.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBS
Perfluoropentanoate	PFPeA
Perfluorohexanonate	PFHxA
Perfluoroheptanonate	PFHpA
Perfluorooctanonate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoroundecanoate	PFUnDA
Perfluorododecanoate	PFDoDA
Perfluorotridecanoate	PFTrA
Perfluorotetradecanoate	PFTeA
Perfluorobutane sulphonate	PFBS
Perfluoropentane sulphonate	PFPeS
Perfluorohexane sulphonate	PFHxS
Perfluoroheptane sulphonate	PFHpS
Perfluorooctane sulphonate	PFOS
Perfluorodecane sulphonate	PFDS
6:2 Fluorotelomer sulphonate	6:2 FTSA
6:2 Fluorotelomer sulphonate	8:2 FTSA
6:2 Clorinated perfluorinated ether sulphonate	6:2 Cl-PFESA
8:2 Clorinated perfluorinated ether sulphonate	8:2 Cl-PFESA

D.2 Summary

Freeze-dried sludge samples (0.5–1 g) are spiked with 25 ng of isotopically labelled internal standard solution containing mPFOS, mPFBA, mPFHxA, mPFOA, mPFDA, mPFUnDA, mPFDoDA, m6:2 FTSA, m6:2 FTCA, m8:2 FTCA, m8:2FTUCA and m10:2 FTUCA. Then add 4.65 mL of 10 mM NaOH in methanol and sonicate the sample for 30 min and shaken overnight

for 12h. Extracts are neutralized with 100 µL of glacial acetic acid and cooled on ice. Five (5) mL of extract is then transferred to a 15-mL polypropylene (PP) tube before adding 100 mg of C18 and 50 mg of primary secondary amine (PSA) to remove interfering compounds. (C18 is the most popular solid phase extraction sorbent because of its extreme retention of non- polar compounds). Extracts are agitated for approximately 1 min, and then centrifuged at 10,000 rpm, 10^{o}C, for 10 min. This process is repeated twice. Finally, filter the extracts using a 45-µm polyether sulfone (PES) syringe filter (pre-rinsed with LC-MS grade methanol) into a PP chromatography vial with a PP lid for analysis.

D.3 Sample Collection, Preservation and Storage

D.3.1 Sample bottle preparation

Sampling bottle: Samples (combined sludge liquors) are collected in a PP bottle (250 - 1000 mL) fitted with a PP screw-cap (Shoemaker and Tellenhorst 2018).

Sample preservation: The preservation reagent recommended by EPA (Analytical Method 537.1 approved for drinking water) can be added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

D.3.2Sample collection

Precaution: Sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. During sampling, PFAS contamination can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves aid in minimizing accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Combined sludge liquors or biosolids are collected at a wastewater treatment plant (WWTP) using a sludge and biosolids grabber (pre-conditioned with Milli-Q water and methanol). Samples are collected in 50-mL polypropylene (PP) centrifuge tubes, sterilized with a 2 % w/w sodium azide solution. Temperature of the sample is expected to be the same temperature inside the WWTP (Coggan et al. 2019).

After samples are collected, cap the PP centrifuge tubes and agitate them by hand until preservative is dissolved (Shoemaker and Tellenhorst 2018).

D.3.3 Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) for sludge and biosolids is not mentioned by Coggan et al. [2019]. Further research is needed in this subject.

D.3.4 Sample shipment and storage

The collected liquor samples are air-dried and sludge is ground with a mortar and pestle. The ground sludge is refrigerated at -20°C until extraction.

D.4 Procedure

D.4.1 Sample preparation

Initial preparation: – Freeze-dried sludge samples (0.5–1 g) are spiked with 25 ng ofisotopically labelled internal standard solution containing mPFOS, mPFBA, mPFHxA, mPFOA, mPFDA, mPFUnDA, mPFDoDA, m6:2 FTSA, m6:2 FTCA, m8:2 FTCA, m8:2FTUCA and m10:2 FTUCA. Then add 4.65 mL of 10 mM NaOH in methanol, and sonicate the samples for 30 min

and shaken overnight for 12h. Extracts are neutralized with 100 µL of glacial acetic acid and cooled on ice.

D.4.2 Sample extraction

In the method employed by Coggan et al. [2019], no solid phase extraction (SPE) phase was performed. Instead, their method is following: 5 mL of extract is transferred to a 15 mL polypropylene (PP) tube before adding 100 mg of C18 sorbent and 50 mg primary secondary amine (PSA) to remove interfering compounds. Extracts are agitated for approximately 1 min and centrifuged at 10,000 rpm, 10^{o}C for 10 min. This process is repeated twice.

Sample bottle and cartridge elution: – Since no SPE phase is performed; no sample bottle and cartridge elution is needed.

Extract concentration: - No extract concentration is needed.

Finally, extracts are filtered using a 45-µmpolyethersulfone (PES) syringe filter (pre-rinsed with LC-MS grade methanol) into a polypropylene (PP) chromatography vial with polyethylene (PE) lid for analysis.

D.4.3 Extract Analysis

The extract analysis is common to all PFAS in non- drinking water matrices. The procedure for HPLC-MS/MS analysis can be found in Section H.4.3.

Appendix E: Analytical method for soil [Meng et al. 2015]

E.1 Scope and Application

This is a vortexed-shaken, centrifuged, solid phase extraction (SPE), filtered liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of per- and polyfluorinated alkyl substances (PFAS) in soil. The compounds for which this method targeted are presented in Table E.1.

Table E.1: Name and abbreviation/acronym of the PFASs in Soil.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoroundecanoate	PFUnDA
Perfluorododecanoate	PFDoDA
Perfluorobutane sulfonate	PFBS
Perfluorohexane sulfonate	PFHxS
Perfluorooctane sulfonate	PFOS
Perfluorononane sulfonate	PFDS
Perfluorodecane sulfonate	PFDS

E.2 Summary

A soil sample (2.5 g) is transferred to a 50-mL polypropylene (PP) tube, and moistened by 2 mL Milli-Q water with vortexing. Then 1 mL of 0.5 M tetra-butyl ammonium hydrogen sulfate(TBAHS), 2 mL of 25 mM sodium acetate and 1 ng of mass-labeled internal standards (PFOA [1, 2, 3, 4^{13}C] and PFOS [$^{18}O_2$]) are added into the PP tube. The mixture is shaken at 700 rpm/min for 5 min. Subsequently, 5 mL of methyl tertiary butyl ether (MTBE) is added and shaken for 20 min. After centrifuging for 20 min at 3500rpm, the supernatant MTBE is collected. This process is repeated twice which produced a final volume of 15 mL MTBE wash. The supernatant

is evaporated to dryness under a gentle flow of high-purity nitrogen, and reconstituted in 1 mL methanol. The 1 mL elution was transferred to 50-mL PP tube, brought to50 mL with Milli-Q water and extracted with a SPE cartridge. The SPE cartridge is preconditioned with 4 mL of 0.1%ammonia in methanol, 4 mL of methanol and 4 mL of Milli-Q water. Fifty mL sample is loaded into the cartridge. The cartridge is washed with 20 mL of Milli-Q water, 4 mL of 25 mM sodium acetate allowed to run dry, and eluted with 4mL methanol and 4mL of 0.1%ammonia in methanol. The extracts are collected, combined, and concentrated to 1 mL under a gentle stream of high purity nitrogen, and then filtered through a 2-mm nylon filter into a 1.5 mL auto sampler vial fitted with a PP cap for HPLC analysis.

E.3 Sample Collection, Preservation and Storage

E.3.1Sample bottle preparation

Sampling bottle: Soil samples must be collected in a polypropylene (PP) bag. Then the samples are transfer to a PP bottle (250 -1000 mL) fitted with a PP screw-cap (Shoemaker and Tellenhorst 2018).

Sample preservation: The preservation reagent recommended by EPA (Analytical method 537.1for drinking water) is added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

E.3.2 Sample collection

Precaution: Sample handlers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. During sampling, PFAS contamination can occur from a number of common sources such as food packaging, and certain foods and beverages. Proper hand

washing and wearing nitrile gloves aid in minimizing this type of accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Topsoil sample is collected from the ground surface (top 0 to 20 cm) using a stainless steel trowel rinsed with methanol. Each sample is a composite of five sub-samples (each weight about 500 g). Meng et al. [2015] collected from the center and four corners of an area of 100 × 100 m^2. Samples are transferred and stored in clean polypropylene (PP) bags. These samples are dried in air, homogenized with a porcelain mortar and pestle, sieved with a 2-mm mesh, and stored in250 mL PP bottles.

E.3.3 Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) for soil is not mentioned by the author Meng et al. [2015]. Further research is needed in this area.

E.3.4 Sample shipment and storage

Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C when the samples are received at the laboratory (Shoemaker and Tellenhorst 2018). Samples are stored in the dark at room temperature until extraction.

E.4 Procedure

E.4.1 Sample preparation

Initial preparation: – Soil sample (2.5 g) is transferred to a 50-mL PP tube, and moistened by 2 mL Milli-Q water with vortexing. Then 1 mL of 0.5 M tetra butyl ammonium hydrogen sulfate

(TBAHS), 2 mL of 25 mM sodium acetate and 1 ng mass-labeled internal standards (PFOA [1, 2, 3, $4^{13}C$] and PFOS [$^{18}O2$]) are added into a PP tube. The mixture will be shaken at 700 rpm/min for 5 min. Subsequently, 5 mL of methyl-tertiary-butyl-ether (MTBE) will be added and shaken for 20 min. After centrifuging for 20 min at 3500rpm/min, the supernatant MTBE will be collected. This process will be repeated twice which produced a final volume of 15 mL MTBE wash. The supernatant will be evaporated to dryness under a gentle flow of high-purity nitrogen, and reconstituted in 1 mL methanol. The 1 mL elution was transferred to 50 mL PP tube, brought to50 mL with Milli-Q water and extracted with the SPE cartridge.

E.4.2 Sample Extraction

The SPE cartridge will be preconditioned with 4 mL of 0.1%ammonia in methanol, 4 mL of methanol and 4 mL of Milli-Q water. Fifty mL sample is loaded into the cartridge. Turn on the vacuum, and add the sample to the cartridge. The entire sample is passed through the cartridge under vacuum at approximately one drop per second (Shoemaker and Tellenhorst 2018).

Sample bottle and cartridge elution: – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect extracts as they are eluted from the cartridges (Shoemaker and Tellenhorst 2018). The cartridge is washed with 20 mL of Milli-Q water, 4 mL of 25 mM sodium acetate, is allowed to run dry, and eluted with 4mL of methanol and 4mL of 0.1%ammonia in methanol. Use a low vacuum such that the solvent exits the cartridge in a drop wise fashion. Repeat sample bottle rinse and cartridge elution with a second 4-mL aliquot of methanol (Shoemaker and Tellenhorst 2018).

Extract Concentration: – Extract is collected, combined, andconcentratedto1 mL dryness under vacuum or a gentle stream of nitrogen in a heated water bath at 60-65°C for 20 min to remove all the water/methanol mix. Then the extract is filtered through a 2-mm nylon filter into a 5-mL auto sampler vial fitted with a PP cap for HPLC analysis.

E.4.3 Extract analysis

The extract analysis is common to all PFAS in non- drinking water matrices. The procedure for HPLC-MS/MS analysis is found in Section H.4.3.

Appendix F: Analytical method for PFAS in sediment [Lee et al. 2019]

F.1 Scope and Application

This is a sieved, sonicated, centrifuged and solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of per- and polyfluorinated alkyl substances (PFAS) in sediment. The compounds for which this method targeted are presented in Table F.1.

Table F.1: Name and abbreviation/acronym of the PFASs in sediment.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoro-n-undecanoate	PFUdA
Perfluoro-n-dodecanoate	PFDoA
Perfluorotetradacanoate	PFTeDA
Perfluorotridacanoate	PFTrDA
Perfluoro-n-undecanoate	PFHxDA
Perfluoro-n-dodecanoate	PFODA
Perfluorobutanesulfonate	PFBS
Perfluorohexanesulfonate	PFHxS
Perfluoroheptanesulfoate	PFHpS
Perfluorooctanesulfonate	PFOS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Perfluorooctanesulfonamide	FOSA
N-Methyl-perfluorooctane-sulfanamido	MeFOSAA
N-Ethyl-perfluorooctane-sulfanamido	EtFOSAA

F.2 Summary

Sediment samples are freeze-dried, ground using a mortar and pestle, sieved using a 40-mesh (425 μm) sieve, placed in polypropylene (PP) conical tubes, and stored in a desiccators. Each samples (1g dry weight) is spiked with 200 μL of the mixed working internal standard solution containing

MPFHxA, M_5PFHxA, M_4PFHpA, MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M_7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA.The concentration of each compound is 10 μg/mL. The target PFAS are extracted with 20 mL methanol in a bath sonicator at 45°C for 30 min. After centrifugation at 3000 rpm for 10 min, supernatants is transferred into new PP conical tubes and concentrated to 2 mL using a rotary evaporator. The concentrated extracts (using rotary evaporator) are used for SPE on WAX cartridges. The cartridges are preconditioned by sequentially passing through 4 mL of 1% (v/v) NH_4OH in methanol, 4 mL of methanol, and 4 mL of de-ionized water. After concentrated extracts loading, the cartridges are washed with 4 mL of 25 mM sodium acetate buffer solution, and then dried under vacuum for 5-10 min. The extracts are sequentially eluted with 3 mL of methanol and 3 mL of 0.5% NH_4OH in methanol. Finally, the elutes are evaporated to dryness, reconstituted in 1 mL methanol, filtered using a syringe filter (0.2 μm, Nylon), and transferred to PP injection vials prior to analysis by LC-MS/MS.

F.3 Sample Collection, Preservation and Storage

F.3.1 Sample bottle preparation

Sampling containers: Sediment must be collected in a polypropylene (PP) tube or bottle fitted with a PP screw cap.

Sample preservation: The preservation reagent recommended by EPA (Analytical Method 537.1) for drinking water is added to each sample bottle as a solid prior to shipment to the field or prior to sample collection (Shoemaker and Tellenhorst 2018).

F.3.2 Sample collection

Precaution: Sample handlers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. During sampling PFAS contamination can occur from a number of common sources, such as food packaging, and certain foods and beverages. Proper hand washing and wearing nitrile gloves aid in minimizing of accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Sediment samples are collected in triplicate from each sampling station using a stainless steel sediment grabber (pre-cleaned with Milli-Q water and methanol). Surface sediment (top 10 cm) are collected and stored in PP tubes. After collecting samples, cap the bottles or tubes and agitate by hand until preservative is dissolved (Shoemaker and Tellenhorst 2018).

F.3.3 Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) for sediment is not mentioned by the author Lee et al. [2019]. Further research is needed in this area.

F.3.4 Sample shipment and storage

Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C when the samples are received at the laboratory (Shoemaker and Tellenhorst 2018). Samples are stored in the dark at 4°C until analysis.

F.4 Procedure

F.4.1 Sample preparation

Initial preparation: – Sediment samples are freeze-dried, ground using a mortar and pestle, sieved using a 40-mesh (425 μm) sieve, placed in PP conical tubes, and stored in a desiccators. Sediment samples (1 g dry weight) are spike with 200 μL of the mixed working internal standard solution containing MPFHxA, M_5PFHxA, M_4PFHpA, MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M_7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA. The concentration of each PFAS is 10 μL/mL. These compounds are extracted with 20 mL of methanol in a bath sonicator at 45 °C for 30 min. After centrifugation (at 3000 rpm for 10 min), the supernatants is transferred into new pp conical tubes and concentrated to 2 mL using a rotary evaporator.

F.4.2 Sample Extraction

The concentrated extracts (using a rotary evaporator) can be used for SPE on WAX cartridges. The cartridges are preconditioned by sequentially passing through 4 mL of 1% (v/v) NH_4OH in methanol, 4 mL of methanol, and 4 mL of de-ionized water. Turn on the vacuum and add sample to the cartridge. The entire sample is passed through the cartridge under vacuum at approximately one drop per second (Shoemaker and Tellenhorst 2018) or adjust the vacuum so that the approximate flow rate is 10-15 mL/min.

Sample bottle and cartridge elution: – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges (Shoemaker and Tellenhorst 2018). After concentrated extracts

loading, the cartridges are washed with 4 mL of 25 mM sodium acetate buffer solution, and then dried under vacuum for 5-10 min. Rinse the sample bottles with 4 mL of methanol and elute the analytes from the cartridges by passing the 3 mL of methanol and 3 mL of 0.5% NH_4OH in methanol through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a drop wise fashion. Repeat sample bottle rinse and cartridge elution with a second 4-mL aliquot of methanol (Shoemaker and Tellenhorst 2018).

Extract concentration: – Concentrate the extract to dryness under vacuum or a gentle stream of nitrogen in a heated water bath at 60-65°C for 20 min to remove all the water/methanol mix. Add the appropriate amount of 96:4% (vol/vol) methanol: water solution to the collection vial to bring the volume to 1 mL and vortex (Shoemaker and Tellenhorst 2018). The sample is filtered using syringe filter (0.2 µm, Nylon), and transferred to PP injection vials prior to analysis.

F.4.3 Extract Analysis

The procedure for high performance liquid chromatography interfaced with tandem mass spectrometry (HPLC-MS/MS) is found in Section H.4.3. The extract analysis is common to all PFAS in non-drinking water matrices.

Appendix G: Analytical method for PFAS in air [Lee et al. 2019]

G.1 Scope and Application

This is a sonication, centrifugation, filtration, liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in air. The compounds for which this method targeted are presented in Table G.1.

Table G.1: Name and abbreviation/acronym of the PFASs in air

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoro-n-undecanoate	PFUdA
Perfluoro-n-dodecanoate	PFDoA
Perfluorotetradacanoate	PFTeDA
Perfluorotridacanoate	PFTrDA
Perfluoro-n-undecanoate	PFHxDA
Perfluoro-n-dodecanoate	PFODA
Perfluorobutanesulfonate	PFBS
Perfluorohexanesulfonate	PFHxS
Perfluoroheptanesulfoate	PFHpS
Perfluorooctanesulfonate	PFOS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Perfluorooctanesulfonamide	FOSA
N-Methyl-perfluorooctane-sulfanamido	MeFOSAA
N-Ethyl-perfluorooctane-sulfanamido	EtFOSAA

G.2 Summary

In the study by Lee et al. [2019],16 target analytes were extracted from air sampling medium (pre-cleaned quartz fiber filters and pre-cleaned PUF/XAD-2/PUFs) by sonication, with a solvent consisting of acetone and hexane (7:3, v/v). In this method, filters and PUF/XAD-2/PUFs are

spiked with 400 µL of the mixed working internal solution containing MPFHxA, M_5PFHxA, M_4PFHpA, MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M_7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA).The concentration of these constituents are 10 ng/mL. The filters and PUF/XAD-2/PUFs are immersed in the extraction solvent (20 mL/filter and 100 mL/PUF) in a bath sonicator for 30 min. The filters are centrifuged at 3000 rpm for 10 min after extraction, and the supernatants are then transferred into new polypropylene (PP) conical tubes. These steps of extraction and centrifugation are carried out twice. The supernatants are concentrated to 4 mL using a rotary evaporator. For cleanup, the concentrated extracts are loaded onto a cartridge. Prior to loading the extract, the cartridges are preconditioned with 4 mL methanol. One mL of the extracts and 1 mL methanol are sequentially passed through the cartridge, and this process is repeated four times. Total 8 mL of extracts are evaporated to dryness, reconstituted in 1 mL methanol, and transferred to PP injection vials prior to analysis by LC-MS/MS.

G.3 Sample Collection, Preservation and Storage

G.3.1 Sample equipment preparation

Air samples are collected 1m above ground level using a high volume air sampler. The air sampler must be pre conditioned before sample collection.

G.3.2 Sample collection

Precaution: Sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample equipment. During sampling PFAS contamination can occur from a number of common sources such as food packaging and certain foods and beverages. Proper hand

washing and wearing nitrile gloves aid in minimizing this type of accidental contamination of the samples (Shoemaker and Tellenhorst 2018).

Sampling: Samples are collected over 24 h at a flow rate of 0.20m^3/min. The volume of air collected for each sample is approximately 283m^3. Particles can be collected on prebaked (450 °C, 12 h) quartz microfiber filters (QM-A filter, 10.16 cm diameter, 2.2 μm pore size).Gaseous species are collected in PUF/XAD-2/PUF cartridges (ORBO 2500 pre cleaned Large PUF/Amberlite XAD-2/ PUF Cartridge, Supelco, 6.5 cm OD ×125mm length). Meteorological parameters are measured using the wireless Vantage Pro2 Weather Station (e.g., Davis Instruments, Hayward, CA, USA).

G.3.3 Field reagent blanks (FRB)

The procedure for Field Reagent Blanks (FRB) for air is not mentioned by the author Lee et al. [2019]. Further research is needed in this area.

G.3.4 Sample shipment and storage

After sampling, the filters and PUF/ XAD-2/PUF cartridges are packed in aluminum foil and placed in pre-cleaned (methanol-washed) glass jars. Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6°C until extraction.

G.4 Procedure

G.4.1Sample preparation

Initial preparation: – Analytes are extracted from the air sampling medium (pre-cleaned quartz fiber filters and pre-cleaned PUF/XAD-2/PUFs) by sonication, with a solvent consisting of acetone and hexane (7:3, v/v). Filters and PUF/XAD-2/PUFs can be spiked with 400 μL of the mixed working internal standard solution containing MPFHxA, M_5PFHxA,M_4PFHpA, MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M_7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA. The concentration of each PFAS constituent is 10 ng/mL. The filters and PUF/XAD-2/PUFs are immersed in the extraction solvent (20 mL/filter and 100 mL/PUF) in a bath sonicator at room temperature for 30 min. Sampleare centrifuged at 3000 rpm for 10 min after extraction, and the supernatants are then transferred into new PP conical tubes. These steps of extraction and centrifugation are carried out twice. The supernatants are concentrated to 4 mL using a rotary evaporator.

G.4.2 Sample extraction

For cleanup, the concentrated extracts are loaded onto an ENVI cartridge (e.g., Supelclean, Sigma-Aldrich, USA) for best possible air filtration. Prior to loading the extract, the cartridges are preconditioned with 4 mL of methanol.

Sample bottle and cartridge elution: – One mL of the extracts and 1 mL methanol are sequentially passed through the cartridge, and this process must be repeated four times. For air sample extraction, no solid phase extraction by SPE cartridge isperformed. Thus, sample bottle and cartridge elution procedure are different from the methods for drinking water.

Extract concentration: – Total 8 mL extracts are evaporated to dryness under a gentle stream of nitrogen in a heated water bath at 60-65°C to remove all the water/chemicals mix, reconstituted in 1 mL methanol, and transferred to PP injection vials prior to analysis by LC-MS/MS(Shoemaker and Tellenhorst 2018).

G.4.3 Extract analysis

The extract analysis is common to all PFAS in non- drinking water matrices. The procedure for HPLC-MS/MS analysis is found in Section H.4.3.

Appendix H: Analytical method for PFAS in fish [Lee et al. 2019]

H.1 Scope and Application

This is a sonication, centrifugation, liquid-liquid extraction, liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination and quantification of selected per- and polyfluorinated alkyl substances (PFAS) in fish (biota). The compounds for which this method targeted are presented in Table H.1.

Table H.1: Name and abbreviation/acronym of the PFASs in fish.

Name	Abbreviation/Acronym
Perfluorobutanoate	PFBA
Perfluoropentanoate	PFPeA
Perfluorohexanoate	PFHxA
Perfluoroheptanoate	PFHpA
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Perfluoro-n-undecanoate	PFUdA
Perfluoro-n-dodecanoate	PFDoA
Perfluorotetradacanoate	PFTeDA
Perfluorotridacanoate	PFTrDA
Perfluoro-n-undecanoate	PFHxDA
Perfluoro-n-dodecanoate	PFODA
Perfluorobutanesulfonate	PFBS
Perfluorohexanesulfonate	PFHxS
Perfluoroheptanesulfoate	PFHpS
Perfluorooctanesulfonate	PFOS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Perfluorooctanesulfonamide	FOSA
N-Methyl-perfluorooctane-sulfanamido	MeFOSAA
N-Ethyl-perfluorooctane-sulfanamido	EtFOSAA

H.2 Summary

Fish muscle is well-mixed using a hand mixer. One gram wet weight (ww) of homogenized fish muscle are mixed with de ionized water (DIW) (w/w; 1:1).The sample is then spiked with 20 μL of the mixed working internal standard solution containing MPFHxA, M_5PFHxA, M_4PFHpA,

MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA.The concentration of each constituent is 100 ng/mL. Next, add 350 µL of protease and 350 µL of lipase to the sample to hydrolyze PFAS bound to fats and proteins. Then, incubated heat 37°C for 16 hr. After incubation, the samples are shaken with 5 mL of hexane for 15 min, and centrifuged at 4000 rpm for 5 min. These steps are repeated twice to remove fats and proteins. Discarded supernatant and sonicate remaining sample for 10 min after adding 1 mL of 0.5 M tetra butyl ammonium hydrogen sulfate (TBAHS) and 2 mL of 0.25 M sodium carbonate (Na_2CO_3)/sodium bicarbonate ($NaHCO_3$).Then, add 5 mL of methyl-t-butyl ether (MTBE) to the sample for liquid-liquid extraction. After stirring and rotating for 30 minutes, the sample is centrifuged at 4000 rpm for 5 min. The supernatant is then transferred into new polypropylene (PP) conical tube. Finally, the supernatant is evaporated to dryness, reconstituted in 200 µL acetonitrile, and transferred to a PP injection vials prior to analysis by LC-MS/MS.

H.3 Sample Collection, Preservation and Storage

H.3.1 Sample collection equipment preparation

Specific number of fish specimen is collected from a particular area using a gill net. This gill net must be pre-conditioned before use.

H.3.2 Sample shipment and storage

After sampling, wrap all fish samples individually in aluminum foil and pack in a zipper bag. All collected samples are stored in an icebox and transported to the laboratory. All fish samples are stored at -20 °C prior to analysis.

H.4 Procedure

H.4.1 Sample preparation

Initial preparation: –Fish muscle is well-mixed using a hand mixer. Homogenized samples of fish muscle (1 g wet weight) is mixed with de ionized water (w/w; 1:1) and spiked with 20 µL of the mixed working internal standard solution containing MPFHxA, M_5PFHxA, M_4PFHpA, MPFOA, M_8PFOA, MPFNA, M_9PFNA, MPFDA, M_6PFDA, MPFUdA, M_7PFUdA, MPFDoA, MPFHxS, MPFOS, M_8PFOS, d_3-N-MeFOSAA and D_5-N-EtFOSAA). The concentration of each constituent is 100 ng/mL. Next, add 350 µL of protease and 350 µL of lipase to the samples in order to hydrolyze PFAS bound to fats and proteins. Then, the samples are incubated at 37°C for 16 hr. After incubation, the samples are shaken with 5 mL of hexane for 15 min, and centrifuged at 4000 rpm for 5 min. These steps to remove fats are repeated twice.The supernatant is discarded and the remaining sample is sonicated for 10 min after adding 1 mL of 0.5 M tetra butyl ammonium hydrogen sulfate (TBAHS) and 2 mL of 0.25 M sodium carbonate (Na_2CO_3)/sodium bicarbonate ($NaHCO_3$).

H.4.2Sample extraction

For liquid-liquid extraction, add 5 mL of methyl-t-butyl ether (MTBE) to the samples. After stirring and rotating in liquid-liquid extraction for 30 minutes, the sample is centrifuged at 4000 rpm for 5 min. The supernatant is then transferred into new PP conical tubes.

Extract concentration: – The supernatant is evaporated to dryness under a gentle stream of nitrogen gas in a heated water bath at 60-65°C to remove all the water/chemical mix, reconstituted

in 200 μL acetonitrile, and transferred to PP injection vials prior to analysis by LC-MS/MS (Shoemaker and Tellenhorst 2018).

H.4.3 Extract Analysis: – The procedural steps for determination of PFASs by high performance liquid chromatography interfaced with tandem mass spectrometry (HPLC-MS/MS) arequality control, calibration and standardization. These procedural steps are found in the EPA Method 537.1 approved for drinking water. The analytes are separated and identified by HPLC-MS/MS by comparing the mass spectra and retention times (under identical HPLC-MS/MS conditions) to the reference spectra and retention times for calibration standards. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to all field and QC samples to monitor the extraction efficiency of the method (Shoemaker and Tellenhorst 2018). For High Performance Liquid Chromatography interfaced with Ion Mobility Quadruple Time of Flight Mass Spectrometry, additional steps and calculationscalculations are needed. The required steps are given in Section H.5.

H.5 Classification of fragment ions and selection of fragmentation flags

In the analysis by Lee et al. [2019], Milli-Q water containing 5 mM ammonium acetate and acetonitrile was used as the mobile phase for Liquid Chromatography (LC). The PFASs were separated by a Zorbax Eclipse Plus C_{18} column and examined by Dual Agilent Jet Stream negative electrospray ionization (ESI) mode.

The tuning is conducted for mass calibration and checking ion mobility resolution using an 85,001 solution (reference mass mixture for the reference mass) before all analysis. In this step, the mass

calibration is checked in real-time by using the lock mass chemicals (a compound of known composition that is added to the MS analysis**);** trifluoroacetic acid (TFA), 1H, 1H, 3H-tetrafluoropropoxy (Hexakis) and phosphazine (HP-0921).

In this instrument the m/z (mass to charge ratio) of each target molecular ion is assumed as $[M-H]^-$ for target analysis and collision energy (CE) at four different conditions (0,10,20 and 40 V) for the same analysis can be considered to cover various types of PFASs. The fragment ions will be detected by full scanning at m/z = 50–1700.

The fragmentation flags can be classified into four classes: fluorinated fragment ions (Class 1 $[CxFy]^-$, Class 2 $[CxFyO]^-$, Class 3 $[CxFyO3S]^-$) and others (Class X, non-fluorinated ions, with neither C nor F, e.g., $[O3S]^-$(see Table H.2).

Table H.2: Classification of PFASs fragment ions used for fragmentation flagging.

No.	Compunds	$[M-H]^-$ Chemical formula	$[M-H]^-$ Exact mass m/z	Fragment ions
1	PFBA	$C_4F_7O_2$	212.9787	$[C_3F_7]^-$
2	PFPeA	$C_5F_9O_2$	262.9755	$[C_4F_9]^-$, $[C_2F_5]^-$
3	PFHxA	$C_6F_{11}O_2$	312.9723	$[C_5F_{11}]^-$, $[C_3F_7]^-$
4	PFHpA	$C_7F_{13}O_2$	362.9691	$[C_6F_{13}]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
5	PFOA	$C_8F_{15}O_2$	412.9659	$[C_7F_{15}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
6	PFNA	$C_9F_{17}O_2$	462.9627	$[C_8F_{17}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$
7	PFDA	$C_{10}F_{19}O_2$	512.9595	$[C_9F_{19}]^-$, $[C_6F_{13}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
8	PFUnDA	$C_{11}F_{21}O_2$	562.9563	$[C_{10}F_{21}]^-$, $[C_6F_{13}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
9	PFDoDA	$C_{12}F_{23}O_2$	612.9531	$[C_{11}F_{23}]^-$, $[C_7F_{15}]^-$, $[C_6F_{13}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
10	PFTrDA	$C_{13}F_{25}O_2$	662.9499	$[C_{12}F_{25}]^-$, $[C_8F_{17}]^-$, $[C_6F_{13}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
11	PFTeDA	$C_{14}F_{27}O_2$	712.9467	$[C_{13}F_{27}]^-$, $[C_9F_{19}]^-$, $[C_6F_{13}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
12	PFHxDA	$C_{16}F_{31}O_2$	812.9403	$[C_{15}F_{31}]^-$, $[C_{11}F_{23}]^-$, $[C_{10}F_{21}]^-$, $[C_9F_{19}]^-$, $[C_6F_{13}]^-$, $[C_5F_{11}]^-$, $[C_4F_9]^-$, $[C_3F_7]^-$, $[C_2F_5]^-$
13	PFBS	$C_4F_9O_3S$	298.9424	$[FO_3S]^-$, $[O_3S]^-$
14	PFPeS	$C_5F_{11}O_3S$	348.9392	$[C_5F_9O_3S]^-$, $[C_2F_5]^-$, $[FO_3S]^-$, $[O_3S]^-$, $[CF_3]^-$
15	PFHxS	$C_6F_{13}O_3S$	398.9361	$[C_6F_9]^-$, $[FO_3S]^-$
16	PFHpS	$C_7F_{15}O_3S$	448.9329	$[C_3F_7]^-$, $[CF_3O_3S]^-$, $[C_2F_5]^-$, $[FO_3S]^-$, $[O_3S]^-$
17	PFOS	$C_8F_{17}O_3S$	498.9297	$[C_8F_{17}]^-$, $[C_2F_5]^-$, $[FO_3S]^-$, $[O_3S]^-$
18	PFNS	$C_9F_{19}O_3S$	548.9265	$[C_8F_9O_3S]^-$, $[C_3F_7]^-$, $[CF_3O_3S]^-$, $[FO_3S]^-$, $[O_3S]^-$
19	6:2 diPAP	$C_{16}H_8F_{26}O_8P$	788.9745	$[C_8H_7F_{13}O_4P]^-$, $[C_8H_4F_{12}O_4P]^-$, $[H_2O_4P]^-$, $[O_3P]^-$
20	8:2 diPAP	$C_{20}H_8F_{34}O_8P$	988.9617	$[C_{10}H_7F_{17}O_4P]^-$, $[C_{10}H_4F_{16}O_4P]^-$, $[H_2O_4P]^-$, $[O_3P]^-$
21	6:2/8:2 diPAP	$C_{18}H_8F_{30}O_8P$	888.9681	$[C_{10}H_7F_{17}O_4P]^-$, $[C_8H_7F_{13}O_4P]^-$, $[C_8H_4F_{12}O_4P]^-$, $[H_2O_4P]^-$, $[O_3P]^-$
22	4:2 FTS	$C_6H_4F_9O_3S$	326.9737	$[C_6H_3F_9O_3S]^-$, $[HO_3S]^-$, $[O_3S]^-$
23	6:2 FTS	$C_8H_4F_{13}O_3S$	426.9674	$[C_8H_3F_{13}O_3S]^-$, $[C_3F_9]^-$, $[HO_3S]^-$, $[O_3S]^-$
24	8:2 FTS	$C_{10}H_4F_{17}O_3S$	526.9610	$[C_{10}H_3F_{16}O_3S]^-$, $[C_9F_{13}]^-$, $[HO_3S]^-$
25	6:2 FTCA	$C_8H_5F_{13}O_2$	376.9847	$[C_7F_{13}]^-$, $[CFO_2]^-$
26	8:2 FTCA	$C_{10}H_5F_{17}O_2$	476.9783	$[C_9F_{13}]^-$

Class 1 $[C_xF_y]^-$, *Class 2* $[C_xF_yO]^-$, *Class 3* $[C_xF_yO_3S]^-$, *Class X* Others

H.6 Selection of peaks of suspected PFAS by fragmentation flagging

All extracted ion chromatograms (EICs) of fragmentation flags are described in the mass error range of ±20 ppm, and the value of the blank of methanol is subtracted.

The conceptual image of fragmentation flagging is developed to select peaks of suspected PFASs. Four extract ion chromatogram (EIC) of fragment ions including $[C7F7]^-$ (m/z 216.9888), $[C7F9]^-$ (m/z 254.9856), $[C8F9]^-$ (m/z 266.9856), and $[C7F11]^-$ (m/z 292.9824) at 40 V are assumed as example. These are common fragment ions of PFASs according to the classification explained in the classification of fragment ions and the selection of fragmentation flags step.

A specific retention time (RT), multiple fragment ions (i.e. fragmentation flags) are overlapped. The overlapping might be due to fragment ions derived from the same molecular ion, as fragment ions from a specific molecular ion can be observed in the same range of retention time. For example, $[C7F7]^-$ (m/z 216.9888), $[C7F9]^-$ (m/z 254.9856), and $[C7F11]^-$ (m/z 292.9824) might be derived from the same molecular ion. This approach has been reported as fragmentation flagging or precursor ion searching (see Figure H.1).

It can be assumed that the peaks of fragmentation flags are found within the range of ±0.1 min retention time derived from the same molecular ion. Therefore, fragmentation flags at each retention time as fragment sets are regrouped from the extract ion chromatograms (EICs) to determine suspected PFASs.

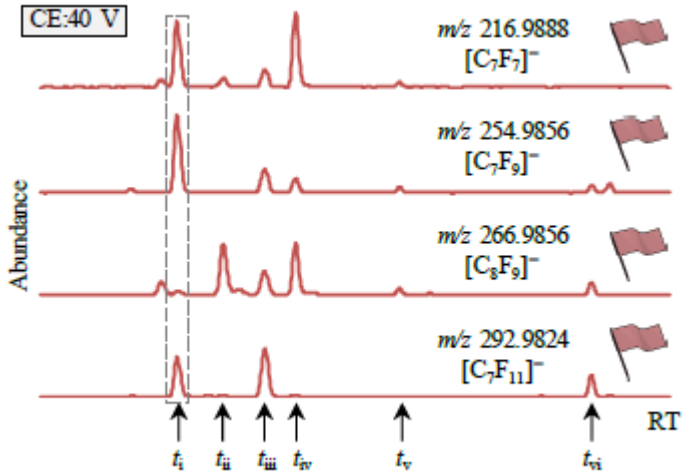

Figure H.1: Conceptual image of fragmentation flagging**.**

H.7 Linking fragmentation flags with their molecular ions by drift time using ion mobility spectrometry

Ion mobility spectrometry can be used to search for molecular ions of fragmentation flags. There are some challenges in conventional analytical methods because many candidates of molecular ions exist in the full-scan spectrum at specific retention time. Therefore, it is difficult to link molecular ions with fragmentation flags according to the information of retention time (RT) only.

PFAS species are separated in a liquid chromatography (LC) column, and their ions are further separated in a drift tube by ion mobility spectrometry. In the new non-target method use ion mobility spectrometry. The specific characteristics (i.e., ions size, shape, charge, and mass) are used to separate molecular isomers**.** Ion mobility spectrometry is evaluated by an index, “drift time”.

When the molecular ions pass into a drift tube, fragment ions are generated in a collision cell, and the switching collision energy (ms) is faster than the drift time (ms) (see Figure H.2). Therefore, the molecular ion and fragment ions can be observed in the same range of drift time, which thus links them.

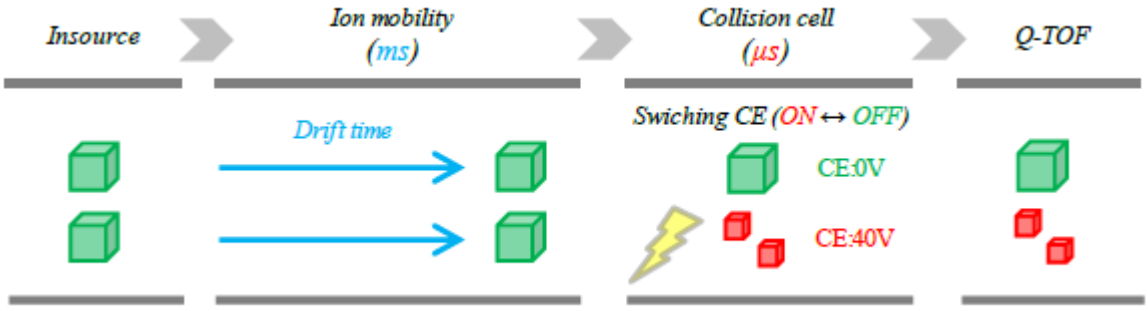

Figure H.2: The structure of LC/IM-QTOF-MS