NATIONWIDE MONITORING OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER IN SOUTH AFRICA
Volume III: Summary report on distribution, sources and health effects of per- and polyfluoroalkyl substances in water

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WATER RESEARCH COMMISSION

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Volume III: Summary report on distribution, sources and health effects of per- and polyfluoroalkyl substances in water

Report to the
Water Research Commission

by

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This report forms part of a set of three reports, viz:
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*. Volume I: Development, optimisation and validation of an LC-MS method for detection and quantification of per- and polyfluoroalkyl substances in water (WRC Report No. TT 931/1/23)
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*.Volume II: Provincial data on the presence, levels and sources of per- and polyfluoroalkyl substances (PFAS) in water sources (WRC Report No. TT 931/2/23)
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*.Volume III: Summary report on distribution, sources and health effects of per- and polyfluoroalkyl substances in water (this report)

EXECUTIVE SUMMARY

BACKGROUND

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemicals used in textiles, packaging, papers, carpets and building and construction materials. Other usage includes, but not limited to, cosmetic formulation, insecticides, paints, non-stick cookware, firefighting foams, hydraulic fluids, waxes and others. Their widespread usage is because of their unique thermal stability and excellent surfactant capacity. During usage or disposal of products treated with PFASs, these chemicals can be released from products into the environment. Other routes of releases into the environment include, among others, during production, military and firefighting operations, discharge of treated effluent and sludge, as well as leachate from landfills. The presence of polyfluoroalkyl substances (PFASs) in water resources is of concern because of their bioaccumulative, persistent, long-range transport and toxic characteristics. Their presence in the environment, particularly water, therefore, needs to be monitored.

PROJECT AIMS

The overall aims of the project were to:

1. Monitor the concentrations of legacy and emerging PFASs in different water sources in pre-selected cities and towns from all the nine provinces in South Africa;
2. Use an appropriate model to identify the PFASs sources and assess the amounts of pollution by resolving the measured mixture of chemical species into the contributions from the individual source types;
3. Develop a nationwide database on PFASs concentrations in different water sources from different parts of the country, and
4. Apply a test battery of bioassays covering a range of endpoints commonly responsive to drinking water to monitor water quality of source and drinking water.

METHODS

1. Selection of sampling sites and collection of water samples

For the purposes of this study, water samples were collected in selected sites in all 9 provinces of South Africa, and the following water sources were sampled for analysis:

- Wastewater effluent (final treated wastewater effluent from a wastewater treatment plant)
- Surface water (from rivers and dams)
- Groundwater
- Drinking water – final treated water from a drinking water treatment plant and household tap water samples from the suburban areas
- Bottled water
- Rainwater

Two sampling approaches were evaluated in this study; grab and passive sampling.

Grab samples were collected from all the sampling sites in all 9 provinces during the dry and wet season. At each site, water samples were collected in clean high-density polyethylene bottles from the various water sources. After collection, the samples were kept in ice and transported to laboratory and prepared for analysis.

Passive sampling was also conducted using Polar Organic Chemical Integrated Sampler (POCIS). The POCIS was deployed at a wastewater treatment plant in the Gauteng Province for two weeks and POCIS extracted on day 7 and 14. Grab samples were also taken on similar days from the same spot where the POCIS was mounted.

2. Monitoring the concentrations of legacy and emerging PFASs in different water sources

Prior to monitoring, it was necessary to first develop, optimize, and validate an appropriate analytical method to determine the presence and concentrations of PFASs in various water sources in South Africa. Details of the procedures followed for the development, optimisation and validation of an LC-MS method for the detection and quantification of PFASs in high and low concentration samples is presented in **Volume I**. Two LC-MS methods were optimised and validated for use in this nationwide PFASs monitoring programme, one for high and another for low PFASs concentration samples.

For the development, optimisation and validation of a method for high PFASs concentration samples, water samples collected from Gauteng province were used for this exercise because of the various industrial activities in the province and hence high levels of known and unknown PFASs may be present in the water samples. For target and non-target PFASs analysis, an LC-MS-8030 triple quadrupole system and a TripleTOF 6600, SCIEX were used, respectively. Targeted provided some specificity and sensitivity for the quantitative analysis; whereas non-targeted analysis leveraged the power of high-resolution modern mass spectrometers to analyse both targeted and undiscovered PFASs. The quantitation of the target compounds was based on internal standard method calibration with concentrations ranging from 1.0-1000 ng/L. An R2=0.99 was achieved in all the calibrations with good precision of the internal standard. The method was then applied to spiked water samples.

Water samples collected from all other provinces were used for the development, optimisation and validation of an LC-MS method for low PFAS concentrations. For the purposes of this project, a target LC-MS/MS (LCMS-8030, Shimadzu) method for PFASs detection and quantification was optimized and validated. Following the identification of emerging PFASs compounds using non-target analysis, more PFASs standards including the sulphonates and alcohol telomers were added to the pool, resulting in the development of four different chromatographic methods comprising **A, B, C** and **D** to ensure good separation of PFASs compounds.

The SPE SupelcoTM Envi18 cartridges purchased from SIGMA Aldrich Ltd were used for all PFASs extraction from all the water samples. Cartridges were first conditioned. Thereafter, the cartridges were then allowed to dry under vacuum for 1 h. The solvent extract was then concentrated under the gentle steam of nitrogen. The reconstituted extract was then transferred to a 2 mL centrifuge tubes and 950 µL of the extract and a 50.0 µL of internal standard added to an autosampler vial. A 10.0 µL of the samples was then injected to the LC-MS/MS.

3. Source apportionment

Multivariate analysis was used to establish inter-relationships between different groups of PFASs, and sample sites and to establish possible sources.

4. Assessing the health effects of PFASs in water using a bioassay method

Samples collected from Northern Cape and Gauteng Provinces were used for this portion of the study. The Yeast bioassay was conducted to determine estrogenic activity in water samples.

RESULTS

Method optimisation and validation

All isomers calibration curves showed linearity, based on correlation coefficients (r) and correlation of determination (r^2) that were greater than 0.99 with good precision of the internal standard. The chromatograms were well separated. The LOD and LOQ values were >0.001 ng/L. The percentage recoveries of the labelled surrogate standards were within the acceptable range.

Distribution of PFASs in water sources in South Africa – grab samples

Analysis using the non-target approach showed that the fluorotelomers were the prominent new compounds. 4:2 Fluorotelomer sulfonic sulfonate, 6:2 Fluorotelomer sulfonate and 8:2 Fluorotelomer Sulfonate were the

most dominant fluorotelomers. Their percentage detection ranged from 30-100 and 0-80 for 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) respectively. Other emerging PFASs identified included: perfluorooctane sulphonamide, N-Methyl perfluorooctane sulphonamide, N-Ethyl perfluorooctane sulphonamide; 6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA, 8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA, 6:2 Fluorotelomer carboxylic acid, 8:2 Fluorotelomer carboxylic acid and 10:2 Fluorotelomer carboxylic acid; Perfluorohexyl Iodide and Perfluorooctyl Iodide; 8:2 Fluorotelomer acrylate, 6:2 Fluorotelomer methacrylate and 8:2 Fluorotelomer methacrylate; Perfluoro-2-methoxyacetic acid, Perfluoro-3-methoxypropanoic acid, Perfluoro-4-methoxybutanoic acid, Perfluoro-2-propoxypropanoic acid, Perfluoro(3.5-dioxahexanoic) acid, Perfluoro(3.5.7-trioxaoctanoic) acid and Perfluoro (3.5.7.9-tetraoxadecanoic) acid.

The telomers sulphonates and alcohols were also detected in a number of the water samples including drinking water. 8:2 FTS and 6:2 FTS featured very prominently in a large number of water samples. Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) percentage detection in drinking water treatment plant ranged from 50-83 and 33-100 respectively. However, their detections were less than 60% in bottled and tap drinking water.

Analysis using the target method indicated the presence of PFASs in most of the samples, some at high and some at low levels. Short chain PFASs were more dominant than the long chain in some cases, albeit long chain PFASs such as PFOA and PFOS was one of the most prevalent compounds detected. Among the short chains, PFBS, PFBA, PFPeA, PFHxA, PFHpA and PFHxS were prevalent in numerous water samples. Due to unavailability of the standards of most of these emerging PFASs, they could not be quantified under the target analysis.

Seasonal influence on the concentrations of PFASs in the samples across most of the provinces was noticeable. Higher concentrations were observed in dry season compared to wet season. PFHxA, PFPeA, 8:2 FTS, PFHpA, LPFBS, PFOA, 6:2 FTS, FOET, FHET and PFBA were all detected in the rainwater samples collected in the Gauteng Province.

Octanol/water partition coefficient (K_{ow}) values obtained from the literature were used to assess the tendency of the PFASs detected (KwaZulu-Natal samples) in the present report to move from the aqueous phase into organic. In some drinking water/tap water, PFOA, L-PFHxS, PFHxA, PFHeA, 8:2 FTS, PFNA, PFHpA, PFUdA, 6:2 FTS, FOET, FHET and PFBA were detected in all the samples. These PFASs compounds have K_{OW} values of 2.829-6.82, indicating their tendency to move from the aqueous phase to the organic phase, hence their detection in most of the water samples.

Monitoring of PFASs concentrations in water using passive sampling method

All PFASs targeted were detected except 6:2 FTS, PFDOA, 8:2 FTS, and PFHxDA. 4-2 FTS had the highest concentration of 81.67 ng/L. The same trend was also observed in grab samples, although FOET exhibited the highest concentration of 22.36 ng/L in this case. Generally, on day 7, the PFASs concentrations recorded for POCIS higher than the grab samples except for FOET. On 14 day, the mean PFASs concentrations for POCIS-HLB ranged 0.94-98.86 ng/L. PFNA had the highest concentration of 94.04 ng/L. On the other hand grab sample had mean concentration range of LOD-30.55 ng/L. PFHxA had the highest concentration of 30.55 ng/L. The PFASs concentrations in POCIS were significantly higher than that of grab samples. The difference between the concentrations recorded for the two sampling method was because grab samples provided only snap shot concentrations, while POCIS-HLP provided time weighted average concentrations.

Source apportionment

Multivariate statistical analysis (PCA) was used to establish inter-relationships between different groups of PFASs, and sample sites and to establish possible sources. From the PCA analysis, some PFASs showed similar sources; while others showed different sources. This trend was also observed with the sampling sites.

Therefore, based on the land use activities around the sampling sites, the presence of PFASs detected in the water samples may have originated from the current/historical usage of PFASs in various activities.

Assessing the human health effects of PFASs using a bioassay method

Estrogenic activity was detected in 12 of the 14 samples tested, whereas cytotoxicity was determined in 9 of the 14 samples. The water samples EEq ranged from below limit of quantification to a maximum of 718 ng. These are significantly higher than the recommended trigger value for drinking water. Compared to dry season, a higher EDC concentration was observed during wet season, notably in wastewater and drinking water treatment facilities. This suggested that the current treatment techniques are unable to remove EDC chemicals. Of the 18 standard PFASs chemicals subjected to bioassay test, only PFOS exhibited cytotoxicity. Therefore, the observed estrogenic activity and cytotoxicity in the water samples may have been caused by PFOS, which demonstrated estrogenic action in yeast bioassays. However, other contaminants in the water samples such as trace metals may have also contributed to the observed estrogenic action since metals that exert metalloestrogens were detected in the water samples.

CONCLUSIONS

Based on the findings, the following conclusions can be made:

- Non-target and target methods for identification and quantification of PFASs in various water sources were successfully developed, validated and used for monitoring the distribution and sources of PFASs in water in South Africa.

- The concentrations of PFASs observed in the present study are, in some cases, higher than the values reported by other researchers in water samples. The PFASs detected in the bottled drinking water in the present study are higher than the IBWA operational limits of 5 ng/L for a single PFASs and 10 ng/L for more than one PFASs. Compared to the health advisory levels at 70 ng/L, by the USEPA to protection its sensitive populations, from a lifetime of exposure to PFOA and PFOS from drinking water, the concentrations of PFOS and PFOA in drinking water in the present study are generally much lower.

- PFASs compounds were detected in both grab and passive samples. However, the PFASs concentrations in the POCIS passive sampler were higher than the grab samples collected on the same days. The observed difference suggested the cumulative time-weighted concentrations of PFASs with passive samplers compared to one-off grab sampling method.

- The PFASs detected in the bottled drinking water in the present study are higher than the IBWA operational limits of 5 ng/L for a single PFASs and 10 ng/L for more than one PFASs. Compared to the health advisory levels at 70 ng/L, by the USEPA to protection its sensitive populations, from a lifetime of exposure to PFOA and PFOS from drinking water, the concentrations of PFOS and PFOA in drinking water in the present study are generally much lower except in few drinking water samples.

- Some PFASs showed similar sources; while others showed different sources. This trend was also observed with the sampling sites. It is, therefore, possible that all the land-use activities around the sampling sites, may have contributed to the observed PFASs in the water samples.

- Estrogenic activity was detected in 12 of the 14 samples tested, whereas cytotoxicity was determined in 9 of the 14 samples. Of the 18 standard PFASs chemicals subjected to bioassay test, only PFOS exhibited cytotoxicity. However, other contaminants in the water samples such as trace metals may have also contributed to the observed estrogenic action since metals that exert metalloestrogens were detected in the water samples.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

AFFF	Aqueous Film Forming Foam
ASTM	Association of Standardized Test Method
ATSDR	Agency for Toxic Substances and Disease Registry
BFRs	Brominated Flame Retardants
CCD	Charge-Coupled Devices
DCM	Dichloromethane
DEA	Department of Environmental Affairs
DWS	Department of Water & Sanitation
EDC	Endocrine disrupting compound
EFSA	European Food Safety Authority
EtOH	Ethanol
FIA	Flow Injection Analysis
FTCA	Fluorotelomer Carboxylic Acids
FTOH	Fluorotelomer Alcohol
GC-MS	Gas Chromatography-Mass Spectrometer
HDPB	High Density Polyethylene Bottles
HPLC	High Pressure Liquid Chromatography
L-FABP	Liver Fatty Acid-Binding Protein
LC-MS	Liquid Chromatography-mass spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MRLs	Minimum Risk Levels
MRM	Multiple Reaction Monitoring
MTBE	Methyl Tert-Butyl Ether
NTMP	National Toxicant Monitoring Programme
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PCF	Propyl Chloroformate
PES	Polyethersulfone
PFAAs	Per- fluoroalkyl acids
PFASs	Per- polyfluorinate Alkyl substances
PFCAs	Per- fluoroalkyl Carboxylic Acids
PFHxS	Per- fluorohexane Sulfonic Acid
PFNA	Per- fluorononanoic Acid
PFOSE	Per- fluorooctane Sulfonamidoethanol
POPs	Persistent Organic Pollutants
POSF	Per- fluorooctanesulfonyl Fluoride
PP	Polypropylene

PPARs	Activate Peroxisome Proliferator-Activated Receptors
R^2	Correlation Coefficients
ROS	Reactive Oxygen Species
RQS	Resource quality services
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
St	Saint
STP	Sewage Treatment Plants
TCM	Trichloromethane
TDIs	Tolerable Daily Intakes
TFE	Tetrafluoroethylene
TIC	Total Ion Chromatogram
ToF-HRMS	Time of flight – High Resolution Mass Spectrometry
TUT	Tshwane University of Technology
USEPA	United States Environmental Protection Agency
WAX	Weak Anion Exchange
WTPs	Water Treatment Plants
WVDEP	West Virginia Department of Environmental Protection
WWTPs	Wastewater Treatment Plants

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

PFASs are man-made chemicals which have been commercially produced since the 1960s (Ahrens *et al*., 2015). The strength of the carbon/fluorine bond makes the molecules chemically very stable and highly resistant to biological degradation. Per- and polyfluoroalkyl substances (PFASs) are highly persistent to natural degradation due to the high electronegativity of fluorine. They are also resistant to heat and hydrolysis (Taniyasu *et al*., 2013). Per- and polyfluoroalkyl substances (PFASs) are all anthropogenic organic chemicals (Kissa, 2001; Lindstrom *et al*., 2011). Some of their properties include water, oil and grease repellence. Due to the carboxylic or sulfonic acid groups, PFASs have high water solubility and can be transported across long distances via water (Yamashita *et al*., 2005; Ahrens, 2011). Per- and polyfluoroalkyl substances (PFASs) bioaccumulate and biomagnify (Martin *et al*., 2003) and some studies have confirmed toxic and bioaccumulative effects of two representative PFASs namely, PFOS and PFOA (Lau *et al*., 2007; EPA, 2009). PFASs are highly persistent due to their resistance to photolysis, pyrolysis and biotransformation (Kissa, 2001). Due to their persistence and abiotic degradation properties, they are used widely in industrial and commercial applications (OECD, 2002, OECD, 2005; Washburn *et a*l., 2005; Fromme *et al.*, 2009).

Per- and polyfluoroalkyl substances (PFASs) are used in textiles, packaging, papers, carpets and building and construction materials. A number of perfluorinated compounds have been used for household and industrial applications. Furthermore, PFAS are used in cosmetic formulation, insecticides, paints, firefighting foams, hydraulic fluids and waxes. Their widespread usage is because of their unique thermal stability and excellent surfactant capacity. During usage or disposal of products treated with PFASs, these chemicals can leach into the environment. They can also be released during production, military and firefighting operations, discharge of treated effluent and sludge, as well as landfill leachates.

The presence of PFASs in source waters is, in most cases, not removed by conventional water treatment processes due to the design and treatment processes. Water users and consumers can, therefore, be exposed unintentionally to PFASs with their concomitant toxic effects in such instances. It is for these reasons that monitoring of PFASs in South African source waters are particularly important. Therefore, by conducting a large-scale monitoring programme that would provide a nation-wide inventory of the concentrations of PFASs in South Africa source waters is a step in the right direction to safeguard public health. In addition, this exercise would contribute towards critically reviewing the current drinking water guidelines in order to address the challenges that may be posed by the presence of PFASs in South African source waters. Data generated on PFASs will contribute towards the National Toxicant Monitoring Programme (NTMP).

Two approaches were employed in this nation-wide PFASs monitoring programme namely, **targeted** and **non-targeted**. Targeted provides an unparalleled level of specificity and sensitivity for the quantitative analysis. However, for new and emerging compounds, this approach is not effective in detecting species that may be of interest, regardless of their chemistry or concentration. Non-targeted analysis leverages the power of high-resolution modern mass spectrometers to analyse both targeted and undiscovered chemicals.

1.2 PROJECT AIMS

The objectives of the overall project were to:

1. Monitor the concentrations of legacy and emerging PFASs in different water sources in pre-selected cities and towns from all the nine provinces in South Africa;

2. Use appropriate model to identify the PFASs sources and assess the amounts of pollution by resolving the measured mixture of chemical species into the contributions from the individual source types;
3. Develop a nation-wide database on PFASs concentrations in different water sources from different parts of the country and
4. Apply a test battery of bioassays covering a range of endpoints commonly responsive to drinking water to monitor water quality of source and drinking water.

1.3 DEVELOPMENT OF A FRAMEWORK FOR NATIONWIDE MONITORING OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER

The overall project was focused on nationwide monitoring of PFASs in different water systems from all the provinces in South Africa in order to present an overview of the presence, levels, sources, as well as human health risks of PFASs. In line with the aims of the study, a framework for nationwide PFASs monitoring was developed as means for environmental protection and public health. This framework included several aspects, which are covered in the sub-sections below.

1.3.1 Target Analytes Selection

Identification of a list of specific PFASs to monitor, considering those with known environmental persistence (legacy), emerging ones, as well as those with potential health risks.

1.3.2 Sampling Strategy

Development of a strategic sampling plan that considers the geographical distribution of monitoring sites, including surface water, groundwater, drinking water sources, wastewater discharges, rainwater and bottled water. An attempt was also made to include routine monitoring sites and locations with known or suspected PFAS contamination sources. The samples were collected over different seasons and under various hydrological conditions to account for temporal variations.

1.3.3 Sample Collection

PFASs monitoring was conducted using both a grab and passive sample collection method. In both instances, standardized sampling techniques were used to ensure consistency across monitoring sites. Thereafter, strict sample handling and chain of custody procedures were to prevent contamination.

1.3.4 Sample Analysis

Prior to sample analysis, a liquid chromatography-mass spectrometry (LC-MS) method for PFAS analysis was optimised and validated through the analysis of calibration standards and selection of multiple reaction monitoring (MRM) to ensure sensitivity and specificity. V**olume I of this report** addresses this aspect. The optimised method was then used for sample analysis, and the results obtained are presented in Chapters 3-11 (volume II).

1.3.5 Risk Assessment

The monitoring data obtained was used to assess risks associated with PFAS exposure, particularly in regions with elevated PFAS concentrations.

1.3.6 Data management

Data management will involve creating database that can easily be accessed by interested users. Two types can be considered namely, local or cloud-based environments? With local database, platform can be created where permission can then be granted to the potential users of the database. There are a host of structured query language softwares that one can use to undertake this task. On the other hand, where cloud-based system is expected/preferred, this will require some additional costs for subscription. In order to minimize cost, a local database that requires some permission for the potential users presents a better option.

1.4 PROJECT LIMITATIONS

Seasonal (wet and dry seasons) samples were collected from all the following provinces, Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North west and Northern Cape except western Cape where only dry season sample was collected. This was because the Western Cape Province monitoring programme was later assigned to the late Dr Rehana Malga-Enus research group at Stellenbosch University. Sample collection from pristine areas was not possible because of inaccessibility of the identified areas.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Over the years, PFASs have been reported to spread globally in various environmental matrices, such as air, surface water, sediments, aquatic invertebrates, fish, and wildlife and predominantly in the aqueous environment (Martin *et al*., 2003). PFASs can travel long distances in air and water current due to their chemical structure. The aquatic ecosystem has been found to be an important and major medium for PFASs transportation, since these chemicals have been found often detected in environmental waters and strongly proved to accumulate in aquatic biotas (Prevedouros *et al*., 2006). Rivers are an important pathway for transport of contaminants from land to oceans, and PFASs levels in rivers are up to thousands of ngL^{-1} (Skutlarek *et al*., 2006).

PFASs have also been detected in influents and effluents from WWTPs around the world (Becker *et al*., 2008; Guo *et al*., 2010). The discharge from industrial WWTPs has been said to be one of the significant point sources of PFASs pollution of aquatic environment in several studies (Bossi *et al*., 2008; Lin *et al*., 2009c; Lin *et al*., 2010; Kim *et al*., 2012). Since the levels of sewage sludge reflect the releases from use of PFASs from consumer products and industrial use, they can be monitored from sewage sludge and a number of studies have monitored PFASs in sewage treatment plants in industrial countries (Ahrens *et al*., 2009; Yoo *et al*., 2010; Guo *et al*., 2010; Washington *et al*., 2010). The contamination of sewage sludge by PFOS and PFOA from the application of soil fertilisers has led to the contamination of soils and runoffs from these sludge and contamination of surface water and drinking water reservoirs (Skutlarek *et al*., 2006; Kröfges *et al*., 2007; USEPA, 2012).

PFASs have also been detected in drinking water in continents such as the US, Europe, Asia (Loos *et al*., 2007) with concentrations exceeding 10 ngL^{-1} for PFOA and PFOS and in China with a median of 4.2-5.4 ngL^{-1} which were comparable to those in US, Europe and Japan (Saito *et al*., 2004; Jin *et al*., 2009). Relatively high concentrations of PFASs have been observed in human serum (e.g. median 374 ngL^{-1} PFOA) among populations who consume drinking water, highly contaminated by PFASs (e.g. 1900-18600 ngL^{-1} PFOA) (USEPA, 2001; LHWA, 2005), indicating that water as the dominant source for the population residing near contaminated areas. From the compilation and evaluation of results from different toxicological studies in 2008 carried out by the European Food Safety Authority (EFSA), it was established that the tolerable daily intakes (TDIs) for PFOS and PFOA are at 0.150 ng g^{-1} and 1.50 ng g^{-1} respectively (EFSA, 2008). Due to their possible negative implications in human health there is an urgent need to continuously assess PFASs in drinking water.

The detection of PFASs in surface and groundwater has raised concern over human exposure risk, as these are both sources of drinking water (Vierke *et al*., 2012). Several countries have set guideline values for PFASs in surface water, groundwater and drinking water supplies to reduce human exposure to PFASs (European Commission, 2002; MDH, 2007; USEPA, 2009; USEPA, 2016). The Provision Health Advisory for PFOA and PFOS is 400 ngL^{-1} and 200 ngL^{-1} respectively, as established by the Office of Water (OW) from the EPA (USEPA, 2009) and they are also susceptible to be introduced into the Safe Drinking Water Act.

In summary, the presence of PFASs has been reported worldwide in general populations of different regions, mostly in developed countries (Sinclair and Kannan, 2006; Olsen *et al*., 2009; Steenland *et al*., 2010;Wang *et al*., 2011; Mudumbi *et al*., 2014; Ahrens *et al*., 2015). The extent of PFASs contamination in the environment is commonly associated with industry and economic development (Wang *et al*., 2012a; Cai *et al*., 2012). Because of that, many previous studies on PFASs concentrated mainly on areas around emission origins including WWTPs and fluorochemical plants (Wang *et al*., 2011; Chen *et al*., 2017; Lu *et al*., 2015). In addition, PFOS and PFOA are found to be the most abundant; hence, most studies have focused on them.

2.2 OCCURRENCE OF PFAS IN WATER

2.2.1 Occurrence of PFAS in water in the Unites States of America

A monitoring study of the Tennessee River near a 3M production plant, manufacturing fluorochemicals in Decatur, Alabama showed elevated concentrations PFOA and PFOS at 598 ngL^{-1} and 144 ngL^{-1}, respectively (Hansen *et al*., 2002). High PFOA concentrations ranging from 361-1050 ngL^{-1} were detected from one of the six wastewater treatment plants that were investigated in New York State, U.S. Domestic and industrial waste were concluded to be the main contributors to the PFOA concentrations in that plant (Sinclair and Kannan, 2006). In late 2014, PFASs released from municipal and industrial sources were assessed from eight WWTPs discharging in the San Francisco Bay. The effluent concentrations reported were with highest median concentrations of PFHxA (24 ngL^{-1}), followed by PFOA (23 ngL^{-1}), PFBA (19 ngL^{-1}), and PFOS (15 ngL^{-1}). The short chain PFCAs had shown increase when compared to samples collected in 2009. Of the eight WWTPs sampled, two had significantly higher total concentrations (390-2900 ngL^{-1}). The elevated levels detected were concluded to be in relation with aqueous firefighting foam (AFFF) sources impacting the influent (Houtz *et al*., 2016).

According to the USEPA (2021) new analyses indicate that negative health consequences from PFOA and PFOS exposure can occur at considerably lower levels than previously thought and that PFOA is a potential carcinogen. The EPA has stated that enforceable drinking water limits for PFOA and PFOS will be established by 2023, and the recent draft documents have the role to achieve this aim (press@epa.gov).

2.2.2 Occurrence of PFAS in water in Europe

Surface waters samples from the Ruhr and Moehne Rivers in Germany showed high total concentrations of PFASs (446-4385 ngL^{-1}). The source of contamination was localized to an area of agricultural land, which came from industrial waste with high concentrations of PFASs that was applied as a 'soil improver' on agricultural land (Skutlarek *et al*., 2006). The industrial effect on elevated concentrations of PFASs was observed in River Tanaro, a tributary of the Po River. In the study, high concentrations of PFOA up to 1270 ngL^{-1} were detected (Loos *et al*., 2008). Effluents of some sewage treatment plants (STP) were detected with high PFOA levels (4.2-2600 ngL^{-1}) followed by PFOS (0.4-123 ngL^{-1}) in Yodo River, Japan (Lein *et al*., 2008).

AFFFs were found to be a problematic source of PFASs in water sampled near a fire training facility at Stockholm Arlanda Airport in Sweden, high concentrations of PFASs were detected at approximately 4000 ngL^{-1} (Ahrens *et al*., 2015). In the same study, concentrations ranging from 146-344 ngL^{-1} for PFASs were detected in Lake Halmsjön of which there was no direct stream connecting the fire training facility to the Lake, giving an indication of sub-surface transport of PFASs to the Lake (Ahrens *et al*., 2015). High PFOS concentration (2710ngL^{-1}) related to industrial activities were detected in the Llobregat River basin, Spain (Campo *et al*., 2015).

Total concentrations of PFASs were found in the range <LOD-725 ngL^{-1} in a nationwide scale investigation of rivers and lakes in mainland France (Munoz *et al*., 2015). The most polluted sites were found near large urban areas and industrial sites. Maximum concentration was detected for PFBA up to 251.3 ngL^{-1} in Ebro and 742.9 ngL^{-1} in Guadalquivir River basins, Spain. High contamination levels were found downstream WWTPs, near a military camp and in ski resorts (Lorenzo *et al*., 2016). Total concentrations of PFASs ranging from <LOD-77 ngL^{-1} for surface water were detected in samples collected in urban and industrial sites in the tropical areas constituted by the French Overseas Territories (Munoz *et al*., 2017).

2.2.3 Occurrence of PFAS in water in Asia

For the first time PFASs were reported in the coastal areas of Bangladesh which is exclusively riverine agricultural country that is undergoing rapid industrialisation, urbanisation, and economic development (Habibullah-Al-Mamum

et al., 2016). The total concentrations of PFASs detected in surface water ranged from 10.6-46.8 ngL^{-1} (Habibullah-Al-Mamum *et al*., 2016).

Rostkowski *et al*. (2006) and Naile *et al*. (2010) detected high PFASs concentrations, particularly for PFOS ranging from 2.24-651 ngL^{-1} and 4.11-450 ngL^{-1} resulting from industrial areas on the coast of Korea (Rostkowski *et al*., 2006; Naile *et al*., 2010). The rapid increase in industry and production of PFASs has been observed in waters from China as well, as high concentrations of PFASs have been detected in some studies. Wang *et al*., 2011 found high levels of PFOS and PFOA in estuarine and coastal waters of North Bohai Sea, which has been a region of significant urbanisation and industrialisation. In Daling River and Daliao River, total PFASs concentrations ranged from 370-713 ngL^{-1} and 44.4-781 ngL^{-1}, respectively (Shao *et al*., 2016;Chen *et al*., 2015). The effect of industrial wastewater in the Bohai Sea region was observed in Chen *et al*., 2017 study, where PFASs were detected at high concentrations up to 69.238 ngL^{-1}.

In surface waters of Eastern China, Lu *et al*., 2015 observed a decrease in PFASs concentrations and greater concentration in shorter chain length PFASs such as PFHxA, confirming the global restrictions on manufacture and use of PFOS imposed by the Stockholm Convention (Lu *et al*., 2015; Yu *et al*., 2013). Although, PFOA still continues to dominate in many areas around the world including remote areas and developing countries (Doung *et al*., 2015; Sammut *et al*., 2017; Lu *et al*., 2015; Essumang *et al*., 2017; Wang *et al*., 2011; Mudumbi *et al*., 2014; Shao *et al*., 2016). The shift in concentrations of long chain PFASs was also observed in the coastal areas of Bangladesh, where low concentrations of PFOS were detected than the shorter chain PFASs (Habibullah-Al-Mamum *et al*., 2016). PFASs levels detected in San Francisco Bay effluent samples also reflected the manufacturing shifts towards shorter chained PFASs (Houtz *et al*., 2016). Sharma *et al*. (2016) study in Ganges River basin, India, reported short chain PFASs exceeding longer chain analogues. Moreover, the use of PFASs containing products has been observed as a source of PFASs in surface water in developing countries as they have been detected in areas with little or no PFASs manufacturing industries.

2.2.4 Occurrence of PFAS in water in Africa

In Africa, the first data generated on PFASs was a preliminary screening of PFASs levels in human cord blood in South Africa (Hanssen *et al*., 2010). Since then, a few more studies on the detection of PFASs in African environment have been reported (Dalahmeh *et al*., 2018). Some of these studies have mainly focused on PFOS and PFOA (Dalahmeh *et al*., 2018). An appreciable level of PFASs contamination was observed in surface waters in Ghana, even though the country does not manufacture PFASs (Essumang *et al*., 2017). Mudumbi *et al*. (2014); Mudumbi *et al*. (2014) also detected high concentrations of PFOS and PFOA in river water samples and sediments in the Western Cape, South Africa. Detectable levels of PFASs were found in wastewater and surface water in Uganda (Dalahmeh *et al*., 2018) and in the Vaal River, South Africa (Groffen *et al*., 2018). The studies by (Hanssen *et al*., 2010; Mudumbi *et al*., 2014; Essumang *et al*., 2017; Dalahmeh *et al*., 2018) give an indication that PFASs are present in the continent and there is a need for more information.

Also the frequent detection of short-chain PFASs in some studies together with a decline in levels of long-chain PFASs has shown an indication of the effectiveness of the shift from long-chain to short-chain PFASs and regulations (Karásková *et al*., 2016; Yao *et al*., 2018). The shift from long-chain to short-chain PFASs has been seen to increase the levels of equally persistent short-chain PFASs in the environment (Yao *et al*., 2018). These changes have been observed in many studies around the world including remote and Mediterranean areas (Wang *et al*., 2011; Mudumbi *et al*., 2014; Doung *et al*., 2015; Lu *et al*., 2015; Shao *et al*., 2016; Habibullah-Al-Mamum *et al*., 2016; Houtz *et al*., 2016; Sammut *et al*., 2017; Essumang *et al*., 2017).

In addition, similar concentration was reported in two Ghana Rivers for PFOA (86.2-321.1 ngL^{-1}) and PFOS (77.2-276.6 ngL^{-1}) (Essumang *et al*., 2017). In some areas, lower concentrations have been detected with short-chain PFASs dominating (Ahrens *et al*., 2016; Dalahmeh *et al*., 2018; Groffen *et al*., 2018). The total PFASs

concentrations ranged from 0.073-5.6 ngL^{-1} in Lake Tana, Ethiopia, and PFBA and PFHxA were reported dominant (Ahrens *et al*., 2016).

In South Africa, there has been a lack of reported and documented studies on the occurrence of PFASs (Mudumbi *et al*., 2014; Hanssen *et al*., 2010). Mudumbi *et al*. (2014) detected PFOA and PFOS in three South African rivers namely, Diep, Salt and Eerste Rivers (Western Cape) with concentrations ranging from 0.7-390 ngL^{-1} and <LOD-181.8 ngL^{-1}, respectively. PFPeA was the highest detected PFAS in the Vaal River, South Africa at 32.3-45.0 ngL^{-1} (Groffen *et al*., 2018). PFHxS also dominated in surface water in Uganda (Dalahmeh *et al*., 2018). These findings confirm the dominance of the use of C6 and shorter-chain length alternatives compared to longer chain length in industrial production.

2.3 PATHWAYS OF EXPOSURE TO PFAS AND HEALTH EFFECTS

2.3.1 Sources of exposure to PFASs

The occurrence of PFASs was first reported in global wildlife (Giesy and Kannan, 2001) and in human serum (Hansen *et al*., 2001). PFASs have been reported to be present in various biological and environmental media, food products and human tissues (Yaun *et al*., 2016; Hansen *et al*., 2010; Prevedouros *et al*., 2006) and in remote areas (Vierke *et al*., 2012) due to their bioaccumulative properties (Zhao *et al*., 2017). PFASs have been reported to accumulate in blood, liver and kidney of living organisms due to their high water-solubility, in comparison with other well-studied organic pollutants that are of adipose-accumulative toxicity (Ahrens *et al*., 2009; Kärrman *et al*., 2010; Murakami *et al*., 2011).

Multiple sources of potential exposure to PFAS have been previously identified in the general population. These sources include diet (Tittlemier *et al*., 2007; Trudel *et al*., 2008; Vestergren and Cousins, 2009; Haug *et al*., 2011a; Domingo and Nadal, 2017), drinking water (Post *et al*., 2009; Thompson *et al*., 2011; Hu *et al*., 2016; Domingo and Nadal, 2019), air and dust (Piekarz *et al*., 2007; Haug *et al*., 2011b; Goosey and Harrad, 2012; Fromme *et al*., 2015; Karásková *et al*., 2016) and consumer products (Begley *et al*., 2005; Bradley *et al*., 2007; Trier *et al*., 2011; Hill *et al*., 2017; Lee *et al*., 2017). The widespread production of PFAS, their use in common commercial and household products, their improper disposal and their resistance to degradation have led to daily human exposures via oral ingestion, inhalation and dermal contact. Different sources and pathways of human exposure are summarised in Table 2-1. Furthermore, the releases of PFASs to the environment can occur next to chemical manufacturing locations, at industrial sites where PFASs are used, and at various stages of product use and disposal (Figure 2-1).

Table 2.1: Sources and pathways of human exposure to PFASs

Sources	Pathways
Dietary sources, such as:	Environment / Ingestion
• Fish and shellfish	Ingestion
• Drinking water	Ingestion
• Food-packaging materials	Ingestion
• Non-stick cookware	Ingestion
• Others (including dairy products, eggs, beverages and vegetables)	
Non-dietary sources, such as:	Inhalation
• Indoor air	Inhalation/ingestion
• Indoor dust	Environment
• Soil and sediment	Inhalation/dermal absorption
• Impregnation spray (for furniture and carpet)	Dermal absorption
• Cosmetics	Dermal absorption
• Other consumer products (including skin waxes, leather samples and outdoor textiles)	

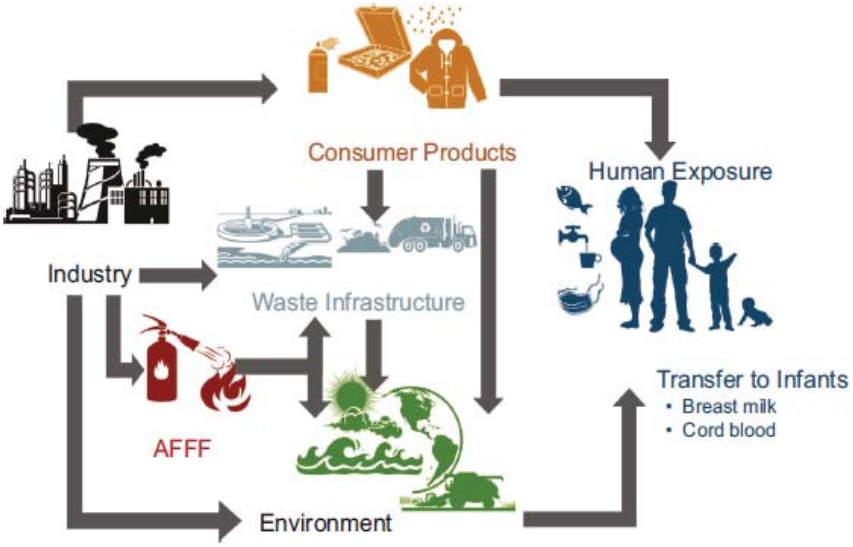

Figure 2.1: Overview of PFASs exposure pathways for different human populations (Source: Sunderland *et al.*, 2018)

2.3.1.1 Dietary sources

The highest exposures to PFASs are often from dietary intake, particularly to PFOS and PFOA (Tittlemier *et al*., 2007; Trudel *et al*., 2008; Vestergren and Cousins, 2009; Haug *et al*., 2011a; Domingo and Nadal, 2017). Fish and shellfish generally exhibit the highest PFAS concentrations and detection rates among all types of foodstuffs (Domingo and Nadal, 2017; Jian *et al*., 2017). Other potential dietary sources of PFAS include dairy products, eggs, beverages and vegetables (Haug *et al*., 2010; Zhang *et al*., 2010; Noorlander *et al*., 2011; Domingo *et al*., 2012; Eriksson *et al*., 2013). However, these foodstuffs have generally low concentrations and low detection frequencies compared to fish and shellfish (Jian *et al*., 2017). In addition, food can become contaminated with PFAS through transfer from food packaging and/or processing (Schaider *et al*., 2017) because PFAS are used as in grease- and water-repellent coatings for food-contact materials and non-stick cookware (Begley *et al*., 2005).

2.3.1.2 Drinking water as a medium of exposure to PFASs

Surface waters such as rivers, lakes and groundwater are one of the main sources of drinking water (Thompson *et al*., 2011; Kim *et al*., 2011). The contamination of these streams by pollutants such as PFASs usually affects drinking water levels of these pollutants, which in turn exposes the general population. Contamination of drinking water by PFASs, particularly, PFOA and PFOS can cause a serious problem because of their high water solubility and poor efficiency to be removed during purification processes (Takagi *et al*., 2008; Skutlarek *et al*., 2006). Recent studies show decreased PFASs levels and have been reported following industrial treatment processes (Stubleski *et al*., 2017; Essummang *et al*., 2017). Due to health risks to human, the detection of these compounds in drinking water has raised some concerns to the exposure of the general population.

As a result, the USEPA issued out a lifetime health advisory level for PFOA and PFOS of 70 ngL^{-1} (USEPA, 2016a). Drinking water has been identified to be the major source of exposure of PFASs in some countries such as Sweden, Italy, and the United States (Stubleski *et al*., 2017; Ingelido *et al*., 2018; Daly *et al*., 2018). However, this has not been the case in other studies as reported by Haug *et al*. (2011); Vestergen *et al*. (2012) on drinking water to be the second contributor source after dietary intake in Norway and Sweden. However, Hemat *et al*. (2010); Thompson *et al*. (2011); Schwanz *et al*. (2016) reported that in other countries, PFASs has been a minor contributor to the exposure. Water used for cooking is also known to contribute to the amount of PFASs assumed through food

(USEPA, 2016b), particularly in foods such as pasta, rice and cereal which absorb a considerate amount of water during cooking (Ingelido *et al*., 2018).

PFASs contamination have been identified to have resulted from long term exposures of populations residing in highly contaminated areas and individuals that have been residing in the areas for a longer period (Ingelido *et al*., 2018; Daly *et al*., 2018). Though PFASs have been detected in a number of countries and populations, the extent of human exposure of PFASs by drinking water seems to differ.

2.3.1.3 Wastewater as point sources for PFASs environmental pollution

Wastewater treatment plants (WWTPs) are the well-studied point source of PFASs to surface and drinking water. Becker *et al*. (2008) concluded that, in several small rivers in Germany, the majority of PFASs enter the rivers via municipal WWTPs. Several studies revealed that one of the significant point sources for PFASs pollution of aquatic environment is the discharge from industrial WWTP (Bossi *et al*., 2008; Lin *et al*., 2009c, Lin *et al*., 2010; Kim *et al*., 2012). Atmospheric deposition has been considered as a major source of PFAS contamination (Ahrens and Bundschuh, 2014), whereas other point sources to the aquatic ecosystem are sewage treatment plants (STP) and landfills. The use of products containing PFASs such as cookware, sports clothing, plastics and others has been reported to affect PFASs levels in surface water (Essumang *et al*., 2017).

Medical devices such as tetrafluoroethylene copolymer (ETFE) layer and radio-opaque ETFE production, in vitro diagnostic medical devices and charge-coupled devices (CCD) colour filters are also known to contain PFOS (Stockholm Convention, 2009). Chirikona *et al*. (2015) suggested that polishing equipment or plastic and elastomers where PFASs are used as dispersants could be a source.

Point sources, such as industrial emission from PFASs production sites were observed to have notable impact on surface water used for tap water production in the USA (Quiñones and Snyder 2009). Around commercial and military airfields, high levels of PFASs can be detected in surface waters. This contamination is connected with the use of aqueous film forming foam (AFFF) containing PFASs and their precursors, such as 6:2 FTSA which can degrade to short chain PFASs (Prevedouros *et al*., 2006). Aqueous film forming foams (AFFFs) containing PFASs were also reported to be a source of contamination in Swedish drinking water from a groundwater well downstream a military airport, where high concentrations of PFHxS were found (Stubleski *et al*., 2017). In China, landfill sites were suggested to be a major source of PFASs to groundwater (Xiao *et al*., 2012). In Ghana, elevated concentrations of PFASs were observed in the Pra and Kakum Rivers (Essumang *et al*., 2017). The pollution of Pra River was from mine wastes, but it was unclear if the pollution contributed to the elevated concentrations. In addition, both rivers were impacted by agricultural activities.

The impact of agricultural activities was also cited in Nascimento *et al*. (2018) study, where the source of PFASs was found to be sufluramid applied in eucalyptus plantations. Rural area sources of PFASs may simply originate from domestic and farming wastewater and from atmospheric precipitation. However, rural areas in close proximity to cities may be affected by urban industrial pollution (Chen *et al*., 2016).

2.3.1.4 Air and dust (indoor environments) as sources of exposure to PFASs

Some PFASs polymers such as FTOHs were frequently used for impregnation treatment of furniture and floor coverings and as intermediates in manufacturing various household products (e.g. paints, carpet and cleaning agents). These neutral PFAS, mainly FTOHs, FOSA and FOSEs, are volatile compounds that are easily released into indoor environments (air and dust) due to their low water solubility and high vapour pressure (Langer *et al*., 2010; Haug *et al*., 2011b; Yao *et al*., 2018). Perfluoroalkyls have also been detected in indoor air and dust (Kubwabo *et al*., 2005; Barber *et al*., 2007; Strynar and Lindstrom, 2008). In the study of 67 houses in Canada, carpeted homes had higher concentrations of PFOA, PFOS and PFHxS in dust, possibly due to the use of stain-repellent coatings (Kubwabo *et al*., 2005). The use of aqueous firefighting foams at military installations and the

production of fluorochemicals at industrial facilities have resulted in widespread contamination in soil and sediment (Xiao *et al*., 2015; Anderson *et al*., 2016). Many consumer products, such as ski waxes, leather samples, outdoor textiles and cosmetics products including hair spray and eyeliner, also contain PFASs (Kotthoff *et al*., 2015; Danish EPA, 2018).

FTOHs and Perfluorooctane sulphonamide (PFOSA) are considered major precursors of PFCAs and PFSAs, respectively (Fasano *et al*., 2009; D'eon and Mabury, 2011). These precursor compounds may degrade to form PFASs via biotic and abiotic pathways (Dinglasan *et al*., 2004; Ellis *et al*., 2004). PFASs are frequently found in indoor air and dust and have been confirmed to have a slight contribution to the overall exposure of PFASs to humans. FTOHs have been found to be highest in shops selling outdoor wear and textiles (Langer *et al*., 2010; Schlummer *et al*., 2013), which is the major indoor source to PFASs (Yao *et al*., 2018). Another source of FTOH indoors are shoes, textiles and leather care products used indoors by consumers which may add to the FTOH concentrations in indoor air (Schlummer *et al*., 2013).

Treated carpets may also be a source of significantly increased indoor levels of PFASs (Gewurtz *et al*., 2009). Fraser *et al*. (2013) reported elevated PFAS concentrations in dust from homes that more frequently clean carpets. However, that was not the case in the study by Karásková *et al*. (2016) where more cleaning of carpets was reported to result in greater removal of PFASs. However, Yao *et al*. (2018) later reported that PFASs concentrations can be triggered by washing and abrasion in products. Concentrations of PFAS and related compounds in products and indoor air also depend on the region and usage, as some studies have reported low levels of the compounds in households (Jogsten *et al*., 2012; Shoeib *et al*., 2016) indicating low usage of PFASs products in countries such as Spain and Egypt compared to the US and China. There are no serious health concerns associated with PFASs contamination in indoor air and dust as the contribution of the exposure is low (Jogsten *et al*., 2012; Schlummer *et al*., 2013; Fromme *et al*., 2015; Shoeib *et al*., 2016).

2.3.2 Transport and clearance of PFASs in the human body

Whereas most persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs), are lipophilic, the substitution of carbon-hydrogen bonds for the strongest carbon-fluorine counterparts coupled with a charged functional group confers unique dual hydrophobic and lipophobic surfactant characteristics to PFAS molecules (Banks and Tatlow, 1994; Kissa, 2001). Most of the available data on transport and clearance of PFAS is based on studies with PFAAs (primarily PFOA and PFOS). In contrast to other persistent organic pollutants, PFAAs are not stored in adipose tissue but undergo extensive enterohepatic circulation. The presence of PFAAs has been confirmed primarily in liver and serum (Pérez *et al*., 2013; Falk *et al*., 2015).

The hydrophobic nature of fluorine-containing compounds can also lead to increased affinity for proteins (Jones *et al*., 2003). Once consumed, PFAAs tend to partition to the tissue of highest protein density, with ~90 to 99% of these compounds in the blood bound to serum albumin (Ylinen and Auriola, 1990; Han *et al*., 2003). Due to the ability of albumin to pass the blood follicle barrier (Hess *et al*., 1998; Schweigert *et al*., 2006), it is suggested that PFAAs can easily be transported into growing follicles. PFAAs have been detected in human follicular fluid and could alter oocyte maturation and follicle development in vivo (Petro *et al*., 2014; Heffernan *et al*., 2018).

The primary route of elimination of PFAAs is through the kidney in the urine (Han *et al*., 2008). Other important clearance pathways include menstruation (Harada *et al*., 2005; Taylor *et al*., 2014; Park *et al*., 2019; Ding *et al*., 2020), pregnancy (Monroy *et al*., 2008) and lactation (Bjermo *et al*., 2013). Sex hormones have been identified as a major factor in determining the renal clearance of PFAAs. One study examined the role of sex hormones and transport proteins on renal clearance and observed that, in ovariectomised female rats, oestradiol could facilitate the transport of PFAAs across the membranes of kidney tubules into the glomerular filtrate, resulting in lower serum concentrations (Kudo *et al*., 2002).

Serum concentrations of PFOA, PFOS, PFHxS and PFNA appear to be higher in males than in females across all age groups (Calafat *et al*., 2007). It has been found that ~30% of the PFOS elimination half-life difference between females and males is attributable to menstruation (Wong *et al*., 2014a). The differences by sex narrows with aging, suggesting that PFAS may reaccumulate after cessation of menstrual bleeding in postmenopausal women (Wong *et al*., 2014b; Dhingra *et al*., 2017; Ruark *et al*., 2017). Decreased serum concentrations have also been shown in premenopausal versus postmenopausal women and, analogously, in men undergoing venesections for medical treatment (Lorber *et al*., 2015).

PFASs are considered metabolically inert and remain in the human body for many years. Estimation of human elimination half-lives (or population halving time) for PFOA, PFOS, PFHxS and PFNA have been reported in previous studies (Olsen *et al*., 2007, 2012; Spliethoff *et al*., 2008; Bartell *et al*., 2010; Brede *et al*., 2010; Glynn *et al*., 2012; Yeung *et al*., 2013a, 2013b; Zhang *et al*., 2013; Wong *et al*., 2014a; Worley *et al*., 2017; Eriksson *et al*., 2017; Li *et al*., 2018; Ding *et al*., 2020). Comparing the estimated half-lives of PFAS among populations is difficult as they differ by sampling time intervals, duration of exposure, sex and age of study subjects. Despite these challenges, most of the aforementioned studies have reported that the half-life in humans of PFOA is around 2-3 years and that of PFOS is ~4-5 years.

2.3.3 Human health risk assessment (epidemiological) studies

The available epidemiology studies do not provide any consistent birth outcomes associated with some PFASs (PFOA, PFOS, PFHxS, PFNA, PFDeA, and PFBA) (ATSDR, 2018). Most studies conducted on women living near a PFOA facility and the general population did not association serum PFASs levels and adverse pregnancy outcomes such as miscarriages (Stein *et al*., 2009; Savitz *et al*., 2012b; Darrow *et al*., 2014; Jensen *et al*., 2015), stillbirths (Savitz *et al*., 2012b) or pre-term birth (Hamm *et al*., 2010; Chen *et al*., 2012a; Darrow *et al*., 2013). In addition, no associations were found between cord blood PFASs levels and child growth (de Cock *et al*., 2014; Wang *et al*., 2016) and weight alterations in adults (ATSDR, 2018; Halldorsson *et al*., 2012).

Hepatic studies reported no associations between serum PFOA levels of workers and residents exposed to PFOA (Anderson-Mahoney *et al*., 2008; Steenland *et a*l., 2015; Darrow *et al*., 2016). At the 3M facility, no associations were observed between cholesterol and serum PFOA and PFOS levels (Olsen and Zobel, 2007). Although, Costa, (2004); Costa, (2009) and Olsen, (2000) observed increased total cholesterol in workers exposed to PFOA.

The NTP, (2016b) concluded that the available human studies provide moderate and low confidence that exposure to PFOA and PFOS is associated with suppression of the antibody and increased incidence of infectious disease. In addition, PFHxS and PFNA were found not to be immunotoxic (ATSDR, 2018). In a cohort study of PFOS-exposed workers in a fluorochemical manufacturing facility, no significant effect of mortality caused by most types of cancer were observed (Alexander *et al*., 2001a; Alexander *et al*., 2003). However, based on a finding of three cases that followed, bladder cancer mortality was elevated among the male workers (Alexander and Grice, 2006). The EPA and IARC also concluded that PFOA and PFOS have carcinogenic potential in humans (USEPA, 2016e; USEPA, 2016f; IARC, 2017).

2.3.4 Animal studies

PFOS and PFOA have been suggested to have affinity for binding to β-lipoproteins, albumin and liver fatty acid-binding protein (L-FABP), resulting in high concentrations of these compounds in liver than in serum (Jones *et al*., 2003; Hundley *et al*., 2006). A study conducted in mice with C6-C9 chain length PFASs indicated a high accumulation in the liver with increasing chain length (Kudo *et al*., 2006). An increase in liver and kidney weight were observed in a toxicity studies of rats dosed with PFBS (3M Company, 2005), PFBA (Lieder et al., 2007) and in male rats dosed with PFOA (Lau *et al*., 2007). Developmental toxicity in mice (Lau *et al.,* 2003; Wolf *et al*., 2007), rats (Mylchrest *et al*., 2005) and mammary gland (White *et al*., 2007) dosed with PFOA and PFOS resulted in

reduced postnatal survival with impaired growth and increased stillbirths among the survivors. Cardiac abnormalities, maternal weight loss and mortality were observed in rats and mice exposed to high PFOS concentrations (Lau *et al*., 2003; Xia *et al*., 2011; Chen *et al*., 2012b). In mice, both PFOS and PFOA induced immunological alterations (Yang *et al*., 2001; Yang *et al*., 2002a; Yang *et al*., 2002b; Qazi *et al*., 2012).

Toxicity studies on humans and laboratory animals do not provide sufficient evidence to allow for comparisons between the two species (Steenland *et al*., 2010). Although, laboratory animal studies suggest toxicity effects caused by high doses of PFOS and PFOA, only a few studies have observed PFASs to be toxic to humans (ATSDR, 2018).

A recent study reported that administration of 10 µg/mL Perfluoro-n-nonanoic acid (PFNA) on bovine oocytes in vitro for 22 h has a negative effect on oocyte developmental competence during their maturation (Hallberg *et al*., 2019). This decrease in oocyte survival was attributed to PPAR-α (Hallberg *et al*., 2019), leading to the disturbance of lipid metabolism and increased lipid accumulation in the ovaries (Bjork and Wallace, 2009; Wang *et al*., 2012). Lending further support, another study showed that excessive lipids in the ooplasm correlated with impaired oocyte developmental competence and low oocyte survival rates (Prates *et al*., 2014). Because PFAS can bind and activate peroxisome proliferator-activated receptors (PPARs) and play an important role in PPAR signalling during ovarian follicle maturation and ovulation, it is plausible that persistent activation of ovarian PPARs through PFAS exposure could disrupt the ovarian cell function and oocyte maturation.

PFAS may also induce oxidative stress with increased generation of reactive oxygen species (ROS) production, increased DNA damage and decreased total antioxidant capacity (Wielsøe *et al*., 2015). Pregnant mice administered 10 mg PFOA/kg/day from gestational days 1-7 or 1-13 exhibited inhibited superoxide dismutase and catalase activity, increased generation of ROS and increased expression of p53 and Bax proteins (important in apoptotic cell death) in the maternal ovaries (Feng *et al*., 2015; Chen *et al*., 2017; Xie *et al*., 2017). Similarly, another study reported significantly increased ROS production in rats exposed to PFOA, which interfered with the activities of complexes I, II and III in the mitochondrial respiratory chain and led to oocyte apoptosis (Mashayekhi *et al*., 2015; López-Arellano *et al*., 2019).

2.4 HEALTH BASED LIMITS AND REGULATION OF PFASS IN DRINKING WATER

The 3M company announced a phase out of PFOS and PFOA and their longer chain homologs in 2000 (Lindstrom *et al*., 2011). In the USA and Europe, the production of PFOS and similar perfluorooctyl products was phased out in 2000-2002 (OECD, 2002). The European Union started to ban PFOS along with its precursor, perfluorooctanesulfonyl fluoride (POSF) and the use of PFOS in consumer products in 2000 (Habibullah-Al-Mamun *et al*., 2016).

PFOS and its precursor compound, POSF were added in May 2009 to the Annex B as new persistent organic pollutants (POPs) in the Stockholm Convention (UNEP, 2010). The European Commission also prohibited the general use of PFOS after June 2008 (European Union Directive, 2006). In addition, European Commission (2002) proposed Maximum Allowance Concentration in 2012 for PFOS and its salts in inland surfaces. Other countries such as Canada have developed similar restrictions and regulations (Environmental Health Canadian, 2006). States such as Minnesota, North Carolina also set guidelines for acceptable concentrations of PFOS and PFOA in drinking water (Table 2.2). However, the guideline acceptable values in Table 2.2 are, in most parts, wide apart from those in Table 2.3. The range of “safe” levels in drinking water ranges from 13-1000 ng L^{-1}. This variation suggests responses to scientific uncertainty in risk assessment, technical decisions and capacity, and social, political, and economic influences from involved stakeholders. It is well documented that health risk assessment requires many assumptions and estimates in order to predict a safe exposure for humans.

Table 2.2: Acceptable concentration of PFOS and PFOA in drinking water

State	PFOS (μgL^{-1})	PFOA (μgL^{-1})
Minnesota	0.2	0.3
New Jersey	-	0.04
North Carolina	-	2
Agency		
USEPA	0.2	0.4
UK HPA	0.3	10
Canada	0.3	0.7
German	0.1 (sum for both PFOS+PFOA)	

*UK HPA – United Kingdom Health Protection Agency; *USEPA – United States Environmental Protection Agency

Table 2.3: PFOA and PFOS drinking water guidelines

	Advisory level	Reference dose	Water ingestion rate
	ng/L	ng/kg-day	L/kg-day
U.S. EPA[a], Health Advisory Level	70(70)	20(20)	0.054(0.054)
Alaska[b], 2016, Groundwater cleanup level	400(400)	20(20)	0.78(0.78)
Maine DEP, Remedial action guideline	130(560)	6(80)	2(2)
Minnesota DOH, Noncancer health-based level	35(27)	18(5.1)	-
New Jersey DEP, Maximum contaminant Level	14(13)	2(1.8)	2(2)
North Carolina DENR[b], Interim maximum Allowable concentration (proposed)	1000	N/A	2
Texas CEQ, Protective concentration level	290(560)	15(20)	0.64(0.64)
Vermont[a] DEC/DOH, Primary groundwater enforcement standard	20(20)	20(20)	0.175(0.175)

Adapted from ITRC (Interstate Technology and Regulatory Council), ITRC PFAS Regulations, Guidance and Advisories Fact Sheet. In ITRC PFAS Regulations Section 5 Tables, Ed. 201[7a]Applies to PFOA and PFOS individually, as well as the sum of PFOA and PFOS; () = PFOS

These include identifying critical effects, addressing interspecies and intra-species variation, quantifying other uncertainties, and selecting exposure parameters. Another important consideration in these and future assessments is the consideration of epidemiological evidence. While risk assessments on PFAS water guidelines are, in most cases, presented as being based solely on scientific considerations, this process is also influenced by political, social, and economic factors. Much like other high-value chemicals, the landscape of what is scientifically known and unknown about their health and environmental impacts is influenced by the context of knowledge production. There is a wealth of evidence that suggests a broad “science-based defence strategy” to “command the science” on chemicals such as PFAS, ranging from suspected influence on various government departments, to the selective peer review publication of internal research.

Economically, invested corporations are bound to indirectly influence the development of PFAS drinking water guideline levels for chemicals of emerging concern such as PFOA and PFOS in drinking water through the strategic production and dissemination of industry-friendly research.

PFASs are prime candidates for chemicals that will need authorisation within the REACH regulation (European Commission, 2002). The watch list of priority substances under the European Union Water Framework Directive was also extended to include PFOS and its derivatives (European Union, 2013). After the phase out and ban of PFOS, the production shifted to PFAS precursors, due to their higher degradation potential and short-chain PFASs due to their lower bioaccumulation potential (Ahrens et al., 2015).

The first oral benchmark dose for PFOA was determined by the C8 Assessment of Toxicity Team (CATT) to be 4.0 μg kg-bw^{-1}d^{-1}, at the West Virginia Department of Environmental Protection (WVDEP, 2002). The Health Risk Assessment Unit in the Minnesota Department of Health developed a protective dose for PFOA and PFOS of 0.14 and 0.075 μg kg-bw^{-1} d^{-1}, respectively (MDH, 2007).

The Agency for Toxic Substances and Disease Registry (ATSDR) further lowered the Minimum Risk Levels (MRLs) for both PFOS and PFOA in 2018, making the drinking water advisory levels 11 ngL^{-1} for PFOA and 7 ngL^{-1} for PFOS (Sunderland *et al*., 2018).

CHAPTER 3: SAMPLING STRATEGY AND METHODS OF SAMPLE ANALYSIS

3.1 SELECTION AND DESCRIPTION OF SAMPLING SITES

For the purposes of this study, water samples were collected in selected cities across all 9 provinces of South Africa (Figure 3.1).

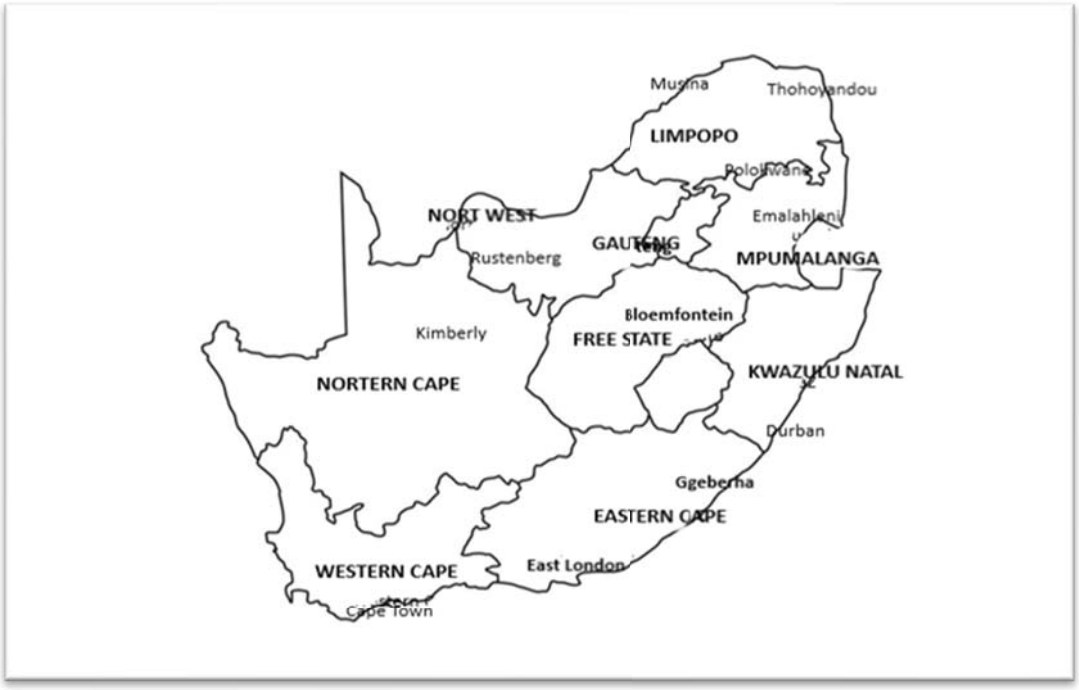

Figure 3.1: Map showing the nine province and sampling locations in South Africa.

Monitoring sites were selected in 9 provinces based on the criteria stated below:

- **Eastern Cape province** – Monitoring in the Eastern Cape Province was conducted in East London and Gqeberha areas. These areas were chosen because they are big metros, highly populated and industrialized. Also, the water quality within both cities has been of great concern to the municipality and, therefore, the communities living in these metropolitan areas.

- **Gauteng province** – Water samples were collected from Pretoria and the Vaal (southern of Johannesburg) in the Gauteng province during both the wet (October-April) and dry (June-August) seasons. Vaal River has, over the years, been at the centre of pollution discussion in the public space because of the various human activities surrounding the river. Pretoria is the administrative centre of South Africa with relatively small industrial activities and, therefore, it presents an important study area.

- **KwaZulu-Natal province** – Water samples were collected from Durban and Umgeni River in the KwaZulu-Natal province during both the wet (October-April) and dry (June-august) seasons. Durban is the most cosmopolitan city in KZN with many industrial and other human activities. Therefore, it represents a good

study area. Furthermore, water samples were also collected from Umgeni River which has been dogged with pollution problems over the years.

- **Limpopo province** – Water samples were collected from Polokwane which is the most cosmopolitan city in the province and Musina and Thohoyandou, are fast growing urban towns in the far north in the Limpopo province during both the wet (October-April) and dry (June-August) seasons. Once again, the selected areas have been in identified as areas with serious water quality problems.

- **Mpumalanga province** – Water samples were collected from eMalahleni and surrounding areas and Oliphant and Zaalklip Rivers in the Mpumalanga province during both the wet (October-April) and dry (June-august) seasons. These areas are highly industrialized with many mining activities such as coal and energy generation.

- **Northern Cape province** – Water samples were collected from Kimberly and the surrounding area in the Northern Cape province during both the wet (October-April) and dry (June-august) seasons. Kimberly is the most populated city in the Northern Cape and, therefore, will have a fair share of pollution problems.

- **North West province** – Water samples were collected from Rustenburg and surrounding areas in the North West province during both the wet (October-April) and dry (June-August) seasons. Rustenburg and its environ is inundated with mining activities and the mines have the potential to generate chemical pollutants such as PFAS during the various stages of mining.

- **Western Cape province** – Water samples were collected from Cape Town and Diep River in the Western Cape province during both the wet (October-April) season.

- **Free State province** – Water samples were collected from Bloemfontein and surrounding area in the Free State province during both the wet (October-April) and dry (June-August) seasons. Once again, Bloemfontein is the most populated city in the province and, therefore, presents a good sampling area.

3.2 SAMPLE COLLECTION

3.2.1 Overview

The following water sample types were collected during this study:

- Wastewater
- Surface water
- Groundwater
- Treated drinking water (final water from a water treatment plant)
- Tap water – Treated drinking water collected from household taps
- Bottled water
- Rain water

Two sampling approaches were evaluated in this study; grab and passive sampling. Grab samples were collected from all the sampling sites in all 9 provinces during the dry and wet season. For investigating PFASs concentrations using a passive sampling method, Gauteng Province was used as a case study.

3.2.2 Grab sampling

Grab samples were collected from all the sampling sites in all 9 provinces during the dry (June-August) and wet October-April) seasons. At each site, water samples were collected in clean high-density polyethylene bottles from the various water sources. After collection, the samples were kept in ice and transported to laboratory and prepared for analysis.

3.2.3 Passive sampling

3.2.3.1 Laboratory Sampling Rate (Rs) method

Pre-cleaned Polar Organic Chemical Integrated Sampler (POCIS) containing Oasis hydrophilic-lipophilic balance (HLB) purchased from (EST – Environmental Sampling Technologies, USA) was used in the study. Before field deployment, the sampling rates were determined in the laboratory in a tap water-filled 50-L aquarium under dark conditions, at 20.5°±2°C. The calibration study was conducted according to Gobelius *et al*. (2019) with minor modification, over 14 days in the laboratory using a modified flow through system consisting a test and a reservoir 20-L glass tanks. Both tanks were wrapped with aluminium foil and with a black lid to prevent UV light penetration. The two glass tanks were fitted with two air pumps to ensure uniform distribution of PFASs and continuous circulation of the water body. Water temperature was controlled by maintaining the room temperature using air conditioning. The tap water in the test and reservoir tanks was spiked with 21 mix PFASs at concentration of 100 ng/L. Before starting the uptake experiment the tank reservoir and tank passive samplers were left to equilibrate overnight to stabilize the sorption of PFASs to the glass walls of the tanks. Every day, 3 L of spiked water sample was removed from the test tank and replaced with the same volume from the reservoir on day 1, 7 and 14 using a peristaltic. In total 3 POCIS-HLB were placed in the test tank. A blank POCIS was exposed in the laboratory environment as laboratory blank. All POCIS-HLB samples were vacuum sealed in polypropylene bags and stored in the refrigerator (4°C) until analysis.

3.2.3.2 Field deployment

Passive samplers were deployed for 14 days in the effluent of a wastewater treatment plant in Pretoria. Passive samplers were retrieved on day 7 and 14. Grab samples were also taken from the same point on the same day. Composite samples comprising influent, aerobic digestion, secondary settlement tank and effluent was also collected. Samples were stored inside a cooler box and stored in the refrigerator (4°C) until analysis. The process of accumulation in the POCIS is essentially adsorption on the internal solid phase after contaminants passively diffuse through the hydrophilic membrane. In order to assess the time-averaged ambient concentration of POCIS-available contaminants, the POCIS is exposed during the linear-phase (phase I) regime, after which a calculation is made based on Equation 3.1:

$$Cwater = Cpocis \,.\, Mpocis / Rs.t \quad \text{(Equation 3.1)}$$

Where:
Cwater = mean contaminant concentration (over the sampling period) in the ambient water (µg/L);
Cpocis = concentration in the POCIS (µg/g);
Mpocis = mass of adsorbent phase in the POCIS (g);
Rs = sampling rate (L/d), which corresponds to the volume of water purified per unit-of-time; and
t is the total exposure time (d).

3.3 PREPARATION OF SAMPLES FOR PER- AND POLYFLUOROALKYL SUBSTANCES ANALYSIS

3.3.1 Grab samples extraction

The SPE SupelcoTM Envi18 cartridges purchased from SIGMA Aldrich Ltd were used for all PFASs extraction from all the water samples. Cartridges were first conditioned. Thereafter, the cartridges were then allowed to dry under vacuum for 1 h. The solvent extract was then concentrated under the gentle steam of nitrogen. The reconstituted extract was then transferred to a 2 mL centrifuge tubes and 950 µL of the extract and a 50.0 µL of internal standard added to an autosampler vial. A 10.0 µL of the samples was then injected to the LC-MS/MS.

3.3.2 Extraction of PFASs from POCIS

PFASs adsorbed on POCIS-HLB retrieved from the laboratory and field set up was extracted using 6 mL SPE cartridge which was fitted with polyethylene frits at the bottom. The HLB sorbent was transferred from the POCIS into the cartridges, through a glass funnel, and rinsed using ultrapure water. Excess water was dried under vacuum for approximately 30 min and then another frit was placed on top of the sorbent. The cartridge was spiked with 100 uL of surrogate standard mixture. The HLB sorbent was eluted with 8 mL methanol. The eluent was collected in 50 mL polypropylene tubes. The POCIS-HLB field blanks underwent the same extraction procedure as the sample. The samples were concentrated under gentle nitrogen at room temperature and 950 µL of the sample was transferred into 1 mL LC glass vials and spiked with 50 µL internal standard. The samples were then analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

3.4 WATER SAMPLE ANALSYSIS USING LC-MS

3.4.1 LC-MS Method For Analysis of High PFAS Concentration Samples

Prior to application, target and non-target methods for analysis of high PFASs concentration samples using an LC-MS was optimised and validated using samples collected from Gauteng province. Details are provided in Volume I of this report. For target and non-target PFASs analysis, an LC-MS-8030 triple quadrupole system (Table 3.1) and a TripleTOF 6600, SCIEX (Table 3.2) were used, respectively. The quantitation of the target compounds was based on internal standard method calibration with concentrations ranging from 1.0-1000 ng/L. An R^2=0.99 was achieved in all the calibrations with good precision of the internal standard. The method was then applied to the extracted water samples.

Table 3.1: Instrument conditions for the target analysis of high PFASs concentration samples

LC-MS/MS instrument	Shimadzu, LC-MS-8030 triple quadrupole system
Analytical column	Kinetex® 2.6 µm XB-C18 100 Å, LC Column 50 x 4.6 mm
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.3000 mL/min
Mobile Phases	A 20 mM Ammonium Acetate B 50:50 Methanol: Acetonitrile

Gradient conditions	
Time (min)	% Mobile phase B
1	20
4	90
7	20
12	0
Acquisition time	12 min

Table 3.2: Instrument conditions for non-target PFASs identification using TOF-MSW

Instrument name	TripleTOF 6600, SCIEX
Analytical column	Luna Omega 3 µm polar C18 100Å LC column 100 x 2.1 mm, Phenomenex
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.50 mL/min
Mobile Phases	A 2 mM Ammonium Acetate, 0.1% Formic Acid B 100% Methanol
Gradient conditions	
Time (min)	% Mobile phase B
1	5.0
16	95
20	5.0
26	0
Acquisition	Information Dependent Acquisition
Acquisition time	26 min

Identification of emerging and legacy PFASs was done using non-targeted analysis (Figure 3.2).

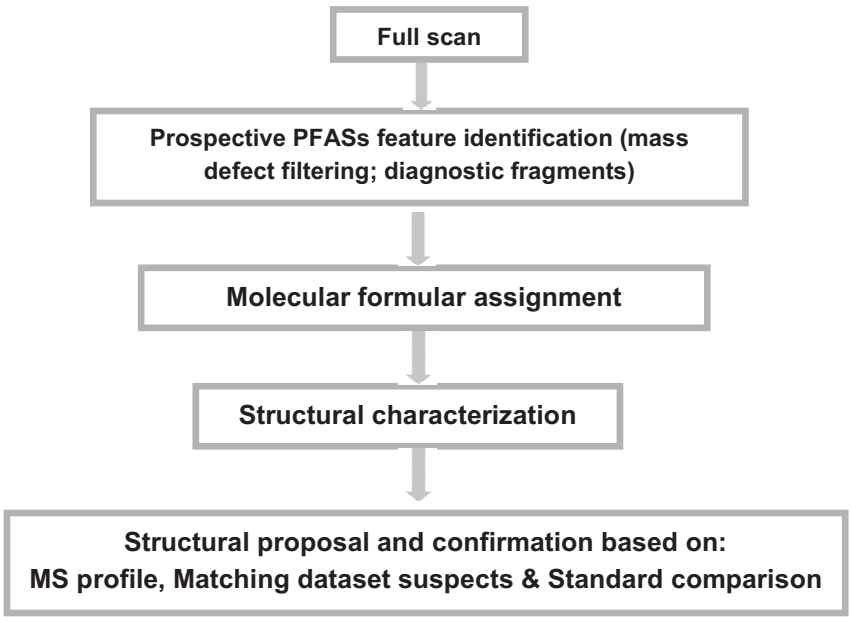

Figure 3.2: General schematic workflow for non-target PFAS by TOF-MS.

The workflow used in this study involved suspect screening and considered evidence reported in the literature to identify legacy and emerging PFASs in different water samples, and such evidence was based on the actual mass, library score of >70%, the presence of fragment ions, homologues mass difference, mass error (mDa) and retention times.

3.4.2 Target analysis of low PFASs concentration samples using LC-MS

Water samples collected from all other provinces were used for the development, optimisation and validation of an LC-MS method for low PFAS concentrations (Volume I). For the purposes of this project, a target LC-MS/MS (LCMS-8030, Shimadzu) method for PFASs detection and quantification was optimized and validated. Four different chromatographic methods comprising **A, B, C** and **D** (Table 3.3) were used for sample analysis to ensure good separation of PFASs compounds.

Table 3.3: Instrument and optimization conditions for targeted analysis low PFASs concentration samples

LC-MS/MS instrument	Shimadzu, LCMS-8030
Analytical column	Kinetex 2.6 um Polar C18 100 A LC Column 100 x 2.1 mm, Unit
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.3000 mL/min

	Method A
Mobile Phases	A: 10 mM Ammonium Formate
	B: 20:80 Methanol: Acetonitrile
	Gradient Conditions
Time (min)	Mobile Phase B (%)
1	45
3	50
4	60
4.5	70
5	65
5.5	68
6	80
7.5	70
10	0
16	Stop

	Method B
Mobile Phases	A: 10 mM Ammonium Formate
	B: 50:50 Methanol: Acetonitrile
	Gradient conditions
Time (min)	Mobile Phase B (%)
1	
4	20
6.5	55
7	75
7.2	95
9	0
10	20
12	Stop

	Method C
Mobile Phases	A: 20 mM Ammonium Acetate
	B: 95:5 Methanol: Water
	Gradient Conditions
Time (Min)	Mobile Phase B (%)
1	20
2	75
3	85
4	70
6	95
7.5	100
10	90
16	Stop

	Method D
Mobile Phases	A: 10 mM Ammonium Formate
	B: 20:80 Methanol: Acetonitrile
	Gradient Conditions
Time (Min)	Mobile Phase B (%)
1	20
2	55
3.5	70
4	0
5	Stop

3.4.3 Quantification of PFASs using targeted analysis

The chromatographic conditions developed were used to calculate the final concentrations of PFASs in the water samples using the following formula:

$$A_{nat}/A_{IS} \times 1/RRT \times M_{IS}/SS \quad \text{(Equation 3.2)}$$

where: A_{nat} = area of surrogate standard; A*is* = area of internal standard; M_{IS} = mass of internal standard (ng); RRF = slope or gradient in the calibration curves; SS = sample size (mL).

The RRF is obtained when the ratio of response for the unit amount of the contaminant of interest to the response of the IS and is expressed in equation below:

$$RRF = A_{NAT}/A_{IS} \times C_{IS}/C_{NAT} \quad \text{(Equation 3.3)}$$

where:
A_{NAT} is peak area of the native ($^{13}C_2$) compound; A_{IS} is the peak area of the internal standard in the standard
C_{NAT} is the concentration of the native standard; C_{IS} is the internal standard concentration.

3.5 SOURCE APPORTIONEMNT

To address Aim 2 of this project, a multivariate statistical analysis was used to establish inter-relationships between different groups of PFASs, and sample sites and to establish possible sources of PFAS. Principal Component Analysis (PCA) was used to identify patterns, potential sources of variation and relationships within the obtained datasets of PFASs concentrations in the different sampling sites. Data interpretation was done in conjunction with knowledge on the land uses within the catchment area of the sampling sites.

3.6 ASSESSING POTENTIAL ESTROGENIC ACTIVITY USING YEAST ESTROGENIC ASSAY

3.6.1 Materials

Yeast was obtained from Prof JP Sumpter's laboratory, in the Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex, United Kingdom. Potassium dihydrogen phosphate (KH_2PO_4), ferric sulphate ($Fe_2(SO_4)_3$, pantothenic acid, 17β-Estradiol, HPLC grade ethanol and pyroxidine were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Anhydrous ammonium sulphate ($(NH_4)_2SO_4$,, potassium hydroxide (KOH) pellets anhydrous magnesium sulphate ($MgSO_4$), L-leucine), L-histidine ,adenine, L-arginine, hydrochloric acid (HCL), L-methionine, L-tyrosine, L-isoleucine, L-lysine-HCl, L-phenylalanine, L-glutamic acid, L-serine, L-valine, D(+)-glucose, L-aspartic acid , L-threonine , thiamine, inositol , anhydrous, copper (II) sulphate ($CuSO_4$), ethanol (HPLC grade) , and Chlorophenol red-β-D-galactopyranoside (CPRG) were obtained from Roche Diagnostics (Mannheim, Germany), (Sigma-Aldrich), glycerol (Sigma), Agar (, Sigma), parafilm, pH indicator strips, HPLC grade MeOH and 32% hydrochloric acid (HCL) were all purchased from Merck (Darmstadt, Germany).

Cryovials and 96-well assay plates were purchased from Thermo Fisher Scientific (Denmark). Autoclave tape was from 3 M Health Care (Neuss, Germany). Polyethersulfone (PES) membrane syringe filters were from Merck (Darmstadt, Germany). Disposable serological pipettes were obtained from Corning Incorporated (Corning, New York, USA); whereas tin foil, glass wool filters were all purchased from Macherey-Nagel. Glass microfiber filter papers (0.45 µm), Supelco ENVI-18™ SPE cartridges (500 mg, 6 mL) and polypropyline bottles (1 L) were all

purchased from Whatman (New jersey, United states), Sigma-Aldrich (Aston Manor, South Africa) and Plastillon (Gezina, South Africa) respectively.

3.6.2 Sample collection and preparation for analysis

Water samples collected from the Northern Cape and Gauteng provinces were used in the yeast bioassay estrogenic assay to test for potential estrogenic activities of PFASs compounds. The pH of the water samples was adjusted to 3 using pH indicator strips and 32% hydrochloric acid before extraction. Samples extraction involved the use of Supelco ENVI-18™ SPE cartridges (500 mg, 6 mL) loaded onto SPE 12-position vacuum manifold (Phenomenex, Torrance, California, USA. After preconditioning the cartridge using 5 mL of distilled water, followed by 5 mL of HPLC grade methanol, the cartridges were allowed to equilibrate with 5 mL of double distilled water (DDH_20). After extraction, the cartridges were dried for 1 h. The samples were then eluted from the cartridges with 5 mL of MeoH. Furthermore, the extracts were evaporated to dryness at 37°C using a reacti-vap and Reacti-therm unit under a mild stream of nitrogen. Afterwards, the sample residues were reconstituted in sterile glass amber vials (4 mL volume) with 1 mL ethanol and stored in a freezer at -20°C prior to bioassay analysis.

3.6.3 Yeast estrogenic bioassay analysis

Yeast bioassay analysis to determine estrogenic activity in water samples were carried out in the EDC laboratory at the University of Pretoria according to the protocols developed by Aneck-hahn *et al*. (2008). According to Routledge & Sumpter (1996), the Genetics Department of Glaxo Group Research Ltd created the YES bioassay to assess the estrogenic activity of compounds. Human oestrogen receptor alpha (hER) and expression plasmids carrying the reporter gene lac-Z, encoding the enzyme β-galactosidase, were genetically added to a yeast strain (Sacchromyces cerevisiae). The reporter gene Lac-Z is expressed in response to substances that bind to and activate the ER, which causes the synthesis of -galactosidase in a dose-dependent mode. The enzyme is released into a media containing the chromogenic substrate chlorophenol red – D-galactopyranoside (CPRG). CPRG is typically yellow, however, it is converted by β-galactosidase into a red product that can be detected by measuring the absorbance at a particular wavelength.

3.6.4 Preparation of Medium and Stock solution

Minimal medium was prepared by adding the following chemicals: 13.6 g KH_2PO_4, 1.98 g $(NH_4)_2SO_4$, 4.2 g KOH pellets, 0.2 g $MgSO_4$, 1 mL $Fe_2(SO_4)_3$ solution (40 mg/50 mL water), 50 mg/L-leucine, 50 mg/L-histidine, 50 mg adenine, 20 mg/L-arginine-HCl, 20 mg/L-methionine, 30 mg/L-tyrosine, 30 mg/L-isoleucine, 30 mg/L-lysine-HCl, 25 mg/L-phenylalanine, 100 mg L-glutamic acid, 375 mg L-serine and 150 mg/L-valine. About 1L of ddH_2O was added to the components and the pH adjusted to 7.1. Then the medium was sterilized by autoclaving for 20 min at 121°C and 15 psi and stored at 4°C. Thereafter, a stock solution of glucose (200 g/L), L-aspartic acid (4 g/L) and L-threonine (24 g/L) was prepared in ddH_2O. The solutions were autoclaved for 20 min at 121°C, 15 psi to sterilize and later stored at 4°C. Vitamin solution was prepared by adding 8 mg thiamine, 8 mg pyroxidine, 8 mg pantothenic acid, 40 mg inositol and 20 mL biotin solution (0.02 g/L in ddH_2O) to 180 mL ddH_2O. The solution was sterilized by filtering through a 0.2 µm syringe filter. Stock solutions of $CuSO_4$ (0.3192 g/L) and CPRG (10 g/L) were also filtered, sterilized and stored at 4°C. Thereafter, a growth medium was prepared by adding 45 mL minimal medium, 5 mL glucose, 1.25 mL L-aspartic acid, 0.5 mL vitamin solution, 0.4 mL L-threonine and 125 µL $CuSO_4$ together. A 54.58 µg/L stock solution of the E2 positive control was prepared in EtOH and stored at -20°C.

3.6.5 Preparation and Storage of Yeast Stock Cultures

Agar slopes were used to prepare long-term yeast stock cultures and a 1% agar solution was prepared in minimal medium. Thereafter, the solution was autoclaved and the following growth medium components were added to 90

mL of the agar solution once it cooled down to 50°C, and 10 mL glucose, 2.5 mL L-aspartic acid, 1 mL vitamin solution, 0.8 mL L-threonine and 250 µL $CuSO_4$ were added to the solution. Using sterilized glass tubes, the solution was poured directly into the tubes and allowed to set at 45° angle. Approximately, 2 µL of the original yeast stock was spread over the surface of the agar slopes and were incubated for 3 days at 32°C. Then, the yeast cells were resuspended in 1 mL sterile glycerol and stored in aliquots in cryovials at -80°C. Short term 10x concentrated stock cultures were prepared by adding, 125 µL of the long-term yeast stock to 50 mL growth medium and incubated at 28°C in a rotating water bath (at 155 upm). After 24 h incubation, 1 mL of the 24 h yeast culture was added to two flasks containing 50 mL growth medium each. The flasks were subsequently incubated for another 24 h in a water bath (28°C, 155 upm). After incubation, the yeast cultures were transferred to 50 mL centrifuge tubes and centrifuged for 10 min at 4°C and 2000 psi. (Sigma 4K15 centrifuge from Sigma Laborzentrifugen, Germany). The pellets were resuspended in 5 mL of 15% glycerol minimum medium after the supernatant was decanted. For a maximum of four months, aliquots of the 10x concentrated stock cultures were kept in cryovials at -20°C.

3.6.6 Yeast Assay Procedure

A volume of 125 µL of 10x concentrated short term yeast stock was inoculated into 50 mL growth medium as outlined in Section 3.6.6. Thereafter, the yeast was incubated overnight in a rotating water bath (Grant OLS 200, Grant Instruments, Cambridge, UK) at 155rpm until turbid for approximately 24 h. At 620 nm, an absorbance reading of at least 1 indicated sufficient yeast growth to proceed with the assay. In a 96 well microtiter plate, a serial dilution of the water sample extract, controls (ethanol), and E2 positive control was performed by transferring 100 µL of ethanol in well 2-12, followed by an addition of 200 µL of the sample extract/control/blank into the first well, then a serial dilution was performed by transferring 100 µL across the plate to determine estrogenic activity. Thereafter, 10 µL of the dilution series was transferred across new triplicate 96 µL plates and allowed to evaporate. Then, a growth medium was prepared as outlined earlier. Furthermore, 200 µL of the seeded assay medium containing the CPRG was dispensed into each sample well of the triplicate plate using a multichannel pipette. The plates were then sealed with an autoclave plate and kept for 3-5days in an incubator at 32°C (Scientific Series 2000 incubator from Lasec, South Africa). In order to obtain data with best contrast between positive and solvent controls and to allow for slow reacting chemicals, the plates were read over 3 days. A Multiskan Spectrum 96-well plate reader (Thermo Fisher Scientific, Vantaa, Finland) was used to determine the colour development of the medium after the plates had been incubated for three days. The absorbance was measured at 540 nm for colour change and 620 nm for turbidity of the yeast growth.

3.6.7 Data Analysis

Turbidity correction was carried out using the following equation:

Corrected value = **test absorbance** (540 nm) – [**test absorbance** (620 nm) **– median blank absorbance** (620 nm)] (Equation 3.4)

Graphpad Prism (version 4) was used to fit the E2 standard curve (sigmoidal function, variable slope), which calculated the minimum, maximum, slope, EC50 value, and 95% confidence limits. The absorbance induced by the solvent control (blank) plus three times the standard deviation was used to calculate the detection limit of the yeast test. Cytotoxic concentrations were defined as sample concentrations having absorbance values less than the solvent control minus three times the standard deviation. The samples' estradiol equivalents (EEq) were interpolated from the estradiol standard curve and corrected with the appropriate dilution factor.

3.6.8 Assessment of trace metals in water samples using inductively coupled plasma-mass spectrometry (ICP-MS)

3.6.8.1 Reagents and materials

All chemicals and reagents used were of analytical grade. Deionized water (-18MΩ-cm water) prepared using an Elga water purification system (Woodridge, USA) was used throughout the experiment for preparations and dilutions of solutions. Nitric acid was purchased from Sigma-Aldrich (St. Loius MO, USA). Indium internal standard (1000 mg/L) was purchased from Inorganic Ventures (Christiansburg, Virginia, USA). Multi standard solution (250 mg/L) was purchased from Sigm-Aldrich (St. Loius MO, USA).

3.6.8.2 Sample preparation and chemical analyses

The collected samples were analysed for 21 trace metals including Lithium (Li), Beryllium (Be), Titanium (Ti) , Vanadium (V), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Rubidium (Rb), Strontium (Sr), Molybdenum (Mo), Cadmium (Cd), Tin (Sn), Antimony (Sb), Tellurium (Te), Cesium (Cs), Barium (Ba), Lanthanum (La), Tungsten (W), Platinum (Pt), Thallium (Ti), Lead (Pb), Bismuth (Bi) and Uranium (U). An Inductively coupled plasma mass spectrometry (ICP-MS-7700X (Agilent Technologies Inc., Tokyo, Japan) was used to analyse the samples. The samples were introduced into the nebulizer using a peristaltic pump. Prior to analysis, the field samples were spiked with 10 ng/L of indium. Operating parameters of the instrument are presented in Table 3.4. The ICP-MS was calibrated for every set using multi standard solution (250 mg/L). Typical concentration calibration set was within a range of 5-50ppb. Indium (10 µg/L) was added to all solutions, including the calibration blank, to verify the performances of the methods. The analyte recovery was at least 90%. Nitric acid (3%) and deionized water was pumped through the nebulizer between all samples to avoid cross contamination.

Table 3.4: ICP-MS operating parameters

ICP parameters	
RF power	155W
RF matching	0.30V
Nebulising pump	0.10rps
Carrier Gas	1.03L/min
Sample depth	10.0mm
S/C Temperature	2°C
Sampling period	0.31sec

CHAPTER 4: IDENTIFICATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER

4.1 INTRODUCTION

Characterizing PFAS is essential for assessing the health and environmental risks associated with these persistent chemicals, identifying contamination sources, and developing effective strategies to manage and mitigate their presence in our environment and protect public health. Identification of PFAS in water typically involves a combination of sample collection, preparation, and analysis using both target and non-target analysis.

4.2 IDENTIFICATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER

4.2.1 Target and non-target analysis of water samples for PFASs identification

Water samples collected from Gauteng province were used to initiate the monitoring of PFASs exercise because of the various industrial activities in the province and hence high levels of known and unknown PFASs may be present in the water samples. Tables 4.1 to 4.5 show targeted and non-targeted PFASs identified in surface water, wastewater treatment plant, drinking water treatment plant and bottled and tap drinking water using TOF-MS. New PFASs were picked up in addition to those in the mixed standard. The fluorotelomers were the prominent new compounds. It is also worth noting that unlike many other PFASs, fluorotelomer alcohols are highly volatile compounds. Consequently, volatilization is a primary transport pathway for these compounds. As they oxidize in the atmosphere, they break down into perfluorinated carboxylic acids, such as PFOA.

The obtained results show that 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) are the most dominant fluorotelomers. Their percentage detection ranged from 30-100 and 0-80 for 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) respectively. Table 3.11 shows the percentage detection of targeted and non-targeted PFASs in wastewater treatment plant. In addition, 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer sulfonate (8:2 FTSA) can be seen to be prominent. As shown in Table 3.12, 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) are the most dominant and their percentage detection range from 50-83 and 33-100 for 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer sulfonate (8:2 FTSA) respectively in drinking water treatment plant . However, in Table 3.13, their detections were less than 60% in bottled and tap drinking water.

Congeners such as 6:2 Fluorotelomer sulfonate (6:2 FTSA) and 8:2 Fluorotelomer Sulfonate (8:2 FTSA) are one of the primary and relevant subgroups of fluorotelomers. Fluorotelomer alcohols (FTOH): The n:2 fluorotelomer alcohols (n:2 FTOHs) are key raw materials in the production of n:2 fluorotelomer acrylates and n:2 fluorotelomer methacrylates. Fluorotelomer sulfonic acids (FTSA): The n:2 fluorotelomer sulfonic acids (n:2 FTSAs) are associated with aqueous film forming foam (AFFF), wastewater treatment plant effluents, and landfill leachate. Fluorotelomer carboxylic acids (FTCA). These compounds are known to form through the biodegradation of FTOHs. Other emerging PFASs identified included: perfluorooctane sulphonamide, N-Methyl perfluorooctane sulphonamide, N-Ethyl perfluorooctane sulphonamide; 6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA, 8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA, 6:2 Fluorotelomer carboxylic acid, 8:2 Fluorotelomer carboxylic acid and 10:2 Fluorotelomer carboxylic acid; Perfluorohexyl Iodide and Perfluorooctyl Iodide; 8:2 Fluorotelomer acrylate, 6:2 Fluorotelomer methacrylate and 8:2 Fluorotelomer methacrylate; Perfluoro-2-methoxyacetic acid, Perfluoro-3-methoxypropanoic acid, Perfluoro-4-methoxybutanoic acid, Perfluoro-2-propoxypropanoic acid, Perfluoro(3.5-dioxahexanoic) acid, Perfluoro(3.5.7-trioxaoctanoic) acid and Perfluoro (3.5.7.9- tetraoxadecanoic) acid.

Table 4.1: Targeted and non-targeted PFASs in surface water samples

Surface water		API D	API UP	HEN UP	HEN D	C-VD1I	C-VD21	C-VD3I	C-VD4I	90236	90176	
Compound Name	Formula	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detection frequency%
(PFBA)	$C_4HF_7O_2$	√	√	√	√	√	√	√	√	√	√	100
(PFPeA)	$C_5HF_9O_2$	√	√	√	√	√	√	√	√	√	√	100
PFHxA	$C_6HF_{11}O_2$	√	√	√	√	√	√	√	√	√	√	100
PFHpA	$C_7HF_{13}O_2$	√	√	√	√	√	√	√	√	√	√	100
PFOA	$C_8HF_{15}O_2$	√	√	√	√	√	√	√	√	√	√	100
PFNA	$C_9HF_{17}O_2$	√	√	√	√	√	√	√	√	√	√	100
PFDA	$C_{10}HF_{19}O_2$	√	√	√	√	√	√	√	√	√	√	100
PFUdA	$C_{11}HF_{21}O_2$	√	√	√	√	N/A	√	√	√	√	√	90
PFDoA	$C_{12}HF_{23}O_2$	√	√	√	√	√	N/A	√	N/A	N/A	√	70
PFTrDA	$C_{13}HF_{25}O_2$	N/A	√	√	√	√	N/A	√	N/A	N/A	√	60
PFTeDA	$C_{14}HF_{27}O_2$	√	√	√	√	√	√	√	√	√	√	100
L-PFBS	$C_4HF_9O_3S$	√	√	√	√	√	√	√	√	√	√	100
L-PFHxS	$C_6HF_{13}O_3S$	√	√	√	√	√	√	√	√	√	√	100
L-PFOA	$C_8HF_{17}O_3S$	√	√	√	√	√	√	√	√	√	√	100
L-PFDS	$C_{10}HF_{21}O_3S$	√	√	√	√	√	√	√	√	√	√	100
L-PFHpS	$C_7F_{15}SO_3H$	√	√	√	√	√	√	√	√	√	√	100
L-PFNS	$C_9F_{19}SO_3H$	√	N/A	√	√	N/A	N/A	√	√	N/A	√	60
L-PFDoS	$C_{12}HF_{25}O_3S$	N/A	√	N/A	N/A	N/A	N/A	N/A	√	N/A	N/A	20
L-PFPeS	$C_5F_{11}SO_3H$	√	√	√	√	√	√	√	√	√	√	100

Non target compounds												
4:2 Fluorotelomer sulfonic sulfonate 4:2 FTSA	$C_6H_5F_9O_3S$	√	√	√	√	N/A	√	√	N/A	√	√	80
6:2 Fluorotelomer sulfonate (6:2 FTSA)	$C_8H_5F_{13}O_3S$	√	√	√	√	√	√	√	√	√	√	100
8:2 Fluorotelomer Sulfonate (8:2 FTSA)	$C_{10}H_4F_{17}O_3S$	√	√	√	√	N/A	√	√	N/A	√	√	80
Perfluorooctane sulfonamide (FOSA)	$C_8H_2F_{17}NO_2S$	√	√	√	√	√	√	√	√	√	√	100
N-Methyl perfluorooctane sulfonamide (MeFOSA)	$C_9H_4F_{17}NO_2S$	N/A	√	N/A	√	N/A	√	√	√	√	√	70
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	$C_{10}H_6F_{17}NO_2S$	√	√	√	√	N/A	√	√	√	√	√	90
6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA	$C_8H_2F_{12}O_2$	√	√	√	√	N/A	√	√	√	√	√	90
8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA	$C_{10}H_2F_{16}O_2$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
6:2 Fluorotelomer carboxylic acid (6:2 FTCA)	$C_6F_{13}CH_2COOH$	√	√	√	√	√	N/A	N/A	N/A	N/A	N/A	50
8:2 Fluorotelomer carboxylic acid (8:2 FTCA)	$C_8F_{17}CH_2COOH$	√	N/A	√	√	√	N/A	N/A	N/A	N/A	N/A	30
10:2 Fluorotelomer carboxylic acid (10: 2 FTCA)	$C_{10}F_{21}CH_2COO H$	√	N/A	√	√	N/A	√	√	N/A	√	√	70
6:2 Fluorotelomer alcohol (6:2 FTOH)	$C_8H_5F_{13}O$	√	N/A	√	√	N/A	√	√	N/A	√	√	30
Perfluorohexyl Iodide PFHxI	$C_6F_{13}IH$	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluorooctyl Iodide PFOI	$C_8F_{17}IH$	N/A	N/A		√	√	√	N/A	N/A	√	N/A	50

8:2 Fluorotelomer acrylate (8:2 FTAC)	$C_{13}H_7F_{17}O_2$	√	N/A	√	√	√	√	√	N/A	√	√	80
6:2 Fluorotelomer methacrylate (6:2 FTAC)	$C_{12}H_9F_{13}O_2$	√	N/A	√	√	√	√	√	N/A	√	√	80
8:2 Fluorotelomer methacrylate (8:2 FTMAC)	$C_{14H9}F_{17}O_2$	√		√	√	√	√	√	N/A	N/A	√	80
Perfluoro-2-methoxyacetic acid (PFMOAA)	$C_3HF_5O_3$	√	N/A	√	√	N/A	√	√	√	√	√	80
Perfluoro-3-methoxypropanoic acid (PFMOPrA)	$C_4HF_7O_3$	√	N/A	√	√	√	√	√	√	√	√	90
Perfluoro-4-methoxybutanoic acid (PFMOBA)	$C_5HF_9O_3$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluoro-2-propoxypropanoic acid (PFPrOPrA)	$C_6HF_{11}O_3$	√	N/A	√	√	N/A	√	√	N/A	√	√	70
Perfluoro(3,5-dioxahexanoic) acid (PFO2HxA)	$C_4HF_7O_4$	√	N/A	√	√	N/A	√	√	N/A	√	√	70
Perfluoro(3,5,7-trioxaoctanoic) acid (PFO3OA)	$C_5HF_9O_5$	√	N/A	N/A	√	N/A	N/A	√	N/A	√	√	50
Perfluoro(3,5,7,9-tetraoxadecanoic) acid (PFO4DA)	$C_6HF_{11}O_6$	√	N/A	√	√	N/A	√	√	√	√	√	80

Table 4.2: Targeted and non-targeted PFASs in surface water

Surface water		**90293**	**MLT**	**90174**	**90236**	**90286**	193663	**90260**	**195445**	**195443**	
Compound Name	Formula	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detection frequency %
(PFBA)	$C_4HF_7O_2$	√	√	√	√	√	√	√	√	√	100
(PFPeA)	$C_5HF_9O_2$	√	√	√	√	√	√	√	√	√	100
PFHxA	$C_6HF_{11}O_2$	√	√	N/A	√	√	√	√	√	√	88.89
PFHpA	$C_7HF_{13}O_2$	√	√	√	√	√	√	√	√	√	100
PFOA	$C_8HF_{15}O_2$	√	√	√	√	√	√	√	√	√	100
PFNA	$C_9HF_{17}O_2$	√	√	√	√	√	N/A	N/A	N/A	N/A	55.56
PFDA	$C_{10}HF_{19}O_2$	√	√	√	√	√	√	√	N/A	N/A	77.78
PFUdA	$C_{11}HF_{21}O_2$	√	√	√	√	√	√	N/A	√	√	88,89
PFDoA	$C_{12}HF_{23}O_2$	N/A	N/A	√	√	√	√	N/A	N/A	N/A	44.44
PFTrDA	$C_{13}HF_{25}O_2$	N/A	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	11.11
PFTeDA	$C_{14}HF_{27}O_2$	√	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	22.22
L-PFBS	$C_4HF_9O_3S$	√	√	√	√	√	N/A	√	√	N/A	77.77
L-PFHxS	$C_6HF_{13}O_3S$	√	√	√	√	√	N/A	N/A	N/A	N/A	55.56
L-PFOA	$C_8HF_{17}O_3S$	√	√	√	√	√	N/A	N/A	N/A	N/A	55.56
L-PFDS	$C_{10}HF_{21}O_3S$	√	N/A	√	√	√	N/A	√	N/A	N/A	55.56
L-PFHpS	$C_7F_{15}SO_3H$	√	√	√	N/A	N/A	N/A	√	N/A	N/A	44.44
L-PFNS	$C_9F_{19}SO_3H$	√	√	√	N/A	N/A	N/A	√	√	√	66.67
L-PFDoS	$C_{12}HF_{25}O_3S$	√	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	22.22
L-PFPeS	$C_5F_{11}SO_3H$	√	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	22.22

Non-targeted PFASs											
4:2 Fluorotelomer sulfonic sulfonate 4:2 FTSA	$C_6H_5F_9O_3S$	N/A	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	11.11
6:2 Fluorotelomer sulfonate (6:2 FTSA)	$C_8H_5F_{13}O_3S$	√	√	√	N/A	N/A	√	√	√	√	77,77
8:2 Fluorotelomer Sulfonate (8:2 FTSA)	$C_{10}H_4F_{17}O_3S$	N/A	√	N/A	√	√	√	√	√	√	77.77
Perfluorooctane sulfonamide (FOSA)	$C_8H_2F_{17}NO_2S$	√	√	N/A	N/A	N/A	√	√	√	√	66.67
N-Methyl perfluorooctane sulfonamide (MeFOSA)	$C_9H_4F_{17}NO_2S$	√	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	22.22
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	$C_{10}H_6F_{17}NO_2S$	√	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	22.22
6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA	$C_8H_2F_{12}O_2$	√	N/A	N/A	√	√	√	√	N/A	N/A	55.56
8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA	$C_{10}H_2F_{16}O_2$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	√	√	22.22
6:2 Fluorotelomer carboxylic acid (6:2 FTCA)	$C_6F_{13}CH_2COOH$	N/A	√	N/A	√	√	N/A	√	N/A	N/A	44.44
8:2 Fluorotelomer carboxylic acid (8:2 FTCA)	$C_8F_{17}CH_2COOH$	N/A	N/A	N/A	N/A	N/A	N/A	√	N/A	N/A	11.11
10:2 Fluorotelomer carboxylic acid (10: 2 FTCA)	$C_{10}F_{21}CH_2COOH$	N/A	N/A	√	√	√	N/A	√	N/A	N/A	44.44
6:2 Fluorotelomer alcohol (6:2 FTOH)	$C_8H_5F_{13}O$	N/A	√	N/A	N/A	N/A	N/A	N/A	√	√	33.33

Perfluorohexyl Iodide PFHxI	$C_6F_{13}IH$	N/A	√	N/A	N/A	N/A	N/A	√	N/A	N/A	22.22
Perfluorooctyl Iodide PFOI	$C_8F_{17}IH$	N/A	√	N/A	√	√	√	√	√	√	22.22
8:2 Fluorotelomer acrylate (8:2 FTAC)	$C_{13}H_7F_{17}O_2$	N/A	√	N/A	N/A	N/A	N/A	√	√	√	44.44
6:2 Fluorotelomer methacrylate (6:2 FTAC)	$C_{12}H_9F_{13}O_2$	N/A	√	N/A	N/A	N/A	√	√	√	√	55,56
8:2 Fluorotelomer methacrylate (8:2 FTMAC)	$C_{14}H_9F_{17}O_2$	N/A	N/A	N/A	N/A	N/A	N/A	√	√	√	33.33
Perfluoro-2-methoxyacetic acid (PFMOAA)	$C_3HF_5O_3$	√	√	√	N/A	N/A	N/A	N/A	N/A	N/A	33.33
Perfluoro-3-methoxypropanoic acid (PFMOPrA)	$C_4HF_7O_3$	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	11.11
Perfluoro-4-methoxybutanoic acid (PFMOBA)	$C_5HF_9O_3$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluoro-2-propoxypropanoic acid (PFPrOPrA)	$C_6HF_{11}O_3$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluoro(3,5-dioxahexanoic) acid (PFO2HxA)	$C_4HF_7O_4$	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluoro(3,5,7-trioxaoctanoic) acid (PFO3OA)	$C_5HF_9O_5$	N/A	√	N/A	√	√	N/A	N/A	√	√	55.56
Perfluoro(3,5,7,9-tetraoxadecanoic) acid (PFO4DA)	C6HF11O6	√	√	N/A	N/A	N/A	N/A	N/A	N/A	N/A	22.22

Table 4.3: Targeted and non-targeted PFASs in wastewater treatment plant

Wastewater Treatment plants		**FINAL D**	**FINAL S**	**SST D**	**SST S**	**RAW D**	**RAW S**	
Compound Name	Formula	Detected	Detected	Detected	Detected	Detected	Detected	Detection frequency %
(PFBA)	$C_4HF_7O_2$	√	√	√	√	√	√	100
(PFPeA)	$C_5HF_9O_2$	√	√	√	√	√	√	100
PFHxA	$C_6HF_{11}O_2$	√	√	√	√	√	√	100
PFHpA	$C_7HF_{13}O_2$	√	√	√	√	√	√	100
PFOA	$C_8HF_{15}O_2$	√	√	√	√	√	√	100
PFNA	$C_9HF_{17}O_2$	√	√	√	√	√	√	100
PFDA	$C_{10}HF_{19}O_2$	√	√	√	√	√	√	100
PFUdA	$C_{11}HF_{21}O_2$	√	√	√	√	√	√	100
PFDoA	$C_{12}HF_{23}O_2$	N/A	√	N/A	√	√	√	66.7
PFTrDA	$C_{13}HF_{25}O_2$	N/A	√	N/A	√	√	√	66.7
PFTeDA	$C_{14}HF_{27}O_2$	√	√	√	√	√	√	100
L-PFBS	$C_4HF_9O_3S$	√	√	√	√	√	√	100
L-PFHxS	$C_6HF_{13}O_3S$	√	√	√	√	√	√	100
L-PFOA	$C_8HF_{17}O_3S$	√	√	√	√	√	√	100
L-PFDS	$C_{10}HF_{21}O_3S$	√	√	√	√	√	N/A	83.3
L-PFHpS	$C_7F_{15}SO_3H$	√	√	√	√	√	√	100
L-PFNS	$C_9F_{19}SO_3H$	N/A	√	√	√	√	√	83.3
L-PFDoS	$C_{12}HF_{25}O_3S$	N/A	N/A	√	N/A	√	N/A	33,3
L-PFPeS	$C_5F_{11}SO_3H$	√	√	√	N/A	√	N/A	66.7
				Non-targeted PFASs				

4:2 Fluorotelomer sulfonic sulfonate 4:2 FTSA	$C_6H_5F_9O_3S$	√	√	N/A	√	√	√	83.3
6:2 Fluorotelomer sulfonate (6:2 FTSA)	$C_8H_5F_{13}O_3S$	√	√	√	√	√	√	100
8:2 Fluorotelomer Sulfonate (8:2 FTSA)	$C_{10}H_4F_{17}O_3S$	√	√	N/A	√	√	√	83.3
Perfluorooctane sulfonamide (FOSA)	$C_8H_2F_{17}NO_2S$	√	√	√	√	√	√	100
N-Methyl perfluorooctane sulfonamide (MeFOSA)	$C_9H_4F_{17}NO_2S$	√	√	√	√	√	√	100
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	$C_{10}H_6F_{17}NO_2S$	√	√	√	√	√	√	100
6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA	$C_8H_2F_{12}O_2$	√	√	√	N/A	√	√	83.3
8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA	$C_{10}H_2F_{16}O_2$	N/A	N/A	N/A	N/A	√	N/A	16.7
6:2 Fluorotelomer carboxylic acid (6:2 FTCA)	$C_6F_{13}CH_2COOH$	N/A	N/A	N/A	√	N/A	√	33.3
8:2 Fluorotelomer carboxylic acid (8:2 FTCA)	$C_8F_{17}CH_2COOH$	N/A	N/A	N/A	N/A	N/A	N/A	0
10:2 Fluorotelomer carboxylic acid (10: 2 FTCA)	$C_{10}F_{21}CH_2COO$ H	√	√	N/A	N/A	N/A	N/A	16.7
6:2 Fluorotelomer alcohol (6:2 FTOH)	$C_8H_5F_{13}O$	√	√	N/A	√	N/A	N/A	50
Perfluorohexyl Iodide PFHxI	$C_6F_{13}IH$	N/A	N/A	N/A	√	N/A	N/A	16.7
Perfluorooctyl Iodide PFOI	$C_8F_{17}IH$	√	N/A	N/A	√	N/A	√	50
8:2 Fluorotelomer acrylate (8:2 FTAC)	$C_{13}H_7F_{17}O_2$	√	√	N/A	√	N/A	√	66,7
6:2 Fluorotelomer methacrylate (6:2 FTAC)	$C_{12}H_9F_{13}O_2$	√	√	N/A	√	N/A	√	66.7
8:2 Fluorotelomer methacrylate (8:2 FTMAC)	$C_{14}H_9F_{17}O_2$	√	√	N/A	N/A	N/A	√	50

Perfluoro-2-methoxyacetic acid (PFMOAA)	$C_3HF_5O_3$	√	√	√	√	N/A	√	83.3
Perfluoro-3-methoxypropanoic acid (PFMOPrA)	$C_4HF_7O_3$	√	√	√	N/A	N/A	N/A	50
Perfluoro-4-methoxybutanoic acid (PFMOBA)	$C_5HF_9O_3$	N/A	N/A	N/A	N/A	N/A	N/A	0
Perfluoro-2-propoxypropanoic acid (PFPrOPrA)	$C_6HF_{11}O_3$	√	√	N/A	N/A	N/A	√	50
Perfluoro(3,5-dioxahexanoic) acid (PFO2HxA)	$C_4HF_7O_4$	√	√	N/A	N/A	N/A	√	50
Perfluoro(3,5,7-trioxaoctanoic) acid (PFO3OA)	$C_5HF_9O_5$	√	√	N/A	√	N/A	√	66.7
Perfluoro(3,5,7,9-tetraoxadecanoic) acid (PFO4DA)	$C_6HF_{11}O_6$	√	√	√	√	N/A	N/A	66.7

Table 4.4: Targeted and non-targeted PFASs in drinking water treatment plant

Drinking Water treatment		DWTP-I	DWTP-F	DWTP-E	
Compound Name	Formula	Detected	Detected	Detected	Detection frequency %
(PFBA)	$C_4HF_7O_2$	√	√	√	100
(PFPeA)	$C_5HF_9O_2$	√	√	√	100
PFHxA	$C_6HF_{11}O_2$	√	√	√	100
PFHpA	$C_7HF_{13}O_2$	√	√	√	100
PFOA	$C_8HF_{15}O_2$	√	√	√	100
PFNA	$C_9HF_{17}O_2$	√	√	√	100
PFDA	$C_{10}HF_{19}O_2$	√	√	√	100
PFUdA	$C_{11}HF_{21}O_2$	N/A	√	√	66.7
PFDoA	$C_{12}HF_{23}O_2$	√	N/A	√	66.7
PFTrDA	$C_{13}HF_{25}O_2$	N/A	N/A	N/A	0
PFTeDA	$C_{14}HF_{27}O_2$	N/A	N/A	N/A	0
L-PFBS	$C_4HF_9O_3S$	√	√	N/A	66,.
L-PFHxS	$C_6HF_{13}O_3S$	√	√	N/A	66.7
L-PFOA	C8HF17O3S	√	√	N/A	66,7
L-PFDS	$C_{10}HF_{21}O_3S$	N/A	N/A	N/A	0
L-PFHpS	$C_7F_{15}SO_3H$	N/A	N/A	N/A	0
L-PFNS	$C_9F_{19}SO_3H$	√	N/A	N/A	33.3
L-PFDoS	$C_{12}HF_{25}O_3S$	N/A	N/A	N/A	0
L-PFPeS	$C_5F_{11}SO_3H$	√	√	N/A	66.7
		Non-targeted PFASs			
4:2 Fluorotelomer sulfonic sulfonate 4:2 FTSA	$C_6H_5F_9O_3S$	N/A	N/A	N/A	0
6:2 Fluorotelomer sulfonate (6:2 FTSA)	$C_8H_5F_{13}O_3S$	√	√	N/A	66.7
8:2 Fluorotelomer Sulfonate (8:2 FTSA)	$C_{10}H_4F_{17}O_3S$	√	√	N/A	66.7
Perfluorooctane sulfonamide (FOSA)	$C_8H_2F_{17}NO_2S$	N/A	√	N/A	33.3

N-Methyl perfluorooctane sulfonamide (MeFOSA)	$C_9H_4F_{17}NO_2S$	N/A	√	N/A	33,3
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	$C_{10}H_6F_{17}NO_2S$	√	√	N/A	66.7
6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA	$C_8H_2F_{12}O_2$	N/A	√	N/A	33.3
8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA	$C_{10}H_2F_{16}O_2$	√	N/A	N/A	33.3
6:2 Fluorotelomer carboxylic acid (6:2 FTCA)	$C_6F_{13}CH_2COOH$	N/A	√	√	66.7
8:2 Fluorotelomer carboxylic acid (8:2 FTCA)	$C_8F_{17}CH_2COOH$	√	√	N/A	66.7
10:2 Fluorotelomer carboxylic acid (10: 2 FTCA)	$C_{10}F_{21}CH_2COO\ H$	√	N/A	√	66.7
6:2 Fluorotelomer alcohol (6:2 FTOH)	$C_8H_5F_{13}O$	√	N/A	N/A	33.3
Perfluorohexyl Iodide PFHxI	$C_6F_{13}IH$	N/A	N/A	N/A	0
Perfluorooctyl Iodide (PFOI)	$C_8F_{17}IH$	N/A	√	√	66.7
8:2 Fluorotelomer acrylate (8:2 FTAC)	$C_{13}H_7F_{17}O_2$	N/A	N/A	N/A	0
6:2 Fluorotelomer methacrylate (6:2 FTAC)	$C_{12}H_9F_{13}O_2$	N/A	N/A	N/A	0
8:2 Fluorotelomer methacrylate (8:2 FTMAC)	$C_{14}H_9F_{17}O_2$	N/A	√	√	66.7
Perfluoro-2- methoxyacetic acid (PFMOAA)	$C_3HF_5O_3$	N/A	N/A	N/A	0
Perfluoro-3- methoxypropanoic acid (PFMOPrA)	$C_4HF_7O_3$	N/A	N/A	N/A	0
Perfluoro-4- methoxybutanoic acid (PFMOBA)	$C_5HF_9O_3$	N/A	N/A	N/A	0
Perfluoro-2- propoxypropanoic acid (PFPrOPrA)	$C_6HF_{11}O_3$	N/A	N/A	N/A	0
Perfluoro(3,5- dioxahexanoic) acid (PFO2HxA)	$C_4HF_7O_4$	N/A	N/A	N/A	0
Perfluoro(3,5,7- trioxaoctanoic) acid (PFO3OA)	$C_5HF_9O_5$	N/A	N/A	√	33.3
Perfluoro(3,5,7,9- tetraoxadecanoic) acid (PFO4DA)	$C_6HF_{11}O_6$	N/A	N/A	N/A	0

Table 4.5: Targeted and non-targeted PFASs in bottled and tap drinking water

Bottled water and tap water		**Product A**	**Product B**	**Product C**	**Product D**	**Tap water**	
Compound Name	Formula	Detected	Detected	Detected	Detected	Detected	Detection freq (%)
Targeted PFASs							
(PFBA)	$C_4HF_7O_2$	√	√	√	√	√	100
(PFPeA)	$C_5HF_9O_2$	√	√	√	√	√	100
PFHxA	$C_6HF_{11}O_2$	√	√	√	√	√	100
PFHpA	$C_7HF_{13}O_2$	√	√	√	√	√	100
PFOA	$C_8HF1_5O_2$	√	√	√	√	N/A	80
PFNA	$C_9HF_{17}O_2$	√	√	√	√	√	100
PFDA	$C_{10}HF_{19}O_2$	√	√	√	N/A	√	80
PFUdA	$C_{11}HF_{21}O_2$	N/A	N/A	√	N/A	N/A	20
PFDoA	$C_{12}HF_{23}O_2$	N/A	N/A	N/A	N/A	N/A	0
PFTrDA	$C_{13}HF_{25}O_2$	N/A	√	N/A	N/A	N/A	20
PFTeDA	$C_{14}HF_{27}O_2$	N/A	N/A	N/A	√	N/A	20
L-PFBS	$C_4HF_9O_3S$	√	√	N/A	N/A	√	60
L-PFHxS	$C_6HF_{13}O_3S$	√	√	N/A	N/A	√	60
L-PFOA	$C_8HF_{17}O_3S$	√	√	N/A	N/A	√	60
L-PFDS	$C_{10}HF_{21}O_3S$	√	N/A	N/A	√	√	60
L-PFHpS	$C_7F_{15}SO_3H$	√	√	N/A	√	N/A	60
L-PFNS	$C_9F_{19}SO_3H$	√	N/A	N/A	N/A	√	40
L-PFDoS	$C_{12}HF_{25}O_3S$	N/A	N/A	N/A	N/A	N/A	0
L-PFPeS	$C_5F_{11}SO_3H$	√	√	N/A	N/A	N/A	40
Non-targeted PFASs							
4:2 Fluorotelomer sulfonic sulfonate 4:2 FTSA	$C_6H_5F_9O_3S$	√	√	N/A	N/A	N/A	40
6:2 Fluorotelomer sulfonate (6:2 FTSA)	$C_8H_5F_{13}O_3S$	√	√	N/A	N/A	√	60
8:2 Fluorotelomer Sulfonate (8:2 FTSA)	$C_{10}H_4F_{17}O_3S$	N/A	N/A	N/A	N/A	N/A	0
Perfluorooctane sulfonamide (FOSA)	$C_8H_2F_{17}NO_2S$	N/A	N/A	√	N/A	N/A	20

N-Methyl perfluorooctane sulfonamide (MeFOSA)	$C_9H_4F_{17}NO_2S$	N/A	√	√	N/A	N/A	40
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	$C_{10}H_6F_{17}NO_2S$	N/A	N/A	N/A	N/A	N/A	0
6:2 Fluorotelomer unsaturated carboxylic acid 6:2 FTUCA	$C_8H_2F_{12}O_2$	N/A	N/A	N/A	N/A	N/A	0
8:2 Fluorotelomer unsaturated carboxylic acid 8:2 FTUCA	$C_{10}H_2F_{16}O_2$	N/A	N/A	N/A	N/A	N/A	0
6:2 Fluorotelomer carboxylic acid (6:2 FTCA)	$C_6F_{13}CH_2COOH$	N/A	N/A	N/A	√	N/A	20
8:2 Fluorotelomer carboxylic acid (8:2 FTCA)	$C_8F_{17}CH_2COOH$	N/A	N/A	N/A	N/A	N/A	0
10:2 Fluorotelomer carboxylic acid (10: 2 FTCA)	$C_{10}F_{21}CH_2COO$ H	√	N/A	N/A	N/A	N/A	20
6:2 Fluorotelomer alcohol (6:2 FTOH)	$C_8H_5F_{13}O$	N/A	N/A	N/A	N/A	N/A	0
Perfluorohexyl Iodide PFHxI	$C_6F_{13}IH$	N/A	N/A	N/A	N/A	N/A	0
Perfluorooctyl Iodide PFOI	$C_8F_{17}IH$	N/A	N/A	N/A	N/A	N/A	0
8:2 Fluorotelomer acrylate (8:2 FTAC)	$C_{13}H_7F_{17}O_2$	N/A	N/A	N/A	N/A	N/A	0
6:2 Fluorotelomer methacrylate (6:2 FTAC)	$C_{12}H_9F_{13}O_2$	N/A	N/A	N/A	N/A	N/A	0
8:2 Fluorotelomer methacrylate (8:2 FTMAC)	$C_{14}H_9F_{17}O_2$	√	N/A	√	N/A	N/A	40
Perfluoro-2- methoxyacetic acid (PFMOAA)	$C_3HF_5O_3$	√	N/A	√	N/A	√	60
Perfluoro-3- methoxypropanoic acid (PFMOPrA)	$C_4HF_7O_3$	N/A	√	N/A	N/A	N/A	20
Perfluoro-4- methoxybutanoic acid (PFMOBA)	$C_5HF_9O_3$	N/A	N/A	N/A	N/A	N/A	0
Perfluoro-2- propoxypropanoic acid (PFPrOPrA)	$C_6HF_{11}O_3$	N/A	√	N/A	N/A	√	40

Perfluoro(3,5- dioxahexanoic) acid (PFO2HxA)	$C_4HF_7O_4$	N/A	√	N/A	N/A	N/A	20
Perfluoro(3,5,7- trioxaoctanoic) acid (PFO3OA)	$C_5HF_9O_5$	N/A	N/A	N/A	N/A	N/A	0
Perfluoro(3,5,7,9- tetraoxadecanoic) acid (PFO4DA)	$C_6HF_{11}O_6$	N/A	√	N/A	N/A	N/A	20

4.2.2 Distribution of legacy and emerging PFASs in water samples

Shown in Figure 4.1 is a summary of legacy and emerging PFASs in water samples in Gauteng province, obtained using non-target approach. As can be seen in Figure 2.1, the following emerging along with legacy PFASs were identified, Perfluoro-2-methoxyacetic acid (PFMOAA), Perfluoro-3-methoxypropanoic acid (PFMOPrA), Perfluoro-4-methoxybutanoic acid (PFMOBA), Perfluoro-2-propoxypropanoic acid (PFPrOPrA), Perfluoro(3,5-dioxahexanoic) acid (PFO2HxA), Perfluoro(3.5.7-trioxaoctanoic) acid (PFO3OA) and Perfluoro (3,5,7,9-tetraoxadecanoic) acid (PFO4DA). It is worth noting that qualitative method was used to identify these emerging PFASs. Quantitative analysis of these new PFASs should be conducted in order to establish their actual concentrations and, thereafter, conduct risk assessment to ascertain whether there is any risk posed by these emerging PFASs.

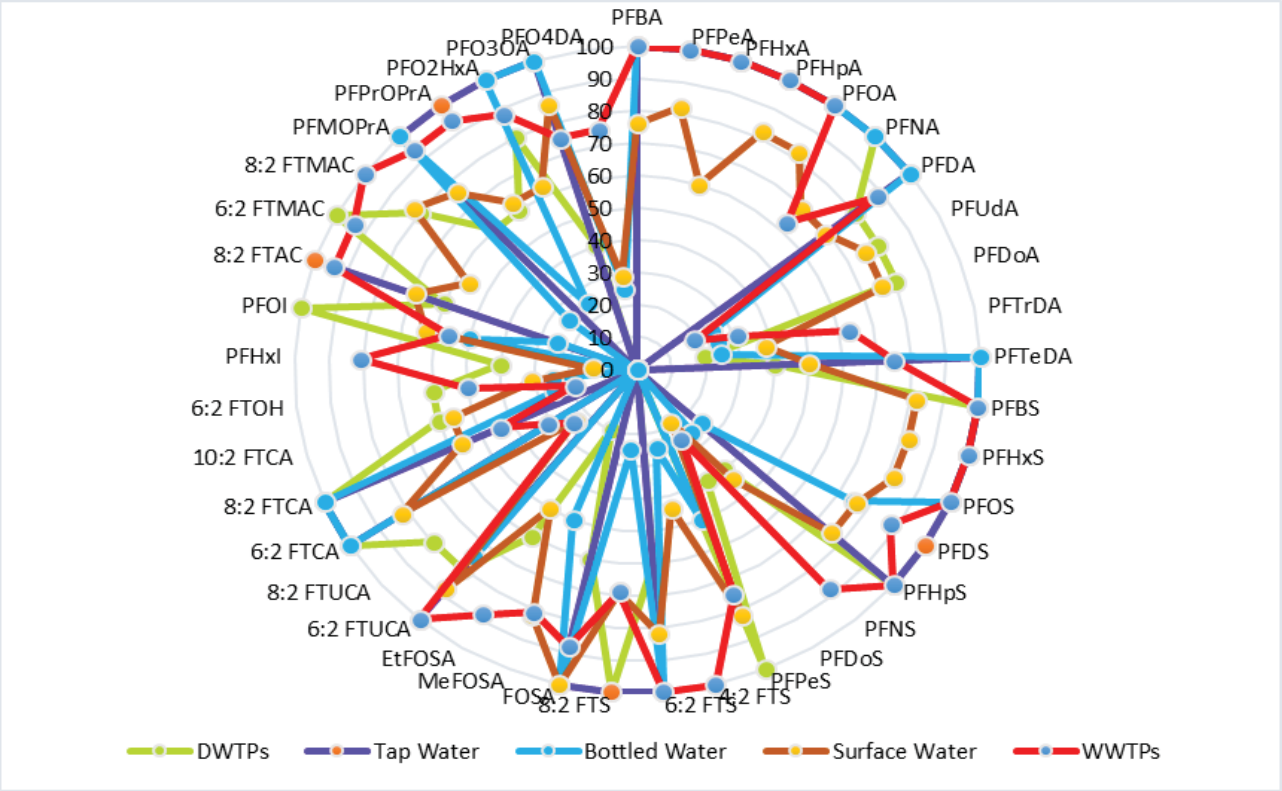

Figure 4.1: Summary of legacy and emerging PFASs in water samples in Gauteng Province obtained using non-target approach

4.3 SPATIAL AND TEMPORAL DISTRIBUTION OF PER- AND POLYFLUOROALKYL SUBSTANCE CLASSES IN VARIOUS WATER SOURCES

PFASs chemicals are classified into different classes such as PFCA, PFSA and others. The contributions of these to the PFASs quantified in different water sources were assessed and these are shown in Figures 4.2 to 4.9.

4.3.1 Class contribution of PFASs in the Eastern Cape province

Figure 4.2 shows a box plot of spatial and temporal contributions of classes of PFASs in various water sources in the Eastern Cape province. During the dry season, fluorotelomers had more contributions in drinking water treatment plant, surface water and wastewater treatment. In tap water, however, the PFSAs had the highest contributions indicated by L-PFOS by followed by L-PFHxS. During the wet season, PFCAs had more contribution in drinking water treatment plant followed by the Fluorotelomer class, exhibited by PFPeA and 6:2 FTS, respectively. The PFCAs group had lesser contribution in both surface and tap water compared to the other groups. In surface water, the Fluorotelomers had the highest contribution as shown by 6:2 FTS followed by PFHxS which belongs to the PFSAs class. PFSAs had the highest contribution in tap water, represented by PFOS in the plot.

4.3.2 Class contribution of PFASs in the Free State province

Figure 4.3 illustrates the contributions of PFASs classes from various water sources in Free State Province during the dry and wet seasons. Drinking water treatment plant (DWTP) was dominated by telomers which is represented by squares. 6-2 FTS exhibited the highest % contribution compared to other telomers. PFCAs had lower % contribution which was lower than Log 20. In surface water, 6-2 FTS, FOET and FHET were the most dominant compared to PFCAs with log contribution of more than 20. Similar trend was also observed with tap water and wastewater treatment plant. On the other hand, in wet season drinking water treatment plant (DWTP) was mostly dominated by telomers and with equal contributions of PFCAs and PFSAs; similar trend was also observed in surface water. In tap water, PFCAs classes were dominant compared to PFASs class. PFBA and PFHpA were the highest compound detected in tap water. While wastewater treatment plant (WWTP) was dominated by PFCAs and telomers, comprising PFPeA and PFHpA and (6-2 FTS, FHET and FHEA) respectively.

4.3.3 Class contribution of PFASs in the Gauteng province

Shown in Figure 4.4 are the contributions of the various classes of PFASs in different water sources in Gauteng during the dry and wet seasons. As can be seen in Figure 4.4, all the classes were detected in drinking and surface water and scattered in borehole samples during the dry season. However, the PFCAs appear to be the most dominant PFASs compared to the PFSAs and fluorotelomers. Similarly, all the PFASs classes were detected during the wet season, with the fluorotelomers the most dominant compared to other PFASs classes.

4.3.4 Class contribution of PFASs in the KwaZulu-Natal province

Figure 4.5 shows that PFSAs class contributed most of the PFASs detected in surface water and wastewater treatment plant collected from KZN in dry season. The compounds that contributed the most to class of PFSAs were PFOS in surface water and PFBS in WWTP. This was followed by the Fluorotelomer class in both water sources. In tap water, however, the fluorotelomers had more contribution with FOET contributing more followed by PFSAs. PFCAs had less contribution to the results in all the water sources collected. For the wet season, fluorotelomers contributed most in surface water collected from KZN. The compound that contributed the most to the class was 6:2 FTS. This was followed by the PFSAs class indicated by L-PFOS. In tap water and wastewater treatment plants, the PFSAs had more contribution with L-PFOS and L-PFHxS, respectively. PFCAs had less contribution to the results in all the water sources collected.

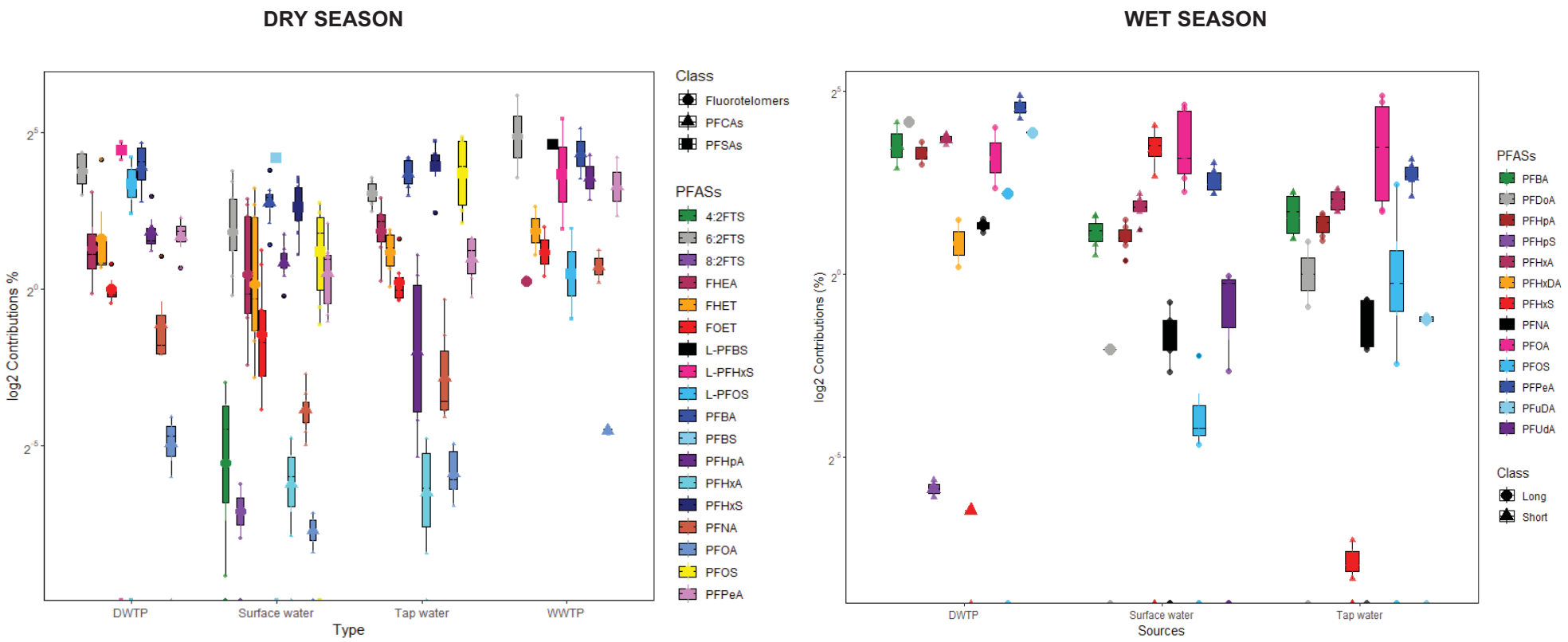

Figure 4.2: Spatial and temporal PFASs class contributions in various water sources in the Eastern Cape province.

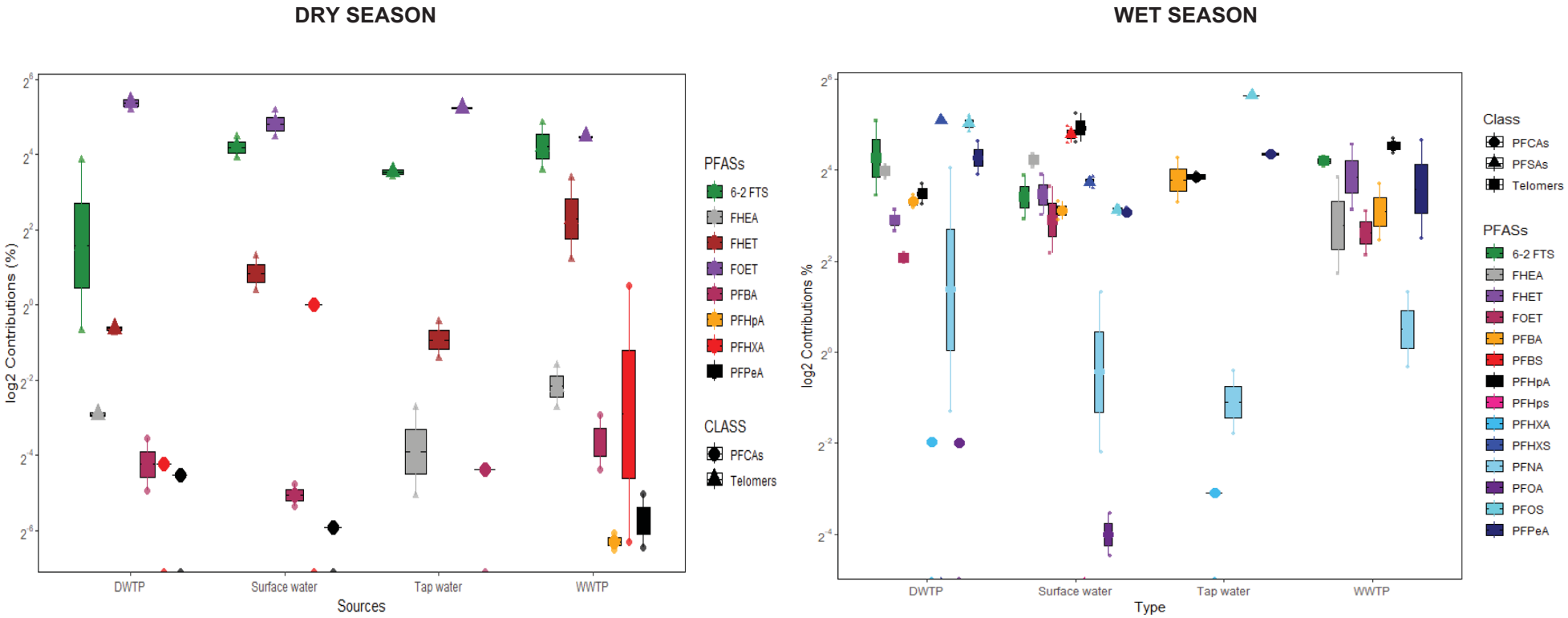

Figure 4.3: Spatial and temporal PFASs class contributions in various water sources in the Free State province.

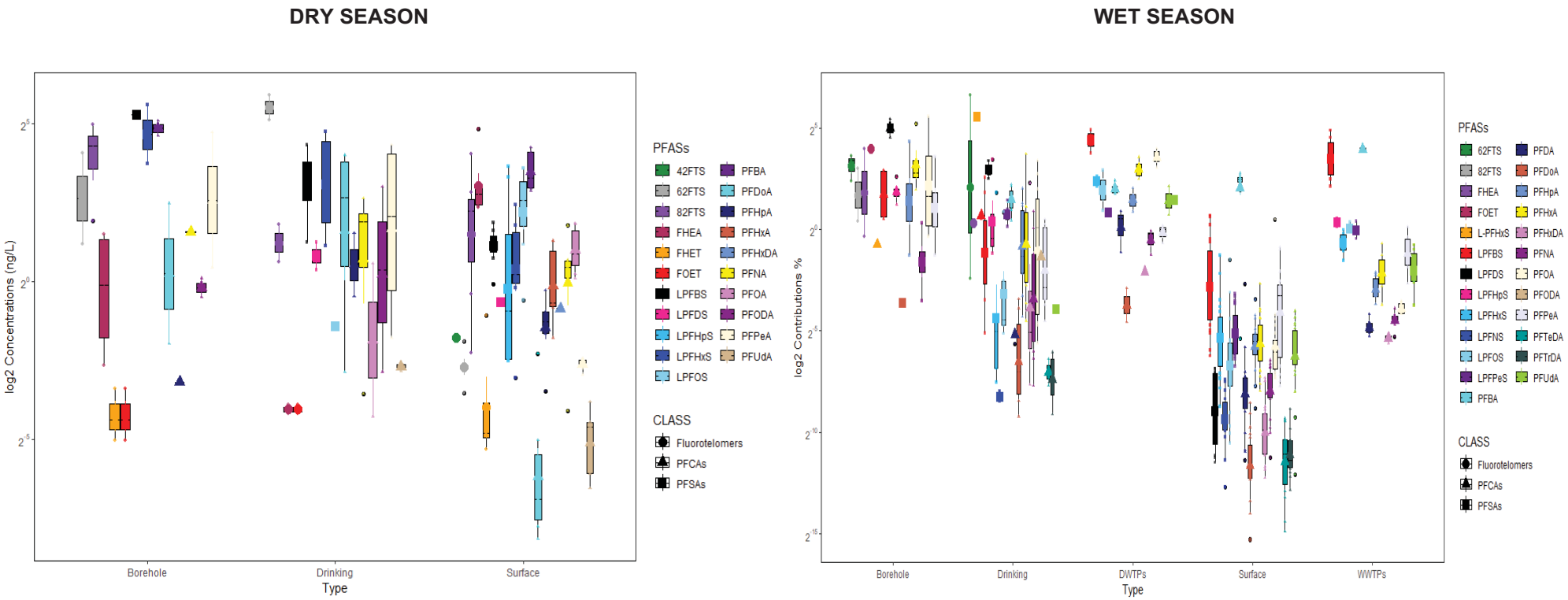

Figure 4.4: Spatial and temporal PFASs class contributions in various water sources in the Gauteng province.

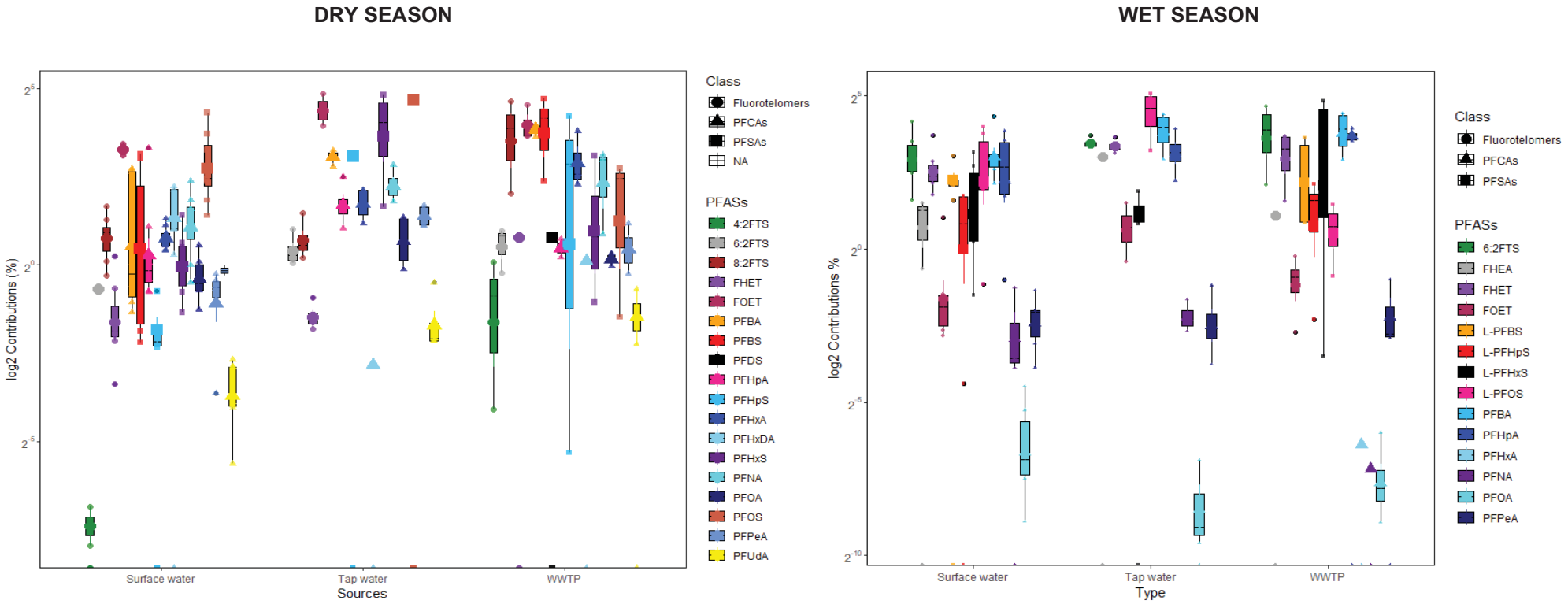

Figure 4.5: Spatial and temporal PFASs class contributions in various water sources in the KwaZulu-Natal province.

4.3.5 Class contribution of PFASs in the Limpopo province

Shown in Figure 4.6 are the PFASs class contributions for various water sources in Limpopo during the dry and wet seasons. PFCAs can be seen to be the most dominant in all the water sources, particularly in the WWTP samples where they are clustered compared to surface water. PFSAs also follow the same trend. The Fluorotelomers are much more in landfill borehole, drinking water and surface water. It can clearly be seen in Figure 4.6 that the PFCAs are the dominant PFASs in all the water sources, although they are mostly clustered in WWTP. This is indicative of use of PFASs products containing PFOA which belongs to PFCAs class of PFCAs. It is used in several industrial applications, including carpeting, upholstery, apparel, floor wax, textiles, firefighting foam and sealants.

4.3.6 Class contribution of PFASs in the Mpumalanga province

The three major classes of PFASs, Fluorotelomers, PFCAs and PFSAs were detected in all the water sources from Mpumalanga in dry season as can be seen in Figure 4.7. However, PFCAs appear to be most dominant. Furthermore, the PFASs classes are more congested in drinking water, DWTP, surface water and WWTP compared to borehole. This is probably expected since contaminants will have to travel through soil before polluting groundwater. With respect to PFASs classes contribution, it can be seen that all the three classes, Fluorotelomers, PFCAs and PFSAs are well clustered in DWTP, surface water and WWTP and scattered in borehole and drinking water.

4.3.7 Class contribution of PFASs in the Northern cape province

Figure 4.8 presents PFASs classes contribution in various water sources in Northern Cape during the dry and wet seasons. The telomers class exhibited the highest contribution, followed by PFSA. The class contribution of PFASs during the wet season, with a focus on the concentration of specific compounds in both DWTPs and WWTPs is shown in Figure 4.8. The data revealed that PFSA was the most dominant class in both DWTPs and WWTPs, while PFCAs had a lower contribution. Furthermore, in WWTPs, telomers were more prevalent than PFCA.

4.3.8 Class contribution of PFASs in the North West province

The three classes of PFASs, PFCAs, PFSAs and Fluorotelomers were all present in the different sourced of water from North West in dry and wet seasons (Figure 4.9). FOET, a telomer, was prominent in all borehole water samples. This was followed by PFOA, a PFCA. Generally, PFCAs were most prevalent in WWTPs and borehole samples, while fluorotelomers contributed to high concentrations in DWTPs, and drinking water samples. All the classes, PFCAs, PFSAs and telomers were present in the water sources analysed.

As can be seen in Figure 4.9, the three classes are most prevalent in the WWTP. This is not surprising since WWTP receives wastewater from domestic and storm water discharged which may contain PFASs compounds leached from PFASs-containing products. Some congestion of the PFASs classes can also be seen in the borehole samples. PFCAs are the most prevalent, although telomers such as FOET exhibited the highest concentration in one of the wastewater samples, NW-W2S in wet season (Figure 4.9). That PFASs classes were detected in borehole samples suggested pollution probably from the use of WWTP effluent to recharge the aquifer or via transport of landfill leachate from unlined landfill sites.

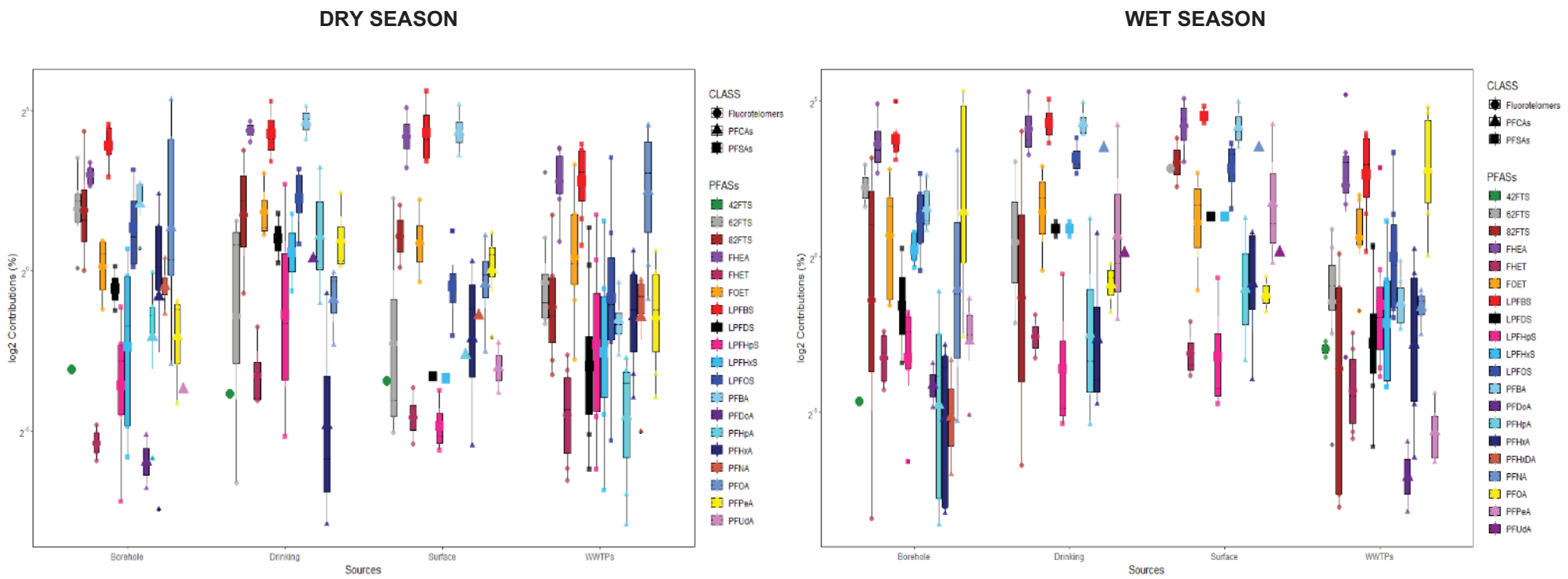

Figure 4.6: Spatial and temporal PFASs class contributions in various water sources in the Limpopo province.

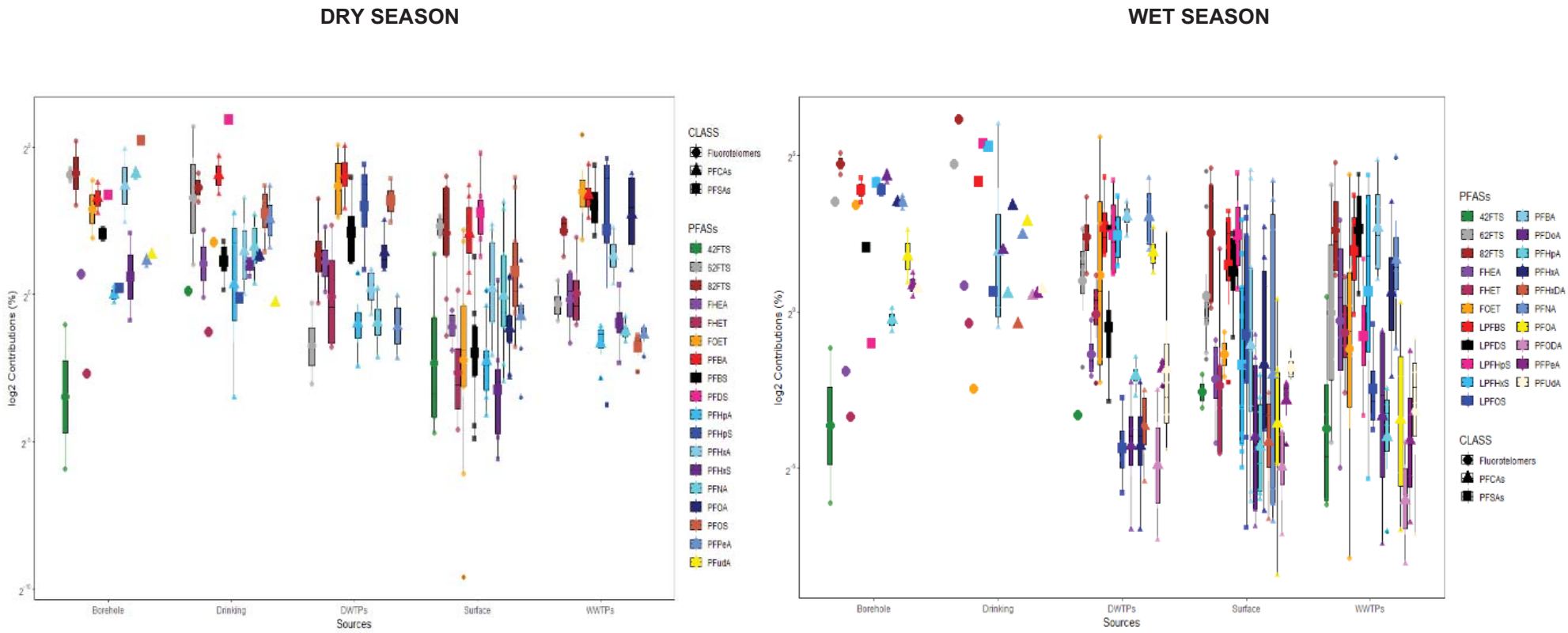

Figure 4.7: Spatial and temporal PFASs class contributions in various water sources in the Mpumalanga province.

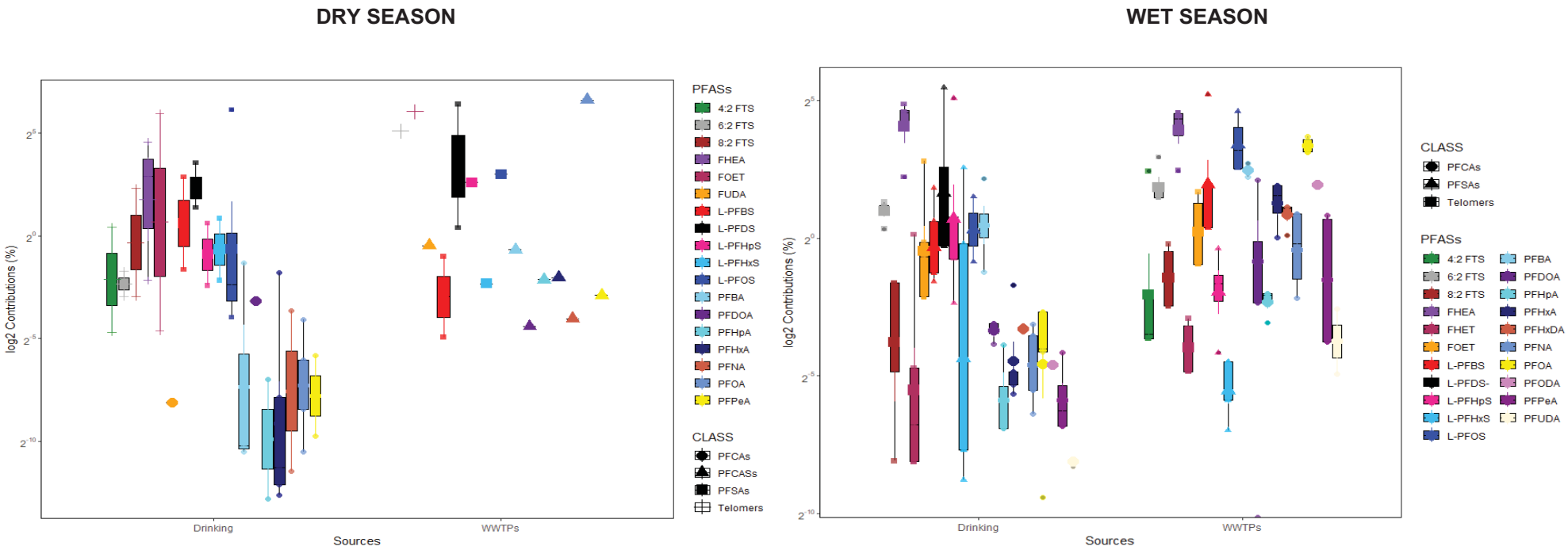

Figure 4.8: Spatial and temporal PFASs class contributions in various water sources in the Northern Cape province.

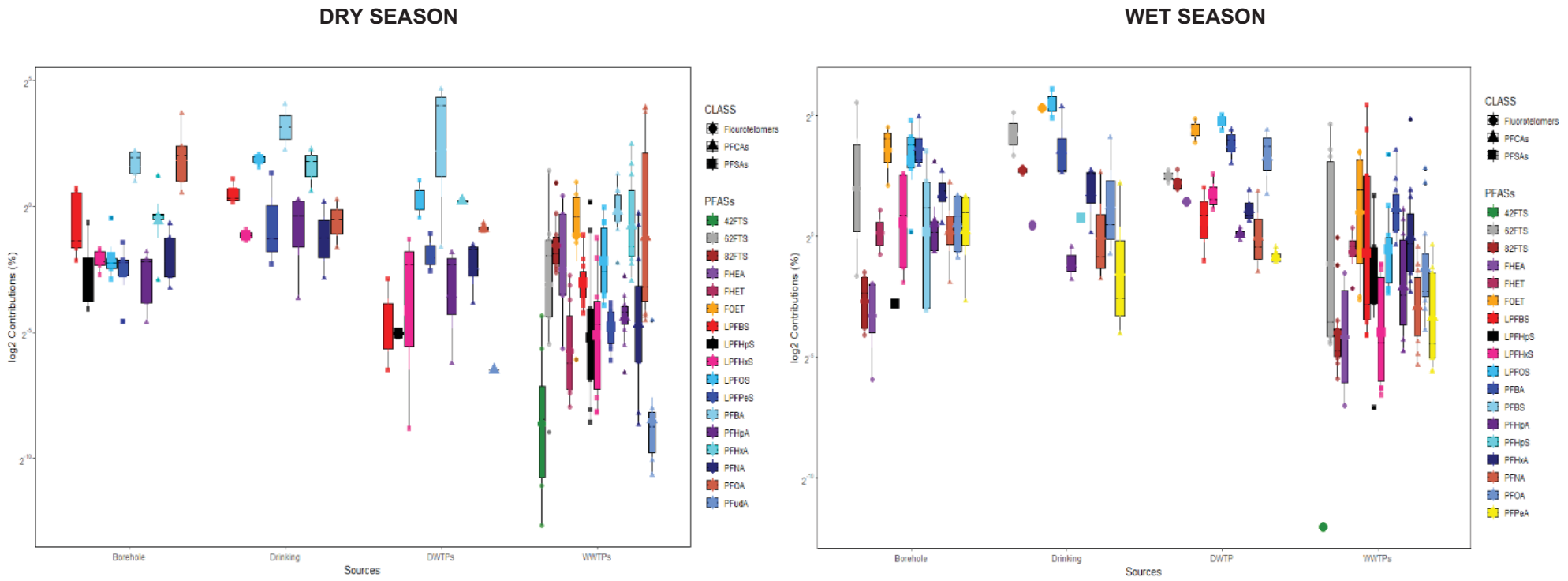

Figure 4.9: Spatial and temporal PFASs class contributions in various water sources in the North West province.

4.4 SPATIAL AND TEMPORAL DISTRIBUTIONS OF LONG AND SHORT CHAIN PER- AND POLYFLUOROALKYL SUBSTANCE IN WATER

Figures 4.10 to 4.17 show the contributions of long and short chain PFASs in water sources in the different provinces. Generally, the short chains had more contribution in the drinking water treatment plant. In both surface water and tap water, the long chains were observed to have more contribution and PFOA was the compound with the highest contribution. The observed pattern is indicative of either more use of short chain PFASs or the breakdown of long chains into short.

4.4.1 Distributions of long and short chain PFASs in water sources in the Eastern Cape

Figure 4.10 represents the contributions of the long and short chain PFASs in various water sources from Eastern Cape during the dry and wet seasons. During the dry season, the short chains had more contribution in the drinking water treatment plant and PFBA was the compound with the highest contribution. In surface water, tap water and wastewater treatment plants the long chains had more contribution and PFNA was the compound with the highest contribution. During the wet season, the short chains had more contribution in the drinking water treatment plant. In both surface water and tap water, the long chains were observed to have more contribution and PFOA was the compound with the highest contribution. The observed pattern is indicative of either more use of short chain PFASs or the breakdown of long chains into short.

4.4.2 Distributions of long and short chain PFASs in water sources in the Free State

In Figure 4.11, it is evident that the short chain PFASs were the most dominant compared to long chain PFASs water samples from Free State in dry season. PFHpA was the only long chain detected in wastewater treatment plant (WWTP). PFBA, PFHxA, PPFeA, were the most contributing short chain PFASs in drinking water treatment plant. Similar trend was also observed in surface water; however, the % contribution of short chain PFASs were lower in DWTP. On the other hand, in the tap water, PFBA was the only short chain detected with log contribution of 2^6. In wet season, it can be seen that the short chains PFASs were detected more than long chain PFASs in drinking water treatment plant. The compounds detected were PFHxS, PFPeA and PFBA with log concentration above log 20 and other remaining compounds were detected at lower concentrations. Additionally, surface water was also dominated by short chain PFASs. In tap water, there was equal contribution between long and short chain. Wastewater treatment plant was dominated by short chain. The prevalence of short chain PFASs in water sources is due to their high mobility, this result in a fast distribution of these short chain compounds to water sources.

4.4.3 Distributions of long and short chain PFASs in water sources in Gauteng

The contributions of short and long-chain PFASs in various water sources from Gauteng during the dry and wet season are shown in Figure 4.12. During the dry season, both short and long chains feature prominently in drinking water compared to borehole and surface water samples. However, long chains appear to have contributed more than short chains. The contributions of long and short chain PFASs in wet season are shown in Figure 4.12 (left). With respect to wastewater treatment plant, all the short chain contributed more than long chains. The detection frequencies of the other long chain PFASs ranged from 33-50%. Similar trend was observed in drinking water treatment plant where the contribution of all the short chain were high. In the borehole water samples, PFPeA short chain was the highest contributor. As for bottled and tap drinking water, all the short chain PFASs namely, PFHxA, PFBA, PFpeA and PFHpA were prominent. In contrast with long chain PFASs, only PFOA contributed the highest. Generally, short contribute more than the long chain.

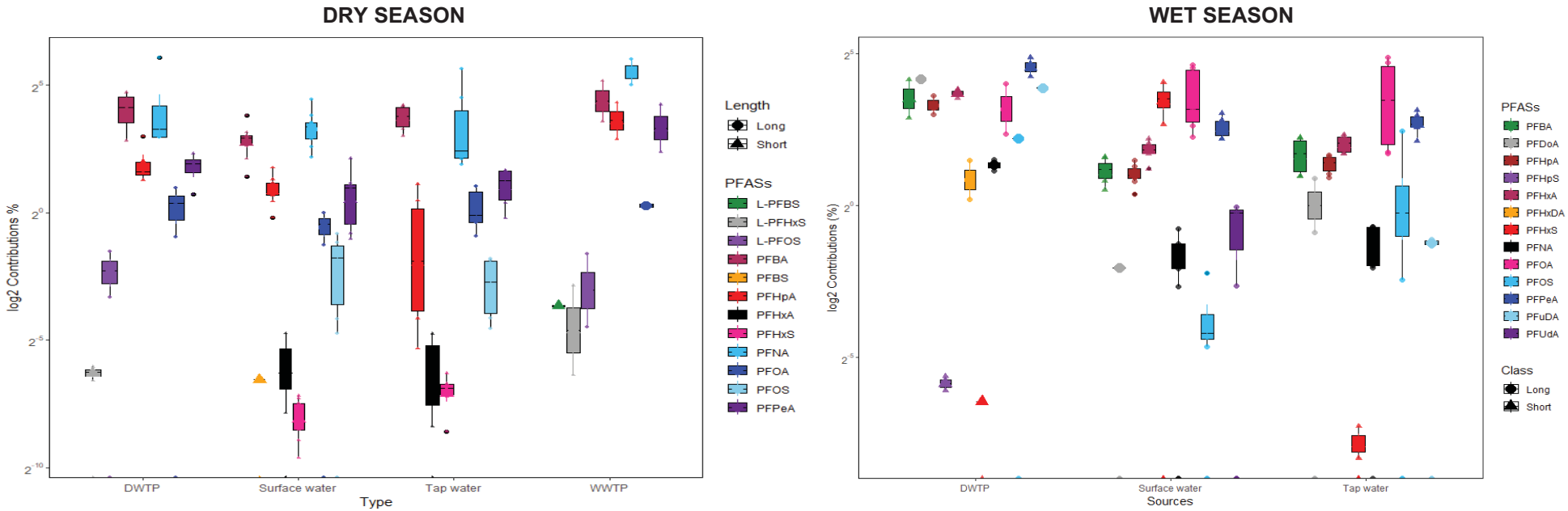

Figure 4.10: Spatial and temporal distributions of long and short chain PFASs in water sources in the Eastern Cape province.

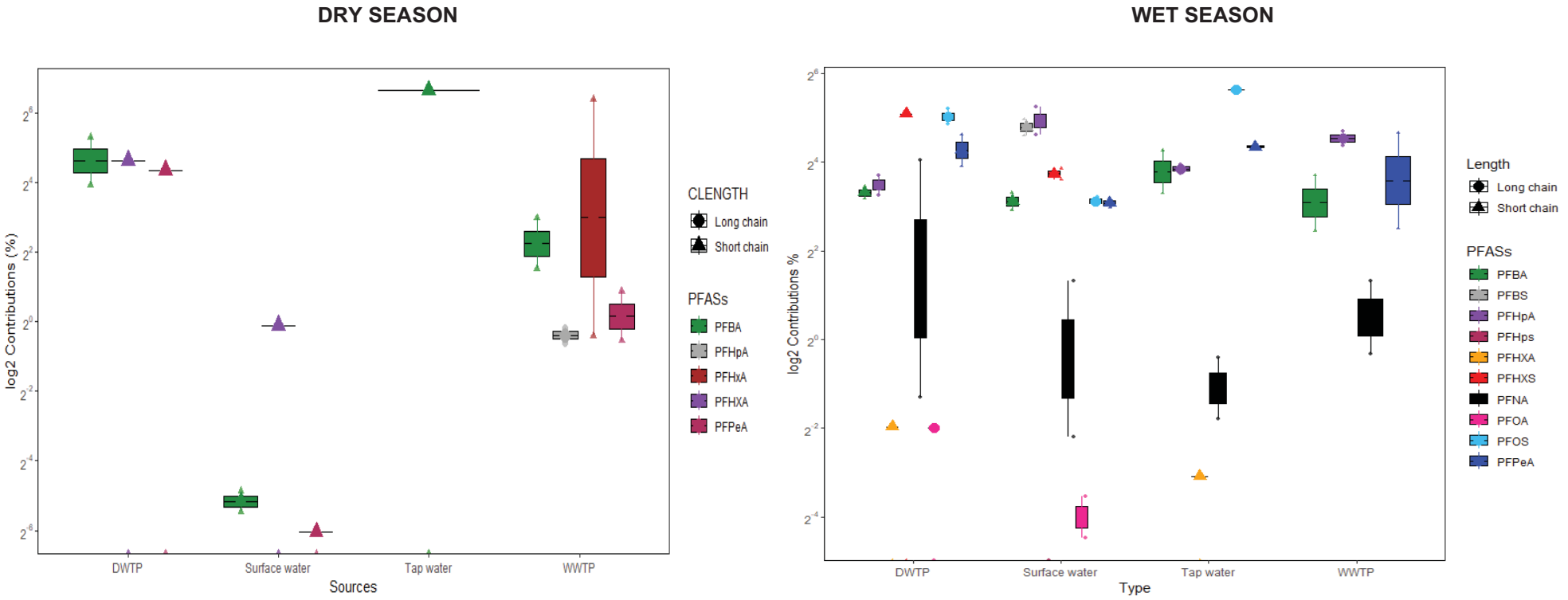

Figure 4.11: Spatial and temporal distributions of long and short chain PFASs in water sources in the Free State province.

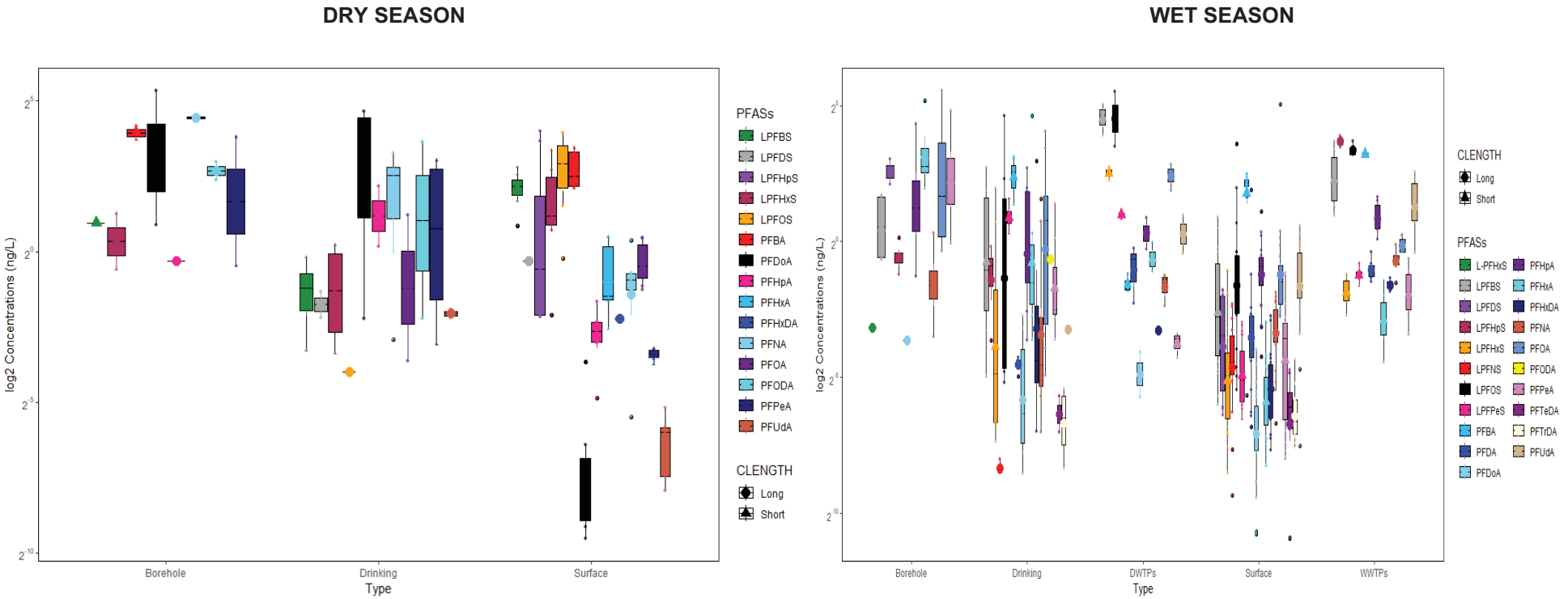

Figure 4.12: Spatial and temporal distributions of long and short chain PFASs in water sources in the Gauteng province.

4.4.4 Distributions of long and short chain PFASs in water sources in KwaZulu-Natal

In KwaZulu-Natal, the long chain PFASs had more contribution than the short chains during the dry season (Figure 4.13). PFOS contributed the most in surface water and in wastewater treatment plant samples, and in tap water PFNA had the most contribution. In all the water sources collected in wet season, the long chain PFASs had more contribution than the short chains. PFNA contributed most in surface water and in tap water. In wastewater treatment plant samples, L-PFOS had the most contribution.

4.4.5 Distributions of long and short chain PFASs in water sources in Limpopo

In Limpopo, the contributions of short and long chain PFASs in dry season are shown in the box scatter plot in Figure 4.14. Short chains are more dominant in all the water sources. This can be attributed to 1) discontinued use of long chain PFASs in products 2) the possible break down of long chain into short chains and 3) more use of short chains in products. Once again, short chain PFASs were more dominant than long chain in wet season as shown in Figure 4.14. Their frequency of detection can be attributed to their ability to be more soluble in water than their long chain analogues.

4.4.6 Distributions of long and short chain PFASs in water sources in Mpumalanga

Figure 4.15 shows the contributions of short and long chain PFASs to the observed concentrations during the dry and wet seasons in Mpumalanga. During the dry season, the short chains appeared to be more prominent. During the wet season, the contributions of long and short chain were well clustered in DWTP, surface water and WWTP and scattered in borehole and drinking water.

4.4.7 Distributions of long and short chain PFASs in water sources in the Northern Cape

In the case on Northern Cape, the contributions of short and long chain PFASs in different water sources in dry season are shown in Figure 4.16. The findings revealed that long chain PFASs were more prevalent in drinking water treatment. In contrast, short chain PFASs were found in higher proportions in wastewater treatment plants, likely due to their lower affinity to bind to solid particles and their greater mobility in water. The results obtained from analysis of samples collected during the wet season showed that long chains were more prevalent than short chains in both DWTPs and WWTPs. This may be due to the fact that long chain PFASs are more stable and resistant to degradation, making them more likely to persist in the environment. Similarly, short chain PFASs have a greater mobility in water and a lower affinity to bind to solid particles, resulting in a higher proportion in WWTPs.

4.4.8 Distributions of long and short chain PFASs in water sources in the North West

In North West, the contributions of short and long chain PFASs during the dry and wet seasons are shown in Figure 4.17. During the dry season, long-chain PFASs dominated the WWTPs and borehole water samples at 65% and 52%, respectively. However, they show similar contributions in DWTP influent and effluent, indicating low removal efficiencies of PFASs by conventional DWTPs. During the wet season, short chains were more in the samples compared to long chain. Nearly all the short chains were detected in all the samples. The dominance of short chains may be due to 1) break down of telomers and long chain PFASs into short chains and 2) use of more short chain-containing products. Furthermore, long chains are less soluble in water and tend to adhere to solids than short chain and as a result, they are not readily available in water. However, PFOA and PFOS which are PFASs chain were detected in almost all the samples. PFOA and PFOS contributed high concentrations of PFASs amongst long-chain PFASs.

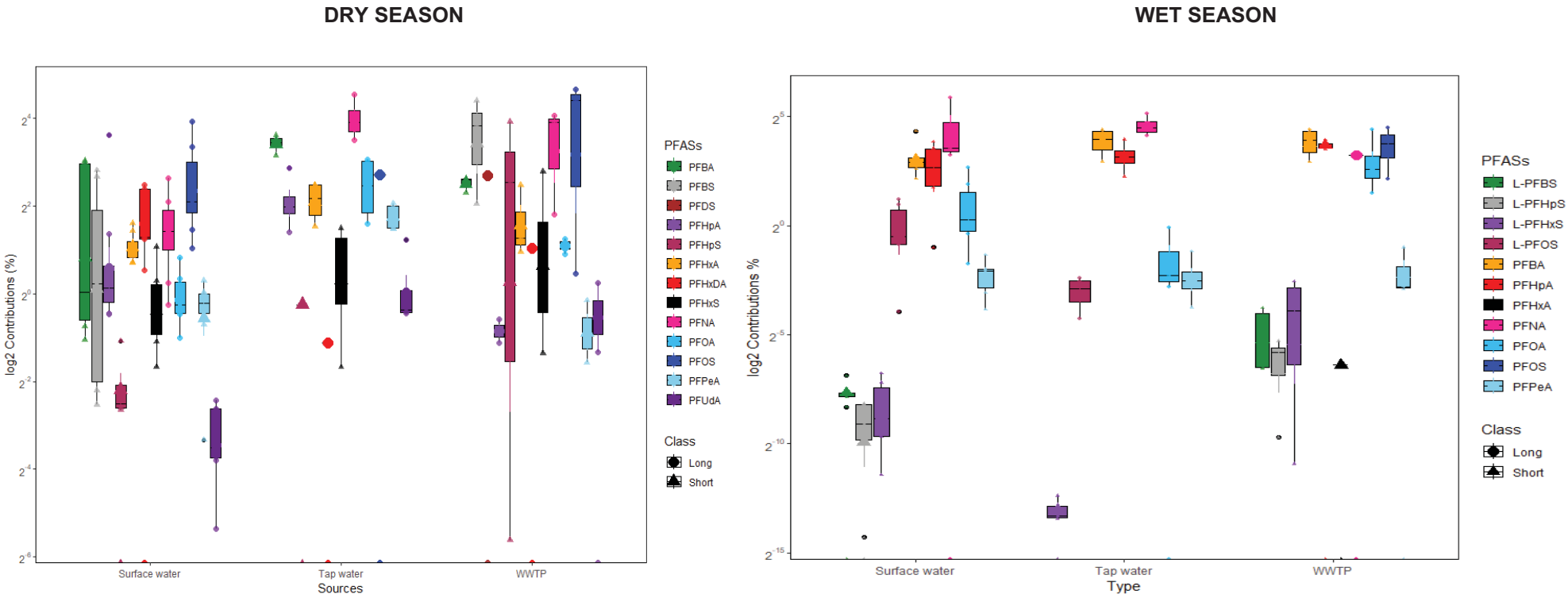

Figure 4.13: Spatial and temporal distributions of long and short chain PFASs in water sources in the KwaZulu-Natal province.

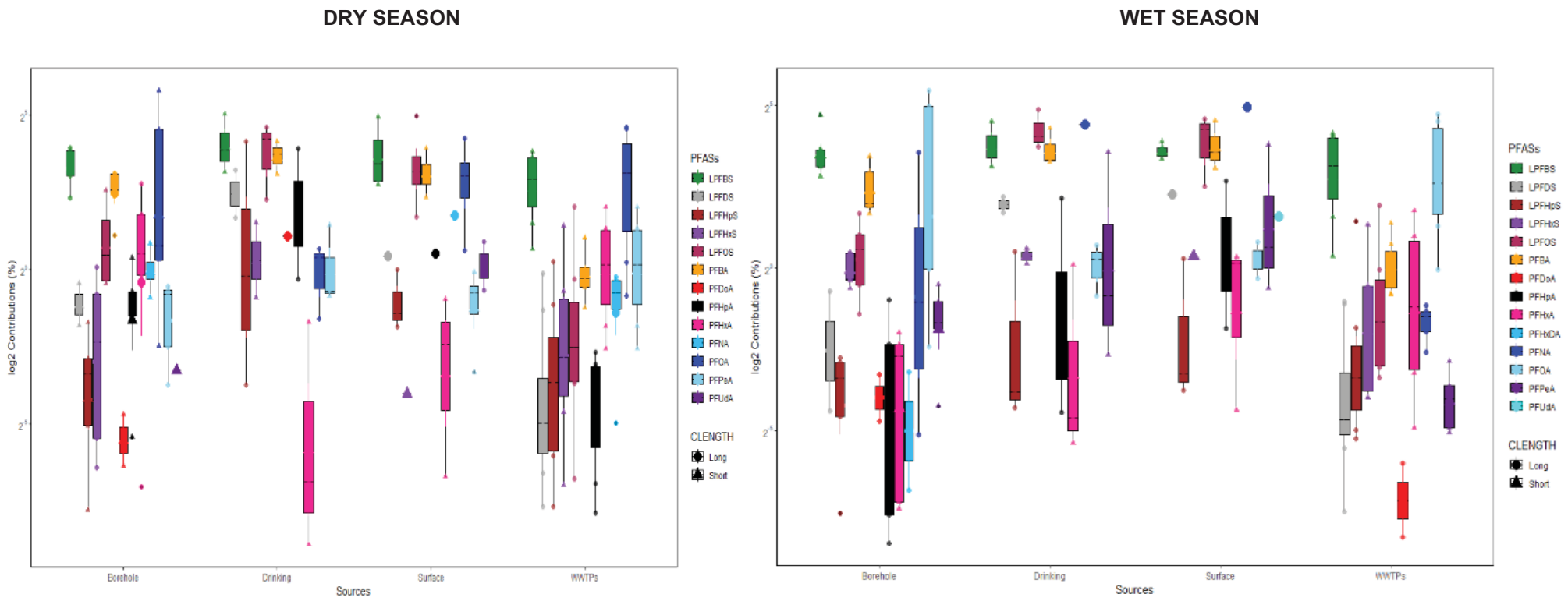

Figure 4.14: Spatial and temporal distributions of long and short chain PFASs in water sources in the Limpopo province.

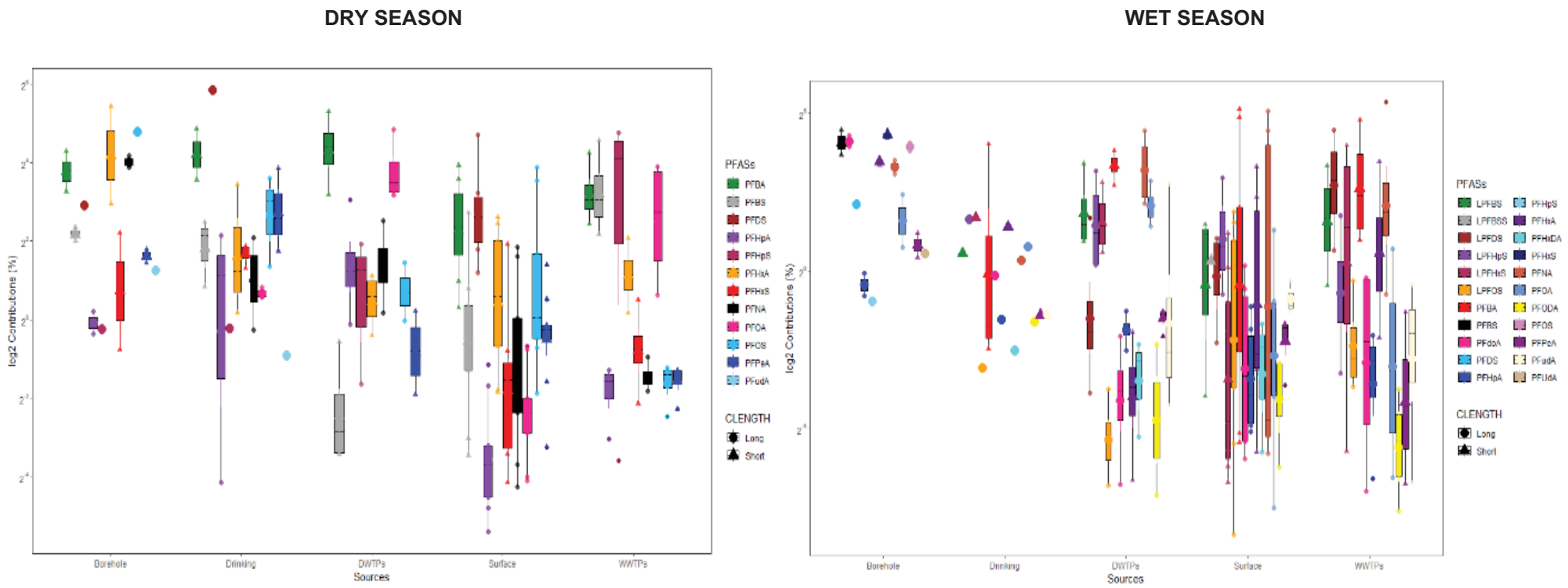

Figure 4.15: Spatial and temporal distributions of long and short chain PFASs in water sources in the Mpumalanga province.

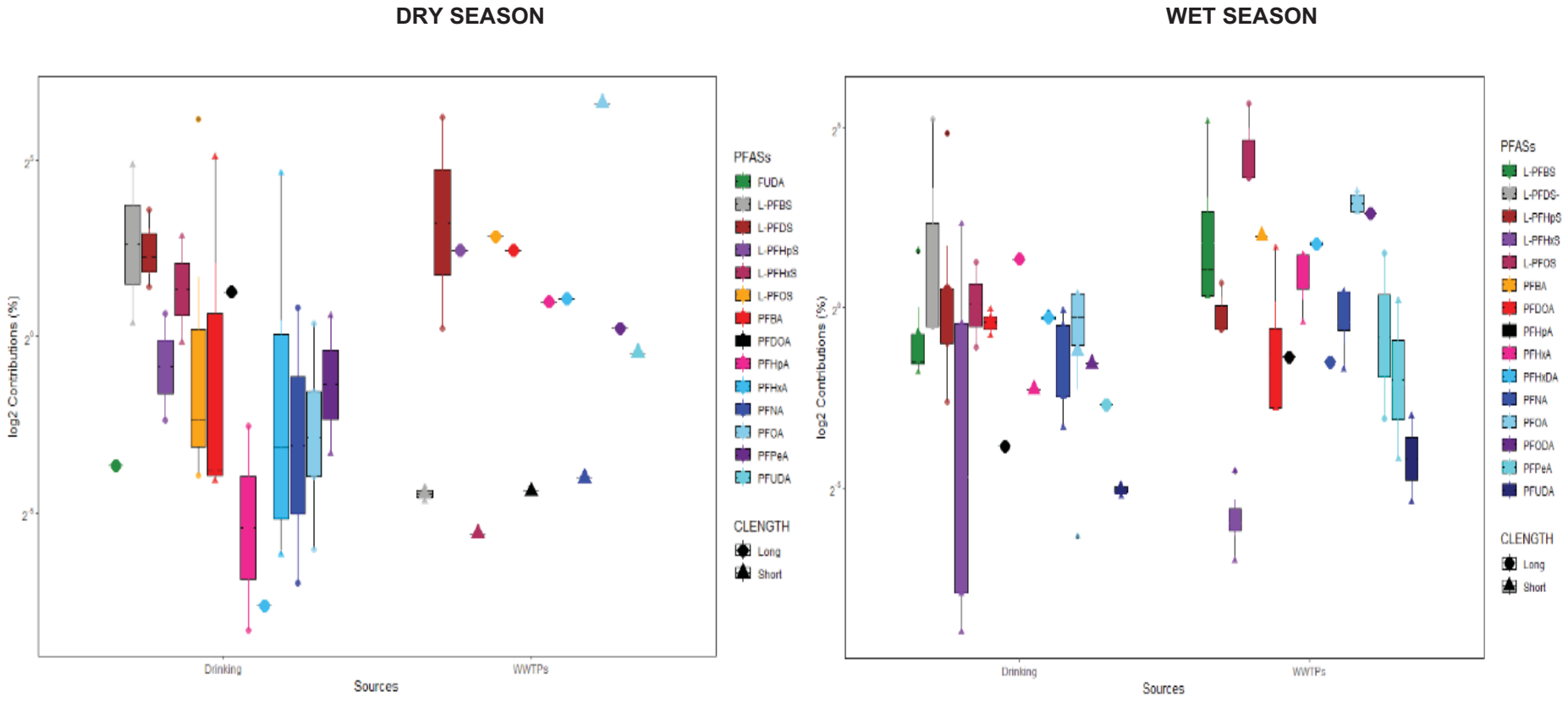

Figure 4.16: Spatial and temporal distributions of long and short chain PFASs in water sources in the Northern Cape province.

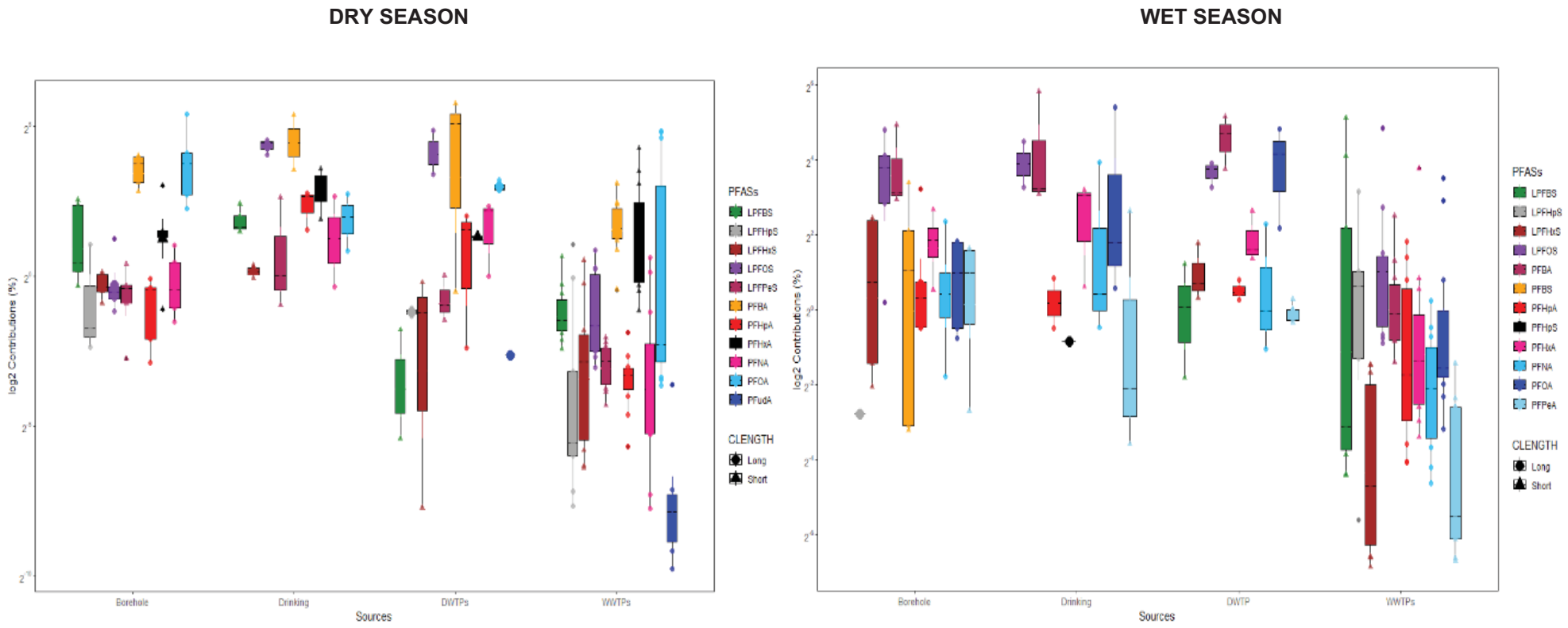

Figure 4.17: Spatial and temporal distributions of long and short chain PFASs in water sources in the North West province.

4.5 SUMMARY

Using the non-target approach, the following emerging PFASs were identified, Perfluoro-2-methoxyacetic acid (PFMOAA), Perfluoro-3-methoxypropanoic acid (PFMOPrA), Perfluoro-4-methoxybutanoic acid (PFMOBA), Perfluoro-2-propoxypropanoic acid (PFPrOPrA), Perfluoro(3.5-dioxahexanoic) acid (PFO2HxA) Perfluoro(3.5.7-trioxaoctanoic) acid (PFO3OA) and Perfluoro (3.5.7.9- tetraoxadecanoic) acid (PFO4DA).

In Eastern Cape, the fluorotelomers had more contributions in drinking water treatment plant, surface water and wastewater treatment in dry season. In tap water, however, the PFSAs had the highest contributions indicated by L-PFOS followed by L-PFHxS. During the wet season, PFCAs had more contribution in drinking water treatment plant followed by the Fluorotelomer class, exhibited by PFPeA and 6:2 FTS, respectively.

It was evident that the short chain PFASs were more dominant compared to long chain in water samples from the Free State in dry season. In wet season, it was observed that the short chains PFASs were detected more than long chain PFASs in drinking water treatment plant.

During the dry season, both short and long chains featured prominently in drinking water compared to borehole and surface water samples in Gauteng province. However, long chains appeared to have contributed more than short chains. With respect to wastewater treatment plant, all the short chain contributed more than long chains. As for bottled and tap drinking water, all the short chain PFASs namely, PFHxA, PFBA, PFpeA and PFHpA were prominent.

In KwaZulu-Natal, the long chain PFASs had more contribution than the short chains during the dry season. In all the water sources collected in wet season, the long chain PFASs had more contribution than the short chains. In Limpopo, short chains were more dominant in all the water sources in dry season. Once again, short chain PFASs were more dominant than long chain in wet season.

In Mpumalanga, short chains appeared to be more prominent in dry season; whereas during the wet season, the contributions of long and short chain were well clustered in DWTP, surface water and WWTP and scattered in borehole and drinking water.

In the case of Northern Cape, the findings revealed that long chain PFASs were more prevalent in drinking water treatment. In contrast, short chain PFASs were found in higher proportions in wastewater treatment plants, likely due to their lower affinity to bind to solid particles and their greater mobility in water.

In North West, long-chain PFASs dominated the WWTPs and borehole water samples at 65% and 52%, respectively. However, they showed similar contributions in DWTP influent and effluent, indicating low removal efficiencies. During the wet season, short chains were more in the samples compared to long chain. Nearly all the short chains were detected in all the samples.

CHAPTER 5: DITRIBUTION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DIFFERENT WATER SOURCES IN SOUTH AFRICA

5.1 INTRODUCTION

Monitoring the presence and levels of per- and polyfluoroalkyl substances (PFAS) in different water sources is crucial to assess potential contamination, protect public health, and inform regulatory actions. The comprehensive sampling plan that outlines the sampling sites, frequency of sampling, the number of samples, and the methods to be used is provided in Chapter 3. This plan considers seasonal variations and potential sources of PFAS contamination. Samples were collected using a grab method. Sample analysis was performed using two LC-MS methods that were optimised and validated for use in this nationwide PFASs monitoring exercise.

5.2 PER- AND POLYFLUOROALKYL SUBSTANCES IN WASTEWATER

5.2.1 Eastern Cape

In Eastern Cape, WWTP samples collected in wet season (Figure 5.1), 6:2 FTS exhibited the highest concentration in the influent samples; followed by PFHpA, PFBA. The other PFASs detected were lower than 25 ng/L. However, in the effluent, there was a general decrease except PFBA and PFPeA which increased. The observed increase could be attributed to the breakdown of fluorotelomers which are regarded as precursors of most PFASs.

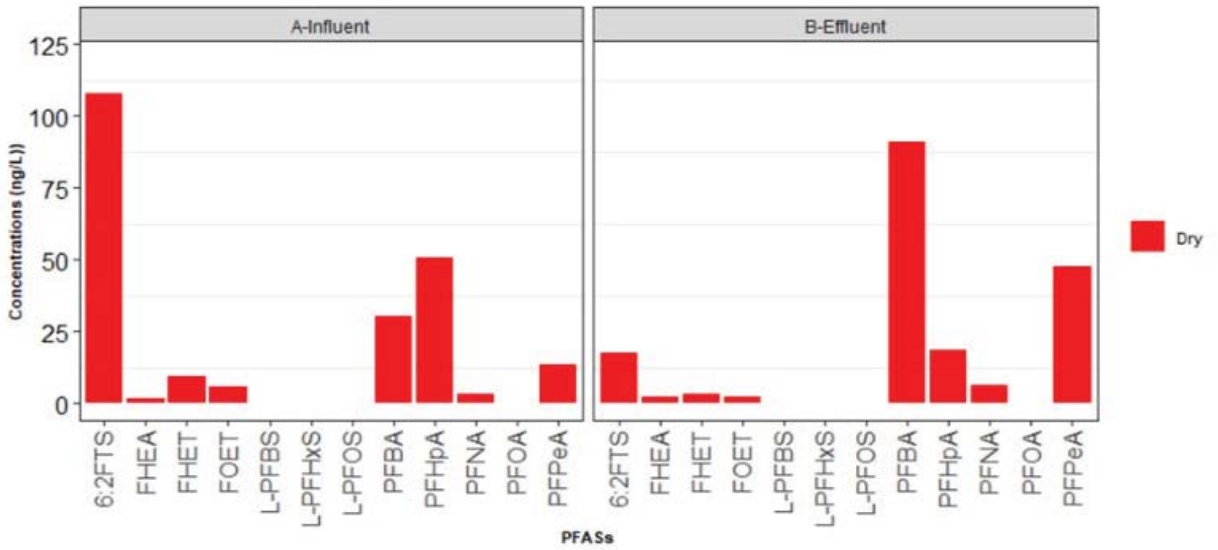

Figure 5.1: PFASs concentrations in wastewater treatment plant sources in the Eastern Cape province.

5.2.2 Free State province

In the case of Free State, there was a general decrease from dry to wet season (Figure 5.2). It can be seen also that PFASs were detected more in dry season than in wet season. Furthermore, the PFASs detected decreased in wet season. The observed difference can be explained as follows: dry season is depicted with little or no rain and, therefore, contaminants are more concentrated in this season. On the other hand, the rain in wet season tend to dilute contaminants.

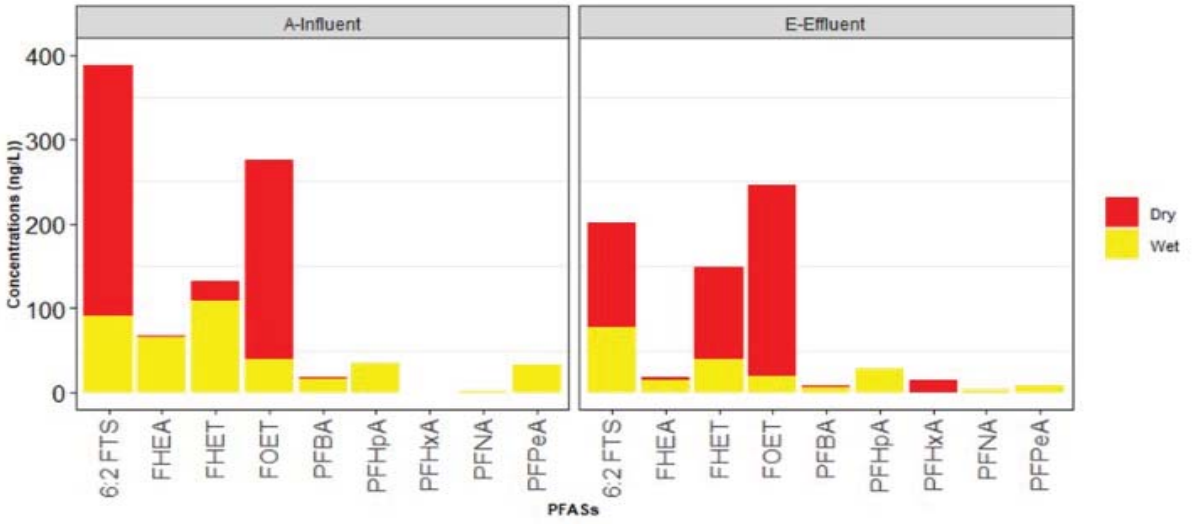

Figure 5.2: PFASs concentrations in wastewater treatment plant sources in the Free State province.

5.2.3 Gauteng province

Water samples collected from influent, secondary settling tank (SST) and effluent in Gauteng only in wet season can be seen in Figure 5.3. PFBA exhibited the highest concentration in the influent sample with a decrease in the SST with the exception of L-PFBS which increased. The concentration of PFBA remained the same in both SST and effluent; whereas PFBS decreased. The treatment processing at different stages in the WWTP may have influenced the concentrations of PFASs detected.

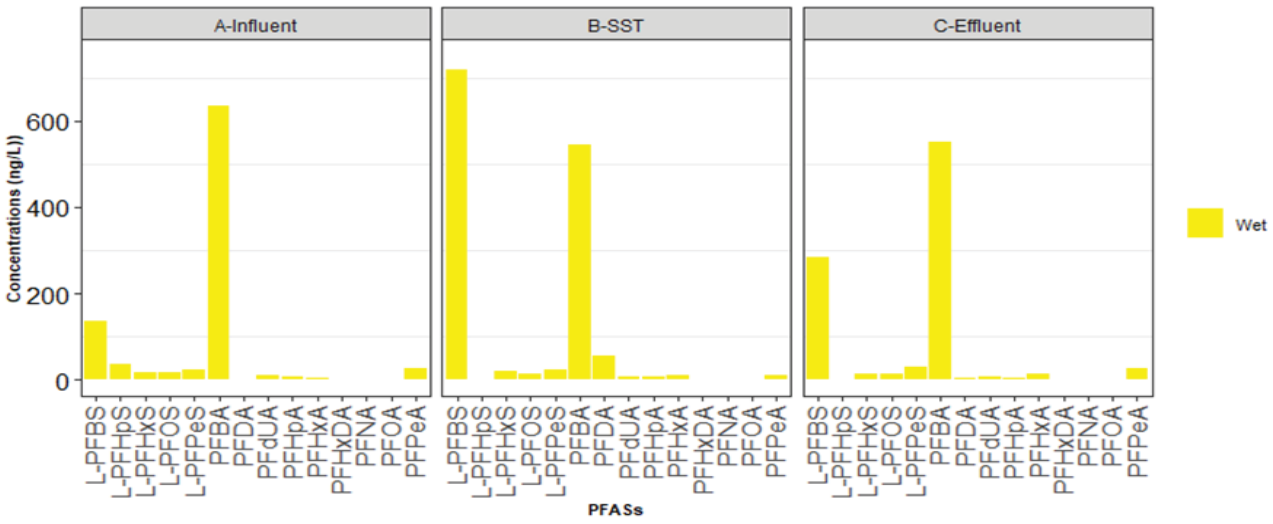

Figure 5.3: PFASs concentrations in wastewater treatment plant sources in the Gauteng province.

5.2.4 KwaZulu-Natal province

In the case of KwaZulu-Natal, PFBA exhibited the highest concentration in the influent followed by PFHpA and 6:2 FTS (Figure 5.4). 6:2 FTS remained the same in the effluent, albeit PFHpA decreased. Seasonally, the wet season contributed more PFASs than dry season. Before sample collect, KwaZulu-Natal experienced high flooding and this may have brough along with it more contaminants including PFASs.

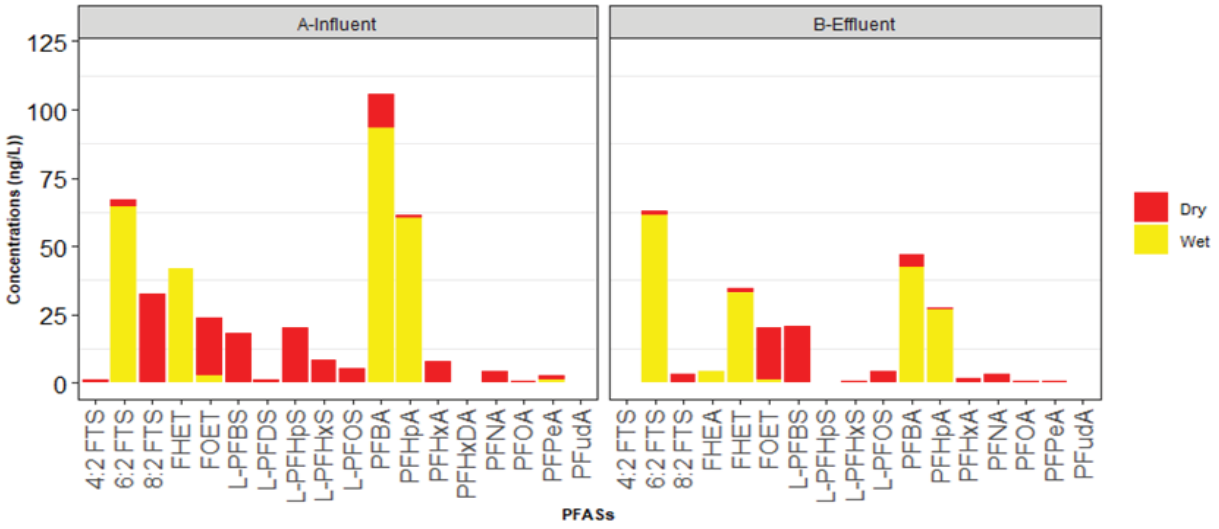

Figure 5.4: PFASs concentrations in wastewater treatment plant sources in the KwaZulu-Natal province.

5.2.5 Limpopo province

In Limpopo, wastewater samples were collected from the influent, primary settling tank (PST), secondary settling tank (SST) and the effluent (Figure 5.5). PFOA exhibited the highest concentrations at the different WWTP stages, although a general decrease from the influent to the effluent was observed. Seasonally, the dry season contributed higher PFASs than the wet season. The concentrations of PFBS remained almost the same in the different stages of treatment.

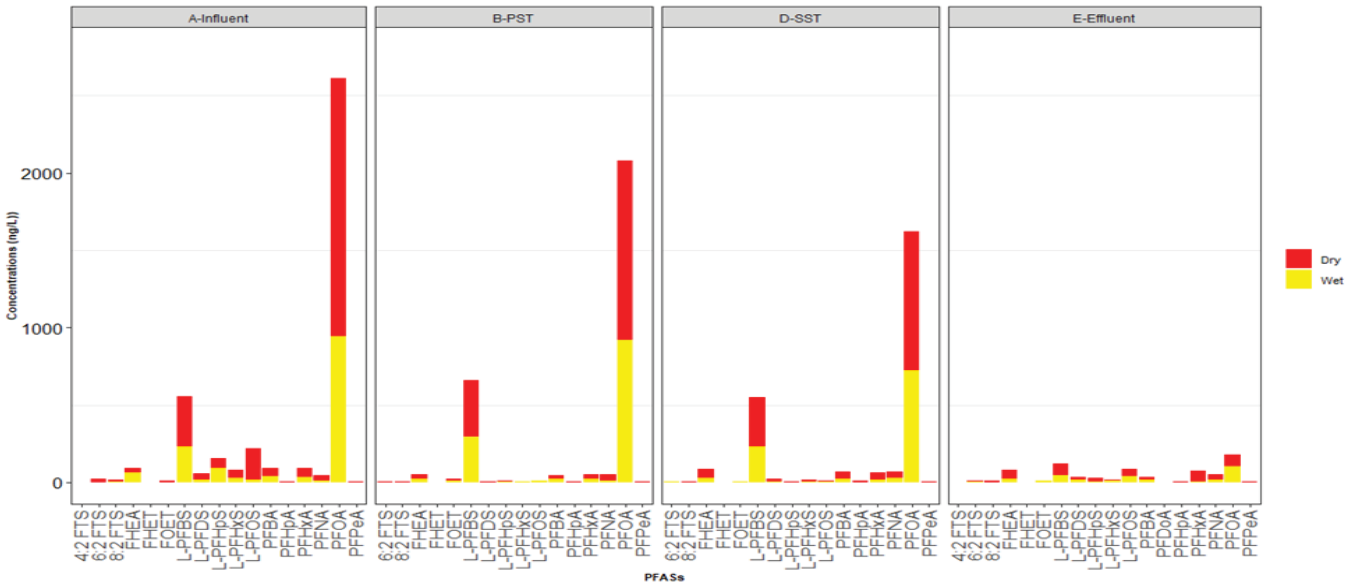

Figure 5.5: PFASs concentrations in wastewater treatment plant sources in the Limpopo province.

5.2.6 Mpumalanga province

Wastewater samples were collected from the influent and effluent in Mpumalanga. As can be seen in Figure 5.6, the PFASs detected generally decreased in the effluent. Wet season contributed more PFASs than the dry season.

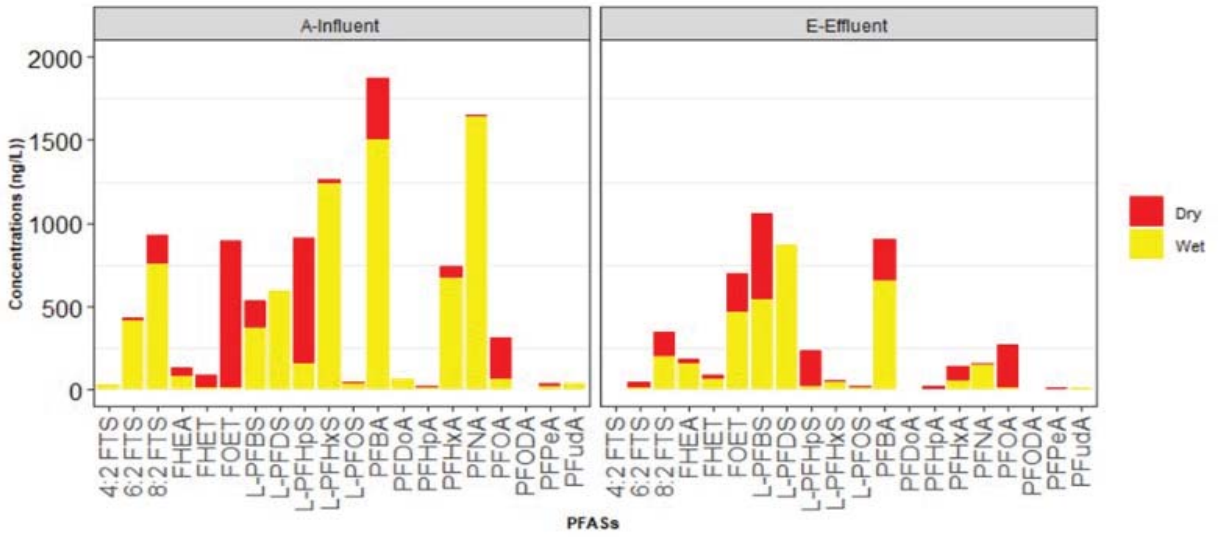

Figure 5.6: PFASs concentrations in wastewater treatment plant sources in the Mpumalanga province.

5.2.7 Northern Cape province

In the case of Northern Cape, FHEA followed by LPFOS and PFOA showed the highest concentrations in the influent (Figure 5.7). These decreased in the effluent except L-PFBS which increased. The two seasons showed almost equal contributions.

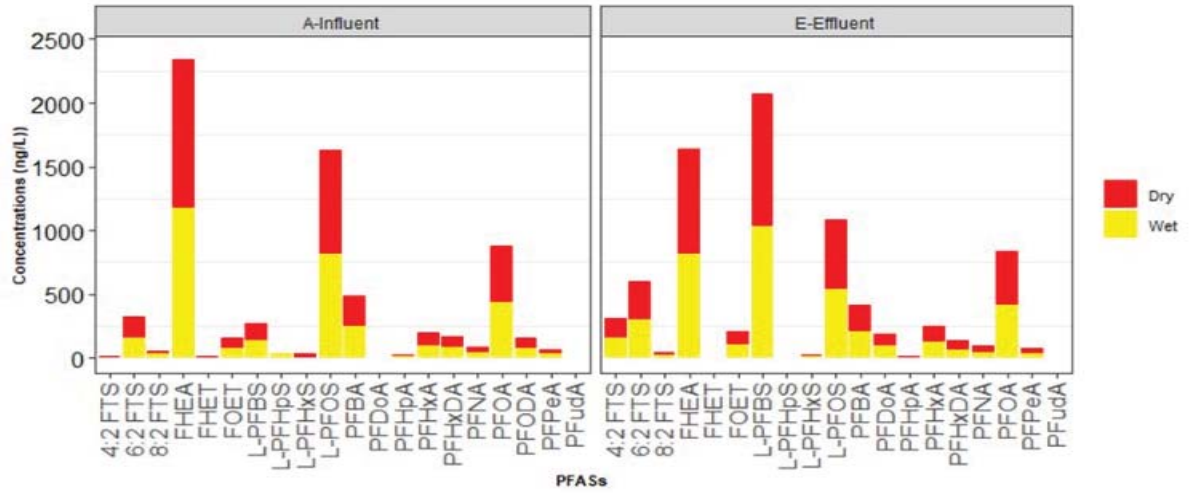

Figure 5.7: PFASs concentrations in wastewater treatment plant sources in the Northern Cape province.

5.2.8 North West province

Wastewater samples were collected from the influent, biological nutrient removal (BNR), secondary settling tank (SST) and the effluent in North West (Figure 5.8). The highest concentration was exhibited by FOET followed by 6:2 FTS in the influent. However, 6:2 FTS increased in the BNR and SST and decreased marginally in the effluent; whereas that of FOET decreased. More PFASs were detected in wet season than in dry.

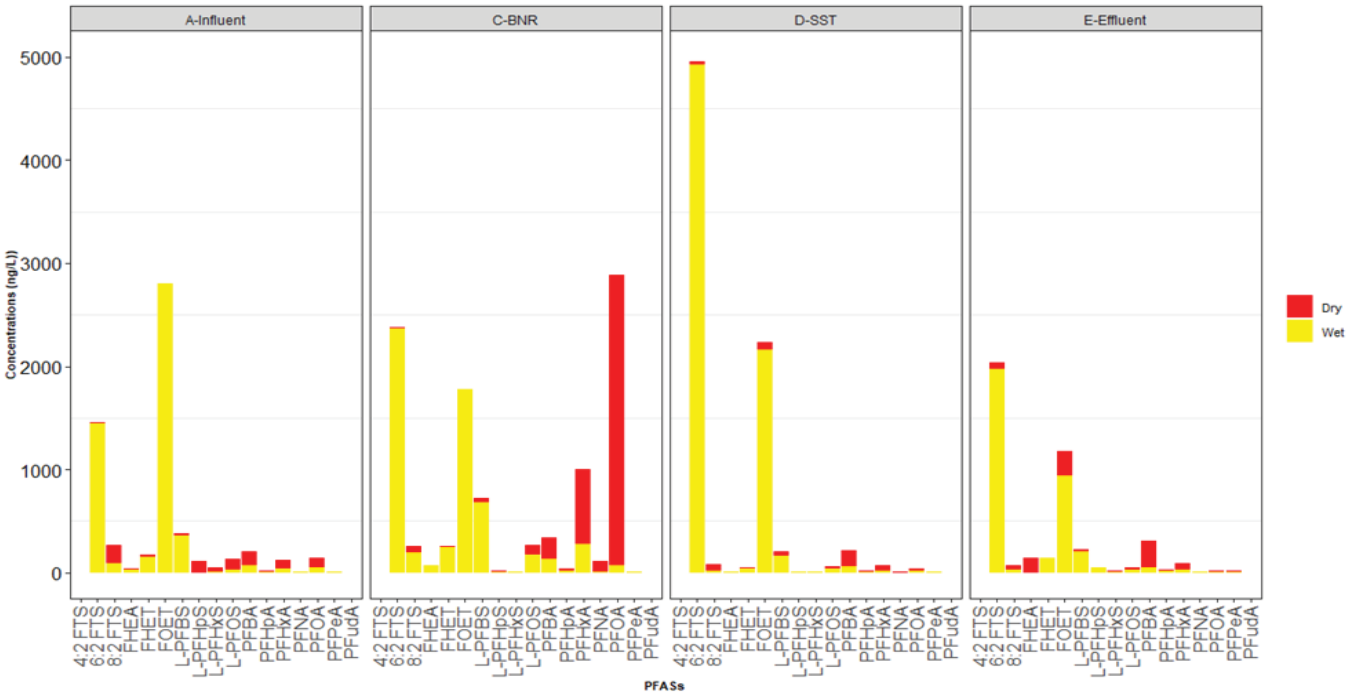

Figure 5.8: PFASs concentrations in wastewater treatment plant sources in the North West province.

5.3 PER- AND POLYFLUOROALKYL SUBSTANCES IN SURFACE WATER

The PFASs concentrations in surface water collected in dry and wet seasons from all the provinces are shown in Figure 5.9.

In Gauteng, PFBA showed the highest concentration (Figure 5.9 C), and more PFASs were observed in wet season.

In KwaZulu-Natal (Figure 5.9 D), the highest concentrations were observed for PFBA followed by FOET and PFHpA. PFBA, PFHpA, 6:2 FTS and FHET were more dominant in wet season; whereas the high concentration exhibited by FOET was in dry season.

In Limpopo (Figure 5.9 E), the order of PFASs detection were as follows: LPFBS>PFBA>PFOA=PHEA. More PFASs were detected in dry season.

In Mpumalanga (Figure 5.9 F), PFBA and PFNA showed the highest concentrations. Wet season contributed more PFASs detected.

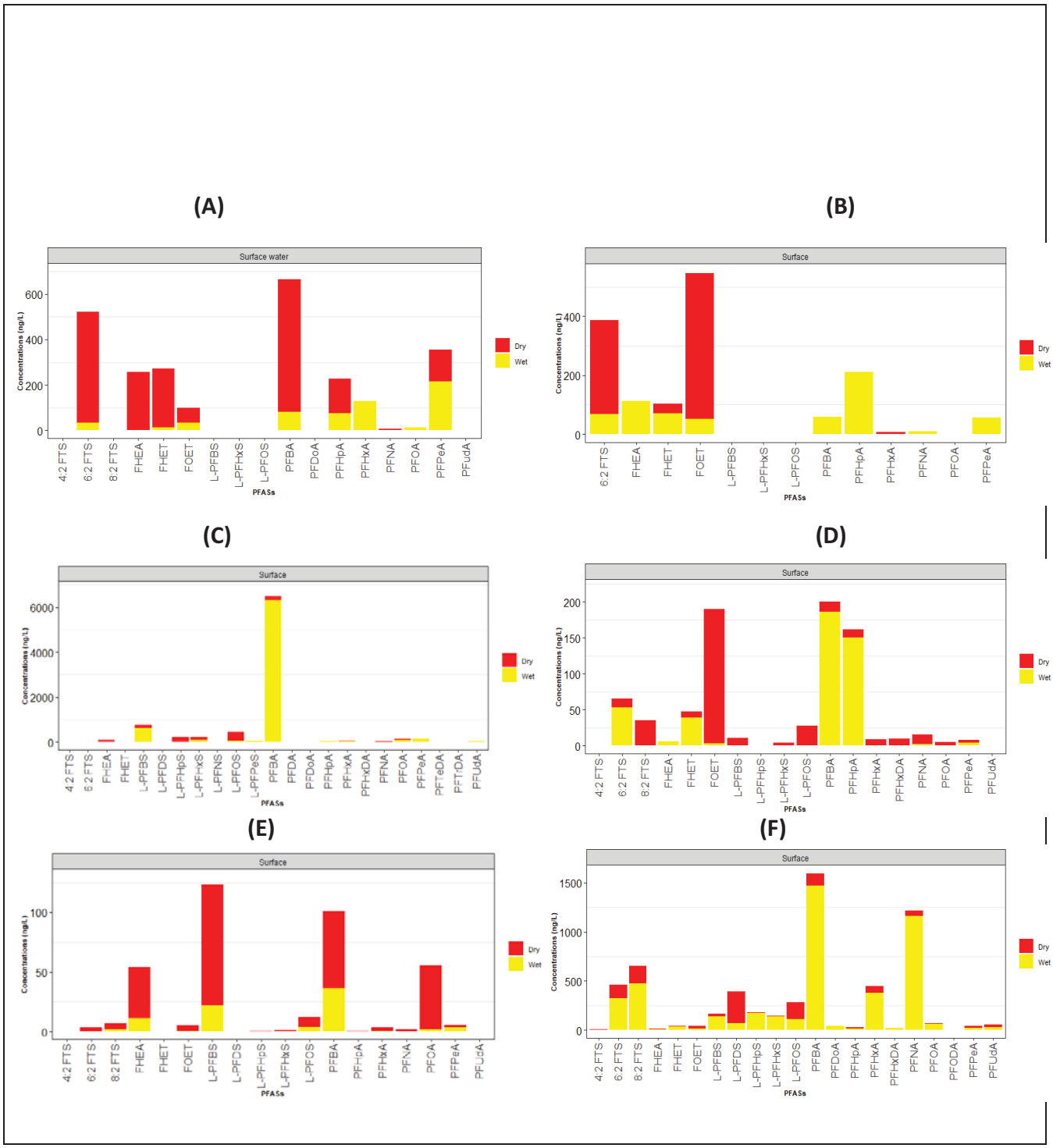

Figure 5.9: PFASs in surface water (rivers and dams) sources (A) Eastern Cape (B) Free State (C) Gauteng (D) KwaZulu-Natal (E) Limpopo and (F) Mpumalanga.

5.4 PER- AND POLYFLUOROALKYL SUBSTANCES IN GROUNDWATER

PFASs concentrations in borehole (groundwater) samples in dry and wet seasons are shown in Figure 5.10.

In Gauteng (Figure 5.10 A), PFBA showed the highest concentration in dry season, followed by LPFOS and PFHxS. PFOA was the most dominant in wet season. More PFASs were detected in dry season compared to wet season.

Figure 5.10 B, shows the concentrations of PFASs detected in borehole water samples from Limpopo. As can be seen in Figure 5.10 B, PFOA followed by PFBS exhibited the highest concentrations. Other PFASs congeners were less than 100 ng/L. Seasonally, more PFASs congeners were detected in dry season.

In Mpumalanga (Figure 5.10 C), 8:2 FTS followed by PFOS and PFNA showed the highest concentrations. More PFASs were detected in wet season compared to dry season.

In North West (Figure 5.10 D), the order of detection was: FOET>8:2 FTS>PFOA>PFBA. Wet season accounted for more PFASs than dry season.

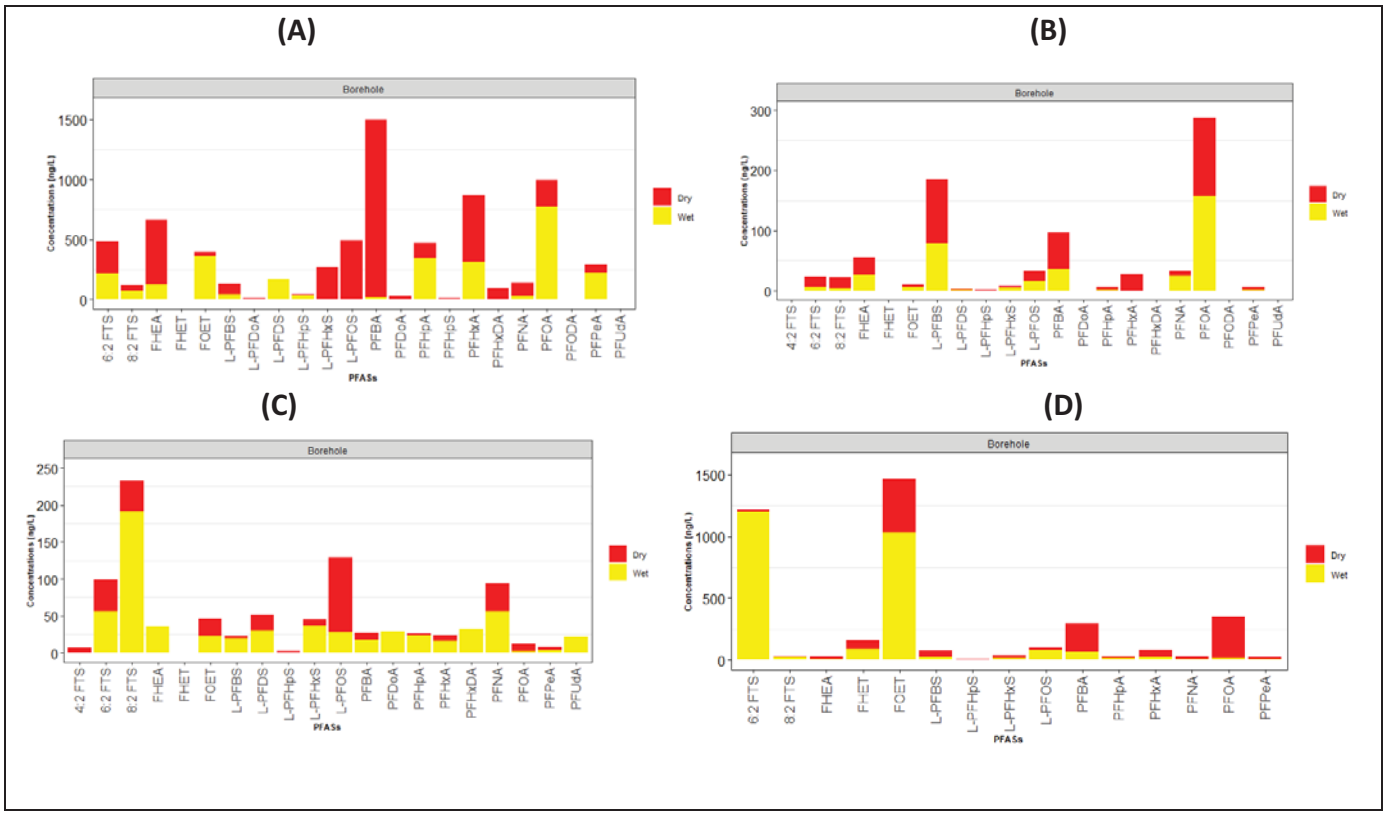

Figure 5.10: PFASs in borehole (groundwater) (A) Gauteng (B) Limpopo (C) Mpumalanga and (D) North West.

5.5 PER- AND POLYFLUOROALKYL SUBSTANCES IN RAINWATER

Table 5.1 shows the mean PFASs concentrations in rainwater collected in February, November and December 2021. The PFASs concentrations ranged from <LOD-38.5 ng/L. PFBA exhibited the highest concentration (173.9 ng/L) in the rainwater sample collected in February 2021, followed by FOET with a concentration of 67.9 ng/L. PFHxA, PFPeA, 8:2 FTS, PFHpA, LPFBS, PFOA, 6:2 FTS, FOET, FHET and PFBA were all detected in the rainwater samples collected in February, November and December 2021. Rainwater sample collected in February exhibited the highest number of PFASs compounds. It was possible that the stench that clouded Gauteng Province for some days in February 2021 may have contributed to the observed high PFASs compounds detected. FOET and PFBA showed high concentrations in all the rainwater samples.

Table 5.1: Mean concentrations (ng/L) of PFASs and standard deviations in rainwater (Feb-Dec 2021)

Compounds	February	November	December
PFUdA	0.333±0.12	<LOD	0.593±0.13
PFHxA	38.5±13.59	3.70±0.69	3.29±1.27
PFPeA	2.65±0.09	1.201±0.17	1.064±0.01
4:2 FTS	0.08 ±0.01	<LOD	<LOD
8:2 FTS	6.25±0.70	0.128±0.62	0.787±1.09
PFHpA	0.881±0.83	0.728±0.11	0.438±0.07
PFNA	2.48±0.62	<LOD	0.832±0.08
L-PFBS	21.9±4.74	0.122±0.05	0.062±0.03
L-PFHxS	0.0957±0.11	<LOD	2.83±2.59
L-PFOS	10.5±0.74	9.20±1.45	<LOD
PFHpS	<LOD	<LOD	<LOD
PFOA	31.2±1.69	1.02±0.46	3.28±1.02
PFDoA	<LOD	<LOD	<LOD
PFODA	<LOD	<LOD	<LOD
L-PFDS	<LOD	<LOD	<LOD
PFHxDA	<LOD	<LOD	<LOD
FHEA	<LOD	<LOD	2.487±1.05
6:2 FTS	1.62±0.02	11.6±0.66	5.62±0.27
FOET	67.9±7.28	37.6±11.66	52.1±6.429
FHET	0.283±0.02	0.107±0.01	0.177±0.03
PFBA	173.9±42.14	17.6±2.01	38.7±11.01

5.6 PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER

Figures 5.11 to 5.16 show the PFASs concentrations in drinking water treatment water samples collected during the dry and wet seasons.

5.6.1 Presence and levels of PFAS in drinking water in the Eastern Cape

Shown in Figure 5.11 are the PFASs congeners detected in river water samples collected from Eastern Cape. PFBA followed by 6:2 FTS exhibited the highest concentrations. More PFASs were observed in dry season than in wet season.

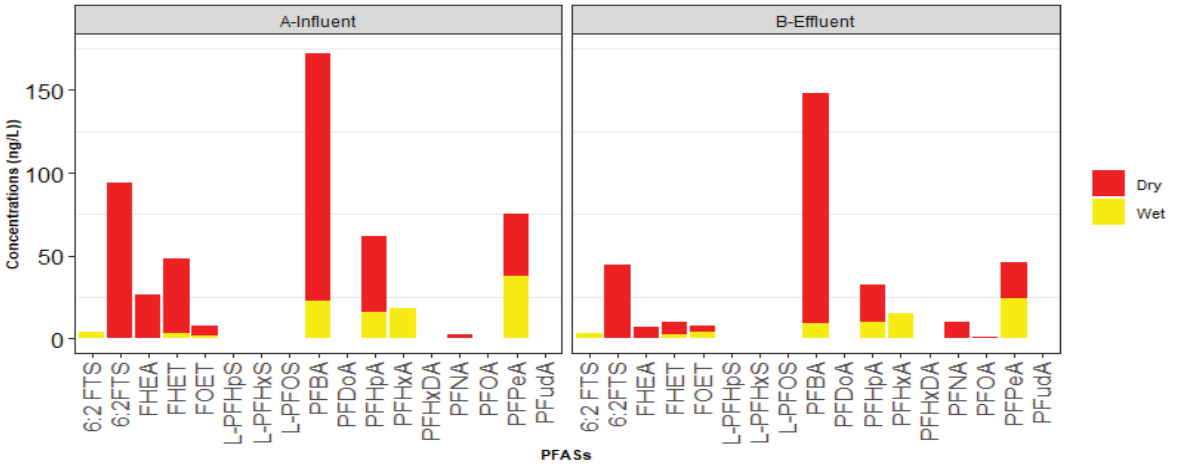

Figure 5.11: PFASs concentration in drinking water samples from the Eastern Cape province.

5.6.2 Presence and levels of PFAS in drinking water in the Free State

In the case of Free State (Figure 5.12), FOET followed by 6:2 FTS showed the highest concentrations. More PFASs were observed in dry season.

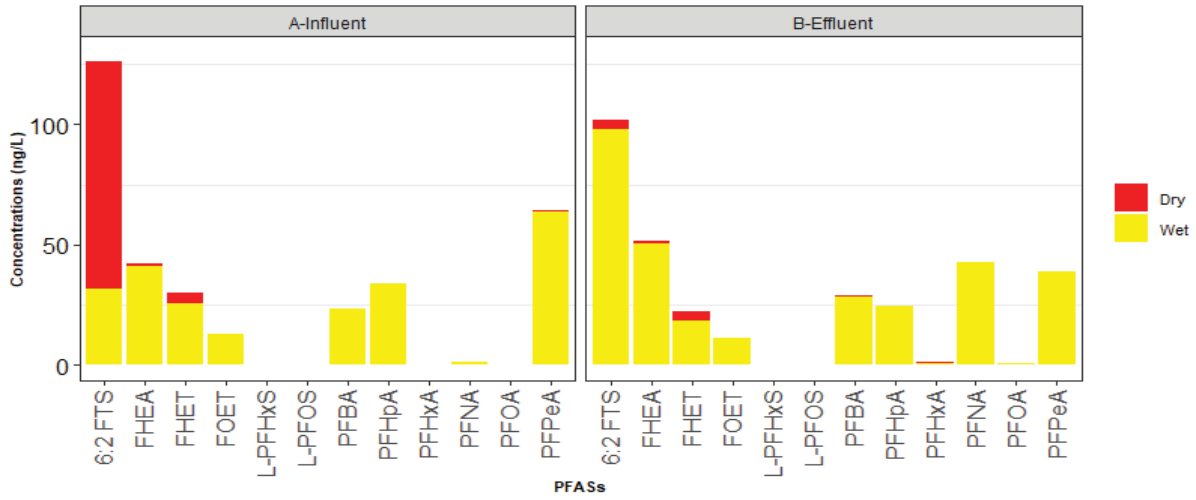

Figure 5.12: PFASs concentration in drinking water samples from the Free State province.

5.6.3 Presence and levels of PFAS in drinking water in Gauteng

Water samples were collected from the influent, filters and effluent from Gauteng drinking water treatment plant (Figure 5.13) only in wet season. The concentrations of PFBS increased from the influent to the filters and decreased greatly in the effluent. Also PFBA decreased in the following order: effluent<filter<influent. PFHxA which was not marginally detected in the influent and filter increased significantly in the effluent. The observed trend indicated the removal/non-removal of PFASs congeners in different treatment stages.

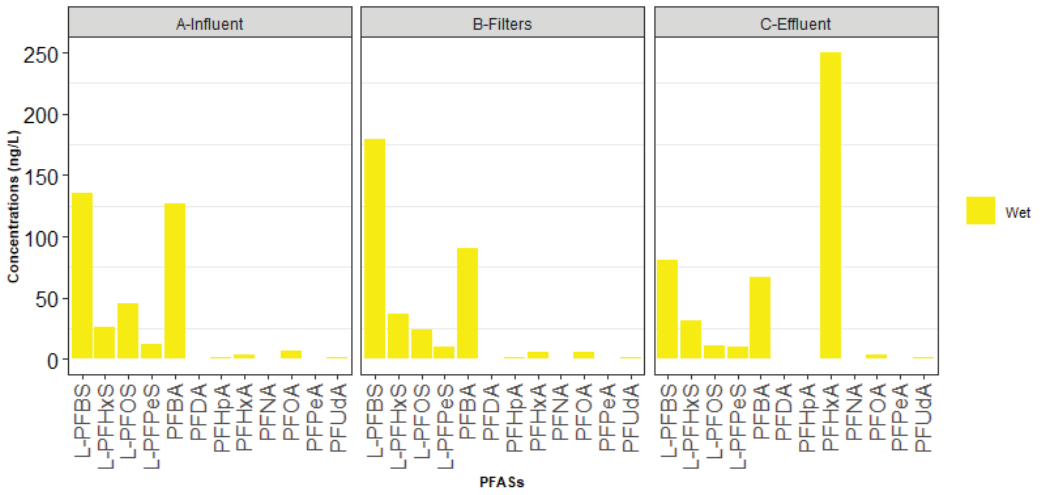

Figure 5.13: PFASs concentration in drinking water samples from the Gauteng province.

5.6.4 Presence and levels of PFAS in drinking water in Mpumalanga

In Mpumalanga (Figure 5.14), 8:2 FTS, FOET, PFBA and PFNA increased in the effluent with a general decrease of other PFASs. More PFASs were observed in wet season than in dry.

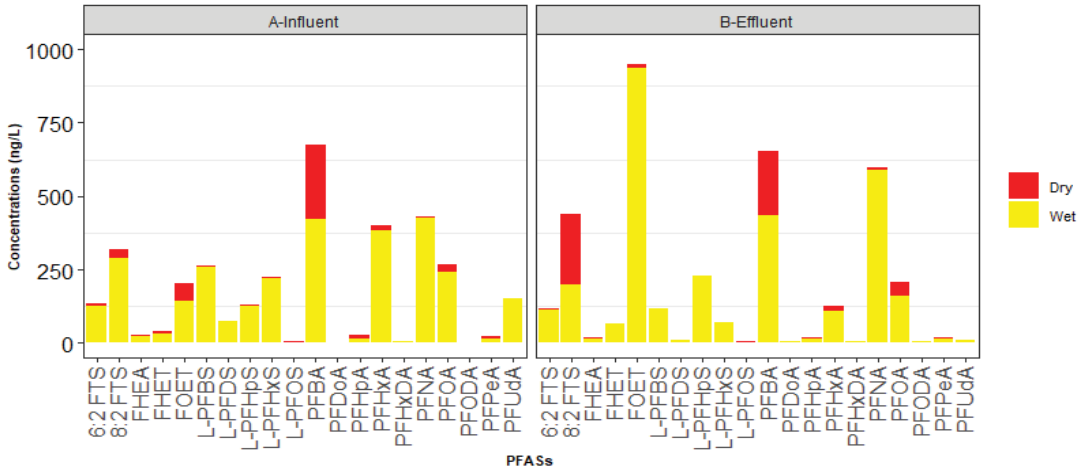

Figure 5.14: PFASs concentration in drinking water samples from Mpumalanga province.

5.6.5 Presence and levels of PFAS in drinking water in the Northern Cape

There was a general increase in PFASs concentrations from the influent to the effluent with the exception of PFOS obtained from Northern Cape (Figure 5.15). Dry season accounted for more PFASs than wet season.

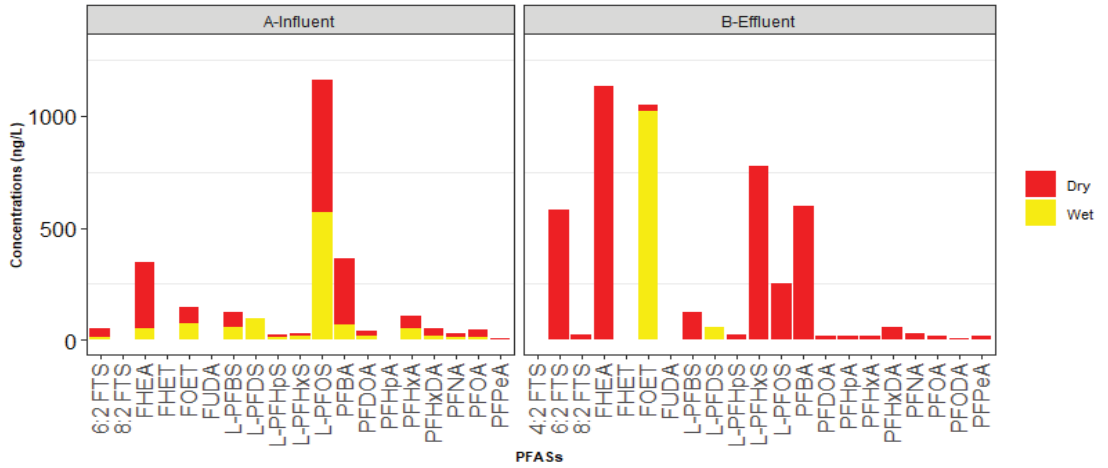

Figure 5.15: PFASs concentration in drinking water samples from the KwaZulu-Natal province.

5.6.6 Presence and levels of PFAS in drinking water in North West

PFBA increased in the following order in drinking water samples collected from North West (Figure 5.16): effluent>filter>influent. However, FOET increased as follows: influent>filters and, thereafter, decreased in the effluent. Once again, more PFASs were observed in dry season than in wet season.

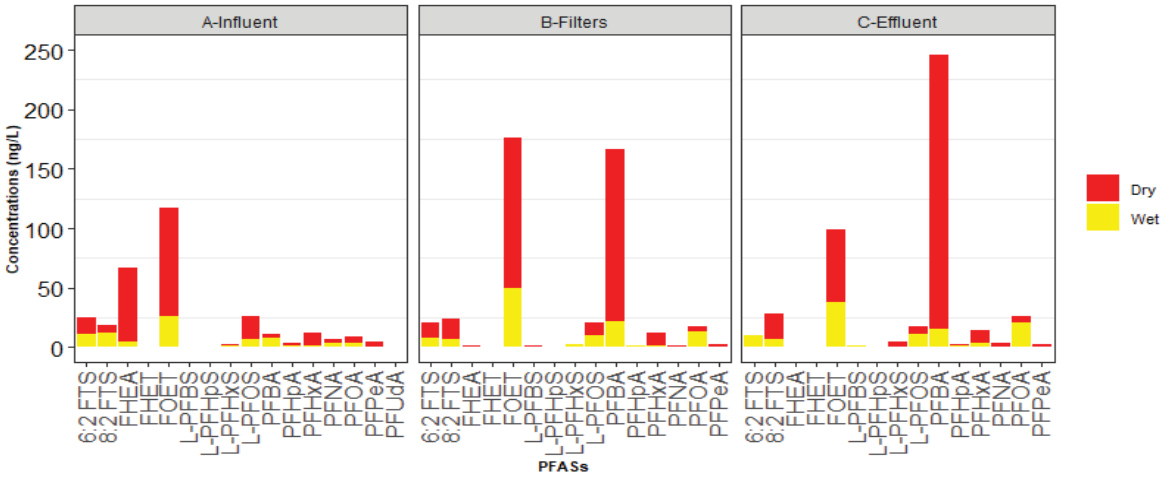

Figure 5.16: PFASs concentration in drinking water samples from the KwaZulu-Natal province.

5.7 PER- AND POLYFLUOROALKYL SUBSTANCES IN TAP AND BOTTLED WATER

Shown in Figure 5.17 are the PFASs concentrations in tap and bottled water. From the results obtained it is clear that the highest concentrations of PFASs were detected in tap water from Eastern Cape (Figure 5.17 A). PFBA was the dominant PFASs followed by PFPeA. Dry season contributed more PFASs than wet season.

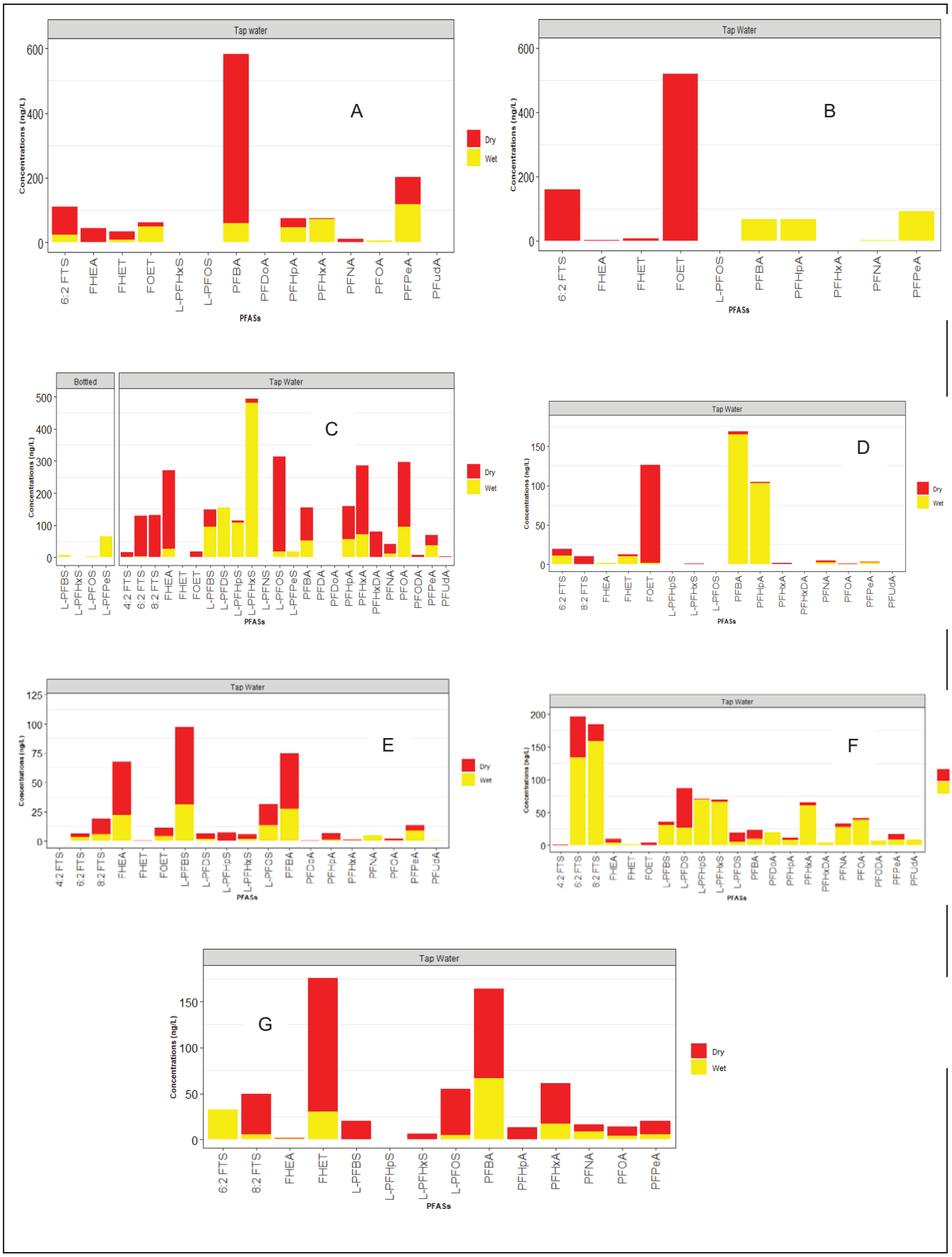

Figure 5.17: PFASs in tap and bottled water (A) Eastern Cape (B) Free State (C) Gauteng (D) KwaZulu-Natal (E) Limpopo (F) Mpumalanga (G) North West.

In Free State (Figure 5.17 B), FOET was the most dominant PFASs followed by 6:2 FTS. More PFASs were observed in dry season compared to wet.

In Gauteng (Figure 5.17 C), the order of detection were as follows: PFHxS>PFOS>PFOA>PFHxA>PHEA. Dry season contributed more PFASs congeners than wet season. Bottled water concentration was <100 ng/L.

Figure 5.17 D, shows the concentrations of PFASs congeners detected in tap water from KwaZulu-Natal. The order of detection were as follows: PFBA>FOET>PFHpA. Wet season contributed more than dry season.

In Limpopo (Figure 5.17 E), PFBS was the most dominant PFASs congeners followed by PFBA, PHEA and PFOS. More PFASs were detected in dry season.

In Mpumalanga (Figure 5.17 F), 8:2 FTS and 6:2 FTS were the most dominant PFASs congeners detected. More PFASs were observed in wet season compared to dry.

Figure 5.17 G, PHEA and PFBA were the dominant PFASs congeners detected in tap water from North West. More PFASs were detected in dry season than in wet.

5.8 PER- AND POLYFLUOROALKYL SUBSTANCES IN THE WESTERN CAPE PROVINCE

Water samples were collected from the following water sources from the Western Cape:

- Tap water (WC-BT)
- Surface water (river water) and
- Wastewater treatment plant (WC-SE effluent)

As can be seen in Table 5.2, PFDoA, PFODA, L-PFDS and PFHxDA were not detected in any of the samples. The overall range of concentrations observed ranged from <LOD-123 ng/L with 8:2 FTS showing the highest concentration of 123.2 ng/L in Stellenbosch WWTP.

Table 5.2: Mean concentrations (ng/L) of PFASs in tap and river water, WWTP effluent

Compounds	Blankenburg River	WC-BT	WC-SE	Diep River
PFUdA	0.270 ±0.06	<LOD	2.82±0.71	0.0269±0.02
PFHxA	24.7±1.89	5.95±1.33	64.9±3.14	35.05±1.04
PFPeA	3.30±0.80	0.528±0.11	1.25±0.09	2.51±0.08
4:2 FTS	<LOD	<LOD	1.29±0.04	<LOD
8:2 FTS	15.61±1.14	0.662±1.03	123.2±1.41	14.7±1.34
PFHpA	<LOD	1.04±0.17	3.72±0.79	14.8±0.58
PFNA	4.23±0.09	<LOD	3.51±0.98	12.8±0.50
L-PFBS	<LOD	<LOD	9.47±1.96	2.43±0.76
L-PFHxS	<LOD	3.21±1.60	27.0±15.15	42.8±8.71
L-PFOS	<LOD	<LOD	63.06±19.26	24.3±0.44
PFHpS	<LOD	<LOD	72.43±10.02	<LOD
PFOA	<LOD	3.77±1.14	93.4±9.14	34.3±0.75
PFDoA	<LOD	<LOD	<LOD	<LOD
PFODA	<LOD	<LOD	<LOD	<LOD
L-PFDS	<LOD	<LOD	<LOD	<LOD
PFHxDA	<LOD	<LOD	<LOD	<LOD

5.9 SUMMARY

In Eastern Cape, the WWTP samples collected in wet season, 6:2 FTS exhibited the highest concentration in the influent samples; followed by PFHpA, PFBA. The other PFASs detected were lower than 25 ng/L. However, in the effluent, there was a general decrease except PFBA and PFPeA which increased. PFBA followed by 6:2 FTS exhibited the highest concentrations. More PFASs were observed in dry season than in wet season. 6:2 FTS exhibited the highest concentration in the influent samples; followed by PFHpA, PFBA. The other PFASs detected were lower than 25 ng/L. However, in the effluent, there was a general decrease except PFBA and PFPeA which increased.

In Free State, FOET was the most dominant PFASs followed by 6:2 FTS. More PFASs were observed in dry season compared to wet. PFASs were detected more in dry season than in wet season. Furthermore, the PFASs detected decreased in wet season.

In Gauteng, the order of detection were as follows: PFHxS>PFOS>PFOA>PFHxA>PHEA. Dry season contributed more PFASs congeners than wet season. Bottled water concentration was <100 ng/L. The concentrations of PFBS increased from the influent to the filters and decreased greatly in the effluent. Also PFBA decreased in the following order: effluent<filter<influent. PFHxA which was not marginally detected in the influent and filter increased significantly in the effluent. The observed trend indicated the removal/non-removal of PFASs congeners in different treatment stages. PFBA exhibited the highest concentration (173.9 ng/L) in the rainwater samples collected in February 2021, followed by FOET with a concentration of 67.9 ng/L. PFHxA, PFPeA, 8:2 FTS, PFHpA, LPFBS, PFOA, 6:2 FTS, FOET, FHET and PFBA were all detected in the rainwater samples collected in February, November and December 2021. In groundwater, PFBA showed the highest concentration in dry season, followed by LPFOS and PFHxS. PFOA was the most dominant in wet season. More PFASs were detected in dry season compared to wet season. PFBA showed the highest concentration, and more PFASs were observed in wet season in surface water. PFBA exhibited the highest concentration in the influent sample with a decrease in the SST with the exception of L-PFBS which increased. The concentration of PFBA remained the same in both SST and effluent; whereas PFBS decreased.

In KwaZulu-Natal, the order of detection were as follows: PFBA>FOET>PFHpA. Wet season contributed more than dry season. The highest concentrations in surface water were observed for PFBA followed by FOET and PFHpA. PFBA, PFHpA, 6:2 FTS and FHET were more dominant in wet season; whereas the high concentration exhibited by FOET was in dry season. PFBA exhibited the highest concentration in the influent followed by PFHpA and 6:2 FTS. 6:2 FTS remained the same in the effluent, albeit PFHpA decreased. Seasonally, the wet season contributed more PFASs than dry season.

In Limpopo, PFBS was the most dominant PFASs congeners followed by PFBA, PHEA and PFOS. More PFASs were detected in dry season. In groundwater, PFOA followed by PFBS exhibited the highest concentrations. Other PFASs congeners were less than 100 ng/L. Seasonally, more PFASs congeners were detected in dry season. The order of PFASs detection in surface water were as follows: LPFBS>PFBA>PFOA=PHEA. More PFASs were detected in dry season. PFOA exhibited the highest concentrations at the different WWTP stages, although a general decrease from the influent to the effluent was observed. Seasonally, the dry season contributed higher PFASs than the wet season.

In Mpumalanga, 8:2 FTS and 6:2 FTS were the most dominant PFASs congeners detected. More PFASs were observed in wet season compared to dry. 8:2 FTS, FOET, PFBA and PFNA increased in the effluent with a general decrease of other PFASs. More PFASs were observed in wet season than in dry. 8:2 FTS followed by PFOS and PFNA showed the highest concentrations. More PFASs were detected in wet season compared to dry season in groundwater. PFBA and PFNA showed the highest concentrations in surface water and, wet season contributed more PFASs detected. The PFASs detected in wastewater samples generally decreased in the effluent. Wet season contributed more PFASs than the dry season.

There was a general increase in PFASs concentrations from the influent to the effluent with the exception of PFOS obtained from Northern Cape. Dry season accounted for more PFASs than wet season. FHEA followed by LPFOS and PFOA showed the highest concentrations in the influent. These decreased in the effluent except L-PFBS which increased. The two seasons showed almost equal contributions.

PHEA and PFBA were the dominant PFASs congeners detected in tap water from North West. More PFASs were detected in dry season than in wet. PFBA increased in the following order in drinking water samples: effluent>filter>influent. However, FOET increased as follows: influent>filters and, thereafter, decreased in the effluent. Once again, more PFASs were observed in dry season than in wet season.

In North West, the order of detection in groundwater was as follows: FOET>8:2 FTS>PFOA>PFBA. Wet season accounted for more PFASs than dry season. The highest concentration was exhibited by FOET followed by 6:2 FTS in the influent. However, 6:2 FTS increased in the BNR and SST and decreased marginally in the effluent; whereas that of FOET decreased. More PFASs were detected in wet season than in dry.

In the Western Cape, PFDoA, PFODA, L-PFDS and PFHxDA were not detected in any of the samples. The overall range of concentrations observed ranged from <LOD-123 ng/L with 8:2 FTS showing the highest concentration of 123.2 ng/L in Stellenbosch WWTP.

CHAPTER 6: MONITORING OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER USING PASSIVE SAMPLING

6.1 INTRODUCTION

Passive sampling is a useful technique for monitoring per- and polyfluoroalkyl substances (PFAS) in various environmental media, including water. It allows for the continuous collection of PFAS over an extended period, providing valuable information on their presence and distribution. Before deployment, the POCIS-HLB used was calibrated in using 21 PFASs mix standards compounds for 14 days (details are in Chapter 3). The calibration plots of POCIS-HLB adsorption of PFASs are shown in Figure 6.1. As can be seen, the uptake of PFASs are linear.

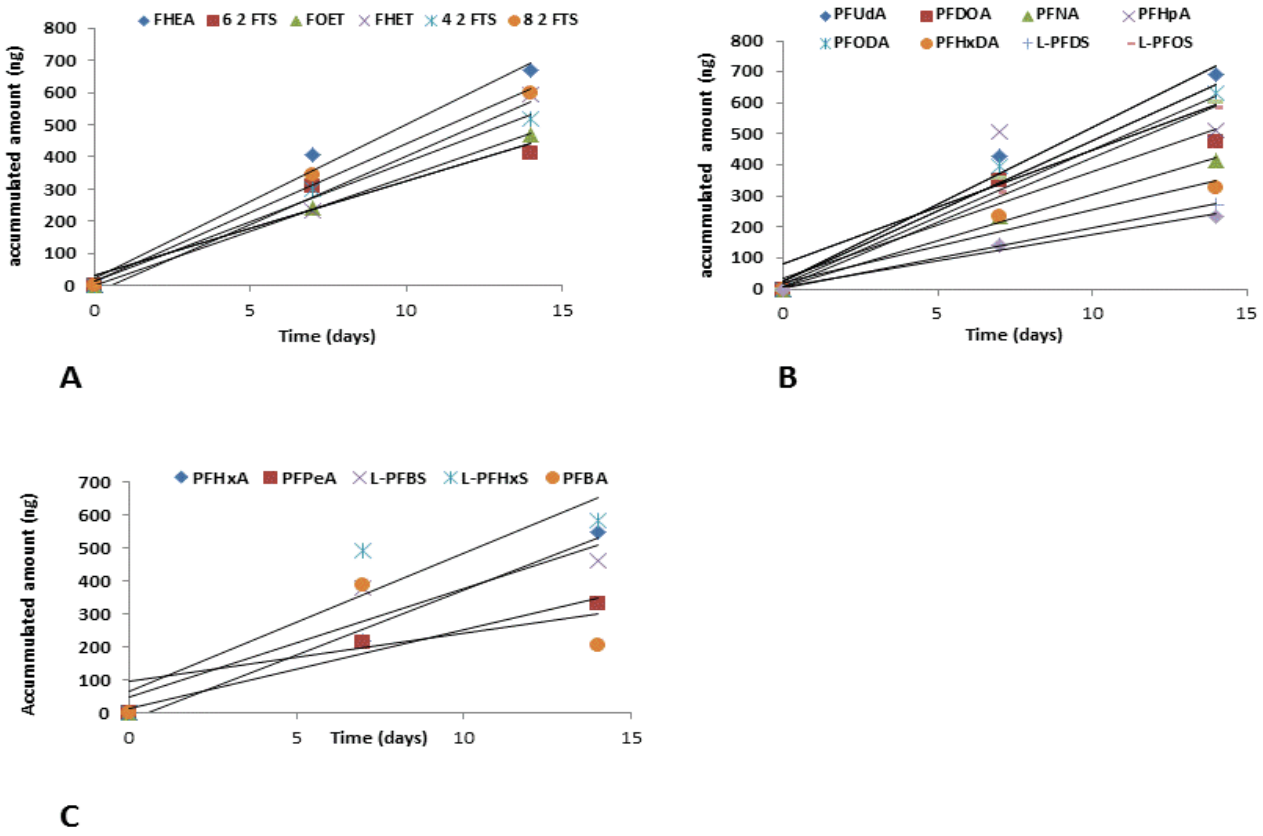

Figure 6.1: Uptake profile of individual PFASs for POCIS-HLB samplers over 14-day period (A) Fluorotelomers, (B) Long chain and (C) short chain PFASs

From the linear plots, the sampling rates were determined as reported (Arditsoglou and Voutsa, 2008; Morin *et al.*, 2012; Weiss *et al.*, 2015). The sampling rates were calculated using the following equation:

$$C_w = C_s M_s / R_s t \quad \text{(Equation 6.1)}$$

where C_w and C_s are the concentrations of PFASs the water (ng/L) and in the POCIS (ng/g) respectively, M_s is the mass of the sorbent in the POCIS (g), Rs is the sampling rate (L/day) and t is the sampling period (days) .

The determined sampling rates were in the following range 0.0029-0.099 L/day as can be seen in Table 6.1.

Table 6.1: Sampling rates for the calibration of POCIS-HLB samplers

Compound	*R_S (L days^{-1})	*R^2
FHEA	0.099±0,023	0.9842
6 2 FTS	0.010±0,002	0.9239
FOET	0.044±0,184	0.9997
FHET	0.054±0,046	0.9843
PFUdA	0.012±0,901	0.9817
PFDOA	0.052±0,006	0,9299
PFHxA	0.020±0,036	0.985
PFNA	0.076±1,10	0.9941
PFPeA	0.084±0,045	0.2824
4 2 FTS	0.0523±0,066	0.9923
8 2 FTS	0.041±0,033	0.9921
PFHpA	0.077±0,043	0.7576
PFODA	0.061±0,061	0.9806
PFHxDA	0.050±0,056	0.941
L-PFBS	0.036±0,043	0.8759
L-PFDS	0.0029±0,023	0.9995
L-PFHxS	0.031±0,01	0.8654
L-PFOS	0.0081±0,14	0.9984
L-PFHpS	0.018±0,632	0.9886
PFOA	0.087±0,001	0.9892
PFBA	0.004±0,14	0.9755

*R_S= sampling rate, R^2 = regression

6.2 MEAN CONCENTRATIONS OF PER- AND POLYFLUOROALKYL SUBSTANCES

Shown in Table 6.2 are the mean concentrations of PFASs detected in GP-W, a domestic wastewater treatment plant in Pretoria. The passive samplers were deployed at the effluent of the GP-W for 14 days. Thereafter, the samplers were retrieved and grab samples collected at the same intervals on day 7 and 14. The mean concentrations of PFASs recorded on 7 day were in the range of 0.51 to 81.67 ng/L. All PFASs targeted were detected except 6:2 FTS, PFDOA, 8:2 FTS, and PFHxDA. 4-2 FTS had the highest concentration of 81.67 ng/L. The same trend was also observed in grab samples, although FOET exhibited the highest concentration of 22.36 ng/L in this case.

Furthermore, FHET and PFHpA were not detected in the grab samples. Generally, on day 7, the PFASs concentrations recorded for POCIS higher than the grab samples except for FOET. On 14 day, the mean PFASs concentrations for POCIS-HLB ranged 0.94-98.86 ng/L. PFNA had the highest concentration of 94.04 ng/L. On the other hand grab sample had mean concentration range of LOD-30.55 ng/L. PFHxA had the highest concentration of 30.55 ng/L. Once again, 6:2 FTS, 8:2 FTS, PFODA and PFHxDA were not in POCIS samples. This trend was seen in grab samples in addition to PFDOA.

It can be seen from Table 6.2, that the PFASs concentrations in POCIS were significantly higher than that of grab samples. The difference between the concentrations recorded for the two sampling method was because grab samples provided only snap shot concentrations, while POCIS-HLP provided time weighted average concentrations (Godlewska, Stepnowski and Paszkiewicz, 2021). The PFASs concentrations detected in the current study are higher than the concentrations reported by Gobelius *et al.* (2019) with total sum of all PFASs of 7.1 ng/L in drinking water treatment samples. The higher PFASs concentrations observed in POCIS-HLB indicated the ability of the sampler to adsorb more PFASs compounds compared to grab samples.

Table 6.2: Mean concentrations of PFASs in POCIS-HLB and grab samples.

PFASs (ng/L)	Day 7		Day 14	
	POCIS HLB sampler	Grab sample	POCIS HLB sampler	Grab sample
FHEA	32.61±0.123	1.61±1.02	38.14±0.09	0.63±0.075
6 2 FTS	ND	ND	ND	ND
FOET	8.67±0.11	22.36±0.03	75.10±0.05	7.50±0.25
FHET	57.98±0.15	ND	35.17±0.125	14.93±0.36
PFUdA	25.60±0.002	3.00±0.05	73.29±0.96	0,18±0.46
PFDOA	ND	ND	0.94±0.05	ND
PFHxA	14.23±0.01	21.13±0.521	55.96±0.09	30.55±0.65
PFNA	38.84±0.23	0.34±0.56	94.04±0.36	2.96±0.09
PFPeA	13.04±0.3650	5.62±048	20.58±0.06	6.31±0.02
4 2 FTS	81.67±0.15	0.31±0.03	9.86±0.09	5.16±0.05
8 2 FTS	ND	ND	ND	ND
PFHpA	0.51±0.655	LOD	3.07±1.5	LOD
PFODA	ND±0.35	ND	ND	ND
PFHxDA	ND	ND	ND	ND
L-PFBS	29.77±0.100	10.56±0.05	47.56±0.02	0.99±0.08
L-PFDS	36.16±1.05	0.56±0.125	54.42±0.06	4.20±0.156
L-PFHxS	48.52±0.80	2.91±0.89	64.55±0.05	0.51±0.03
L-PFOS	7.58±0.92	0.80±02.712	23.18±0.65	15.08±0.06
L-PFHpS	36.42±0.15	0.03±0.14	46.53±0.985	0.08±0.089
PFOA	12.79±0.02	4.64±0.125	33.73±0.062	0.83±1.01
PFBA	7.1±0.05	0.68±0.05	15.84±0.03	5.8±0.46

6.3 SUMMARY

All PFASs targeted were detected except 6:2 FTS, PFDOA, 8:2 FTS, and PFHxDA. 4-2 FTS had the highest concentration of 81.67 ng/L. The same trend was also observed in grab samples, although FOET exhibited the highest concentration of 22.36 ng/L FHET and PFHpA were not detected in the grab samples. Generally, on day 7, the PFASs concentrations recorded for POCIS were higher than the grab samples except for FOET. On 14 day, the mean PFASs concentrations for POCIS-HLB ranged 0.94-98.86 ng/L. PFNA had the highest concentration of 94.04 ng/L. On the other hand, grab sample had mean concentration range of LOD-30.55 ng/L. PFHxA had the highest concentration of 30.55 ng/L. Once again, 6:2 FTS, 8:2 FTS, PFODA and PFHxDA were not in POCIS samples. This trend was seen in grab samples in addition to PFDOA. The difference between the concentrations recorded for the two sampling method was because grab samples provided only snap shot concentrations, while POCIS-HLP provided time weighted average concentrations. The higher PFASs concentrations observed in POCIS-HLB indicated the ability of the sampler to adsorb more PFASs compounds compared to grab samples.

CHAPTER 7: ESTABLISHING THE POSSIBLE SOURCES OF THE DETECTED PER- AND POLYFLUOROALKYL SUBSTANCES

7.1 INTRODUCTION

To address Aim 2 of this project, a multivariate statistical analysis was used to establish inter-relationships between different groups of PFASs, and sample sites and to establish possible sources of PFAS. Principal Component Analysis (PCA) was used to identify patterns, potential sources of variation and relationships within the obtained datasets of PFASs concentrations in the different sampling sites. Data interpretation was done in conjunction with knowledge on the land uses within the catchment area of the sampling sites.

7.2 PER- AND POLYFLUOROALKYL SUBSTANCES CONGENER CONTRIBUTIONS AND THEIR RELATIONSHIPS IN SOURCE APPORTIONMENT

In Eastern Cape, positive correlation was observed for 6:2 FTS, PFHxS, and FOET suggesting similar sources (Figure 7.1 A (I). FHET was negatively associated, suggesting different sources. Since some fluorotelomers are known to be a source of PFCAs, the high detection of the fluorotelomers may explain the prevalence of PFBA detected in all the water sources. The high PFBA could be attributed to some anthropogenic activities, as the site is very close to a beach. In addition, at the time of the sampling, there was many plastic fragments observed inside the water which might also be a contributor in addition to the degradation of Fluorotelomer. Congeners PFNA, PFHpA, PFHxA, and PFPeA were positively associated with each other (Figure 7.1 A (II). Although PFOA is on the same score plot with the aforementioned PFASs, however, it is not close to others. This behaviour suggests different pattern/source. The same can be said for 6:2 FTS and FOET. On the other hand, FHEA, PFBA and FHET were negatively associated with each.

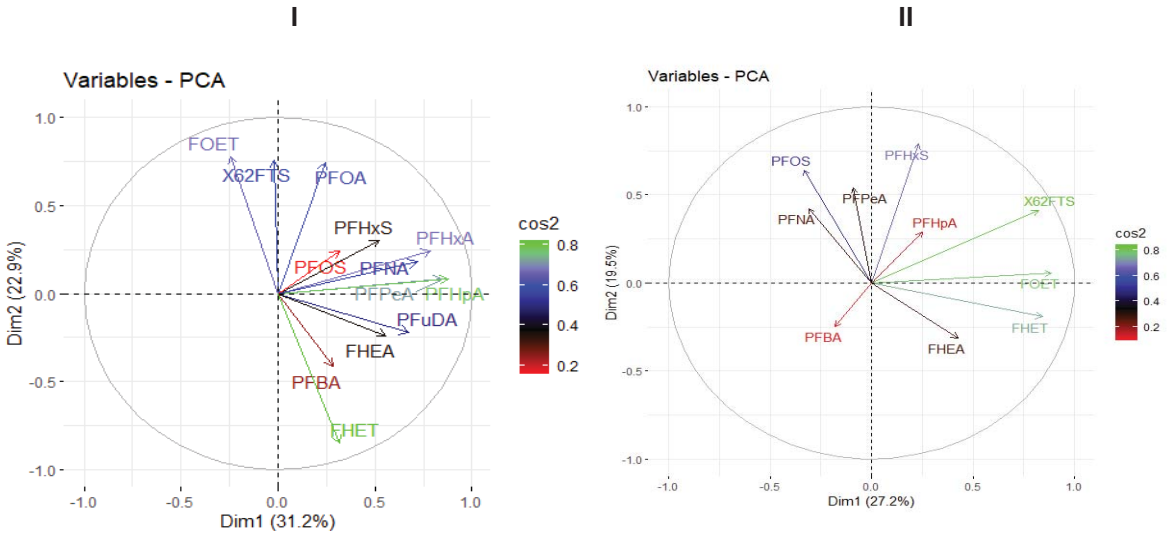

Figure 7.1: PFASs congener contributions and their relationships in source apportionment in the Eastern Cape province (I = dry and II = wet season).

For Free State, it can be seen in Figure 7.2 (I), the following PFASs were correlated: PFHpA, LPFOS, PFBA, 6:2 FTS, PFHxS, PFOA, PFPeA and FHEA (first quadrant – clockwise) in dry season; suggesting possible similar sources; whereas PFHxA and FOET were outlayers. Significant industrial sectors in Bloemfontein include retail & trade, manufacturing and transport. For example, PFBS is used as a surfactant in a variety of applications such as pesticides, textile and others. As can be seen in Figure 7.2 (I), the following PFASs are correlated: PFBA and PFPeA (group 1), PFHpA and FHEA; FOET and FHET (group 2) suggesting possible similar sources; whereas PFNA and 6-2 FTS are outliers, suggesting different sources. Once again, the detection of PFASs in the water samples from the sampling sites may have originated from the use of PFASs-containing products from any of the aforementioned sources. For example, fluorotelomers are used in firefighting foams, grease resistant food packaging, anti-fogging sprays, textile and others. In wet season, Figure 7.2 (II), the following PFASs are correlated: PFBA and PFPeA; PFHpA and FHEA; FOET and FHET suggesting possible similar sources; whereas PFNA and 6-2 FTS are outliers, suggesting different sources.

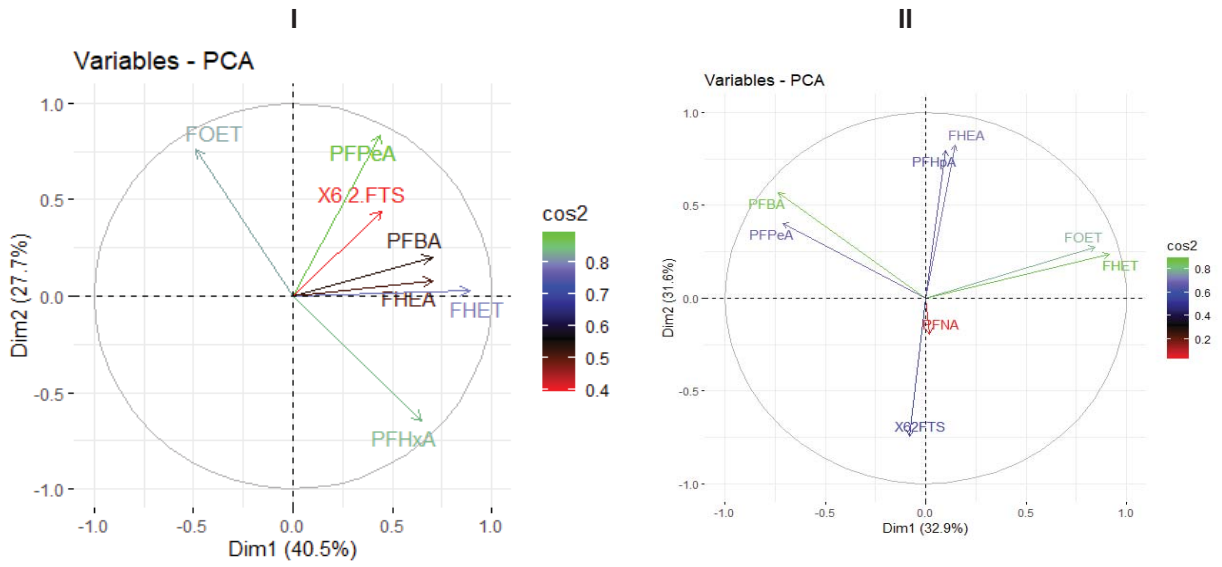

Figure 7.2: PFASs congener contributions and their relationships in source apportionment in the Free State province (I = dry and II = wet season).

Figure 6.1 C (V) shows PFASs congener contributions and their relationships. The first quadrant (clockwise) shows a mix of fluorotelomers, PFCAs and PFSAs. The same also applies to the second quadrant. However, the third and fourth quadrants are dominated by the sulfonates. PFASs in the same quadrants would suggest similar sources. PFASs. Shown in Figure 6.1 C (VI) are the contributions of PFASs compounds detected in the water samples during the wet season. The results show detectable concentrations especially for the short chain PFASs. Lower to none detectable levels were detected for the long chain PFAS, suggesting that they were less produced and consumed. Another reason could be due to the low water solubility of the long chain. Compounds LPFPeS, PFDA, FHEA, PFPeA, PFNA and PFOA, all show positive strong contributions in the first quadrant (clockwise). The same applied to FOET, PFDoA, PFTrDA and 8:2 FTS in the second score plot, albeit in the negative quadrant. PFBA is in its own in the third score plot; whereas the sulphonates are dominant in the fourth score plot. The clustering of PFASs in different may suggest different sources for different groups in different score plots.

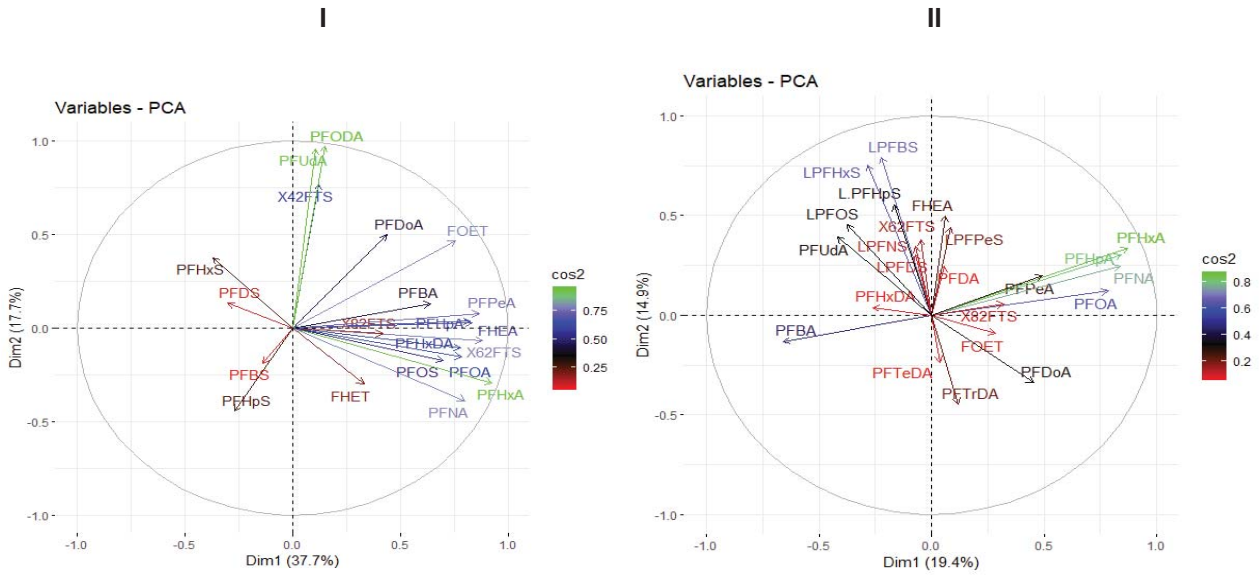

Figure 7.3: PFASs congener contributions and their relationships in source apportionment in the Gauteng province (I = dry and II = wet season).

In KwaZulu-Natal Province water samples (Figure 7.4 (I), PFOA, PFOS, PFDoA, PFNA, PFBS and PFHxA all show positive strong contributions in dry season. The observed pattern suggests similar sources. PFBA, PFHxS, 8:2 FTS, PFHpS, PFPeA and PFUdA also showed similar behaviour in the second quadrant. 6:2 FTS, FOET and FHET are in the third and fourth quadrants respectively. In wet season, (Figure 7.4 (II), L-PFHpS, L-PFHxS, L-PFBS, 6:2 FTS and FHET showed a positive strong contributions. PFHpA, PFBA and PFPeA also showed similar behaviour. PFHpA and PFBA had the highest concentrations detected in most of the sampling sites. The high detection of perfluoroalkyl carboxylic acids (PFCAs) detected in these samples might be attributed to the degradation of fluorotelomers.

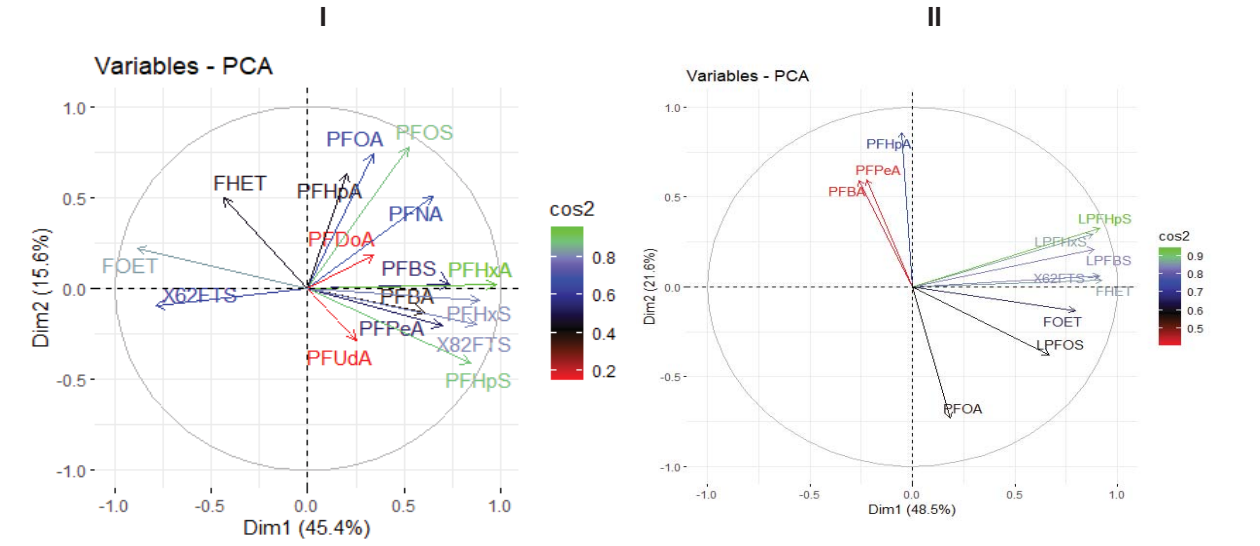

Figure 7.4: PFASs congener contributions and their relationships in source apportionment in the KwaZulu-Natal province (I = dry and II = wet season).

Shown in Figure 7.5 (I), there is a strong correlation between 8:2 FTS, 6:2 FTS, L-PFOS, L-PHET, L-PFHxS, PFHpS and L-PFDS, all found in the same quadrant, suggesting that these compounds have similar pattern/ sources. Same applies for PFBA, PFNA, PFHPA, L-PFBS, FHEA, FOET and PFOA. Figure 7.5 (II) are the contributions of PFASs compounds detected in the water samples during the wet season. Similarly, congeners in the same score plot, probably receive PFASs from similar sources.

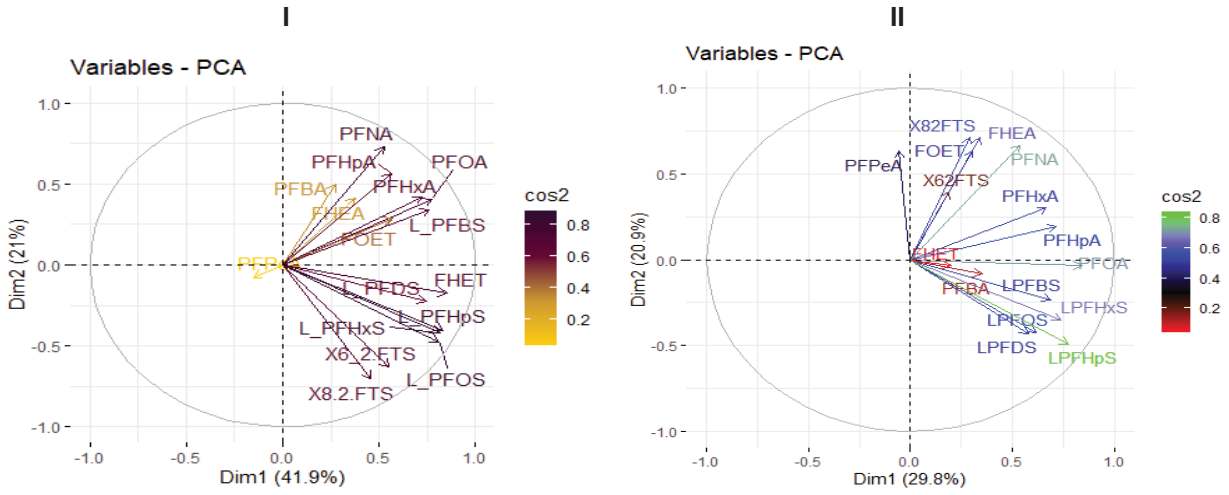

Figure 7.5: PFASs congener contributions and their relationships in source apportionment in the Limpopo province (I = dry and II = wet season).

In Mpumalanga, PFPeA, FHET, PFBS, FHEA, 8:2 FTS and PFHpS are all located on the top right of the score plot close to each; whereas the long chain PFASs, PFHxS, PFNA, and PFOS are located on the bottom right of the score plot close to each other (Figure 7.6 (I). This observation suggests similar pattern/sources. The arrows in this diagram show that PFOS, PFHxS, 8:2 FTS, PFNA and PFPeA are the variables that explain the separation among the samples. Figure 7.6 (II), all the PFASs congeners in the same quadrants are correlated to one another and, therefore, share similar sources. PFOS is in the 4th quadrant on its own suggesting difference sources from the other PFASs congeners.

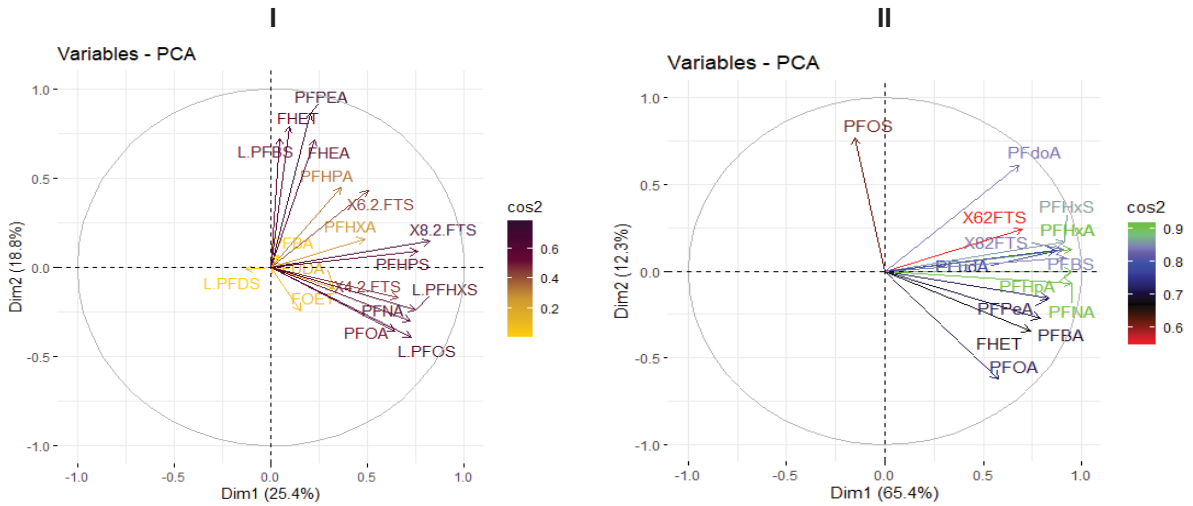

Figure 7.6: PFASs congener contributions and their relationships in source apportionment in the Mpumalanga province (I = dry and II = wet season).

Shown in Figure 7.7 (I), is the principal component analysis of contributions and their relationship in Northern Cape in dry season. FHEA, PFHXDA, PFODA, PFHPA, PFBA, 8:2 FTS, PFOA, 6:2 FTS, PFNA, FHET AND PFPeA are all clustered in the first quadrant, suggesting positive correlation and hence similar source. About 60% of the PFASs compounds in the first quadrant were detected in all the samples. In wet season, analysis revealed that L-PFBS, PFHxA, PFBA, and L-PFOS in the first quadrant (clockwise) had a shared occurrence source (Figure 7.7 (II). These compounds were found to cluster heavily with short-chained compounds, which may suggest a preference for shorter-chained compounds over longer ones. Another group comprising 6:2 FTS, PFHpA, PFOA, PFPeA, and PFHxS also showed a similar origin. The dominance of PFCA in this group is attributed to its frequent use in carpentry, surfactants, and firefighting foams.

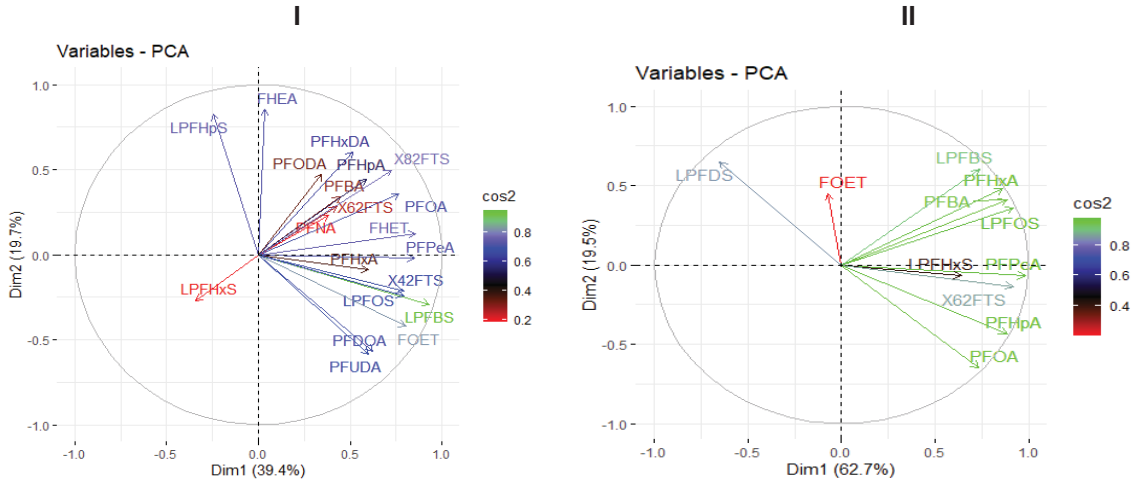

Figure 7.7: PFASs congener contributions and their relationships in source apportionment in the Northern Cape province (I = dry and II = wet season).

In North West, Figures 7.8 (I), PFBS, PFHxA, PFOS, PFHpS, PFBA and 8:2 FTS were strong and positively associated. These high loadings in quadrant 1 (clockwise) are associated with medical devices such as endoscopes and woven and non-woven surgical drapes and gowns, radio-opaque ethylene tetraethylene copolymer uses, metal plating, paints, waxes, inks and industrial coatings, metal plating, polyvinylidene fluorides, coatings, carpets, couches and food packaging. Their association, therefore, suggested the same source. Quadrant 2 is populated with PFHpA, PFOA, FHET and PFNA. The content of the quadrant is mainly used in textile, coatings, fluorinated surfactants fields and food packaging industries. PFPeA is the only occupant of quadrant 3, suggesting different sources from the other PFASs. Quadrant 4, was characterised by PFHpS, PFHxS, 6:2 FTS and FOET. These compounds are used in stain-resistant fabrics, coatings, firefighting foams, fabrics and food packaging.

As shown in Figure 7.8 (II), quadrants 1 and 2 explained show high loadings of PFPeA, PFBA, 8:2 FTS, PFOA, PFHxA, PFBS and PFHpA and FHEA, 6:2 FTS, FOET, FHET, PFHxS, PFOS, and PFNA respectively. A component with high loadings of so much of PFASs could suggest a mixture of pollutants. This can be justified by the precipitation and storm-water run-off during the wet season, resulting in a mixture of sources.

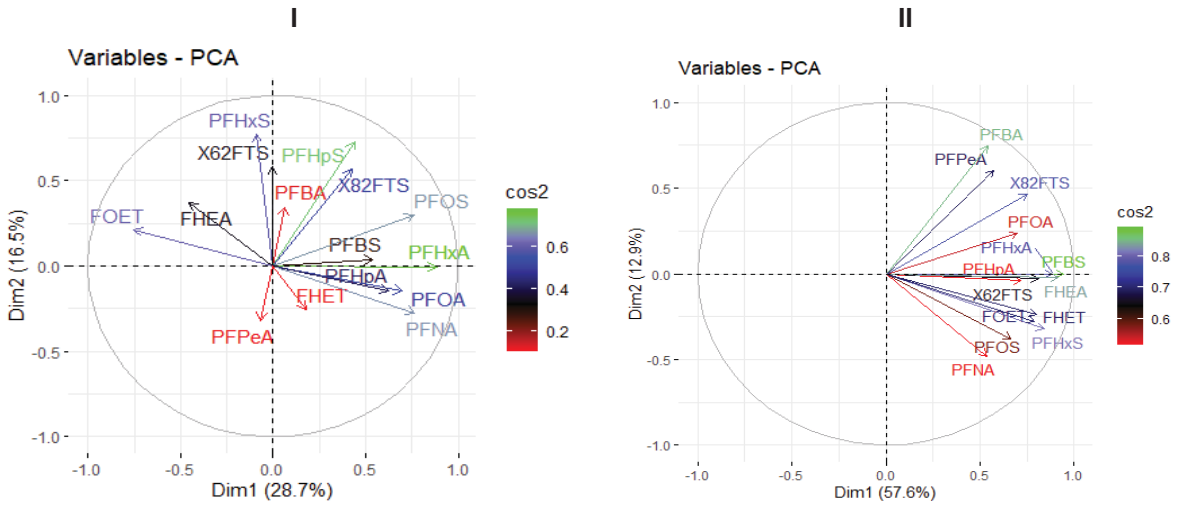

Figure 7.8: PFASs congener contributions and their relationships in source apportionment in the North West province (I = dry and II = wet season).

7.3 SAMPLING SITES AND THEIR RELATIONSHIPS IN SOURCE APPORTIONMENT

In Eastern Cape, (Figure 7.9 (II)), more sampling sites for drinking water treatment plants and tap water are clustered on the left side of the quadrant, which suggests a similar pattern. Whereas surface water sites are more scattered on the right, including EC-WI1 from a WWTP. Therefore, suggesting a different pattern from the others. Figure 7.1 A (II), shows the observation of the sampling sites in wet season. As can be seen in Figure 7.9 (II), with the exceptions of AP and MW on one hand and LD1 and RW on the other, all the other sampling sites are scattered, particularly EC-D1. This behaviour suggested different pattern from others. These were all in line with high PFASs concentrations observed at these sampling sites. Considering the fact that the predominant PFASs detected in the water samples were the PFAAs and these have a wide industrial applications, it is possible that the various industrial and agricultural activities around the sampling areas may have used or still using PFASs in their activities.

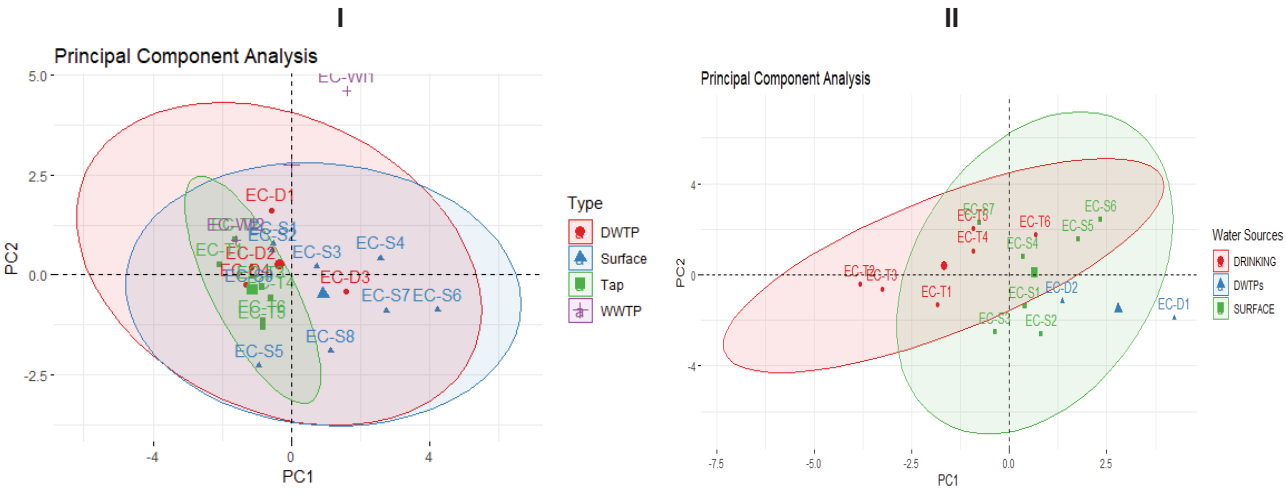

Figure 7.9: Sampling sites and their relationships in the Eastern Cape province (I = dry and II = wet season).

Figure 7.10 (I) shows BF-D1/ BF-S1, BF-T1/BF-T2/BF-DE, BF-S2/BF-WE, and BF-W1 formed a single cluster in sampling sites and their relationship in dry season. This clustering suggested that the site had similar sources of contamination and it was noted that the sites had similar concentrations of PFASs, and may be receiving PFASs from the same source, storm water, domestic wastewater and others. Figure 7.10 (I), shows the formation of BF-D1/ BF-DE, BF-S1/BF-S2, BF-T1/BF-T2 and BF-WI/BF-WE, in four separate clusters. This clustering suggests that the sites had similar sources of contamination and it was also noted that the sites had more or less the same concentrations of PFASs, which may be receiving PFASs from the same sources, storm water, domestic wastewater and others.

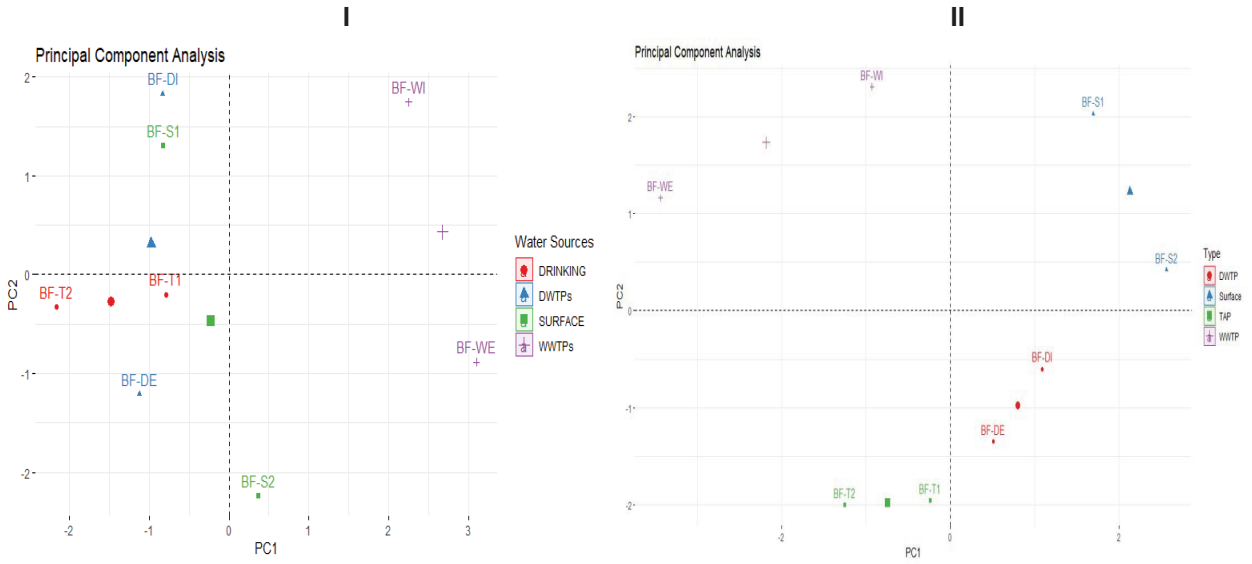

Figure 7.10: Sampling sites and their relationships in the Free State province (I = dry and II = wet season).

With respect to samples and their relationships in Gauteng in dry season, a PCA plot was constructed and it is shown in Figure 7.11 (I). The surface samples are clustered and well isolated from the other samples. Some of the borehole samples are also clustered, whereas the tap water are well separated. The clustering may suggest share of the same sources of PFASs contamination. On the other hand, non-clustering would suggest different sources. Figure 7.1I (II) shows the PCA of the sampling sites in wet season. As can be seen in Figure 7.11 (II), GP-T1, GP-BL2 and GP-BT1 are clearly the outlayers compared to the other sites. The surface samples are clustered. This suggested that the sources of PFASs may be different for these sites. These PFASs may have originated from any of the following: 1) Chemical industry (application of fluorochemicals in production of materials); 2) Wastewater treatment plant (PFASs-containing domestic cookware and food raps that are flushed into the sewerage system thereby ending in wastewater treatment plants); 3) Landfill sites (dumping of PFASs-containing wastes); 4) Waste dump sites (PFASs-containing wastes that are dumped indiscriminately that can be washed into water bodies); 5) Use of fire-fighting foams that may contain PFASs – airport, fire-fighting stations and others); 6) Storm water and 7) mining sources.

Shown in Figure 7.12 (I) is the PCA plot of sampling sites and their relationships in KwaZulu-Natal in dry season. The water sources are all clustered indicating similar sources of PFAS. The concentrations detected in these sites may also be due to the discharge from WWTPs into river samples, since a high concentration was also detected at point KZN-S8. Although the wastewater sites are not clustered as observed for tap and river water, they occupy the second quadrant suggesting similar sources. The high concentrations detected at KZN-S4 may be due to the agricultural activities as there are sugar cane field around the site and the concentration at KZN-S8 may be due to the discharge of WWTPs into the Umngeni River as mentioned during dry season. Figure 7.12 (II) shows the observation of the sampling sites wet season. Samples from surface water and tap water

were clustered together, suggesting a similar sources of PFASs contamination excluding KZN-S6. Wastewater treatment plant samples also showed similar sources for the PFAS concentrations detected, except for KZN-WE2.

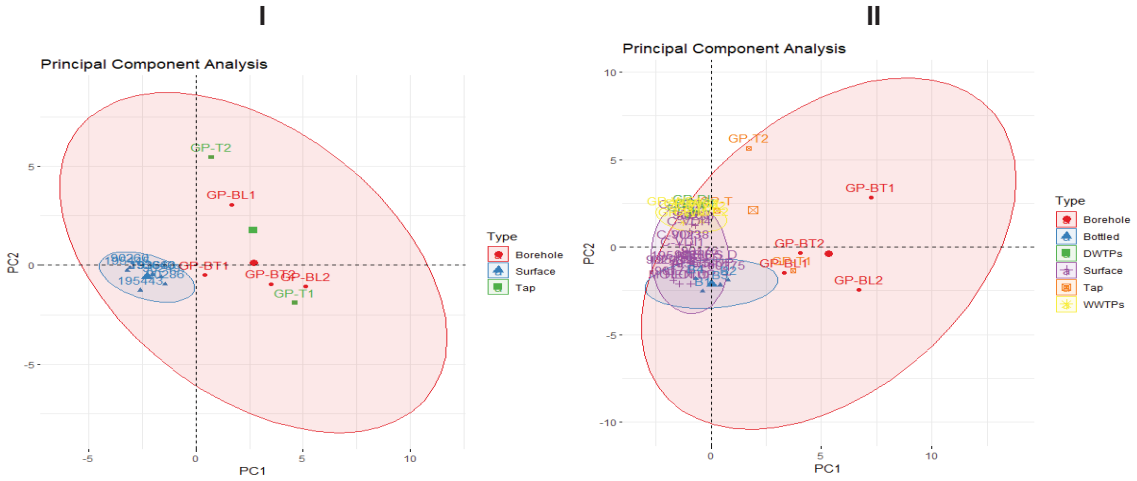

Figure 7.11: Sampling sites and their relationships in the Gauteng province (I = dry and II = wet season).

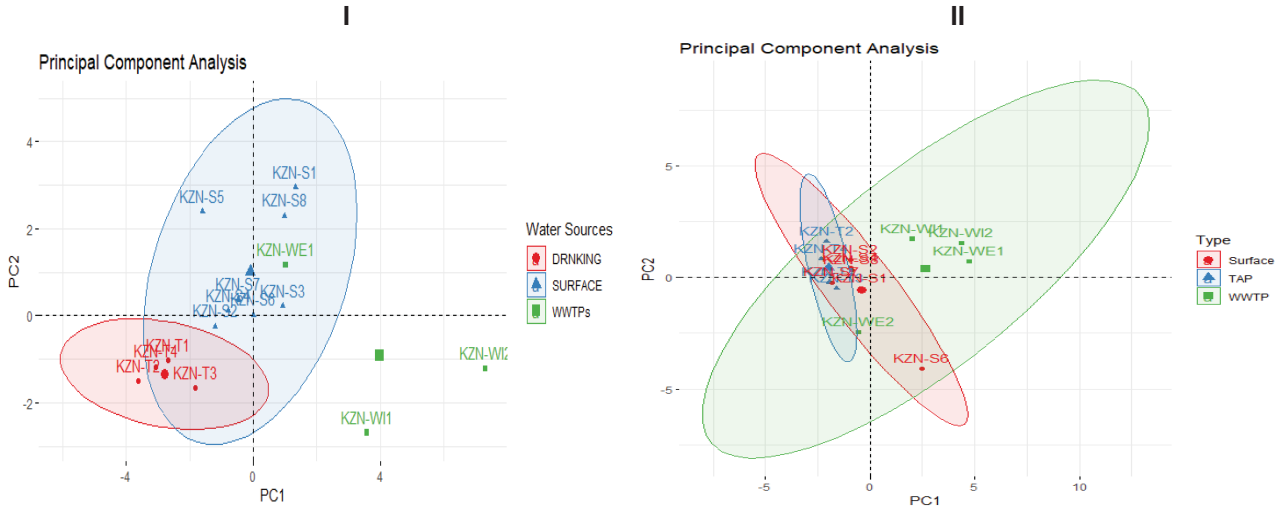

Figure 7.12: Sampling sites and their relationships in the KwaZulu-Natal province (I = dry and II = wet season).

Figure 7.13 (I) shows the PCA of the sampling sites in Limpopo in dry season. As can be seen, most of the sampling sites are clustered in the 1st, 2nd and 3rd quadrants of the score plot. This suggests that the sources of PFASs are similar for these sites. However, LP-WI1 showed different sources from the rest. Figure 7.13 (II) shows the PCA of the sampling sites in wet season. Similarly, the sites that are in the same cluster might have similar sources of PFAS contamination. Sample LP-WI2 and LP-WI2 are expressed similar to PCA observed during the dry season. LP-B5 and LP-B2 showed a negative correlation.

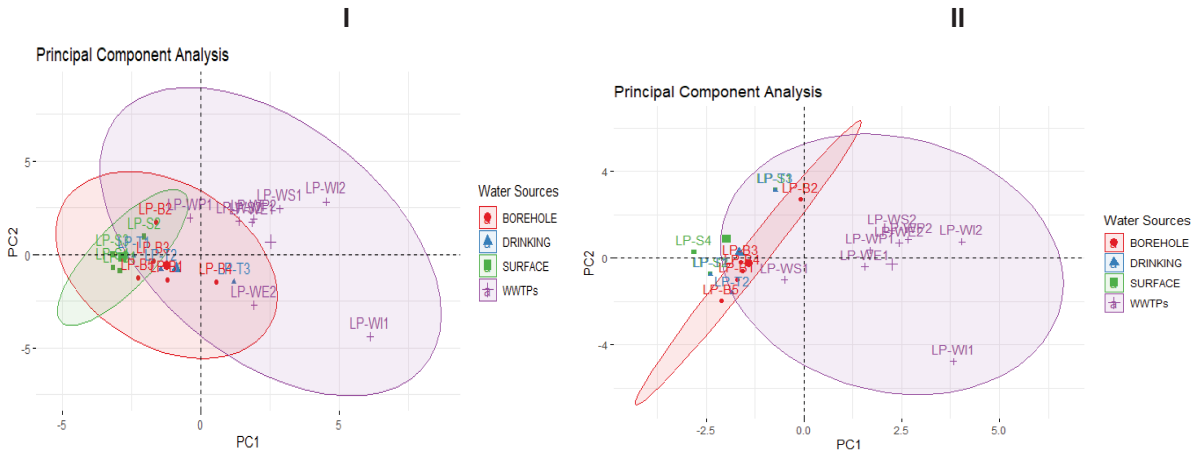

Figure 7.13: Sampling sites and their relationships in the Limpopo province (I = dry and II = wet season).

With respect to the sampling sites (Figure 7.14 (I), sampling sites in Mpumalanga in dry season, MP-S8 and MP-S2 showed no correlation, and this may be as a result of their distance and their sources of pollution. This suggests that the contamination in the analysed water samples from these sites have different pattern/sources. The same suggestion can also be extended to sampling sites MP-T3 and MP-T2. MP-T2 and MP-T4 are negatively correlated. MP-B1, MP-B2 and MP-S5 showed positive correlation. It is important to note that MP-B1 and MP-B2 were collected from different areas but were clustered together. These groups: MP-S3, S4 and T3, MP-S6, S7, T4 and S8, DWTPs and WWTPs samples were grouped together, suggesting similar sources of pollution within these groups. Figure 7.14 (II) shows the PCA plot for samples in various water samples wet season. Clusters can be observed on the plot with borehole samples clustered closely showing a strong correlation which could be as a result of similar behavioural patterns of PFASs or similar sources. The same was observed between MP-T2 and MP-T3. Surface water samples showed a strong and negatively association with each other, except MP-S2. MP-W2E and MP-W2I were clustered while MP-W1E and MP-W1I were responsible for stretching the WWTPs ellipses on to overlap. These samples show a great variation in PFASs sources of pollution or behavioural patterns.

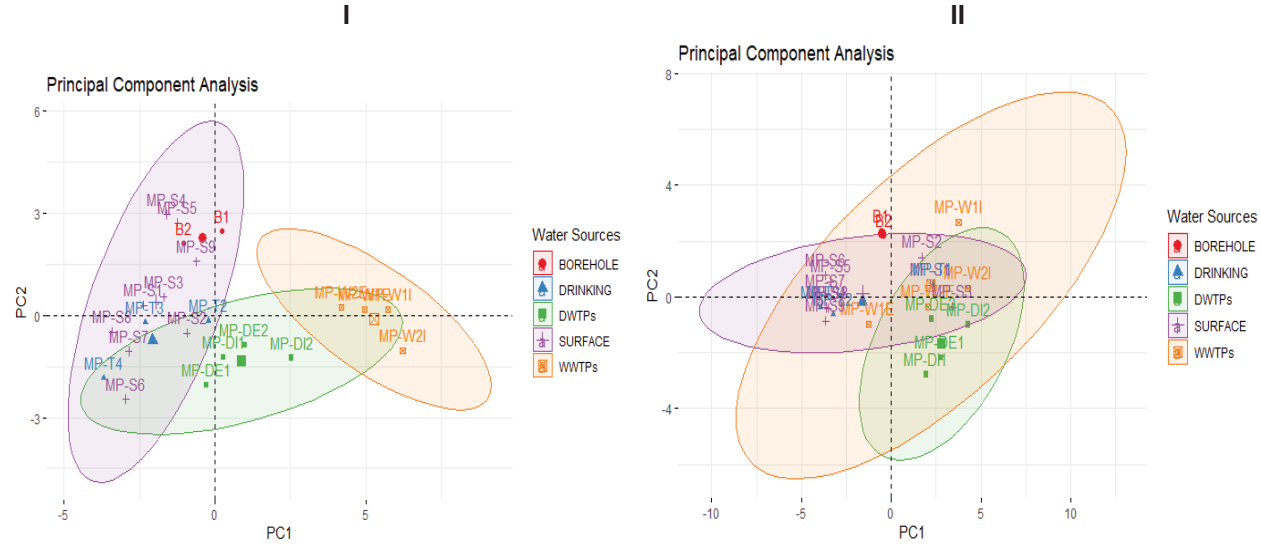

Figure 7.14: Sampling sites and their relationships in the Mpumalanga province (I = dry and II = wet season).

In Northern Cape grouping of NC-DW2 and NC-DW1; NC-W2 and NC-WW1 can be seen Figure 7.15 (I). This indicated shared source of contamination. NC-DW5, NC-DW4, NC-DW3 and NC-WW4 are staggard and this pattern suggests different sources of contamination. Each of these locations is close to an airport, a mine, a landfill and a firefighting station, all of which have been connected with the use of PFASs in their operations. The findings presented in Figure 7.15 (II). The clustering of NC-WW4 and NC-WW3 suggested that they had a comparable source of contamination, possibly from the same industrial activity. Similarly, the grouping of NC-DW1, NC-DW2, NC-WW1, and NC-WW2 indicated a possible shared source of contamination. The close proximity of these sites to an airport, a mine, a landfill, and a firefighting station, all of which are known to use PFASs in their operations, could explain the contamination.

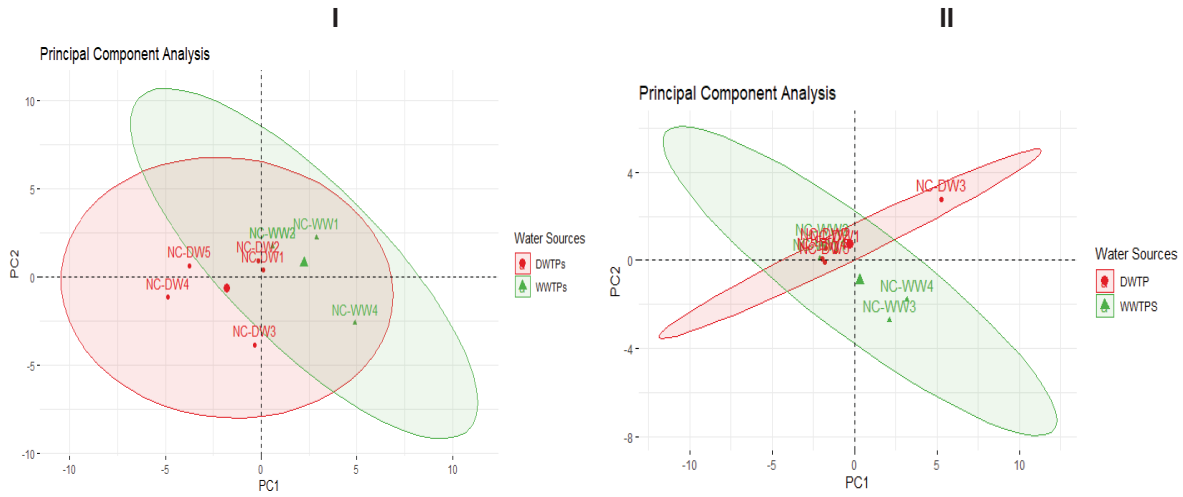

Figure 7.15: Sampling sites and their relationships in the Northern Cape province (I = dry and II = wet season).

In North West, NW-W2R and NW-2R are responsible for the variability as shown in Figure 7.16 (I). However, the groupings of samples collected from different samples within the WWTPs show that PFASs follow a particular pattern which may be similar between the two WWTPs. Borehole water samples: NW-B1, B2, B3 and B5 were negatively correlated with NW-B4. DWTP samples were grouped all together, and this could suggest less variability in the PFASs behaviour in and out of the treatment plant system. NW-T1 and NW-T2 were closely related, which could suggest they share similar sources of PFASs packaging industries and firefighting, while 8:2 FTS in coatings, paper and carpets and cleaning agents industries. As shown in Figure 7.16 (II), NW-B3 and NW-B4 were closely related. This pattern can also be seen with NW-T3 and NW-B5; NW-W2B and NW-W2A; NW-B1 and NW-2S. These closely related may share similar sources.

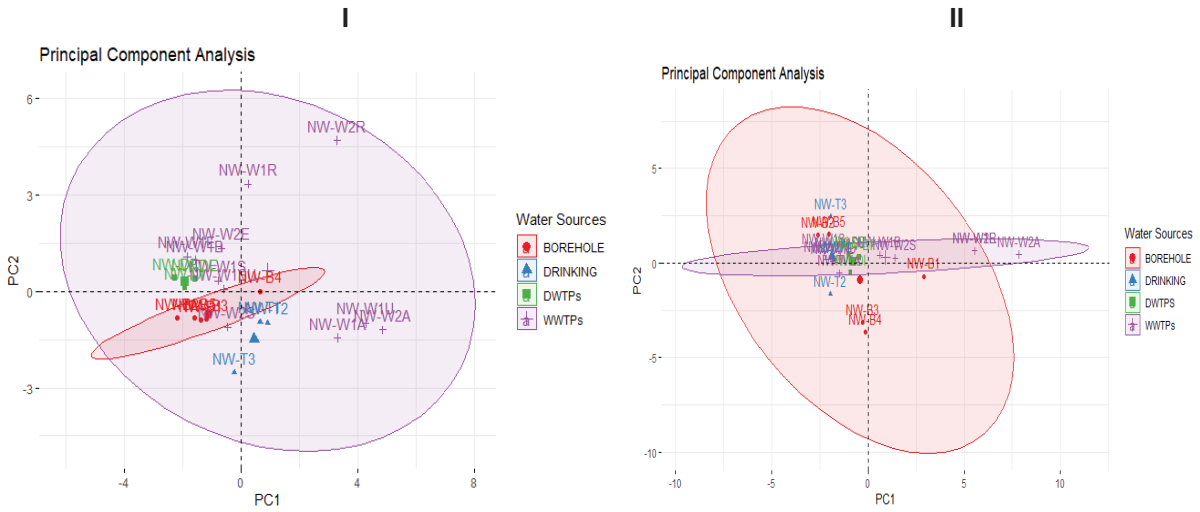

Figure 7.16: Sampling sites and their relationships in the North West province (I = dry and II = wet season).

7.4 SUMMARY

In Eastern Cape, positive correlation was observed for 6:2 FTS, PFHxS, and FOET suggesting similar sources. FHET was negatively associated, suggesting different sources. Since some fluorotelomers are known to be a source of PFCAs, the high detection of the fluorotelomers may explain the prevalence of PFBA detected in all the water sources. With the exceptions of AP and MW on one hand and LD1 and RW on the other, all the other sampling sites are scattered, particularly EC-D1. This behaviour suggested different pattern from others. These were all in line with high PFASs concentrations observed at these sampling sites. Considering the fact that the predominant PFASs detected in the water samples were the PFAAs and these have a wide industrial applications, it is possible that the various industrial and agricultural activities around the sampling areas may have used or still using PFASs in their activities.

For Free State, the following PFASs were correlated: PFHpA, LPFOS, PFBA, 6:2 FTS, PFHxS, PFOA, PFPeA and FHEA in dry season; suggesting possible similar sources; whereas PFHxA and FOET were outlayers. BF-D1/ BF-S1, BF-T1/BF-T2/BF-DE, BF-S2/BF-WE, and BF-W1 formed a single cluster in sampling sites. This clustering suggested that the site had similar sources of contamination and it was noted that the sites had similar concentrations of PFASs, and may be receiving PFASs from the same source, storm water, domestic wastewater and others.

In Gauteng, a mix of fluorotelomers, PFCAs and PFSAs were clustered suggesting similar trend. Lower to none detectable levels were detected for the long chain PFAS, suggesting that they were less produced and consumed. Compounds LPFPeS, PFDA, FHEA, PFPeA, PFNA and PFOA, all show positive strong contributions. The same applied to FOET, PFDoA, PFTrDA and 8:2 FTS. Some of the borehole samples were clustered, whereas the tap water are well separated. The clustering may suggest share of the same sources of PFASs contamination. On the other hand, non-clustering would suggest different sources.

In KwaZulu-Natal, PFOA, PFOS, PFDoA, PFNA, PFBS and PFHxA all show positive strong contributions in dry season. The observed pattern suggests similar sources. PFBA, PFHxS, 8:2 FTS, PFHpS, PFPeA and PFUdA also showed similar behaviour. In wet season, L-PFHpS, L-PFHxS, L-PFBS, 6:2 FTS and FHET showed a positive strong contributions. PFHpA, PFBA and PFPeA also showed similar behaviour. PFHpA and PFBA had the highest concentrations detected in most of the sampling sites. The high detection of perfluoroalkyl carboxylic

acids (PFCAs) detected in these samples might be attributed to the degradation of fluorotelomers. The water sources are all clustered indicating similar sources of PFAS.

In Limpopo, there was a strong correlation between 8:2 FTS, 6:2 FTS, L-PFOS, L-PHET, L-PFHxS, PFHpS and L-PFDS, suggesting that these compounds have similar pattern/ sources. Same applied to PFBA, PFNA, PFHPA, L-PFBS, FHEA, FOET and PFOA.

In Mpumalanga, PFPeA, FHET, PFBS, FHEA, 8:2 FTS and PFHpS were all located close to each other; whereas the long chain PFASs, PFHxS, PFNA, and PFOS were located differently. This observation suggests similar pattern/sources. Sampling sites MP-S8 and MP-S2 showed no correlation, and this may be as a result of their distance and their sources of pollution. This suggested that the contamination in the analysed water samples from these sites showed different pattern/sources. The same suggestion can also be extended to sampling sites MP-T3 and MP-T2. MP-T2 and MP-T4 which were negatively correlated.

In Northern Cape in dry season, FHEA, PFHXDA, PFODA, PFHPA, PFBA, 8:2 FTS, PFOA, 6:2 FTS, PFNA, FHET AND PFPeA were all clustered, suggesting positive correlation and hence similar source. In wet season, analysis revealed that L-PFBS, PFHxA, PFBA, and L-PFOS had a shared occurrence source. Another group comprising 6:2 FTS, PFHpA, PFOA, PFPeA, and PFHxS also showed a similar origin. Grouping of NC-DW2 and NC-DW1; NC-W2 and NC-WW1 indicated shared source of contamination. NC-DW5, NC-DW4, NC-DW3 and NC-WW4 were staggard and this pattern suggested different sources of contamination.

In North West, PFBS, PFHxA, PFOS, PFHpS, PFBA and 8:2 FTS were strongly and positively associated. Their association, therefore, suggested the same source. The same applied to PFHpA, PFOA, FHET and PFNA; PFHpS, PFHxS, 6:2 FTS and FOET; PFPeA, PFBA, 8:2 FTS, PFOA, PFHxA, PFBS and PFHpA and FHEA, 6:2 and FTS, FOET, FHET, PFHxS, PFOS, and PFNA. The groupings of samples collected from different samples within the WWTPs showed that PFASs follow a particular pattern which may be similar between the two WWTPs. Borehole water samples: NW-B1, B2, B3 and B5 were negatively correlated with NW-B4. DWTP samples were grouped all together, and this could suggest less variability in the PFASs behaviour in and out of the treatment plant system.

CHAPTER 8: ASSESING POTENTIAL HUMAN HEALTH RISKS ASSOCIATED WITH EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER

8.1 INTRODUCTION

Assessing potential human health risks associated with exposure to per- and polyfluoroalkyl substances (PFASs) in water is a critical undertaking. This is important, considering the widespread use and persistence of these synthetic chemicals. After use, these substances find their way into water sources, whether through industrial discharges, runoff, or groundwater contamination. Therefore, understanding the potential health implications becomes paramount. Chronic exposure to PFAS has been linked to adverse health effects, including reproductive and developmental issues, immune system disruption, and an increased risk of certain cancers. The main aim of this section is to assess potential human health risks associated with exposure to PFASs. Timely and comprehensive assessments are essential for informing regulatory measures, implementing effective water treatment strategies, and safeguarding public health against the potential hazards posed by PFAS contamination in water sources.

8.2 USING THE YEAST ESTROGEN SCREEN ASSAY TO DETECT ESTROGENIC ACTIVITY IN WATER

As shown in Table 8.1, Estrogenic activity was assessed using YES in 14 samples collected from various matrices in the Northern Cape and Gauteng provinces. As can be seen in Table 8.1 the following samples were <LOD for both the dry and wet seasons, NC-DW5, NC-WW2; whereas samples NC-DW2 and GP-BTW2 were also below the <LOD in wet season. Additionally, only GP-BW1 was < LOD throughout the dry season.

Table 8.1: Estrogenic activity of water extracts collected from selected sites in Northern Cape and Gauteng using Yeast estrogenic Bioassay

Province	Sample code	Sample type	Dry season	Wet season
			Estradiol equivalents (EEq) in ng/L	
Northern Cape	NC-WW1	Raw Wastewater	117±1.4*	150.6±35.8*
	NC-WW2	Treated wastewater	<LOD	<LOD/<LOQ
	NC-WW3	Raw wastewater	593±23*	718±15.8*
	NC-WW4	Treated wastewater	5.451±1.0*	5.8±0.22
	NC-DW1	Raw Drinking water	0.329±0.008	0.712±0.090
	NC-DW2	Treated drinking water	0.163±0.026	<LOD/<LOQ
	NC-DW3	Raw water	0.154±0.02*	0.543±0.033

Province	Sample code	Sample type	Dry season	Wet season
	NC-DW4	Treated drinking water	2.374±0.35*	13.32±3.09*
	NC-DW5	Treated drinking water	<LOD/<LOQ	<LOD/<LOQ
	Blank	Control	<LOD/<LOQ	<LOD/<LOQ
Gauteng	GP-BTW1	Borehole tap water	0.179±0.020*	0.149±0.016
	GP-BTW2		0.584±0.044	0.890±0.125
	GP-TW1	Tap water	0.334±0.0078	<LOD/<LOQ
	GP-TW2		5.00±.0.5*	0.500±0.023
	GP-BW1	Landfill borehole monitoring water	<LOD/<LOQ	3.26±0.37
	GP-BW2		0.608±0.053*	0.357±0.015
	Blank	Control	<LOD/<LOQ	<LOD/<LOQ

*Cytotoxicity observed; below limit of detection(<DL)

Estrogenic activity in raw wastewater for samples NC-WWI (150.6±35.8 ng/L) and NC-WW3 (718±15.8 ng/L) was higher in wet season, compared to the dry season which had EEq values of 117±1.4 ng/L (NC-WW1) and 593±23 ng/L (NC-WW3). A similar pattern was seen in treated wastewater (NC-WW4), with a concentration of 5.451±1.0 ng/L (dry season) and 5.8±0.22 ng/L (wet season). In general, estrogenic activity was higher in influent than in effluents. Bistan *et al*. (2013) observed a similar pattern. The decrease of EDC in effluents can possibly be attributed to the removal of estrogens and xenoestrogens during treatment processes in WWTPs.

Increase in estrogenic activity was observed in raw drinking water samples, NC-DW1 (from 0.329±0.008 to 0.712±0.090 ng/L) and NC-DW3 (from 0.154±0.02 to 0.543±0.033 ng/L) during the wet season. Similarly, estrogenic activity increased in treated drinking water for samples NC-DW4 (from 2.374±0.35 to 13.32±3.09 ng/L) during the wet season. In contrast, a decrease in treated drinking water was detected for sample NC-DW2 (0.163±0.026-<LOD). The decrease of estrogen activity in the final treated drinking water possibly indicates the ability of some treatment plant to remove EDC. Dias *et al*. (2015) reported that chlorination was effective in reducing EDCs in water treatment plants.

For sample GP-BTW1 (0.1490±.016 ng/L), a decrease was seen in borehole tap drinking water samples compared to the dry season. In contrast to the dry season, there was an increase at sampling site GP-BTW2 (from 0.584±0.044 to 0.890±0.125 ng/L) during the rainy season. Additionally, a decline in estrogenic activity was recorded for samples GP-TW1 and GP-TW2 in the tap water sample. An increase in estrogenic activity was observed in the landfill monitoring borehole samples at sampling site GP-BW1 (from <LOD to 3.26±0.37 ng/L), whereas the estrogenic activity in sample GP-BW2 (from 0.608±0.053 to 0.357±0.015 ng/L) declined in wet season. Zhai *et al*. (2010); Beck *et al*. (2005) also observed higher estrogenic activity during the dry season compared to the wet season. The highest EEq value was measured in wastewater for sample NC-WW2 (718±15.8 ng/L). While a prevalent EEq value of 13.32±3.09 ng/L was measured as in drinking water at for sample NC-DW4. In tap water a dominant EEq value of 5.00±.0.5 ng/L*(GP-TW2) and in landfill monitoring borehole water a greater EEq value of 3.26±0.37 ng/L was observed at sampling point GP-BW1.

Out of the 14 samples, five water samples from the Northern Cape and one from Gauteng both exhibited some cytotoxicity, which resulted in an inhibition of yeast growth within a range of 8.3-50% (Table 8.2). Per- and polyflouro alkyl substances present in the sample may be the cause of the inhibition. It is noteworthy that cytotoxicity may conceal or cause estrogenic activity to be underestimated. Because of the cytotoxicity, estrogenic activity exhibited by samples NC-WW1 (dry and wet), NC-WW3 (dry and wet), NC-WW4 (dry), NC-DW3 (dry), NC-DW5 (dry), and GP-TW2 (dry) may have been underestimated.

Table 8.2: Inhibition growth of yeast in water samples

Sample code	Cytotoxicity (inhibition %)	
	Dry season	Wet season
NC-WW1	42	33
NC-WW3	50	50
NC-WW4	8.3	
NC-DW4	8.3	
NC-DW5	17	
GP-TW2	25	

8.3 ASSESSMENT OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASS) EFFECT IN SAMPLES USING THE YES ASSAY

Estrogenic activity and cytotoxicity were evident in most of the samples subjected to bioassay test. Over time of the two sampling periods, estrogenic activity, was detected in 12 of the 14 samples. Whereas cytotoxicity was determined in 9 of the 14 samples. Out of all the compounds, only PFOS showed estrogenic activity. Moreover, none of the compounds showed toxicity at the tested concentrations. Plots of positive estrogenic responses of PFOS is shown in Figure 8.1 and an example of the non-responsive curve in Figure 8.2 using 4:2 FTS.

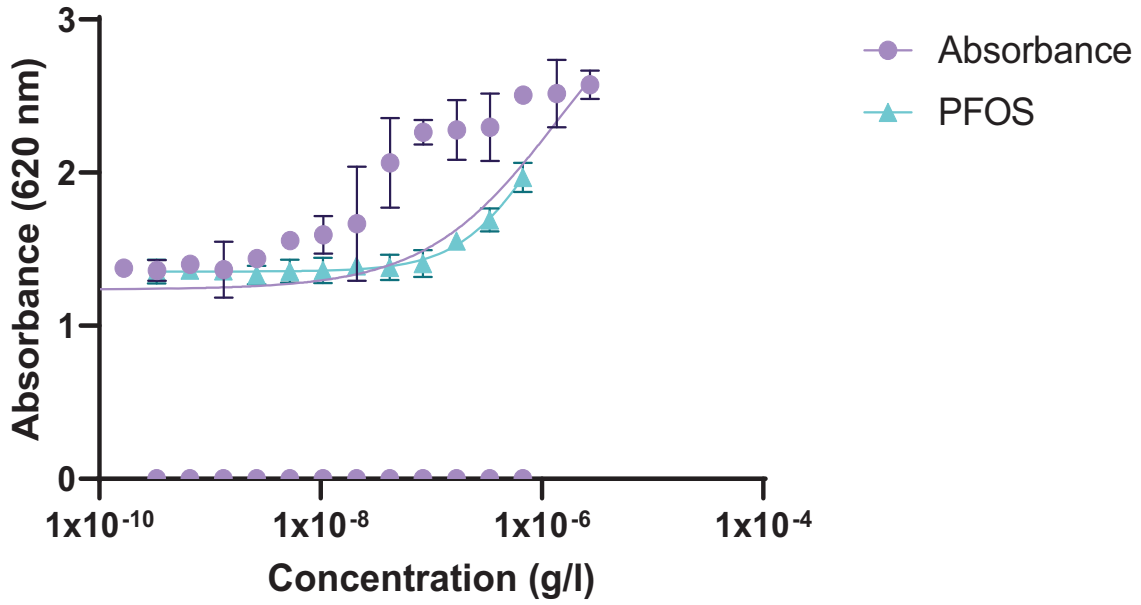

Figure 8.1: Estrogenic response of PFOS in the YES bioassay (Data points represent the average ± SD (n=3)

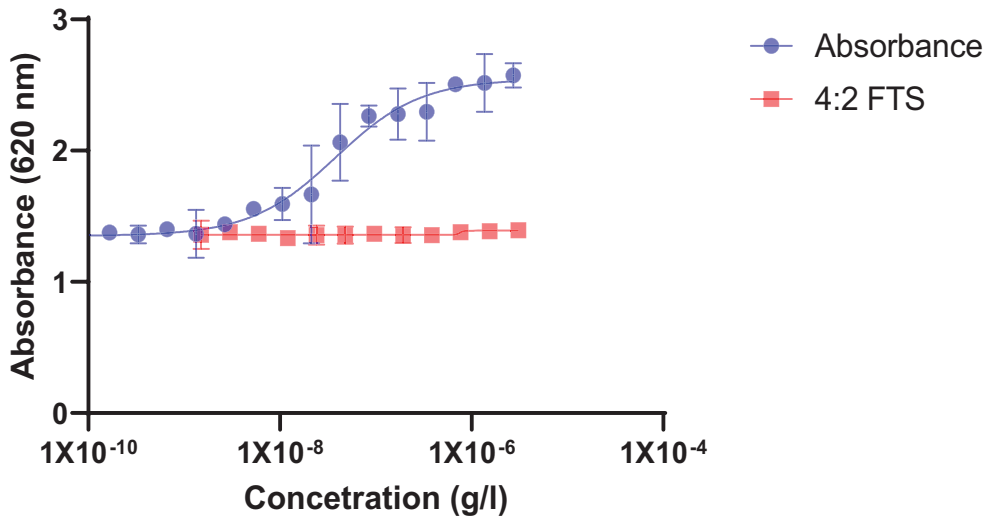

Figure 8.2: Estrogenic response of 4:2 FTS in the YES bioassay (Data points represent the average ± SD (n=3)

8.4 TRACE METAL ANALYSIS

Because PFOS was the only PFAS chemicals with positive estrogenic response, it was deemed necessary to subject the water samples to trace metal analysis. A number of trace metals such as cadmium (Cd), arsenic (As), lead (Pb) and others are known to have estrogenic/cytotoxic characteristics and therefore, their presence in the water samples may have also contributed to the observed estrogenic/cytotoxic activities in the samples. Sample preparation for trace metal analysis is as described in **VOLUME I SECTION 3.3.7.**

Table 8.3 shows the concentrations of the targeted trace metals in the samples. The concentration range across all the sample was 0.01-513 ug/L. WHO trace metals concentrations considered safe in drinking water range up to 0.003-3.0 ug/L (WHO 2006). Although not all the water samples analysed were drinking water, the concentrations for most of the trace metals exceeded the WHO range. Trace metal concentrations across all the samples, was in the following descending order:

Sr<Zn<Ba<Mn<Rb<Ni<Cr<Li<Cu<Vn<Ti<Se<Ar<As<Pb<La<Pt<Mo<U<Co<Sn<Ti<Te<W<Sb<Cs<Bi<Cd<Be.

For examples, trace metals such as cadmium, antimony, barium, chromium, copper, zinc, lead, mercury, nickel, arsenic, aluminium, cobalt, and mercury, have shown to exert metalloestrogens with estrogenic activity (Denier *et al.,* 2009). Due to the presence of trace metals in the water samples, it is possible that the estrogenic activity identified in the water samples as mentioned in Table 8.3, may have also been caused by the trace metals. A number of these trace metals are used for nanoparticle production and are found in a variety of consumer products such as cosmetics, household items, and processed foods and others. These metals can be released into the water environmental via several routes such as disposal of trace metal containing wastes, illegal dumping of trace metal-containing waste on river banks and others. Of the “representative metals” listed in Table 8.3, cadmium has been shown to be estrogenic and is equipotent to the effects elicited by estradiol.

Table 8.3: Concentrations (µg/L) of heavy metals in environmental water samples

	NC-WW4	NC-DW1	NC-DW2	NC-WW3	NC-WW1	NC-DW3	NC-DW4	GP-TW4	GP-BW2	GP-BW1	GP-TW1	GP-TW2
Elements												
Lithium (Li)	5.31	4.86	4.98	18.2	10.9	3.28	3.38	1.91	0.912	5.58	1.90	1.81
Beryllium (Be)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
Titanium (Ti)	2.53	0.31	0.30	6.36	6.00	1.00	1.54	0.83	< 0.1	< 0.1	< 0.1	< 0.1
Vanadium (V)	3.28	1.43	1.62	8.14	4.13	13.0	5.47	3.88	0.128	0.235	3.72	3.55
Chromium (Cr)	9.61	6.51	4.90	7.88	6.35	4.31	6.02	4.56	6.83	6.99	3.89	3.03
Manganese (Mn)	116	1.29	1.70	30.08	9.00	1.34	1.34	1.46	1.57	4.68	1.36	0.701
Cobalt (Co)	0.556	0.238	0.193	0.448	0.874	0.146	0.153	0.149	0.496	0.272	0.132	0.125
Nickel (Ni)	6.79	3.41	2.78	7.61	11.3	11.7	8.29	3.17	4.91	3.54	5.36	6.64
Copper (Cu)	3.33	5.54	1.40	6.62	7.81	4.31	11.2	6.02	2.27	3.30	1.77	1.82
Zinc (Zn)	39.4	182	19.3	42.5	86.7	47.7	36.8	43.2	11.7	5.44	12.0	11.1
Arsenic (Ar)	0.797	1.78	1.19	0.442	1.00	0.275	0.241	0.764	0.290	< 0.1	0.311	0.364
Selenium (Se)	< 1.4	< 1.4	< 1.4	< 1.4	1.53	1.75	5.35	< 1.4	< 1.4	< 1.4	< 1.4	< 1.4
Rubidium (Rb)	10.0	1.72	1.68	28.5	29.0	2.26	1.99	1.92	3.87	3.09	1.44	1.55
Strontium (Sr)	209	163	34.2	350	268	378	513	88.5	138	48.3	85.8	83.9
Molybdenum (Mo)	0.376	0.165	0.137	0.768	0.982	0.0743	0.101	0.691	0.616	0.359	0.672	0.609
Cadmium (Cd)	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07	< 0.07
Tin (Sn)	0.191	0.179	< 0.09	0.507	0.286	0.222	0.345	0.174	0.306	0.301	< 0.09	0.174
Antimony (Sb)	0.140	< 0.03	< 0.03	0.181	0.212	0.043	< 0.03	0.111	0.0767	0.0676	0.0789	0.101
Tellurium (Te)	< 0.09	0.145	< 0.09	0.148	0.285	0.342	0.175	0.165	0.265	0.285	0.374	0.171
Cesium (Cs)	0.134	0.127	0.116	0.156	0.144	0.0674	0.0407	0.119	0.0404	0.0617	0.0182	0.0353
Barium (Ba)	56.5	45.5	15.1	41.5	21.4	57.8	79.7	52.0	34.8	< 0.08	13.2	29.1
Lanthanum (La)	0.400	0.318	0.098	0.660	0.0574	0.870	0.130	2.96	0.0224	0.291	0.0157	0.390
Tungsten (W)	0.296	0.0408	0.0511	0.324	0.231	0.077	0.120	0.0775	0.146	0.0935	0.104	0.0587
Platinum (Pt)	0.406	0.379	0.784	0.725	0.325	0.500	0.347	0.778	0.406	0.543	0.654	0.264
Thallium (Ti)	0.286	0.209	0.199	0.201	0.225	0.165	0.167	0.173	0.254	0.236	0.161	0.133
Lead (Pb)	0.704	0.414	0.222	1.040	0.819	0.774	0.818	0.722	0.405	0.245	0.172	0.256
Bismuth (Bi)	0.0126	0.0101	0.0154	0.0292	0.0224	0.0189	0.0178	< 0.01	0.0203	0.0106	0.0116	0.0201
Uranium (U)	0.528	0.607	< 0.01	0.841	0.213	1.01	1.51	0.102	0.0732	0.0139	0.107	0.102

8.5 SUMMARY

Estrogenic activity and cytotoxicity were evident in most of the samples subjected to bioassay test. Over time of the two sampling periods, estrogenic activity, was detected in 12 of the 14 samples. Whereas cytotoxicity was determined in 9 of the 14 samples. Out of all the compounds, only PFOS showed estrogenic activity. Moreover, none of the compounds showed toxicity at the tested concentrations. Trace metals such as cadmium (Cd), arsenic (As), lead (Pb) and others are known to have estrogenic/cytotoxic characteristics and therefore, their presence in the water samples may have also contributed to the observed estrogenic/cytotoxic activities in the samples.

CHAPTER 9: CONCLUSIONS & RECOMMENDATIONS

9.1 CONCLUSIONS

- Generally, the order of PFASs concentrations in different water samples were as follows: WWTP>DWTP>Surface Water>Borehole Water (groundwater)> Tap Water> Rainwater>Bottled Water;

- The following emerging PFASs were identified, Perfluoro-2-methoxyacetic acid (PFMOAA), Perfluoro-3-methoxypropanoic acid (PFMOPrA), Perfluoro-4-methoxybutanoic acid (PFMOBA), Perfluoro-2-propoxypropanoic acid (PFPrOPrA), Perfluoro(3.5-dioxahexanoic) acid (PFO2HxA) Perfluoro(3.5.7-trioxaoctanoic) acid (PFO3OA) and Perfluoro (3.5.7.9- tetraoxadecanoic) acid (PFO4DA);

- Seasonal influence on the concentrations of PFASs in the samples across most of the provinces was noticeable. Higher concentrations were observed in dry season compared to wet season;

- Short chain PFASs were more dominant than the long chain in some cases, albeit long chain PFASs such as PFOA and PFOS was one of the most prevalent compounds detected;

- The concentrations of PFASs observed in the present study are, in some cases, higher than the values reported by other researchers in water samples. The PFASs detected in the bottled drinking water in the present study are higher than the IBWA operational limits of 5 ng/L for a single PFASs and 10 ng/L for more than one PFASs. Compared to the health advisory levels at 70 ng/L, by the USEPA to protect its sensitive populations, from a lifetime of exposure to PFOA and PFOS from drinking water, the concentrations of PFOS and PFOA in drinking water in the present study are generally much lower;

- PFASs compounds were detected in both grab and passive samples. However, the PFASs concentrations in the POCIS passive sampler were higher than the grab samples collected on the same days;

- Some PFASs showed similar sources; while others showed different sources. This trend was also observed with the sampling sites. It is, therefore, possible that all the land use activities around the sampling sites, may have contributed to the observed PFASs in the water samples and Estrogenic activity was detected in 12 of the 14 samples tested, whereas cytotoxicity was determined in 9 of the 14 samples. Of the 18 standard PFASs chemicals subjected to bioassay test, only PFOS exhibited cytotoxicity. However, other contaminants in the water samples such as trace metals may have also contributed

9.2 RECOMMENDATIONS

The current nation-wide exercise has shown that these chemicals are in our water system. The importance of monitoring of POP chemicals such as PFASs, without any doubt, is an expensive exercise. However, regular monitoring of these chemicals is extremely important, particularly in water system because it is via this process that proper informed decision can be made to regulated and control the presence of these in water systems.

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APPENDIX A: VALIDATION OF EXTRACTION METHOD – PFASS RECOVERIES

Table A1: Percentage recoveries of PFASs in procedural blanks

Procedural blanks (n = 4)						
Target analytes	**Low spike (5 ng/L)**		**Medium spike (800 ng/L)**		**High spike (1600 ng/L)**	
	Mean%Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	106.5	5.94	83.6	15.7	116.1	2.3
PFudA	82.6	3.57	99.2	24.4	71.7	2.9
8:2 FTS	103.0	0.46	101.0	2.0	86.4	1.6
PFHpA	113.8	14.99	94.2	14.2	98.1	9.7
4:2 FTS	97.6	20.42	89.2	1.9	85.9	14.4
PFDoA	97.3	17.81	94.2	2.5	95.6	11.9
PFHxA	93.2	21.37	90.9	23.0	95.8	2.16
PFPeA	73.9	13.27	83.4	6.0	86.4	5.6
PFODA	115.4	1.75	73.5	6.8	97.2	0.0
PFBA	104.8	9.74	102.0	13.8	81.9	1.7
PFHxDA	77.5	4.74	77.0	6.6	78.7	0.5
FHET	124.7	3.52	97.	16.5	88.6	2.5
FOET	126.1	1.52	104.3	6.4	94.8	7.4
6:2 FTS	102.7	23.50	110.5	1.0	89.9	7.9
FHEA	116.6	2.75	76.3	3.3	80.1	0.3
PFDS	109.7	22.03	88.7	30.3	86.2	17.1
PFBS	67.5	14.60	103.5	2.8	80.7	4.1
PFHxS	120.5	3.60	97.2	13.2	100.9	6.1
PFOS	107.1	23.08	77.2	3.2	98.3	6.2
PFHps	75.7	3.79	94.5	11.4	90.7	12.4
PFOA	85.0	3.09	105.1	16.1	92.3	1.9

Table A2: Percentage recoveries of spiked PFASs standards from drinking, surface and borehole water samples

Target analytes	Drinking water (n =4)						Surface water (n=4)						Borehole (n=4)					
	Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)		Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)		Low spike (5 ng/L)		Medium spike		High spike	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	76.0	3.3	100.3	10.5	109.6	7.3	84.0	5.5	85.1	8.0	72.0	3.3	93.9	10.9	100.5	9.2	89.0	3.2
PFudA	102.7	15.0	98.8	7.4	104.3	10.7	85.2	20.7	57.1	0.9	77.8	2.0	111.3	16.2	98.6	1.8	83.7	0.1
8:2 FTS	80.9	16.8	98.6	2.2	90.5	23.9	91.0	18.3	87.3	3.3	84.1	1.2	109.1	1.2	101.1	2.1	101.1	1.0
PFHpA	100.5	16.8	68.8	1.4	91.2	6.4	85.5	13.1	77.9	1.2	81.6	2.2	96.2	4.2	83.0	11.8	100.0	3.2
4:2 FTS	117.1	8.4	82.4	4.2	83.6	2.4	113.4	7.5	83.4	2.9	88.1	8.3	110.1	7.9	105.6	2.6	92.0	7.3
PFDoA	104.1	28.5	87.0	18.8	92.0	9.4	**51.4**	11.8	72.5	2.0	96.7	7.4	135.7	5.8	85.1	1.3	87.6	6.1
PFHxA	101.1	1.4	106.4	5.3	89.0	5.0	86.7	14.4	91.8	15.0	99.5	6.2	123.0	6.0	91.1	12.7	102.7	2.1
PFPeA	108.2	5.2	89.1	3.3	74.6	0.4	98.8	1.1	74.7	10.3	88.8	19.1	123.5	5.3	90.4	17.3	91.1	6.3
PFODA	69.0	0.5	85.9	9.1	83.4	16.7	128.6	6.3	99.9	10.5	83.1	16.5	106.6	0.6	94.4	12.4	98.5	0.4
PFBA	101.6	18.7	88.5	8.7	71.0	1.5	98.0	22.0	102.2	7.3	83.8	2.0	80.2	26.0	105.2	4.0	70.0	2.1
PFHxDA	102.5	13.6	77.1	6.7	69.6	1.2	97.6	23.3	77.4	1.7	83.5	23.3	82.5	27.1	104.9	22.7	70.2	1.3
FHET	105.1	9.8	98.7	10.7	86.4	1.0	108.1	16.5	100.8	9.4	75.8	4.2	88.0	25.9	108.8	12.9	94.3	7.3
FOET	106.0	12.4	95.4	13.7	90.1	17.4	116.4	7.4	80.6	0.6	86.0	1.7	78.0	3.5	99.6	6.4	96.3	0.5
6:2 FTS	70.9	9.6	85.9	6.3	69.8	0.7	94.4	3.1	83.9	8.7	92.3	6.4	117.5	6.1	85.4	9.2	93.6	10.6
FHEA	75.2	26.6	76.3	1.4	88.4	12.9	114.3	4.1	90.7	7.5	96.5	13.6	144.0	6.8	119.6	2.6	99.7	1.1
PFDS	106.2	6.4	80.8	6.0	80.2	20.3	103.1	2.8	83.2	8.5	98.0	16.2	84.8	3.2	93.3	0.2	83.1	2.1
PFBS	84.1	5.6	108.9	7.3	90.1	4.1	119.4	4.8	92.9	3.5	89.5	15.1	106.5	6.6	89.1	10.1	80.7	3.3
PFHxS	90.3	17.1	88.2	7.4	78.0	8.2	116.5	16.3	95.6	9.9	89.7	9.1	106.3	10.2	88.5	12.0	85.4	6.4
PFOS	100.9	5.5	99.4	5.3	80.3	2.4	74.8	17.8	82.4	4.9	91.9	0.7	93.4	8.2	105.1	6.5	100.7	0.4
PFHps	109.7	1.6	84.7	11.6	72.7	15.1	103.6	24.5	96.4	1.1	98.8	2.8	100.6	1.8	103.4	8.6	97.2	4.1
PFOA	91.8	8.9	93.1	17.8	92.4	6.9	73.5	3.7	93.5	3.3	88.3	23.8	106.7	24.6	95.2	4.9	86.4	4.5

Table A3: Percentage recoveries of spiked PFASs standards from drinking water influent and effluent samples

Target analytes	DWTP-Influent						DWTP-Effluent					
	Low spike		Medium spike		High spike		Low spike		Medium spike		High spike	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	124.4	19.7	97.5	17.7	108.7	6.8	66.5	9.9	92.5	12.3	102.8	7.8
PFudA	92.9	10.7	72.5	11.7	60.1	8.6	110.7	3.1	105.9	0.3	93.2	2.1
8:2 FTS	79.8	13.1	93.2	3.6	94.5	7.4	78.6	33.5	103.1	2.6	107.6	3.3
PFHpA	105.3	2.4	67.8	0.7	78.7	11.8	128.6	12.2	70.1	3.5	85.0	7.1
4:2 FTS	124.7	1.1	108.7	2.9	74.0	2.1	111.1	15.9	90.8	5.4	84.9	13.0
PFDoA	130.1	15.1	88.4	4.6	101.2	15.4	93.9	0.1	113.3	3.6	103.2	11.2
PFHxA	103.2	26.6	74.9	12.9	104.4	8.1	106.4	6.2	107.9	0.1	102.9	8.6
PFPeA	63.5	26.8	78.8	1.7	75.3	6.6	70.3	20.1	76.9	4.8	94.6	10.7
PFODA	77.4	5.5	70.3	17.5	79.2	7.6	64.3	2.1	83.5	0.1	76.8	10.7
PFBA	93.6	19.3	84.9	20.2	96.9	5.2	104.8	3.5	80.2	4.2	84.0	12.1
PFHxDA	73.7	3.3	88.0	6.4	122.8	3.9	119.1	8.3	91.5	6.4	85.1	10.8
FHET	104.2	25.7	103.9	3.7	88.0	4.2	88.7	27.6	74.8	15.2	97.2	11.1
FOET	127.8	22.1	95.0	7.3	76.3	11.4	112.1	3.5	91.2	1.8	80.6	0.0
6:2 FTS	110.9	2.4	75.5	2.6	73.5	1.7	98.5	19.4	93.6	1.1	80.8	3.7
FHEA	125.0	1.8	108.5	6.7	91.6	24.9	112.8	7.2	83.6	8.0	86.2	12.3
PFDS	78.8	24.7	66.8	3.9	89.1	28.4	101.9	13.8	68.0	0.4	88.2	14.4
PFBS	115.2	6.7	92.4	9.2	105.5	4.3	94.2	29.9	90.8	3.1	83.9	2.1
PFHxS	116.2	6.3	107.5	5.4	91.4	4.0	110.2	10.6	89.5	14.4	89.1	9.0
PFOS	124.7	5.1	67.8	12.9	93.5	4.3	97.4	27.8	77.4	0.1	91.5	22.8
PFHps	101.0	16.9	85.9	2.6	97.5	3.9	92.5	30.4	91.2	3.8	93.8	25.2
PFOA	108.4	2.8	93.4	10.6	84.0	7.8	59.3	15.9	94.6	5.6	83.5	14.9

Table A4: Percentage recoveries of spiked PFASs standards from wastewater influent and effluent samples

Target analytes	WWTP-Effluent						WWTP- influent					
	Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)		Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec	%RSD	Mean %Rec	%RSD	Mean %Rec.	%RSD
PFNA	94.5	25.5	95.6	16.9	84.6	0.1	100.4	1.3	107.6	13.0	78.0	4.1
PFudA	71.5	20.1	96.0	2.1	92.2	20.8	93.0	4.1	71.3	2.7	50.4	11.2
8:2 FTS	84.4	20.2	80.2	13.7	83.9	3.3	80.6	29.7	107.7	1.0	108.3	2.5
PFHpA	71.8	2.5	80.1	19.2	89.1	21.4	116.1	20.0	92.3	19.3	82.6	19.6
4:2 FTS	123.0	4.5	109.5	3.0	69.4	5.5	100.5	5.1	95.9	3.9	93.5	25.9
PFDoA	95.7	23.1	100.1	15.3	**43.3**	2.5	86.0	8.7	79.1	5.0	**48.8**	24.9
PFHxA	99.5	**32.5**	104.4	5.0	100.0	1.4	110.4	13.8	111.5	3.6	98.8	5.2
PFPeA	113.1	19.8	99.2	4.9	93.2	11.6	128.6	9.8	95.0	4.3	77.9	8.3
PFODA	58.5	4.3	81.3	25.0	78.9	8.9	84.4	**37.0**	75.4	0.0	76.8	14.9
PFBA	72.2	22.1	87.8	3.8	83.0	2.9	111.5	5.2	93.3	28.9	86.6	1.5
PFHxDA	109.7	9.0	69.9	9.3	79.1	19.4	106.0	14.6	76.3	0.7	73.4	14.9
FHET	95.2	21.9	95.3	5.5	87.7	7.2	83.4	19.0	101.0	0.1	96.8	19.6
FOET	105.0	1.8	90.9	5.5	71.0	16.3	83.4	19.0	101.0	0.1	96.8	19.6
6:2 FTS	98.9	2.4	95.6	4.8	96.1	14.1	87.1	3.4	104.2	12.4	104.7	10.0
FHEA	87.2	21.4	87.0	2.6	93.8	13.5	109.9	25.7	98.3	2.0	82.5	13.0
PFDS	78.0	4.6	86.4	12.0	81.3	7.1	87.6	13.1	110.7	8.4	101.5	7.4
PFBS	123.6	2.3	94.3	15.0	80.9	8.7	83.7	20.0	78.0	3.9	107.6	6.9
PFHxS	81.8	18.3	94.1	10.2	98.6	19.4	96.3	1.9	100.8	3.9	101.6	0.4
PFOS	93.3	12.4	79.7	9.9	94.7	0.4	82.1	10.1	76.8	11.7	80.2	4.7
PFHps	96.4	10.8	91.4	18.0	99.0	5.1	90.5	5.8	95.8	10.2	80.6	0.9
PFOA	111.8	3.2	99.6	5.8	102.0	2.2	79.5	20.9	99.5	12.5	84.4	2.6

TableA5: Certified reference material recoveries (CRM IRMM-428)

	Certified value concentration (ng/L)	Uncertainty concentration (Certified value) (ng/L)	Instrument concentration (ng/L)	%RSD	%Recovery
PFBS	5.5	1.4	5.4±1.1	19.5	98
PFHxS	3.6	1.0	4.0±0.7	17.6	112
PFOS	9.6	1.7	11.6±0.7	6.0	121
PFPeA	4.0	1.0	3.1±0.5	15.5	78
PFHxA	7.4	1.0	8.2±1.0	12.5	110
PFHpA	3.7	0.7	3.5±0.4	10.5	97
PFNA	3.9	1.4	4.2±0.5	12.0	109