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Bioaccumulation of Perfluoroalkyl Substances in African marigold (*Tagetes erecta* L.) used for Diabetes *mellitus* Management and in Diabetic Serum of a South African Population

By

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"It always seems impossible until it is done" –

Nelson R. Mandela

DECLARATION

I, ***John Baptist Nzukizi Mudumbi***, declare that the contents of this dissertation represent my own work, and that the dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology and the National Research Foundation of South Africa.

In addition, all intellectual concepts, theories, and methodologies used in this thesis and under review in various journals were derived solely by the candidate and first author of the submitted manuscripts under review. Where appropriate, the intellectual property of others was acknowledged by using appropriate references. The contribution of co-authors, for conference papers and manuscripts under review, was in a research assistance and supervisory capacity to meet the requirements of the degree.

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Signed **Date**

ABSTRACT

Polyfluoroalkyl substances (PFASs), including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are anthropogenic chemicals. For more than half a century, these long-chain compounds have been used in a wide range of industrial applications, such as the manufacturing of consumer products, ranging from grease-proof food packing to aqueous fire-fighting foams and to stain repellents such as Teflon®. Subsequently, these ubiquitous contaminants which are environmentally persistent, toxic, and bioaccumulative, have been a focus of public concern worldwide. Hence, due to public health apprehensions and environmental risks posed by PFASs, their manufacturers and various environmental agencies decided on restricting their use, and whereby the use of these chemicals could not be stopped, their replacement by other alternative chemicals was suggested. Therefore, alternatives to long-chain PFASs was suggested, i.e. to replace the compounds with shorter per- or polyfluorinated carbon chains, e.g. perfluorobutane sulfonate (PFBS), which has been regarded as one of the most important short-chain PFASs and less harmful to the environment at large. However, a systematic review from the current work reveals that physicochemical properties of short-chain PFASs are not different from their predecessors thus suggesting that short-chain PFASs are as harmful as their homologues. Similarly, the literature reviewed demonstrated how novel technologies have also been proven to be incapable of removing these substances, including to short-chain PFASs, from various environmental matrices.

Moreover, plant species have extensively been susceptible to PFASs, and various other POPs accumulation. However, the mechanisms that led to their uptake and storage by plants stayed unknown until proteins belonging to the family of major intrinsic proteins (MIPs) and later named as Aquaporins (AQPs) were discovered. Hence, the present work has reported that there are diverse AQPs in plants than in mammals, with specific functions, even though first reports on these proteins suggested that their significant impact was water for transportation only. To date, it is well known that plant AQPs possess subclasses or isoforms. Some of these include SoPIP2;1 and AtTIP2;1, prevalent in *Spinacia oleracea* and *Arabidopsis thaliana*, respectively. We report that these two isoforms have individual pore diameters or sizes: SoPIP2;1 (2.1 Å) and AtTIP2;1 (3 Å), which might play a role in the selectivity process of molecules which pass through the water transportation channels of the concerned plants. This ultimately suggested SoPIP2;1 pore diameter serving as a pathway of smaller molecules, while AtTIP2;1 pore diameter would serve as a conduit for both smaller and larger compounds. As

such, the pore diameters of these two isoforms made them potential conduits of PFASs whose carbon–fluorine bond typical size is 1.35 Å, much smaller than that of AtTIP2;1_2.1 Å and PIP2s, i.e. SoPIP2;1_3 Å, thus substantiating the uptake and ultimate storage of PFASs by plant species. Subsequently, the uptake and storage of PFASs and other POPs by plants have been proven to lead to unprecedented environmental and human risks. As plants with the potential to heal or manage certain ailments, such as Diabetes *mellitus* (DM), when exposed to PFASs, it was necessary to substantiate such a phenomenon.

This current study further determined the propensity of PFASs, such as PFOA, PFOS and PFBS, to accumulate in a plant commonly used in the management of DM, namely the African marigold (*Tagetes erecta* L.). The study was important as this plant is used in diabetes management in the Western Cape, South Africa, thus implying the plant being a pathway through which humans might be exposed to PFASs and its precursors. Accordingly, the target analytes of the study, PFOA, PFOS and PFBS, were identified and quantified in samples collected from the said plant, i.e. *Tagetes erecta* L., in contaminated river water used to irrigate the studied plant, as well as diabetic serum samples from patients likely to use the plant. The analysis was done using a liquid chromatography coupled with tandem mass spectrometry (Shimadzu LCMS-8030, Canby, OR, USA). The MS operational conditions were sourced with an MS interface electrospray ionisation in negative ion mode. A multiple reaction monitoring (MRM) mode of analysis was used to quantify the targeted PFASs in samples. Hence MRM transition for PFOA, PFOS and PFBS being of 413.00 > 368.95 (acquisition time: 8.6 min), 499.00 > 80.15 (8.9 min) and 299.00 > 80.10 (6.8 min), respectively. A Luna® Omega Polar C18 column (2.1 × 100 mm, 3.0 µm, Phenomenex, Aschaffenburg, Germany), with 40 °C in temperature, assisted in the separation of the analytes. The mobile phase at a flow rate of 0.3 L/min was made of 20 mM ammonium acetate and MeOH (100%). The process followed (for solid samples, i.e. plants) (n = 8) was: 1) sample drying, 2) milling, 3) screening, 4) digestion, 5) sonication, 6) filtration, 7) Solid phase extraction (SPE), 8) analyte elution and 9) analysis; for water samples (n = 20) the process was: 1) filtration, 2) SPE, 3) analyte elution and 4) analysis; while for serum samples (n = 179) the process was: 1) sample uptake, 2) buffers, 3) Mix, 4) centrifuge, 5) Dissolve, 6) filtration, 7) SPE, 8) conditioning, 9) elution, 10) reconstitute, 11) analysis.

PFOA, PFOS and PFBS were observed in all the plant samples and were found in concentrations of up to 94.83 ng/g, 5.03 ng/g, and 1.44 ng/g, for PFOA, PFOS and PFBS, respectively. Similarly, PFOA, PFOS and PFBS were identified in all the river water samples and were found in concentrations ranging between 1.15 to 107.82, 1.24 to 20.75 and ND to 0.06

ng/L for PFOA, PFBS and PFOS, respectively, for regime A (winter/wet season) and <LOQ to 4.35, 1.89 to 5.29, and <LOQ to 0.06 ng/L for PFOA, PFBS and PFOS, respectively, for regime B (summer/dry season). As the river water analysed in the current study showed concentration levels of PFOA, PFOS and PFBS in comparison to the studied plant (i.e.*Tagetes erecta* L.), the prevalence of these substances in river water samples which was used to irrigate the studied plant suggests that contaminated water sourced for plant irrigation purposes such as in impoverished communities in South Africa, will ultimately result in the irrigated plant's contamination. Hence, the bioconcentration factor (BCF) in the present study has indicated the African marigold's affinity to PFAS accumulation. The BCF for PFOA, PFOS and PFBS was in the range 0.48 to 2.52, 4.00 to 167.67 and 0.05 to 0.31, respectively. Thus, the studied plant, i.e. *Tagetes erecta* L., demonstrated a high bioaccumulation potential for PFOS.

Furthermore, PFOA, PFOS and PFBS were detected in all the serum samples (n = 179) of individuals suffering from DM, who are likely to use *Tagetes erecta* L. in order to determine whether there is a direct correlation between PFOA, PFOS, PFBS with known cases of DM. The patients are from a Bellville South population, in Cape Town, South Africa, who are of mixed-ancestry origin with the second highest prevalence of diabetes in South Africa. PFOA, PFOS and PFBS concentrations of up to 4.74, 0.77 and 1.27 ng/L were detected in males, respectively; and 10.73, 1.06 and 1.77 ng/L in females, respectively; with PFBS being the second most abundant PFAS in the sera, after PFOA; albeit, no significant association was found between the investigated PFASs and DM, but a significant correlation trend was detected between PFOA and individual anthropometric and biochemical measurements.

PUBLICATIONS

CONFERENCEPAPERS/JOURNALS/BOOK CHAPTERS

Conference proceedings

- Removal of Perfluoroalkyl Compounds using *Agave Sisalanan* Microporous Activated Carbon Fibre. "12th *International Phytotechnologies Conference*", from September 27 – 30, 2015. Manhattan, Kansas, USA
- Concentrations of Perfluorooctanoate and Perfluorooctane Sulfonate in Sediment of Western Cape Rivers, South Africa. "*11th International Phytotechnologies Conference*", from September 30 – October 3, 2014. Heraklion, Greece.
- Susceptibility of riparian wetland plants to perfluorooctanoic acid (PFOA) accumulation. "*10th International Phytotechnologies conference*", from 1 – 4 October 2013. State University of New York, USA.
- Perfluorooctanoate (PFOA) and Perfluorooctane sulphonate (PFOS) in South African river water. "*3rd Regional Conference of Southern African Young Water Professionals (YWP)*", from 16 – 18th July 2013. Stellenbosch, Western Cape, South Africa.

Articles published for/from this thesis

- **Mudumbi, J.B.N.**; Daso, A.P.; Okonkwo, O.J.; Ntwampe, S.K.O.; Matsha, T.E.; Mekuto, L.; Itoba-Tombo, E.F.; Adetunji, A.T.; Sibali, L.L. 2019. Propensity of Tagetes erecta L., a medicinal plant commonly used in diabetes management, to accumulate perfluoroalkyl substances. Toxics, 7, 18. DOI: 10.1007/s10661-018-6634-2
- **John Mudumbi**, Seteno Karabo Obed Ntwampe, Lukhanyo Mekuto, Tandi E. Matsha, Elie Fereche Itoba-Tombo. 2018. The role of pollutants in Type 2 Diabetes Mellitus (T2DM) and their prospective impact on phytomedicinal treatment strategies. Environmental Monitoring and Assessment 190: 262. https://doi.org/10.1007/s10661-018-6634-2
- **John Baptist Nzukizi Mudumbi**, Seteno Karabo Obed Ntwampe, Lukhanyo Mekuto, Elie Fereche Itoba-Tombo, Tandi E. Matsha, 2017, Are aquaporins (AQPs) the gateway that conduits nutrients, persistent organic pollutants and perfluoroalkyl substances (PFASs) into plants? Springer Science Reviews, 5(1–2), pp. 31–48, DOI: 10.1007/s40362-017-0045-6
- **John Baptist Nzukizi Mudumbi**, Seteno Karabo Obed Ntwampe, Tandi Matsha, Lukhanyo Mekuto, Elie Fereche Itoba-Tombo. 2017. Recent developments in polyfluoroalkyl compounds research: A focus on human/environmental health impact,

suggested substitutes and removal strategies. Environmental Monitoring and Assessment, 189(8), p.402.

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John B.N.Mudumbi

2019

DEDICATION

To My Late Wife

Veronica Mwange Koli Nyembo

To My Late Father

Mudumbi Kasunga (Paul)

To My Mom

Ntabanyere Maramuke M'Kahalalo (Espérance)

To my older brother and his wife

Ikong Mweze Mudumbi & Maman Jeanne Bashige

To the future Mother of My children

BIOGRAPHICAL SKETCH

John Baptist Nzukizi Mudumbi was born in Bukavu, in the Democratic Republic of Congo (DRC). He attended Kakumbo Primary School and matriculated from Nyamokola High School in 1996. During the same year, he enrolled at ISP Bukavu (l'Institut Supérieur Pédagogique de Bukavu) where he obtained a Bachelor of Education degree in Pedagogy Applied in Geography and Natural Sciences in 1999. He has taught both at primary and high school levels in Bukavu, DRC.

In 2006, he enrolled at Cape Peninsula University of Technology, Cape Town, South Africa and obtained a National Diploma in Environmental Management in 2008. In 2009 he completed a Bachelor of Technology degree in Environmental Management at the same University. He was awarded a Master of Technology degree in Environmental Management by the Cape Peninsula University of Technology in 2013 with the thesis being passed with a distinction.

He also completed a certificate in Environmental Law from the University of Pretoria in 2014. At the Cape Peninsula University of Technology, he has worked as a student assistant, student tutor, part-time lecturer, and lecturer and has co-supervised several Environmental Management and Biotechnology in-service training students for their research projects.

He enrolled for his doctoral degree in Environmental Health in 2013 under the supervision of Prof. Seteno Karabo Obed Ntwampe and Prof. Tandi E. Matsha.

His research was based on the potential of medicinal plants as the source of Polyfluoroalkyl substances intake in South Africa, from which four (4) articles have been published, and one (1) submitted and under review. He has published several peer-reviewed scientific papers in international journals and has presented his work at local and international conferences. He was awarded a merit scholarship during his tenure as a postgraduate student from the National Research Foundation of South Africa.

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GLOSSARY

Abbreviations	Definition
Å	ångström
AFFFs	Aqueous film forming foams
ammonia	NH_3
Ammonium	NH_4^+
AOPs	Advanced Oxidation Processes
AQPs	Aquaporins
As	arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
Ba	Barium
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BCF	bioconcentration factor
BFRs	Brominated flame retardants
BMF	Biomagnification factor
BMI	body mass index
Cd	Cadmium
Co	Cobalt
CO_2	Carbon dioxide
CPUT	Cape Peninsula University of Technology
Cr	Chromium
Cu	Copper
CVDs	cardiovascular diseases
DAF	dissolved air flotation
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEOS	Department of Environmental and Occupational Studies
diSAmPAP	Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamido) ethyl] phosphate
DM	Diabetes *mellitus*

DNA	Deoxyribonucleic acid
Dr.	Doctor
DRC	Democratic Republic of Congo
EDCs	endocrine disrupting chemicals
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
ER	endoplasmic reticulum
ESI	electrospray ionization
ESI	electrospray ionization
EtBr	ethidium bromide
EtFOSA	N-ethyl perfluorooctane sulfonamide
EtFOSE	*N*-ethyl perfluorooctane sulfonamido ethanol
Fe	Iron
Fe^{2+}	Fenton
FO	Forward Osmosis
FSAs	fluorotelomer sulfonaminds
FTOHs	fluorotelomer alcohols
FTs	Fluorotelomers
FTS	fluorotelomer sulphonic acids
GAC	Granular Activated Carbon
GIPs	GlpF-like intrinsic proteins
H_2O_2	hydrogen peroxide
HCB	Hexachlorobenzene
HDTMAB	hexadecyltrimethylammonium bromide
HIPs	hybrid intrinsic proteins
HIV	human immunodeficiency virus
HO*	hydroxyl radicals
HSO_5^-	peroxymonosulfate
IDF	International Diabetes Foundation
ISP	Institut Supérieur Pédagogique de Bukavu
ISTD	internal standard
ITRC	Interstate Technology and Regulatory Council Remediation

JICSTDA	Joint International Conference on Science and Technology for Development in Africa
K_{ow}	Octanol-water partition coefficient
LC	liquid chromatography
LC–MS/MS	liquid chromatography/tandem mass spectrometry
LN_2	liquid nitrogen
LOD	Limit of detection
LOQ	limit of quantification
LRT	Long range transport
MDB	Membrane Desalting Buffer
MDH	Minnesota Department of Health
MIPs	major intrinsic proteins
Mn	Manganese
MPa	megapascal
MPFNA	perfluoro-n-[1,2,3,4, 5-$^{13}C_5$] nonanoic acid
MPFOA	perfluoro-n-[1,2,3,4-^{13}C4] octanoic acid
MPFUnDA	perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid
MRM	multiple reaction monitoring
ND	Not Detected
NF	Nanofiltration
NHANES	National Health and Nutrition Examination Survey
Ni	Nickel
NIPs	nodulin 26-like intrinsic proteins
NPA	asparagine, proline, alanine
NRF	National Research Foundation
OECD	Organisation for Economic Cooperation and Development
PAC	powdered, activated carbon
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PBTs	persistent, bioaccumulative and toxicants
PCBs	Polychlorinated biphenyls
PCBs	Polychlorinated biphenyls
PCMAs	permanently-confined micelle arrays

PFASs	Per-polyfluoroalkyl substances
PFBS	Perfluorobutane sulfonate
PFCAs	Perfluorocarboxylic acids
PFCs	Polyfluoroalkyl compounds
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane Sulfonate
PFSAs	Perfluorosulfonic acids
PIPs	plasma membrane intrinsic proteins
p*K*a	acid-dissociation constant
POPs	Persistent organic pollutants
POU	Point-of-Use
PP	polypropylene
PPA	Point-of-use Plasma Abatement
RCF	root concentration factor
RNA	Ribonucleic acid
RO	reverse osmosis
RT	retention times
RW	River Water,
S/N	signal-to-noise
S1	Supplementary one
S2	Supplementary two
$S_2O_8^{2-}$	persulfate
S3	Supplementary three
S6	Supplementary six
SAmPAP	Sodium 2-(N-ethylperfluorooctane-1-sulfonamide) ethyl phosphate
SD	standard deviation
Se	Selenium
SF	selectivity filter
SIPs	small basic intrinsic proteins
SO^{*}_4	oxidative sulphate radical anions
SOCs	synthetic organic compounds
SPE	Solid phase extraction
SPM	suspended particulate matter
Sr	Strontium

ß-ME	ß-mercaptoethanol
T1D	type 1 diabetes
T2D	Type two diabetes
T2DM	type 2 diabetes *mellitus*
TIPs	Tonoplast intrinsic proteins
TSCF	transpiration stream concentration factor
TUT	Tshwane University of Technology
UNEP	United Nations Environment Programme
USA	United States of America
UV	Ultraviolet
w.w	wet weight
WCP	Western Cape Province
WCPs	Water Channel Proteins
WHO	World Health Organisation
XIP	uncategorized (X) intrinsic proteins
Zn	Zinc
ZVI	zero-valent ion

PREFACE TO THE THESIS

The research work presented in this dissertation was conducted at the Department of Environmental and Occupational Studies (DEOS); with the support and instrumentation from the laboratories of the Bioresource Engineering Research Group, the Department of Biotechnology, and the Department of Bio-Medical Sciences (all on the District six and Bellville campuses, respectively) of the Cape Peninsula University of Technology, as well as the Department of Environmental, Water and Earth Sciences, Faculty of Science, of the Tshwane University of Technology (TUT). These institutions are both located in South Africa, specifically in Cape Town and Pretoria, respectively.

This dissertation is presented in a format of fives (5) articles. Overall four (4) have been published in peer-reviewed journals (Mudumbi *et al*., 2017a, b; Mudumbi *et al., 2018;* Mudumbi *et al., 2019)*.

Chapter 1 gives a brief introduction of this research, the research questions, the objectives of the study, the significance and delineation of the research, as well as the dissertation framework.

Chapter 2 is the first section of the literature review published in 2017, thus overviewing the recent developments in per-and polyfluoroalkyl compounds (PFCs) research and their substitutes, including PFOA, PFOS and PFBS, and highlights the shortcomings and challenges in removing f these substances, as well as the environmental impacts of short-chain PFCs, previously regarded as harmless, in substitution of long-chain PFCs (Mudumbi *et al*., 2017a).

Chapter 3 covers the second section of the literature review published in 2017, which investigated the potential role that proteins such as AQPs play in facilitating the translocation and storage of POPs and other pollutants, such as PFCs, into plants (Mudumbi *et al*., 2017b)

Chapter 4 relates the third part of the literature review published in 2018, and surveyed the possible threat that these emerging POPs (e.g. PFCs) and heavy metals represent to the success of medicinal plants usage in the treatment of human ailments such as diabetes *mellitus* (Mudumbi *et al*., 2018).

Chapter 5 is the original research article published in 2019 and dedicated to the susceptibility of medicinal plants to PFCs, and suggests the potential of medicinal plants (e.g. *Tagetes erecta* L) as the pathway of PFCs into humans (Mudumbi *et al*., 2019).

Chapter 6 is the final version of a manuscript submitted for peer-review and dedicated to the susceptibility determination of diabetic patients to PFASs. Thus, known DM cases were analysed in this chapter to determine their PFASs concentration levels.

Chapter 7 provides conclusions of this study and further suggests recommendations for supplementary research to be conducted.

CHAPTER 1

Introduction

1.1 Introduction

Since the publication of the book titled "*Silent Spring*", in 1962, by Rachel Carson, a book that pedantically described how DDT (Dichlorodiphenyltrichloroethane) enters the food chain through bioaccumulation processes in soil, plants, and subsequent storage in the fatty tissue of animals, including human beings, numerous other persistent organic pollutants (POPs) remained undocumented. Thus, newly identified POPs have emerged during the current century, which have resulted in human health and environmental concerns, similar to those reported for DDT and polychlorinated biphenyls (PCBs).

Polyfluoroalkyl compounds (PFCs) have topped the list of these emerging POPs, and have been listed as such, ever since the Stockholm Convention (Wang *et al*., 2009; 2014). It has been indicated that, there are several hundred PFCs (and Ellis *et al*., 2004; Martin *et al*., 2006; Ahrens *et al*., 2009b). However, the most studied and documented had been perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) (Stahl *et al*., 2009; Ahrens *et al*., 2009b; Lechner and Knapp, 2011). Meanwhile, other studies have indicated the predominance of perfluorobutane sulfonate (PFBS) in various matrices (Ahrens *et al*., 2009a, 2009b; Möller *et al*., 2010), as it has similar human and environmental health consequences as those associated with PFOA and PFOS.

Polyfluoroalkyl compounds were anthropogenically manufactured since the 1950s (Renner, 2001; Ahrens, 2009), suggesting they do not occur naturally in the environment.

These compounds have hydrophilic (Lee, 2005), oleophobic (Han and Steckl, 2009) and hydrophobic (Chandler, 2005) properties, and are also moderately soluble in water (Möller *et al*., 2009). These properties have led to these compounds being used in various industrial

applications to manufacture products that humans heavily rely on (Kissa, 2001; Möller *et al*., 2009), including packaging products, paper, as well as leather, textile coatings, fire-fighting foam and cooking utensils. The prevalence of these compounds in soil (Stahl *et al*., 2009), sediment (Higgins and Luthy, 2006; Mudumbi *et al*., 2014c), bottled water (Heo *et al*., 2014), river water used for agricultural purposes (Mudumbi *et al*., 2014b), and plants (Stahl *et al*., 2009; Mudumbi *et al*., 2014a), have been the reason why PFCs (that is PFOA and PFOS) accumulate in the food chain, ending up in human body tissues (Fromme *et al*., 2009; Hanssen *et al*., 2010).

Thus, humans get exposed to PFCs via food and water consumption (Emmett *et al*., 2006a; Zhang *et al*., 2010; Heo *et al*., 2014), as well as the inhaled air (Harada *et al*., 2006; Kim *et al*., 2012; Hong *et al*., 2013; Dreyer *et al*., 2015). Additionally, various studies have indicated the distribution of PFCs in different plant compartments. For example, PFOA was found to be higher in vegetative compartments of potatoes, cucumbers and carrots than in other parts of the same crops (Lechner and Knapp, 2011), while in a similar study on wheat, the concentrations of PFOS and PFOA in roots were higher (Zhao *et al*., 2013). Correspondingly, PFCs distribution was indicated to be high in tomato root, leaf and stem, respectively, in a study by Felizeter *et al*. (2014). This suggests varying transportation mechanisms for different plants. According to recent studies, PFCs have been detected in various foodstuffs (Ericson *et al*., 2008; Schecter *et al*., 2010; Zhang *et al*., 2010; Noorlander *et al*., 2011) and vegetables (Clarke *et al*., 2010; Ji *et al*., 2012; Herzke *et al*., 2013; Lü *et al*., 2014; Zabaleta *et al*., 2014).

Moreover, there have been studies conducted on the uptake of PFCs by plants (Lechner and Knapp, 2011; Mudumbi *et al*., 2014a), most of which had positively detected these compounds in plants, including agricultural produce, suggesting that, PFCs can bioaccumulate in plants and plant-based products, and subsequently be ingested by humans. However, to our knowledge, there is very little scientific evidence to suggest plants (including agricultural produce) are a source of ingested PFCs in developing countries such as South Africa. This is also the case for medicinal plants and/or products, particularly for developing countries, such as South Africa, where these plants are commonly used (Davids *et al*., 2016). For instance, in the sub-Saharan African region, in particular, medicinal plants have played a major role in combating several diseases, including diabetes *mellitus* (DM) (Davids *et al*., 2016), due to prohibitive cost of orthodox medicine and the low income of the populations (Mounanga *et al*., 2015). This suggests phytomedicines to be more accessible and affordable by local communities in this African region (Mahomoodally, 2013).

Furthermore, little is also known on how living plants uptake PFCs, from contaminated soil and/or water. In other words, the mechanism used by plants to translocate and store PFCs in plant tissue and/or to the different plant compartments remains unclear, although preponderant studies reporting on the uptake of these pollutants by plants.

Additionally, Renner (2001) and Ostertag *et al*. (2009) have since indicated that, PFCs, particularly PFOA and PFOS, have caused environmental degradation and human health problems over the past decades. Thus, although, PFOA and PFOS have been the focus of most studies related to PFCs, it has been recently indicated that there are various types of PFCs, suggesting that the threat of these undocumented PFCs to the environment and humans at large still remains unknown.

Moreover, phytomedicines have gained tremendous attention recently due to their reputable medicinal benefits (Kim *et al*., 1999; Youn *et al.,* 2004; Eshun and He, 2004; Shibano *et al*., 2008; Bing *et al*., 2009). Irrespective of medicinal plants approval from overseers and users in particular, it has been debated that environmental contamination of these plants is a major concern (Street *et al*., 2008); for this reason, a recent study has suggested that, medicinal plants from which phytomedicine products are manufactured should be harvested from areas free of any contamination sources (Gjorgieva *et al*., 2010). In fact, a study by Fennell *et al*. (2004) has indicated that, although phytomedicinal products are widely assumed to be safe, many are potentially toxic. For example, a study in Macedonia investigated Barium (*Ba*), Chromium (*Cr*), Cadmium (*Cd*), Iron (*Fe*), Strontium (*Sr*), Lead (*Pb*), and Zinc (*Zn*) content in commonly used medicinal herbs -*Urtica dioica* L., *Taraxacum officinale*, and *Matricaria recutita* in two areas (that is a polluted and an unpolluted area). From the results, it was concluded that, quality assurance and monitoring of toxic metals should be conducted for plants intended for human use and consumption (Gjorgieva *et al*., 2010).

From a South African perspective, Street *et al*. (2008) mentioned that, herbal medicines are commonly harvested from the wild and consumed as such, with consumers ignoring and/or not being aware of the safety of these products, as industrial development has led to the contamination of water sources, such rivers (Mudumbi *et al*., 2014b), from which some of the medicinal plants are grown. In addition, various studies have reported on the prevalence of heavy metals, including PFCs, in the South African environment (Okonkwo and Mothiba, 2005; Mudumbi *et al*., 2014a, 2014b, 2014c). Similarly, it has been indicated that, poor farming methods, coupled with unregulated application of pesticides and fertilizers may lead to

phytomedicines being contaminated by recalcitrant contaminants, heavy metals, toxic substances and adulterants including PFCs (Chan, 2003; Street *et al*., 2008).

Furthermore, another study has suggested that, POPs have the potential to interact and induce several stress responses in the plants (Gjorgieva *et al*., 2013), producing metabolites that are deemed to have health benefits, such as antioxidants. Thus, a study was conducted to investigate POP stress on total antioxidants level in *Urtica dioica*, (also known as the Common Nettle) leaves and stems, a well-known medicinal plant. It was found that POP contents in stems changed synchronously with those in leaves of the plant, which led to imbalance of mineral nutrient elements and increased antioxidants in the plant (Gjorgieva *et al*., 2013). Consequently, the abovementioned study indicated that POP concentrations damaged the deoxyribonucleic acid (DNA) stability of the studied plant, which is *Urtica dioica* (Gjorgieva *et al*., 2013). Therefore, the mechanism allowing the transportation and subsequent storage of POPs, such as PFCs in medicinal plants must be investigated.

Additionally, plant proteins (that is Aquaporins-AQPs) play an important role in plant growth. For examples, AQPs and vacuoles are known for facilitating the transport nutrients and proteins in plants (Kaldenhoff and Fischer, 2006 and Chrispeels, 1991). Vacuoles are further known of storing organelles for sugars (Rausch, 1991), polysaccharides (Wagner *et al*., 1983), organic acids (Ting, 1985), and act as micro-kidneys inside each plant cell; suggesting they sequester potential toxic pollutants (Taiz, 1992). Thus, it has been indicated that, most of the flavours we get from fruits and vegetables are due to the compounds stored in the vacuoles (Taiz, 1992). This ultimately suggests that consumer intake of compounds stored in plant vacuoles, is a major exposure pathway of these compounds for humans – particularly if POPs are stored in these plants. As such, a study in Mali has further indicated high levels of toxic metals in commonly used plants for medicine and food purposes. In this study, metals such as *Zn, Cr*, Nickel (*Ni*), *Pd*, and Cooper (*Cu*) were found in seven medicinal and edible plants from the aforementioned country (Maiga *et al*., 2005). In addition, maximum concentration of *Cd* occurred in Pea (*Pisum sativum* L. cv. Azad) compartments, including roots, stems and leaves (Dixit *et al*., 2001). Most of these compounds have many common characteristics with new emerging POPs such as PFCs.

Subsequently, plant studies have been conducted, revealing plants predisposition to PFCs uptake (Stahl *et al*., 2009; Mudumbi *et al*., 2014a). However, to our knowledge, the mechanism employed by plants and which facilitates the uptake of PFCs by plants haven't been scientifically reported and documented. Additionally, the redundancy of phyto-

degradation of POPs as reported by Barac *et al*. (2004), suggests that non-biodegradable pollutants can easily be stored in medicinal plants and products, in particular POPs such as PFCs, suggesting a feasible intake route for humans, thus increase the risk of diseases such as diabetes *mellitus* (DM). At this stage, there are no information including the link between consumption of PFC contaminated medicinal plants - PFCs in overweight human sera – and DM, from a South African perspective, where it has recently been demonstrated diabetic patients have used medical plants as a therapy (Davids *et al*., 2016). This includes the link between AQPs in medicinal plants/products and concentration of POPs such as PFCs.

1.2 Research questions

It is hypothesised that the concentration of PFCs (that is PFOA, PFOS and PFBS) is high in medicinal plants and products, and this suggests these plants might be a potential PFCs human exposure pathway, consequently linking PFCs to diseases such as diabetes. Furthermore, it is hypothesised that there is a direct link between PFC levels in overweight humans, their propensity to consume medicinal plants/products including their PFC transportation/storage mechanisms and DM. Therefore, this study will subsequently answer the following questions:

a) What are the current developments surrounding emerging POPs, including PFASs?
b) Is there a correlation between studied medicinal plant's consumption and the prevalence of PFOA, PFOS and PFBS in these plants?
c) Which PFAS is more predominant in the selected studied medicinal plant?
d) Does the plant's root system, on its own, sufficiently explain its uptake of chemical substances?
e) Do AQPs facilitate the dissemination of chemical compounds in plants?
f) Are there any variations in the concentrations of the identified PFCs contaminants in the selected and studied medicinal plant?
g) What are the possible health threats or risks of medicinal plants being contaminated by PFCs?
h) Is there a correlation between PFCs concentrations and blood samples of individuals diagnosed with diabetes?
i) What are the concentration variation levels of PFCs in the sera of non-diabetic and diabetic individuals?
j) What is the relationship between age, gender, body weights and PFCs prevalence in blood samples?

1.3 General objectives

The following were the objectives of the proposed study:

- To quantify PFOA, PFOS and PFBS in a common medicinal plant used for Diabetic *mellitus* (DM) management in South Africa, and
- To determine the plant's vulnerability to accumulate PFOA, PFOS and PFBS when irrigated with PFC-contaminated river water,
- To elucidate the role of AQPs in PFCs uptake by medicinal plants, and
- To determine whether there is a direct link between sera PFCs (that is PFOA, PFOS and PFBS) concentration in DM sufferers and their anthropometric and biochemical measurements.

1.4 Significance of the research

Most studies on medicinal plants have focused typically on their healing properties. Currently the focus has been orientated on the market values of products made from these plants. No studies have been conducted, in South Africa in particular, and internationally, in general, on the prevalence of emerging pollutants, such as PFCs, in medicinal plants/products and the impact that this might cause on individuals who rely on products made from these crops. Furthermore, there is limited information on the prevalence of PFCs in diabetic patients, which is one of the primary focuses of this study.

1.5 Delineation of the research

The focus of the proposed study will be the analysis and quantification of PFOA, PFOS and PFBS in selected South African traditional medicinal plants, and how AQPs influence the uptake of these compounds by these plants. Furthermore, the study will look into the association between PFCs (that is PFOA, PFOS and PFBS) and body weight diseases, such as DM, and the environmental impacts in relation to phytomedicinal product use. This study will not cover the healing ability of the selected medicinal plant, the sources of PFCs present in the selected medicinal plant, the causes of diabetes, or the quantification and functions of AQPs present in the selected medicinal plant.

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CHAPTER 2

Recent developments in polyfluoroalkyl compounds research: a focus on human/environmental health impact, suggested substitutes and removal strategies

Mudumbi *et al*., *Environmental Monitoring and Assessment*, 189: 402;

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2.1 Abstract

Between the late 1940s and early 1950s, humans manufactured polyfluoroalkyl compounds (PFCs) using electrochemical fluorination and telomerisation technologies, whereby hydrogen atoms are substituted by fluorine atoms, thus conferring unnatural and unique physicochemical properties to these compounds. Presently, there is a wide range of PFCs, and owing to their bioaccumulative properties, they have been detected in various environmental matrices and in human serum, but also in other types of human samples. It has thus been suggested that they are hazardous. Hence, this review aims at highlighting the recent developments in PFC research, with a particular focus on perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), the most studied and predominantly found PFCs in various environmental matrices. We also included perfluorobutane sulfonate (PFBS), which was previously regarded as innocuously harmless, when compared to its counterparts, PFOA and PFOS. As such, proper investigations are thus required for a better understanding of short-chain PFC substitutes, which have been suggested as suitable replacements to long-chained PFCs, although these substitutes have also been suggested to pose various health risks comparable to those associated with long-chain PFCs. Similarly, several novel

technologies, such as PFC reduction using zero-valent iron, including removal at point of use, adsorption and coagulation, have been proposed. However, regardless of how efficient removers some of these techniques have proven to be, short-chain PFCs remain a challenge for scientists to overcome.

Keywords: Polyfluoroalkyl compounds, PFOA, PFOS, PFBS, Substitutes.

2.2 Introduction

Polyfluoroalkyl compounds (PFCs) are a wide assortment of anthropogenic chemicals, manufactured between the late 1940s and early 1950s (Niu *et al*., 2016) using electrochemical fluorination and telomerisation (Benskin *et al*., 2012; Banks *et al*., 2013). Thus, $F(CF_2)_xR$ is regarded as the general molecular formula for these chemicals, with two distinctive subsets characterising them; namely, PFCs, in which the head group contains no C-H bonds and fluorotelomers (FT) in which the R-group contains an even-numbered alkyl-chains, resulting in the general formula of $F(CF_2)_x(CH_2\text{-}CH_2)_yR$ and $F(CF_2)_x(CH{=}CH)_yR$) (Møskeland, 2010). Table 2.1 provides a general illustration of PFCs that have been of interest for the global scientific community.

Table 2.1: PFCs of interest, including their chemical structures and general formula (Butt *et al*., 2014; Kwon *et al*., 2016; Zhou *et al*., 2016)

Class	Compound	Abbreviation	General formula
Polyfluorinated sulfonamides (FSAs)	*N*-methyl perfluorobutane sulfonamidoethanol	NMeFBSE	$F(CF_2)_4SO_2N(CH_3)\ CH_2CH_2OH$
	N-ethyl perfluorobutane sulfonamidoethanol	NEtFBSE	$F(CF_2)_4SO_2N(CH_2CH_3)\ CH_2CH_2OH$
	Perfluorooctane sulfonamide	PFOSA	$F(CF_2)_8SO_2NH_2$
	N-methyl perfluorooctane sulfonamide	NMeFOSA	$F(CF_2)_8SO_2N(CH_3)H$
	N-ethyl perfluorooctane sulfonamide	NEtFOSA	$F(CF_2)_8SO_2N(CH_2CH_3)H$
	N-methyl perfluorooctane sulfonamidoethanol	NMeFOSE	$F(CF_2)_8SO_2N(CH_3)\ CH_2CH_2OH$
	N-ethyl perfluorooctane sulfonamidoethanol	NEtFOSE	$F(CF_2)_8SO_2N(CH_2CH_3)\ CH_2CH_2OH$
Fluorotelomer Alcohols (FTOHs)	4:2 fluorotelomer alcohol	4:2 FTOH	$F(CF_2)_4CH_2CH_2OH$
	6:2 fluorotelomer alcohol	6:2 FTOH	$F(CF_2)_6CH_2CH_2OH$
	8:2 fluorotelomer alcohol	8:2 FTOH	$F(CF_2)_8CH_2CH_2OH$
	10:2 fluorotelomer alcohol	10:2 FTOH	$F(CF_2)_{10}CH_2CH_2OH$
	12 :2 fluorotelomer alcohol	12 :2 FTOH	$F(CF_2)_{12}CH_2CH_2OH$

Table 2.1: *Continues*

Perfluorosulfonates (PFSAs)	Perfluorobutane sulfonate	PFBS	$F(CF_2)_4SO_3^-$
	Perfluorohexane sulfonate	PFHxS	$F(CF_2)_6SO_3^-$
	Perfluorooctane sulfonate	PFOS	$F(CF_2)_8SO_3^-$
	Perfluorodecane sulfonate	PFDS	$F(CF_2)_{10}SO_3^-$
Perfluorocarboxylates (PFCAs)	Perfluorohexanoate	PFHxA	$F(CF_2)_5CO_2$-
	Perfluoroheptanoate	PFHpA	$F(CF_2)_6CO_2^-$
	Perfluorooctanoate	PFOA	$F(CF_2)_7CO_2^-$
	Perfluorononanoate	PFNA	$F(CF_2)_8CO_2^-$
	Perfluorodecanoate	PFDA	$F(CF_2)_9CO_2^-$
	Perfluoroundeconate	PFUA	$F(CF_2)_{10}CO_2^-$
	Perfluorododecanoate	PFDoA	$F(CF_2)_{11}CO_2^-$
	Perfluorotridecanoate	PFTriA	$F(CF_2)_{12}CO_2^-$
	Perfluorotetradecanoate	PFTetA	$F(CF_2)_{13}CO_2^-$
	Perfluoropentadecanoate	PFPA	$F(CF_2)_{14}CO_2^-$
	Perfluorohexadecanoate	PFHxDA	$F(CF_2)_{15}CO_2^-$

Table 2.1: *Continues*

Fluorotelomer carboxylates (FTCAs, FTUCAs)	6:2 fluorotelomer carboxylate	6:2 FTCA	$F(CF_2)_6CH_2CO_2^-$
	6:2 fluorotelomer unsaturated carboxylate	6:2 FTUCA	$F(CF_2)_6CHCO_2^-$
	8:2 fluorotelomer carboxylate	8:2 FTCA	$F(CF_2)_8CH_2CO_2^-$
	8:2 fluorotelomer unsaturated carboxylate	8:2 FTUCA	$F(CF_2)_8CHCO_2^-$
	10:2 fluorotelomer carboxylate	10:2 FTCA	$F(CF_2)_{10}CH_2CO_2^-$
	10:2 fluorotelomer unsaturated carboxylate	10:2 FTUCA	$F(CF_2)_{10}CHCO_2^-$
Fluorotelomer sulfonates (FTSs)	6:2 fluorotelomer sulfonate	6:2 FTS THPFOS	$F(CF_2)_6CH_2CH_2SO_3^-$
	8:2 fluorotelomer sulfonate	8:2 FTS	$F(CF_2)_8CH_2CH_2SO_3^-$
	10:2 fluorotelomer sulfonate	10:2 FTS	$F(CF_2)_{10}CH_2CH_2SO_3^-$

Moreover, there are various PFCs, of which two types have been widely utilised by a variety of industries. These are perfluorocarboxylic acids (PFCAs), identifiable by their structures, $F(CF_2)_xCOOH$, and perfluorosulfonic acids (PFSAs), $F(CF_2)_xS(O_3)H$. These PFCAs and PFSAs are acids which are readily ionised and thus can be negatively charged due to the loss of a proton, leading to their being referred to as perfluorocarboxylates and perfluorosulfonates, respectively (Schröter-Kermani *et al*., 2013). The most researched and reported of these compounds, particularly in ecotoxicology studies, are perfluorooctanoic acid (PFOA, $F(CF_2)_7COOH$) and perfluorooctosulfonic acids (PFOS, $F(CF_2)_8S(O_3)H$) (Mudumbi *et al*., 2014a,b,c; Zhao *et al*., 2015; Shoeib *et al*., 2016; Yang *et al*., 2016). Recently, perfluorobutane sulfonate (PFBS, $C_4HF_9O_3S$) has also been suggested to be a persistent organic pollutant (POP) once it enters the environment (Zhao *et al*., 2015; Shoeib *et al*., 2016; van den Dungen *et al*., 2016). In production techniques for these fluorocarbons, the substitution of hydrogen atoms by fluorine atoms from suitable precursors allows for the conferring of particular physicochemical properties to these compounds (Hidalgo and Mora-Diez, 2016), such as chemical stability, non-wetting, fire, including weather resistance, and hydrophobicity and oleophobicity. They can lower the surface tension of viscous matrices, are irradiation-resistant and biologically non-biodegradable (Ludwicki *et al*., 2015; Bennett *et al*., 2015; Niu *et al*., 2016), thus, persist in the environment.

2.3 Molecular structure of polyfluoroalkyl compounds

Polyfluoroalkyl compounds are characterised by a perfluorinated carbon chain coupled with one special functional group at the end of the molecular chain, which can be either a carboxylic (-COOH) or a sulfonic group. The fluorinated carbon chain of PFCs directly influences their hydrophobicity, while the functional group permits the molecules to be hydrophilic. Different functional groups have shown diversified behaviour once introduced into different environments (Senevirathna, 2010). Thus, some PFCs, that is, predominantly PFOA and PFOS, have been detected in various environmental matrices; although current research has abundantly indicated that other PFCs, such as PFBS, should not be ignored.

2.4 Diversified application of polyfluoroalkyl compounds

Since PFCs have been manufactured for various applications due to their unique physical properties (Hagenaars *et al*., 2011), to date, numerous industries have used these molecules as building blocks to form fluorinated polymers such as perfluoralkylpolymers.

These polymers should not be confused with fluoropolymers, such as polytetrafluoroethylene, that is, Teflon™, which are aliphatic compounds (Møskeland, 2010; Ebnesajjad, 2013). In certain cases, PFCs are used in the manufacturing process of fluoropolymers and later appear as residues in the final product (Herzke *et al*., 2007; Møskeland, 2010). This, in our opinion, has diversified their utilisation, which exacerbates their prevalence, even in areas presumed free of such contaminants, for example the Canadian Arctic region (Butt *et al*., 2008).

PFC-generated fluoropolymers are used as additives in hydraulic fluids, photographic emulsifiers and paints, to lower their surface tension, and/or as coating in carpets, and textiles to allow stain and water repellency (Herzke *et al*., 2007; Møskeland, 2010; Martens, 2013). Furthermore, an exceptional and important application of PFCs has been in specialised aqueous film-forming foams (AFFFs) due to their ability to form films even at high temperatures, a requirement when extinguishing fires (Place and Field, 2012; Sha *et al*., 2015). Due to their versatility, various other industrial applications and processes have since been developed, thus giving rise to new products such as lubricants and motor oil additives, sports clothing, medical equipment, extreme weather military uniforms, and waterproof breathable fabrics (Bao *et al*., 2014; Wang *et al*., 2014a,b; Niu *et al*., 2016). PFCs have also been used as polymerisation aids in the production of components for electronic products (Senevirathna, 2010). Therefore, such diversified applications of these materials can result in far-reaching consequences, including consistent and prolific release, as well as transportation into living organisms. Table S1 and S2 highlight various polymers and non-polymers which have been extensively used in several industry applications worldwide (provided as supplementary material, together with Table S3-S6).

2.5 Polyfluoroalkyl compounds in the environment: discharge, transportation, occurrence and persistence

2.5.1 Discharge of PFCs directly into the environment

As a result of excessive use, PFCs have found ways into the environment. As such, it has been reported that PFCs are discharged into the natural environment both directly and indirectly (Wang *et al*., 2014a, b, 2015a, b). Thus, direct discharge has been regarded as the primary mechanism by which PFCs enter the environment from their life cycle (that is, manufacture, usage and disposal) when assessing their products, derivatives, residues or as

unintentional by-products, that is, impurities in consumer products (Li *et al*., 2015; Kotthoff *et al*., 2015). Their indirect discharge is suggested to be through transformation and/or degradation resulting in their presence in wildlife, and humans (Guzmàn *at al*., 2016; Gomis *et al*., 2016); as well as from fluorotelomer-made products through abiotic or biotic processes (Butt *et al*., 2014).

It has further been indicated that PFCs and their by-products, including precursors, may enter the environment via various other routes, such as (a) spilled discharge or through solid waste, for example exhaust/fuel gases from combustion, domestic wastewater, sludge, and from manufacturing premises (Li *et al*., 2015; Kwon *et al*., 2016; Bečanová *et al*., 2016); (b) either by volatilisation along the supply chain from manufacturers to downstream industrial or end-consumers (OECD, 2013; Oliaei *et al*., 2013); (c) or through fugitive release by end-users, especially where PFCs containing products (for example, fluoropolymer manufacturing sites, paper and textile factories) including their precursors have been processed into final products (Kotthoff *et al*., 2015). Furthermore, their incorporation into raw materials/consumer products can result in their wash-off directly into the environment (Kotthoff *et al*., 2015; Bečanová *et al*., 2016). In most cases, unsuitable treatments methods are applied. For instance, the use of sewage sludge as a fertiliser, untreated outgassing from landfills or insufficient wastewater treatment, can further exacerbate contamination of PFC-free environments or the food chain (Gallen *et al*., 2016; Kwon *et al*., 2016).

2.5.2 Occurrence, transportation and persistence of polyfluoroalkyl compounds

Polyfluoroalkyl compounds, especially PFOA and PFOS, have been known to display both persistence and long-range transportation (LRT) once they have entered the environment. This has been confirmed by their ubiquitous presence in various environmental matrices far away from anthropogenic activities (Stock *et al*., 2010). However, the fact that PFCs have different properties than their counter parts, that is BFRs and PCBs for which models of environmental persistence and LRT have been developed, result in the complexity of developing suitable models for their persistence and LRT, which can conclusively explain the mechanisms of how PFCs are transported in the environment (Møskeland, 2010). This is because PFCs (that is PFOA and PFOS) are strong ionic acids and surface wetting agents, as opposed to being hydrophobic apolar compounds, characteristics associated with BFRs and PCBs (Fliedner *et al*., 2012). The p*K*a (or acid-dissociation constant) of these substances has been estimated to be near 0 for PFCAs, for example, PFOA, and around -3 for PFSAs, for

example PFOS (Campbell *et al*., 2009; Møskeland, 2010), making them some of the most effective surfactants.

Additionally, it has been indicated that the perfluoroalkyl tail of these substances is one of the most hydrophobic molecular fragments and anionic/acidic functional groups (CO_2^-, SO_3^-). Consequently, it has been suggested that PFCAs and PFSAs have a strong affinity to water with a hydrophilic head, whereas the rest of the molecule is hydrophilic (Xiao *et al*., 2013). Thus, these molecules are likely to have a high LRT in the environment through water transportation (for example, by dispersion in lakes and rivers, including sorption to atmospheric moisture) as previously indicated by some studies (Schindler *et al*., 2013; Shan *et al*., 2015; Kirchgeorg *et al*., 2016).

According to Yao *et al*. (2015) and Guo *et al*. (2015), the uniqueness of PFCs and the mechanism of their transportation into the environment has remained an active area of research; and for this reason, part of the recommendations proposed include scientists being able to deal with the unique environmental transportation and partitioning processes of PFCs; that is, that researchers need an additional set of model parameters to account for the ionic and surfactant nature of these compounds, their p*K*a (the acid-dissociation constant), surface-water sorption coefficients, including their critical micelle and aggregate-formation concentrations (Zhou *et al*., 2010a; Zareitalabad *et al*., 2013).

Recent research has since reported the distribution of PFCs globally (Rankin *et al*., 2015; Washington and Jenkins, 2015a; Routti *et al*., 2015). Overall, PFCs have been found in surface river waters, or alternatively, in wastewater treatment plants in South Africa (Mudumbi *et al*., 2014b; Adeleye, 2016; Chen *et al*., 2016; Pitarch *et al*., 2016; Shiwaku *et al*., 2016; Lopez *et al*., 2015; Lescord *et al*., 2015; Lu *et al*., 2016; Hu *et al*., 2016; Zhang X *et al*., 2016). It has also been indicated that water currents and evaporation/precipitation have facilitated the transportation of these substances into remote areas, such as the arctic, remote islands and other remote inland environments, for example alpine lakes, etc. (Lescord *et al*., 2015 ; Wang Z *et al*., 2015 ; Yamazaki *et al*., 2016). Additionally, evidence suggests that among all environmental media, the ocean is likely to be the largest global reservoir of PFCs such as PFOA (Cousins *et al*., 2011), thus, inland deposition through the water cycle is inevitable.

PFOA and PFOS have dominated most reports, with PFOS being found in higher concentration levels, that is, 271.10 g/L (Llobregat river water), in a recent study from Spain (Campo *et al*., 2015). Moreover, PFBS has recently received attention among the list of PFCs

with researchers believing that it should not be overlooked, with Zhou *et al*. (2013) indicating that although PFBS has a lower adsorption potential than PFOA and PFOS, which suggests its lower potential to bioaccumulate in aquatic biota, its aquatic and ecological risk must be assessed, because of the substance's increasing usage, release and transportation.

Over the past two decades, research projects have demonstrated the susceptibility of living organisms to PFCs. They have thus been detected in human sera (Ludwicki *et al*., 2015; Shrestha *et al*., 2015), in animals (Filipovic *et al*., 2015; Koponen *et al*., 2015), and plants (Mudumbi *et al*., 2014c; Blaine *et al*., 2014a b; D'Hollander *et al*., 2015; Yang *et al*., 2015). Additionally, some reports have indicated that, although fluorotelomer alcohols (FTOHs), fluorotelomer sulfonamindes (FSAs) and fluorotelomer sulphonic acids (FTS) have been regarded as the most substantial PFC precursors, several hundred other PFCs are considered capable of conversion into PFCAs and PFSAs (Gomis *et al*., 2015; Sun *et al*., 2016). Additionally, precursors to PFCs, such as FTOHs, are volatile and can be released from products under ambient conditions and later be transformed into PFCs (EPA, 2014). As a result, it has been argued that the occurrence of PFCs and its salts is not only due to direct release of these compounds into the environment, but is also due to the indirect conversion of many other PFCs (Kim *et al*., 2015). It has also been indicated that both direct and indirect sources of these compounds were considered in multimedia models that account for the occurrence of these substances (Kim *et al*., 2015; Gomis *et al*., 2015), with the modelling of PFOA distribution and its higher homologues being reported in a review (Cousins *et al*., 2011). The models were generally found to support the conclusion that direct use of PFOA and PFOS-based products was the dominant global environmental contributor for these two PFCAs (OECD, 2013).

2.5.3 Polyfluoroalkyl compounds' precursors of concern

Various reports have suggested that PFCs enter the environment by either direct or indirect sources. Direct sources are regarded as the discharge of PFCs into the environment as such, regardless of whether it is intentional release or otherwise (Buck *et al*., 2011; Liu, 2015); while indirect sources imply the formation of PFCs by means of biotic or abiotic degradation from other perfluoroalkyl and polyfluoroalkyl substances (PFASs), regarded, in this case, as precursors to PFC (pre-PFCs), as they enter various environmental mediums (Buck *et al*., 2011; Liu, 2015). Thus, researchers believe the indirect sources play a significant role in the prevalence of PFCs in humans and the environment (Benskin *et al*., 2013; Lee *et al*., 2014; Liu, 2015; Avendaño and Liu, 2015). The OECD released a list of 615 pre-PFCs that have the

potential to degrade into PFCA (OECD, 2007). Table S3 depicts examples of these types of substances, and most of which there is limited data available on their pathways into the environment.

Furthermore, examples of pre-PFCs have included mono-and di-esters such as Sodium 2-(N-ethylperfluorooctane-1-sulfonamide) ethyl phosphate (SAmPAP), Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamido) ethyl] phosphate (diSAmPAP), N-ethyl perfluorooctane sulfonamide (EtFOSA), etc. According to Wellington (2014), not only are SAmPAP esters persistent in the ecosystem, but they are precursors of PFOS, and very little evidence is available on their lifetime and transformation. Hence, it has been indicated that most PFAS-containing products that humans rely on daily contain pre-PFCs (Herzke *et al*., 2012; Gebbink *et al*., 2013; Liu, 2015), which, according to available data, have not been investigated (Wellington 2014; Liu, 2015), suggesting a potential threat to consumers. For instance, the PFOS-precursor EtFOSA is used in the manufacturing of sulfluramid, a pesticide for controlling leaf-cutting ants (Löfstedt Gilljam *et al*., 2015). Ultimately, this explains why PFOS has largely been detected in the environment, with its plant concentration levels higher in certain countries, like in South Africa (Mudumbi *et al*., 2014b), where agriculture is an integral part of the economy. Similarly, a lengthy biodegradation half-life of *N*-ethyl perfluorooctane sulfonamido ethanol (EtFOSE), another pre-PFOS, and recalcitrant nature of SAmPAP were recently reported by Benskin *et al*. (2013), and which, according to the authors, explains the elevated concentrations of PFOS-precursors in the environment. However, it is argued that clarity is needed on whether SAmPAP can be a potential significant source of PFOS in benthic and higher trophic level organisms (Benskin *et al*., 2013). It has been further suggested that the development of enhanced (i.e., residual-free) SAmPAP standards would be of great assistance to scientists who assess the stability and environmental behaviour of these substances (Benskin *et al*., 2013).

On the other hand, recent data has revealed the potential of fluorotelomer-based polymers to degrade and to form PFOA and related compounds (Washington *et al*., 2015b). Hence, researchers have suggested that elevated concentrations of pre-PFCs observed in studied samples explain the large distribution of PFCs in the natural environment and beyond, i.e. to areas far from their production (Benskin *et al*., 2013; Washington *et al*., 2015b), and these precursors thus might constitute the major sources of PFOA, PFOS, etc. (Washington and Jenkins, 2015a) but have also called for more investigations to be conducted (Washington *et al*., 2015b).

2.5.4 Bioaccumulation of PFCs in biota and humans

Bioaccumulation potentials are estimated using what is known as the partition coefficient (K_{ow}) between octane-water phases (OECD, 2013). However, because PFCs are surfactants, an emulsion can be formed during measurements. It has been reported that K_{ow} is unknown for most PFCs (OECD, 2013). Therefore, to determine the bioaccumulation potential of PFCs in environmental media, either a bioaccumulation factor (BAF) or a bioconcentration factor (BCF), which is the extent to which pollutants concentrate from water into other matrices (Chiou, 2003), can be estimated by dividing the average concentrations in matrices by the concentrations of PFCs in a water environment (Senevirathna, 2010). BAF or BCF should not be confused with biomagnification factor (BMF) used to refer to the ratio of contaminant concentration in biota to that in the surrounding water when the biota was exposed via contaminated food (Nowell *et al*., 1999). It is determined by dividing the average concentrations in predators to those in prey (Senevirathna, 2010).

As a result, BMF has been quantified globally in various species, particularly in fish (Lescord *et al*., 2015; Ahrens *et al*., 2015; Hong *et al*., 2015; Bossi *et al*., 2015; Svihlikova *et al*., 2015, Ahrens *et al*., 2016), polar bears (Letcher *et al*., 2014; Jenssen *et al*., 2015), including albatross (Chu *et al*., 2015), and seals (Routti *et al*., 2015), to name a few, with results indicating that, long chained PFCs are bioaccumulative (Kakuschke and Griesel, 2016; Zhai *et al*., 2016), and can ultimately biomagnify in the food chain (Zhang *et al*., 2015; Franklin, 2015) and in humans (Fujii *et al*., 2015; Goudarzi *et al*., 2016). Table S4 reports on the bioaccumulation potential (BMF) of selected PFCs in certain aquatic organisms.

As such, various PFSAs and PFCAs have been detected in human sera in the general population (Bennett *et al*., 2015; Gomis *et al*., 2016) of which PFOA, PFOS and PFBS are the most frequently detected substances (Li *et al*., 2011; Arbuckle *et al*., 2013; Bao *et al*., 2014; Zeng *et al*., 2015, Lorber *et al*., 2015), with both PFOA and PFOS having an estimated 1000 days residence time in human blood (OECD, 2013). Nevertheless, uncertainties remain among scientists as to what the possible health effects on humans, exposed to PFCs could be, since, of the PFCs that have been found to accumulate in the human body, the levels of accumulation have been seen decreasing slowly over time (ATSDR, 2015, 2016). Conversely, available data have indicated that the ability of PFCs to bioaccumulate in the human body, also referred to as body burden, has increased concerns about the possibility of these compounds to cause detrimental health effects in humans (ATSDR, 2015, 2016). Hence, a number of human studies

have reported that certain PFCs may affect foetus and child development, including child growth, learning and behaviour (Ek *et al*., 2012; ATSDR, 2015, 2016); while others have found inconsistent associations between PFOA or PFOS serum levels and changes in reproductive hormone levels (Raymer *et al*., 2012; Specht *et al*., 2012; Joensen *et al*., 2013). On the other hand, conflicting results were found in studies investigating the association of sperm parameters (Toft *et al*., 2012; Raymer *et al*., 2012; Joensen *et al*., 2013) and impaired fertility (Fei *et al*., 2012; Vestergaard *et al*., 2012; Whitworth *et al*., 2012). Similarly, evidence has further indicated that exposure to PFCs, such as PFOA, increases cholesterol (Frisbee *et al*., 2010; Eriksen *et al*., 2013), and affects the immune system (ATSDR, 2015, 2016). In addition, increases in the incidence of prostate, kidney, and testicular cancers have been reported in workers and communities living near PFCs manufacturing facilities (ATSDR, 2015). Nonetheless, there are limited data on whether PFCs exposure can cause cancer in humans, suggesting that more research is needed in this regard. Additionally, reproductive toxicity studies have also revealed a possible associations between serum PFC levels and changes in reproductive hormone levels in men. Nevertheless, there has been inconsistencies in the reported results. For instance, Raymer *et al*. (2012) found significant positive correlations between PFOA levels and free testosterone and LH levels, but not with other reproductive hormones; while, in a similar study by Joensen *et al*. (2013) no significant associations between reproductive hormone levels and serum PFOA, and other PFCs, such as PFHxS, or PFHpS were found. In contrast, no associations between serum PFOS levels and reproductive hormones were found by Raymer *et al.* (2012); while, a significant negative correlation between PFOS and testosterone, free testosterone, and free androgen levels was found by Joensen *et al*. (2013) in young men. Table S5 provides a brief toxicological summary of available epidemiological data present in the reviewed literature on reproductive effects in humans exposed to PFCs.

Furthermore, even though PFCs have been studied in a number of human epidemiological studies and their prevalence reported in human tissues, including blood samples (Genuis *et al*., 2013), there are still no reports of human deaths from accidental or intentional acute exposure to high concentrations of PFOA or PFOS (ATSDR, 2015). However, most studies have indicated the potential associations between mortality and long-term exposure to these substances. For example, a study by Alexander *et al*. (2003) found no death increases from all causes led by being exposed to PFOS, and Leonard *et al*. (2008) indicated the same for all illnesses related to PFOA's exposure.

2.5.5 Polyfluoroalkyl compounds pathways into humans

The presence of chemical compounds in the environment does not automatically translate into human exposure. Typical exposure depends on a number of parameters, including, but not limited to, the degree of exposure. Thus, a growing body of evidence suggests that, human exposure to PFCs and their potential precursors can be divided into three major categories, namely, occupational exposure, general human exposure, and exposure from mother to foetus or infants.

2.5.5.1 Occupational exposure

This form of exposure occurs during the performance of normal and legally delegated job requirements/responsibilities. Thus, workers in facilities that manufacture PFCs or in the formulation and production amenities that use products containing PFCs, direct exposure is through the handling of these preparations, having contact with processing liquids, wastewater or treated products, or when carrying out maintenance, sampling, testing, or other procedures. For example, high level of PFOS and PFOA were found in workers at PFCs production sites (Freberg *et al*., 2010; OECD, 2013).

2.5.5.2 General human exposure

A growing body of scientific evidence has also revealed that, general human exposure to PFCs and its precursors occurs by way of (i) indoor and outdoor air and aerosols, (ii) contaminated drinking water, (iii) food, and (iv) dust (D'Hollander *et al*., 2014, 2015; Pérez *et al*., 2014; Duong *et al*., 2015; Brambilla *et al*., 2015; Filipovic *et al*., 2015; Koponen *et al*., 2015; Liu *et al*., 2015; Schlummer *et al*., 2015). Accordingly, PFCs and their precursors can be found in various food items (Post *et al*., 2012; Yeung *et al*., 2013; OECD, 2013). In addition, it has been argued that, exposure via dust particles might be a minor exposure pathway for adults in comparison to dietary intake (Xu *et al*., 2013; OECD, 2013), although, it may be a significant pathway for infants and toddlers (Fromme *et al*., 2009; D'Hollander *et al*., 2010; OECD, 2013). Overall, tap water and agricultural produce, irrigated with contaminated river water, have been found to be a significant source of exposure for humans (Tabtong *et al*., 2015; Chen S et al., 2016; Hurley *et al*., 2016). Recent research has indicated that paper and packaging for food, as well as different materials used for food contact, play a contributory role in the

contamination of food from PFCs (Surma *et al*., 2015; Shoeib *et al*., 2016). Table 2.2 depicts evidence of PFCs in wrappers from different food contact paper, food brands and beverages.

Table 2.2: Evidence of PFC content in fast food wrapper (Schaider *et al*., 2017)

	Brands tested (*n*)	**Samples tested (*n*)**	**PFC content (%)**
Food contact wrapper (by type)			
Sandwich/burger	20	138	38
Dessert/bread	9	69	56
Tex-Mex	3	42	57
Food contact wrapper (all)	27	248	46
Food contact paperboard	15	80	20
Noncontact paper	9	15	0
Paper cups	9	30	0
Other beverage containers	10	25	16
Miscellaneous	7	9	0

2.5.5.3 Foetal and/or infant exposure to PFCs

The exposure of foetuses and/or infants to PFCs has been of particular concern and is not well understood. Foetuses and infants have a higher risk of PFC exposure (Fromme *et al*., 2009; OECD, 2013). However, from mammalian studies, it is known that PFCs are able to transcend the placenta and enter the foetus (Gützkow *et al*., 2012). From a human perspective, it is suggested that this exposure occurs in two ways, namely, (i) through the placenta to the foetus (Cariou *et al*., 2015), and (ii) from lactating mothers to their infants through breast-feeding (Mogensen *et al*., 2015; Kang *et al*., 2016).

However, Fromme *et al*. (2009) have argued that the mechanism by which PFCs are transferred from the mother's blood to breast milk remains unclear, although further evidence has suggested that PFCs are strongly bound to the protein fraction in the blood (Han *et al*.,

2003; Li J *et al*., 2013). In addition, it was previously reported that, PFCs, that is, PFOA, levels in maternal blood decreased from 54 to 7% after six months and 12 months, of breast-feeding, respectively, compared to their levels in the child's blood (Thomsen *et al*., 2010), while, PFOA levels in the serum of six-month-old infants were 4.6 times higher than maternal blood levels at birth (Fromme *et al*., 2010), suggesting that other exposure pathways had contributed to the sudden increase. Similarly, breast-fed infants of around six months of age take up 4.1 ng kg^{-1}bw d^{-1} of PFOA, which is 15 times higher than the uptake in adults (Haug *et al*., 2011). The question is: "did age-related exposure play a role in this instance?" It is unclear at this point, simply because the majority of studies that have studied the correlation between age and PFC concentrations in blood have not observed any significant effects (Calafat *et al*., 2007; Fromme *et al*., 2009); although, PFCs such as PFOA and PFOS do not biodegrade. It might be expected that the BMF would rise with age, just as it was reported with other POPs in Duarte-Davidson and Jones (1994) and Knower *et al*., (2014).

2.6 Toxicity and health risks associated with perfluoroalkyl compounds

The toxicity of PFCs differ from other POPs, and their toxicokinetic mechanisms are still unknown (Senevirathna, 2010). Nevertheless, medium and long-chained PFCs are believed to be more toxic than short-chained PFCs (Renner, 2006; Senevirathna, 2010). Accordingly, both PFOA and PFOS seem to be readily absorbed through oral intake (that is ingestion or gaseous), but are poorly eliminated from the human body (Lau *et al*., 2007; Møskeland, 2010). Both PFOA and PFOS do not biodegrade substantially, due to their stability, and thus, tend to accumulate into the kidney, liver or possibly other organs, as a result of attaching to certain proteins, such as β-lipoproteins, albumin and fatty acid binding proteins in the liver, as it has been demonstrated to be the primary organ targeted by PFCs (Fang *et al*., 2015; Midgett *et al*., 2015; Li *et al*., 2016). To elaborate on this, PFCs have previously been regarded as peroxisome proliferators (PPs), suggesting that they can lead to a variety of toxicological effects on the liver, including carcinomas (Vaughn *et al*., 2013; Krafft and Riess, 2015). PPs include certain hypolipidaemic drugs, phthalate ester plasticisers, industrial solvents, herbicides, food flavourings, leukotriene D4 antagonists and hormones (Reddy, 2004). Furthermore, PFOS and PFOA have half-lives in humans ranging from two to nine years, but it has been argued that, this half-life coupled with continued exposure can increase the humans' body burden and ultimately lead to levels that would result in long-term adverse

health outcomes (EPA, 2014; ATSDR, 2015). Chronic toxicity reports have associated PFOA exposure with tumours (Rosen *et al*., 2009; Wan *et al*., 2013) while severe and intermediary duration oral studies on rodents have indicated risks associated with potential stunted development, reproductive and other systemic growth defects (EPA, 2014). It was also been suggested that, PFOA and PFOS are able to compete with thyroxin, which is linked with the human thyroid hormone transport protein transthyretin (Weiss *et al*., 2009; Møskeland, 2010). In general, this appears to be the effect of longer-chained PFCs than shorter-chained PFCs (for example, PFBS). This finding has prompted a shift in industry practice to favour shorter-chain PFCs (Renner, 2006), which is detrimental to the efforts to eradicate PFC usage worldwide (Jensen *et al*., 2015). Table 2.3 depicts a brief summary of the results from various studies on PFCs' toxicities in biota.

Moreover, recent studies have demonstrated that PFCs may induce reactive oxygen species (ROS) generation and induce deoxyribonucleic acid (DNA) damage in the cells of humans and livers of wildlife animal (Reistad *et al*., 2013; Mashayekhi *et al*., 2015). Additionally, in a retrospective cohort mortality study in which more than 6000 PFOA-exposed employees were involved, results reported elevated standardised mortality ratios for kidney cancer, as well as a significant increase in diabetes mortality for male workers, although the study indicated that further investigations were required to substantiate the findings (Lau *et al*., 2007; EPA, 2014). Evidence from Melzer *et al*. (2010) and White *et al*. (2011) also reported that higher concentrations of PFOA and PFOS in human sera were associated with thyroid disease in elderly persons. However, the study suggested that further analysis was required to identify the mechanisms allowing this association (Melzer *et al*., 2010).

In addition, PFOS exposure was also associated with bladder cancer (Chang *et al*., 2014; Grandjean and Clapp, 2015). *In vitro* and *in vivo* epidemiologic and immunotoxicologic studies reported that high levels of PFCs in adults and children correlated with decreases in IgE levels, coupled with increases in antinuclear antibodies, asthma, influenza, and gastroenteritis (Keil, 2015). To mitigate the health effects associated with long-chain PFCs, it was suggested that commercially available alternative short-chain chemicals should replace these long-chain PFCs (Poulsen *et al*., 2005).

Table 2.3: Brief summary data on PFOA and PFOS toxicities (Stahl *et al*., 2011)

Compound	Exposure time	Spices type	Organ tested	Effect	Dosage	NOAEL	Reference
PFOA	7 days	Japanese guppies	n.i.	Activity of peroxisomal acyl-CoA-oxidase ↑	2 to 20 mg/kg feed	n.r.	Yang, 2010
	14 days	Minnows	n.i.	Changes in the expression of Apo lipoproteins and upstream genes	n.r.	n.r.	Fang *et al*., 2010
	90 days	Rats (male)	Liver	Liver mass ↑ and hepatocellular necrosis	1.7	0.6	Cui *et al*., 2009
PFOS	28 days	Rats	Liver & other	Body weight ↓ , liver mass ↑, and altered gene expression and fatty acid metabolism in the liver, T_3 and T_4 ↓	2 to 20 mg/kg feed	n.r.	Curran *et al*., 2008
	14 weeks	Rats (male)	Liver	Hypertrophy and vacuolization of the liver	n.r.	0.37	Seacat *et al*., 2003

Table 2.3: *Continues*

PFOS	26 weeks	Cynomolgus monkey	Liver & other	Centrilobular vacuolization, hypertrophy of the liver, T 3 ↓, TSH ↑, HDL ↓, and bilirubin, cholesterol concentrations ↓	n.r.	0.03	Seacat et al., 2002
	1 and 4 months	Fresh water larvae	n.i.	Deterioration of behavioural and activity parameters (larvae were less active, less able to avoid attackers, or less efficient in foraging)	> 10 µg/L	10 µg/L	Van Gossum *et al*., 2009

T_3: tri-iodo thyronine; T_4: thyroxin; Upward arrow: increased; downward arrow: decreased; n.r.: not reported; n.i.: not indicated

2.7 Commercially available alternatives to long-chain perfluoroalkyl compounds

For decades, long-chain PFCs, including PFOA and PFOS, were used in various industrial applications (Wang *et al*., 2014a, b; Taniyasu *et al*., 2015; Niu *et al*., 2016). However, concerns over the effect of these compounds in humans and the environment led to an interest in exploring suitable alternatives (Jenssen *et al*., 2015). Thus, there are three types of available alternatives to long-chain PFCs, namely, (i) substances with shorter per- or polyfluorinated carbon chains; (ii) non-fluorine-containing substances; and (iii) non-chemical techniques (OECD, 2013).

2.7.1 Substances with shorter per- or polyfluorinated carbon chains

The discontinuity of "C_8-chain"-fluorinated compounds manufacturing was agreed upon between the manufacturers of these chemicals and regulatory agencies (for example, the Stockholm Convention on POPs) decades ago. Hence, equivalent "short-chain" fluorinated substances were suggested as alternative replacements, with indications suggesting that they were less hazardous and can be manufactured as substitutes for applications in which long-chain PFCs were used (Holt, 2011; OECD, 2013; Jenssen *et al*., 2015). Thus, examples of suggested replacement compounds included (i) 6:2 fluorotelomer-based chemicals; (ii) perfluorobutane sulfonyl fluoride (PBSF)-based derivatives; (iii) mono- and polyfluorinated-ether-functionality compounds; (iv) fluorinated oxetanes; and (v) other fluorinated polymers (Buck *et al*., 2011; OECD, 2013).

Furthermore, it has been indicated that the most important short-chain PFCs were perfluorobutane sulfonate (C_4, PFBS) and perfluorohexane sulfonic acid (C_6, PFHxS) (Jenssen *et al*., 2015). Table 2.4 depicts some of the commonly known commercially available short-chain alternatives.

Table 2.4: Some of the commonly known commercial alternatives to long-chain PFCs' (Jenssen *et al.*, 2015)

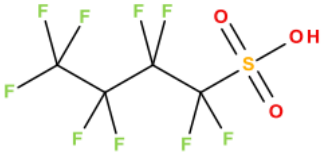
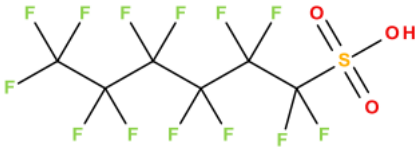
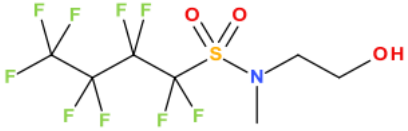
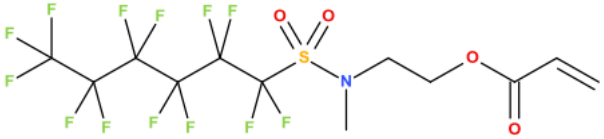
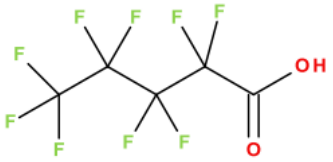
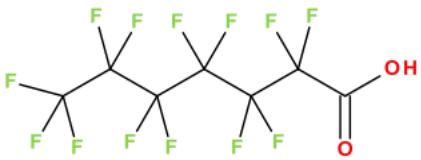
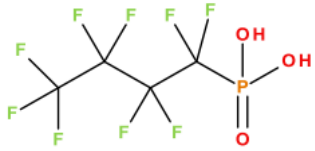
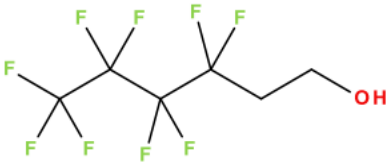
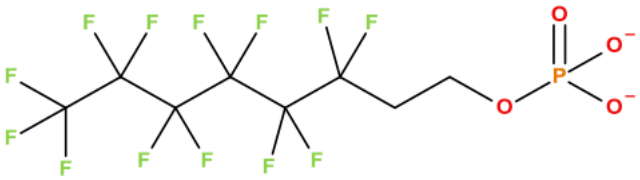
Compound and Acronyms	Chemical structure
Perfluorobutane sulfonic acid (PFBS)	
Perfluorohexane sulfonic acid (PFHxS)	
N-Methyl perfluorobutane sulfonamidoethanol (MeFBSE)	
N-Methyl perfluorohexane sulfonamidoethyl acrylate	
Perfluorobutanoic acid (PFBA)	
Perfluorohexanoic acid (PFHxA)	

Table 2.4: *Continues*

Perfluorobutyl (PFBPA)	
Perfluorohexyl phosphonate (PFHxPA)	
4:2 Fluorotelomer alcohol (4:2 FTOH)	
6:2 Fluorotelomer phosphate/mono[2-(perfluorohexyl)ethyl] phosphate	

2.7.2 Non-fluorine-containing substitutes

Non-fluorine containing compounds with similar properties to those seen in PFCs are available commercially and some have been used in various industrial applications (OECD, 2013), namely (i) naphthalenes or biphenyls used as water repelling agents for rust protection systems, marine paints and coatings, amongst others; (ii) fatty alcohol polyglycol ether sulphate used as a levelling and wetting agent; (iii) sulfosuccinates used for surface coating, paints and varnish; (iv) hydrocarbon surfactants used in the photographic industry; (v) siloxanes and silicone polymers used for impregnation of textiles, leather and carpets; (vi) stearamidomethyl pyridine chloride which is also used for the impregnation of textiles, leather and carpets; and (vii) polypropylene glycol ether, amines, and sulphates. However, it has been noted that these alternatives may have limited usability when compared to their long-chain predecessors (Holt, 2011; OECD, 2013). Conversely, some of these alternatives have been determined to be hazardous to humans (Dong *et al*., 2013; Gorrochategui *et al*., 2014), although conclusive results are still required. In addition, critics suggest the health and environmental profiles of these substitutes to be fully tested before their large scale commercialisation.

2.7.3 Potential health impact associated with short-chain perfluoroalkyl compound alternatives

Firstly, a recent study has indicated that various known short-chain PFCAs and PFSAs have similar physicochemical properties as those seen in long-chain PFCs, such as high water solubility, persistency, amongst others (Gomis *et al*., 2015). Two decades ago, a trend driven by concerns over long-chain PFCs and their undesired impact on humans and environmental health, resulted in the development of alternative compounds worldwide among PFC producers, in order to replace C_8-fluorocarbons (Wang *et al*., 2013).

However, information on their impact, including their bioaccumulative potential in the environment, has generally remained limited and is not readily available (Wang. *et al*., 2013). The OECD (2013) has indicated that this lack of information has been due to confidentiality and trade secret concerns, while Wang. *et al*. (2013) have argued that these alternatives to long-chain PFCs, applied similar production techniques such as polymerisation which suggested that they may enter the environment, including surrounding production

sites where they were produced and used, which, in the long term, will mimic similar distributary mechanisms observed for long-chain PFCs.

Accordingly, various studies have reported short-chain alternatives to PFCs in several matrices using similar research techniques to those applied for long-chain PFCs. For instance, elevated levels of PFBS and other precursors have been detected in water samples from Germany (Möller *et al*., 2010), Japan (Ahrens *et al*., 2010) and the Northwest Pacific Ocean (Cai *et al*., 2011).

Nevertheless, published research has argued that due to concerns over intellectual property rights, required data to assess the safety of these substitutes has not yet been established (OECD, 2013; Wang. *et al*., 2013). The lack of such information has made it possible for critics to question whether these alternatives have been fully scrutinised prior to their commercialisation (Wang. *et al*., 2013). There has been no focus on the environmental health impact of PFC substitutes in countries with lower or non-existent regulatory requirements, therefore, regulatory monitoring and reporting mechanisms are non-existent even for long-chain PFCs; for example, in South Africa. This reality has further inhibited researchers, regulators and other civil society stakeholders, from assessing and developing strategies that can minimise the risks associated with these substitutes; without monitoring activities and studies into the environmental fate and potential adverse effects of PFC substitutes. It is therefore difficult to mitigate their impact in the long term (Goldstein *et al*., 2013; Wang. *et al*., 2013).

There are suggestions which indicate that short-chain PFCs alternatives are less bioaccumulative (Wang. *et al*., 2013) and toxic (Borg and Hakansson, 2012), although recent scientific evidence has suggested that short-chain PFCs have shown a higher uptake into the leaves, stems and fruits of plants (Krippner *et al*., 2014, 2015). This ultimately suggests that these contaminated florae will constitute a major exposure pathway for humans. Among the PFC alternatives, that is, PFBS, PFBA, PFHxS and PFHxA (Krippner *et al*., 2015), PFBS has been shown to be persistent in the environment, a characteristic observed for C_8-homologues (Wang. *et al*., 2013). Although, PFBA, PFHxS and PFHxA, including PFBS, have shorter half-lives in both humans and biota than their longer-chain homologues (Iwai 2011; Borg and Hakansson, 2012), current studies have reported that some PFHxAs can even have longer serum half-lives than long-chain PFCs, such as PFOS, suggesting the unsuitability of using these compounds as alternatives (Wang. *et al*., 2013).

Additionally, the Asahi Glass Company (2006), described PFHxA as being acutely toxic, three to five more than PFOA, with PFBS being reported to cause disruptive effects on cell membranes (Oldham *et al*., 2012; Jensen *et al*., 2015), and having the potential to act as an aromatase inhibitor in placental cells (Gorrochategui *et al*., 2014). PFBS and PFHxS have been suggested to have an effect on how lipids are metabolised (Bijland *et al*., 2011; Jensen *et al*., 2015). Thus, PFHxS lead to liver weight increases. Relevant data on the content of short-chain PFCs in human organ tissues and PFOA/PFOS are shown in Table 2.5. Hence, in order to reduce the potential impact of both long-chain and their suggested substitutes, some novel technologies have been developed for either the decomposition and/or treatment of these compounds, particularly at the point of use. Currently, these technologies are still at laboratory level, and have yet to be implemented on a larger scale.

Table 2.5: Concentration of short-chain PFCs in five human organ tissues (Pérez *et al*., 2013; Jensen *et al*., 2015)

	Mean concentrations ng/g w. w.				
PFC substance	**Liver**	**Bone**	**Brain**	**Lung**	**Kidney**
PFBS	0.9	3.2	<LOD	17.8	8
PFBA	12.9	<LOD	13.5	304	464
PFPeA	1.4	0.8	<LOD	44.5	<LOD
PFHxA	11.5	35.6	18.0	50.1	5.6
PFHxS	4.6	1.8	3.2	8.1	20.8
FHEA (metabolite of 6:2 FTOH)	92.6	42.5	18.6	2.4	23.7
PFOA	13.6	60.2	<LOD	29.2	2.0
PFOS	102	<LOD	4.9	29.1	75.6

LOD: Limit of detection, w.w.: wet weight

2.8 Some of the novel technologies used for the treatment and/or removal of polyfluoroalkyl compounds in water

Concerns over the prevalence of PFCs in the environment have increased during recent decades. However, the treatment and removal of these compounds from contaminated water have remained a challenge. The unique physicochemical properties, including strong fluorine-carbon bonds in PFCs, have contributed to these compounds being resistant to most conventional treatment technologies (Arvaniti and Stasinakis, 2015).

Currently, advanced treatment technologies have emerged with regard to reduction processes and advanced oxidation (Arvaniti and Stasinakis, 2015), including electrochemical treatment (Schaefer *et al*., 2015), processes which have been proven to be suitable for the treatment of PFCs in environmental matrices. Furthermore, treatment at the point of use can be harnessed to reduce PFCs. Some well-established PFC treatment/removal processes include the use of adsorption and advanced membrane filtration systems. Overall, all these processes are designed for the treatment of potable water and wastewater.

2.8.1 Granular Activated Carbon adsorption

For well-established processes, adsorption has been the most common remediation technology used for PFCs, which is based on PFCs adsorption into GAC (Shih and Wang, 2013; Arias-Espana *et al*., 2015). Thus, four steps, namely (i) diffusion from the liquid phase, (ii) mass transfer on to the solid phase, (iii) internal diffusion (pore and surface diffusion) inside an adsorbent, and (iv) electrostatic and/or hydrophobic interaction with the exchange site, were identified by Yong (2007) as being critical in the adsorption mechanism using activated carbon. Thus, Vecitis *et al*. (2009) reported that GAC is utilised to remove PFCs, in this case PFOA and PFOS, and has been proven effective in removing both substances at more than 90% mass of PFC removal/mass of GAC used (mg/g GAC) subsequent to the thermal treatment of GAC, with results indicating minimal residual PFC post-thermal treatment (Watanabe *et al*., 2015). However, controversial views have been raised in the literature on the ability of GAC to remove PFOS and PFOA. For instance, although GAC has been demonstrated to remove PFOS at µg/L levels, this is not the case for PFOA (Senevirathna *et al*., 2010; Appleman *et al*., 2013). Several other studies have indicated that factors such as carbon-fouling and pre-washing, as well as the presence of organic matter and high salinity, can decrease PFC removal which affects adsorption and the modification of surface properties

of the GAC (Yu and Hu, 2011; Appleman *et al*., 2013). Additionally, Hansen *et al*. (2010) have indicated that commercial GAC has been mainly used to investigate PFOS and PFOA removal, with the removal of other PFCs, including proposed short-chain substitutes remaining unknown.

Recently, GACs/PFC removal has also been achieved using natural sources such as Bambusoideae (bamboo) and *Agave sisalana* (Deng *et al*., 2015a; Mudumbi *et al*., 2015). Furthermore, various other adsorbents have been utilised in the treatment and removal of PFCs, and have included powdered, activated carbon (PAC), carbon nanotubes, mesoporous carbon nitride commercial resins, polymers, maize straw-derived ash, alumina, chitosan, goethite, silica, montmorillonite, organo-clay, hexadecyltrimethylammonium bromide (HDTMAB)- immobilised hollow mesoporous silica spheres, cetyltrimethyl ammonium bromide-modified sorbent, permanently-confined micelle arrays (PCMAs) sorbents and electrospun fibre membranes (Senevirathna *et al*., 2010; Hansen *et al*., 2010; Zhou *et al*., 2010b; Tang *et al*., 2010; Deng *et al*., 2010; Yu and Hu, 2011; Chen *et al*., 2011; Wang and Shih, 2011; Zhang *et al*., 2011; Deng *et al*., 2012; Das *et al*., 2013; Zhou *et al*., 2013; Dai *et al*. 2013; Xu *et al*. 2013; Yan *et al*. 2013; Bei *et al*. 2014; Chularueangaksorn *et al*. 2014a; Yao *et al*., 2014; Li and Zhang, 2014; Wang *et al*., 2014; Deng *et al*., 2015b). However, when comparing PAC and GAC, evidence has reported higher and faster removal of PFOS and PFOA using PAC rather than GAC (Arvaniti and Stasinakis, 2015); whereby the adsorption equilibrium was reached in 6 h during PAC treatment, which escalated to 168 h during GAC treatment (Senevirathna *et al*., 2010; Arvaniti and Stasinakis, 2015). A similar trend was also reported by Arias-Espana *et al*. (2015). This suggests that exchange sites in PAC are more suited to PFC removal than those in GAC. Therefore, ion/site exchange effectiveness can effectively determine the success of a treatment strategy and thus the development of resin based treatment methods.

2.8.2 Anion resin ion exchange adsorption

Numerous studies have indicated the suitability of ion-exchange for the removal of pollutants (Alesi and Kitchin, 2012; Shkolnikov *et al*., 2012). According to Helfferich (1962), ion-exchange resins are the most important class of ion exchangers, thus, can be used to adsorb POPs.

It has been reported how ion-exchange resins can be utilised to exchange unwanted ions with hydrogen or hydroxyl group to remove contaminants, including PFCs (Deng *et al*., 2010; Senevirathna *et al*., 2010; Alesi and Kitchin, 2012; Shkolnikov *et al*., 2012;

Chularueangaksorn *et al*., 2014b). It was reported that an anion-exchanger was better than GAC in the removal of PFOA (Chularueangaksorn *et al*., 2014b), while Appleman *et al*. (2014) demonstrated the effectiveness of an anion exchange in removing PFOS (>92%), PFOA (74%) and PFNA (>67%).

Nevertheless, regardless of the success of ion-exchange resins, Chularueangaksorn *et al*. (2014b) have indicated that resins are expensive. Thus, the report suggests that they should be periodically regenerated for re-use in the removal of PFCs. This suggestion, however, did not consider the effect of cross and cumulative contamination as some resin beads may contain residual PFCs even after regeneration. Additionally, it has been reported that the rate of removal using an anion exchange treatment is largely dependent on the concentration level of the contaminant, the concentration of competing ions and the treatment system design (that is, flow rate and the size of the resin bed) and the nature of the exchange ions within the resin (ITRC, 2008; Cummings *et al*., 2015). Additionally, Appleman *et al*. (2014) and Rahman *et al*. (2014) have recommended that further research is needed to effectively comprehend and identify the most suitable resins for removal of various pollutants in general, and PFCs in particular. These studies also noted that it is necessary to frequently change the resins to completely eradicate residual PFCs in the beads. The ITRC (2008) has further suggested that, both the management of the resin and that of the brine should also be taken in consideration when anion resin is used.

2.8.3 Removal of PFCs by combination of adsorption and coagulation

Coagulation has been reported as another technique that can be utilised for the removal of PFCs. However, its efficacy has been questioned in most cases. For instance no removal occurred even after coagulation processes were coupled with sedimentation and sand filtration in a study by Takagi *et al*. (2011). This was consistent with the results that were observed by Thompson *et al*. (2011), Eschauzier *et al*. (2012) and Xiao *et al*. (2013). Similarly, Appleman *et al*. (2014) further indicated that coagulation followed by sedimentation did not remove PFCs, but when sedimentation was replaced by dissolved air flotation (DAF), a 49% removal of PFOS was achieved, although, shorter-chain PFCs, such as PFCAs and PFSAs, were not well removed (Appleman *et al*., 2014). This suggests that coagulation on its own is likely not to yield positive results. Thus, a study by Deng *et al*. (2011) found that coagulation can remove most PFOA from water, but high residual PFOA concentrations remained in the water. In this regard, the study combined adsorption and coagulation and the removal was

enhanced. Similarly, recent evidence has reported that the combination of adsorption by powdered activated carbon (PAC) and coagulation increased the removal ratios up to >90% for PFCs, such as PFOX with an initial concentration of 1 mg/L (Bao *et al*., 2014). Hence, this further implies how adsorption enhances coagulation. Nevertheless, it has further been indicated that, in a PFC-adsorption technique where fulvic acid (FA) is used, its concentration (i.e. FA) increase decreases the removal ratio of PFOS and PFOA, simply due to the steric hindrance effect of this acid's molecules and the competitive adsorption of these PFCs (Bao *et al*., 2014), suggesting that, the selection of coagulants, as well as that of adsorbents to be used during the coagulation/adsorption technique, etc., is also paramount. Du *et al*. (2014) reviewed PFC removal using various adsorbents, and reported that adsorption not only removed PFCs effectively, but also affected PFC distribution in different environments. However, Du *et al*. (2014) have argued that, on the basis of C–F chain substances having hydrophobic and oleophobic properties, this implies that PFCs are likely display different adsorption behavior as compared to their counterparts, e.g. the hydrocarbon substances. Thus, the authors have suggested that this aspect, coupled with the competitive adsorption of PFCs with other traditional POPs present in various environments, warrants further investigation (Du *et al*., 2014; Bao *et al*., 2014).

Nevertheless, the stubbornness of shorter-chain PFCs in resisting removal, as indicated by Appleman *et al*. (2014), remains a cause for concern, particularly, since there is not enough data available reporting on these new emerging POPs, even though their use as substitutes to long-chain PFCs is increasing (Rahman *et al*., 2014). This suggests that improved removal techniques for shorter-chains PFCs are required. On the other hand, Yang *et al*. (2016a, b) have suggested that, to improve scaling-up PFC removal techniques, more understanding of the mechanisms that have been proven effective is required, as well as testing these mechanisms on various PFCs.

2.8.4 Advanced filtration: membrane-based treatment processes

Filtration has been broadly defined as a technique that separates suspended particles from a liquid phase by causing the latter to pass through a porous filter, with the purpose of either removing the impurities and/or collecting them from the solution where they are concentrated (Crittenden *et al*., 2012). In the case of PFCs, sand filtration cannot be used for the removal of PFCs (Takagi *et al*., 2011; Eschauzier *et al*., 2012; Arvaniti and Stasinakis, 2015). However, most potable water treatment works in developing countries, such as South Africa,

still use sand filters. Conversely, it was reported that the usage of advanced filtration techniques such as nanofiltration (NF) and reverse osmosis (RO) achieved a significant reduction of PFCs (Schröder *et al*., 2010; Appleman *et al*., 2013; Stasinakis *et al*., 2013).

2.8.4.1 Nanofiltration

Introduced during the late 1980s (Mohammad *et al*. 2015), NF is another form of membrane technology process used with the purpose of softening and removing synthetic POPs (Rahimpour *et al*., 2010). Thus, Izadpanah and Javidnia (2012) have indicated that this method of filtration provides high water flux at low operating pressure. It has been shown that NF can be effective in the removal of PFCs. Similarly, Tang *et al*. (2007) and Schröder *et al*. (2010) reported 90% and 99% removal of PFCs using NF. However, lower removal rates (that is, 44% to 86%) were reported by Rattanaoudom (2011), suggesting that the technique is inefficient. As such, Arias-Espana *et al*. (2015) indicated that pH is an important factor that affects nano-membrane retention rates for POPs. Similarly, at a pH ≤ 3, Steinle-Darling and Reinhard (2008) and Wang *et al*. (2015a, b) observed a decline in the rejection of PFC (35%) and Wang *et al*. (2015a, b) also observed that PFOS rejections improved from 91.17% to 97.49% with an increase in pH from 3.2 to 9.5 at 4×10^5 Pa. However, a similar study reported that PFOS removal using NF was higher than for PFOA (Rattanaoudom, 2011), a result that was also observed by Yu *et al*. (2014) with a removal efficiency of 77.4% for PFOS and 67.7% for PFOA. Additionally, Appleman *et al*. (2013) observed a 93% removal for all target PFCs through the usage of NF.

Moreover, recent research has focused on ways of improving NF effectiveness by modifying membrane materials used, with the purpose of increasing the strength, heat resistance, functionality and other factors (Luo *et al*., 2016). As such, several inorganic fillers, for example, zeolites (Gevers *et al*., 2005), ceramic oxides (Pages *et al*., 2013; Schmidt *et al*. 2014; Zhang *et al*., 2014), and inorganic compounds (Fang and Duranceau, 2013; Namvar-Mahboub and Pakizeh, 2013; Gholami *et al*., 2014 and Chen *et al*., 2014), and layered silicates have been used. The reason being that their dispersion is possible in polymeric matrices at the nanoscale (Luo *et al*., 2016), which can further enhance membrane electro-chemical properties that are essential in filtration systems, particularly for the removal of compounds with unique properties, such as PFCs, compounds containing a hydrophobic backbone and hydrophilic functional groups.

2.8.4.2 Reverse osmosis

Reverse osmosis (RO), as a POP treatment process, uses high pressure to force water through a semi-permeable membrane (Lee *et al*., 2010). Hence, Letterman (1999) indicated the removal of salts from brackish water and seawater, as the primary usage of RO; although, the same technique can also be used for high rejection of synthetic organic compounds (SOCs), such as PFCs. Thus, Vecitis *et al*. (2009) reported that RO has shown its effectiveness in PFCs removal. Another study showed ≥99% removal of PFOS and PFOA (Flores *et al*., 2013). Similarly, it was revealed in a study by Tang *et al*. (2007) that, RO had a higher efficacy in PFCs removal than NF. This was attributed to the smaller pores and thicker rejection layers of the RO membranes used. In a hybrid membrane experiment where the reduction of turbidity from fire-fighting foam wastewaters was used, a 71% to 77% removal of fluorinated surfactants was reported. However, from a pilot fire-fighting foam wastewater treatment plant where RO was used, rejection rates >99% were achieved (Baudequin *et al*., 2011; Arias-Espana *et al*., 2015).

Nevertheless, regardless of the high efficiency of the RO, criticism about its use is based on the relatively high operational costs associated with the technology due to energy-intensified requirements of the system (Joo and Tansel, 2015). Additionally, it also has been indicated that the RO is susceptible to biofouling, for which an improvement is required to enhance its usability in communities with minimal investment capital (Henthorne and Boysen, 2015).

Furthermore, recent evidence indicates the versatility of RO systems and their effectiveness in new applications with proponents suggesting that RO can outperform other desalination technologies (McGovern and Lienhard, 2014). As such, Forward Osmosis (FO) has been investigated in the past decade, not to replace RO, but to be utilised to process feed waters that cannot be treated by RO (Shaffer *et al*., 2015). This further suggests that, to date, there is no generally accepted technique that is readily available for the removal of PFCs, and other perfluoroalkyl pollutants. Ultimately, the degradation and/or decomposition of PFCs might be the only viable option, with advanced oxidation processes having been reported to be suitable.

2.8.5 Advanced oxidation processes

According to Arias-Espana *et al*. (2015) the chemical structure of PFCs, mostly PFOA and PFOS, allows them to resist oxidation owing to the complete substitution of hydrogen (C–H bond) for fluorine (C–F bond). Fluorine atoms resist oxidation because it is the most electronegative element. This has been explained by Wardman (1989), who argues that fluorine with a reduction potential of 3.6V is thermodynamically unsuitable to be substituted with any other oxidant (Arias-Espana *et al*., 2015).

Furthermore, Advanced Oxidation Processes (AOPs), coupled with hydroxyl radicals in combination with ozone (or O-atom), were determined to be suitable for the reduction of recalcitrant POPs (Arias-Espana *et al*., 2015). However, for POPs such as PFOA and PFOS, the AOPs/OH/O_3 was determined to be ineffective, as PFOA and PFOS do not contain hydrogen atoms, which can be reduced at pH commonly prevalent in the ecosystem (Arias-Espana *et al*., 2015). Hence, Schröder and Meesters (2005) argued that compounds such as PFOA and PFOS become inert to advanced oxidation mechanisms due to the substituted hydrogen by fluorine atoms in these POPs. Moreover, in-situ advanced oxidation has been explored as a possible mechanism to treat PFCs in the environment (Liu *et al*., 2012a, b). As such, oxidation processes have on several occasions, been tested against recalcitrant contaminants (Arvaniti and Stasinakis, 2015), during which the in-situ formation of highly oxidizing species, mainly free radicals, was involved.

Therefore, it was suggested that a variety of reagents have to be supplemented in AOPs in an attempt to enhance these oxidation processes. These supplementary compounds include activated persulfate, Fenton's agent, subcritical water, zero-valent metal, and/or a combination of these agents (Arias-Espana *et al*., 2015). Supplementation with hydrogen peroxide (H_2O_2) has been commonly used, due to its capability to generate hydroxyl radicals (HO*), as well as persulfate ($S_2O_8^{2-}$), Fenton's reagent (Fe^{2+} + H_2O_2) (Rayne and Forest, 2009) and peroxymonosulfate (HSO_5^-) (Antoniou and Andersen, 2015; Arvaniti and Stasinakis, 2015).

Hydrogen abstraction allows hydroxyl radicals to attack the organic substances by forming carbon centre radicals during the oxidation processes (Antoniou and Andersen, 2015). Thus, because of the nonexistence of hydrogen atoms in PFCs that can be abstracted, this limits hydroxyl radicals' ability to react with these POPs, reducing the direct electron transfer (Vecitis *et al*., 2009; Arvaniti and Stasinakis, 2015).

Additionally, a significant number of photolytic methods have been reported to effectively degrade PFCs into fluoride ions, carbon dioxide and shorter chain PFCAs in aquatic samples (Arvaniti and Stasinakis, 2015). Photolytic methods such as H_2O_2 photolysis and photocatalysis (Hori *et al*. 2004), direct photolysis (Chen and Zhang, 2006; Yamamoto *et al*., 2007), persulfate photolysis (Hori *et al*., 2005; Chen and Zhang, 2006), alkaline isopropanol photolysis (Yamamoto *et al*., 2007) and photo-Fenton (Hori *et al*., 2007; Wang *et al*., 2008; Tang *et al*., 2012), are examples which can be used for PFC reduction. New methods have emerged such as thermal- or microwave-activated persulfate oxidation (Liu *et al*., 2012a), heat-persulfate oxidation (Hori *et al*., 2008; Rayne and Forest, 2009; Lee *et al*., 2012), and ultrasonic treatment (Cheng *et al*., 2008; Lin *et al*., 2015). These methods have been applied and proven to be effective in degrading PFCs. Thus, Hori *et al*. (2005) and Wang *et al*. (2010) revealed that the usage of persulfate produced highly oxidative sulphate radical anions (SO^{*}_4) which significantly degraded PFOA to F^- and CO_2 as major by-products. However, it was reported that shorter chain perfluorocarboxylic acids (PFCAs) were formed, that is, compounds which were proposed as replacements for long-chain PFCs, suggesting the inadequacy of the method. This inadequacy suggested a secondary treatment stage is required. Similarly, PFOA degradation was achieved using a photocatalytic AOP persulfate at 50 mM $[S_2O_8]^{2-}$ and a 4 h irradiation with PFOA at a concentration being 1.35 mM (Arias-Espana *et al*., 2015).

Moreover, others have demonstrated that a sulphite/UV process was efficient in reductive degradation of PFOA (Song *et al*., 2013). Accordingly, 100% removal of PFOA and an 88.5% defluorination was completed after 1 h and a reaction time of 24 h respectively, under a nitrogen atmosphere. Similarly, the use of a UV–Fenton process achieved a 95% PFOA removal (Tang *et al*., 2012). Due to the success of these processes, other reductive processes such as Zero-valent ion processes have been developed.

2.8.6 Reduction processes using zero-valent iron

Although the removal and/or treatment of PFCs by means of reduction processes using zero-valent ion (ZVI) has remained limited (Arvaniti and Stasinakis, 2015), a study by Hori *et al*. (2006) has reported that a partial degradation of PFOS by microsized ZVI coupled with high temperature (>250 °C) and pressures of up to 20 MPa can be achieved. Similarly, Lee *et al*. (2010) demonstrated that PFOA was susceptible to degradation up to 68 and 73% after 2 and 8 h, respectively, using persulfate activated by ZVI. In addition, a recent study by Arvaniti *et al*. (2015) investigated the removal and/or treatment of various PFCs in water

using nanoscale ZVI (nZVI), using the nZVI uncoated and coated with Mg-aminoclay (MgAC). This method reportedly has PFC removal ability ranging from 30 to 96% (from 10 mg/L) under acidic conditions (pH = 3), low temperature (20 °C) and high doses of synthesised nanomaterials (1000 mg nZVI/L). According to Arvaniti and Stasinakis (2015), both sorption and degradation mechanisms are responsible for PFCs' removal when coated nZVI was used, a process used to achieve higher removal rates. In order to improve the effectiveness of processes using specialised materials such as ZVI, electrochemical cells can also be used.

2.8.7 Electrochemical treatment of polyfluoroalkyl compounds

Recently, the use of an electrochemical cell and a Ti/RuO_2 anode in laboratory experiments was assessed, demonstrating an increase in both PFOA and PFOS decomposition with increased current density (Schaefer *et al*., 2015). Thus, at a current density of 10 mA/cm^2, the electrochemical treatment rate of both PFOA and PFOS was 46×10^{-5} and 70×10^{-5} [(min^{-1}) $(mA/cm^2)^{-1}$ (L)], respectively (Schaefer *et al*., 2015), with a defluorination ratio of 58% and 98% recovery for both PFOA and PFOS, respectively. Similarly, a study by Lin *et al*. (2012) investigated the electrochemical degradation of PFOA in aqueous solution over anodes, such as $Ti/SnO_2—Sb$, $Ti/SnO_2—Sb/PbO_2$, and $Ti/SnO_2—Sb/MnO_2$. The results revealed a 98.8% degradation ratio of the substance (i.e. PFOA), with a 73.9% defluorination ratio, which is inconsistent with that of Schaefer *et al*. (2015). Nevertheless, both studies (i.e. Lin *et al*., 2012; Schaefer *et al*., 2015) have reported that short-chain PFCs remained recalcitrant to electrochemical degradation mechanism, suggesting a poor performance of the electrochemical treatment of PFCs as previously reported by Zhuo *et al*. (2011), and the need for an enhanced technology in this regard. In addition, previous studies that used this treatment method have indicated that the electrochemical treatment of PFCs can be efficient and yield significant results, in divided electrochemical cells rather than in undivided cells (Agladze *et al*., 2007; Schaefer *et al*., 2015). However, minimal research data are available in this regard; that is, the evaluation of divided cells (Schaefer *et al*., 2015). The application of an inert environment, high temperature and pressure can further enhance electrochemical treatment.

On the other hand, electrocoagulation using a stainless steel rod as cathode has recently emerged as an efficient PFC removal technique, achieving a removal ratio of 99.7%/98.1% and 98.9%/97.3%, using stainless steel and aluminium rods as cathodes in the

presence of different anions (e.g. Cl^-/NO^-_3), respectively (Wang *et al*., 2016). Previously, Lin *et al*. (2015) demonstrated that the hydrophobic interaction was a prime role player in PFCs sorption and removal, a condition under which zinc anode proved to be more efficient than the other three anode materials, with 96.7% removal capacity. Hence, both these studies, i.e. Lin *et al*. (2015) and Wang *et al*. (2016) are evidence that electrocoagulation technique under various driving forces is an effective and alternative method to remove PFOA from aqueous solution. Nevertheless, it remains unclear what would be the removal effectiveness of this technique on short-chain PFCs. Similarly, different influencing factors, including pH, etc., can also be contributing factors in the removal of PFCs in various environments. Hence, Table S6 provides an overview comparison summary of results for PFCs removal using different techniques.

Although technologically advanced, these methods require specialised knowledge, which limits practical application compared to cheaper options that rely on removal at the Point-of-Use (POU).

2.8.8 Removal of PFCs at the point-of-use

This technique uses PoU treatment devices, which are applied and/or installed at an individual or single tap, faucet or outlet for the purpose of reducing contaminants at that point-of-use (Lee, 2005; MDH, 2008). As such, a study by MDH reported that when applied, installed, operated and maintained according to the manufacturer's specifications, PoU treatment devices effectively remove PFCs (MDH, 2008). In the report, it is suggested that devices were evaluated for their PFC removal capabilities, using an assessment classified into two categories; that is, (i) those using GAC and (ii) those using a combination of multiple methods for the removal. From the results, it was revealed that some devices (n = 11) were found to remove PFCs in field tests to below the employed detection limits (50 ng/L) (MDH, 2008). Additionally, in the late 90s, a Point-of-use Plasma Abatement (PPA) method was reported as one way to effectively eliminate PFCs at PoU (Fiala *et al*., 1999).

2.9 Conclusion

Perfluoroalkyl compounds (PFCs) are a group of chemical substances that fall under recalcitrant POPs. They consist of a fully fluorinated hydrophobic alkyl chain attached to a hydrophilic-end group. The unique physicochemical properties of these substances led to their extensive industrial and household applications, particularly in surfactants, fire-fighting

foams and food-packing paper, as well as in textile, carpet and leather treatment. There are many types of PFCs, but the most widely used have included PFOA and PFOS. Recently, there have been studies reporting on PFBS as a potential replacement, as it has PFC characteristics and similar health risks as those associated with PFOA and PFOS. Thus, notwithstanding the role they have played in industrial and household applications, PFCs have been regarded as bioaccumulative, persistent and potentially precarious to humans and wildlife. For this reason, the development of alternatives to these compounds is underway. Ultimately, this has led various manufacturers to utilise short-chain PFCs in substitution of long-chain PFCs. However, like their homologues, short-chain PFCs have also been associated with various health risks. This finding suggests that further investigations are needed in this regard, since most studies have mostly focused on health-related risks of long-chain PFCs. To mitigate associated health risks to humans and animals, numerous treatment methods have been suggested, although treatment at point-of-use is currently the only viable option available to the general population. In our opinion, it is worth indicating that short-chain PFCs are recalcitrant, even to highly efficient removal techniques; this is a challenge that requires the attention of researchers.

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CHAPTER 3

Are aquaporins (AQPs) the Gateway that Conduits Nutrients, Persistent Organic Pollutants and Perfluoroalkyl Substances (PFASs) into plants?

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3.1 Abstract

Besides water and sunlight, plants and/or crops also require an assortment of dissimilar nutrients/elements to grow. Thus, some of these nutrients have been classified as essential or macronutrients [e.g. calcium (Ca), magnesium (Mg) and sulfur (S)], for they facilitate plant growth; while others, such as copper (Cu), iron (Fe), zinc (Zn), etc., are considered as micronutrients. However, it is apparent now that plants are exposed to a variety of other chemical compounds, including a range of persistent organic pollutants (POPs) and perfluoroalkyl substances (PFASs), which have been found in several plants. Hence, it has been common knowledge that mechanisms such as mass flow, diffusion, etc., facilitated by plant root systems, have allowed the translocation of these nutrients and pollutants into plants; although, other researchers have argued that roots on their own cannot elucidate the dissemination of these chemical constituents into plants. This dissension remained until the discovery of Aquaporins (AQPs), which ultimately led to numerous AQPs being identified in plants. Thus, the aim of this review is to present an overview on the progress made thus far in attempting to understand the possibility of these proteins (i.e. AQPs) being the gateway that conduits nutrients, POPs and PFASs into plants; although, the gathered evidence currently, remains rudimentary and limited,

suggesting that further research is required to elucidate plant AQPs involvement at this stage in POP transportation and storage in plants.

Keywords: Aquaporins, Plants, POPs, PFASs, Nutrients.

3.2 Introduction

Persistent organic pollutants (POPs) are synthetic man-made organic chemical substances, produced intentionally and unintentionally, through various anthropogenic activities, with their release into the environment being through direct or indirect sources [178]. Since the industrial revolution, after World War II, a large quantity of these chemical compounds have been commercially produced and used, as they have proven to be beneficial in various economic sectors, including in agriculture whereby they are used in pesticides and fertilisers to increase crop yield. Plants and/or crops do not only need sunlight and water, but also require an assortment of metals, to grow. Some of which are heavy metals, including chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se) [17, 82, 177]. Similarly, it has been indicated that when these substances become insufficient in the soil, farmers manually apply them onto the land to mitigate against arable soil nutritional deficiencies [31]. The demand in agricultural produce to meet the food need of the rapidly growing global population [95] has resulted in the excessive application of synthetic products, leading to an upsurge in the prevalence of POPs in fresh produce. Thus, there is compelling evidence that plants accumulate and partially metabolize some environmental contaminants, which suggests that plants act as reservoirs for numerous persistent pollutants [65, 136, 191, 193].

Research reports have indicated that POPs persist for extended periods in the environment, and thus bioaccumulate and biomagnify through the food chain [49, 95, 128]. Hence, various researchers have recounted the prevalence of POPs and/or heavy metals in edible crops [7, 8, 15, 38, 51, 71, 72, 154, 163], as well as in arable soil and plants [8, 56, 168, 173]. Table 3.1 depicts 12 POPs or the "Dirty Dozen", of which nine are pesticides [141].

Table 3.1: The "Dirty Dozen" and their sources

Source	POP	Main use	References
Pesticides	Aldrin & Dieldrin	Insecticides: on crops such as corn and cotton; also to control termites.	[49, 141, 144, 169, 183]
	Chlordane	Insecticide: on crops, including vegetables, small grains, potatoes, sugarcane, sugar beets, fruits, nuts, citrus, and cotton. Also used on garden pests, and extensively on termites.	[46, 49, 141, 144, 169, 183]
	Dichlorodiphenyltrichloroethane DDT	Insecticide: on agricultural crops, such as cotton, and anopheles mosquitoes that carry diseases such as malaria and typhus.	[46, 49, 141, 144, 169]
	Endrin	Insecticide: on field crops, such as cotton and grains; can also be used to control rodents.	[49, 141, 144, 169, 178]

Table 3.1: *Continues*

Pesticides	Mirex	Insecticide: used to combat fire ants, termites, and mealybugs. Also utilised as a fire retardant in plastics, rubber, and electrical products.	[46, 49, 141, 144, 169, 178]
	Heptachlor	Insecticide: used primarily against soil insects and termites. Also used against some crop pests and to combat malaria	[49, 141, 144, 169, 183]
Industrial Chemicals	Hexachlorobenzene (HCB)	Fungicide: used for seed treatment. Used in industrial chemical to make fireworks, ammunition, synthetic rubber, and other substance.	[46, 49, 141, 144, 169, 178]
	Polychlorinated biphenyls (PCBs)	Utilised in a variety of industrial uses, including as dielectrics in transformers and large capacitors, as heat exchange fluids, as paint additives, in carbonless copy paper and in plastics.	[49, 144, 169]

Table 3.1: *Continues*

Unintended products	Toxaphene	Insecticide: used primarily to control pests on crops, such as cotton, cereal grains, fruits, nuts and vegetables, and on livestock. Also used to kill unwanted fish in lakes	[49, 141, 144, 169, 178]
	Dibenzodioxins & Dibenzofurans	Unknown. However, both are related to a variety of incineration reactions and use of a variety of chemical products	[35, 49, 144, 169, 178]

Therefore, humans are exposed to these substances on a daily basis, through various pathways, including consumption of contaminated food and water [150, 179, 185, 187, 188].

Recently, new POPs have emerged, namely the per-and polyfluoroalkyl substances (PFASs) (see relevant section in this review), which have been added to the list of POPs by the Stockholm Convention [67, 174]

Plants are known for up-taking and storing these nutrients and pollutants using various complex mechanisms, which have been largely reported in the literature reviewed. For example, Collins et al. [41] suggested that a number of processes facilitate the uptake of nutrients by plants, including transfer from soil and water to the roots; roots to the shoots; as well as sorption from the atmosphere through vapour. These mechanisms are herein suggested to be similar to those involved in POP uptake by plants [160]. To maintain the aim of this review, details on these mechanisms have been separately and briefly reported on in a supplementary file (SM1), with Table S1 depicting the primary uptake mechanisms for nutrient transport to root systems. However, researchers remain uncertain about the role of these mechanisms, with some even suggesting that roots on their own, were insufficient to effectively substantiate the translocation and storage of nutrients and/or pollutants by plants [130]. Decades ago, this uncertainty became clear with the discovery of aquaporins (AQPs) by Peter Agre [2, 9, 33, 90]. Thus, the discovery of AQPs shed some insight into the mechanism of water-transmembrane transportation [190].

Therefore, the main purpose of this review is to present an overview on the progress made thus far in attempting to understand the possibility of these proteins (i.e. AQPs) being the gateway that conduits nutrients, POPs and PFASs into plants.

3.3 Aquaporins: what are they?

The name 'aquaporin' (AQP) of Latin words: *aqua* which means water, and *porus* meaning passage, and was proposed by Agre and his team of researchers in 1993 resulting in the substitution of the traditional name, i.e. Water Channel Proteins (WCPs) [1, 3, 21]. AQPs belong to the class of major intrinsic proteins (MIPs) [14, 90, 113, 180], and have been defined as a family of minute, integral membrane proteins that are expressed generally in all living cells [113], including animals [90, 180, 184], plants [79, 80, 184], archaea, eubacteria and fungi [61, 90, 171].

3.3.1 Mammalian AQPs classes

Compelling evidence has suggested that there are 13 types of AQPs in mammals [156, 172], commonly divided into four subgroups: (a) orthodox or classical AQPs (AQP0, 1, 2, 4, 5, 6) which are selectively known to be water permeable [47, 132, 153]; and (b) aquaglyceroporins (AQP3, 7, 9, 10), which are believed to be permeable not only to water, but also to glycerol, urea and/or other small solutes [47, 64, 107, 132]; (c) water and ammonium AQPs (AQP8) [52, 156], and (d) super AQPs (AQP11, 12) which are dissimilar to other AQPs as they have been reported to have exceptional intracellular localization [47, 52, 64, 132, 156], with recent reports suggesting the permeation of water and glycerol through AQP11 [47, 106, 181], although their transport properties and/or functional selectivity are still not clearly elucidated [47, 64, 153]).

3.3.2 Plant AQPs classes

Plant AQPs on the other hand, have been classified by various sequencing techniques, into seven subfamilies, namely (i) nodulin 26-like intrinsic proteins (NIPs), (ii) plasma membrane intrinsic proteins (PIPs), (iii) tonoplast intrinsic proteins (TIPs), (iv) small basic intrinsic proteins (SIPs) [14, 75, 79, 90, 184], b; [22, 69, 81, 96, 112, 114, 116, 180], (v) the uncategorized (X) intrinsic proteins (XIP) [13, 43, 69, 84, 96, 100, 116, 120], (vi) the GlpF-like intrinsic proteins (GIPs) and (vii) the hybrid intrinsic proteins (HIPs) [13, 43, 69, 96, 184]. According to Li et al. [96], these subfamilies correspond to distinct and multiple subcellular compartments, a characteristic that explains the diversity of plant AQPs isoforms [190]. Table 3.2 depicts the classification of AQPs in cell membranes from selected edible plants, as they are presumed to be largely responsible for human POP exposure, thus suggesting research is required focusing on the relationship of these identified AQPs and the susceptibility of these crops to pollutants.

Furthermore, reports have suggested that AQPs are abundant and diversified in plants than in any other form of life [22, 43, 79–81, 90, 117, 147], with, AQPs of higher plants exhibiting a high diversity. For example, Sade et al. [145] suggested that 37 aquaporins are available in Solanum lycopersicum (i.e. 18 PIP, 9 TIP, 6 NIP, 3 SIP, and 1 XIP), while Park et al. [131] reported 71 in Gossypium hirsutum (i.e. 28 PIP, 23 TIP, 12 NIP, 7 SIP and 1 XIP), Zhang et al. [185, 187, 188] recounted 66 in Glycine max (i.e. 22 PIP, 23 TIP, 13 NIP, 6 SIP, 2XIP). As a typical example of this diversity, Figure 3.1 shows a phylogenetic tree of flax AQPs in comparison with those from

A. thaliana, *O. sativa*, *P. trichocarpa* with five distinct clusters representing a different class of AQPs [152]. The figure clearly indicates that in the plant kingdom, a single plant can have multiple AQPs, implying their various functions.

Table 3.2: Classification of AQP sequences from selected edible plants

Plant family	Plant species	Common name	Expressed AQP types	References
Poaceae	*Oryza sativa*	Rice	NIPs, PIPs, TIPs, SIPs	[105, 147]
			PIPs, TIPs	[99, 146]
			PIPs, TIPs, NIPs, SIPs	[57, 126, 147]
Poaceae	*Zea mays*	Maize	PIPs	[59, 111]
Fabaceae	*Phaseolus vulgaris*	Green bean	PIPs	[11]
Amaranthaceae	*Spinacia oleracea*	Spinach	PIPs, TIPs	[39, 108]
Solanaceae	*Nicotiana tabacum*	Tobacco	PIPs	[94, 110]
Poaceae	*Triticum aestivum*	Wheat	PIPs	[12]
Asteraceae	*Lactuca sativa*	Lettuce	PIPs	[45, 138]
Solanaceae	*Solanum lycopersicum L.*	Tomato	PIPs, TIPs, NIPs, SIPs, XIPs	[143]
Vitaceae	*Vitis vinifera*	Grapevine	PIPs, TIPs	[139, 151, 165]

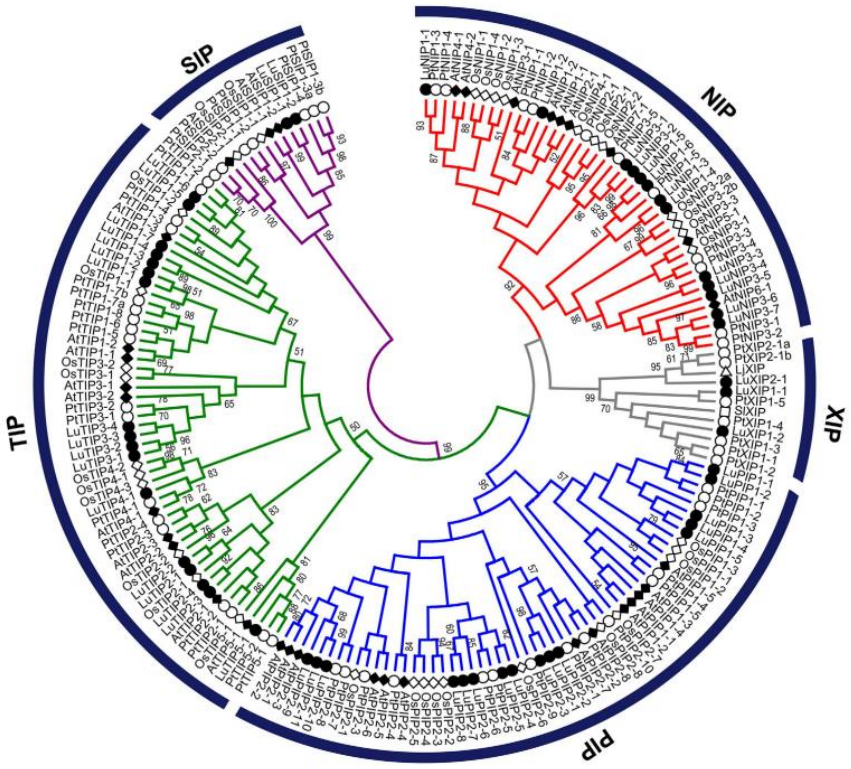


Figure 3.1: Phylogenetic tree analysis of plant AQPs. Different AQPs encoded in flax (Lu) are shown in comparison with the genes from rice, Arabidopsis, Solanum, Lotus, and Populus indicated with the prefixes Os, At, Sl, Lj, and Pt, respectively. The first and the last digit in the protein name, identify the group and the individual gene product, respectively [76]. Hence, in flax genome 16 PIPs, 17 TIPs, 13 NIPs, 2 SIPs and 3 XIPs were identified, and all the 51 AQPs are grouped into five different classes (i.e. PIPs, TIPs, NIPs, SIPs, and XIPs). Adapted from Shivaraj et al. [152].

In addition, from these statistics, it is evident that PIPs and TIPs are representative of AQPs in plants. According to Li et al. [96], PIPs are frequently shared among plants, which suggests characteristics that are inherited from their ancestors during the evolution of terrestrial plants; while Pérez Di Giorgio et al. [135] have further suggested that PIPs have functional constraints than their homologues, TIPs. Table 3.3 summarizes the diversity of AQPs in selected plant species.

Table 3.3: Diversity of aquaporin gene family in selected plant species

				Plant Aquaporin Subfamilies								
Plant family	**Plant Species**	**Common name**	**POP uptake potential**	**PIPs**	**TIPs**	**NIPs**	**SIPs**	**XIPs**	**HIPs**	**GIPs**	**Total**	**References**
Selaginellaceae	*Selaginella moellendorffii*	Spike moss	-	3	2	8	1	3	2	*n/r*	19	[10, 114]
Funariaceae	*Physcomitrella patens*	Moss	+	8	4	5	2	2	1	1	23	[43, 114, 148]
Poaceae	*Oryza sativa*	Rice	+	11	10	10	2	*n/r*	*n/r*	*n/r*	33	[68, 114, 147]
Brassicaceae	*Arabidopsis thaliana*	Mouse ear cress	+	13	10	9	3	*n/r*	*n/r*	*n/r*	35	[76, 114, 140, 189]
Solanaceae	*Solanum lycopersicum*	Garden tomato	+	14	11	12	4	6	*n/r*	*n/r*	47	[98, 114, 143]
Salicaceae	*Populus trichocarpa*	Black cottonwood	+	15	17	11	6	6	*n/r*	*n/r*	55	[16, 58, 114]

Table 3.3 : *Continues*

Fabaceae	*Glycine max*	Soybean	+	22	23	13	6	2	*n/r*	*n/r*	66	[44, 114, 185, 187, 188]
Malvaceae	*Gossypium hirsutum*	Upland cotton	+	28	23	12	7	1	*n/r*	*n/r*	71	[19, 114, 131]

n/r not reported, + detected, — undetected

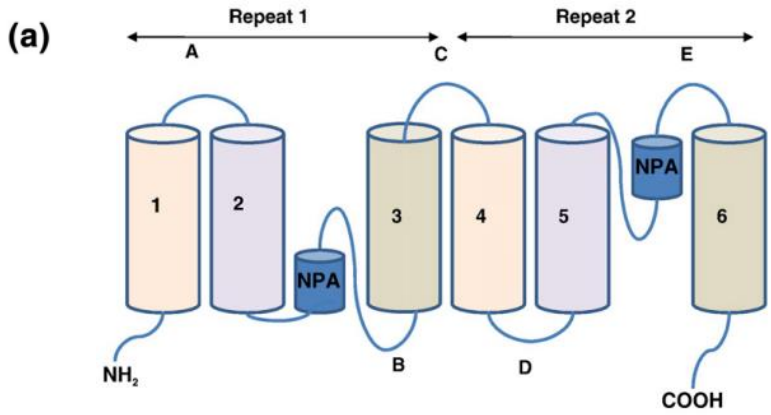
3.4 Structure and Transport Mechanism of AQPs

3.4.1 Aquaporins Common Structure

To date, there are several reports that have provided and discussed the structure and functional selectivity of AQPs [61, 87, 114, 152, 161]. Commonly, AQPs are 23–31 kDa proteins [117] sharing a common structural feature [37, 61, 117]. They consist of six transmembrane spanning helices [61, 85, 86] linked by five loops (A to E) located on the intra- (B, D) or extracytoplasmic (A, C, E) side of the membrane [114, 117]. As demonstrated in Figure 3.2a, adopted from Gomes et al. [55]. The amino (N-) and carboxyl (C-) termini extremities of the polypeptide are located on the cytoplasmic side of the membrane [61, 85, 86, 117], and the two halves of the polypeptide present a significant similarity to each other [192], and each half has hydrophobic loops (i.e. loop B and E), both containing the highly conserved signature motif asparagine, proline, alanine (NPA) signature motif [61, 117, 192] characteristic of most AQPs [192]. Structurally, loop B and E overlap in the centre of the lipid bilayer to form two hemipores, culminating in a narrow water-filled channel, which are crucial for water selectivity [192], thus rep-resenting a key feature for water permeation [61, 192]. In addition, AQPs contain an outer aromatic/arginine (ar/R) constriction with a width of ~ 2.8 ångström (Å) [see Figure 3.2b (iii)], which creates the narrowest section of the channel and constitutes a major restriction point for either solute and/or pollutant permeability [61]. This channel functions as a main selectivity filter [114] and is thought to have substrate specificity [87]. Thus, structural and simulation studies have indicated that the seventh transmembrane domain is intimately involved in facilitating an aqueous pathway for solutes through the AQP [42, 78, 124, 157]. Three-dimensional structure analyses in various organisms, including plants, i.e. spinach [55, 161, 92] have shown that AQPs share typical but conserved structural properties [42]; and are able to form tetramers in the membrane, with each subunit defining its own pore. The four subunits are arranged in parallel, forming a fifth pore in the centre of the tetramer [42, 55], as shown in Figure 3.2b (i), adopted from Gomes et al. [55]. Each monomer functions independently as a single pore channel [55].

Therefore, it is worth indicating that the structure of the channels (i.e. AQPs) is important, because it determines: (1) which molecules permeate and/or are excluded from the channels, and (2) at which rate molecules are translocated through the pores [61]. This suggests that both the

size and/or volume of the compound and that of the AQP pore are interdependent to facilitate the uptake process of nutrients and other compounds, even pollutants. In this regard, Da Ines [42] reported that the pore can narrow to approximately 3 Å in diameter, which can limit the transportation of large uncharged molecules through the AQPs, which suggests that such a pore is just large enough to accommodate a single water molecule [42]. This is in agreement with a study by Ye et al. [182] whose findings formerly suggested that AQPs of either a bigger and smaller diameter (volume) will present different translocation selectivity between osmolytes, leading to small solutes being permeable across bigger AQPs, but not across the small pores, with large solutes being completely excluded from all pores; a trend which concured with observations by Hub and de Groot [66]. The study further suggested large osmolytes splitting, which ultimately allowed the researchers to evaluate the size of large and small AQP channels (i.e. AQPs).



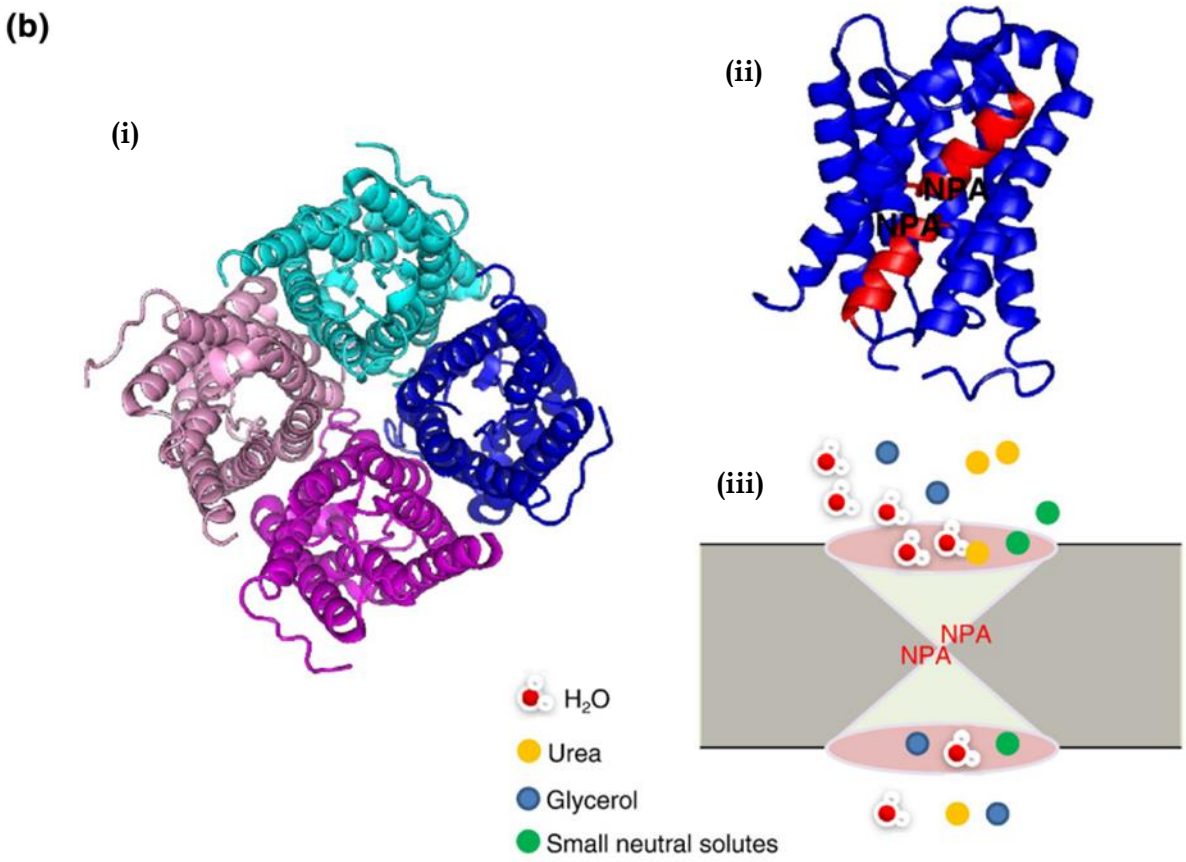


Figure 3.2: (a) AQP structure topology. The primary structure of AQPs comprises 6 transmembrane domains (1–6) connected by five loops (a–e), with cytoplasmic N- and C-termini, is shown. Highly conserved NPA (Asn-Pro-Ala) motifs are located at the loops B and E and form short hydrophobic helices that fold back into the membrane from opposite sides. **(b)** Three-dimensional structure of spinach SoPIP2;1 (adopted from [55]). AQPs are grouped as tetramers in biological membranes **(i)**. Each monomer **(ii)** functions as a single channel pore. The intracellular loop B (blue) and the extracellular loop E (red) fold into the membrane and interact with each other through the NPA motifs, forming a central constriction and participating to the pore selectivity (**iii)**. IC intracellular; EC extracellular. In addition to water, several small molecules, such as glycerol and urea, and small neutral solutes and ions, are reported to permeate some AQPs. Adapted from [55].

3.5 Plant AQP Isoforms: Their Different Structure and Selectivity

Recent events in genetic techniques have demonstrated and elucidated species-specific differentiation for each of the abovementioned AQPs subclasses [112, 114]. Hence, two isoforms, i.e. SoPIP2;1 and AtTIP2;1, prevalent in Spinacia oleracea and Arabidopsis thaliana, respectively, have been widely studied as they represent two plant AQPs structures with very different

substrate specificity and pore profile, attributes which have either facilitated or restricted different molecules.

3.5.1 PIP Isoforms

Expressed mainly in the plasma membrane [55], PIPs represent the largest subfamily of plant AQPs as previously indicated, consisting of numerous members, with 13 members being identified in Arabidopsis, 14 in maize, 11 in *Oryza sativa*, and 14 in *Populus trichocarpa* [4, 36, 76]. They are phylogenetically divided into two subgroups, i.e. PIP1 and PIP2 [27, 114] and [156], with PIP1s having a longer N-terminal section, a shorter C-terminal section and a shorter extracellular loop A than PIP2s [27, 76]. Unlike in the PIP1 subgroup, higher water channel activity has been reported in PIP2 members [27], although, when PIP1s are co-expressed with PIP2s, a synergistic effect on water channel activity is observed [20, 27]. In the case of *Arabidopsis thaliana*, PIP1 and PIP2 have five and eight isoforms [76], respectively, as depicted in Figure 3.1, with SoPIP2;1 being a typical example isoform in *Spinacia oleracea* (spinach). For example, an in vivo analysis demonstrated two phosphorylated serine residues in response to an increase in the apoplastic water potential [91], with phosphorylation being suggested to be responsible in regulating the water channel activity of SoPIP2;1, and thus regulating the water channel activity in this protein [77]. In this regard, a study by Törnroth-Horse-field et al. [161] presented evidence of an X-ray structure of SoPIP2;1 depicting both a closed conformation at a resolution of 2.1 Å (Figures 3.3 and 3.4) and an open conformation at 3.9 Å. Thus, SoPIP2;1 is the only plant AQP for which an atomic resolution at 2.1 Å based on X-ray crystallography is available [161]. Generally, SoPIP2;1 is a water-specific protein [87], but Gomes et al. [55] have suggested that, its 2.1 Å pore diameter makes it susceptible to serve as a pathway for molecules smaller than water.

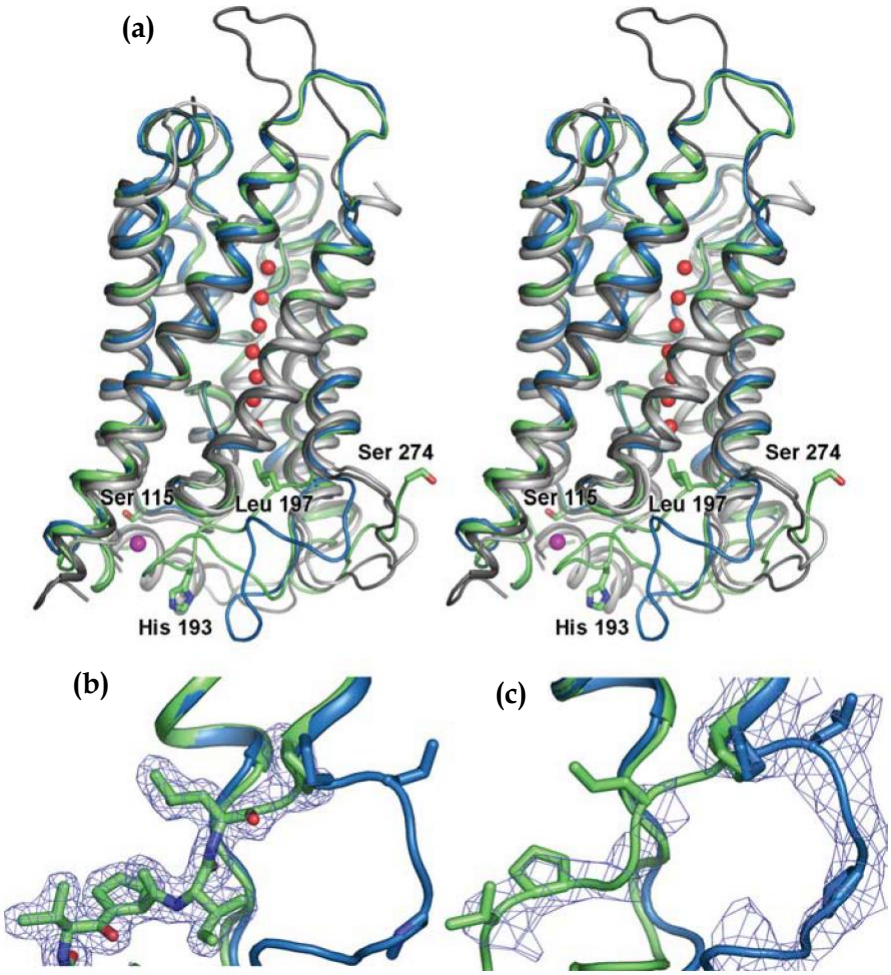


Figure 3.3: Structures of the closed and open conformations of SoPIP2;1 [161]. **(a)** Stereo models of SoPIP2;1 in its open (blue) and closed (green) con-formations overlaid on that of AQP0 (light grey; Protein Data Bank (PDB) entry 1YMG) and AQP1 (grey; PDB entry 1J4 N). **(b)**, **(c)** Electron density for loop D in the closed (b, green) and open (c, blue) conformations. Residual electron density in c indicates that the closed conformation is also present in partial occupancy.

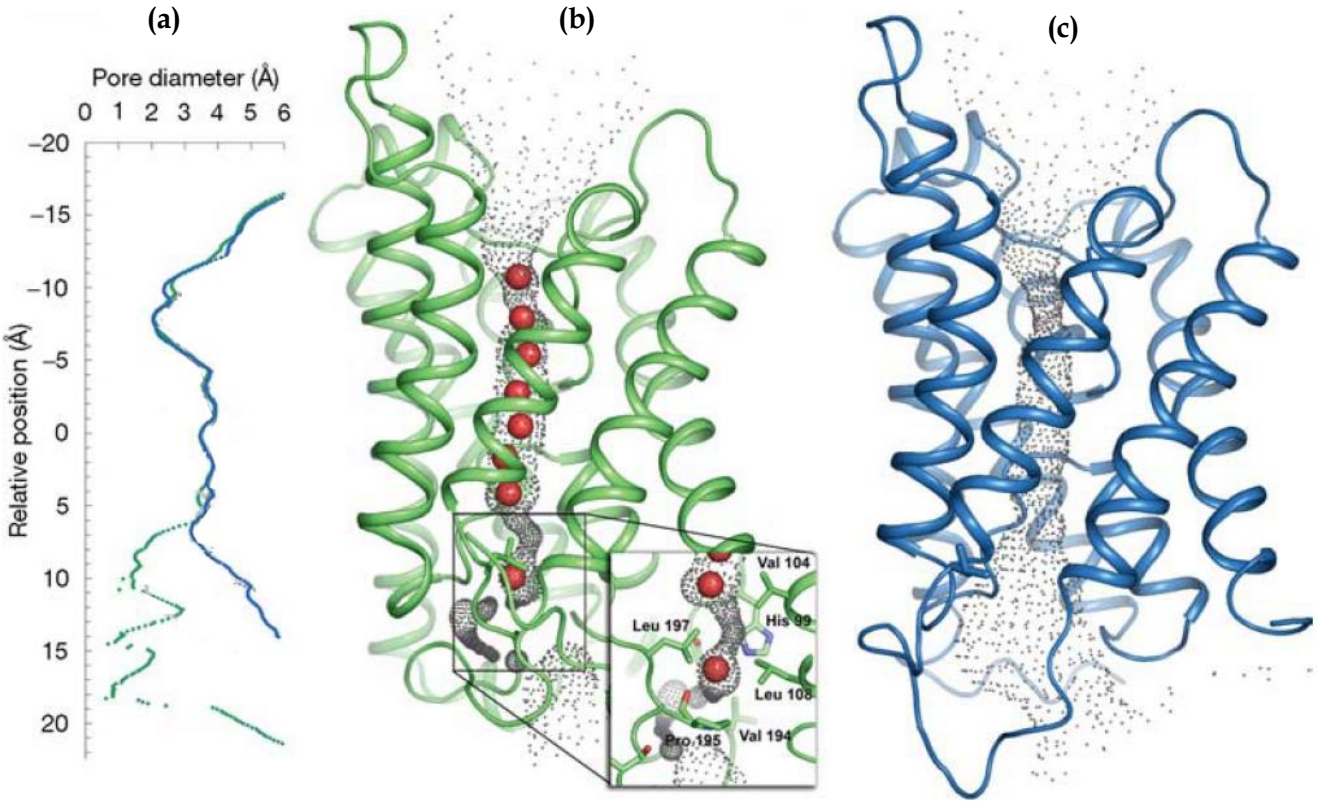


Figure 3.4: Characterizing the SoPIP2;1 isoform. **(a)** The pore diameter of the closed conformation of SoPIP2;1 (green), and the open conformation of SoPIP2;1 (blue), represented as a function of the distance from the NPA signature sequence calculated with HOLE32. **(b)** The same information for the closed conformation of SoPIP2;1 as in a but represented as a funnel illustrating the pore boundaries. The inset shows the pore near the gating region of loop D characterized by Leu 197, Pro 195 and Val 194. **(c)** The same representation as in **b** but corresponding to the open conformation of SoPIP2;1. Adapted from [161].

3.5.2 TIP Isoforms

TIPs are expressed primarily in the tonoplast membrane, although other subcellular locations cannot be ruled out [55]. AQPs are the most abundant proteins of the tonoplast, which explains why the water permeability of the tonoplast is higher than that of the plasma membrane [55, 109]. Based on their sequence homology [142], TIPs are divided into five subfamilies [114, 142, 156]: TIP1, TIP2, TIP3, TIP4 and TIP5 [83, 147], and are believed to have several isoforms, i.e. TIP1 (TIP1;1, TIP1;2, and TIP1;3), TIP2 (TIP2;1, TIP2;2, and TIP2;3), TIP 3 (TIP3;1 and TIP3;2), TIP4 (TIP4;1), and TIP5 (TIP5;1) [76, 142], with their diversity as a guarantee for their survival [83]. In addition to their role as water channel proteins, TIPs also transport hydrogen peroxide (H_2O_2), besides glycerol [109, 142] and exhibit functional characteristics associated with water flow regulation in response to drought and salinity stresses, as evidenced in *Arabidopsis thaliana* [6, 32, 79]. Furthermore, they have been reported to enhance nitrogen-uptake efficiency and detoxification by acid entrapment of ammonium ions in vacuoles [87, 102]. A study by Kirscht et al. [87] became the first in establishing an understanding of the structural features that confer ammonia selectivity for the AtTIP2;1 isoform, and for *Arabidopsis thaliana*. In this regard, the current study has presented a crystal structure of AtTIP2;1 (see Figure 3.5) determined at an atomic resolution of 1.18 Å using X-ray diffraction coupled with molecular dynamics (MD) simulations in order to study functional properties of mutants, thus providing new insights into the molecular basis of substrate selectivity in the AQP superfamily [87]. Hence, this became indicative of (a) an extended selectivity filter (SF), a section out of which a narrowest region of the channel lumen is formed due to the conserved ar/R, with the former providing the AQP its selectivity towards water molecules, and ultimately, its ability to distinguish the molecule from protons (Figure 3.6a); (b) the presence of a water-filled side pore [83, 87], which extends from the loop C near the extracellular side of the protein directly into the main pore into the SF (see Figure 3.5). This provides a rare second means of entry into the permeation conduit. Hence, such an insight has shown that the SF region is the narrowest part of the channel, while the pore diameter of AtTIP2;1 (3 Å) was determined to be uniform throughout the channel (see Figure 3.6a); ultimately, this is in contrast with previously reported structures of other AQPs, as proposed by Kirscht et al. [87]. This recent revelation further suggested that the AtTIP2;1 isoform has the ability to serve as a mode of translocation for compounds larger than water [83, 87].

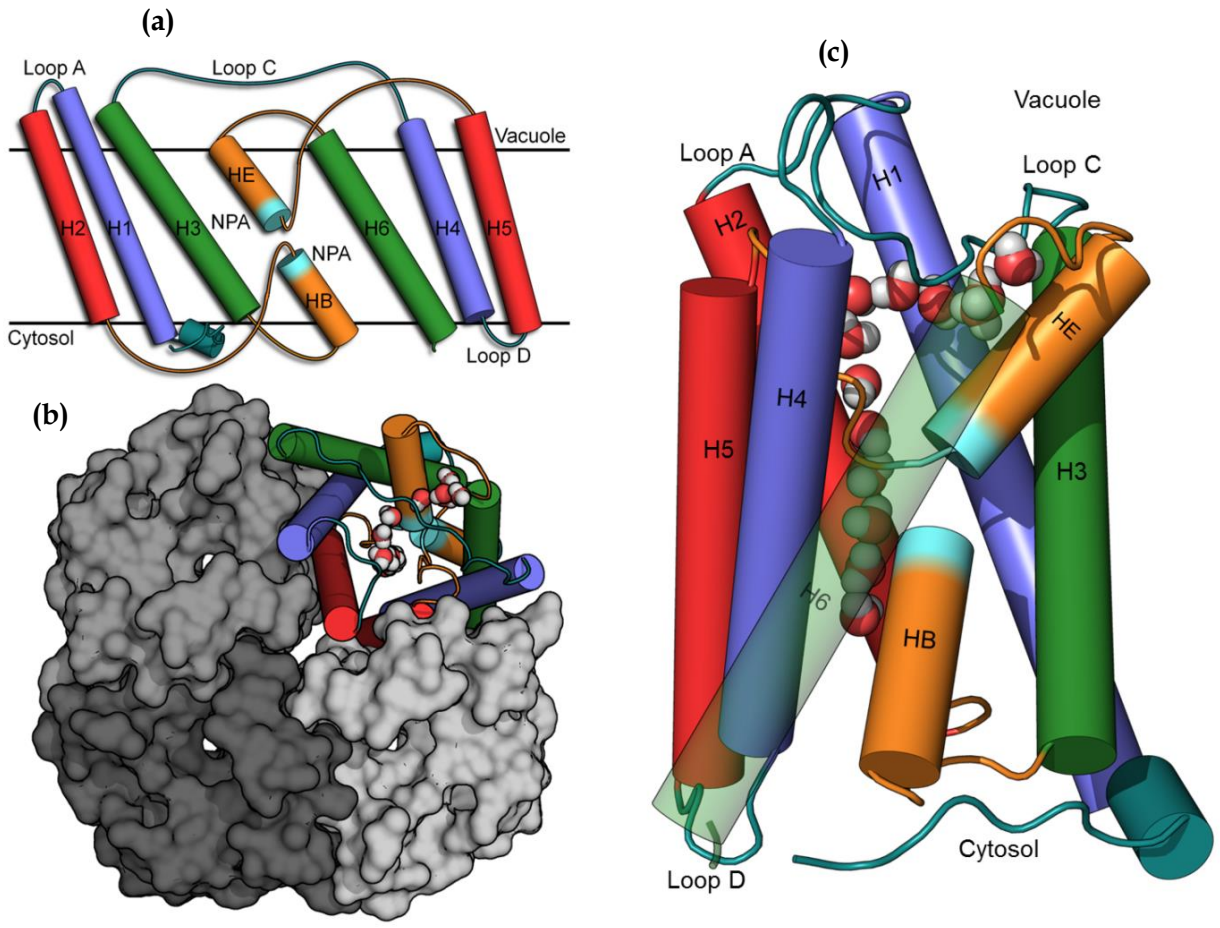


Figure 3.5: Structure of AtTIP2;1 [87]. **(a)** Membrane spanning helices (H1-H6) and two half helices (HB and HE), connected via conserved NPA-motifs, form a pore through the vacuolar membrane. Homologous helices in the internal repeat are indicated in colour. **(b)** AtTIP2;1 tetramer viewed from the vacuolar side. **(c)** Side view of the monomer with the same orientation as in a. Eight water molecules form a single file in the main pore, and five water molecules are seen in a side pore underneath loop C.

In this regard, Figure 3.6b depicts pore and SF differential comparisons between water-specific proteins, e.g. SoPIP2;1 and AtTIP2;1, but only those which have been proven to be different at the level of their individual pore diameters. Hence, the former has a smaller pore diameter, which is wide enough to facilitate the permeation of smaller but not larger molecules into the cell membrane of the plant; while the latter, has a wider pore capable of facilitating the translocation of both smaller and larger compounds.

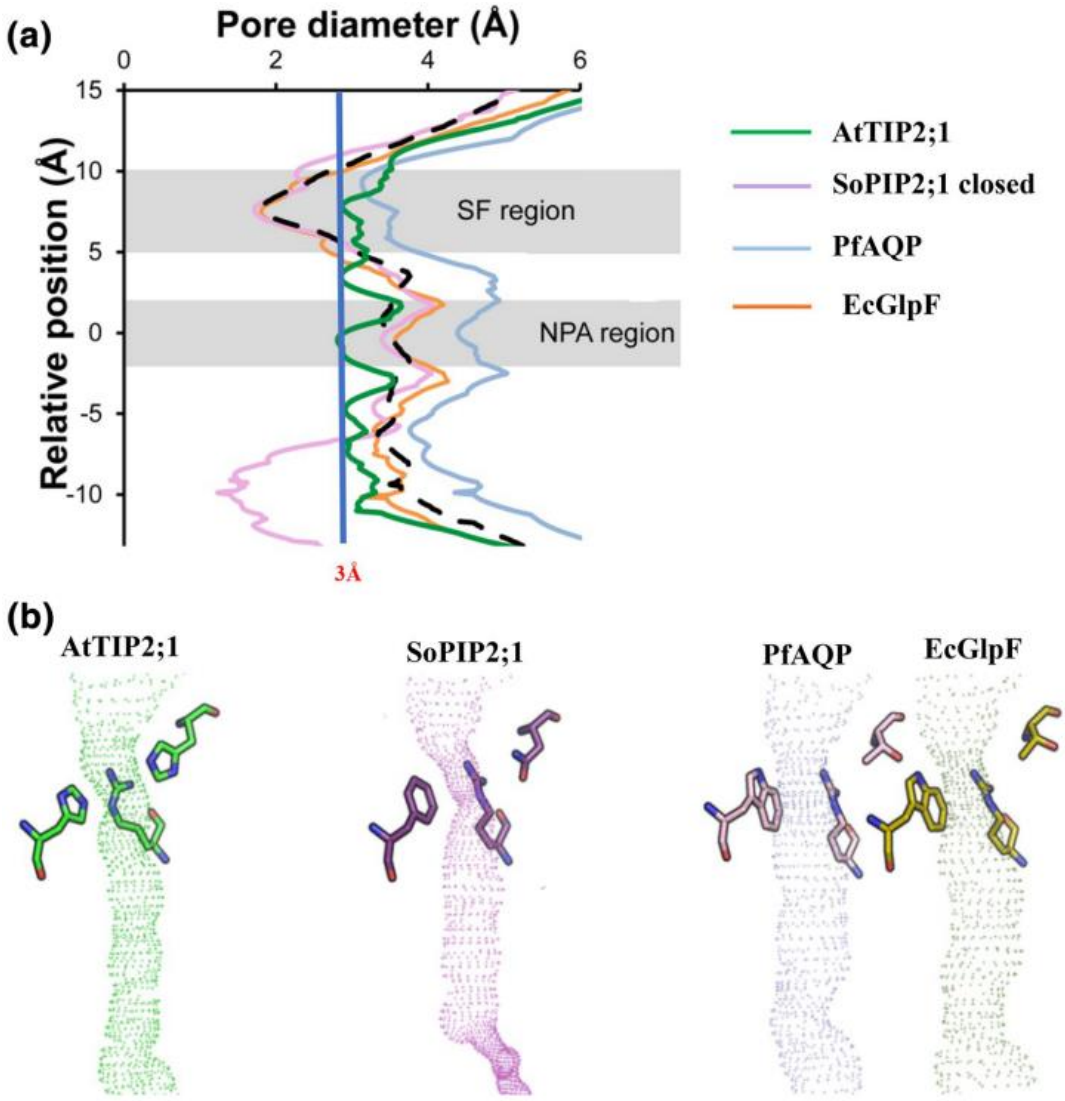


Figure 3.6: Comparison of pore diameter and the extended selectivity filter of different AQPs. Individual isoforms of AtTIP2;1 (green), water-specific SoPIP2;1 in closed conformation (purple), as well as average diameter of other open water-specific AQP structures are shown. AtTIP2;1 (green) provides a more or less constant/uniform pore diameter at 3 Å (blue), thus suggesting its ability to serve as a conduit for molecules and/or compounds larger than water **(a)**. AtTIP2;1 presents the narrowest NPA, but a much wider SF region; only glycerol-containing structures such as PfAQP and EcGlpF have a larger diameter at the SF **(b)**. AtTIP2;1 (green) is compared to the water-specific SoPIP2;1 (purple) and two other AQPs (e.g. glycerol-permeable EcGlpF) (b). Adapted from [83] and [87].

3.6 Plant AQPs Translocate Nutrients and Facilitate Uptake

When AQPs were first discovered, it was reported that their significant impact was unique for water transportation in living cells [3], for example, of plants [79, 184] and animals [90, 180, 184]. To date, compelling evidence has indicated that some plant AQPs facilitate the transport of small solutes or gases and nutrients [156]. For example, most PIPs are characterized to facilitate water diffusion; while TIPs are primarily for the diffusion of water, urea, ammonia, and H_2O_2, with NIPs being associated the diffusion of metalloids (boric acid and arsenite) in addition to glycerol and water [53]. In addition, boron is an essential nutrient for plants, which in its boric acid form, is also structurally related to water [112]. It has since been reported that AtNIP5;1 (a NIP isoform in the case of *Arabidopsis thaliana* plant type) transports boric acid in *Xenopus oocytes* and significantly contributes to the root uptake of boron [117]. Similarly, AtNIP6;1 and AtNIP7;1, which are selectively expressed in leaf nodes and floral anthers, respectively [96, 97, 158], were also reported as boric acid translocation facilitators [96]. It is worth mentioning that, despite boron being an essential metalloid for plants, its excessive presence into the environment is undesirable, particularly in the agricultural sector [34, 158].

Moreover, abundant evidence has identified OsNIP2;1 (a NIP isoform identified in *Oryza sativa*, rice) as the first silicon transportation protein in plants [96, 104]. Like its homologue boron, silicon is another essential mineral component for certain plants [96]. Hence, OsNIP2;1 functions as an influx channel for silicic acid, allowing in the process, the uptake of silicon from the soil into the root stele and vascular tissues [96, 103, 104, 119]. A study by Mitani-Ueno *et al*. [118] also reported on the role played by the residue at the H5 position of the ar/R filters of both OsLsi1 and AtNIP5;1 in the permeability and uptake of arsenic by rice, a staple food for several communities worldwide, implying arsenic accumulation in rice grain as a serious threat to human health [118, 191, 193].

Furthermore, recent evidence has suggested that other plant AQPs expressed in plant tissues, where water flow dynamics appear to be low and/or less needed, have been responsible for solutes and other chemical compounds' acquisition by the plants evaluated [26, 86, 133, 134, 156]. This was explained in the case whereby the AQP in question is found to be hydrophobic [152]. For instance, the ar/R selectivity filter in XIPs from different plants is more hydrophobic in

nature, so is NIP1s. The hydrophobic nature of these AQPs has recently been reported to facilitate the transportation of bulky and hydrophobic molecules such as glycerol, urea and boric acid, in crops [24, 152]. Similarly, ammonium/ammonia (NH_4^+/NH_3) is an important nitrogen fertilizer for crops [96]. Carriers of NH_4^+ have been documented in several studies, with diffusion being suggested to be the primary transporter of NH_3 into cell membranes [73, 96]. New evidence has indicated that various TIP2 isoforms of Arabidopsis and wheat, were suitable for the permeability of this compound (i.e. NH_3), with some AQP isoforms having an ability to distribute NH_3 in various crop compartments [73, 96, 102]. The aforementioned could not be confirmed in a study by Loqué *et al*. [102], as evidence could not be found to suggest that NH_4^+/NH_3 uptake is facilitated by AtTIP2;1 and AtTIP2;3 although these AQPs were over-expressed in Arabidopsis. This was clarified by Kirscht et al. [87] who revealed new features that were not predicted by homologue modelling [53], such as the one used by Loqué *et al*. [102]. These features, include an extended selective filter, due to a fifth residue of the ar/R and a wider pore diameter, i.e. 3 Å [53, 87], highlighting for the first time that NH_4^+ might be deprotonated by the interaction with this His, while NH_3 then moves through both the main pore and protons through a side pore to the vacuolar surface [53, 87]. This suggested the furtherance of the AQPs research field, since we are still far from a fully integrated view of the function profile of AQPs [96]. In addition, excessive levels of NH_4^+ in the environment can lead to NH_4^+ toxicity, which can lead to crop-growth suppression [60] and yield reduction [164].

3.7 Plant AQPs and Their PFASs and POPs Potential Acquisition

3.7.1 PFASs Structural Manufacturing Process

Per- and polyfluoroalkyl substances (PFASs) are a class of man-made chemical compounds, implying they are not naturally found in the environment [50]. Available evidence has indicated that, various types of PFASs have been manufactured, with PFOA ($C_7F_{15}COO^-$) and PFOS (PFOS; $C_8F_{17}SO_3^-$) being predominantly used [5, 93, 155]. Their production processes have involved the use of electrochemical fluorination technologies, which have conferred unique physicochemical properties to these compounds (see Figure 3.7), not observable in many other synthetic compounds. Their structural integrity is associated with hydrogen atoms substitution by fluorine atoms [122]. Due to these properties, PFASs are stable, heat resistant, water- and fat repelling; and for this reason, PFASs have become popular in numerous industries and the

manufacturing of consumer products [62, 88]. The excessive application of these compounds by several economic sectors has led to their widespread distribution within the ecosystem. Thus, to date, compelling evidence as documented by scientists, clearly indicates the accumulation of PFASs in several environmental matrices, including several plants, some of which are edible crops [28, 29, 40, 125, 137, 155, 175, 186]. The consumption of crops, contaminated by these substances has been suggested, as the main cause of PFASs exposure to humans [28–30, 63]. In addition, some plants have proven to be more susceptible to PFASs than others [121]; this trend has not yet been explained.

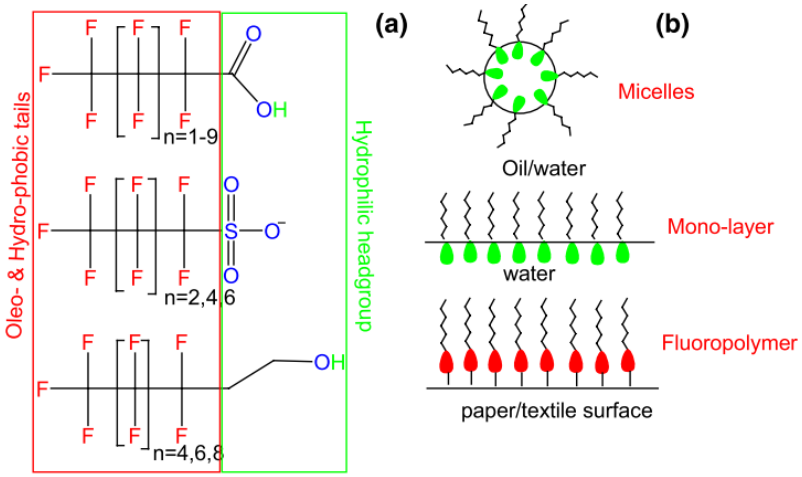


Figure 3.7: Chemical structure of PFASs [123]. **(a)** PFASs physicochemical properties are shown. They have a fluorinated tail and a hydrophilic head, thus making chain that can vary in chain length (n, represents the number of carbons in the perfluorocarbon chain). **(b)** Schematic diagram for hydrophobic interaction in different environments.

3.7.2 Why are AQPs the Potential Reason for Plant PFASs and POPs Uptake?

To our knowledge, not much has been said in the literature about the possibility of AQPs being the gateway that facilitates PFASs and other POPs into plants. Recently, a study by Wen *et al*. [176] reported, for the first time, that protein and lipid presence within plants, plays a role in the accumulation and distribution of PFOS and PFOA in plants. However, the authors suggested that an exact explanation for the observed effect remains to be proven. Similarly, Mudumbi *et al*. [121] suggested that different plants variably accumulate PFASs, but this study had not justified the observed trend.

In addition, available evidence has indicated that PFASs have carbon–fluorine bonds (C–F) with a typical size of about 1.35 Å [89, 149]. This size (i.e. 1.35 Å) is smaller in comparison to pore diameter associated with numerous isoforms, TIP2s, i.e. AtTIP2;1_2.1 Å and PIP2s, i.e. SoPIP2;1_3 Å. Recently, it was suggested that the AQP pore-length determines which molecules permeate and/or are excluded from the channels, while regulating the rate at which molecules can move through the pores [61]. Hence, this, in our view, suggests that PFASs are likely to be absorbed, translocated and distributed by AQPs whose pore diameter matches the C-F bond size in conjunction with other smaller compounds.

Furthermore, distribution and accumulation of PFASs (i.e. PFOA and PFOS) in plants have been suggested to be species-dependent [176]; so is the expression of AQPs in plant species. Available evidence has indicated that, AQP proteins are expressed in multiple isoforms [4], including 35 in Arabidopsis and 33 homologues in rice [76], of which some might have different functional aspects as elucidated in this review (see Table 3.4). A complete understanding of AQP functions requires a precise knowledge of their expression, structural properties in specific tissues, cell types and compartments [42]. Moreover, to our knowledge, these characteristics have not been reported, particularly in the case of PFASs, and various other POPs, suggesting that, this field of research still requires more attention from researchers.

Table 3.4: Summary of functional expression and substrates uptake specificity of typical plant aquaporins

Subclass	Isoform	Substrate	Expression System	Transport Assay	References
PIP	*At*PIP2;1	Water	Proteoliposome	Shrinkage	[114, 170]
	*At*PIP2;1	H_2O_2	Yeast	Toxicity growth assay	[48, 114]
	*At*PIP2;2	Water	*Xenopus oocyte*	Swelling	[114, 162]
	*Nt*AQP1	Glycerol	*Xenopus oocyte*	Radiolabeling	[23, 114]
	*Nt*AQP1	CO_2	*Xenopus oocyte*	Intracellular pH	[114, 166]
	*Nt*AQP1	CO_2	Yeast	Intracellular pH	[114, 129]
	*Nt*AQP1	CO_2	Planar lipid bilayer	Local pH	[114, 167]
TIP	*At*TIP1;1	Water	*Xenopus oocyte*	Swelling	[114, 115]
	*Nt*TIPa	Urea	*Xenopus oocyte*	Radiolabeling	[54, 114]
	*Nt*TIPa	Glycerol	*Xenopus oocyte*	Radiolabeling	
	*At*TIP1;2	H_2O_2	Yeast	Intracellular fluorescence	[25, 114]

Table 3.4 : *Continues*

TIP	*Ta*TIP2	NH_3	Yeast	Extracellular pH	[73, 114]
	*Zm*TIP1;1	H_2O_2	Yeast	Toxicity growth assay	[18, 114]
	*At*TIP2.3	NH_3	*Xenopus oocyte*	Radiolabeling	[102, 114]
NIP	*At*NIP5;1	$B(OH)_3$	*Xenopus oocyte*	Intracellular dosage	[114, 159]
	*Os*NIP2;1	$Si(OH)_4$	*Xenopus oocyte*	^{68}Ge-radiolabeling	[104, 114]
	*Zm*NIP2;1	GeO_2	Yeast	Toxicity growth assay	[114, 118]
NIP	*At*NIP5;1	$As(OH)_3$	*Xenopus oocyte*	Intracellular dosage	[74, 114]
	*Bj*NOD26	Water	Proteoliposome	Shrinkage	[74, 114]
	*Bj*NOD26	NH_3	Proteoliposome	Internal pH	
SIP	*Vv*SIP1	Water	Yeast	Shrinkage	[70, 114]
	*Vv*SIP1	Water	Proteoliposome	Shrinkage	
XIP	*Nt*XIP1;1	H_2O_2	Yeast	Toxicity growth assay	[24, 114]
	*Pt*XIP2;1	Water	*Xenopus oocyte*	Swelling	[101, 114]

3.7 Conclusion

Plants play a major role in the environment and need not only sunlight and water to grow, but also nutrients. Thus, nitrogen, potassium and phosphorus are some of the nutrients referred to, and are reported to be essential for plant growth; while Cr, Mn, Fe, Co, Cu, Zn, and Se are examples of heavy metals and/or toxicants, identified in various plants. It is also now well known that plants also come into contact with an amalgam of other toxic chemical elements, such as POPs and PFASs, present in the environment, some of which have been extensively detected in plants. In addition, although the plant root systems being previously regarded as the major contributors to these chemical compounds translocation and storage in plants, recent evidence has reported that plants make use of a variety of mechanisms (see SM1 and Table S1) to uptake and store these nutrients and other toxicants in plant cell membranes. Hence, the mechanism that facilitates the uptake of water, nutrients and other essential minerals, as well as toxicants such as POPs and PFASs, was thought to be limited to the physical and diffusive mechanisms until the discovery of AQPs—proteins that expedite water permeability in living cells, including those in plants, in which these proteins (i.e. AQPs) have been said to be more diversified than in animals. Some structural studies have revealed that AQPs share a common fold and a narrow substrate-conducting channel. Hence, to date, there are numerous AQPs that have been identified in an assortment of plants' living cell membranes. Thus, plants' AQPs were previously classified into four groups or subfamilies, i.e. NIPs, PIPs, TIPs and SIPs, to which three additional subfamilies (i.e. XIP, GIPs and HIPs) have recently been added. Research studies have revealed that plants' AQPs are not only contributors to water and mineral nutrients' translocation in plant cell membranes, but also act as pathways facilitating the transport of toxic trace metals such as arsenic (As), antimony (Sb) metalloids, etc. Thus, various plants with specific AQPs have recently tested positive for the uptake and storage of some POPs, some of which include emerging POPs, such as PFASs. For instance, positive translocation correlations were found in membrane proteins present in maize (*Zea mays*) and PFOA and PFOS. However, despite the alleged evidence that has emerged demonstrating the role that plant proteins and/or AQPs might play in the uptake, translocation and tissue dissemination of nutrients, POPs, and thus PFASs by plants, researchers have indicated that it is too soon to consider this recent observation as an explanation. This further suggests that the question remains unresolved as to whether AQPs are the gateway which

conduits these chemical compounds into plants. Furthermore, to answer this question, more studies in this field are required.

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CHAPTER 4

The role of pollutants in type 2 diabetes mellitus (T2DM) and their prospective impact on phytomedicinal treatment strategies

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4.1 Abstract

Type 2 Diabetes *Mellitus* (T2DM) is the most common form of diabetes and it is characterised by high blood sugar and abnormal serum lipid levels. Although the specific reasons for the development of these abnormalities are still not well understood, traditionally, genetic and lifestyle behaviour have been reported as the leading causes of the disease. In the last three decades, the number of diabetic patients has drastically increased worldwide, with current statistics suggesting the number is to double in the next two decades. To combat this incurable ailment, orthodox medicines, to which economically disadvantaged patients have minimal access, have been used. Thus, a considerable amalgamation of medicinal plants have recently been proven to possess therapeutic capabilities to manage T2DM; and this has prompted studies primarily focusing on the healing aspect of these plants, and ultimately, their commercialization. Hence, this review aims to highlight the potential threat of pollutants, i.e. polyfluoroalkyl compounds (PFCs), endocrine disrupting chemicals (EDCs) and heavy metals, to medicinal plants, and their prospective impact on the phytomedicinal therapy strategies for T2DM. It is further suggested that auxiliary research be undertaken to better comprehend the factors that influence the uptake of these compounds by these plants. This should include a comprehensive risk assessment of phytomedicinal products destined for the treatment of T2DM. Regulations that control the use of PFC-precursors in certain developing countries are also long overdue.

Keywords: Diabetes *mellitus*, Medicinal plants, PFCs, EDCs, Synergy

4.2 Introduction

The 21st century has seen an increase in chronic and lifestyle related diseases worldwide, some of these being associated with high mortality rates, including diabetes *mellitus* (DM). In fact, it has been indicated that chronic diseases are the leading cause of death in the world (Yach et al. 2004), with these diseases becoming the dominant burden on health systems in many developing countries (Nugent 2008). From a South African perspective, chronic diseases were reported to be the main cause of death in 2000, and these included cardiovascular diseases and diabetes (Reddy 2003). Similarly, CVD were reported as the second leading cause of death in South Africa after HIV/AIDs (Matsha et al. 2012); and recently, diabetes has been added as a major risk factor for people infected by the virus (Dimala et al. 2016; Isa et al. 2016; Moreira et al. 2016). Hence, DM has been described as a chronic (Zimmet et al. 2001) and metabolic disorder (ADA 2014) with compound aetiology and characterized by a raised blood sugar, medically referred to as hyperglycemia (Rehman et al. 2011). Accordingly, hyperglycemia is said to be accompanied, in most cases, by changing degrees of disrupted carbohydrate and fat metabolism (Waugh and Grant 2014), and should not be confused with normoglycemia, which is the normal blood sugar concentration (ADA 2014). The World Health Organization (WHO), had previously indicated that DM is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO 1999). Moreover, in the human body, blood glucose levels are controlled by two hormones, namely insulin and glucagon (Waugh and Grant 2014). Both these hormones are secreted by the pancreas, and are believed to perform opposing actions (Bell et al. 1983; Ahrén et al. 2004). Thus, insulin primary function is to lower raised blood nutrient levels, including glucose, amino acids, and fatty acids (Waugh and Grant 2014), while the glucagon, on the other hand, unlike its counterpart, the insulin, increases blood glucose levels by means of glycogenolysis, i.e., the conversion of glycogen to glucose (Bell et al. 1983; Ahrén et al. 2004; Waugh and Grant 2014). Additionally, glucose is seen as a source of energy for the cells that make up muscles and other body tissues, and comes from one major source, namely food (including plants). According to Rodriguez (2004), carbohydrates that are consumed become blood glucose and are used by the body. It is thus understood that, if we do not use this glucose,

the body stores it, and ultimately becomes fat (Rodriguez 2004), leading to obesity and a risk of developing diabetes (Russell-Jones and Khan, 2007; Daniele et al. 2014). Similarly, when the body becomes incapable of making sufficient quantities of insulin, or, is unable to use insulin effectively, or the combination of both, this can potentially culminate into diabetes. Additionally, DM has been recently referred to as an endocrine-related disease and disorder (Bergman et al. 2013; Birnbaum 2013), suggesting it is related to the functioning of the endocrine system. For example, Alberti and Zimmet (1998) indicated that various pathogenic processes are involved in the development of diabetes, among which some processes related to the destruction of beta cells in organs such as the pancreas, are included (Bloom 2012; Petzold et al. 2015).

Furthermore, it has been indicated that there are different cases of DM, of which fall into two wide etiopathogenetic categories, namely type 1 diabetes (T1D) and type 2 diabetes (T2D) (ADA 2014). Accordingly, available data has suggested that a deficiency in insulin secretion leads to T1D, while a combination of resistance to insulin action and an insufficient compensatory insulin secretory response are allegedly responsible for T2D (WHO 1999; ADA 2014). Similarly, there is a strong link between type 2 diabetes *mellitus* (T2DM) with overweight and obesity, age increase, ethnicity, and family history (IDF 2017). On the other hand, recent evidence on dietary factors has further reported an association between excessive consumption of sugar-sweetened beverages and risk of T2DM (Malik et al. 2010; Imamura et al. 2015; IDF 2017). Hence, Table SM1 and Figure SM1 depict disorders of glycemia: etiological types and clinical stages and etiological classification of disorders of DM (provided as supplementary material).

4.3 Type 2 diabetes mellitus and the role of pollutants

4.3.1 Polyfluoroalkyl compounds and diabetes

Polyfluoroalkyl compounds (PFCs) have been described as new emerging persistent organic pollutants (POPs) (Corsini et al. 2014), and they cover a wide assortment of anthropogenic chemicals that were manufactured between the late 1940s and to date (Jiang et al. 2015; Niu et al. 2016) using electrochemical fluorination and telomerization (Banks et al. 2013; Jiang et al. 2015). These compounds have unique physicochemical properties, such as chemical stability, hydrophobicity, oleophobicity, etc. (Gao et al. 2015; Zhang et al. 2015; Hidalgo and Mora-Diez 2016). Hence, owing to these properties, PFCs have been widely used in many consumer

products, including carpets, textiles, packaging products, leather, home furnishings, paper products, non-stick cookware, and numerous cleaning products (Kotthoff et al. 2015; Bečanová et al. 2016). Additionally, there are several hundred types of PFCs (Martin et al. 2006; Ahrens 2009), of which perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are the most studied and documented (Stahl et al. 2009; Lechner and Knapp 2011). Consequently, various PFCs have been found to bioaccumulate and persist in numerous environmental matrices (Naile et al. 2013) including plants and freshwater sources (Naile et al. 2013; Mudumbi et al. 2014a, b) and fish species (Shi et al. 2012; Naile et al. 2013). Subsequently, as a result of excessive use and the persistence of PFCs, the compounds have now been detected in human serum (Whitworth et al. 2012a; Guerranti et al. 2013; Bao et al. 2014; Predieri et al. 2015; Manzano-Salgado et al. 2016), which has led to worldwide concerns, particularly since the compounds ' probability of causing disease has emerged. Hence, new evidence has indicated a strong relationship between POPs, obesity, and the development and/or leading to T2DM and other life-threatening diseases (Airaksinen et al. 2011; Bourez et al. 2012, 2013; La Merrill et al. 2013; Taylor et al. 2013; Ljunggren et al. 2014; Magliano et al. 2014; Myre and Imbeault 2014; Pereira-Fernandes et al. 2014; Reaves et al. 2015). Recent evidence has shown a global increment in obesity/overweight cases by 27.5% in adults and 47.1% in children between 1980 and 2013 (Whitworth et al. 2012a, b; Ng et al. 2014). As such, the rising rate of obesity is regarded as an unequivocal contributor to the global diabetes epidemic and its sequel. The fact that the increase in obesity and diabetes worldwide is occurring over a period of a few decades underscores the interplay between the various factors that relate to the development of diabetes. Lately, there has been increased evidence suggesting polyfluoroalkyl compounds, i.e., PFOA and PFOS, as possible contributors to diabetes development (Chen et al. 2012a, b, c; Whitworth et al. 2012a, b; Eriksen et al. 2013; Karnes et al. 2014), in particular T2DM. For example, studies by Chen et al. (2012a, b, c) reported an association between the levels of PFCs and infant birth weight in relation to childhood DM development. Previously, it has been argued that low birth weight may be linked to adult diseases, including diabetes (Barker and Osmond 1986; Chen et al. 2012a, b, c). Additionally, a positive association was observed between PFCs (i.e., PFOA and PFOS) and a high total cholesterol in humans by Eriksen et al. (2013), as well as in a similar study by Fletcher et al. (2013); with cholesterol levels being significantly associated to diabetes development (Patel et al. 2010; Costacou et al. 2011; Seneff et al. 2012), in particular T2DM (Booe 2016), although a recent study examining the relationship between exposure to PFOA and T2DM concluded that there is minimal direct

association between PFCs and T2DM (Karnes et al. 2014). However, it should be indicated that PFOA concentrations used in this study were estimated, which, in our view, suggests inaccuracy, while the compound's half-life in humans was not indicated, and the investigation did not state whether the participants were on medication, and what were the implications of this aspect on the outcomes being reported. Thus, all of the aforementioned limitations have suggested inconclusive relatedness between PFCs and diabetes. Similarly, this research niche requires further investigations. In fact, it was argued that PFCs have capabilities to interfere with fatty acid metabolism, which suggest possible risk factors for metabolic disorders (Costa et al. 2009; Steenland et al. 2010; Corsini et al. 2014).

Additionally, Eriksen et al. (2013) revealed that DM which may trigger cholesterol synthesis was associated with PFOA and PFOS, but the study warned that, for an accurate interpretation, similar studies were required. Moreover, an association was found between the in vivo expression of genes involved in cholesterol metabolism and exposure to PFOA including PFOS; an indication of feasible links between exposure to these chemicals and chronic diseases such as T2DM (Fletcher et al. 2013). Furthermore, it was previously reported that PFCs were significantly correlated with DNA hypomethylation (Guerrero-Preston et al. 2010), which is regarded as the loss of the methyl group in the 5-methylcytosine nucleotide (Peinado 2012). Consequently, DNA hypomethylation has been previously associated with chronic diseases, including diabetes (Pogribny and Beland 2009; Guerrero-Preston et al. 2010).

In another study, it was revealed that there is an association between high concentration levels of PFOS and PFOA in blood serum and body mass index (BMI) (Ji et al. 2012). Although, the analysis of diabetes risk was not reported in this study, it is however important to indicate that the correlation between BMI and diabetes had previously been investigated in other studies, including a study by the World Health Organization (Barba et al. 2004), Berrington de Gonzalez et al. (2010), Taylor et al. (2010), Zheng et al. (2011), Ogden et al. (2014), and Ng et al. (2014). Recently, it was also revealed that higher serum levels of PFOS may be a contributing factor for individuals being susceptible to developing T1DM (Predieri et al. 2015). These results were consistent with those reported the following year by Su et al. (2016) as far as exposure to PFOS in workers was concerned. However, the study further indicated that those exposed to PFOA, PFNA, and PFUA showed a lower risk of developing T2DM, although, a cross-sectional study found that higher PFC levels were associated with higher insulin levels, higher beta cell activity,

higher insulin resistance (HIR), and higher triglycerides, in overweight children, than in those with normal weights (Timmermann et al. 2014). Indicatively, HIR is a sign that T2DM patients are insulin resistant, which makes their body tissues to respond sluggishly to the insulin (Booe 2016). Similar studies have reported that perfluorononanoic acid (PFNA) was significantly related to T2DM in a non-linear manner, with PFOA being related to insulin secretion, while none of these compounds were associated to insulin resistance (Lind et al. 2014). From this study, it is believed that the significant non-linear relationship between PFNA and diabetes supports the view that this substance, i.e., PFNA, has the potential to influence glucose metabolism in humans (Lind et al. 2014).

Generally, it has been indicated that environmental PFC exposure has the potential to influence the risk of metabolic syndrome (Wang et al. 2017), which has previously been identified as a multiplex risk factor for CVD by the Treatment Panel III report (ATP III) (Grundy et al. 2004) and characterized by six components, namely obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance and/or glucose intolerance, and proinflammatory and prothrombotic states (Grundy et al. 2004). Based on these components, Wang et al. (2017) further suggested that PFCs could increase the metabolism syndrome risks including T2DM. Additionally, a study from Korea has indicated that intense vitamin C supplementation to patients reversed the effects of PFC levels which are associated with insulin resistance (Kim et al. 2016). Thus, these authors suggested that enriched diets with vitamin C are to be part of the patients' diet, as it has a potential to reduce the adverse effect of PFCs. However, the risks of such an intensive treatment are real, and can ultimately lead to hypoglycemia, also called low blood glucose (NIH, 2008), further suggesting that precautionary measures are required when managing DM.

As for endocrine disrupting chemicals (EDCs), Su (2016) has positively linked PFCs as one of the contributory synthetic chemicals which significantly influence the risk of T2DM and subclinical CVD, a suggestion echoed by Lee (2016). Previously, Casals-Casas and Desvergne (2011) reported on the possibility of PFCs acting as EDCs, a report which was consistent with that of Du et al. (2013) who argued that PFOS had the capability to act as an endocrine disruptor both in vitro and in vivo by disrupting the function of nuclear hormone receptors. This argument was elucidated by Bergman et al. (2012) suggesting that, indeed, PFCs must be categorized EDCs. Hence, Lind and Lind (2016) suggested that environmental contaminants with endocrine disrupting properties could be potential classical risk factors for CVD (Kirkley and Sargis 2014),

such as diabetes, hypertension, obesity, etc. This can be attributed to new evidence from current reports that have indicated the prevalence of EDCs and PFCs in products used daily by humans, including plastic bottles, cans, cosmetics and pesticides, and processed foods manufactured using processes in which EDCs and PFCs have been used in one form or another (Lind and Lind 2012; Nohynek et al. 2013; Rousselle et al. 2013; Chevalier and Fénichel 2015; Rosenmai et al. 2016; Bečanová et al. 2016). As such, some studies, including that of Chevalier and Fénichel (2015), have suggested prolonged exposure to EDCs as a new DM emerging contributing factor; although previously, Polyzos et al. (2012) established the link between EDCs and insulin resistance. Similarly, it was recently argued that a wide range of environmental contaminants with endocrine disrupting properties has the potential of leading to the development of several classical risk factors of CVD, including diabetes, hypertension, obesity, hyperlipidemia, and the metabolic syndrome (Lind and Lind 2016). Hence, a higher intake of nitrates, nitrites, and N-nitroso compounds, as well as higher serum levels of PCBs, and 2,3,7,8-tetrachlorodibenzo-p-dioxin have all been associated with diabetes (Vasiliu et al. 2006; Navas-Acien et al. 2006, 2008).

Additionally, recent cross-sectional and prospective studies have reported that serum concentrations of dioxins, PCBs, and chlorinated pesticides were significantly associated with T2DM risk (Song et al. 2016), with other studies associating chlorinated dibenzo-p-dioxins, chlorinated dibenzofurans, and PCBs to diabetes. Evidence has emerged from Thompson (2014) and Mori et al. (2014) demonstrating that all three POPs were found to be associated with diabetic nephropathy. Additionally, a study by Lignell et al. (2013) indicated a significant association between POPs, i.e., PCBs and polybrominated diphenyl ethers (PBDEs) and birth weight, while high dioxin levels have been linked to increased risk of diabetes (Palioura and Diamanti-Kandarakis 2015). Also, bisphenol A and phthalate metabolites were associated with diabetes in a study conducted by Sun et al. (2014). There is an indication that mankind ' s daily life is subjected to the exposure of a wide range of EDCs, some being present in the air, water, and soil on which our food is cultivated, prepared, and served (Kabir et al. 2015). Similarly, various studies have detected PFCs in these abovementioned environments (Miralles-Marco and Harrad 2015). Thus, Table 4.1 depicts the use of some common EDCs and PFCs, while Figure 4.1 illustrates the EDC pathways into humans, conduits which can also be associated with PFCs as well.

Table 4.1: Examples of some common EDCs and their uses

Human commonly used EDCs	Uses	References
DDT, chlorpyrifos, atrazine, 2,4-dichlorophenoxyacetic acid, glyphosate	Pesticides	Kabir et al. 2015 ; de Arcaute et al. 2016
Lead, cadmium	Children's products	Exley et al. 2016; Giudice 2016
BPA, phenol	Food contact materials	Kabir et al. 2015; Yurdakoḱ 2015
Brominate flame retardants, PCBs	Electronics and building materials	Peverly et al. 2015; Al-Omran and Harrad 2016
Triclosan	Antibacterials	Renko et al. 2016; Ginsberg and Balk 2016
Perfluorochemicals	Textiles, clothing, food packaging, firefighting foams, photography, etc.	Rosenmai et al. 2016; Bečanová et al. 2016
Parabens, glycol ethers, fragrances, cyclosiloxanes	Cosmetics, personal care products, cleaners	Nicolopoulou-Stamati et al. 2015 ; Gabb and Blake 2016
Tributylin	Antifoulants used to paint the bottom of the ship	Daszykowski et al. 2015; Noring et al. 2016
Phthalates	Personal care products, medical tubing, Cosmetics, personal care products, cleaners, Children's products, Food contact materials	Kabir et al. 2015 ; Schantz et al. 2015 ; Exley et al. 2016; Arbuckle et al. 2014
Nonylphenol (alkylphenols)	Surfactants-certain kinds of detergents used for removing oil and their metabolites	Niu et al. 2015; Xu et al. 2016
Ethinyl estradiol (Synthetic steroid)	Contraceptive	Mennenga et al. 2015; Suvarna et al. 2016

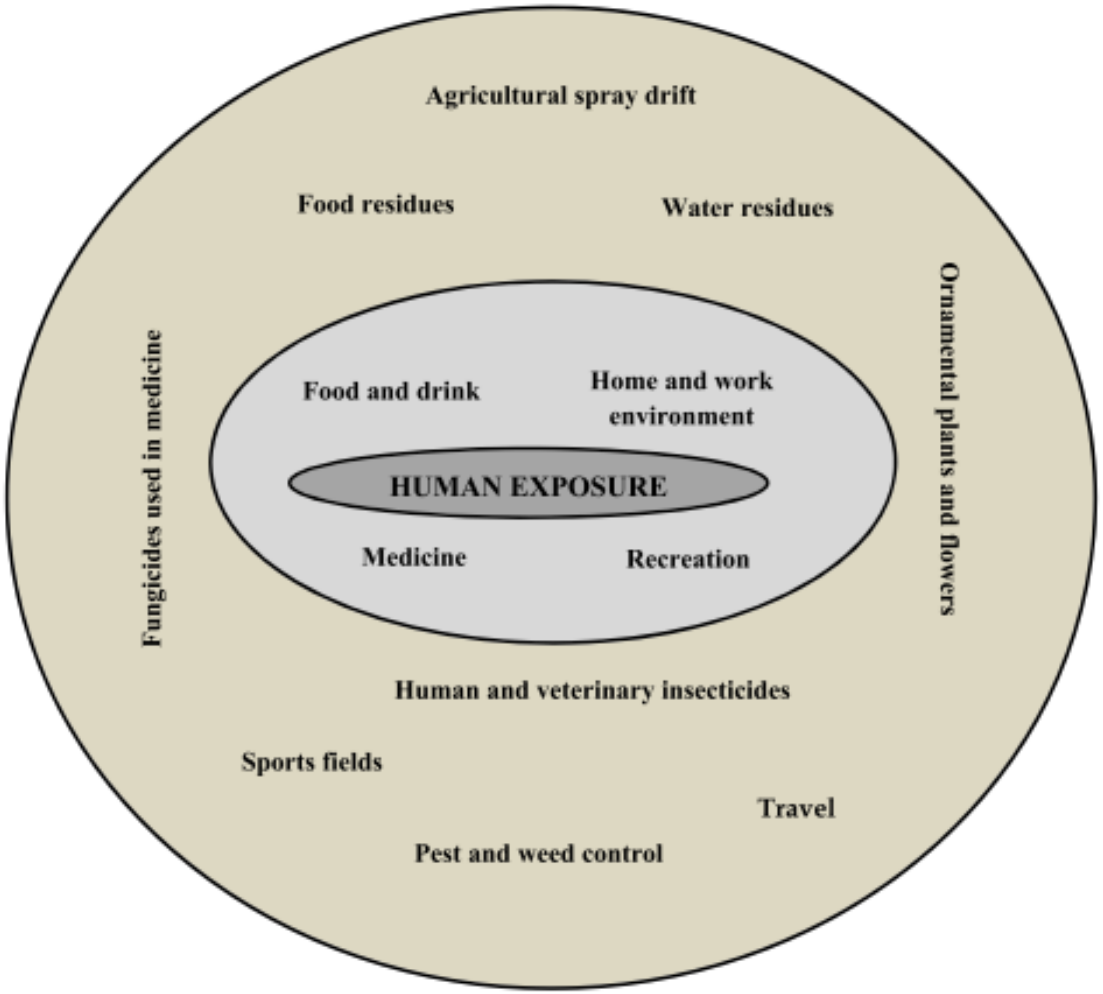

Figure 4.1: Different exposure pathways of EDCs and PFCs into humans (Kabir et al. 2015; Birks et al. 2016)

4.3.2 Type 2 diabetes *mellitus* and heavy metals

Heavy metals are naturally and anthropogenic occurring chemical elements (Tchounwou et al. 2012), known to be persistent in the human body, due to their excretion half-lives that can last for decades (Qu et al. 2012), a statement which has been in contradiction with that of Bergman et al. (2012). Nevertheless, Mattina et al. (2003) have demonstrated that plants can concurrently uptake both heavy metals and POPs present in soil. Heavy metals include compounds such as arsenic (*As*), mercury (*Hg*), lead (*Pb*), cadmium (*Cd*), chromium (*Cr*), etc. Like their counterparts, i.e., PFCs, heavy metals have also been classified as of persistent substances (Casals-Casas and Desvergne 2011; Kim et al. 2014). Humans get exposed to heavy metals through inhalation of dust, direct ingestion of soil and water, dermal contact of polluted soil and water, and consumption of vegetables grown on contaminated lands (Qu et al. 2012). Once they have entered the human body, these chemicals can lead to a wide range of toxic effects, including carcinogenicity, mutagenicity, and teratogenicity (Thomas et al. 2009; Putila and Guo2011; Tchounwou et al. 2012; Qu et al. 2012).

Thus, various epidemiological studies have reported a high correlation between levels of toxic metals exposure and increased risks of diabetes. For example, a study found that levels of all these metals, i.e., *As*, *Cd*, and *Pb*, were significantly higher in women with diabetes and their infants than in the women without diabetes and their new-borns (Kolachi et al. 2011). Similarly, recent evidence found that aluminium (*Al*), titanium (*Ti*), cobalt (*Co*), nickel (*Ni*), copper (*Cu*), zinc (*Zn*), selenium (*Se*), rubidium (*Rb*), strontium (*Sr*), molybdenum (*Mo*), cadmium (*Cd)*, antimony (*Sb*), barium (*Ba*), tungsten (*W*) and lead (*Pb)* were all associated with diabetes (Feng *et al*., 2015), as well as chromium (*Cr)*, iron (*Fe*), manganese (*Mn*), and mercury (*Hg)* (Forte et al. 2013). Liu et al. (2014a, b) have associated *Ni* with T2DM, higher fasting glucose, higher average glucose (HbA1c), higher insulin levels, and increased insulin resistance, a metabolic abnormality that characterizes individuals suffering from T2DM; although Kuo and Navas-Acien (2015) have suggested that the link of *Ni* to diabetes still needs further evaluation. Similarly, type 2 diabetic samples were found to have 0.89 ng/ml of *Ni* in the blood relative to 0.77 ng/ml in the control samples (Forte et al. 2013; Khan and Awan 2014). Additionally, *Zn*, a key role player in the storage and secretion of insulin was linked to T2DM; hence, it was found that *Zn* transporter (*Zn*T8) (Feng et al. 2015), a key protein that regulates insulin secretion from the pancreatic β–cells, was associated with T2DM (Wijesekara et al. 2010; Khan and Awan 2014; Feng et al. 2015). Table 4.2

depicts comparison concentration levels of heavy metals in populations with T1 and T2DM. Recently, data from an epidemiological study found that the levels of urinary *Cu, Zn, As, Se, Mo*, and *Cd* were significantly higher in T2DM cases and those that have been identified as having a high risk of hyperglycemia (Liu et al. 2016a, b). These findings are consistent with those of Li et al. (2017). It was, however, suggested, in both studies, that further investigations that encompass a larger sample size were required to validate the results reported. Hence, an increased obesity due to *Ba* has been recently demonstrated in children, while *Cd*, *Pb* and *Co* led to weight loss in the same study (Shao et al. 2017). Thus, although heavy metals have been proven to be potential risk factors in the development T2DM, the inconsistency observed in various studies has suggested that more research in the era of diabetes and prevention needs to be conducted.

4.3.3 Air pollution and type 2 diabetes *mellitus*

Humans get exposed to pollutants in various ways, such as in- and out-door exposure (Tsakas et al. 2011). Hence, Braniš (2010) has argued that once a pollutant has been discharged and/or formed in the air, ultimately leading to air pollution, it becomes unlikely not to get exposed to this pollutant, for the simple reason that people breathe polluted air continuously. Thus, according to Teichert and Herder (2016), air pollution represents an uncontested environmental risk factor for several health conditions, including CVDs (Miller et al. 2007; Teichert and Herder 2016).

Furthermore, a variety of evidence has suggested that long-term exposure to air pollution and/or pollutants, facilitates the development and progression of T2DM (Chen et al. 2012a, b, c; Liu et al. 2013; Balti et al. 2014; Park and Wang 2014; Eze et al. 2015; Meo et al. 2015; Dzhambov and Dimitrova 2016; Liu et al. 2016a, b; Teichert and Herder 2016; Park 2017). For instance, Liu et al. (2016a, b) reported an increment in $PM_{2.5}$ that was significantly associated with increased T2DM prevalence. From this study, it was suggested that long-term exposure to particulate matter or $PM_{2.5}$ had a potential to increase the risk of T2DM development. Similarly, a strong association between T2DM and $PM_{2.5,}$ PM_{10}, nitrogen dioxide (NO_2) and other pollution related gases was made (Meo et al. 2015). The findings Liu et al. (2016a, b) and Meo et al. (2015) were consistent with those recently reported by He et al. (2017) Esposito et al. (2016), and previously by Liu et al. (2014a, b); Wang et al. (2014); Janghorbani et al. (2014); Pope et al. (2014); Thiering et al. (2013); Coogan et al. (2012) and Xu et al. (2011).

Table 4.2: Heavy metals concentration comparison in both types of diabetes (Forte et al. 2013)

			Heavy Metals (ng/ml)								
			Cr	***Cu***	***Fe* (µ g/ml)**	***Hg***	***Mn***	***Ni***	***Pb***	***Se***	***Zn***
Sample and Gender	**Control**	**F**	0.75	1,046	519	3.63	15.4	0.82	22.1	141	6,627
		M	0.85	977	591	3.12	12.5	1.03	31.7	142	7,317
	T1DM	**F**	0.65↓	1,080	496	2.78	9.30↓	0.72↓	15.9↓	136	5,965
		M	0.71↓	967	571	3.00	8.59↓	0.80↓	22.4↓	143	6,600
	T2DM	**F**	0.76	1,099	498	4.16	12.9	0.80	22.3	136	6,595
		F	0.66	997	522	3.26	9.93	0.75	31.6	145	6,506

F: female; M: male; ↓: significantly lower than in controls (<0.03)

Additionally, evidence demonstrating the prevalence of PFCs in the atmosphere (De Silva et al. 2012; Wang et al. 2013; Dreyer et al. 2015; Kwok et al. 2015; Yao et al. 2016) has further elucidated risks associated with polluted air. For instance, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorobutane sulfonic acid (PFBS), including polyfluoroalkyl phosphate diesters (DiPAPs) and perfluoroalkyl acids (PFAAs) were higher in urban outdoor dust (78−98%), compared to PFHxA, PFHpA and PFBs which were less than 60% (Yao et al. 2016). Nevertheless, despite this amalgamation of evidence linking air pollution and/or pollutants and the risk of T2DM, a recent report has suggested there is insufficient evidence attributing a proportion of the risk of T2DM to air pollution-related immune activation, nor to the extent which the risk of T2DM can be reduced by reducing air pollution levels (Teichert and Herder 2016). This argument was in agreement with what was previously regarded as high risk of bias (Eze et al. 2015). This, has further suggested that more research is required, to assess the impact of air pollution to the prevalence of T2DM cases in certain countries (Liu et al. 2016a, b), in particular, those in which outdoor and indoor air pollution have been reported to be high; for instance, in developing countries (Eze et al. 2015).

4.4 Medicinal plants in the treatment of diabetes and their risk of contamination by pollutants

To date, there has been no generally accepted cure for diabetes. This has led to the disease being regarded as a lifetime ailment, particularly T2DM (Saudek 2009; Blasi 2016). Nevertheless, pancreas or islet transplants have been portrayed as a feasible cure (Buse et al. 2009; Saudek 2009; Blasi 2016). However, the costs associated with such treatment methods for diabetes have been said to be unaffordable, particularly by lower income patients in developing countries; albeit, the procedure that still requires detailed investigation (Tahrani et al. 2011). This has further suggested that an alternative treatment is needed for patients who cannot afford the costs associated with DM management.

Nonetheless, there has been enough evidence suggesting a healthy lifestyle (Meltzer 2014; Coppola et al. 2015; Raidl and Safaii 2015) - including diet and regular physical exercises (Evert et al. 2013; Safaii and Raid 2013) - and insulin intake (Swinnen et al. 2010; Heller et al. 2012) as per clinical recommendations - can make a difference in a diabetic patient's life. Hence, a study by Garber et al. (2012) has argued that, the pharmacokinetic properties of prescribed insulins by physicians should be well understood to avoid risk of hypoglycaemia and its consequences, particularly in T2DM cases. On the other hand, a study by Bartley et al. (2008) has indicated the possibility of weight gain by a patient on insulin therapy, which may complicate the patient's clinical outcomes; while Ali et al. (2006) has suggested that, due to the unbearable side effects associated with the use of insulin, new types of diabetes therapeutics are required. Additionally, oral antihyperglycemic agents, including canagliflozin (Schernthaner et al. 2013; Inagaki et al. 2015), empagliflozin (Zinman et al. 2015), sitagliptin (Green et al. 2015), liraglutide (Marso et al. 2016a), and semaglutide (Marso et al. 2016b), have all been proven to be effective in the management of T2DM. For instance, in T2DM patients who have a high cardiovascular risk, death rates were significantly lowered among those who were on semaglutide treatment than in the placebo group (Marso et al. 2016b). A similar trend was observed in those on liraglutide (Marso et al. 2016a), results which were in agreement with those previously reported by Zinman et al. (2015) on patients receiving empagliflozin for T2DM therapy. It is worth indicating that, although it was suggested that, canagliflozin and sitagliptin were also effective drugs (Schernthaner et al. 2013 and Green et al. 2015), and previous evidence reported that, canagliflozin was associated

with increased genital infections in T2DM patients (Schernthaner et al. 2013). Donath (2014) further indicated that, several antidiabetic drugs are associated with adverse effects, with gastrointestinal symptoms in patients treated with metformin being the most problematic; hypoglycaemia and weight gain in patients treated with sulphonylureas. Currently, it has been indicated that insulin remains the preferred treatment for glycemic control in hospitalized patients (ADA 2016).

Moreover, the use of medicinal plants and/or products has, in the last decade, been suggested to be a potential new breakthrough in the battle against various diseases (Vlietinck et al. 2015), including T2DM (Davids et al. 2016). Thus, numerous studies have highlighted the anti-diabetic potential of several hundred plants (Afolayan and Sunmonu 2010; Chen et al. 2012a, b, c; Keter and Mutiso 2012; Semenya et al. 2012; Street and Prinsloo 2012; Tag et al. 2012; Mahomoodally 2013; Zapata et al. 2013; Arise et al. 2014; Cock 2015). Additionally, it has been further indicated that plants' constituents such as glycosides, alkaloids, tocoherols, flavonoids, carotenoids, polyphenols, steroids, etc., possess anti-diabetic activity (Malviya et al. 2010; Ayeleso et al. 2014; Ayepola et al. 2014a; Oyenihi et al. 2015). The benefits of medicinal plants and/or products and their hypoglycaemic effects in the management of T2DM, have been overwhelmingly confirmed by an assortment of studies (Semenya et al. 2012; Street and Prinsloo 2012; Ayepola et al. 2014a, b).

In the sub-Saharan African region, in particular, medicinal plants have played a major role in combating the disease due to the prohibitive cost of orthodox medicine and the low income of its population (Mounanga et al. 2015), thus suggesting these medicines to be more accessible and affordable by local communities in this African region (Mahomoodally 2013). However, although very promising, sub-Saharan medicinal plants have been subjected to numerous challenges (Moyo et al. 2015). For instance, the conservation of natural resources such as plants remains a worldwide challenge (Moyo et al. 2015), which has been exacerbated in sub-Saharan Africa, where pollution, and other factors, e.g. the overexploitation of these resources for diverse purposes, including medicinal uses (Iwu 2014; Moyo et al. 2015; Davids et al. 2016) have rendered conservation efforts difficult. Table 4.3, , illustrates selected medicinal plants that are believed to be at risk of being contaminated by pollutants in South Africa, for example, where a recent study reported a wide use of medicinal plants by diabetic patients (Davids et al. 2016), although the sufferers were being prescribed allopathic therapy by physicians. This, ultimately, suggests the

trust vested in medicinal plants as compared to orthodox medication. In addition, recent studies have reported that DM, in particular cases of T2DM, which previously were rare in developing countries, have risen recently in these countries, with 80% of new cases of DM worldwide now being reported in developing states, thus including the sub-Saharan region (Chan et al. 2009, Shaw et al. 2010; Chen et al. 2012a, b, c). Therefore, to adequately address this increment in DM cases, Mahomoodally (2013) has suggested that potential risk factors, such as contamination with heavy metals, be addressed, coupled with the development and enforcement of regulatory guidelines, of which one of its aims should be to eradicate and/or keep to a minimum these factors. Additionally, unlike in the developed world, where efforts to control and regulate the use of PFCs and its precursors have been strongly established, in the sub-Saharan region this still is not the case. In South Africa, for instance, PFCs are simply referred to as pollutants of concern in the National Environmental Management Air Quality Act of 2004, but no specificities are provided in terms of their usage in the country. In our opinion, this should urgently be addressed, particularly in a country such as South Africa where agriculture, a major source of PFCs intake (Lӧfstedt Gilljam et al. 2015), plays an important economic role. Subsequently, the lack of adequate regulations on the use of PFCs, in sub-Saharan Africa, also represents challenges to the observed increase in the use of traditional medicinal plants, to treat T2DM, as a substitute for an expensive orthodox therapy.

On the other hand, although in recent years there has been a witnessed increase in the use of medicinal plants and/or products (Eldeen et al. 2016), the abandonment of orthodox medicines, of which some have been reported to be contaminated with excessive or banned pesticides, microbial contaminants, heavy metals, and chemical toxins (Chan 2003), should be a primary concern at this stage. Concerns have been reported over the possibility of medicinal plants and/or products being contaminated with POPs and other new emerging pollutants, if they are grown under a contaminated environment or during collection of these plant materials, as well as if they are treated and stored under unsuitable conditions (Chan 2003). Recent studies have addressed the uptake of PFCs and/or EDCs by plants, some of which have been edible crops (Blaine et al. 2014; Lee et al. 2014; Yang et al. 2015; Bizkarguenaga et al. 2016; Kurwadkar et al. 2017; Zhao and Zhu 2017). Furthermore, results from Lee et al. (2013) provided evidence of soil biodegradation of DiPAPs and their subsequent uptake including their intermediate by-products uptake into plants; while Bizkarguenaga et al. (2016) determined the highest bioconcentration

factors (BCFs) for PFOA and PFOS in carrot (*Daucus carota*); with PFCs being found in all plants grown in biosolids-amended soil (Wen et al. 2016).

Table 4.3: Selected medicinal plants used to treat T2DM and potentially threatened by pollutants in South Africa

Plant species (Family)	Common or vernacular names	Compartments used	References
Sutherlandia frutescens (*Fabaceae*)	Cancer bush (Eng.)	Leaves, and often whole plant	Drewes et al. 2006; van Wyk and Albrecht 2008; Street and Prinsloo 2012
Moringa oleifera (*Moringaceae*)	Makgonatˇsohle (Sipedi), drumstick tree (Eng.)	Seeds and leaves	Semenya et al. 2012
Artemisia afra (*Asteraceae*)	African Wormwood (Eng.)	Leaves and roots	Erasto et al. 2005; Thring and Weitz 2006; Van Wyk 2008; Afolayan and Sunmonu 2010
Cannabis sativa L. (*Cannabaceae*)	Dagga (Afr.)	Leaves	van de Venter et al. 2008
Aloe ferox Mill. (*Asphodelaceae*)	Cape Aloe or bitter Aloe (Eng.)	Leaves	Deutschländer et al. 2009 ; Loots et al. 2011; Street and Prinsloo 2012; Balogun et al. 2016
Pelargonium sidoides (*Geraniaceae*)	Umckaloabo (Zulu)	Tubers and roots	Street and Prinsloo 2012

Table 4.3: *Continues*

Hypoxis hemerocallidea (*Hypoxidaceae*)	Star flower, yellow star, African potato (Eng.); Inkomfe (Zulu); Sterblom and Gifbol (Afr.)	Roots	Musabayane et al. 2005; Ojewole 2006; Street and Prinsloo 2012; Balogun et al. 2016
Sclerocarya birrea (*Anacardiaceae*)	Hochst. subsp. caffra, marula, tree of life	Stem	Gondwe et al. 2008; Street and Prinsloo 2012
Herichrysum nudifolium L. (*Asteraceae*)	Hottentot's tea (Eng.); Hottentotstee (Afr.); icholocholo (Xhosa, Zulu)	Leaves and roots	Erasto et al. 2005; Afolayan and Sunmonu 2010
Herichrysum petiolare H & B.L (*Asteraceae*)	Everlasting (Eng.); Kooigoed (Afr.); Imphepho (Xhosa)	Whole plant	Erasto et al. 2005; Afolayan and Sunmonu 2010
Leonotis leonurus L. (*Lamiaceae*)	Wild dagga or Lion's ear (Eng.); Wildedagga (Afr.); Imvovo (Xhosa)	Leaves, flowers	Thring and Weitz 2006; Afolayan and Sunmonu 2010
Momordica balsamina L. (*Cucurbitaceae*)	Balsam pear (Eng.); Laloentjie (Afr.); Nkaka (Thonga) Intshungu (Zulu)	Stem, flowers	van de Venter et al. 2008 ; Afolayan and Sunmonu 2010

Table 4.3: *Continues*

Momordica foetida Schumach (*Cucurbitaceae*)	Wild cucumber (Eng.)	Leaves, and often whole plant	Oishi et al. 2007 ; van de Venter et al. 2008 ; Afolayan and Sunmonu 2010 ; Acquaviva et al. 2013
Psidium guajava L. (*Myrtaceae*)	Common guava, yellow guava, lemon guava (Eng.)	Leaves, roots, whole plant	van de Venter et al. 2008 ; Afolayan and Sunmonu 2010 ; Sanda et al. 2011
Sclerocarya birrea Hochst (*Anacardiaceae*)	Marula (Eng.) ; Mufula (Venda)	Stem, bark, roots	van de Venter et al. 2008 ; Afolayan and Sunmonu 2010
Vinca major L. (*Apocynaceae*)	Bigleaf periwinkle (Eng.)	Leaves, roots, stem	van de Venter et al. 2008 ; Afolayan and Sunmonu 2010
Vernonia oligocephala Sch. Bip. (*Asteraceae*)	Bicoloured-leaved Vernonia (Eng.); Groenamarabossie (Afr.); Ihlambihloshane (Zulu)	Leaves, twigs, roots	Erasto et al. 2005; Afolayan and Sunmonu 2010
Catha edulis Forrsk. Ex Endl. (*Celastraceae*)	Arabian tea, Abyssinian tea, Bushman's tea (Eng.)	Leaves, stems, roots	van de Venter et al. 2008 ; Afolayan and Sunmonu 2010

Table 4.3: *Continues*

Brachylaena discolor DC. (*Asteraceae*)	Coast silver oak (Eng.) ; Kusvaalbos (Afr.) ; Phahla (Zulu and Xhosa)	Leaves, roots, stem	Erasto et al. 2005 ; van de Venter et al. 2008 ; Afolayan and Sunmonu 2010
Eriocephalus punctulatus (*Asteraceae*)	Roosmaryn or Kapokbos (Afr.) ; wild rosemary (Eng.)	Leaves	Mierendorff et al. 2003 ; Njenga and Viljoen 2006; Sandasi et al. 2011 ; Balogun et al. 2016

Afr.= Afrikaans; Eng.= English

Similarly, the uptake of PFOA led to root growth impairment in wheat seedling process (Zhou et al. 2016a, b); with Zhao et al. (2017) reporting a high root uptake of four perfluorinated carboxylic acids (PFCAs) by wheat.

Moreover, it has been argued that, due to the widespread prevalence of heavy metals in the environment, their residues have reached the entire ecosystem, leading to their assimilation into medicinal plants (Sarma et al. 2012). Thus, *Ba, Cr, Cd, Fe, Sr, Pb,* and *Zn* were found in medicinal plants (Gjorgieva et al. 2010), which prompted the authors to suggest that, these plants should be collected in areas free of any contaminants. A similar study determined *Fe, Ti, Mn, Cr, Cu, Ni, Zn, Sr* and *Ba* in *Hemerocallis minor* Miller, a plant used in folk medicine, using the non-destructive X-ray fluorescence spectrometry (XRF), which suggested that prior to using plants for medicinal purpose, it is vital to assess, the plants heavy metal content (Chuparina and Aisueva 2011). Street (2012) further concluded that exposure to heavy metals in medicinal plant products has the potential to cause countless health implications including liver and kidney failure. Previously, a link between liver and kidney failure and T2DM has been established (Inzucchi et al. 2012; Mudaliar et al. 2013; Kohan et al. 2014). Similarly, another research study indicated that, heavy metal stress has the potential to decrease the total antioxidants level in medicinal plants (Gjorgieva et al. 2013).

In South Africa, various research studies have reported on the contamination of the natural environment – water, soils and sediments, plants - by heavy metals (Olujimi *et al*., 2015), including emerging pollutants, such as PFCs (Mudumbi et al. 2014a, b, c), thus suggesting that the medicinal plants and/or products (see Table 4.3) are at risk of being contaminated by PFCs. This further suggests that these products might constitute a pathway to humans being exposed to these compounds. In addition, recently, a study by Hanssen et al. (2010) reported higher concentrations of PFCs (i.e. PFOA and PFOS) in human serum; of which the exposure pathways in South Africa remain unknown. Thus, the evidence on the contamination of the natural environment in general, and that of medicinal plants and/or products, in particular, by POPs and allegedly by new emerging pollutants, such as PFCs, has brought quality, efficacy and safety concerns with regard to the use of these commodities (Chan 2003; Adewunmi and Ojewole 2004). However, to our knowledge, there is limited information on the threats of emerging POPs, for instance PFCs, to medicinal plants and/or products, and ultimately, to diabetic patients who rely on these plants and/or their products for the management of the disease.

Therefore, it is important that, while many plants are being explored for their anti-diabetic potential, it is also necessary that research studies diversify their investigations on the susceptibility of these plants to emerging pollutants, i.e. PFCs, since, arithmetical projections have demonstrated that the number of diabetes cases will rise in decades to come, suggesting that successful anti-diabetic drugs can be synthesized from extract of medicinal plants and/or by-products; i.e. the development processes for phytomedicinal products must take in too consideration the threat of emerging pollutants (contamination) to these products. Nevertheless, Kuo et al. (2013) have called for cautiousness in the interpretation of results associating diabetes to new chemicals. For this reason, we are of the view that emerging compounds such as PFOA and PFOS and their association to diabetes still requires prolong investigations. This same view applies to the potential contamination of antidiabetic medicinal plants by PFCs.

4.4.1 Synergy in phytomedicinal therapy: challenges and limitations

Recent reviews have reported on the synergy and interactions that exist among and between medicinal plants (Rasoanaivo et al. 2011; Yarnell 2014, 2015; Zhou et al. 2016). Hence, medicinal plants synergy is regarded as the amalgamation of two or more medicinal plants to produce a combined effects greater than the sum of individual plant effects (Chou 2010; van Vuuren and Viljoen 2011; Breitinger 2012; Zhou et al. 2016a, b), in substitution of the "one drug, one target, one disease" approach, which remained the conventional pharmaceutical approach in the development of most medicines and treatment strategies (Zhou et al. 2016a, b). Accordingly, recent evidence has demonstrated the potentiality of combined therapy and/or drugs in the treatment of various diseases, example diabetes (Zhou et al. 2016a, b), pancreatic cancer (Yue et al. 2014), etc. Thus, significant progress has been achieved in medicinal plants synergistic effects.

Nevertheless, despite the prospects of this field looking promising, Zhou et al. (2016a, b) have argued that various challenges have emerged from phytomedicinal synergy techniques, which have led to various limitations in this field, and ultimately making it difficult for herbal synergistic studies to develop suitable phytomedicinal synergistic methods (Zhou et al. 2016a, b). Additionally, evidence supporting synergistic effects of combined medicinal plants and the interactions of their therapeutic components remain controversial (Zhou et al. 2016a, b). For instance, it has been argued that the low/extremely low levels of active components content in certain medicinal plants suggest insignificant synergistic and therapeutic effects of their herbal

formulations (Williamson 2001; Danz et al. 2002; Zhou et al. 2016a, b). Thus, according to Tausk (1998) and Zhou et al. (2016a, b), this kind of scepticism has led to these plants being considered as simple placebos. However, numerous other studies have highlighted the significance of synergistic action present in medicinal plant therapies, by demonstrating that plant extracts of multiple plants in complex formulations have been proven effective than when used alone (Leonard et al. 2002; Scholey and Kennedy 2002; Zhang et al. 2014, Zhou et al. 2016a, b).

Furthermore, it is also not clear, at this stage, whether the combined final product, with potential medicinal plant synergistic interaction between their active components is able to inhibit, reduce and/or keep the contaminants/pollutants at a possible harmless minimum level once they have been uptaken by the identified plants. This aspect needs to be further investigated, although studies by Cantelli-Forti et al. (1994), Zhao et al. (1995) and Chen et al. (2009) suggested plants' synergistic effects led to the reduction of toxicity of one medicinal plant by another. Besides, certain medicinal plants species are naturally known as toxic (Bussmann *et al*., 2011; Nasri and Shirzad 2013; Tamilselvan et al. 2014; Monseny et al. 2015), while others are likely to become toxic as a result of uptaking toxicants and/or contaminants (Plewa 1991), a primary reason why it is advised to collect, use and/or store medicinal plants from uncontaminated environments (Gjorgieva et al. 2010).

4.5 Past, present, and future global DM trends and burden

It is without any doubt that DM can now be found in every population group globally. Documented evidence has suggested that, lack of efficient prevention and control programmes would result in an increase in cases of DM worldwide (WHO, 1994; Amos et al. 1997), with the disease being estimated the 7th leading cause of death in 2030 (Mathers and Loncar 2006). Additionally, Zimmet (2000) and Zimmet et al. (2001), indicated that DM was considered as a disease of minor world health significance, but by the 21st century, the disease has become one of the main threats to human health globally (Zimmet et al. 2001), and thus classified as a lifestyle disease.

The WHO Ad Hoc Diabetes Reporting Group published, using data from 75 communities in 32 countries, the first global estimates and comparable information on the prevalence of DM in 1993 (King and Rewers, 1993; King et al. 1998). However, the data lacked satisfactory research interests, particularly in the area of future trends in the burden of DM. Therefore, a study

combining global database from the WHO with demographic estimates and projections from the United Nations (UN) was undertaken between 1995 and 2025, to estimate the proportion of people with diabetes globally for the above period (King et al. 1998). Accordingly, in 1994, the number of people suffering from DM was estimated to be over 100 million worldwide (IDF 1994; Amos et al. 1997). The data suggested that DM was likely to double to 239 million in 2010 (Amos et al. 1997). From this study, it was revealed that 85 to 90% of all diabetes under the T2DM category was in developed countries. In addition, it has been recently reported that, as many as one-third to one-half of T2DM cases in the population may be undiagnosed because they may remain asymptomatic for many years (IDF 2017).

Furthermore, in 1998, a study by King et al. (1998) indicated that the number of adults with diabetes in the world was 135 million, and was projected to an increase of up to 300 million by the year 2025. These estimates concurred with those reported by Hussain et al. (2007) and Beulens et al. (2010). A proportion of DM increases was projected to be dominantly in developing countries, with 84 to 228 million individual cases, suggesting that, 75% of people with diabetes will reside in developing countries, as compared with 62% in 1995 (King et al. 1998). In 2010, a study indicated that there was 285 million people suffering from diabetes, with the same study estimating that this estimate will likely increase to 439 million by 2030 (Shaw et al. 2010). Moreover, in 2011 there were 366 million people living with diabetes and the probability was that, this number is likely to reach 552 million by 2030 (Whiting et al. 2011). It is important to note that, these estimations were done using different methods. To substantiate this, it has been indicated that, estimates in DM studies vary widely depending on the population groups involved in the study, as well as the methods used to analyze the data (Susan et al. 2010).

There is an indication that, DM, and in particular T2DM, was relatively rare in developing countries some decades ago (Chan et al. 2009; Chen et al. 2012a, b, c). Nevertheless, the burden of DM has now taken place in developing countries rather than in industrialized countries, with 80% of new cases of DM worldwide now being reported in developing countries (Shaw et al. 2010; Chen et al. 2012a, b, c). For the African continent, i.e. one of the contributing factors for new DM cases (Abubakari et al. 2009; Mbanya et al. 2010; Hall et al. 2011; Chen et al. 2012a, b, c) is lifestyle choices and physical inactivity. From projected data, it was indicated that an increment in the number of people with diabetes will be observed, with nearly double the number in the Sub-Saharan Africa region, followed by the Middle-East and North African regions, by the year

2030 (Chen et al. 2012a, b, c). In previous DM hotspot areas, such as in Europe and America, it is suggested that the disease has stabilized. However, little is being said as to what is behind this abrupt control of DM prevalence in these areas.

Also, recent statistics have indicated that, in 2013, 382 million people had diabetes, with these figures expected to rise to 592 million (Guariguata et al, 2014) in 2030. Thus, in this study, it has, once again been suggested that, the proportion of people with DM varied by region and income, and/or both, with the highest proportion being low-income earners (Whiting et al. 2011; Guariguata et al. 2014).

Furthermore, DM has been listed by the IDF as the largest global health emergencies of the 21st century. The organization has indicated that, of the 415 million people who were estimated to be living with diabetes in 2015 (425 million in 2017), 318 million were suffering from impaired glucose tolerance, which, according to the IDF, exposed them at high risk of developing the disease in the future (IDF 2015; 2017). Additionally, the trend and burden of the disease, i.e. diabetes, has been exacerbated by the fact that many countries have remained unaware of the social and economic impact of DM, suggesting that this lack of understanding is becoming the largest barrier factor to effective prevention strategies in halting the inexorable rise of T2DM (IDF 2015).

Besides, enough evidence is available and which has reported on better awareness and new developments in treatment of T1DM and T2DM and, particularly, the prevention of T2DM (ADA 2011; Inzucchi et al. 2012; ADA 2013; Copeland et al. 2013). However, in each edition of the IDF Diabetes Atlas, an unrelenting increase in the number of people living with the disease has been clearly shown. Thus, its seventh edition has indicated that in 2015, there were 415 million diabetic people worldwide, of which more than 14 million were found on the African continent. The institution has projected that by 2040, 642 million would be suffering from DM, should the current growth continue. The number of diabetic patients in Africa is projected to be more than 34 million by 2040 (IDF 2015) and 41 million by 2045 (IDF 2017). Recent data has further indicated that, 2 out of 3 people with diabetes are undiagnosed on the African continent; while 3 out of 4 diabetes related deaths, on the continent, were from people under the age of 60 (IDF 2017).

Moreover, it has been indicated that the use of medicinal plants and/or products has become fundamental worldwide, and particularly in developing countries, including the sub-

Saharan Africa region, where these products are accessible and affordable (Mahomoodally, 2013), unlike the orthodox products. For instance, a recent report suggested that 80% of South Africans use phytomedicinal products (Street and Prinsloo 2012) for various ailments, including DM. Similarly, a study in Morocco reported that 80% of interviewed patients used medicinal plants for the management of DM (Eddouks et al. 2002), while Ocvirk et al. (2013) indicated the use of traditional medicinal plants for the treatment of DM being a common practice in Bangladesh. As such, the WHO has recently recommended the use medicinal plants and/or products (Chikezie et al. 2015) for the management of DM, although their safety being questionable (Haq 2004; Abdel-Azim et al. 2011), currently, due to emerging organic contaminants, such as PFCs.

4.6 Conclusion

During the last century, humanity has witnessed increases in chronic diseases, of which some have deplorably been lethal. In certain countries, such as South Africa, these diseases have been the leading causes of death. Regrettably, diabetes is on the increase, and developing countries are alleged to be more affected in years to come, as their lifestyle improves. Traditionally, it is consistently been reported that unhealthy diets, physical inactivity and family history are the main leading contributing factors to diabetes. However, during the past decades, pollutants, of which some are of anthropogenic sources, affect humans, resulting in exposure through various pathways, including food, water, soil, air and plants. Consequently, research studies have demonstrated that pollutants are also causing diabetes. Currently, there is no cure for diabetes; and although therapy have included antihyperglycemic agents and insulin intake, several studies have indicated that this therapy have limitations, including patients complaining about side effects of agents being used, as well as reports suggesting weight gain by patients who are on insulin treatment. Thus, recently the focus in the attempt to manage diabetes has shifted from orthodox anti-diabetic drugs to medicinal plants and/or products of which anti-diabetic potential have been investigated, reported and extensively documented. However, current research evidence has indicated the susceptibility of these plants to pollutants, including EDCs, and heavy metals such as *Ba, Cr, Cd, Fe, Sr, Pb*, and *Zn*. Moreover, new pollutants have emerged, namely PFCs, such as PFOA, PFOS and PFBS. Unlike their predecessors, PFCs are entirely anthropogenic, and they are widely distributed in the environment. Their prevalence has been reported in various environmental matrices, including water, soil, sediments, plants, etc. Nevertheless, there is little information on the vulnerability of medicinal plants and/or products

to PFCs, and so is the human exposure to these compounds through medicinal plants and/or products intake and subsequent implications, either on short or long-term basis. The lack of appropriate regulations controlling the use of PFCs in regions such the sub-Saharan region is likely to exacerbate the contamination of medicinal plants, unless something is done by respective authorities. Additionally, large scale and promising research studies on medicinal plants anti-diabetic and their activities are still needed; and it is further suggested studies to consider cultivating, harvesting or collecting and storing medicinal plants and/or products in areas free of any contamination. This will enhance the quality, efficacy and safety of medicinal plants and/or products, and ultimately the health of those who rely on these plants.

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CHAPTER 5

Propensity of *Tagetes erecta* L., a Medicinal Plant Commonly Used in Diabetes Management, to Accumulate Perfluoroalkyl Substances

Mudumbi *et al*., *Toxics*, 7, 18; *doi:10.3390/toxics7010018*

5.1 Abstract

It has been extensively demonstrated that plants accumulate organic substances emanating from various sources, including soil and water. This fact suggests the potentiality of contamination of certain vital bioresources, such as medicinal plants, by persistent contaminants, such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorobutane sulfonate (PFBS). Hence, in this study, the propensity of *Tagetes erecta* L. (a commonly used medicinal plant) to accumulate PFOA, PFOS, and PFBS was determined using liquid chromatography/tandem mass spectrometry (LC–MS/MS-8030). From the results, PFOA, PFOS, and PFBS were detected in all the plant samples and concentration levels were found to be 94.83 ng/g, 5.03 ng/g, and 1.44 ng/g, respectively, with bioconcentration factor (BCF) ranges of 1.30 to 2.57, 13.67 to 72.33, and 0.16 to 0.31, respectively. Little evidence exists on the bioaccumulative susceptibility of medicinal plants to these persistent organic pollutants (POPs). These results suggest that these medicinal plants (in particular, *Tagetes erecta* L., used for the management of diabetes) are also potential conduits of PFOA, PFOS, and PFBS into humans.

Keywords: Medicinal plants; Perfluoroalkyl substances (PFASs); Perfluorooctanoic acid (PFOA); Perfluorooctane sulfonate (PFOS); Perfluorobutane sulfonate (PFBS); *Tagetes erecta* L.

5.2 Introduction

Evidence exists which indicates that plants were used for medical purposes long before the industrial epoch. Ancient Egyptian papyrus manuscripts have also reported and suggested the extensive use of medicinal plants. Currently, the World Health Organization (WHO) has estimated that 80% of the global population relies on medicinal plants for aspects of their first-hand health care requirements [1]. African marigold (*Tagetes erecta* L.) is a member of the Asteraceae plant family. Evidence has indicated that *Tagetes erecta* L. is well-known as an important commercial plant utilized mostly for decorative purposes [2–4]. Recently, the plant has been renowned for its industrial and medicinal usage [5–7]; a number of studies have suggested that *Tagetes erecta* L. has the potential to treat ailments such as diabetes mellitus (DM) [8–12]. In South Africa, use of the leaves of *Tagetes erecta* L. in the treatment of DM has been reported [13].

Nevertheless, these phyto-bioresources are believed to be susceptible to environmental effects, including negative externalities such as contamination by toxic substances, especially persistent organic pollutants (POPs). This assertion is based largely on evidence indicating that plants are capable of taking up and accumulating nutrients and a variety of other chemicals to which they are, either directly or indirectly, exposed. Thus, compelling evidence has demonstrated that plants accumulate and metabolize environmental contaminants, ultimately suggesting that plants are reservoirs for chemical substances [14,15]. Some scientists have reported the prevalence of toxic substances and/or heavy metals in plants [16–24]. Moreover, various medicinal plants have previously been reported to be exposed to chemical substances, including heavy metals. For instance, research results have recently suggested that medicinal plants' exposure to chemical substance results in chemo-stress, which influences the antioxidant status of the plant and culminates in damage to its DNA [25].

Previously, heavy metals, including barium (Ba), chromium (Cr), cadmium (Cd), iron (Fe), strontium (Sr), lead (Pb), and zinc (Zn) have been reported in medicinal plants [15,26]. Furthermore, a study by Tian et al. [27] determined that plant leaves are effective in taking up PFASs from the atmosphere, with previous studies by Blaine et al. [28] reporting the

bioaccumulation of various perfluoroalkyl acids (PFAAs) in edible crops, including lettuce (*Lactuca sativa)* and strawberry (*Fragaria ananassa*), suggesting these crops are a potential route of exposure for humans. In most instances, it is contaminated river water and fertilizer, as well as aero-deposition, that results in the contamination of these plants [29,30]. Nevertheless, due to limited available evidence on the contamination of medicinal plants by PFASs [15], the possibility that these plants are a pathway through which humans are likely to be exposed to PFASs is still to be established. It is worth noting that available evidence has reported wide concerns about these substances, and their health safety remains unclear [31–34]. Nevertheless, health advisory standards have been proven [34], and can be used as a benchmark for the establishment of a better safety level for toxicity of these substances for humans. Therefore, the aim of this study was to determine the propensity of *Tagetes erecta* L., a common medicinal plant used by diabetic patients in sub-Saharan Africa, to accumulate PFOA, PFOS, and PFBS.

5.3 Materials and Methods

5.3.1 Chemicals and Reagents

A specific perfluorocarboxylic acid (PFCA) standard (i.e., perfluorooctanoic acid (PFOA)), and singular linear perfluoroalkyl sulfonic acids (PFSAs) such as perfluorobutane sulfonate (PFBS) and perfluorooctane sulfonate (PFOS), were obtained from the laboratory facility of the Department of Environmental, Water and Earth Sciences, Tshwane University of Technology (TUT), South Africa; these were purchased in methanol at 50 µg/mL from Wellington Laboratories (Ontario, Canada). A solution of surrogate mixture of stable isotopically-labelled PFAS standard containing perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid (MPFOA), perfluoro-n-[1,2,3,4, 5-$^{13}C_5$] nonanoic acid (MPFNA), and perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid (MPFUnDA) was also obtained from TUT, and purchased in methanol at 50 µg/mL from Wellington Laboratories (Ontario, Canada). Acetic acid, polypropylene (PP) membrane filters (0.22 µm, Cameo syringe filters) and syringes (Becton Dickinson), LC–MS grade water, acetonitrile, methanol, and ammonium acetate, as well as Supelco-Select HLB SPE cartridges (500 mg), were purchased from Sigma-Aldrich (Aston Manor, South Africa). T Milli-Q water was used throughout the study.

5.3.2 Sample Collection: *Tagetes erecta* L. and River Water

Samples of plant leaves (n = 8) were harvested from main plants (i.e., *Tagetes erecta* L.) separated in cultivation pots. River water samples (n = 20) from the Salt River, Western Cape, South Africa, were used to irrigate the plants. The river water samples were randomly taken during summer months (i.e., dry season—March) and winter months (i.e., wet season—August), with the bulk of the river water being used to irrigate the plants without pre-treatment at a frequency of 120 mL every two to three days for pots containing 0.5 L of loamy soil.

5.3.3 Sample Pre-Treatment and Solid Phase Extraction

5.3.3.1 Plant Samples

Samples were pre-treated using protocols previously used by Tian et al. [27] and Mudumbi et al. [28], with minor changes. Thus, plant leaf samples (n = 8) were harvested using a laboratory scalpel and oven-dried for 24 h at approximately 60 °C, and subsequently milled into a powder form. Thereafter, 2 g from each of the samples was transferred to a clean 15 mL PP centrifuge tube. The tubes were subsequently spiked with a 50 µL surrogate mixture of stable isotopically-labelled PFASs standard (i.e., MPFOA, MPFNA, and MPFUnDA), and the mixture was allowed to equilibrate for about 1 h at ambient temperature (21–26 °C). Subsequently, 15 mL of 0.01 M NaOH/MeOH was added and the mixture was then homogenized by vigorous vortexing (2 min), at ambient temperature. Subsequently, the PP tubes were centrifuged at 3000 rpm for 4 min and the supernatants were emptied into new PP tubes (15 mL) pre-rinsed with analytical LC–MS grade methanol. The cycle was repeated twice, and the supernatants from both cycles were filtered using polypropylene 0.22 µm Cameo syringe filters (Sigma-Aldrich, Darmstadt, Germany). Thereafter, a total volume of 15 mL was recorded, which was used for solid phase extraction (SPE).

5.3.3.2 River Water Samples

River water was randomly collected in PP containers of 25 L capacity, from a local Western Cape river (i.e., Salt River) previously known to be contaminated with PFASs [29], and the PFASs analyses were carried out based on the same source protocols, with negligible changes. Hence, from this water, a total of twenty samples (n = 20) were randomly taken from the river water to

irrigate the plants. The samples contained suspended particulate matter (SPM), which was removed by means of filtration; PP membrane filters (0.22 µm, Cameo syringe filters, Sigma Aldrich, Darmstadt, Germany) were used. Subsequently, the filtered river water samples were spiked with 50 µL of a surrogate mixture of stable isotopically-labelled PFASs standard (i.e., MPFOA, MPFNA, and MPFUnDA), and vortexed (2 min) prior to SPE, without pH adjustment or dilution.

5.3.3.3 Solid Phase Extraction

Supelco-Select HLB SPE cartridges (500 mg solid phase, 12 mL tubes) were used for SPE using procedures as suggested in previous studies, including Mudumbi et al. [28–30], with minor modifications. Hence, the cartridges were preconditioned with 5 mL of analytical LC–MS grade methanol and then 5 mL of Milli-Q water at a flow rate of 1 drop per two seconds. After loading the samples (i.e., a volume of 15 and 20 mL of plant and water extracts, respectively) at a flow rate of one drop per two seconds, Supelco-Select HLB SPE cartridges were washed with 5 mL of 40% (v/v) analytical LC–MS grade methanol in Milli-Q water, as reported by Mudumbi et al. [28,29]. Successively, PP collection tubes were added to the SPE apparatus, and PFASs were eluted from Supelco-Select HLB SPE cartridges into the PP collection tubes, using 10 mL of analytical LC–MS grade methanol. It was extremely pertinent to use PP collection tubes in order to minimize background cross-contamination of the eluents. The tubes were thereafter dried under nitrogen gas, and reconstituted with 0.5 mL of 50 ng/mL M2PFOA internal standards (ISTD) prepared in 10% acetonitrile. Figure 5.1 outlines the scheme of the overall process used. The final aliquots (500 µL) of the supernatants were transferred into PP autosampler vials before analysis using LC–MS/MS.

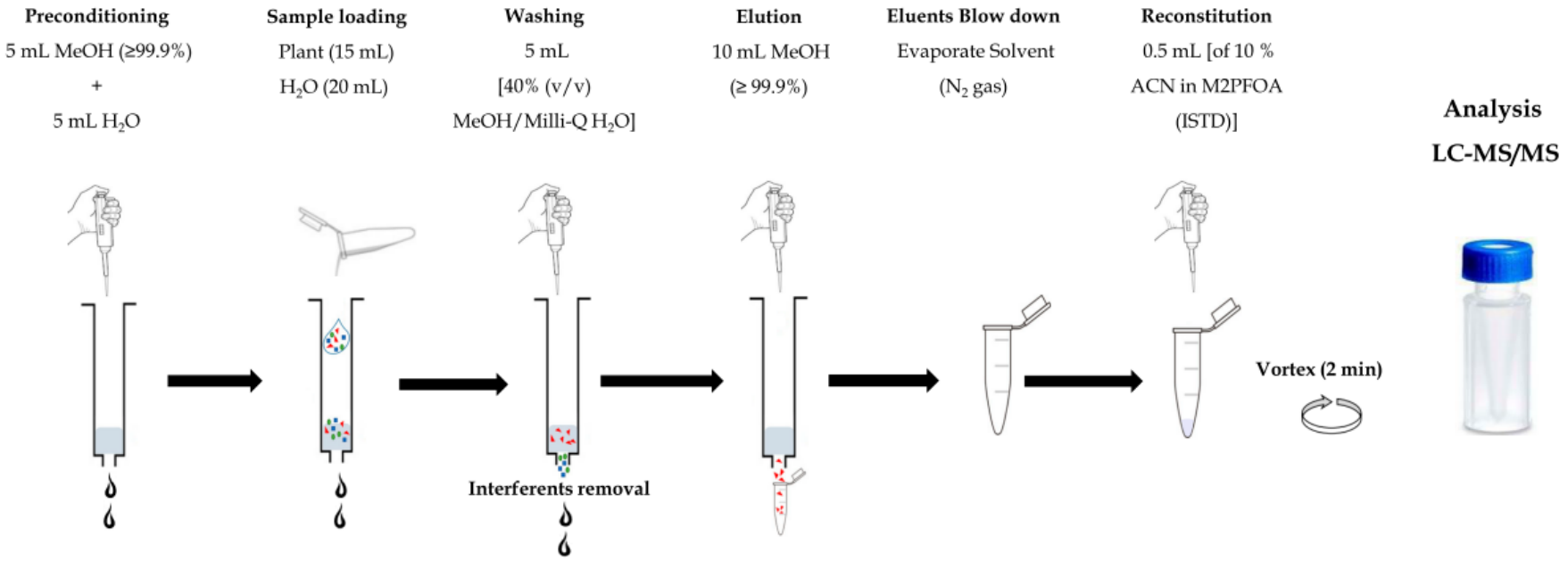

Figure 5.1: Schema for solid phase extraction (SPE) of water and plant samples

5.3.4 LCMS-8030 Analysis

5.3.4.1 LCMS-8030 Configuration for PFOA, PFOS and PFBS Quantification

The analysis of PFASs (i.e., PFOA, PFOS, and PFBS) in plant and river water samples was conducted using a liquid chromatograph (LC) coupled with triple quadrupole linear ion trap tandem mass spectrometer (Shimadzu LCMS-8030, Canby, OR, USA) equipped with an electrospray ionization (ESI) source, which was in a negative ion mode. The targeted PFASs were quantified using multiple reaction monitoring (MRM) mode of analysis. The chromatographic separation of analytes was achieved with a Luna® Omega Polar C18 column (2.1 × 100 mm, 3.0 µm, Phenomenex, Aschaffenburg, Germany). The column temperature was set at 40 °C. A gradient elution program was applied and was made of 20 mM ammonium acetate (solvent A) and 100% MeOH (solvent B), at a flow rate of 0.3 mL/min and an injection volume of 10 µL used for individual samples. The linear gradient elution program started at 20% B and increased to 80% B after 5 min, then increased to 95% B for 15 min; it was kept to 100% B for 17–27 min, before being 20% B for 30–40 min. The total run time for each injection was 40 min. Argon gas was used as the collision gas. The LC system was a LCMS-8030 Shimadzu system with a DGU-20A_{3R} degassing unit, coupled with an LC-20AD liquid chromatograph, a CTO-20AC column oven, a SIL-20AC autosampler and a NM32LA nitrogen gas generator.

5.3.4.2 Validation of Method

To ensure method precision, procedural blanks were prepared during the analysis and were analyzed at an interval of ten samples. This was to assess whether contamination occurred during sample extraction. Hence, solvent blanks comprising MeOH (195 µL) and ISTD (5 µL) were prepared for analyses after every twenty processed samples to monitor for background contamination. To assure the accuracy and precision of each run, duplicate injections and recalibration using appropriate standards were conducted for each run after processing twenty samples. In cases whereby the target analytes were detected in the procedural blanks, their peak areas' average values were subtracted from the peak areas of the target analyte of the actual sample before the final concentrations were calculated. The level of detection (LOD) was defined as the peak signal of a target analyte that needed to yield a signal-to-noise (S/N) ratio of 3:1 and ranged from 0.003 to 0.03 ng/L for all the three investigated PFASs. The limit of quantification

(LOQ), was defined as the standard deviation (SD) of the blanks and was determined to be 0.03 ng/L for PFOA and PFOS, and 0.07 ng/L for PFBS. Additionally, 50 µL of native surrogates were used for matrix spike recovery testing. Hence, recoveries of native standard surrogates spiked in the plant and water matrix were 98, 96, and 93% for PFOA, PFOS, and PFBS, respectively. Furthermore, Equations (5.1) and (5.2) were used to obtain the relative response factors and final concentrations of the targeted PFASs, respectively.

$$RRF = \frac{A_{\mathrm{NAT}}}{A_{\mathrm{IS}}} \times \frac{C_{\mathrm{IS}}}{C_{\mathrm{NAT}}} \tag{5.1}$$

where:

RRF is the relative response factor;

A_{NAT} is peak the area of the native 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.

$$FC = \frac{A_{\mathrm{NAT}}}{A_{\mathrm{IS}}} \times \frac{1}{RRF} \times \frac{V_{\mathrm{IS}}}{V_{\mathrm{S}}} \tag{5.2}$$

where:

FC is the final concentration;

A_{NAT} is the peak area of the target analyte;

A_{IS} represents the peak area of the internal standard used for that particular analyte;

RRF is the calculated relative response factor of the specific analyte;

V_{IS} is the volume of the internal standard added in the sample prior to extraction (mL);

V_{S} is the volume of the sample (mL).

5.5 Results

5.5.1 LCMS Calibration Curves for the Detection and Quantification of PFOA, PFOS and PFBS

A procedural blank matrix free of the 3 PFASs was prepared and used in preparation for post-spiked calibrants, and thus the calibration curves were constructed based on a 10-point

curve at concentrations of 1, 2, 5, 10, 20, 25, 50, 75, 100, and 125 ng/L. The regression coefficients (R^2) of calibration curves for all the target analytes have revealed good linearity ($R^2 > 0.99$), as can be seen in Figure 5.2 which displays the calibration curves of PFOA, PFOS, and PFBS.

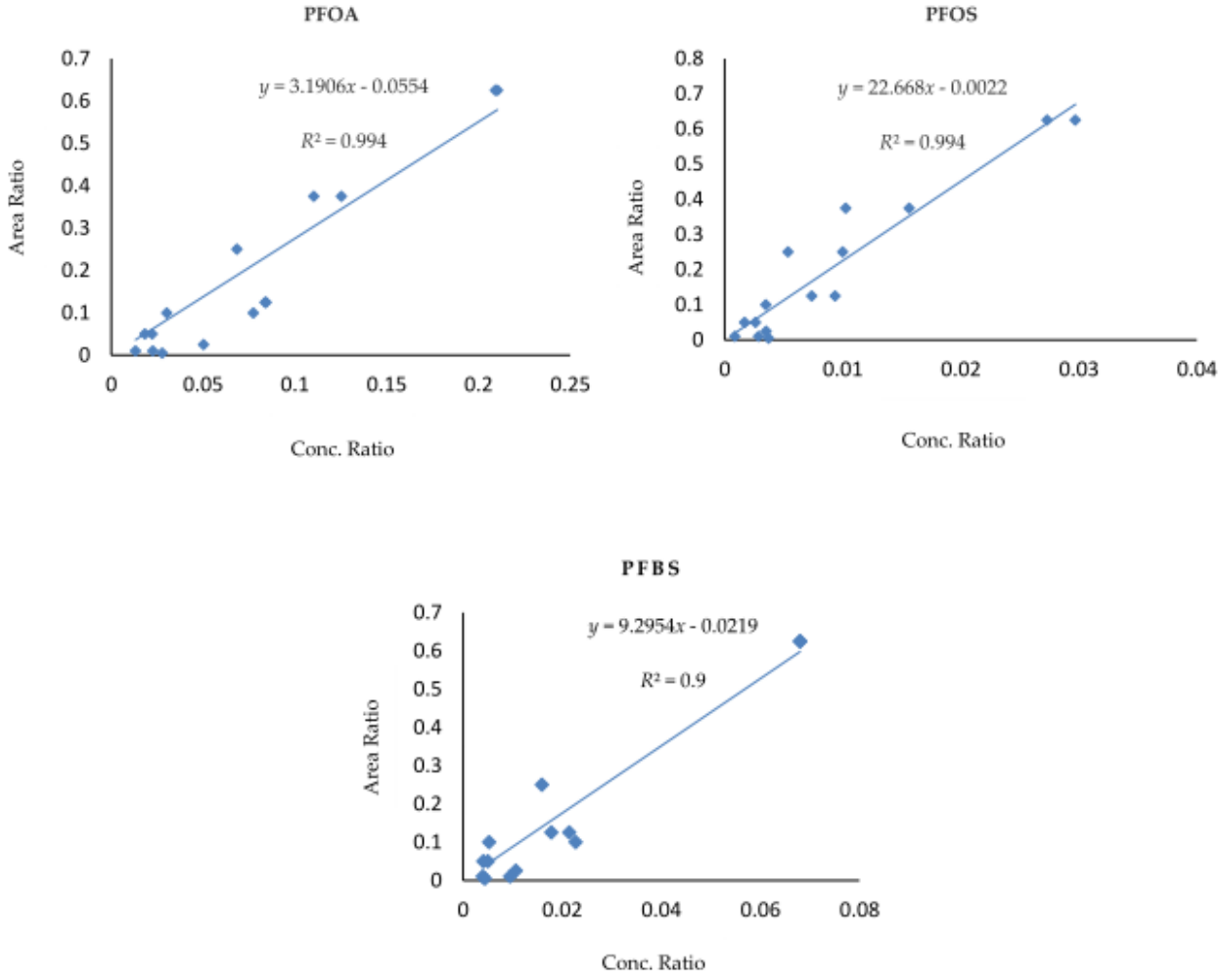

Figure 5.2: Calibration curves (ng/L) of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorobutane sulfonate (PFBS) in procedural blank matrix.

5.5.2 LCMS Chromatographs for PFOA, PFOS, and PFBS

The MRM optimization of three PFASs (i.e., PFOA, PFOS, and PFBS) and one ISTD (i.e., M-PFOA) was carried out, with two MRM transitions being utilized for each PFAS. Thus, one was used as an ion quantifier and the other for confirmation. Table 5.1, as well as Figure S1, shows

the mass transitions used for the identification and quantification of each targeted compound, as well as the ISTD, and their retention times (RT).

Table 5.1: Names and multiple reaction monitoring (MRM) transitions of three perfluoroalkyl substances (PFASs) and one internal standard (ISTD).

Compound	Acronym	Transition Qualifier (*m/z*)	Transition Quantifier (*m/z*)	Retention Time (min)
Targets				
Perfluorooctanoic acid	PFOA	413.00 > 169.05	413.00 > 368.95	8.6
Perfluorooctane sulfonate	PFOS	499.00 > 98.90	499.00 > 80.15	8.9
Perfluorobutane sulfonate	PFBS	299.00 > 99.10	299.00 > 80.10	6.8
ISTD				
Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	M2PFOA	414.80 > 169.00	414.80 > 369.90	8.7

5.5.3 Results of Previously Known Contaminated River Water

Although evidence of PFASs in the South African environment remains limited, a previous study has reported concentrations of PFOA and PFOS in a Western Cape river (i.e., Salt River) of 0.7 to 390 ng/L and <LOD to 50 ng/L, respectively [29]. Of the three rivers that were studied for their PFASs predisposition, the Salt River recorded the highest PFOA concentration. The Salt River also had the second-highest PFOS concentration, although PFBS was not investigated. In this current study, the water that was collected from the Salt River was for the purpose of irrigation of the plants that were studied. Therefore, it was pertinent to first assess the concentration levels of PFASs in the collected water, prior to using the water for irrigation purposes, and to ensure the accuracy of the results. Therefore, three PFASs (i.e., PFOA, PFOS, and PFBS) were quantified in twenty samples (n = 20). Two sampling regimes were implemented with river water: Regime A (n = 10) samples were taken after heavy rain, and constituted winter/wet season conditions, while Regime B (n = 10) samples were taken during the summer/dry season, for which rainfall was absent for the previous five months. The results obtained in this regard are summarized in Table 5.2, and it can clearly be seen from these that the investigated substances have been detected in some samples. From the investigated plant

samples, the concentration of the substances varied markedly between individual samples, as well as the river water regimes. The PFAS concentrations in samples were in the following decreasing order: PFOA > PFBS > PFOS. From the investigated samples, Regime A registered all the highest concentrations in terms of the analyzed substances, while Regime B recorded the lowest. On the other hand, Figure 5.3 demonstrates how each river water sample has contributed to the overall concentrations of each investigated substance.

Table 5.2: Concentration of PFOA, PFOS and PFBS in river water (ng/L).

Compounds				Regimes
Sample ID	**PFOA**	**PFBS**	**PFOS**	
RW1	76.79	8.59	0.08	Regime A
RW2	86.69	20.75	ND	
RW3	66.44	6.78	0.12	
RW4	98.21	3.82	ND	
RW5	107.82	3.88	<LOD	
RW6	97.82	2.59	ND	
RW7	105.12	4.26	0.06	
RW8	95.81	1.72	ND	
RW9	1.15	1.24	<LOQ	
RW10	3.65	2.41	0.06	
RW11	1.56	1.89	0.10	Regime B
RW12	<LOQ	2.99	<LOQ	
RW13	<LOQ	3.49	0.06	
RW14	<LOQ	2.12	<LOQ	
RW15	<LOQ	3.44	0.06	
RW16	3.76	5.29	0.06	
RW17	1.20	4.83	<LOQ	
RW18	<LOQ	5.16	<LOQ	
RW19	0.71	4.61	<LOQ	
RW20	4.35	3.77	<LOQ	

RW: river water; ND: not detected; <LOD: below the limit of detection; <LOQ: below the limit of quantification.

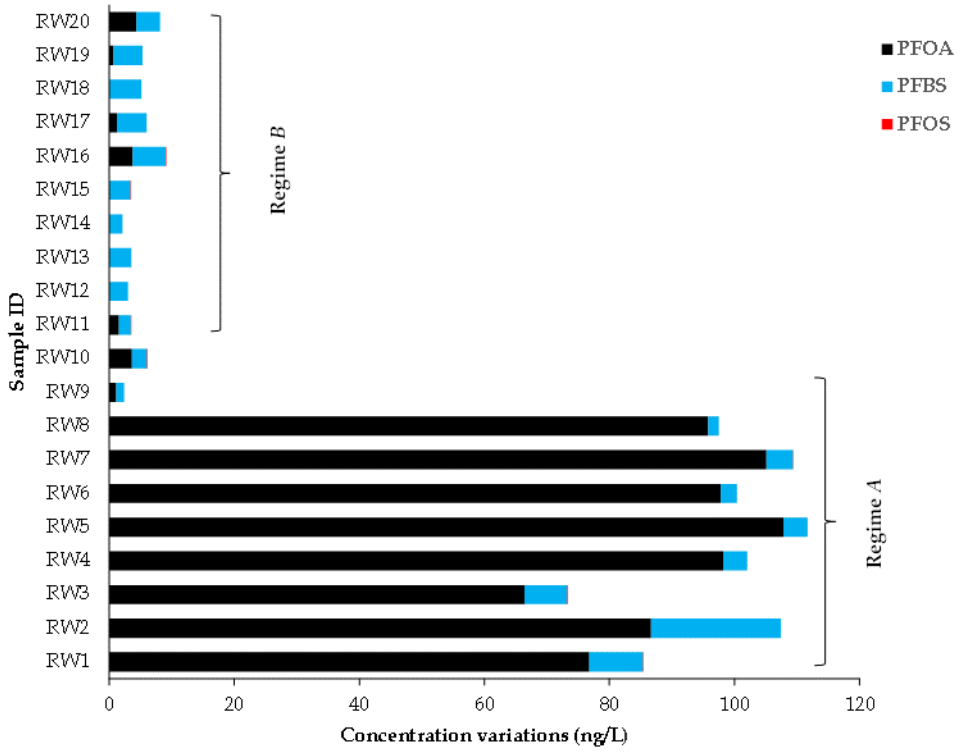

Figure 5.3: Individual PFAS concentration level variations for each sampling regime

5.5.4 PFOA, PFOS and PFBS Accumulation in a Commonly-Used Medicinal Plant

There are various reports that have indicated the prevalence of PFASs (i.e., PFOA, PFOS, and PFBS) in several environmental matrices, including plants. For instance, Mudumbi et al. [28] reported the susceptibility of riparian plants to PFOA accumulation in South Africa, Western Cape Province (WCP), while Krippner et al. [35,36] indicated higher uptake of PFASs, including PFBS, into plant leaves. Recently, Kurwadkar et al. [37], as well as Zhao and Zhu [38], addressed the uptake of PFASs in plants. Similarly, studies by Sznajder-Katarzyńska et al. [39] and Zhao et al. [40] have reported on the vulnerability of edible plants to accumulation of PFASs. Nevertheless, there is little evidence on the vulnerability of medicinal plants to PFASs accumulation [15], as most studies have focused on the therapeutic side of these plants and not on their susceptibility to emerging POPs, such as PFASs, which are a potential risk to human health. For this reason, PFASs (i.e., PFOA, PFOS, and PFBS) were investigated in *Tagetes erecta* L., and traces of the three PFASs were detected in all the plant samples. The concentrations of these

POPs among all the investigated plant samples were in the following decreasing order: PFOA > PFOS > PFBS. Contaminated samples recorded the highest amount of PFOA and PFOS. The summary of these results is depicted in Table 5.3, and Figure 5.4 shows the contribution of each sample to the concentration levels of PFASs that were quantified in the plant under investigation.

Table 5.3: Summary of studied plant samples (*Tagetes erecta* L.), with their PFAS concentrations (ng/g) and bioconcentration factor (BCF).

Average PFAS Conc./*n* = 20/Water (ng/L)	Plant Samples	PFOA/BCF		PFBS/BCF		PFOS/BCF	
PFOA (37.6)	CS1	48.70	1.30	0.75	0.16	0.41	13.67
	CS2	58.96	1.57	1.44	0.31	1.29	43.00
	CS3	94.83	2.52	1.15	0.24	2.17	72.33
PFBS (4.7)	S4	32.36	0.86	1.44	0.31	0.12	4.00
	S5	34.55	0.92	0.25	0.05	3.57	119.00
PFBS (4.7)	S6	37.34	0.99	0.74	0.16	5.03	167.67
	S7	28.49	0.76	0.45	0.10	4.24	141.33
	S8	18.05	0.48	0.51	0.11	1.39	46.33

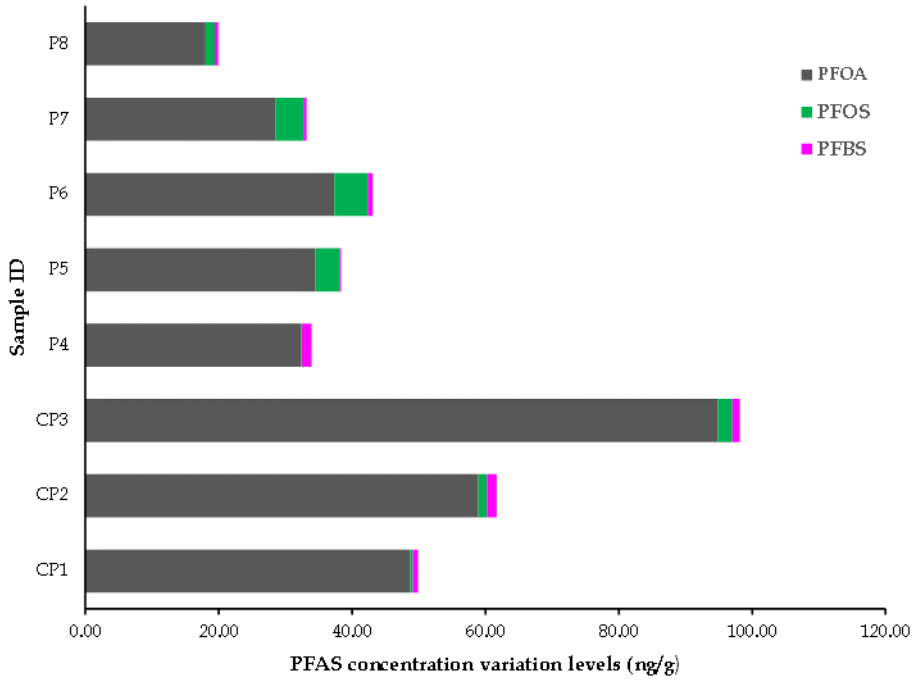

Figure 5.4: Contribution of each sample to the PFASs concentration levels in *Tagetes erecta* L.

5.6 Discussion

5.6.1 New Evidence on the Contamination of Salt River by PFASs

Concentrations of PFOA, PFOS, and PFBS were observed in all the samples, with PFBS being the most dominant PFAS, followed by PFOA. However, concentration levels for PFOS were mostly not detected (ND) for individual samples. The results are summarized in Table 5.2. From the results, it can be seen that the concentrations of PFOA, PFBS, and PFOS were <LOD to 107.82 ng/L; 1.24 to 20.75 ng/L; and ND to 0.12 ng/L, respectively. Overall, Regime A samples had the highest concentrations of PFASs with sample RW5 having 107.82 ng/mL for PFOA, RW2 20.75 ng/L for PFBS, and RW3 0.12 ng/L for PFOS. However, the second sample (i.e., RW11) had the highest PFOS concentration (0.10 ng/L) observed among the Regime B samples. Figure 5.3 shows PFAS concentration variations in samples from the two regimes, A and B, (i.e., for samples taken in two different seasons).

Furthermore, from Table 5.2, it can be seen that two of the three assessed PFASs (PFOA and PFBS) showed a significant increase during Regime A, which was putatively regarded as a result of the rain which might have contributed to run-off of PFAs into the river. This trend substantiates the fact that runoff has been suggested as being a contributing factor to higher concentrations of PFASs in water streams [29,41]. Overall, PFBS was prevalent in most samples, although PFOA was observed to have had the highest concentrations in a few samples, with the PFOA concentrations of most samples being below the LOQ (that is, 0.03 ng/L). Similarly, PFOS concentration levels remained below the LOQ in some samples (n = 7), with only one sample (RW5) being below the LOD (that is, 003 to 0.03 ng/L). Additionally, PFOS was the only PFAS that was not detected in certain individual samples, including sample RW2. PFBS was found to be prevalent in both sampling regimes (A and B), while LOQ for PFOA and PFOS were evenly distributed, in particular for Regime B. As both PFOA and PFOS are classified as long-chained PFASs, while PFBS is identified as a short-chain compound [42], it was previously suggested that PFOA and PFOS prevalence in the Western Cape rivers might be attributed to a highly active agricultural sector [29]. These two PFASs have been the most studied and have predominantly been found in various environmental matrices, both worldwide and in South Africa [14]. Recent reports have now indicated that PFBS, previously thought to be harmless, fits the category of

POPs [14]. In addition, recent reports have now indicated that PFBS, previously known as a harmless PFAS, fits the category of POPs [14,43–45], and it has been found to be the most dominant PFAS in this study—a pattern previously reported by Heydebreck et al. [46] and Pan et al. [47]. This ultimately suggests the use of PFBS in the Western Cape, South Africa, and thus, there is cause for concern with regard to the prevalence of this short-chain PFAS in the South African environmental ecosystem, especially in river water. Accordingly, further studies are required to determine other short-chain PFASs prevalent in the South African environment, and their possible source(s). Nevertheless, Cai et al. [41] and Zhu et al. [48] have reported that the abundance of short-chain PFASs signifies the predominance of the use of perfluorocarboxyl compounds in a study area. Evidence of the prevalence of short-chain PFASs in humans is also limited (if not non-existent) in the Western Cape, and particularly in South Africa.

Furthermore, we compared the concentration levels of the three PFASs investigated in the Salt River with those found in other rivers worldwide (see Table 5.4). As far as the Salt River is concerned, it was found that concentrations of PFOA and PFOS were much lower than they were in previous studies conducted in 2014, and remained the lowest among comparative PFASs studied [29]. This decrease can be attributed to the fact that during the sampling year for this study (2017), the Western Cape Province experienced a severe drought, which led to minimal and/or limited runoffs into the river under investigation. It was further suggested that there has been a decrease in the use of the said substances and/or products containing them in the region. This argument still has to be confirmed by further investigations. Nevertheless, the concentration levels of both PFOA and PFBS, in the current study, were found to be much higher than in other rivers globally, but PFOS concentration remained generally much lower, or undetected. These results are similar to those of the Rhine River (see Table 5.4), and the PFBS concentration determined in this study was also similar to that of the Rhine river [46].

Table 5.4: Comparison of PFOA, PFOS and PFBS levels (ng/L) in rivers from previous studies.

River	Country	Sampling Year	Level	PFOA	PBFS	PFOS	Reference
Salt	South Africa	2017	mean	107.82	20.75	0.12	This study
Salt	South Africa	2014	mean	390.0	n/a	46.8	[29]
Diep	South Africa	2014	mean	314.4	n/a	181.8	[29]
Eerste	South Africa	2014	mean	145.5	n/a	22.5	[29]
Yangtze	China	2016	mean	13.5	1.84	1.83	[47]
Yellow	China	2016	mean	2.05	0.99	1.84	[47]
Pearl	China	2016	mean	7.45	4.49	11.09	[47]
Kakum	Ghana	NI	mean	167.4	n/a	113	[49]
Tai	China	2012	mean	24.7	3.18	9.78	[50]
Liao	China	2016	mean	8.95	0.94	3.46	[47]
Ganges	India	2014	mean	1.2	n/a	1.7	[50]
Guadalquivir	Spain	NI	mean	11.6	10.1	1.8	[51]
Orge	France	2011	mean	9.4	4.4	17.4	[52]
Rhine	Europe	NI	mean	4.72	21.28	ND	[46]
Swedish	Sweden	2013	mean	4.2	n/a	6.9	[53]
Pearl	China	2013	mean	3.13	ND	2.2	[54]

n/a = not analysed; NI: not indicated; ND = not detected.

5.6.2 Traces of PFASs in the Investigated Medicinal Plant

In this study, the propensity of the African marigold (*Tagetes erecta* L.) to accumulate PFOA, PFOS, and PFBS was investigated. *Tagetes erecta* L. is a medicinal plant commonly used for DM therapy [8–13]. Since the study was conducted using a set of plants, we used contaminant-free plant sets as a reference. The soil in which the plants were grown was not assessed for PFASs as they were grown in pristine soil, with the source of the PFAS being the river water.

Subsequently, PFOA, PFOS, and PFBS, as found in the river water, were observed in all the plant samples (n= 8) with PFOA being the most highly accumulated PFAS by *Tagetes erecta* L., followed by PFOS, and then PFBS, with concentrations of up to 94.83 ng/g, 5.03 ng/g, and 1.44 ng/g, respectively. Table 3 displays the overview of these concentrations. In addition, these concentrations were attributed to the highest concentration of both PFOA and PFBS in the river water, hence their prevalence in higher concentration in the plant samples. The accumulation was hypothesised to be facilitated by mass flow translocation, a process through which chemical constituents in water are taken up by the plants [55–57] via the root system of the plant [14,56,57]. Hence, it can be suggested that the higher the concentration of PFASs in the water, the higher the likelihood of these pollutants to accumulate in plant compartments, including leaves. These results are an indication that medicinal plants are at risk of being contaminated by pollutants, including PFASs, and ultimately, constitute a potential pathway through which these substances might be ingested by humans who rely on them for therapeutic purposes. Hence, Table S1 depicts a list of select medicinal plants that are used to treat T2DM in South Africa, which are at risk of being exposed to the prevalence of PFASs, as river water is predominantly used in underprivileged communities which rely heavily on phytomedicines for the management of diseases.

Furthermore, the results obtained in the current study partially concur with the results previously found by Mudumbi et al. [28], Yoo et al. [58], Marchand et al. [59], and Stahl et al. [60], which reported that various plants had the potential to accumulate PFASs, PFOA in particular. However, a slight decrease in the uptake of PFOA was observed in the present study compared to that by Mudumbi et al. [28]. As previously suggested, the contribution of the root system of the studied plant, that is *Tagetes erecta* L., to the uptake of PFASs was not analysed, a factor which

Mudumbi et al. [28] suggested to play a pivotal role in the manner in which a given plant uptakes pollutants, including PFASs.

5.6.3 *Tagetes erecta* L. Sorption Aptitude by Means of Bioconcentration Factor (BCF)

Bioconcentration factor (BCF), according to available evidence, is seen as the capability of a plant to uptake a specific chemical substance with relation to its concentration in the soil [61,62]. Hence, the BCF, in this regard, was calculated as the ratio of the concentrations of the PFASs in the plant samples to those in the river water samples to assess the sorption capacity of *Tagetes erecta* L.:

$$BCF = C_{plant\ samples}/C_{water} \quad (5.3)$$

Consequently, the BCFs of PFASs for the investigated plant species (i.e., *Tagetes erecta* L.) are shown in Table 3. Hence, for PFOA, the BCF for the different plant samples was 1.30 (CS1), 1.57 (CS2), 2.52 (CS3), 0.86 (S4), 0.92 (S5), 0.99 (S6), 0.76 (S7), and 0.48 (S8); for PFBS it was 0.16 (CS1), 0.31 (CS2), 0.24 (CS3), 0.31 (S4), 0.05 (S5), 0.16 (S6), 0.10 (S7), and 0.11 (S8), while for PFOS, it was 13.67 (CS1), 43 (CS2), 72.33 (CS3), 4 (S4), 119 (S5), 167.67 (S6), 141.33 (S7), and 46.33 (S8). Overall the BCF values for PFOS were higher than those of PFOA and PFBS, a trend which suggests that there was a bioaccumulation potential of this particular PFAS in *Tagetes erecta* L., when compared to the other two PFASs. In this regard, individual plant samples demonstrated an accumulation potential of PFOS. Not only plants were determined to accumulate PFASs in South Africa, another previous study indicated the predominance of PFASs in South African drinking water sources [63], suggesting that even when tap water is used for irrigation, there would be a potential of PFAS accumulation in the plants.

Furthermore, PFBS, which is a short-chain PFAS, tends to demonstrate much lower adsorption potential than PFOS and PFOA, which are long-chained PFASs, ultimately suggesting that their bioaccumulation potential in plants might be dependent on their molecular size, as previously suggested by Zhou et al. [64] and Conder et al. [65]. Additionally, it has been indicated that PFBS tend to translocate horizontally and vertically with water diffusion and permeation, making it a much more mobile PFAS than PFOA and PFOS [64]. In addition, the BCF of two (i.e., PFOA and PFBS) of the three investigated PFASs has remained slightly high in the contaminated

plant samples. It has been previously suggested that the distribution and accumulation of PFAS in plants are species-dependent [29,66].

5.6.4 Environmental Implications

Subsequently, the benefits of medicinal plants and their hypoglycemic effects in the management of T2DM have overwhelmingly been confirmed by an assortment of studies [15,67–70]. Nevertheless, evidence on the vulnerability of medicinal plants to pollutant accumulation, including the emerging ones, such as PFASs, remains limited. This constitutes a cause for concern; according to Mudumbi et al. [15], medicinal plants have played a tremendous role in battle against several diseases, particularly in the sub-Saharan African region, due to the prohibitive cost of orthodox medicine and the low incomes of many communities in the region [71]. This suggests that medicinal plants and/or their derived products are accessible and affordable to these communities [1–15]. Hence, Mudumbi et al. [15] suggested that the cultivation, harvest or collection, and storing of medicinal plants and/or their products should be conducted in areas free of any form of contamination, including that of PFASs. The authors further argued that this precautionary measure would ensure enhanced quality, efficacy, and safety of medicinal plants and/or products, and eventually enhanced health for those who rely on these plants as a means of treatment for the ailments they are suffering from, such as T2DM. Moreover, although the future of medicinal plants is promising in the Sub-Saharan region, there is a need for education around conservation, and awareness as to the dangers of using contaminated river water for irrigation purposes [72].

5.7 Conclusions

South Africa is a water-stressed country with uncontrolled contamination of river water, particularly in certain provinces such as the Western Cape, which recently experienced a severe drought. Subsequently, it has been reported that surface and tap water, as well as riparian plants, in the Western Cape region are contaminated with emerging pollutants, such as PFASs. In the present study, river water was used to irrigate a medicinal plant used to manage DM, *Tagetes erecta* L., as is commonly done in local communities. The PFASs levels in this water were also analysed, as well as the tendency of this plant (i.e., *Tagetes erecta* L.) to uptake these compounds. Consequently, PFOA, PFOS, and PFBS were found in the river water, as well as in the plant under

investigation. Individual plant samples demonstrated abundant PFOA concentrations, thus bioaccumulation, and PFBS was observed to be the most predominant in all the river water samples. The BCF suggested that PFBS, a short-chain PFAS, has lower translocation potential into the plant, a trend which allowed this PFAS to remain in the water. In addition, the relatively low accumulation of PFOS in the plant was hypothesized to be dependent on plant species, but future studies still have to be conducted in this regard. Moreover, the prevalence of PFASs in river water used for irrigation, and their subsequent bioaccumulation in medicinal plants, can be considered as a potential pathway through which humans can be exposed to PFASs in communities relying on alternative and unorthodox management of DM. The results from the present study can contribute to the establishment of a database for monitoring the accumulation of PFASs, including PFOA, PFOS, and PFBS, in medicinal plants. There is currently limited information on their susceptibility to PFASs, such as PFOA, PFOS, and PFBS, and there is more that still needs to be established.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Selected medicinal plants under possible threats by PFASs in South Africa.

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CHAPTER 6

Connotation of perfluoroalkyl substances and Diabetes ailments: A case study of a Bellville South population, in Cape Town, South Africa

6.1 Abstract

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a class of chemicals used in several industrial applications and consumer products worldwide. They are anthropogenic and only regulated voluntarily by a few countries, regardless of their environmental persistence and health effects. The aim of this study was to examine serum PFAS levels and their association with diabetes *mellitus* (DM) in a Bellville South, Western Cape population, in South Africa. Therefore, a liquid chromatography/tandem mass spectrometry (LC–MS-8030) was used to measure the PFASs, coupled with Statistica software package, for statistical analysis. PFASs, perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), perfluorobutane sulfonate (PFBS), were detected in all the tested serum samples (n = 179); albeit, there was no direct and significant association between PFOA, PFOS, PFBS and any of the predictors, i.e. overweight and obesity, even in known DM cases for the studied population (p-values < 0.05). In summary, the inconsistency in our findings warrants further investigation.

Keywords: Diabetes *mellitus*, perfluoroalkyl substances (PFASs); perfluorooctanoic acid (PFOA); perfluorooctane sulfonate (PFOS); perfluorobutane sulfonate (PFBS), Serum.

6.2 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a wide collection of synthetic chemicals [1,2]. They have exceptional properties, such as thermal stability and resistance to degradation, including resistance to staining and repellency against oil and water [3,4]. Several industries have extensively used them for decades, both as surfactants and surface protectors, in various industrial processes to manufacture household goods, as well as industrial products [3-5]. These goods and/or products include consumer goods, electronics, textile coatings, surface treatments agents, adhesives and building materials [6]; as well as non-stick coatings, food wrappers, upholstery, firefighting foams, clothing and furnishings [7-11].

Apart from these compounds' prevalent usage, PFASs are highly resilient and persistent once they have entered the natural environment. The latter is due to a strong bond that exists between the carbon and fluorine atoms in the structure of these substances [4], leading to the substances being extremely resistant to environmental and biological degradation [5]. Subsequently, these pervasive characteristics have led to humans being exposed to PFASs [7]. In this regard, the general population is prevalently exposed to PFASs through various routes, including dietary intake, drinking water, indoor air and household dust and food packaging including cookware [10,12,13].

Additionally, Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs), lists, since 2009, some PFASs including perfluorooctane sulfonate (PFOS) and perfluorooctanoic (PFOA) [14]. These chemicals were added on the list of substances that authorities globally should consider to regulate as they pose human health risks [5,15]. Overall, there are thousand types of PFASs that have been reported and documented in numerous studies [11].

Furthermore, exposure to PFASs has been associated with various ailments, including type 2 diabetes (T2D) and other metabolic diseases in various epidemiological studies [16-19]. For instance, higher serum PFOA concentrations were recently associated with a greater adiposity and an increased body mass index (BMI) in children between 2-8 years of age; albeit, this association was not observed with PFOS, perfluorononanoic (PFNA) and perfluorohexane sulfonic (PFHxS) [19]. Similarly, another study found an association between serum PFOA concentrations with increased adiposity, and the risk of weight gain or obesity in adult women

during their pregnancy [20,21]. Similarly, a hasty weight gain was observed in baby girls born to women who were diagnosed with high levels of PFOS while pregnant [21,22]. On the other hand, a recent study has revealed an association between PFNA and an increased risk of metabolic syndromes [7], which are regarded as a cluster of disorders, some of which are exacerbated by obesity, which is one of the leading causes of T2D [23,24], including cardiovascular diseases (CVDs) [7,25,26]. Accordingly, a study by Huang et al. [10] has suggested an association between exposure to PFASs and a risk of CVDs. Consequently, it has been reported that CVDs are some of the leading causes of death worldwide [10,27].

From a South African perspective, PFASs have been detected in potable (drinking) and surface water [28,29], as well as in a number of other environmental matrices [30-33]. Similarly, a recent publication on South Africa (Western Cape) has reported on the prevalence of PFASs, including perfluoroundecanoic acid (PFUnDA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), PFOA, and perfluoroheptanoic acid (PFHpA), in the fillets of fish (*Thyrsites atun*) which is consumed in large quantities by the populace forming part of this study [34]. In addition, CVDs were reported as the second major cause of death, after AIDS [35]; and recently, Pheiffer et al. [36] indicated that T2D was a major source of morbidity and mortality in South Africa, due to increased urbanisation and unhealthy lifestyle habits. Similarly, diabetes has been reported as a leading risk factor for people living with HIV [11,37-39], a virus which has claimed many lives in South Africa. Nevertheless, it is worth indicating that, there has been inconsistency in the evidence reporting on the association of PFASs and DM, suggesting more studies are required.

To our knowledge, there has been evidence on the prevalence of PFASs in the South African population and the environment [40-45]. However, as a country where cases of T1D and T2D have increased due to the country's socio-economic development, there is a need to assess the link between the increasing DM cases and the levels of PFASs in human serum. Currently, no study in South Africa, has investigated the potential relationship between PFASs exposure and DM. Therefore, this study's primary aim was to investigate the concentration levels of three commonly studied PFASs consisting of two long-chained PFCs (i.e. with seven or more perfluorinated carbons, e.g. PFOA and PFOS), and one short-chain PFC (i.e. five or fewer perfluorinated carbons, e.g. PFBS), in the serum of diabetic patients from a Bellville South population, in Cape Town, South Africa. To determine whether there is a direct correlation

between PFOA, PFOS, PFBS with known cases of DM, particularly in this population group, which is of mixed-ancestry origin, and has the second highest prevalence of diabetes in South Africa [35]. Firstly, this study included both long and short-chain PFASs because, firstly, PFBS (short-chain) was recently found to be the most dominant PFAS in water samples collected in the region, compared to long-chain such as PFOA and PFOS [33]. Secondly, PBFS, which was previously regarded as less harmful, is proven as unsafe as its analogues or long-chain PFASs [46-48]. Thirdly, most studies have only focused on long-chain PFASs, such as PFOA and PFOS [18,49-54]. The differences between serum PFAS levels reported and other studies were also determined.

6.3 Materials and methods

6.3.1 Chemicals, reagents and standards

The PFASs standards of perfluorooctanoic acid (PFOA), perfluorobutane sulfonate (PFBS), and perfluorooctane sulfonate (PFOS), were obtained from the laboratory of the Department of Environmental, Water and Earth Sciences, Tshwane University of Technology (TUT), South Africa. All standards were purchased in methanol at 50 μgmL^{-1} from Wellington Laboratories (Ontario, Canada). A surrogate mixture of stable isotopically labelled PFASs standard containing perfluoro-n-[1,2,3,4-$^{13}C4$] octanoic acid (MPFOA), perfluoro-n-[1,2,3,4, 5-$^{13}C_5$] nonanoic acid (MPFNA), and perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid (MPFUnDA), was also obtained from TUT, and purchased in methanol at 2 μgmL^{-1} from Wellington Laboratories (Ontario, Canada). Sodium carbonate, anhydrous extra pure sodium carbonate (Na_2CO_3, 99.5 %) were purchased from Sigma-Aldrich (Aston Manor, South Africa).

Organic solvents, such as Ammonium acetate (NH_4Ac, LC-MS grade, ≥99%), Ammonium hydroxide solution (NH_4OH, LC-MS grade, ≥25 %), Methanol (MeOH, LC-MS grade, ≥99.9) and Acetonitrile (ACN, LC–MS grade; ≥99.9%), Formic acid (CH_2O_2, LC-MS grade, >98), Tetrabutylammonium hydrogensulfate (TBAHS) and Methyl-*tert*-butyl ether (MTBE) of HPLC grade were purchased from Sigma-Aldrich (Aston Manor, South Africa). Only polypropylene (PP) tubes, syringes, filters, and cartridges were used throughout the experiment to avoid any possible cross-contamination to the samples.

6.3.2. Sample preparation and extraction

The procedure used to prepare and extract the serum samples was based on the methods previously used by Bao et al. [5] and Mudumbi et al. [33], with minor modifications. Human sera (n = 179 samples) were pipetted (0.5 mL each) into a sterile and pre-rinsed 15 mL PP tubes, with an isotopically labelled internal standard (50 µL) being added to each sample in the tube. To the mixture, a 1 mL of 0.5 M TBAHS solution (pH was adjusted to 10 with KOH) was added; and prior to mixing each tube gently, a 2 mL of 0.2 M bicarbonate buffer solution (pH 9.2) was added, followed by gentle mixing, prior to adding 5 mL of MTBE. The mixture in PP tubes was then agitated in a shaker (Amerex SK-703, Lafayette, USA) for 25 min at 250 rpm. The separation of the organic and aqueous phases from the matrix was performed by centrifugation at 3500 rpm for 5 min. For each sample, a volume (4 mL) of the aqueous phase was transferred into a new sterile pre-rinsed 15 mL PP tube. The extraction was repeated as described above, and the extracts were combined in a second pre-rinsed 15 mL PP tube. The solvent (i.e. MTBE) was allowed to evaporate using analytical grade nitrogen evaporator at 30°C. The residues were reconstituted using 1 mL of 20% acetonitrile, whereby the PP tubes were centrifuged for 10 min at 10000 rpm. The final extracts were filtered using 0.2 µm PP filters obtained from Sigma-Aldrich (Aston Manor, South Africa) prior to solid phase extraction (SPE).

For SPE, Supelco-Select HLB SPE cartridges (500 mg solid phase, 12 mL tubes) were used. Therefore, cartridges were conditioned with 2 mL of 2% NH_4OH in MeOH/MTBE (1:9, v/v), and left to equilibrate for 10 min. Subsequently, 2 mL of 2% CH_2O_2 in sterile distilled water was used to wash the cartridges. The samples were loaded into the cartridges, and washed with 2 mL of 2% CH_2O_2 in H_2O, coupled with 2 mL of MeOH. Pre-rinsed PP collection tubes were put in place in the SPE cartridge older, prior to a further 2 mL of 2% NH_4OH in MeOH/MTBE (1:9, v/v) being added to the cartridges to elute the PFASs from the anion-exchange sorbents of each SPE cartridge, at a flow rate of, approximately, 1 drop/5sec. the SPE extract was dried under nitrogen gas and reconstituted with 0.5 mL of 10% ACN, prior to the analytes being decanted in PP LC-MS/MS vials, which were thereafter stored in a refrigerator prior to the LC-MS/MS-8030 analysis. Figure 5.1, depicts the overall samples' preparation and extraction schema.

6.3.3. Instrumental analysis

To analyze the concentrations of the targeted PFASs in sera samples, a liquid chromatography (LC) system was used, combined with tripartite quadrupole linear ion trap tandem mass spectrometer (MS/MS-8030), with an electrospray ionization (ESI) source operating in a negative ion mode, with multiple reaction monitoring (MRM). A column used was a Luna Omega 3.0 um Polar C18 100 A LC Column 100 x 2.1 mm (Phenomenex, Aschaffenburg, Germany) set at 40 °C. A mobile phase with a combination of 20 mM ammonium acetate and 20% isopropanol (solvent A) and 100% MeOH (B) was used at a flow rate of 0.3 mL/min, with 10 µL as the appropriate injection volume for distinct samples. The linear gradient elution program started at 20%B and amplified to 80%B after 7 min, then increased to 95%B for 15 min; thereafter, maintaining 100%B for 17-27 min, before being 20%B for 30-40 min (total running time for each injection). Nitrogen was used as the collision gas. The LC system was a Shimadzu (LCMS-8030) system with a degassing unit (DGU-$20A_{3R}$), coupled with a liquid chromatograph (LC-20AD), a column oven (CTO-20AC), an autosampler (SIL-20AC) and a nitrogen gas generator (NM32LA). It was used to attain the chromatographic separation of the targeted analytes.

6.3.4. Quality control and assurance

To ensure the accuracy of the method, preparation of procedural blanks were made which facilitated the analysis of the samples at a range interval of ten (10) for each run. This was done to evaluate whether there was any contamination that occurred during the extraction of the samples. Consequently, solvent blanks made of MeOH (195 µL) and internal standard (ISTD) (5 µL) were prepared for analysis after every twenty (20) samples to control background contamination. To ensure precision and accuracy of each executed analysis, duplicate injections and recalibrations were performed using the appropriate standards for each analysis after processing twenty samples, respectively. In cases whereby the target analytes were detected in the procedural blanks, their average peak areas were deducted from those of the actual samples, prior to the computation of the final analyte concentrations. The LOD were outlined as the peak signal of a target analyte that required yielding a signal/noise (S/N) ratio of 3:1, which ranged from 0.003 to 0.03 ng/mL for all the investigated PFASs, i.e. PFOA, PFOS, PFBS. The limit of quantification (LOQ) was outlined as the variance (SD) of the blanks, selected as 0.03 ng/mL for PFOA and PFOS, and 0.07 ng/mL for PFBS. A 50 µL of native surrogates was used for matrix

spike recovery testing. The recoveries of native customary surrogates spiked within the serum matrix averaged 98, 96 and 93% for PFOA, PFOS and PFBS. Equation 1(S1) was adapted to acquire the standardization curves (Figure S1), respectively.

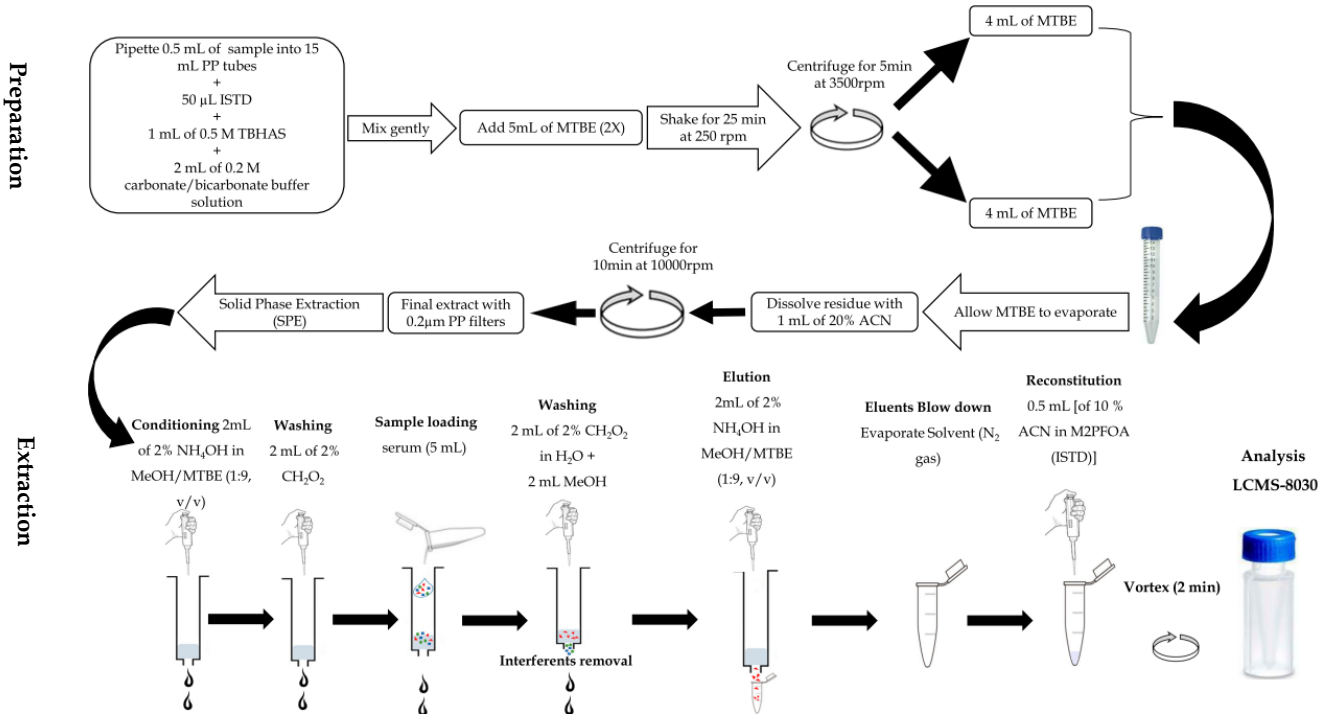

Figure 6.1: Serum samples' preparation and extraction schema. Adapted from Mudumbi et al. [33]

6.3.5. Study population and sample collection

Participants from this study were from a cross-sectional Cape Town Vascular and Metabolic Health (VMH) on-going study, and an extension of the Cape Town Bellville South study previously described in other studies, including Matsha et al. [35], Erasmus et al. [55], Davison et al. [56]; Davids et al. [57] and Zemlin et al. [58]. The present study population comprised of 179 mixed ancestry adults (22% males and 78% females) residing in Bellville South, Cape Town, South Africa. A detailed protocol describing data-collection procedures (questionnaires and physical examination) and interviews were developed as previously described [35, 56]. A team of professional nurses collected clinical, biochemical and anthropometric measurements, i.e. weight, height, and hip and waist circumferences, using standardized techniques as prescribed by WHO [59]. The samples were processed within an

appropriate time and aliquots were stored at -80°C. A Sunbeam EB710 digital bathroom scale, calibrated and standardized at a weight of known mass, was used to determine participants' weights. The measured weights were recorded to the nearest 0.1 kg, after ensuring that each subject wore light clothes, and no shoes or socks. A stadiometer was used to record the height of each subject to one decimal place. Body mass index (BMI) was calculated as weight per square meter (kg/m^2). To measure the waist circumference, a non-elastic tape was utilized for non-obese individuals, but for obese participants this measurement was done between the ribs and the iliac crest. Turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics) was used to assess Glycated haemoglobin (HbA1c). The subjects' present tobacco use was defined as a cotinine level >10 ng/mL [56, 59]. As such, all anthropometric measurements were performed three times and the average measurements were used for analysis.

6.3.6. Statistical analysis

Data were analysed with a statistical analysis system package, STATISTICA software (Statsoft, http://www.statsoft.com). One-way ANOVA was used to determine descriptive statistics and results are presented as mean ± standard deviation (SD), for all variables, including the investigated PFASs (i.e. PFOA, PFOS and PFBS), age, body mass index (BMI), etc. categorized according to glycaemic status and gender. The Spearman rank correlation test was used to determine correlations between PFAS levels and other variables investigated, including age, BMI etc. The statistical significance of both the correlations and differences were set at $p < 0.05$. Additionally, further statistical analyses used in the present study were performed as per previous studies [35,55-58].

6.3.7. Ethics endorsement and consent participation

The Health and Wellness Sciences-REC (Research Ethics Committees) of the Cape Peninsula University of Technology approved ethics endorsement for study (ref. no. CPUT/HWS-REC 2015/H04). The study observed the Code of Ethics of the World Medical Association as incorporated in the Declaration of Helsinki. All the participants were provided with full explanations regarding the study and voluntarily signed written informed agreements.

6.4. Results

6.4.1. Baseline participants' characteristics

A total of 179 samples were analysed and comprised of 140 females and 39 males. Table 6.1 provides the general characteristics and mean concentration levels of sera PFASs according to gender. The mean age (standard deviation) of participants was 55.8 (±2.5) years and was not significantly different between the genders. PFASs levels did not significantly differ between males and females, except for PFOA which was significantly higher in females, p=0.0116, with the BMI, hip circumference and waist to hip ratio being significantly higher in females, p≤0.0009 (Table 6.1). There was an insignificant difference in PFASs concentrations between normotolerant (n=67), screen-detected diabetes (n=58) and individuals with diabetes (n=54), p=0.5475 (Figure 6.2A,B,C). Similarly, there was an insignificant difference in PFASs between normal weight (n=38), overweight (n=49) and obese individual (n=82), p=0.3749 (Figure 6.3A,B,C).

6.4.2. Correlations of PFASs sera levels with anthropometric and biochemical measurements

Table 6.2 shows the correlations between the PFASs and the general characteristics categorised according to gender. There was an insignificant correlation between the PFASs and any of the other measurements in the male group. PFOA (ng/mL) was found to be positively correlated with prevalence of PFBS (ng/mL) (r = 0.21, p=0.01) and PFOS (ng/mL) (r = 0.27, p=<0.01) in females. PFOS (ng/mL) showed a significant positive correlation with anthropometric measurement WHR (r = 0.27, p<0.01), with glycaemic measurements FBG (mmol/L) (r = 0.17, p=0.04) as well as HbA1c (%) (r = 0.19, p=0.02), while PFOS (ng/mL) also showed a significant negative correlation with cotinine (ng/mL) (r = -0.17, p=0.04). Similarly, PFOA (ng/mL) showed a significant negative correlation with PostBG (mmol/L) (r = -0.24, p=0.02), while PFBS (ng/mL) showed an insignificant correlation with any of the anthropometric or biochemical measurements.

Table 6.1. Anthropometric and biochemical measurements and distribution of serum PFASs by gender

Characteristics	**Total group (*n*=179)**	**Males (*n*=39)**	**Females (*n*=140)**	***p***
		Mean±SD		
PFOA (ng/mL)	9.43±13.16	4.74±9.23	10.73±13.80	0.0116
PFOS (ng/mL)	1.00±1.51	0.77±1.24	1.06±1.57	0.2923
PFBS (ng/mL)	1.66±2.39	1.27±1.39	1.77±2.59	0.2483
Age (years)	55.8±12.5	57.4±11.5	55.4±12.8	0.3639
BMI (kg/m^2)	30.5±6.9	27.1±6.4	31.4±6.7	0.0009
WaistC (cm)	98.0±13.8	96.7±16.3	98.3±13.1	0.5282
HipC (cm)	109.9±13.9	101.6±10.2	112.1±13.9	<0.0001
WHR	0.89±0.08	0.94±0.08	0.88±0.07	<0.0001
FBG (mmol/L)	7.50±3.54	7.49±3.24	7.50±3.63	0.9899
PostBG (mmo/L)	9.15±4.95	8.75±4.80	9.25±5.01	0.6615
HbA1c (%)	6.96±1.82	7.04±2.11	6.93±1.74	0.7522
Cotinine (ng/mL)	122.7±177.2	154.6±192.0	113.9±172.6	0.2109

SD: standard deviation; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; PFBS: perfluorobutane sulfonate BMI: body mass index; *p*: *p*-value; WaistC: waist circumference; HipC: hip circumference; WHR: waist to hip ratio; FBG: fasting blood sugar; PostBG: post blood sugar, HbA1c: Glycated haemoglobin.

Table 6.2: Correlation between PFASs and anthropometric and biochemical measurements

	PFOA (ng/mL)				PFOS (ng/mL)				PFBS (ng/mL)			
	Male		*Female*		*Male*		*Female*		*Male*		*Female*	
Characteristics	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
PFOA (ng/mL)	-	-	-	-	-0.12	0.47	0.07	0.44	0.05	0.77	0.21	0.01
PFBS (ng/mL)	0.05	0.77	0.21	0.01	0.28	0.08	0.27	<0.01	-	-	-	-
PFOS (ng/mL)	0.28	0.08	0.27	<0.01	-	-	-	-	-0.12	0.47	0.07	0.44
Age (years)	-0.01	0.94	0.04	0.67	-0.22	0.17	0.06	0.51	0.01	0.94	0.05	0.55
BMI (kg/m^2)	0.10	0.55	0.01	0.89	0.22	0.21	-0.01	0.94	-0.15	0.40	0.01	0.88
WaistC (cm)	0.17	0.30	0.05	0.59	0.27	0.10	0.11	0.19	-0.11	0.52	0.00	0.99
HipC (cm)	0.15	0.37	0.05	0.53	0.19	0.27	-0.05	0.55	-0.19	0.28	0.06	0.48
WHR	0.14	0.41	0.00	0.96	0.16	0.37	0.27	<0.01	0.04	0.83	-0.06	0.50
FBG (mmol/L)	-0.00	1.00	-0.16	0.05	-0.10	0.54	0.17	0.04	-0.03	0.83	-0.07	0.38
PostBG (mmo/L)	-0.08	0.69	-0.24	0.02	-0.08	0.73	0.10	0.32	0.15	0.49	-0.07	0.47
HbA1c (%)	-0.10	0.54	-0.09	0.31	-0.04	0.82	0.19	0.02	-0.30	0.07	-0.06	0.51
Cotinine (ng/mL)	0.01	0.95	-0.10	0.25	0.15	0.36	-0.17	0.04	0.12	0.48	-0.00	0.97

PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; PFBS: perfluorobutane sulfonate; *p*: *p*-value; BMI: body mass index; WaistC: waist circumference; HipC: hip circumference; WHR: waist to hip ratio; FBG: fasting blood glucose; PostBG: post blood glucose, HbA1c: Glycated haemoglobin.

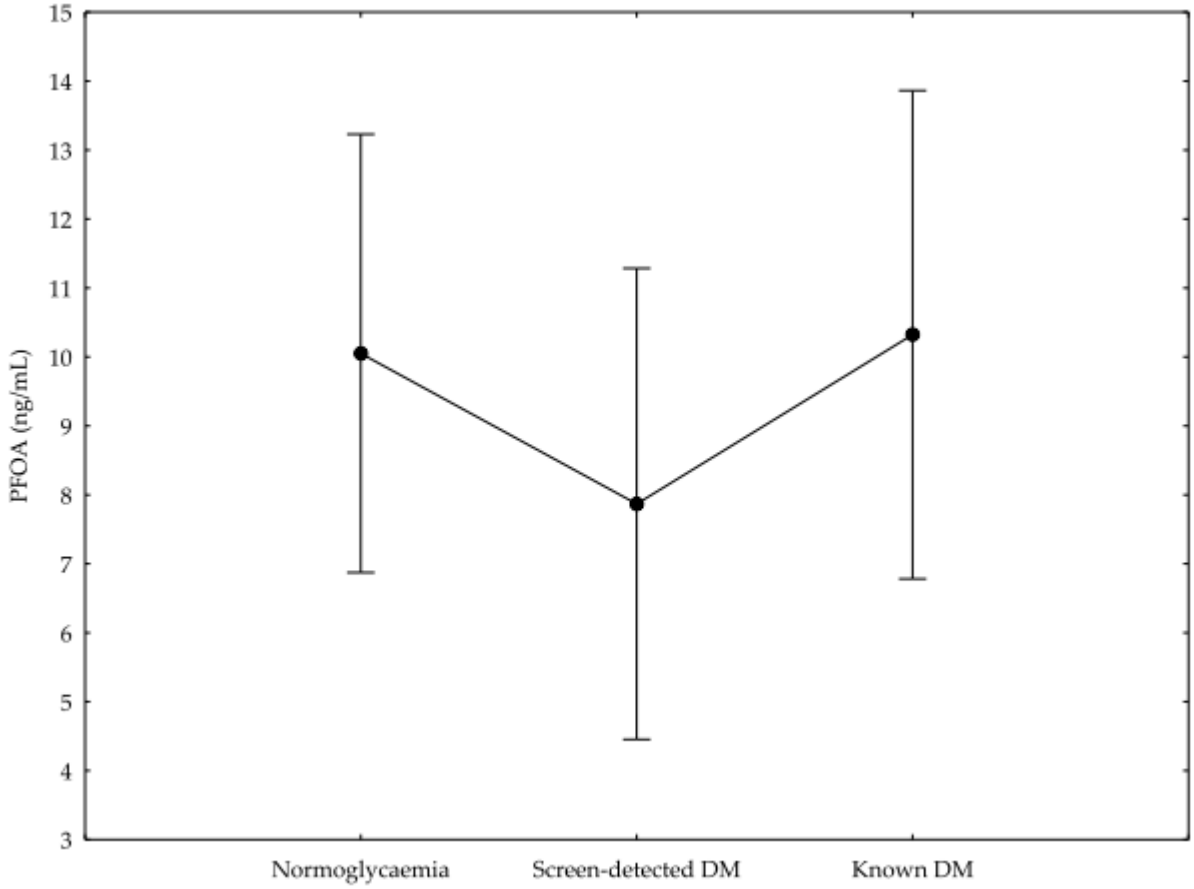

Figure 6.2A. Serum concentration of PFOA (normoglycaemic group compared with screen-detected and known DM groups). There was no significant difference in PFOA (ng/mL) values when categorized by glycaemic status: mean±SD: 10.1±11.0 ng/mL in normoglycaemic subjects (*n*=67), 7.9±10.0 ng/mL in screen-detected DM subjects (*n*=58) and 10.3±17.9 ng/mL in known DM subjects (*n*=54); *p*=0.5475

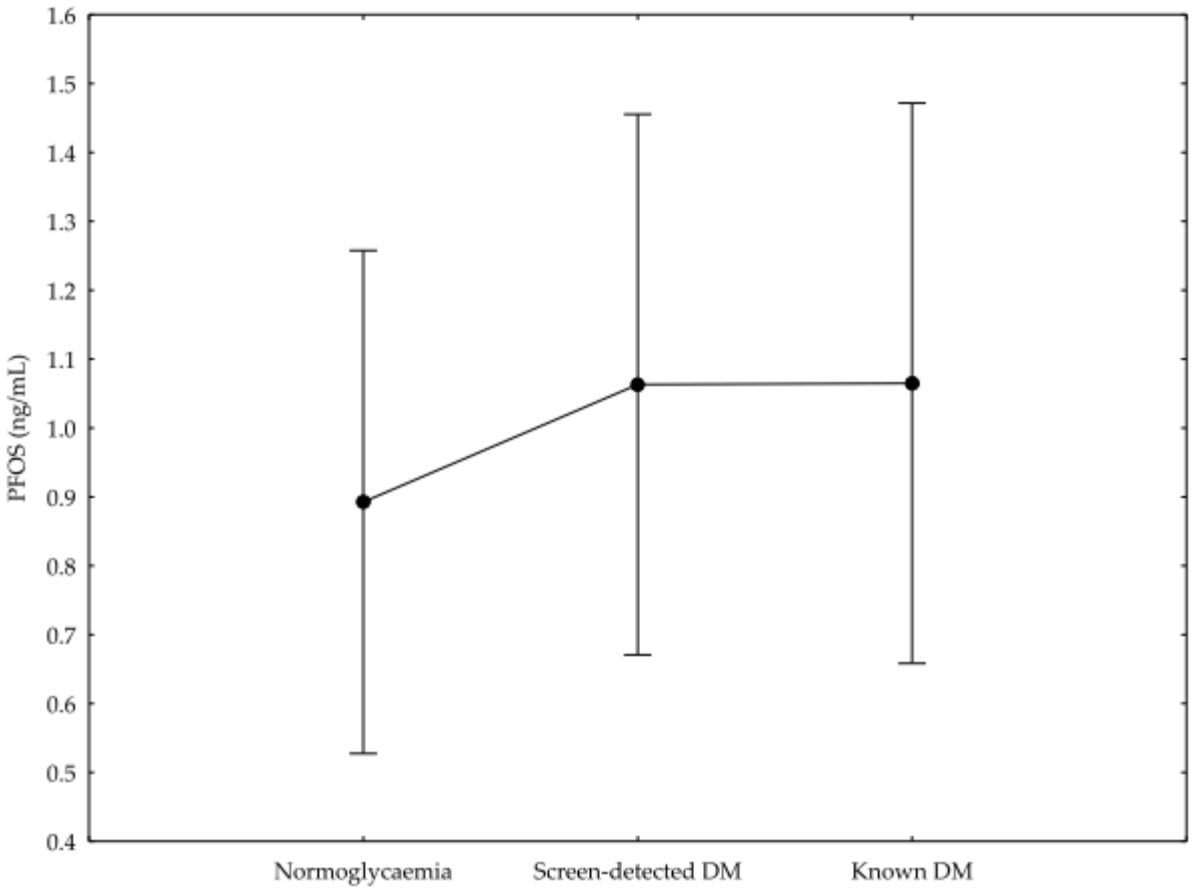

Figure 6.2B. Serum concentration of PFOS (normoglycaemic group compared with screen-detected and known DM groups). There was no significant difference in PFOS (ng/mL) values when categorized by glycaemic status: mean±SD: 0.89±1.51ng/mL in normoglycaemic subjects (*n*=67), 1.06±1.61 ng/mL in screen-detected DM subjects (*n*=58) and 1.06±1.41 ng/mL in known DM subjects (*n*=54); *p*=0.7644.

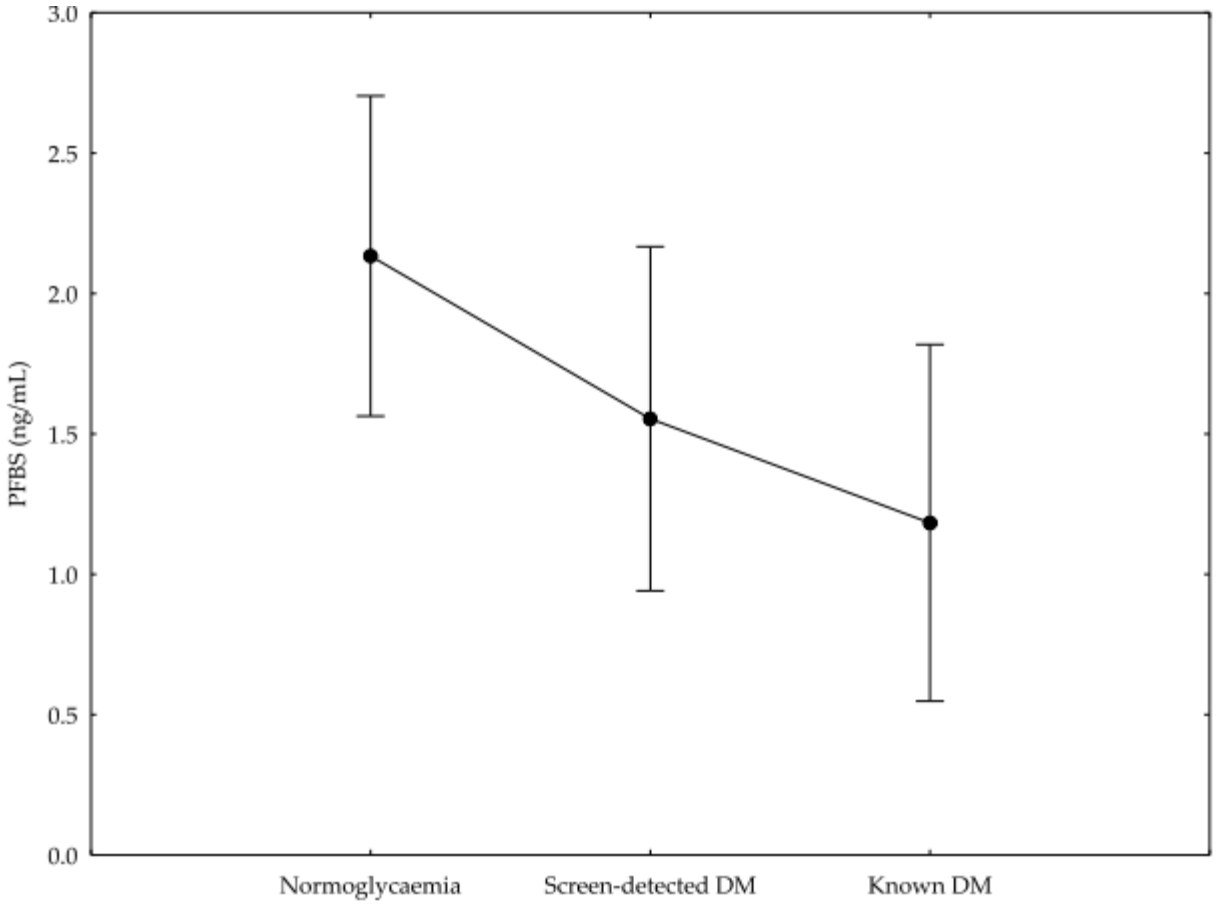

Figure 6.2C. Serum concentration of PFBS (normoglycaemic group compared with screen-detected and known DM groups). There was no significant difference in PFBS (ng/mL) values when categorized by glycaemic status: mean±SD: 2.13±3.36 ng/mL in normoglycaemic subjects (*n*=67), 1.55±1.51 ng/mL in screen-detected DM subjects (*n*=58) and 1.18±1.43 ng/mL in known DM subjects (*n*=54); *p*=0.0851.

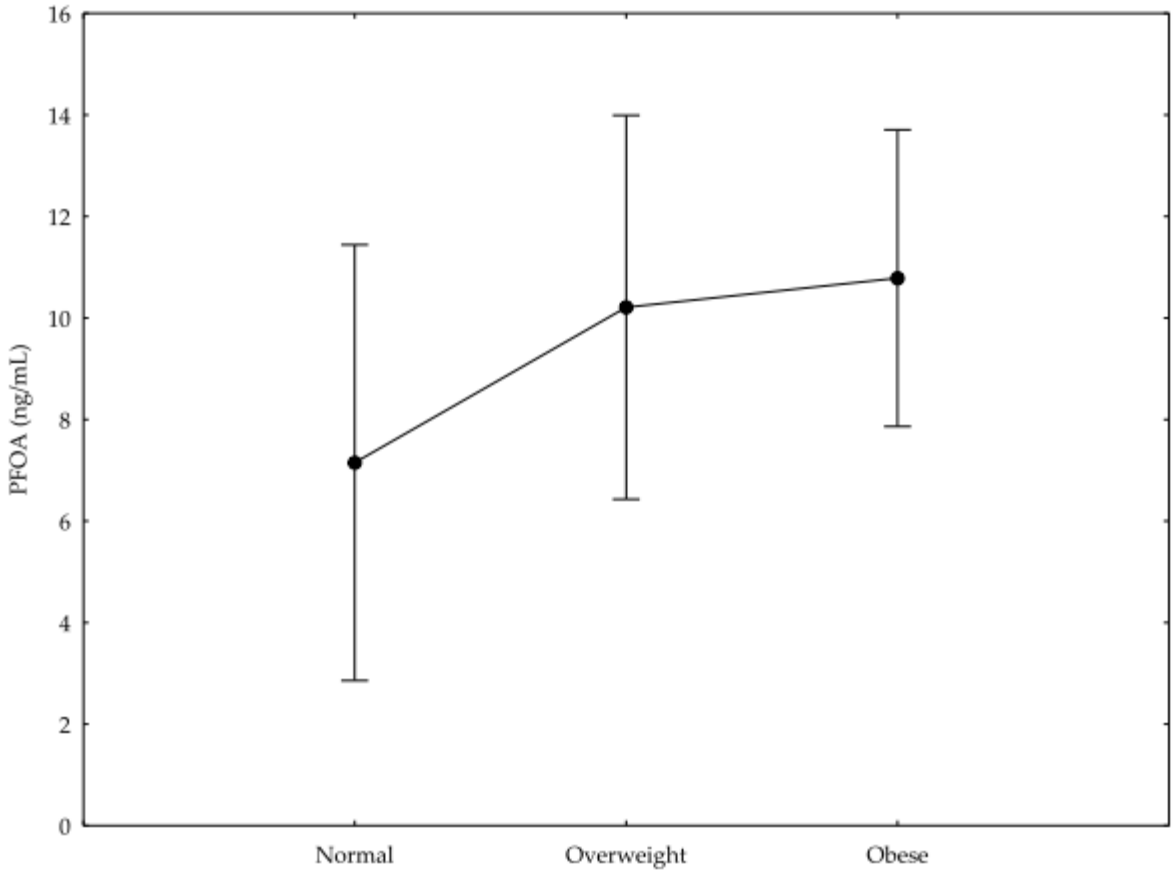

Figure 6.3A. Serum concentration of PFOA (normal group compared with overweight and obese groups). There was no significant difference in PFOA (ng/mL) values when categorized by obesity status: mean±SD: 7.2±9.1 ng/mL in normal weight subjects (n=38), 10.2±16.8 ng/mL in overweight subjects (n=49) and 10.8±12.7 ng/mL in obese subjects (n=82); p=0.3749.

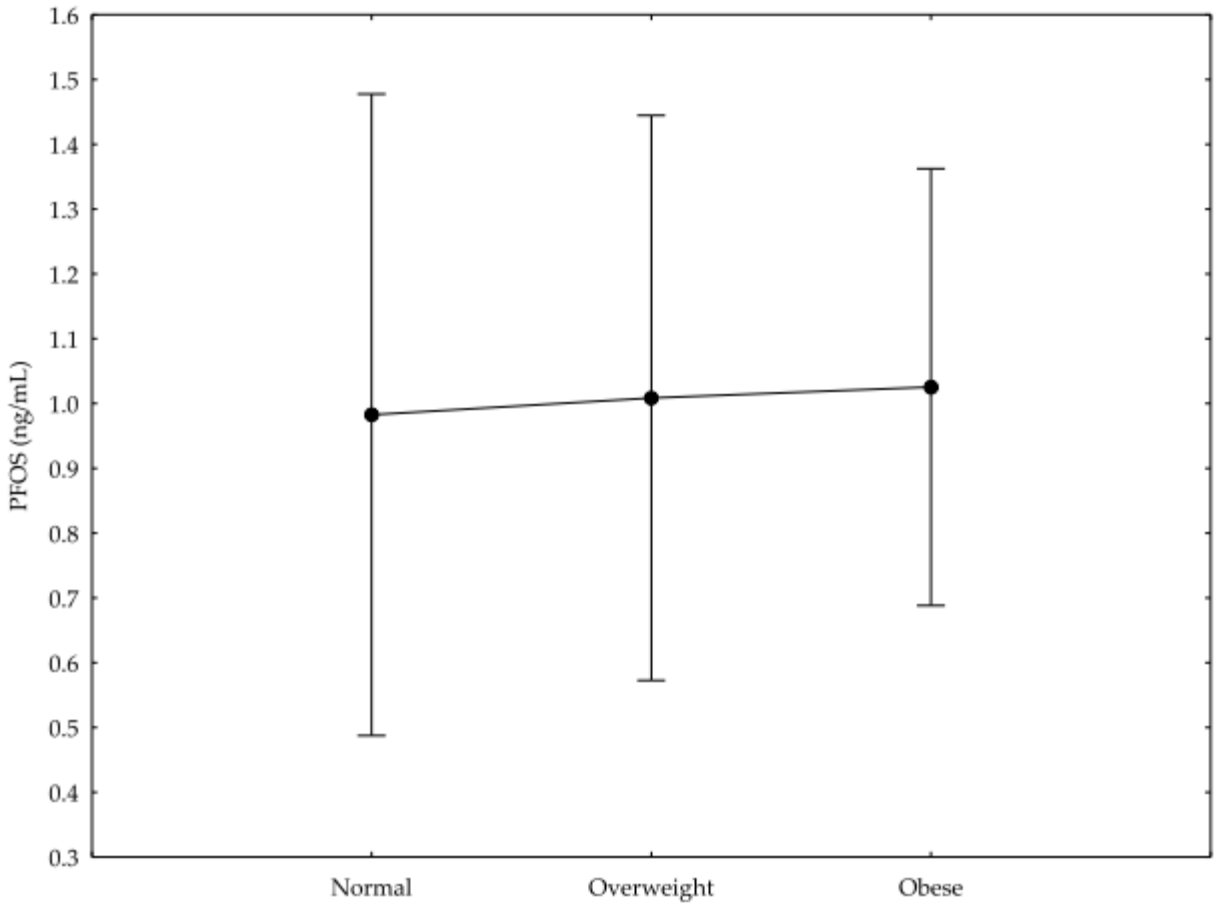

Figure 6.3B. Serum concentration of PFOS (normal group compared with overweight and obese groups). There was no significant difference in PFOS (ng/mL) values when categorized by obesity status: mean±SD: 0.98±1.62 ng/mL in normal weight subjects (*n*=38), 1.01±1.58 ng/mL in overweight subjects (*n*=49) and 1.03±1.49 ng/mL in obese subjects (*n*=82); *p*=0.9901.

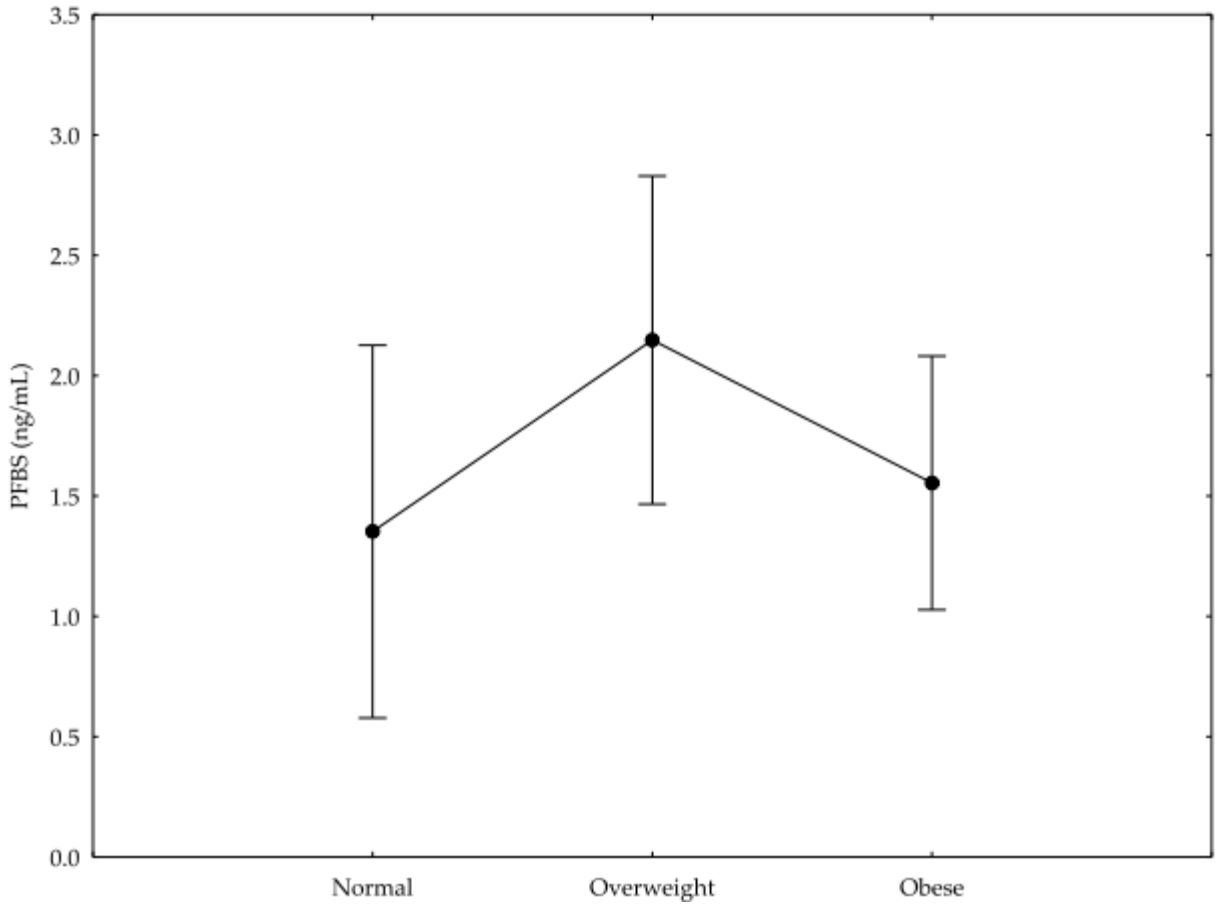

Figure 6.3C. Serum concentration of PFBS (normal group compared with overweight and obese groups). There was no significant difference in PFBS (ng/mL) values when categorized by obesity status: mean±SD: 1.35±1.38 ng/mL in normal weight subjects (n=38), 2.15±3.79 ng/mL in overweight subjects (n=49) and 1.55±1.61 ng/mL in obese subjects (n=82); p=0.2553.

6.5. Discussion

To the best of our knowledge, no study has yet investigated the prospective correlation between PFASs exposure and autoimmune diseases, such as DM, in a South Africa population, in particular, and Africa in general. Hence, this is the first study about the prevalence of serum PFASs in DM patients, and the association of these substances to the ailment. All three investigated PFASs, i.e. PFOA, PFOS and PFBS, were measured in the serum samples analysed, with PFOA being the most abundant PFAS in both females (10.73 ng/mL) and males (4.74 ng/mL), followed by PFBS (1.77 and 1.27 ng/mL), for males and females, respectively. However, the three PFASs were generally higher in females than males, a trend that was previously observed by Li *et al*. [61]. This suggested that women in this region are the most likely to be exposed to these substances. Table 6.3 depicts the differences between serum PFAS levels

reported in previous studies. It can be observed that the PFAS levels from the current study are relatively lower compared to other studies.

We measured cotinine, a chemical that the body makes after you are exposed to nicotine, the reason being that available evidence has reported smoking prevalence among the population group under investigation in this study [62,63]. Cotinine concentrations were higher in males than in females (154.6 and 113.9 ng/mL, respectively). The correlation between PFASs and cotinine was not significant (p= 0.2). This is inconsistent with results from Mamsen et al. [63], who previously found a significant positive correlation between investigated PFASs and cotinine.

Table 6.3. Summary of other studies on the association between PFASs exposure and diabetes

	PFASs levels (ng/mL)			
Studies of reference	**PFOA**	**PFOS**	**PFBS**	**Outcomes**
Present study	9.43	1.00	1.66	No evident association between analysed PFASs and risk of developing diabetes
[18]	0.49	0.95	n/a	High serum levels of PFOS may lead to being susceptible to develop diabetes.
[49]	3.94	13.10	n/a	Higher concentrations of PFOA were significantly associated with an increased risk of diabetes
[51]	1.8	3.4	n/a	No consistent evidence for any positive associations between the PFASs and diabetes
[50]	82.3	23.1	n/a	PFAS levels were negatively associated with diabetes
[52]	5.4	5.2	n/a	Negative association of PFOA with diabetes. Positive association between FPOS and diabetes
[53]	4.96	35.7	n/a	Higher concentrations of PFOS and PFOA were associated with an elevated risk of T2D
[54]	1.30	2.81	n/a	No evident association between PFASs and risk of diabetes

n/a = not analysed

Recent reports have indicated that there has been an increase in PFBS prevalence [7], which might substantiate the reason why PFBS is the second most abundant substance in the current study. Similarly, Mudumbi et al. [33] recently reported a high prevalence of PFBS in both river water and a commonly used South African medicinal plant (Tagetes *erecta* L.), suggesting a link between water, medicinal plants and the susceptibility of humans to not only long-chain PFASs, such as PFOA and PFOS, but also short-chain PFASs, such as PFBS. Ultimately, this further suggests the use of PFBS in industrial applications in the Western Cape Province, South Africa, where the participants reside, as well as the possibility of DM sufferers' being exposed to other short-chain PFASs, such as PFBS. To confirm the latter statement, further studies are required in this regard. Mudumbi et al. [29] has previous indicated that river water is used, countrywide, to irrigate crop lands, including plants used in the management of DM.

Unlike in males, a significant positive correlation between PFOA and PFOS (r = 0.27; p = <0.01), as well as PFOA and PFBS (r = 0.21; p = 0.01) (Table 6.2) was found in females, a trend previously reported by Li et al. [61]. This suggests there is a common exposure pathway of these substances, which allows the exposure of females. It is worth indicating that, in the South African context, women are involved in jobs that are likely to expose them to PFASs, such as cooking. For example, Stats-SA reported in its 2018 report that women dominated the domestic worker market [64]. Subsequently, scientific evidence has reported the prevalence of PFASs in households [65-67].

Common sources of long-chain PFASs, including PFOA and PFOS, have mainly included diet and water [68, 69]. And although not enough evidence of PFOA, PFOS and PFBS is available in South Africa as far as food and/or diet is concerned, recent reports have indicated the prevalence of these three PFASs in both tap and surface water in the country, as well as in a popular plant (i.e. Tagetes *erecta* L.) [28,29,33]. This plant is commonly ingested by locals for the management of diabetes [29,33].

In the present study, serum PFOS and PFBS levels were positively correlated with PFOA; albeit, independent of PFOS levels, which were positively associated with HbA1c, in women. HbA1c develops when haemoglobin, a protein within red blood cells that carries oxygen throughout the human body, joins with glucose in the blood, and thus becoming 'glycated' [70] . The same source indicates that, for people with DM, measuring HbA1c is important, as the higher

it is, the greater the risk of developing diabetes-related complications. Our results showed higher levels HbA1c for both males and females (7.04 and 6.93%, respectively), in comparison to the <5.7% considered as normal by Shah et al. [71]. This ultimately suggests that higher concentrations of PFOS, or any other PFAS, in diabetic sufferers are likely to lead to further complications due to the relationship that might occur between these substances and HbA1c.

Previously, Liu et al. [72] observed a negative association between PFOA and HbA1c. Nevertheless, it has been suggested that the reasons behind such conflicting results between studies remain largely unknown, but putatively, variations can be caused by various perplexing factors, including early or late stage exposure to PFASs, and perhaps the status of insulin resistance [65]. We also strongly believe that the time frame until samples are analysed might have an effect on the final result outcomes; this is so because a study by Blake et al. [4] suggested that, PFAS half-lives may play a role in their temporal stability in biological samples. More research is thus required in this regard.

It was found that there was no difference between PFOA and PFOS concentrations, respectively, in normal subjects and the known DM cases (Figure 6.2A and B). This is inconsistent with previous results from Predieri et al. [18], which reported a similar scenario for PFOS. Thus, this trend suggests, in our view, and as far as this pilot study is concerned, that the levels of PFASs observed in the current study cannot be considered as a leading cause of DM in the studied population of the Western Cape. Nevertheless, we suggest further research to be undertaken to substantiate the observed trend, as this study is a preliminary one in as far as South Africa is concerned. One positive attribute of this study is the high sensitivity equipment used, for it was capable to detect PFAS concentrations in all our samples, even at extremely low concentrations.

Nonparametric correlation coefficient was used to compare PFOA, PFOS, PFBS and obesity status. Each entry in Figure 6.2A, B, and C gives the correlation coefficient estimate, the *p*-value for its significance test, and the number of observations used. The *p*-values are larger than 0.05 in every case. We found that, there was no significant association between PFOA, PFOS, PFBS and any of the three primary predictors (p=0.3749, 0.9901 and 0.2553, respectively), including for known DM cases regardless of the higher concentration levels of these substances; albeit observed to be slightly higher in normal weight and overweight subjects (see Figure 6.3A, B and C). Additionally, our results showed higher PFAS levels in obese and known DM subjects who, to our knowledge, were on oral DM treatment, including insulin. This is in contradiction with report

results from Genuis et al. [73], which suggested that insulin, including cholestyramine (CSM) treatment, had the potential in facilitating the elimination of some PFASs. Hence, this inconsistency requires further investigations to be conducted in this field.

Generally, although no evident association between the analysed PFASs and risk of developing DM was found, of PFASs observed in the current study should be considered as a warning, particularly from a South African context, taking in account the recent findings by Mudumbi et al. [33], a study which reported the susceptibility of *Tagetes erecta* L., a medicinal plant used in South Africa for the management of DM, to PFOA, PFOS, PFBS bioaccumulation. Ultimately, such plants, including those reported by Davids et al. [74] and Mudumbi et al. [75] are important in the management of DM; however they are still a viable pathway through which humans, including DM sufferers, would be exposed to PFASs [33], and which in return can lead to cases of various ailments, including vulnerability to DM development and complications.

Our study had some limitations, including the small sample size used, which might reduce the efficacy of the reported results, as well as the ability to compare the results with previously published results from other studies. The samples were also stored for elongated periods prior to preparation and analysis; which might have compromised the stability of the investigated PFASs; albeit, suitable sample preservation strategies were implemented. The samples had fewer males than females, suggesting that it was not a 50/50 representation in terms of gender.

6.6. Conclusions

In summary, the results from this study indicated that there is human exposure to PFASs in a Bellville South population in Cape Town, South Africa. Of the three PFASs, PFOA and PFBS were the most abundant substances detected in the sera samples in the general population living in the Bellville south zone. Regardless, the study found minimal evident association between analysed substances and the susceptibility to develop DM. Nevertheless, we suggest further investigation be conducted to validate our findings due to limitations associated with the availability of the test subjects.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, S1: Equation. Figure S1: Procedural blank matrix calibration curves for PFOA, PFOS and PFBS (ng/L)

Author Contributions: Conceptualization, John Baptist Nzukizi Mudumbi and Seteno Karabo Obed Ntwampe; Data curation, John Baptist Nzukizi Mudumbi; Formal analysis, John Baptist Nzukizi Mudumbi, Thomas Farrar and Tandi E. Matsha; Funding acquisition, John Baptist Nzukizi Mudumbi, Seteno Karabo Obed Ntwampe and Tandi E. Matsha; Investigation, John Baptist Nzukizi Mudumbi; Methodology, John Baptist Nzukizi Mudumbi, Adegbenro Peter Daso, Justine Oma Angadam and Tandi E. Matsha; Project administration, Seteno Karabo Obed Ntwampe; Resources, Seteno Karabo Obed Ntwampe, Okechukwu Jonathan Okonkwo and Tandi E. Matsha; Software, Okechukwu Jonathan Okonkwo, Thomas Farrar and Tandi E. Matsha; Supervision, Seteno Karabo Obed Ntwampe and Tandi E. Matsha; Validation, Seteno Karabo Obed Ntwampe, Adegbenro Peter Daso, Okechukwu Jonathan Okonkwo, Elizabeth Ife Omodanisi, Thomas Farrar, Lukhanyo Mekuto, Elie Fereche Itoba-Tombo, Justine Oma Angadam and Tandi E. Matsha; Writing – original draft, John Baptist Nzukizi Mudumbi; Writing – review & editing, Seteno Karabo Obed Ntwampe, Adegbenro Peter Daso, Elizabeth Ife Omodanisi, Thomas Farrar, Lukhanyo Mekuto, Elie Fereche Itoba-Tombo, Justine Oma Angadam and Tandi E. Matsha.

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Conflicts of Interest: The authors declare no conflict of interest.

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CHAPTER 7

Overall Discussion and Concluding remarks

7.1 Overall discussion

In this study, the African marigold (*Tagetes erecta* L.), a South African well known medicinal plant that belongs to the *Asteraceae* plant family was found to accumulate perfluoroalkyl substances (PFASs), that is, PFOA, PFBS and PFOS. In certain cases, concentration levels of PFOA and PFBS were found to be higher compared to previous studies in other countries. It is worth indicating that, of the three investigated PFASs, two (i.e. PFOA and PFOS) are known as long-chains or "C_8-chain" PFASs, while PFBS is a short-chain PFAS. Long-chain PFASs have dominated most investigations due to unprecedented reports on their impacts on human health and their bioaccumulative nature in the environment at large. Subsequently, substitutes and/or alternatives to long-chain PFASs were needed, a need which prompted the manufacturing of "harmless" PFASs, the short-chain ones (according to available evidence), including perfluorobutane sulfonate (C_4, PFBS) and perfluorohexane sulfonic acid (C_6, PFHxS), which are regarded as some of the most important short-chain PFASs in existence. Nonetheless, recently, short-chain PFASs that were previously regarded as less harmful have now been proven to be as unsafe as their analogues or long-chain PFASs, by countless scientific literature.

Moreover, these compounds, that is, PFOA, PFBS and PFOS, were investigated in known contaminated river water and in *Tagetes erecta* L. (irrigated with polluted water and grown under laboratory conditions), as well as in serum samples from diabetes sufferers. In river water, PFOA, PFBS and PFOS were found in concentrations of up to 107, 20.75 and 0.12 ng/L, respectively. In plant (*Tagetes erecta* L.) samples, concentrations of PFOA, PFBS and PFOS were, 94, 1.44 and 5.03 ng/g, respectively. In serum samples, PFOA, PFBS and PFOS were observed in all the samples and were found in concentrations up to 9.43, 1.66 and 1 ng/L, respectively; thus making PFBS the second most abundant PFAS in this current study, as far as river water and serum samples are

concerned. For plant samples, PFOS had the highest BCF (167) in this study. These results indicate that there is a potential link between contaminated water to serve as carrier to harmful substances into crops and/or plants, including medicinal plants. The latter will ultimately lead to the uptake of these substances by humans through direct ingestion of these plants for therapeutic purposes, for instance, as it has been the case for *Tagetes erecta* L., used in South Africa for diabetes management, and as presented in the current study. This further suggests that humans who are subjected to any medicinal plant not adequately monitored for its PFASs content or accumulation, are at risk of increased PFASs accumulation as a result of consuming contaminated plants.

In the present study, the correlation between the analysed PFASs and diabetes *mellitus* (DM) was studied. However, a link between the investigated PFASs and DM failed to be substantiated as no significant correlations were found between these PFASs and the possibility of developing DM. However, these findings remain inconclusive due to certain inconsistencies, coupled with a number of limitations which were observed, and which might have reduced the effectiveness of the analysis of the present results, thus implying that more research is required.

Furthermore, evidence of long-chain PFASs, such as PFOA and PFOS, prevalence in the general environment, as well as their potential lead to the development of DM is available. However, this has not been the case as far as the substitutes of these long-chain PFASs, are concerned. Subsequently, to our knowledge, the uptake of these substitutes, including PFHxS, perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA) and perfluorobutyl (PFBPA) by crops, such as medicinal plants, and ultimately, their association to primary predictors (e.g. overweight and obesity) of DM are generally limited worldwide, and particularly in a South African context. However, in light of the inconsistencies and contradictions that the present study has highlighted, it is thus clear that studies investigating the prevalence of short-chain PFASs in the general environment (e.g. surface water), as well as the susceptibility of medicinal plants to these particular substances, that is short-chain PFASs, are long overdue.

7.2 Overall concluding remarks

Water and plants share an undoubted bond driven by how the ecosystem functions. But most importantly, water is a necessity which all living organisms, including humans and plants, require for their survival. However, for decades, it has been proven that contaminated water, either surface water or groundwater, will ultimately contaminate the land and thus the plants

which have been exposed to this contaminated water. This is because plants have the capacity to uptake contaminants through various mechanisms, such as root interception, diffusion, and mass flow, although other reports suggesting that these mechanisms were not conclusive in that regard. This inconclusiveness led to the discovery of plant proteins, the aquaporins (AQPs). These proteins have been reported to be more abundant in plant kingdoms than in mammalian species, and possess unique structural features which determine which pollutant size passes through which protein pore sizes. Hence, AQPs with smaller pore diameters translocate smaller molecules, while those with wide pores move larger molecules through the plant membrane cells.

Accordingly, today, it has abundantly been demonstrated that plants accumulate substances, including POPs such as PFASs (e.g. PFOA, PFOS and PFBS), as well as heavy metals (e.g. copper, manganese, iron, zinc, etc.) through these AQPs.

In the South African context, surface water and plants, in particular, have been proven to be susceptible to PFASs. Consequently, *Tagetes erecta* L., a South African medicinal plant is a typical used plant with a predisposition to accumulate both long and short-chain PFASs, such as PFOA, PFOS and PFBS. There is a cause for concern because there are more than 3000 of these substances that have been reported and documented globally, in the environment in general.

According to the literature reviewed, medicinal plants have played a significant role in the lives of several African households, especially those with low incomes and which, ultimately, are unable to afford themselves orthodox medicines in cases where treatments of certain ailments are required. However, scientific reports have indicated that the role played by these plants is at high risk of being comprised due to several contaminants that have polluted the natural environment. Similarly, there is available evidence that some diseases are the results of sufferers being exposed to these substances, including PFASs, through various pathways, such as direct or indirect ingestion.

Diabetes *mellitus* (DM) has been one of the diseases associated to the exposure of PFASs, including PFOA, PFOS and PFBS. This exposure has been reported to be through either water or food, an example being consuming contaminated crops and/or plants. Thus, like in various other populations in the world, the current study have found these three PFASs in diabetic serum samples taken from a Bellville south population, in the Western Cape, in South Africa, regardless of the absence of a significant correlation between these substances and DM, in the studied population. The results remain worrisome though, because DM has killed millions worldwide,

with no cure available to date. And similarly, DM has been one of the leading causes of death in South Africa, in general.

Consequently, the present research results represent the first study on PFOA, PFOS and PFBS contamination in the South African context. Further scientific scrutiny is warranted to quantify risk contamination of the South African environment in general, and its population in particular, by not only long-chain PFASs, but also their counterparts, short-chain PFASs; and investigate further whether or not these substances, in particular short-chain PFASs, are an independent risk factor for the contamination of any other medicinal plant, and for Diabetes *mellitus* (DM) development.

7.3 Recommendations

The present research study reported on a medicinal plant as a potential source of Polyfluoroalkyl substances intake in South Africa. Nevertheless, there are aspects that still require to be addressed in order for this research topic to be adequately covered, and they include the following:

- The types of Aquaporins (AQPs) present in the studied plant, that is *Tagetes erecta* L., should be identified.
- The profiling of other short-chain PFASs or long-chain PFASs substitutes is required.
- Further research is needed to elucidate the concentration of substitutes to long-chain PFASs in people suffering from Diabetes *mellitus* (DM).
- Potential short-chain PFAS sources in South Africa should be appraised.
- The prevalence of short-chain PFASs in other regions of South Africa should be profiled.
- The concentration levels of other PFASs in additional South African medicinal plants should be profiled.
- The prevalence of PFASs in agricultural products, such as honey, should also be assessed.

APPENDICES

Supplementary Materials: Recent developments in polyfluoroalkyl compounds research: a focus on human/environmental health impact, suggested substitutes and removal strategies

Table S1: Overview of major uses of polymeric polyfluoroalkyl compounds

Industry sector	Polymers		Reference
Automotive	Raw materials for components such as low-friction bearings & seals	Lubricants	Smarts et al. 1994; Kutz 2011
Aviation, aerospace & defence	Insulators; "solder sleeves"		OECD 2013
Cable & wiring	Coating for weathering, flame and soil resistance	Surface-treatment agent for conserving landmarks	Smarts et al. 1994; Kutz 2011
Construction	Coating of architectural materials (fabrics, metals, stone, tiles, etc.); additives in paints		Smarts et al. 1994

Table S1. (*Continued*)

Electronics	Insulators; "solder sleeves";		vapour-phase soldering media	Kleine and Jho 2009; Kutz 2011; Carlson and Schmiegel 2000
Energy	Film to cover solar collectors due to weather ability			Smarts et al. 1994
Fire-fighting	Raw materials for fire-fighting equipment, including protective clothing	fuel repellents for FP & foam stabilizers in AR-AFFF and FFFP;7coating for fire-fighting equipment		Kleine and Jho 2009
Food processing	fabrication materials			Kutz 2011
Household products	non-stick coating			Kutz 2011
Medical articles	surgical patches cardiovascular grafts; raw materials for implants in the human body	stain- and water-repellents for surgical drapes and gowns		Kutz 2011; OECD 2013

Table S1. (*Continued*)

Paper and packaging		Oil and grease repellent	Oil and grease repellent	OECD 2013
Semiconductors	Raw materials for equipment		Working fluids in mechanical vacuum pumps	Smarts et al. 1994; OECD 2013
Textiles, leather and Apparel	Raw materials for highly porous fabrics	Oil and water repellent and stain release	Oil and water repellents	OECD 2013

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Table S2: Overview of major uses of non-polymeric polyfluoroalkyl compounds

Industry sector	Non-polymers			Reference
Aviation, aerospace & defence	Additives in aviation hydraulic fluids			SCPOP 2012
Biocides		Active ingredient in plant growth regulators or ant baits; enhancers in pesticide formulations		SCPOP 2011, 2012
Construction products		Additives in paints and coatings	Additives in paints and coatings	OECD 2013
Electronics	Flame retardants			Miteni 2016
Fire-fighting		Film formers in AFFF	Film formers in AFFF and FFFP	Kleiner and Jho 2009

Table S2. (*Continued*)

Household products	Wetting agent in floor polishes	Wetting agent or surfactant in products such as floor polishes and cleaning agents	Wetting agent or surfactant in products such as floor polishes and cleaning agents	OECD 2013
Metal plating	Wetting agent, mist suppressing agent	Wetting agent, mist suppressing agent	Wetting agent, mist suppressing agent	SCPOP 2012; OECD 2013
Oil and mining production	Surfactants in oil well stimulation	Surfactants in oil well stimulation	Surfactants in oil well stimulation	SCPOP 2012; OECD 2013
Polymerization	(emulsion) polymerization processing aids	(co)monomer of side-chain fluorinated polymers	(co)monomer of fluoropolymers & side-chain fluorinated polymers	Smarts et al. 1994; Kutz 2011; OECD 2013

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Table S3: Examples of fluorinated compounds that can potentially degrade into PFCAs (OECD 2007)

Compound Functional group	CAS No.	Chemical Name	*n*
Perfluoro alcohol compounds	2378-02-1	Perfluoro-tert-butyl alcohol	4
	6189-00-0	3-Pentanol, 1,1,1,2,2,4,4,5,5,5-decafluoro-3-(pentafluoroethyl)	7
Perfluoro amine compounds	311-89-7	1-Butanamine, 1,1,2,2,3,3,4,4,4-nonafluoro-N,N-bis(nonafluorobutyl)	5
	90622-99-4	Amides, C7-19, α-ω-perfluoro-N, N-bis (hydroxyethyl)	7-9
Perfluoro carboxylic compounds	307-55-1	Undecafluorohexanoic acid	5
	307-55-1	Tricosafluorododecanoic acid	11
	72623-77-9	Fatty acids, C 6-18 , perfluoro, ammonium salts	5-17
Perfluoro ester compounds	85681-64-7	2-Propenoic acid, perfluoro-C8-16-alkyl esters	8-16
	125328-29-2	2-Propenoic acid, 2-methyl-, C10-16-alkyl esters, polymers with 2-hydroxyethyl methacrylate, Me methacrylate and perfluoro-C8-14-alkyl acrylate	8-14

Table S3. *(Continued)*

Perfluoro ether compounds	335-36-4	Furan, 2,2,3,3,4,4,5-heptafluorotetrahydro-5-(nonafluorobutyl)	8
	68155-54-4	2H-Pyran, 2,2,3,3,4,4,5,5,6-nonafluorotetrahydro-6-(nonadecafluorononyl)	14
	297730-93-9	Hexane, 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)	7
Perfluoro iodide compounds	307-50-6	Undecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-tricosafluoro-11-iodo	11
	307-63-1	Tetradecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13, 14,14-nonacosafluoro-14-iodo	14
Perfluoro phosphonic/phosphinic compounds	68412-68-0	Phosphonic acid, perfluoro-C6-12-alkyl derives. Phosphonic acid, perfluoro-C6-12-alkyl derivatives (AICS)	6-12
	68412-69-1	Phosphinic acid, bis(perfluoro-C6-12-alkyl) derivatives	6-12

Table S3. *(Continued)*

Partial perfluoro and miscellaneous perfluoro compounds	76-21-1	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononan-1-oic acid	8
	307-43-7	1-bromohenicosafluorodecane	10
Fluoro alcohol compounds	307-30-2	1-Octanol, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-	7
		2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctan-1-ol	
	865-86-1	1-Dodecanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heneicosafluoro-	10
	65104-65-6	1-Eicosanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,	18
		16,16,17,17,18,18,19,19,20,20,20-heptatriacontafluoro-	
Fluoro ammonium compounds	31841-41-5	1-Decanaminium, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-N,N-bis(2-hydroxyethyl)-N-methyl-, iodide	8
	115535-36-9	Quaternary ammonium compounds, trimethyl(δ-ω-perfluoro-C8-14-β-alkenyl), chlorides	5-11

Table S3. *(Continued)*

Fluoro amine compounds	70969-47-0	Thiols, C8-20, γ-ω-perfluoro, telomers with acrylamide	6-18
	97660-44-1	Ethanol, 2-(methylamino)-, N-(γ-ω-perfluoro-C8-14-β-alkenyl) derives.	6-12
Fluoro carboxylic compounds	376-50-1	Hexanedioic acid, octafluoro-, diethyl ester	4
	37881-62-2	Octafluoroadipoyl difluoride	4
	238420-80-9	Propanedioic acid, mono(γ-ω-perfluoro-C8-12-alkyl)erives., bis[4-(ethenyloxy) butyl] esters	6-10
Fluoro ester compounds	307-98-2	2-Propenoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyl ester	7
	1799-84-4	2-Propenoic acid, 2-methyl-, 3,3,4,4,5,5,6,6,6-nonafluorohexyl ester	4
	1996-88-9	2-Propenoic acid, 2-methyl-, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester	8

Table S3. *(Continued)*

Fluoro ether compounds	38565-52-5	Oxirane, (2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptyl)-	6
	52584-45-9	Benzenesulfonic acid, 4-[[4,4,5,5,5-pentafluoro-3-(pentafluoroethyl)-1,2,3-tris(trifluoromethyl)-1-pentenyl]oxy]-, sodium salt	10
	68877-51-0	Poly(oxy-1,2-ethanediyl), α-[1,4,4,5,5,5-hexafluoro-1,2,3-tris(trifluoromethyl)-2-pentenyl]- ω-methoxy-	8
Fluoro iodide compounds	375-50-8	1,1,2,2,3,3,4,4-octafluoro-1,4-diiodobutane	4
	2043-54-1	Dodecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heneicosafluoro-12-iodo-	10
	30046-31-2	Tetradecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-pentacosafluoro-14-iodo-	12
	65104-63-4	icosane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18-heptatriacontafluoro-20-iodo-	18

Table S3. *(Continued)*

Fluoro phosphate compounds	1895-26-7	bis[3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12 henicosafluorododecyl] hydrogen phosphate	10
	54009-73-3	,4,5,5,6,6,7,7,8,8,9,9,10,11,11,11-hexadecafluoro-2-hydroxy-10-(trifluoromethyl) undecyl dihydrogen phosphate	9
	57677-98-2	bis[3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluorododecyl] hydrogen phosphate, compound with 2,2'-iminodiethanol	10
Fluoro sulfate compounds	68516-17-6	Sulfuric acid, mono(γ-ω-perfluoro-C_{6-12} -alkyl) esters, ammonium salts	4-10
	84238-62-0	Sulfuric acid, mono(γ-ω-perfluoro-C_{8-12} -alkyl) esters, ammonium salts	6-12
	85995-90-0	Sulfuric acid, mono(γ-ω-perfluoro-C_{8-14}-alkyl) esters	6-12
Fluoroalkyl silicate compounds	170424-64-3	Siloxanes and Silicones, hydroxy Me, Me octyl, Me (γ-ω-perfluoro C8-14-alkyl) oxy, ethers with polyethylene glycol mono-Me ether	6-12
	182700-77-2	Siloxanes and silicones, di-Me, hydroxy-terminated, polymers with tetradecanedioic acid,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13, 13-tricosafluoro-1-tridecanol-terminated	11

Table S3. *(Continued)*

Fluoro sulfonate /sulfonamide /sulfonyl compounds	27607-61-0	1-Nonanesulfonyl chloride, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluoro-	7
	27619-89-2	1-Octanesulfonyl chloride, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-	6
	27619-91-6	1-Dodecanesulfonyl chloride, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11, 12,12,12-heneicosafluoro- (	10
	297175-71-4	Sulfonic acids, C_{8-20}-alkane, γ-ω-perfluoro, compds. With triethylamine	6-18
	91770-74-0	Sulfonyl fluorides, C1-5-alkane, ω-(ethenyloxy), perfluoro	1-5
Fluoro siloxanes /silicone/silane compounds	78560-44-8	Silane, trichloro(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl)-	8
	78560-45-9	Trichloro(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)silane	6
	160965-19-5	Poly [2-perfluoroalkyl (C 4-8) ethylsiloxane]	4-8
Fluoro thiols compounds	68140-18-1	Thiols, C4-10, γ-ω-perfluoro	2-8
	68140-19-2	Thiols, C4-20, γ-ω-perfluoro	2-18
	68140-21-6	Thiols, C10-20, γ-ω-perfluoro	8-18

Table S3. *(Continued)*

Fluoro thioether compounds	53122-42-2	Carbamic acid, [4-methyl-3-[[(2-methyl-1-aziridinyl)carbonyl]amino]phenyl]-, 2-[[3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 9, 10,10,10- hexadecafluoro-9-(trifluoromethyl) decyl]thio]-1-[[[3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,10-hexadecafluoro-9-(trifluoromethyl)decyl] thio]methyl]ethyl ester	9
	68187-24-6	1,4-Butanediol, 2,3-bis[(γ-ω-perfluoro-C6-20-alkyl)thio] derives	4-18
Fluoro thioester compounds	28506-33-4	2-Propenethioic acid, 2-methyl-, S-[3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,10-hexadecafluoro-9-(trifluoromethyl)decyl] ester	9
	30769-88-1	2-Propenethioic acid, 2-methyl-, S-[3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,12,12,12-eicosafluoro-11-(trifluoromethyl)dodecyl] ester	11
	30769-91-6	2-Propenethioic acid, 2-methyl-, S-[3,3,4,4,5,5,6,6,7,8,8,8-dodecafluoro-7-(trifluoromethyl)octyl] ester	7
	113089-67-1	Thiols, C4-20, γ-ω-perfluoro, reaction products with methylated formaldehyde-1,3,5-triazine-2,4,6-triamine polymer	2-18

Table S3. *(Continued)*

Fluoro urethane compounds	68990-40-9	Fatty acids, C18-unsatd., dimers, diisocyanates, polymers with 2,3-bis(γ-ω-perfluoro-C4-18-alkyl)-1,4-butanediol, 1,6-diisocyanato-2,2,4(or 2,4,4)-trimethylhexane and 2,2'-(methylimino)bis[ethanol]	2-16
	95370-51-7	Carbamic acid, [2-(sulfothio)ethyl]-, C-(γ-ω-perfluoro-C6-9-alkyl) esters, monosodium salts	4-7
Partial fluoro & miscellaneous fluoro compounds	307-70-0	1-Undecanol, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-eicosafluoro-	10
	47795-34-6	[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,11,11,11-icosafluoro-10-(trifluoromethyl) undecyl] oxirane	11
	54009-77-7	[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,15,15,15-octacosafluoro-14-(trifluoromethyl)pentadecyl]oxirane	15
	54009-78-8	[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,13,13,13-tetracosafluoro-12-(trifluoromethyl)tridecyl]oxirane	13
	54009-79-9	[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,17,17,17-dotriacontafluoro-16-(trifluoromethyl)heptadecyl]oxirane	17

n: Length of the perfluorinated carbon chain

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Table S4: Examples of biomagnification factor (BMF) values of PFCs in selected aquatic organisms (Ding and Peijnenburg 2013)

Organism	Substance/ BMF								Reference
	PFOA	PFOS	PFOSA	PFNA	PFDA	PFHxS	PFUnA	PFDoA	
$Seatrout_{whole}/Pinfish_{whole}$	7.2	4.6	24	1.5	3.7	nc	0.9	0.1	Houde et al. 2006
$Dolphin_{whole}/Striped\ mullet_{whole}$	13	2.6	8.3	5	2.9	4	1.9	0.2	Houde et al. 2006
$Dolphin_{whole}/Spotfish_{whole}$	6.4	0.8	4.4	4.6	2.8	6	3.9	0.6	Houde et al. 2006
$Dolphin_{whole}/Red\ drum_{whole}$	2.7	1.2	3.4	1.4	2.4	14	3.2	0.4	Houde et al. 2006
Glaucous gull/Polar cod	–	38.7	–	11.6	–	7.20	–	–	Haukås et al. 2007
$Striped\ mullet_{whole}$ $/Zooplankton_{whole}$	–	23	2.5	–	–	nc	–	89	Houde et al. 2006; Haukås et al. 2007
$Dolphin_{whole}/Atlantic\ croaker_{whole}$	2.3	2.2	1.5	24	2.5	nc	2.1	1.8	Houde et al. 2006
Common mergansers/fish	–	8.9	–	–	–	–	–	–	Sinclair et al. 2006

Table S4. (*Continued*)

Glaucous gull/Black guillemot	–	27.0	–	9.34	–	8.49	–	–	Haukås et al. 2007
$Dolphin_{whole}/Sheephead_{whole}$	–	16	–	–	–	–	–	–	Houde et al. 2006
Black guillemot/Mixed diet	–	5.66	–	–	–	–	–	–	Haukås et al. 2007
Black guillemot/Ice amphipod		1.54	12	–	–	–	–	–	Haukås et al. 2007
$Dolphin_{whole}/seatrout_{whole}$	1.8	0.9	1.3	2.1	2.4	3.3	2.5	0.6	Houde et al. 2006
$Pigfish_{whole}/Zooplankton_{whole}$	–	12	nc	–	–	9.1	–	2.5	Houde et al. 2006

nc: not calculated

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Table S5: Toxicological results of reproductive effects in Humans Exposed to Perfluorinated substances (Stahl et al. 2011; ATSDR 2015)

Significant effects	Population group	Origin	End point	Reference
Birth weight (b.w.)	Exposed women	USA	No correlation between extent of PFOS exposure and b.w	Grice et al. 2007
	General population	Japan	No correlation between PFOS concentration in cord blood and b.w.	Inoue et al. 2004
	General population	Danish	Correlation between the PFOA concentration in mother's plasma and b.w; not detectable for PFOS	Fei et al. 2007
	General population	USA	Weak inverse correlation between concentrations of PFOS and PFOA in cord blood and b.w.	Apelberg et al. 2007
	General population	Canada	No correlation of PFC serum concentrations and b.w.	Monroy et al. 2008
	General population	Japan,	Negative correlation of *in utero* exposure to PFOS b.w.; not detectable for PFOA	Washino et al. 2009
	General population	USA	No indication of a connection between low b.w. and PFOA-contaminated drinking water	Nolan et al. 2009
	General population	USA	Correlation between PFOS contamination and the risk of reduced b.w.	Stein et al. 2009
	General population	Canada	No correlation between PFOA, PFHxS, PFOS serum concentrations and b.w.	Hamm et al. 2009

Table S5. (*Continued*)

Gestation time	General population	Danish	No correlation of PFOA and PFOS concentrations in mother's plasma with time of gestation	Fei et al. 2007
	General population	USA	No indication of premature birth as a result of PFOA contamination via drinking water	Nolan et al. 2009
	General population	USA	No connection of PFOS or PFOA serum concentration with miscarriage or premature birth	Stein et al. 2009
	General population	Canada	No correlation between PFOA, PFHxS, PFOS serum concentrations and gestation time	Hamm et al. 2009
Development	General population	Danish	No difference in the development of new-borns from mothers with high PFOA and PFOS concentrations and children of mothers with low PFOA and PFOS concentrations; sitting without support possibly delayed in children of mothers with high PFOS concentrations	Fei et al. 2008
Fertility	General population	Danish	Fertility disorders related to elevated PFOA and PFOS plasma concentrations	Fei et al. 2009

Table S5. (*Continued*)

Other aspects	General population	USA	Weak inverse correlation between concentrations of PFOS and PFOA in cord blood and the ponderable index or head circumference	Apelberg et al. 2007
	General population	Japan	No correlation between PFOS concentration in cord blood and concentration of thyroid hormones	Inoue et al. 2004
	General population	USA	Weak correlation of PFOA concentrations and occurrence of miscarriages	Stein et al. 2009
	General population	USA	Weak association of PFOA and PFOS serum concentrations with the occurrence of preeclampsia	Stein et al. 2009
	General population		Increased risk of ADHD for children with elevated PFOS, PFOA, PFHxA, and PFNA serum concentrations	Hoffman et al. 2010
	Women	USA	PFOS negatively associated with estradiol concentration in perimenopausal and menopausal groups; no significant association for PFOA	Knox et al. 2011
			Odds of endometriosis diagnosis positively associated with serum PFOA and PFNA, but only with unadjusted model for PFOS No significant association with PFHxS	Louis et al. 2012

Table S5. (*Continued*)

Other aspects	Men	Denmark	Negative association between PFOS and testosterone, free testosterone, free androgen index, testosterone/luteinizing hormone ratio, free androgen/luteinizing hormone ratio Negative association between PFHpS and the % of progressively motile sperm No other significant associations between PFCs and reproductive hormones or sperm parameters observed	Joensen et al. 2013
		USA	Serum PFOA correlated with free testosterone and luteinizing hormone levels No significant associations between sperm parameters and PFOS or PFOA levels or between PFOS and reproductive hormone levels	Raymer et al. 2012

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Table S6: Summary comparison of different techniques used in certain studies for PFCs removal

Study	Used technique	Substance	Removal ratio (%)	Further brief discussions
Bao et al. 2014	Coagulation	PFOA	~47.6%	Ratio were ▲ under acidic conditions as by Arvanit et al. (2015) recently in ZVI, but ▼ (i.e. ~12% and 32% when $FeCl_3.6H_2O$ was added as the coagulant
		PFOS	94.7%	
Xiao et al. 2013	Adsorption and Coagulation	PFOA	≤ 20%	At Alum dosage of 10—60 mg/L and final pH of 6.5–8.0, removal was ▼. Removal was enhanced by increasing the alum dosage (> 60 mg/L), and thus a 10% ↑ was achieved
		PFOS		
Du et al. 2016	Adsorption and degradation	PFOS	93.3%	This study is regarded as the highest efficien adsorption and degradation of PFOS and F53B in wastewater treatment
		F53B	97.6%	
Huang et al.2016	Photoinduced hydrode fluorination	PFOA	58.5%	Various SiC/graphene dosages were determined. Decomposition efficiencies of PFOA with 0.1 g L^{-1}, 0.25 g L^{-1}, 0.5 g L^{-1}, 0.75 g L^{-1}, and 1.0 g L^{-1} SiC/graphene were 40.5%, 45.3%, 58.5%, 51.4%, and 44.4%, respectively.

Table S6. (*Continued*)

				The technique was regarded as another insight in th decomposition of PFCs.
Lin et al. 2012	Electrochemical degradation	PFOA	98.8%	Different conditions played significant roles. Fo instance, A low PFOA degradation efficiency wa observed at high pH value, while PFOA significantly ↑ with ↑ current density. Plate distance also had a effect on the substance. Hence, the degradation ratio of PFOA were 95.9%, 90.3%, 78.0% and 68.9% for th plate distances of 0.5, 1.0, 1.5 and 2.0 cm, respectively
Niu et al. 2012	Electrodeposition technology	PFBA	31.8%	The results from this study demonstrated that PFC chain length appeared to have a significant effect o the observed degradation, on the basis that th treatment capacity of some these substances (e.g. 6.5 mg h^{-1} for PFHpA) was much higher than others (e.g 2.1 mg h^{-1} for PFBA)
		PFPeA	41.4%	
		PFHxA	78.2%	
		PFHpA	97.9%	
		PFOA	96.7%	

Table S6. (*Continued*)

Niu et al. 2013	Electrochemical mineralization mechanism	PFOA	>98%	The results obtained in this study constitute a breakthrough information which the authors believe can be used as an instrument for a comprehensive understanding of the mineralization of PFOA in the electrolysis system. TOC removal ratio was slightly lower (i.e. 94.3%) than the PFOA degradation, thus implying that only a portion of the intermediates had accumulated in bulk solution (Niu et al. 2013). Additionally, short-chain PFC was not be detected.
Dai et al. 2013	Adsorption	PFOS	>75%	Multi-walled carbon nanotube (MWCNT) and electrospun nanofibrous membranes (ENFMs) were prepared by means of electrospinning. The sorption isotherms showed that the maximum adsorption capacities of PFOS onto the pure ENFMs was ▼ (i.e. 0.92 ± 0.06 μmol g^{-1}), but ▲ (16.29 ± 0.26 μmol g^{-1}) with MWCNT-ENFMs.

Table S6. (*Continued*)

				The results thus suggest that the combination o MWCNT-ENFMs are promising sorbents for PFOS removal, even though it was clear that pH led to a significant effect on PFOS sorption, which efficiencie ↓ with the ↑ solution pH.
Lin et al. 2013	Electrochemical mineralization	PFNA	98.7%	The results were achieved in aqueous solutions (0.2 mmol L^{-1}) over anodes, including SnO_2, PbO_2, and BDD. However, it has been indicated that SnO electrode yielded ▼ PFCA removals, and secondary pollution due to Sb ions was noticed, suggesting a risk assessment of used anodes during the treatmen process is paramount.
		PFDA	96.0%	
Lin et al. 2015	Electrocoagulation (EC)	PFOA	98.7%	It is reported in this study that coagulation processe led to aluminium hydroxide flocs or polyaluminum chloride, which ultimately was ineffective in removing the substances, i.e. PFOA/PFOS.

Table S6. (*Continued*)

				Hence, the removal was attributed to suspended solids, in consistency with what was previously suggested by Deng et al. (2011).
Yang et al. 2016	EC	PFOA	99%	In this study various parameters, such as current density, initial aqueous pH, etc. were probed to improve the EC. Fe anode demonstrated the highest PFOA removal efficiency. This removal achievement is relatively closer to that previously reported by Lin et al. (2015).

▲: high/higher; ▼: low/lower; ↑: increases/increased; ↓: decreases/decreased

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Supplementary Materials: Are aquaporins (AQPs) the Gateway that Conduits Nutrients, Persistent Organic Pollutants and Perfluoroalkyl Substances (PFASs) into plants?

Table S1: Primary uptake mechanisms in nutrient/element transport to roots (Walters 2011; Pagani et al. 2013)

Nutrient/element	Root interception	Mass flow	Diffusion	Ionic forms	Mobile (+) / Immobile (-)
Nitrogen (*N*)		■		NO_3^-(nitrate), NH_4^+(ammonium)	+
Phosphorus (*P*)	■		■	K^+	+
Potassium (*K*)			■	$H2PO_4^-$, HPO_4^{2-}(phosphate)	+
Calcium (*Ca*)		■	n/s	Ca^{+2}	-
Chlorine (*Cl*)	n/s	n/s	n/s	Cl^-(chloride)	+
Magnesium (*Mg*)		■		Mg^{+2}	+
Sulfur (*S*)		■		SO_4^{2-}(sulfate)	-
Manganese (*Mn*)			■	Mn^{+2}	-
Zinc (*Zn*)			■	Zn^{+2}	-
Molybdenum (*Mo*)	n/s	n/s	n/s	MoO_4^{2-}(molybdate)	+
Nickel (*Ni*)	n/s	n/s	n/s	Ni^{+2}	-

Table S1 continued

Iron (*Fe*)	■	■	Fe^{+2} (ferrous), Fe^{+3} (ferric)	-
Copper (*Cu*)	■		Cu^{+2}	-
Boron (*B*)		■	H_3BO_3 (boric acid), $H_2BO_3^-$(borate)	-

n/s: not specified

Comments

Persistent organic pollutants: soil to root movement

The movement and uptake of POPs and heavy metals throughout the soil profile to the root system consist of several stages: i) the balance between the compound concentration in the plant and the external environment; ii) the pollutant sorption on to lipophilic root solids (Briggs et al. 1983; Collins et al. 2013). Briggs et al. (1983) have also suggested that lipids present in plants' membranes and cell walls are a typical example of lipophilic solids in plants. In addition, studies by Duarte-Davidson and Jones (1996) and Wild et al. (1992) found higher levels of organic chemicals, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in plant roots, with lipophilic organic compounds demonstrating greater tendency to partition into the root's lipids than hydrophilic pollutants. Briggs et al. (1983) further reported a linear correlation between the octanol-water partition coefficient (K_{ow}) of non-ionised compounds and the observed root concentration factor (RCF). On the other hand, Bromilow and Chamberlain (1995) indicated that the differences in POP uptake potential can further be explained by the varying types and quantity of lipids present in the root cells. However, there are limited research studies available to demonstrate this, suggesting that further studies are required.

Organic pollutants movement from roots to plant compartments

The mechanism involved in organic pollutant movement resulted in the concept of a transpiration stream concentration factor (TSCF), which is the ratio of chemical concentration in the transpiration stream to the concentration found in an external solution (Shone and Wood, 1977; Collins et al. 2013). Hence, it is believed that, after the transport into the stem, water and solutes diffuse laterally into adjacent tissues and thus become concentrated in plant shoots, tubers and fruits (McFarlane 1995); although, Tangahu et al. (2011) suggested that data reporting on this aspect is very limited.

Furthermore, Collins et al. (2013) suggested that, this is a two-phase process which begins with the balance partitioning between water present in the plant vascular system and the aqueous solution in cell tissues, followed by sorption into the cell walls. Thus, a proportional linear partitioning for non-ionised organic compounds to plant stems was previously demonstrated by Briggs et al. (1983) and Barak et al. (1983). Hence, Collins et al. (2013) concluded that, the lipid composition in plant tissues is likely to be an important contributing factor in pollutant uptake and accumulation. On the other hand, Tangahu et al. (2011) have indicated that,

evapotranspiration, the process that influences water to evaporate from plant leaves, serves as a pump to absorb nutrients, pollutants and other soil substances into plant roots; and is thus responsible for moving contaminants into the plant shoots as well.

Nutrients and POPs uptake mechanisms by plants

Tangahu et al. (2011) have argued that crops have evolved highly specific mechanisms to translocate and store nutrients. Hence, these same mechanisms are suggested to also be involved in the uptake, translocation and storage of POPs in plants, depending on individual POP chemical properties, in comparison to those of essential nutrients that crops require to grow. Thus, numerous reports have indicated that nutrients as well as POPs movement in different types of soil can be known and correlated with the structure of the soil, nutrient absorption and mobility, uptake and mass flow in a form of diffusion, mechanisms which are largely responsible for the root uptake of individual nutrients (Walters 2011; Pagani et al. 2013; Schwartz 2015). For example, Su and Zhu (2007) reported the partition of PAHs in rice is dominated by sorption to the crop cell walls.

Overall, plant root systems play a pivotal role in the whole process of plant uptake of nutrients and POPs. Thus, roots absorb nutrients and toxicants depending on root affinity and the bioavailability of these pollutants; as they are the primary transportation systems for constituents in soil and anchor the plant thus furnish physical support to the stem, while serving as storage organs for the plant. They can also act as nutrient transformers, as most plants cannot form or transport some nutrients in their elementary form (Pagani et al. 2013). Thus, before a nutrient and/or POP ion can be absorbed by the plant, it must be in an appropriate form (Walters 2011; Pagani et al. 2013; Haun 2015). As such, three mechanisms have been mentioned as being facilitators of plants nutrients uptake from the soil; namely (i) *root interception*, (ii) *diffusion*, and (iii) *mass flow* (Walters 2011; Pagani et al. 2013; Haun 2015; Schwartz 2015). Table S1 summarizes the primary uptake mechanisms in nutrient transport to root systems. These mechanisms are herein suggested to be similar to those involved in POP uptake (Tangahu et al. 2011).

Structure of soil

Soil structure determines how nutrients and contaminants (e.g. POPs) get to the roots of plants. According to Schwartz (2015), soil compaction can decrease the capability of roots to move toward nutrient or pollutant sources, reducing the ability of water or pollutants to move through the soil to allow nutrients to reach the root system. Soil compaction has been defined as the physical consolidation of soil particles by an applied force that degrades structure, reducing its

porosity, and thus, limiting infiltration, as well as increasing resistance to root penetration, which ultimately results in the reduction of crop yield (Wolkowski and Lowery 2008; DeJong-Hughes 2009).

Nutrients and POPs absorption

The general concentration of nutrients and POPs within the soil has been argued to significantly influence their movement to the root system (Schwartz 2015). Unavoidably, the concentration of nutrients throughout the soil profile was indicated to be directly proportional to the opportunity of chemical constituent movement either as nutrient or POPS to the plant roots (Pagani et al. 2013; Schwartz 2015; Barker and Pilbeam 2015). Thus, Schwartz (2015) and Barker & Pilbeam (2015) have suggested that by monitoring the levels of the constituent and determining their prevalence throughout the season is essential for the estimation of bioaccumulation potential and for uptake. For instance, macronutrients such as phosphorus can be present in the soil as an orthophosphate ion (e.g. dihydrogen phosphate-$H_2PO_4^-$ or $H_2PO_4^{2-}$) but at very low concentrations; resulting in the intensity of its adsorption by the soil particles (Walters 2011). On the other hand, nitrogen sources are commonly found in much higher concentration levels in the soil (usually as nitrate-NO_3^-) and are very poorly adsorbed by soil particles, making this macronutrient available for uptake by plant roots. This will suggest that fertilizers some of which contain trace quantities of POPs, and are rich in phosphorus are suitable and must be placed very close to the seed to ensure effective availability; whereas, nitrogen can be applied over the surface of the soil where it can easily be washed down to plant roots (Walters 2011). A similar phenomenon can also be attributed to POPs, as different forms can occur in the soil resulting in differentiated uptakes.

Nutrients and POP mobility

Available research has indicated that chemical elements (i.e. nutrients and toxic elements) move relatively easily from the root to different plant compartments, in particular when plant growth is unrestricted (Pagani et al. 2013). Pagani et al. (2013) has reported that some absorbed soil constituents can also move from older tissue to newer tissue if there is a substantial differentiation in concentration of nutrients within the plants. Schwartz (2015) has also specified that the mobility varies or differs with different chemical constituents, with some being very mobile, thus suggesting, they can quickly move through the profile of the soil and reach plant

roots easily; while others are immobile, resulting in reduced diffusivity from older to newer plant tissue (Pagani et al. 2013).

Root interception or contact exchange

Nutrients as well as pollutants uptake and exchange by roots is directly proportional to the activity of the root, its ability to absorb both, and their concentration at the surface of the root (Walters 2011; Pagani et al. 2013; Haun 2015; Schwartz 2015). Thus, during root interception (contact exchange) root hairs and small roots growing throughout the soil profile come into direct contact with the soil, including organic matter particles containing either essential plant nutrients or pollutants (Walters 2011).

Furthermore, it has been argued that as the plant root system develops throughout the soil, it comes into direct contact with some available nutrients and POPs (Walters 2011; Pagani et al. 2013; Schwartz 2015). Accordingly, the role of the root interception process in plant nutrient and POP uptake mechanisms has been regarded as insignificant in Walters (2011) and Pagani et al. (2013), suggesting there could be other mechanisms that influence the movement of nutrients and POPs into the plant, (Pagani et al. 2013), with the profile of the soil structure influencing such mechanism (Schwartz 2015).

Mass flow translocation of nutrients and POPs

During the process of mass flow, it is understood that chemical constituents move or migrate to the roots via water (Pagani et al. 2013; Schwartz 2015), which facilitates the uptake of the nutrient (in ionic form) by the plant (Walters 2011; Pagani et al. 2013; Schwartz 2015). Mass flow accounts for a substantial quantity of nutrient and contaminant movement towards the plant root and will largely contribute to the mobility of chemical compounds (Pagani et al. 2013). Additionally, mass flow has been found to account for a large transfer of mobile constituents in soil (e.g. 80% of nitrogen-*N*) into the root system of plants when compared to immobile constituents (e.g. 5% of phosphorous-*P*). Thus, diffusion accounts for the remainder of the migration, thus constituting a mass flow limiting step (Pagani et al. 2013).

Translocation of nutrients and POPs by diffusion

Diffusion has been defined as the process where chemical constituents translocate or migrate from an area of high concentration to an area of low concentration (Walters 2011; Pagani et al. 2013). As the plant root system develops throughout the soil, coming into contact with

chemical elements/compounds, results in the direct contact around the root system, –with diffusion being influenced by the concentration of the constituents around the root. It has been reported that relatively immobile constituents are highly dependent on diffusion to facilitate their movement or migration into plant root systems (Pagani et al. 2013), which further suggested that if they are not exceedingly mobile, facilitation of their translocation will be dependent solely on the high concentration of nutrients and/or toxicants throughout the soil (Pagani et al. 2013; Schwartz 2015).

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Supplementary Materials: The role of pollutants in type 2 diabetes mellitus (T2DM) and their prospective impact on phytomedicinal treatment strategies

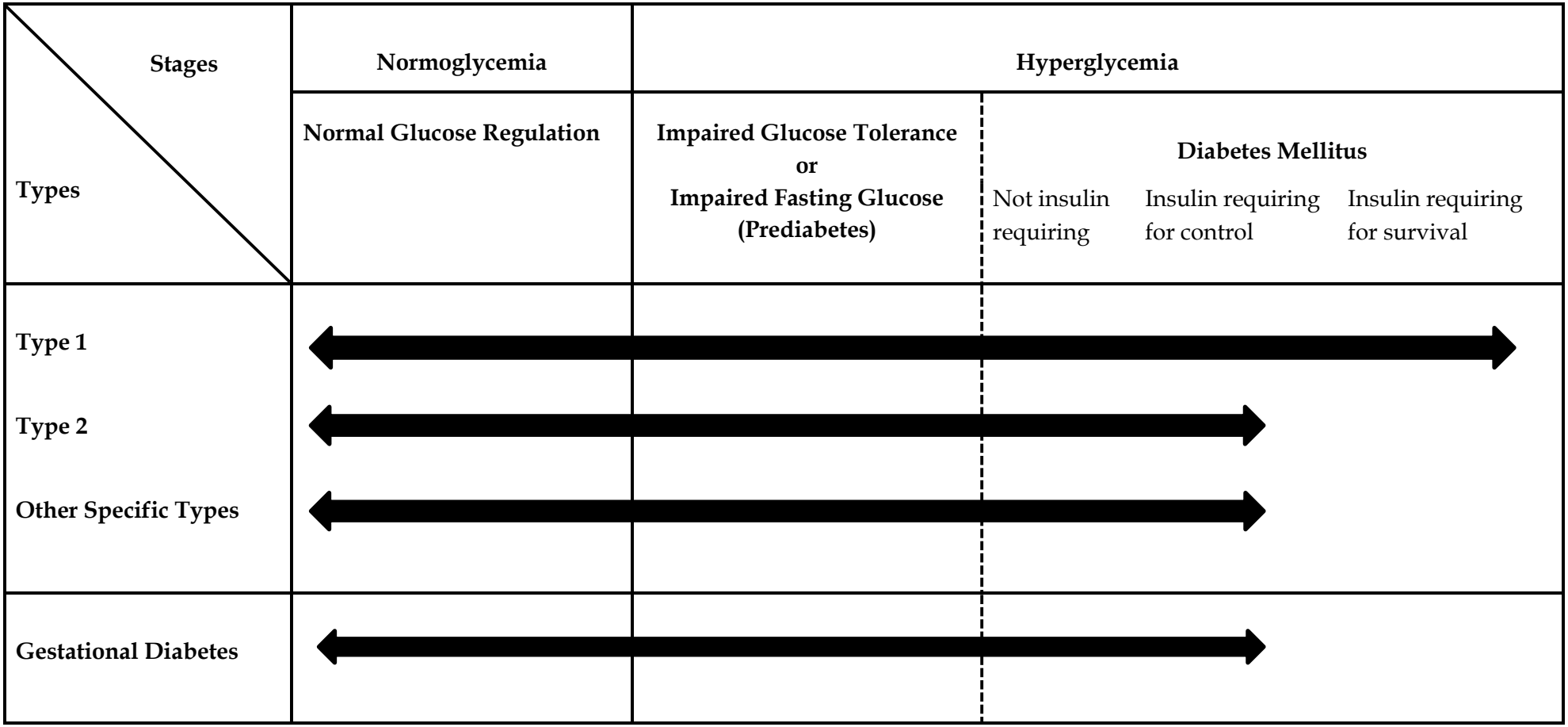

Figure S1: Disorders of glycaemia: etiological types and clinical stages (Alberti and Zimmet, 1998; WHO, 1999; ADA, 2014)

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Table S1: Etiological classification of DM (WHO, 1999; ADA, 2014)

Types		Descriptions
I.	***Type 1 diabetes***	(β -cell destruction, usually leading to absolute insulin deficiency) A. Immune mediated B. Idiopathic
II.	***Type 2 diabetes***	(may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III.	***Other specific types***	A. Genetic defects of β -cell function 1. MODY 3 (Chromosome 12, HNF-1α) 2. MODY 1 (Chromosome 20, HNF-4α) 3. MODY 2 (Chromosome 7, glucokinase) 4. Other very rare forms of MODY (e.g., MODY 4: Chromosome 13, insulin promoter factor-1; MODY 6: Chromosome 2, NeuroD1; MODY 7: Chromosome 9, carboxyl ester lipase) 5. Transient neonatal diabetes (most commonly ZAC/HYAMI imprinting defect on 6q24) 6. Permanent neonatal diabetes (most commonly KCNJ11 gene encoding Kir6.2 subunit of β-cell K_{ATP} channel) 7. Mitochondrial DNA 8. Others B. Genetic defects in insulin action 1. Type A insulin resistance 2. Leprechaunism 3. Rabson-Mendenhall syndrome 4. Lipoatrophic diabetes 5. Others

Table S1 (*Continued*)

C. Diseases of the exocrine pancreas
    1. Pancreatitis
    2. Trauma/pancreatectomy
    3. Neoplasia
    4. Cystic fibrosis
    5. Hemochromatosis
    6. Fibrocalculous pancreatopathy
    7. Others

D. Endocrinopathies
    1. Acromegaly
    2. Cushing ′ s syndrome
    3. Glucagonoma
    4. Pheochromocytoma
    5. Hyperthyroidism
    6. Somatostatinoma
    7. Aldosteronoma
    8. Others

E. Drug or chemical induced
    1. Vacor
    2. Pentamidine
    3. Nicotinic acid
    4. Glucocorticoids
    5. Thyroid hormone
    6. Diazoxide
    7. β -Adrenergic agonists
    8. Thiazides
    9. Dilantin
    10. γ-Interferon
    11. Others

Table S1 (*Continued*)

F. Infections
    1. Congenital rubella
    2. Cytomegalovirus
    3. Others
G. Infections
    1. Congenital rubella
    2. Cytomegalovirus
    3. Others
H. Infections
    1. Congenital rubella
    2. Cytomegalovirus
    3. Others
I. Uncommon forms of immune-mediated diabetes
    1. Stiff-man syndrome
    2. Anti-insulin receptor antibodies
    3. Others
J. Other genetic syndromes sometimes associated with diabetes
    1. Down syndrome
    2. Klinefelter syndrome
    3. Turner syndrome
    4. Wolfram syndrome
    5. Friedreich ataxia
    6. Huntington chorea
    7. Laurence-Moon-Biedl syndrome
    8. Myotonic dystrophy
    9. Porphyria
    10. Prader-Willi syndrome
    11. Others

Table S1 (*Continued*)

IV.	***Gestational diabetes mellitus***	Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient.

References

ADA, Association Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care. 37: S81-S90.

WHO, World Health Organisation Consultation, 1999. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. WHO/NCD/NCS/99.2. Available Online: https://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf. Accessed on 22 June 2017.

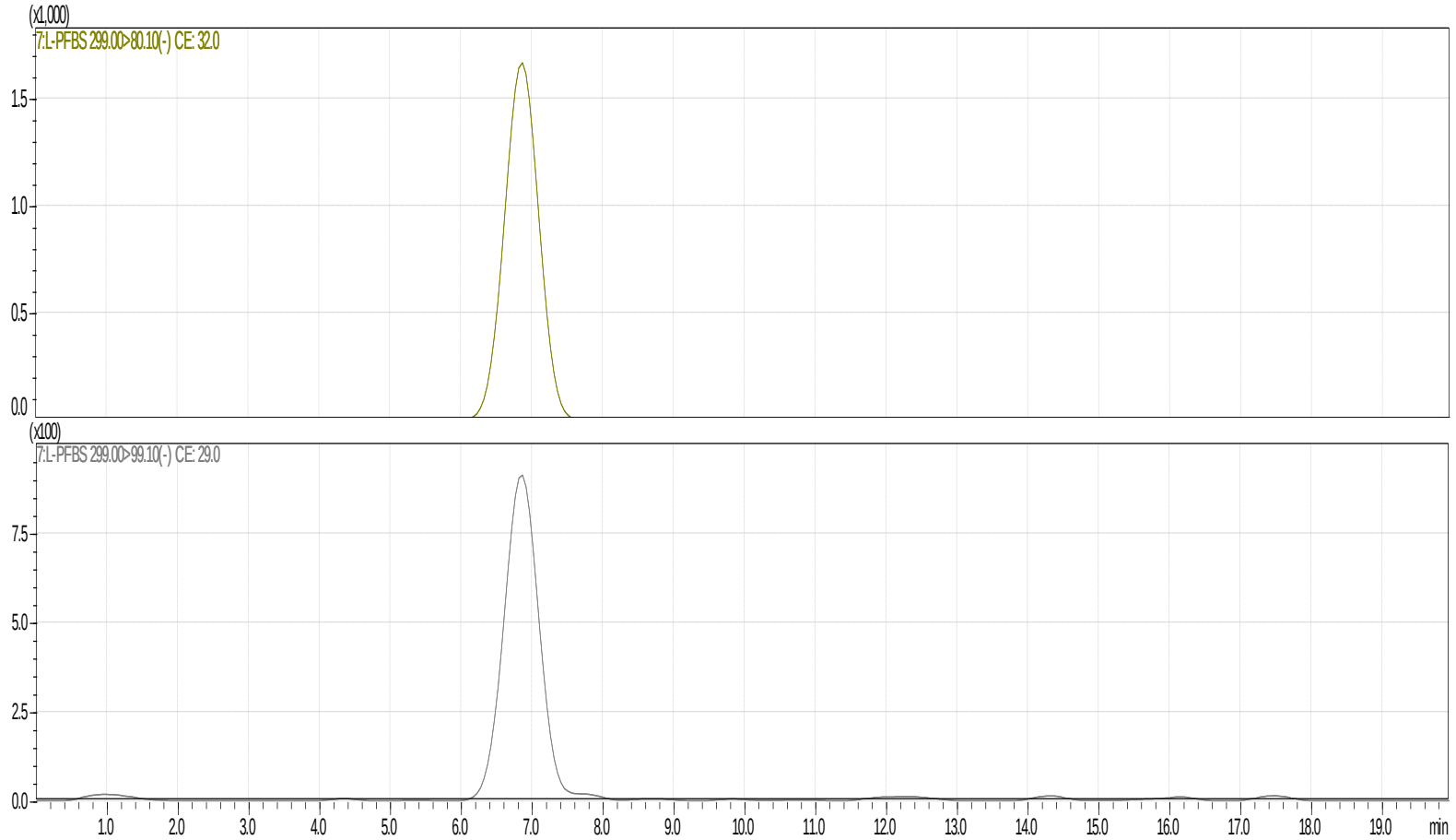
Supplementary Materials: Propensity of *Tagetes erecta* L., a Medicinal Plant Commonly Used in Diabetes Management, to Accumulate Perfluoroalkyl Substances

Table S1: Selected medicinal plants under possible threats by PFASs in South Africa [1].

Plant Species (Family)	Common or Vernacular Names	Compartments Used	References
Tagetes erecta (*Asteraceae*)	African marigold (Eng.)	Leaves and roots	This study, [2–7]
Sutherlandia frutescens (*Fabaceae*)	Cancer bush (Eng.)	Leaves, and often whole plant	[8–10]
Moringa oleifera (*Moringaceae*)	Makgonatˇsohle (Sipedi), drumstick tree (Eng.)	Seeds and leaves	[11]
Artemisia afra (*Asteraceae*)	African Wormwood (Eng.)	Leaves and roots	[8,12–14]
Cannabis sativa L. (*Cannabaceae*)	Dagga (Afr.)	Leaves	[15]
Aloe ferox Mill. (*Asphodelaceae*)	Cape Aloe or bitter Aloe (Eng.)	Leaves	[10,16–18]
Pelargonium sidoides (*Geraniaceae*)	Umckaloabo (Zulu)	Tubers and roots	[10]
Hypoxis hemerocallidea (*Hypoxidaceae*)	Star flower, yellow star, African potato (Eng.); Inkomfe (Zulu); Sterblom and Gifbol (Afr.)	Roots	[10,18–20]
Sclerocarya birrea (*Anacardiaceae*)	Hochst. subsp. caffra, marula, tree of life	Stem	[10,21]
Herichrysum nudifolium L. (*Asteraceae*)	Hottentot's tea (Eng.); Hottentotstee (Afr.); icholocholo (Xhosa, Zulu)	Leaves and roots	[12,14]
Herichrysum petiolare H & B.L (*Asteraceae*)	Everlasting (Eng.); Kooigoed (Afr.); Imphepho (Xhosa)	Whole plant	[12,14]

Leonotis leonurus L. (*Lamiaceae*)	Wild dagga or Lion's ear (Eng.); Wildedagga (Afr.); Imvovo (Xhosa)	Leaves, flowers	[13,14]
Momordica balsamina L. (*Cucurbitaceae*)	Balsam pear (Eng.); Laloentjie (Afr.); Nkaka (Thonga) Intshungu (Zulu)	Stem, flowers	[14,15]
Momordica foetida Schumach (*Cucurbitaceae*)	Wild cucumber (Eng.)	Leaves, and often whole plant	[14,15,22,23]
Psidium guajava L. (*Myrtaceae*)	Common guava, yellow guava, lemon guava (Eng.)	Leaves, roots, whole plant	[14,15,24]
Sclerocarya birrea Hochst (*Anacardiaceae*)	Marula (Eng.); Mufula (Venda)	Stem, bark, roots	[14,15]
Vinca major L. (*Apocynaceae*)	Bigleaf periwinkle (Eng.)	Leaves, roots, stem	[14,15]
Vernonia oligocephala Sch. Bip. (*Asteraceae*)	Bicoloured-leaved Vernonia (Eng.); Groenamarabossie (Afr.); Ihlambihloshane (Zulu)	Leaves, twigs, roots	[12,14]
Catha edulis Forrsk. Ex Endl. (*Celastraceae*)	Arabian tea, Abyssinian tea, Bushman's tea (Eng.)	Leaves, stems, roots	[14,15]
Brachylaena discolor DC. (*Asteraceae*)	Coast silver oak (Eng.) ; Kusvaalbos (Afr.); Phahla (Zulu and Xhosa)	Leaves, roots, stem	[12,14,15]
Eriocephalus punctulatus (*Asteraceae*)	Roosmaryn or Kapokbos (Afr.); wild rosemary (Eng.)	Leaves	[18,25–27]

Afr. = Afrikaans; Eng. = English.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12.0
13.0
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17.0
18.0
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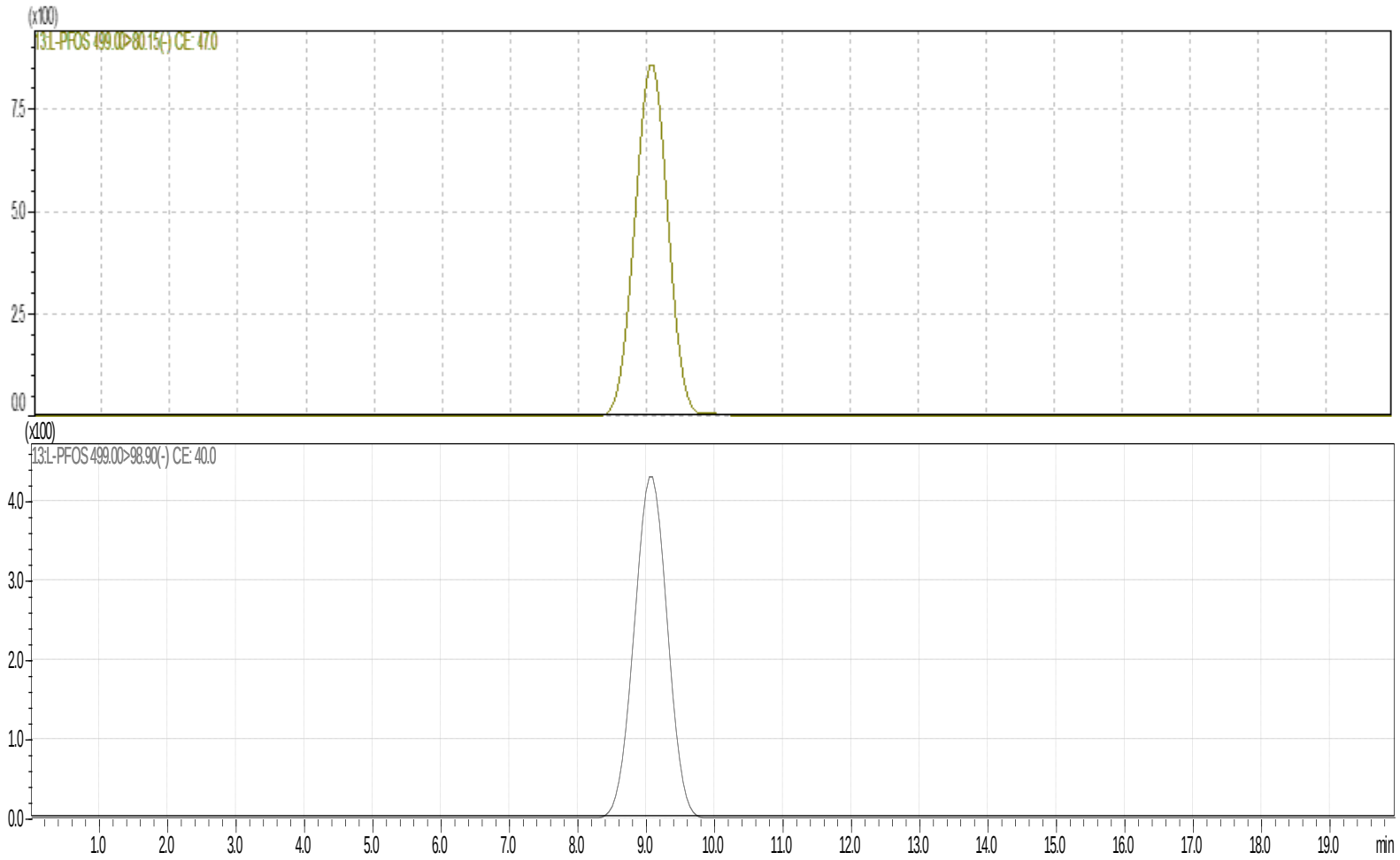
min


(x100)
13:L-PFOS 499.00>80.15(-) CE: 47.0
7.5
5.0
2.5
0.0
(x100)
13:L-PFOS 499.00>98.90(-) CE: 40.0
4.0
3.0
2.0
1.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
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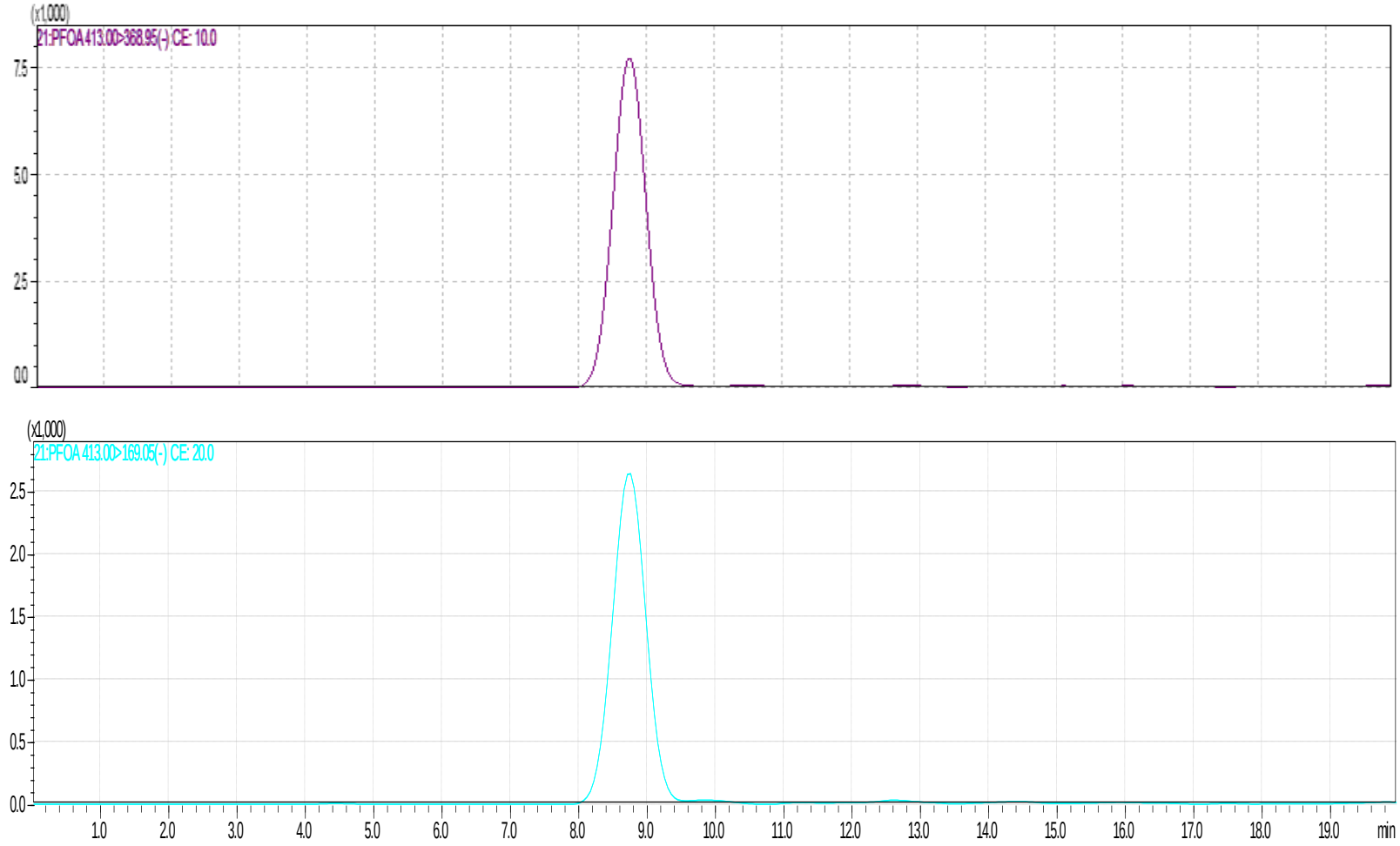

Figure S1: MRM chromatograms of PFBS, PFOS and PFOA.

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