The Detection of Perfluoro Carboxylic Acids in Complex Environmental Matrices Utilising Ultrasound-assisted Extraction and Chromatographic Analysis

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SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

School of Biodiversity, One Health & Veterinary Medicine
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University of Glasgow

Submitted: March 2024

I am not scared of death,

I've got dreams again

It's just me and the curve of the valley

And there is meaning on earth,

I am happy.

...

I'm gripping the wheel,

I'm back between villages,

And everything's still.

– Noah Kahan

Abstract

Anthropogenic chemicals are an essential part of modern life, infiltrating everyday life as well as industrial applications. While those compounds are beneficial in many ways with their intended use, dissemination into the environment has become a significant concern. One class of compounds, per- and polyfluoroalkyl substances (PFASs), have been of particular interest to research, due to their potential adverse effects on non-target species and prolonged residence times in the environment, hence, monitoring of concentration levels is advisable. Wastewater treatment facilities have since been identified as a crucial point source of contamination, recognising that traditional treatment processes are not designed to breakdown complex chemicals and chemical mixtures. By comparison, the practice of sewage sludge (biosolids) application to agricultural land remains less scrutinised despite presenting a more diffuse and unpredictable prospective entry route for contaminants into agriculture and the wider environment, however, readily availability and economic advantage over conventional inorganic fertiliser keeps the application of sludge products relevant.

This thesis describes the method development of a gas chromatographic – mass spectrometric (GC-MS) technique for the detection of perfluoro carboxylic acids (PFCAs) with electron impact ionisation. Optimisation of the methods progressed through the evaluation of instrumental parameters and successfully separated and detected PFCAs (PFHpA (C_7) – PFDoA (C_{12})) with a limit of detection of 25 ng/mL respectively. Efficacy of the method was dependent on the degree of volatility and interaction of compounds with the engaged stationary phase, with smaller PFCAs, ranging from PFBA (C_4) to PFHxA (C_6), ultimately not successfully retained in the process.

A previously implemented ultrasound-assisted extraction (UAE) method for the detection of pharmaceuticals in biosolids was adapted and optimised for the detection of perfluoro carboxylic acids in biosolids and soil matrices. The amount of soil undergoing extraction was investigated in the process, with 1 gram of soil achieving comparably lower recoveries to 0.5 grams of soil, albeit with improved, reduced relative standard deviations, fitting with the previously optimised to 1 gram of biosolids for the process. The implementation of multiplexing, the

simultaneous extraction of three samples, was found to be consistent across the employed sonication device and reduced the sample preparation time by 66 %.

The application of liquid chromatography and orbitrap mass spectrometry (LC-Orbitrap-MS) analysis overcame issues with the detection of smaller PFCAs and overall sensitivity observed GC-MS, however, matrix effects had to be addressed in the process. All investigated PFCAs, ranging from PFBA to PFDoA were detected across six biosolids batches whereas biosolids-amended soils collected over 2 years were found to contain varying levels of predominantly PFHpA and PFDA. Regardless of the continued biannual biosolids application, differences in the PFCA concentration profiles in soil were observed with a spike and successive decrease in concentration within a month of the initial monitored biosolids application. The comparison with agricultural soil that never received biosolids-amendment does not show any significant contamination with PFCAs and hence suggests that the biosolids-amendment is responsible for the introduction of PFCAs into the soil environment receiving the biosolids treatment despite discrepancies in PFCA profiles. The utilisation of UAE and LC-Orbitrap-MS was able to reliably detect PFCA concentrations in both, biosolids and soils, making it a suitable approach for the investigation of environmental trace analysis.

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Abbreviations

Chemicals and Chemical Classes

AAP	Acetaminophen
ACN	Acetonitrile
A-D10	Deuterated (D10) anthracene
ATL	Atenolol
CBZ	Carbamazepine
DDT	Dichlorodiphenyl trichloroethane
di-PAPs	Polyfluoroalkyl diphosphate esters
DPS	Dapsone
EtAc	Ethyl acetate
FOSAs	Perfluorooctane sulfonamides
FOSEs	Perfluorooctane sulfonamido ethanols
FTs	Fluorotelomer
FTCAs	Fluorotelomer carboxylic acids
FTOHs	Fluorotelomer alcohols
HCOOH	Formic acid
HMP	Hexametaphosphate
IBP	Ibuprofen
MeOH	Methanol
MSTFA	N-Methyl-N-(trimethylsilyl) trifluoroacetamide
NH_3	Ammonia
P	Phenanthrene
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PFAAs	Perfluoroalkyl acids
PFASs	Per- and polyfluoroalkyl substances
PFBA	Perfluoro butanoic acid
PFCAs	Perfluoro carboxylic acids
PFDA	Perfluoro decanoic acid
PFDoA	Perfluoro dodecanoic acid
PFHpA	Perfluoro heptanoic acid
PFHxA	Perfluoro hexanoic acid
PFNA	Perfluoro nonanoic acid
PFOA	Perfluoro octanoic acid
PFOS	Perfluoro octane sulfonate
PFPeA	Perfluoro pentanoic acid
PFSAs	Perfluoro sulfonic acids
PFUnA	Perfluoro undecanoic acid
PPCPs	Pharmaceuticals and personal care products
PTFE	Polytetrafluoroethylene

PUF	polyurethane foam
TCL	Triclosan

Analysis and Instrumentation

1TMS	Addition of one TMS group
2TMS	Addition of two TMS groups
^{12}C	Carbon isotope, 12 amu
^{13}C	Carbon isotope, 13 amu
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
C8	Octa(silyl)
C18	Octadecyl(silyl)
CI	Chemical ionisation
D	Derivatisation (derivatised)
DAD	Diode array detector
DOE	Design of experiment
ECD	Electron capture detector
EI	Electron impact ionisation
EM	Electron multiplier
ESI	Electrospray ionisation
FID	Flame ionisation detector
FLD	Fluorescence detector
GC	Gas chromatography
GCxGC	Comprehensive (two-dimensional) gas chromatography
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
IS	Internal standard
LC	Liquid chromatography
LOI	Loss on ignition
MAE	Microwave-assisted extraction
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NCE	Normalised collision energy
NP	Normal phase
PAR	Peak area ratio
PCA	Principal component analysis
PLE	Pressurised liquid extraction
PLOT	Porous-layer open tubular column
PRM	Parallel reaction monitoring
Q	Quadrupole mass filter
RP	Reversed phase
RSD	Relative standard deviation
SCOT	Support-coated open tubular column

SD	Standard deviation
SIM	Selected ion monitoring
SIP	sorbent impregnated polyurethane disk samplers
SPE	Solid phase extraction
SSL	Split/splittless (inlet or injection)
TCD	Thermal conductivity detector
TD	Thermal drying
TH	Thermal hydrolysis
TIC	Total ion count
TMS	Trimethylsilyl group
TOF	Time of flight
UAE	Ultrasound-assisted extraction
WAX	Weak anion exchange
WCOT	Wall-coated open tubular column

General Terms

AFFF	Aqueous film forming foam
CAS	American chemical society
CLP	Classification, labelling and packaging
EC	Emerging contaminant
ECF	Electrochemical fluorination
EDC	Endocrine-disrupting chemical
EEC	European economic community
EPA	Environmental protection agency
ERA	Environmental risk assessment
EU	European Union
IUPAC	International Union of Pure and Applied Chemistry
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentrations
PNEC	Predicted no-effect concentration
POP	Persistent organic pollutant
PPCPs	Pharmaceuticals and personal care products
Q	Quadrupole mass analyser
REACH	Registration, evaluation, authorisation and restriction of chemicals
UN	United Nations
US	United States (of America)

Variables and Units

°C	Degrees centigrade
%v/v	Volume concentration
AC	Alternating current
amu	Unified mass unit
atm	Atmosphere
Bp	Boiling point (°C)

cm	Centimeter	(10^{-2} m)
DC	Direct current	
DW	Dry weight	
eV	Electron volt	(1.6×10^{-19} V)
g	Gram	
ID	Inner diameter	
kDA	Kilodalton	(10^{3} Da)
kHz	Kilohertz	(10^{3} Hz)
kV	Kilovolt	(10^{3} V)
L	Litre	
LOD	Limit of detection	
LOQ	Limit of quantification	
µg	Microgram	(10^{-6} g)
µL	Microliter	(10^{-6} L)
µm	Micrometer	(10^{-6} m)
mg	Milligram	(10^{-3} g)
min	Minutes	
mL	Millilitre	(10^{-3} L)
mm	Millimeter	(10^{-3} m)
mmol	Millimol	(10^{-3} mol)
MHz	Megahertz	(10^{6} Hz)
ms	Millisecond	(10^{-3} s)
MW	Molecular weight	
m/z	Mass to charge ratio	
N	Number of samples	
ND	Not detected	
ng	Nanogram	(10^{-9} g)
OM	Organic matter content	
tR	Retention time	
PA	Peak area	
pH	Negative dekadian logarithm of hydrogen ions	
pKa	Acid dissociation constant	
pKd	Partitioning coefficients	
ppm	Parts per million	
rpm	Revolutions per minute	
V	Volt	
WC	Water content	

Acknowledgements

I would like to thank my supervisory team, Dr. Michelle Bellingham and Prof. Caroline Gauchotte-Lindsay, for their guidance and support during this project. I would also like to acknowledge and thank the University of Glasgow Lord Kelvin and Adam Smith (LKAS) scholarship which allowed me to pursue this degree.

I express my sincere thanks to Dr. Anca Amariei, Dr. Celeste Felion and Dr. Kate Fell, for sharing their time and expertise to help with this project. I would like to thank Dr. Joanne Roberts from the Glasgow Caledonian University for her expertise and help in utilising liquid chromatography used in this project. I am also grateful for the support received from Mara Knapp from Strathclyde University, despite never completing that part of the project due to instrumental issues. In addition, I would like to thank everyone providing additional support in and around the lab, Alysha Hunter, Dr. Ana Monterio, Elizabeth Palmer and Julie Russel.

To my fellow researchers and friends, Dr. Kelly Stewart and Dr. Raminta Kazlauskaité – thank you for your continued support and encouragement, this PhD experience would have been very different without you. To the many researchers in the hiRACE and Water & Environment group, thank you for allowing me to share this experience with you; it was a privilege to work alongside such talented scientists.

I would like to express my deepest gratitude to my family for their support, patience and encouragement throughout my studies. To my parents, Antje Manuela Birgit and Heiko, my grandmothers, Oma Brigitte and Oma Heidrun Ute Bergande, my brother Michael, Josephine and the little Türks, Frida Carlotta, Anton and Oscar, know all my achievements have been for you. To Kevin Andrew, Oliver Cracker, Isla Thea and Oreo Albert, thank you, for existing and everything else on top of that.

Finally, to the brilliant women in my life, my best friends of the last two decades, Dr. Marie-Louise Morkos, Fiona Brand and Malin Meißenburg, thank you for all the adventures, love and support over the years. This degree was only achievable because you made me who I am today just as much as my family has.

Author's Declaration of Originality

Statement of Originality to Accompany Thesis Submission

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I certify that the thesis presented here for examination for a PhD degree of the University of Glasgow is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it) and that the thesis has not been edited by a third party beyond what is permitted by the University's PGR Code of Practice.

I declare that the thesis does not include work forming part of a thesis presented successfully for another degree.

I declare that this thesis has been produced in accordance with the University of Glasgow's Code of Good Practice in Research.

I acknowledge that if any issues are raised regarding good research practice based on review of the thesis, the examination may be postponed pending the outcome of any investigation of the issues.

Chapter 1

Introduction

1.1. Background

Pollution control, irrespective of the applied scope can be challenging, as primary sources are not necessarily easily identified. On one hand, anthropogenic pollution from point sources, such as wastewater treatment facilities or landfills are continuously evaluated, and different types of pollution (e.g. water, soil and air pollution) can be monitored, assessed and addressed, pinpointing to that single origin of pollution. On the other hand, the assessment of diffuse pollution from non-point sources remains challenging: Run-off, from farmland in agriculture or from roads in urbanised areas, and the subsequent movement of pollutants, into the wider environment or seeping into groundwater, poses a complex monitoring problem.

In agriculture, the worldwide application of processed sewage sludge (biosolids) to land first and foremost portrays a cost-effective route for recycling the byproduct of domestic and industrial wastewater and the replenishment of nutrients for farmland. However, biosolids may also provide an entry route for undesired contaminants into the environment. Prior to application, biosolids are treated to minimise the water content, the microbial load and to reduce the potential release of greenhouse gases. During sludge production processes, general specifications (e.g. dry matter, organic matter, nitrogen and phosphorus content) and contamination with heavy metals are assessed, however, no regulations or limits concerning the contamination with other constituent chemicals are in place in the United Kingdom or European Union. As a result, chemicals that were recalcitrant to wastewater treatment processes can bind to sludge particles and engage biosolids as a vector of pollution to enter soil environments.

While pesticides and veterinary medicinal compounds are somewhat expected to be found in an agricultural setting, biosolids potentially carry a complex mixture of pollutants. The assessment of pollution linked to biosolids is a difficult task, as the composition of biosolids themselves depend on the wastewater composition and processes employed during the wastewater and sludge treatments along with

other contributing factors (e.g. compound properties). Compounds of high research interest include chemicals with industrial origin, such as per and polyfluoroalkyl substances (e.g. perfluoro carboxylic acids). Over recent years, increased research efforts have been focused on the impact of chemicals and chemical mixtures on livestock, wildlife, plant and environmental health in general, ranging from monitoring increasing rates of antimicrobial resistance in wastewater and surface water systems (e.g. rivers basins) to physiological changes across various species that are unintentionally exposed to chemical mixtures (e.g. livestock grazing on biosolids-amended farmland).

As a way to assess the dissemination of pollutants through the environment and to causally link origin and effect subsequently, contaminants first have to be extracted from environmental matrices with reliable and robust extraction techniques. Ultrasound-assisted extraction techniques are often investigated for this purpose as they involve low-cost instrumentation and sustainable, minimal waste streams associated with the extraction process. Efficiencies of this undertaking must take all three partakers into account: the pollutants, the sample matrix to be extracted from and the solvent facilitating the mass transfer of the pollutants of interest. Once extraction is successful, the objective falls to identify and apply sensitive analytical methods for the detection at environmental concentrations. Depending on the intrinsic properties of the contaminants of interest, gas and liquid chromatography can be applied, often in conjunction with mass spectrometric detection. However, chromatographic possibilities and limitations have to be considered in the process.

1.2. Research Aims and Objectives

The overall aims of this study were to, firstly, develop a high-throughput ultrasound-assisted extraction technique for the targeted analysis of perfluoro carboxylic acids (PFCAs) in biosolids and soils in conjunction with gas and liquid chromatographic techniques. Secondly, this project aimed to establish prevalent PFCA-contamination levels across biosolids, and temporal profiles of PFCAs in soils relating to the biosolids application to agricultural land to assess the environmental impact of this general, globally used practice. To achieve the aims of this study, the following objectives were set:

I. To increase extraction capabilities of a sonication probe to minimise cost and labour arising from ultrasound-assisted extraction. Pharmaceuticals and personal care products were utilised for the method development and validation on this part of the project as previous developments were taken into account.
II. To evaluate and optimise an ultrasound-assisted extraction method for the extraction of PFCAs from biosolids and soils following the identification as a high-interest group of compounds considering ubiquitous contamination events globally.
III. To investigate the use of gas chromatography – mass spectrometry for the detection of PFCAs in complex samples at environmental concentration levels compared with more commonly employed liquid chromatographical techniques considering the greener credentials of gas chromatography compared to liquid analysis techniques.
IV. To assess concentration ranges between biosolids and soils, to evaluate the mass transfer from one to the other, and the environmental burden associated with PFCAs overall.

1.3. Thesis Outline

This thesis consists of six chapters which detail the approach towards the method development and optimisation for the targeted analysis of perfluoro carboxylic acids in environmental samples (biosolids and soils).

Chapter 2 introduces relevant emerging contaminants; their sources, fate and potentially adverse effects on the environment and its biota within the context of human development and the concept of sustainability. This chapter establishes methodological gaps regarding the treatment and preparation of complex environmental matrices and resulting limitations while also offering a first insight into applied analytical methods for the detection of contaminants.

Chapter 3 produces the technical background corresponding to methods employed in this project. The chapter explains the principle of ultrasound-assisted extraction and details the theory of analytical separation techniques (gas and liquid chromatography) as well as mass spectrometric detection.

Chapter 4 presents the matrix characterisation of studied biosolids and soils, and evaluates the extraction efficiencies within the context of extraction parameter

optimisation. The chapter presents the development of a novel, adequate analysis method for perfluoro carboxylic acids using gas chromatography – electron impact – mass spectrometry.

Chapter 5 presents the study of concentration profiles of perfluoro carboxylic acids in biosolids batches and across soils collected and monitored over 2 years. A comparison is drawn between the apparent prevalence of contaminants in applied biosolids and receiving soils via targeted analysis with liquid chromatography analysis.

Finally, chapter 6 summarises the key findings of this research in relation to the outlined objectives and overall aims of the thesis. The chapter further highlights recommendations for future work.

Chapter 2

Literature Review

2.1. Introduction

Chemicals define modern life and are omnipresent in the 21st century. The discovery of naturally occurring chemicals and synthesis of these and new chemicals started slowly in the early 1900's as technological advanced and capabilities improved. In 1908 Cellophane was discovered by Jaques Brandenberger and produced commercially by 1912, in 1910 the ammonia synthesis using the Haber-Bosch process was commercialised in Germany, Penicillin was discovered by Alexander Flemming in 1928, and slowly the abundance and number of modern chemicals increased. By the 1930's polymers such as polyethylene and Teflon were synthesised and widely used (Feldman, 2008). As knowledge and technical possibilities advanced the number of chemicals increased rapidly. The American Chemical Society (CAS) now lists over 200 million entries in their registry, with an estimated 20 million different known organic compounds, both naturally occurring and purely synthetic (American Chemical Society, 2024). Organics find application as pharmaceuticals and personal care products (PPCPs), biomaterials, pesticides and insecticides, dyes, plastics, food additives, preservatives and processing aids and surface treatments, amongst other uses. However, environmental contamination sources can be difficult to determine, as emerging pollutants disseminate through the environment, originating from industrial production itself, consumer goods, agriculture and livestock, landfills and compost, food and drinking water as well as indoor environments.

While many chemical products have improved modern life, others were found to have adverse effects. Historically, adverse effects of chemicals were primarily assessed in a restricted context, following their intended application. However, the release of chemicals into the environment was eventually identified as a significant risk, negatively impacting non-target species or accumulating in environmental compartments. Public environmental and ecological awareness evolved widely over the past decades and influenced lifestyles and communities to varying levels globally. With time, legislation, policies and regulatory agencies were introduced to ensure safe environments. Environmental protection agencies

(EPAs) were formed as early as 1970 in the US and 1974 in Germany (US EPA, 2023, Umwelt Bundesamt, 2024). The European Environment Agency and EPA UK followed in 1993 and 1996, respectively, amongst other governmental bodies (EU EPA, 2024, UK EPA, 2024). As the gained knowledge on pollution increases and transpires through all levels of society, the importance of the natural environment and global ecosystem as a whole, including its biodiversity and climate stability, is highlighted. Correspondingly, introduced interventions targeting pollution are required to address sustainability on a communal and global level, as well as poverty, economic growth, and the imbalance of resources.

2.2. Sustainable Goals and Conventions

The first world conference on the human environment organised by the United Nations (UN) took place in 1972. As a result of this, 152 countries ratified the Stockholm declaration and action plan for the human environment (United Nations, 1973). In the subsequent years through the 1990's sufficient evidence was gathered to demonstrate hazardous effects for a small number of persistent organic pollutants (POPs), and the Stockholm convention was approved to eliminate, restrict or reduce the production and use of twelve chemicals originally named as "the dirty dozen" (United Nations, 2019). The affected chemicals fell into three categories: pesticides (aldrin, chlordane, Dichlorodiphenyl-trichloroethane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene), industrial chemicals (hexachlorobenzene, polychlorinated biphenyls (PCBs) and by-products (hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, and PCBs) (Secretariat of the Stockholm Convention, 2019). The latest list of chemicals falling under the Stockholm convention now lists 30 POPs for elimination: 15 pesticides, 13 industrial chemicals and two compounds falling under both descriptors (United Nations, 2019).

Similarly, by 1987 the United Nations published the first comprehensive document on global environment and development targets (United Nations, 1987). It states that the aim is to ensure sustainable economic growth and to output policies to safeguard, sustain and expand the environmental resource base. Different versions of that publication followed since, with the sustainable development goals published in 2015 as the current iteration (United Nations, 2015). While environmental pollution is not the dominant theme within the sustainable goals, it does play a key role in achieving a number of goals from protecting, restoring

and sustaining terrestrial and aquatic ecosystems (goal 14 and 15), and sustainable consumption and production (goal 12), as well as the management of water and sanitation (goal 6) amongst others. In total, seven of the 17 sustainable goals and 13 of the outlined 169 targets are affected by environmental pollution (United Nations, 2015).

On a European level, approximately 40 laws regulate aspects of chemicals, from registration, evaluation, authorisation, and restriction of chemicals (REACH) and classification, labelling and packaging (CLP) to regulation on persistent organic pollutants (POPs) and endocrine disrupting chemicals (EDCs). Ratified directives also set frameworks for regulations regarding air, water and soil pollution, however, the extent of included chemicals does vary significantly. As an example, the sewage sludge directive 86/278/EEC was first approved in 1986 and describes and regulates the use of sewage sludge or sludge-derived products in agriculture. As such, it details what sludge is, procedures and time scales correlating to the application and the requirements to analyse sludge regarding composition, pH, and heavy metals at least every 6 months. The directive received amendments since, but still only considers a selection of heavy metals as pollutants (European Commision, 1986). The document furthermore provides information on corresponding soil sampling and testing following sewage sludge application, in addition to specification and testing of the sewage sludge (European Commision, 1986).

2.3. Emerging Contaminants

Contaminants, natural or anthropogenic, have been traced across environmental compartments exploiting different analytical methods. Traditionally, inorganic targets like heavy metals were investigated in environmental matrices and informed governmental documents like the sewage sludge directive (European Commision, 1986). Emerging contaminants (ECs) are not necessarily new to the environment but have not been considered previously (Boxall, 2012, Sauvé and Desrosiers, 2014). However, as evidence of potentially harmful effects is gathered for more pollutants, their elongated presence in the environment needs to be re-assessed (Figure 2-1) (Sauvé and Desrosiers, 2014, Ngoc Han et al., 2018, de Oliveira Santos et al., 2023).

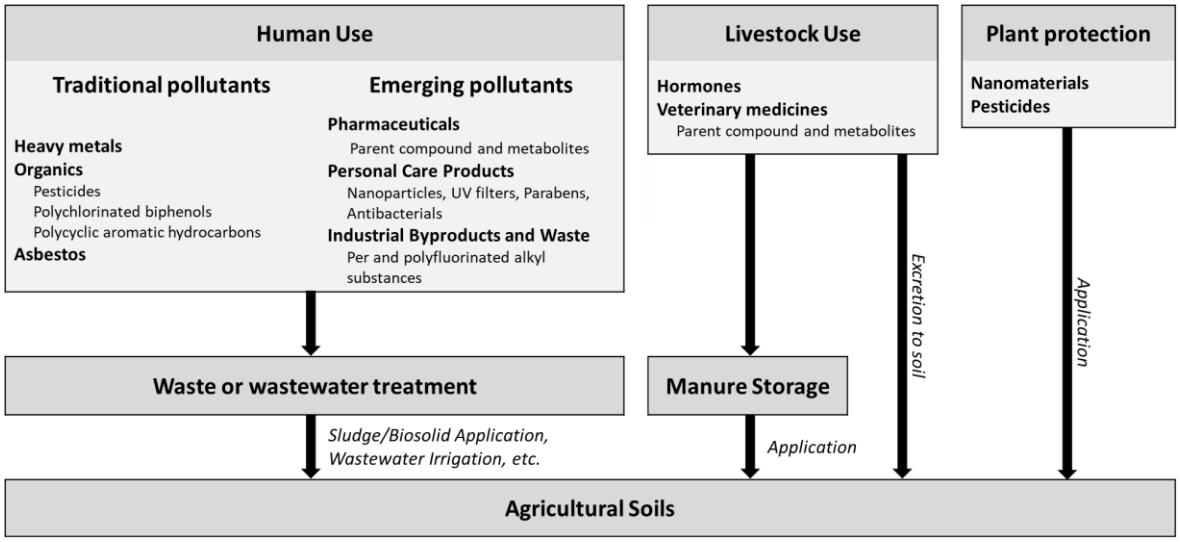


Figure 2-1: Sources of pollutants in agricultural environments depending on applications. Pollutants may be linked to human and livestock use, plant protection or correlate with waste or wastewater treatment processes. Pollutants originating from anthropogenic use in particular present as a highly varied profile of compounds that are entirely undesired within the wider environment. However, wastewater treatment and sewage sludge application to agricultural land pose as a risk to environmental health.

Howard and Muir (2010) analysed different databases to identify potentially persistent and bio-accumulative compounds in items of commerce and identified 610 compounds of concern within 30,000 screened compound details. Beyond this study, the overall estimate of chemicals that enter the environment varies widely but is believed to range between 80,000 and 100,000 contaminants (Megson et al., 2016, Naidu et al., 2016).

2.3.1. Emerging Contaminants and the Environment

The introduction, distribution and toxicity of environmental pollutants is analysed and studied to identify and assess potential adverse effects on ecosystems. However, there is no common ground in research, meaning that publications and studies do not necessarily monitor the same compounds, or use varying analytical techniques and different matrices, potentially have vastly different concentrations, thus generate data that is not necessarily comparable.

2.3.1.1. Wastewater Treatment Processes

The processes surrounding wastewater treatment and sewage sludge have been identified as sources for contamination and gained significant interest in ongoing research into environmental contamination (Boxall, 2012, Kosma et al., 2020, Mejias et al., 2022, Rapp-Wright et al., 2023). Common biological wastewater treatment processes are generally designed to break down easily or moderately degradable compounds containing carbon, nitrogen and phosphorous, leaving

more recalcitrant compounds (e.g. pharmaceuticals and industrial products) unchanged throughout the processes (Boxall, 2012, Constantin et al., 2018, Drillia et al., 2005, Naidu et al., 2016).

In principle, the aim of wastewater treatment is to recover water from industrial and domestic sources with a high enough quality to be released back into the environment, through rivers, lakes and lochs or to be utilised again. The absence of chemical substances and organisms harmful to health are primary quality requirements, irrespective of the use (Von Sperling, 2007). Other characteristics such as turbidity, colour, taste and odour, the hardness and aggressiveness (acidity) of water may be addressed depending on subsequent utilisation (Von Sperling, 2007).

Wastewater treatment processes can be roughly organised into 4 process levels. Firstly, preliminary treatment is only designed to remove coarse suspended materials and sand (Von Sperling, 2007). Primary treatment further removes settleable solids and organic matter, whereas secondary treatment removes most of the organic matter and nutrients through mainly biological processes (i.e. trickling filters, aeration tanks). Finally, tertiary treatment usually involves advanced methods, such as ozonisation and ultraviolet radiation, aiming at the removal of pathogens and pollutants, however, due to the increased cost and complexity tertiary treatment remains optional in most countries (Von Sperling, 2007). It remains difficult to predict the behaviour of organic pollutants during wastewater treatment as the behaviour is governed by multiple factors, such as partitioning coefficients and water solubility. Compounds with hydrophobic and/or lipophilic character, are likely to adsorb to solid particles to be found in sewage sludge subsequently (Boxall, 2012, Drillia et al., 2005).

2.3.1.2. Sewage Sludge Treatment Processes

Throughout the wastewater treatment process, particles and solids are removed. While some particles readily settle to the bottom of treatment vessels, others have to be agitated, undergo flocculation or go through other means of processing to be removed (Tompkins, 2018). The removal of solids through sludge formation during primary and secondary wastewater treatment generates sludges with varying characteristics regarding microbial load, particle sizes of solids, inorganic and organic content but 95 – 98 % of the initial weight is the contribution of residual water (Tompkins, 2018). The objectives of downstream tertiary sludge

treatment processes, after separation from the liquid phase, are the removal of excess water to be returned to the water system (dewatering and thickening), the removal of organic matter prone to volatilisation (stabilisation) as well as the reduction of pathogenic organisms (disinfection), this can be achieved by means of different treatment processes (Von Sperling, 2007).

The main treatment methods for sludge destined for application in agriculture can be divided into conventional and enhanced treatment processes, accounting for approximately 3 and 41 % of the overall tonnage of biosolids produced in Scotland (2017), respectively (Tompkins, 2018). The addition of a thermal hydrolysis (TH) step prior to conventional anaerobic digestion improves the dewaterability and degradability of sludge, increases the rate of biogas (60:40 %v/v methane and carbon dioxide) release and improves the overall digestion process (Tompkins, 2018). TH is achieved through the exposure of sludge to elevated temperatures (130 – 200 °C) within a pressurised vessel for approximately 30 minutes, followed by the sudden release of pressure, causing cell lysis to any contained microorganisms (Tompkins, 2018, Von Sperling, 2007). Additionally, thermal drying (TD, 450 °C) or liming (pH >12) may be used to further treat sludge to reduce the apparent pathogen load, however, TD is an energy intensive process, whereas liming can increase the volume of the product making subsequent haulage more expensive (Tompkins, 2018, Von Sperling, 2007). Conventional treatment achieves a 99% ($2\log_{10}$) reduction in *E. coli* concentrations whereas advanced (enhanced) treated sludge shows a 99.9999 % ($6\log_{10}$) reduction in *E. coli* while also eliminating *Salmonella* from the sludge product (Water UK, 2001, Tompkins, 2018). Overall, different combinations of treatment processes are available, achieving conventional or enhanced treatment outcomes (Table 1-1).

Once sludge processing is completed, the resulting dried product, called biosolids, only contains residual water and significantly reduced amounts of pathogens and is ready for disposal (Water UK, 2001). Since sea disposal of biosolids was phased out the EU in 1998, remaining common disposal routes for biosolids are agricultural reuse and reclamation, incineration and disposal at landfills (Tompkins, 2018, Birchenough, 2024). Overall, biosolids present as a nutrient-rich fertiliser, which serves as a more affordable product than commercially-available inorganic counterparts (Tompkins, 2018). In the UK, approximately 44 % (45,000 tons dry

solid) of the produced biosolids is applied to land, while 36 % are incinerated (Tompkins, 2018).

Table 1-1: Suite of treatment combinations commonly employed for sewage sludge processing, required before land application of sewage sludge products (biosolids) in Scotland. Conventional and enhanced treatments most commonly include anaerobic digestion of sludge, however, thermal drying and liming may also achieve an enhanced product for land application. Modified from Tompkins (2018).

Thermal hydrolysis	Anaerobic digestion	Thermal drying	Liming	Dewatering
Conventional sludge treatment				
	Yes			
	Yes			Yes
Advanced (enhanced) sludge treatment				
		Yes		
			Yes	
	Yes	Yes		
Yes	Yes	Yes		
Yes	Yes			Yes

The behaviour of emerging contaminants during wastewater and sludge treatment steps and in the environment is difficult to predict because of the varying physico-chemical properties of the substances as well as the complexity of the processes employed (Zuloaga et al., 2012). Amongst other factors, molecular structure, size, weight and hydrophobicity or partitioning coefficients (pK_d) have an influence on the behaviour of compounds during treatment, however, available information is limited (National Research Council, 2014, Tompkins, 2018, Dubey et al., 2021).

Tompkins (2018) and Dubey et al. (2021) reviewed the limited number of available studies investigating the fate of pharmaceuticals and personal care products, industrial products and estrogens and found that degradation during sludge treatment varies significantly depending on the compound and employed process. For example, the removal rate of ibuprofen across different studies was found to vary from not being eliminated at all to over 80 % using anaerobic digestion whereas advanced sludge treatment resulted in 20-82 % of removal of the same compound; carbamazepine was found to withstand degradation during conventional anaerobic sludge digestion but decreased 10 – 20 % in concentrations with advanced treatment. The nature of employed wastewater and sludge processes, flow dynamics, additives (e.g. flocculation agents), the percentage of suspended solids as well as their properties may influence the sorption of

pollutants to particles. Ultimately, the general lack of knowledge about the behaviour of pollutants during treatment processes raises concerns regarding both, the release of wastewater effluent into waterways as well as the application of biosolids to agricultural land.

Once micropollutants reach the environment, different interactions and processes can occur irrespective of the entry route. Generally, more hydrophilic compounds reaching the environment are mobilised with liquid fractions, including wastewater effluents, irrigation, rain and run-off (Figure 2-2) (Stevens et al., 2003). By contrast, hydrophobic compounds potentially partition onto small particles such as sludge formed during wastewater treatment, clays in soil or sediments in water, making the transport of such chemicals particle-dependent (Boxall, 2012, Drillia et al., 2005, Singh, 2016).

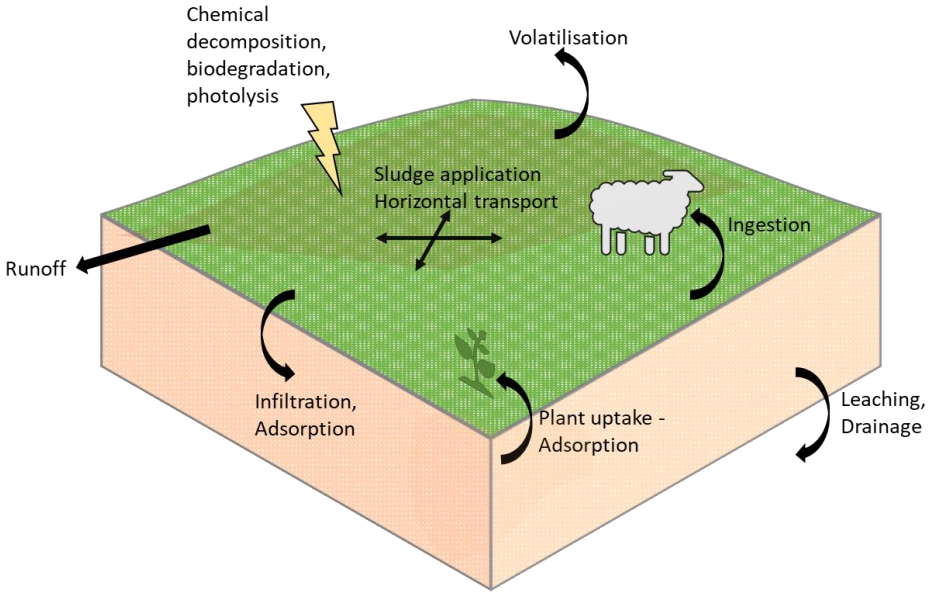


Figure 2-2: Potential pathways for the dissemination of contaminants in the environment following sewage sludge application to agricultural land. Once biosolids break down and micropollutants are released, a myriad of interactions become possible. Behaviour of pollutants varies depending on physico-chemical properties, compounds can infiltrate the horizon and adsorb to particles or plants, and subsequently be ingested by livestock. However, the movement of pollutants with water (e.g. runoff, leaching and drainage may also present pathways to reach wider environments. Degradation processes, such as photodegradation or biological degradation can reduce the presence of a subset of chemicals in soil environments.

While some compounds can undergo complete mineralisation others form transformation products or do not undergo any changes. In some cases, transformation products can be more harmful to the environment (Boxall, 2012). Additionally, adsorption into plant material and ingestion by livestock can be highlighted as risk following the biosolids application. The Sewage Sludge Directive 86/278/EEC defines a no-grazing period of three weeks to minimise effects on

soil, vegetation, animals and man, however, the fate of the pellets themselves and processes involved in the breakdown of the pellets during that period is not well understood. Nevertheless, the environmental protection agencies in the US and Europe promote the use of biosolids in agriculture, and ~55% of the 18 million dry metric tons per year of treated sewage sludge produced in the USA is applied to agricultural land (Sharma et al., 2017).

2.3.1.3. Environmental Risk Assessment

The targeted analysis of organic pollutants in the environment got underway in the 1970's and has crucially increased as a better understanding of the transgression of chemicals from industrial and domestic use to environmental exposure evolved (Biel-Maeso et al., 2018, Garrison and Hill, 1972, Garrison et al., 1976, Karnjanapiboonwong et al., 2011, Kumirska et al., 2015, Maurer et al., 2020). Occasionally, risk assessment advances with increasing evidence and compounds like the pesticides dichlorodiphenyl trichloroethane (DDT), chloropyrifos and chloropyrifos methyl, were subsequently phased out due to adverse effects on human and environmental health (Lintelmann et al., 2003, Garcia-Valcarcel and Tadeo, 2009, de Oliveira Santos et al., 2023). The primary objective of environmental risk assessment (ERA) is to determine the likelihood of adverse effects occurring to organisms or environmental communities (Department for Environment, 2011, Boxall, 2012, Huang et al., 2018, Di Lorenzo et al., 2023).

Standardised endpoint-orientated tests are performed with the help of organisms from different trophic levels (Chapman and Elphick, 2015, Peake et al., 2016). A predicted no-effect concentration (PNEC) is determined based on the organisms' response to a substance, such as a reduction in survival rate and delays in development (Huang et al., 2018). Effects on mortality and deficiency in growth are relatively easy to assess, however, changes in physiology, e.g. reproduction and fertility, are more difficult to evaluate but may have a significant effect on population sizes. Di Lorenzo et al. (2023) highlights that PNECs corresponding to "sub-lethal concentrations" may be beneficial for ERA minimising the overall impact, despite the limited availability of such values for groundwater species.

ERA ideally assesses different species across trophic levels (e.g. algae, daphnia and fish), identifying the likelihood, risk and uncertainty associated with exposure to pollutants of interest (Department for Environment, 2011). Ultimately, the

lowest concentration with measurable adverse effects is set as PNEC, indicating the most sensitive reaction to occur following exposure. The comparison of PNEC values to measured or predicted environmental concentrations (PEC) ultimately informs whether a significant risk of exposure is given, if more information is needed, or if according to current knowledge no risk is associated to the presence of pollutants at their respective concentrations (Peake et al., 2016).

However, the practice of environmental risk assessment requires significant improvements as the assessment of complex mixtures is needed (Elcombe et al., 2022). The effect of low concentration mixtures is mostly unknown and generally unconsidered, since ERA is commonly performed for single compounds under laboratory conditions. Lagunas-Rangel et al. (2022) detailed that the individual concentrations of environmental pollutants may fall below the threshold for adverse effects such as endocrine disruption but synergistic effects in mixtures can have an additive effect exceeding effects of individual chemicals.

2.3.1.4. Endocrine Disrupting Chemicals

The disruption of endocrine systems is an adverse effect of growing concern across different organisms, human and animal, as it is one of the main regulatory structures in bodily systems, besides the nervous and immune system (Lintelmann et al., 2003). Crisp et al. (1997) stated that “an environmental endocrine disruptor is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour”. Irrespective of the increasing interest in endocrine disruption, the report by the US EPA (Crisp et al., 1997) highlighted that the process of endocrine disruption itself is not a valid endpoint during environmental risk assessment rather than an influencing factor towards outcomes (e.g. cancer or death, etc.).

Annamalai and Namasivayam (2015) and Evans et al. (2014) comprehensively reviewed literature on the consequences of EDCs to organisms of different trophic levels. For example, Kunz and Fent (2006) emphasise that different experimental mixtures of UV filters with estrogenic activity showed synergistic effects in *in vitro* yeast estrogen assays, even at no-effect concentrations of individual compounds. Earthworms sampled from biosolids-treated soils showed measurable concentrations of multiple compounds outlining the potential for bioaccumulation

(Stevens et al., 2003). Willingham (2004) investigated the effects of exposure of red-eared slider turtles to four different EDCs, individually as well as in mixtures, and found that exposure during embryogenesis influences the sex of individuals. Rhind (2002) and Rhind et al. (2002) highlighted exposure routes for endocrine disrupting compounds in soil following sewage sludge application as well as in farm animals. *In vivo* animal models utilising sheep have been employed to assess potential adverse effects following the exposure to complex chemical mixtures linked to sludge and biosolids application (Paul et al., 2005, Rhind, 2008, Lind et al., 2009, Bellingham et al., 2012). Lind et al. (2009) reported the disruption of bone tissue homeostasis in sheep, leading to a reduced bone strength. Moreover, different studies have found that maternal exposure of ewes on biosolids-amended pastures can have an adverse effect on gonadal development, in both female and male offspring, however, the impact on male offspring testicular morphological changes was more significant indicating potential downstream shortcomings in adult animal health (Paul et al., 2005, Bellingham et al., 2012, Evans et al., 2014). Interestingly, pregnant ewes across studies did not necessarily display any adverse effects, indicating that the time and route of exposure, especially during development and early life, does have a significant effect on the outcome (Diamanti-Kandarakis et al., 2009). Additionally, Di Nisio et al. (2020) presented results from *in vitro* and *in silico* analysis towards human exposure, providing evidence for the interference of perfluoro octanoic acid in vitamin D, leading to altered bone development in humans.

Ultimately, substances often linked to endocrine disruption persist on different trophic levels, eventually reaching within the food chain due to biomagnification and accumulation in different tissues (Annamalai and Namasivayam, 2015, Rhind et al., 2009, Rhind et al., 2010). However, establishing a causal relationship between adverse effects and the presence of endocrine-disrupting chemicals at environmental concentrations remains difficult (Crisp et al., 1997, Rhind, 2002, Rhind et al., 2002). Due to the complexity of the problem, most published studies focus on specific aspects, such as the compound concentrations in one particular matrix, effects observed in animal models or measuring concentrations in specific tissues of subjects without tracing the movement of contaminants in the environment.

2.3.2. Per- and Polyfluoroalkyl Substances

Due to the diversity of organic contaminants, classification can be achieved by different means. Classically, chemicals with common structures were organised, grouped and named systematically by the International Union of Pure and Applied Chemistry (IUPAC). However, due to the complexity of chemical structures that are commercially used, organising by application can be more meaningful and sub-categorisation and organisation by observed adverse effects, synthesis or other means allows targeting of relevant compounds.

Per- and polyfluoroalkyl substances (PFASs) are a complex class of compounds, with differing definitions in use to date. The Organisation for Economic Co-operation and Development (OECD) and REACH define PFASs as "any substance that contains at least one fully fluorinated methyl (CF_3-R^1) or methylene (R^2-CF_2-R^3) carbon atom (without any H/Cl/Br/I attached to it)" with minor further exceptions (OECD, 2021). While fluorination does occur in some naturally occurring compounds, PFASs are considered to be an entirely synthetic, man-made class of compounds (Allison, 1987).

2.3.2.1. Manufacturing Processes

Production of PFASs began in the 1940's and compounds are found in applications in different industries (Benning et al., 1946, Berry, 1951, Dohany, 1982, Plunkett, 1941). Buck et al. (2011) discussed the two dominant manufacturing processes, electrochemical fluorination (ECF), and telomerisation. Briefly, ECF involves the electrolysis of an organic raw material (e.g. octane-1-sulfonyl fluoride ($C_8H_{17}FO_2S$)) in anhydrous hydrofluoric acid (Figure 2-1**Error! Reference source not found.**). The "free-radical nature of the process" causes breakages and re-arrangements in the raw material, leading to fully fluorinated isomers that can be linear or branched, in an apparent ratio of 70 – 80 % linear and 20 – 30 % branched isomers in the case of perfluoro octanoic acid (PFOA) and Perfluoro octane sulfonate (PFOS) depending on process conditions (Buck et al., 2011). However, Kissa (2001) stated that the mechanism of ECF was not completely understood at the point of their publication and byproducts such as explosive oxygen difluoride were problematic.

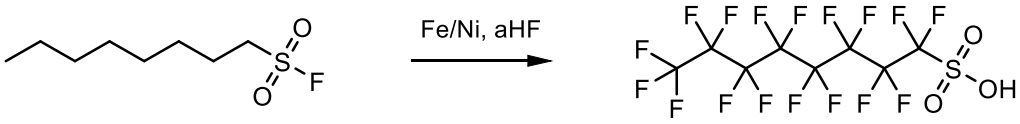


Figure 2-3: Electrochemical fluorination of octane-1-sulfonyl fluoride for the synthesis of perfluorooctane sulfonic acid. The reaction commonly employed iron or nickel electrodes in combination with anhydrous hydrofluoric acid as reaction medium.

Telomerisation on the other hand, uses perfluoro alkyl iodides (e.g. trifluoromethyl iodide (CF_3I), the telogen of the process) and tetrafluoroethene (C_2F_2, the taxogen of the process) as educts, to synthesise products with varying perfluorinated alkyl chains (Figure 2-4) (Buck et al., 2011). Kissa (2001) further elaborated on the telomerisation process stating that the taxogen concentration and reaction conditions dictate the manufacturing process, similar to the polymerisation processes employed in plastics.

Notably, the pairing of a linear two-carbon length telogen and taxogen (given examples above) results in the synthesis of exclusively linear perfluoro alkyl chains. The use of branched or odd numbered carbon chain telogens with the same taxogen results in a mixture of branched and linear products that potentially includes an odd number of carbon (Buck et al., 2011). Furthermore, following this initial stage of perfluoro alkyl iodide formation, employing a second stage of reaction allows for the generation of fluorotelomer iodides, the basis of fluorotelomer-based PFASs as well as the synthesis of perfluorocarboxylic acid (PFCAs).

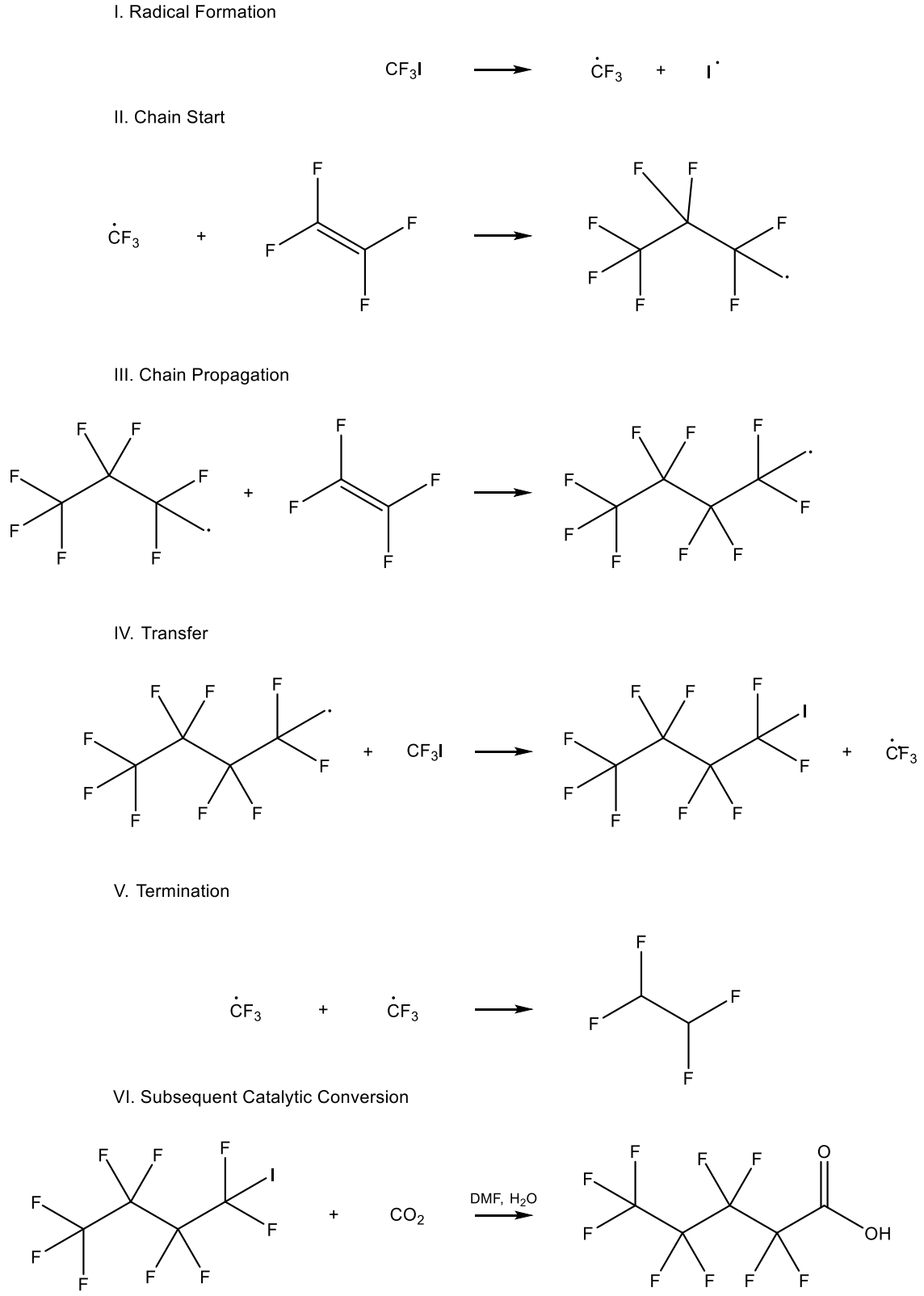


Figure 2-4: Principle of telomerisation. Perfluoroalkyl iodides (e.g. trifluoromethyl iodide, CF_3I) are used as telogens in telomerisation reactions (I). Once reaction conditions allow for the formation of radicals the chain start of the telomerisation process can be initiated in the presence of monomer units (taxogens), like tetrafluoroethene (C_2F_2) (II). The extend of chain propagation to occur in the process depends on the concentration of the taxogen of the reaction as well as overall reaction conditions (III). Once taxogen concentrations are reduced the radical moiety is transferred to a trifluoromethyl iodide (IV), which is readily undergoing radical combination forming tetrafluoroethane (V). The formed elongated perfluoroalkyl iodide can then undergo catalysis to form perfluorocarboxylic acids, amongst other available reaction mechanisms (VI).

2.3.2.2. General Physico-chemical Properties and Applications

Key et al. (1997) specified that fluorinated compounds are distinctively different from their hydrocarbon counterparts as the perfluorinated chains are both, hydrophobic and lipophobic. Allison (1987) further highlighted that the substitution of a single hydrogen in hydrocarbons with a fluorine atom increases the bond length, whereas multiple substitutions decrease the bond length leading to an increased bond strength making more fluorinated molecules more stable. The increase in bond strength and the associated rigidity that is introduced with extensive fluorination lead to excellent innate surface-active properties (Brase et al., 2021). Remarkably, surface treatments, e.g. with perfluoro decanoic acid (PFDA) find wide and unexpected applications, such as the use of coatings in polyurethanes, significantly improving the antithrombogenicity to optimise blood compatibility compared to untreated polyurethane highlighting the application of PFASs in biomaterials (Han et al., 1992).

Brase et al. (2021) further elaborated on properties of fluorinated chemicals, with an increased reactivity compared to non-fluorinated analogues, increased activity of organic acids, low surface tension and exceptional surface activity. Prevedouros et al. (2006) stated that PFCAs are stable to acids, bases, oxidants and reductants. The observed vapour pressure decreases with increasing chain length making volatilisation of larger molecules unlikely (Prevedouros et al., 2006). Due to the overall structure of PFASs they form multiple layers and micelles in an octanol and water mixture making the determination of partitioning coefficients difficult (Kissa, 2001, Prevedouros et al., 2006). An advantage of the formation of micelles in solution however, is the potential solubilisation of substances that would otherwise not dissolve in the used solvent which can be exploited for applications. (Kissa, 2001). Notably, properties that are exploited for industrial applications and consumer products facilitate adverse effects in the environment, with uncertainty regarding long-term sinks like soil and sediments (Prevedouros et al., 2006, Rankin et al., 2016).

Kissa (2001) elucidated the processes associated with the increased surface activities, and while physical adsorption is primarily mediated by van-der-Waals forces, many aspects of surface activity involve dispersion forces, hydrophobic bonding, charge transfer and hydrogen bonding. Ionic sorption through oppositely charged functional groups does occur along with chemisorption, facilitated

through covalent bonds (Kissa, 2001). Overall, the adsorption at solid-liquid interfaces depends on several factors, including the overall structure of the surfactant, the strength of adsorptive bond (ionic > covalent > hydrogen bonds > van-der-Waals forces), the nature of the substrate (solid) (e.g. polarity, geometry) and the composition of the liquid phase along with physical conditions (e.g. temperature, pressure, agitation) (Kissa, 2001).

Hendricks (1953) already described the use of PFASs for applications requiring a lowered surface tension in water. The intrinsic water and oil or solvent repellence finds application with many substrates like textiles, leather, paper and printed circuits, and furthermore, surface treatments perform excellent at levels as low as 25 ppm due to the chemical and thermal stability and high efficiency (Allison, 1987). The comparably lower concentration for surface treatments with fluorinated surfactants is of importance as production prices are usually higher when compared to non-fluorinated surfactants, and cost efficiency is only achieved through the reduced concentrations required (Kissa, 2001). Applications beyond surface treatments include the use as aerosol repellents, anaesthetics, pesticides, plant growth regulators, medicines, adhesives, fire retardants and blood substitutes, amongst others (Key et al., 1997).

2.3.2.3. Classification of Per- and Polyfluoroalkyl Substances

Following the comprehensive systematic approach of Buck et al. (2011) PFASs can be roughly categorised into polymers and non-polymers. Firstly, non-polymers consist of perfluoroalkyl substances, compounds with aliphatic carbons exclusively occupied by fluorine apart from functional groups, and polyfluoroalkyl substances, with at least one aliphatic carbon not completely substituted with fluorine atoms. Secondly, polymers are subdivided into three groups depending on the position of the fluorination occurring (i.e. directly on the carbon backbone of a molecule or fluorinated sidechains) and the incorporation of oxygen in the polymer backbone.

Further differentiation within subcategories is caused by the incorporation of different functional groups, allowing varying physico-chemical properties. Amongst polymer PFASs, Polytetrafluoroethylene (PTFE, also known as Teflon) is the most commonly known compound and finds wide applications in commercial and industrial products and processes. The primary use of polymers is in surface protection or as surfactants (Buck et al., 2011). While a number of non-polymer PFASs are primarily used as raw materials for other PFASs, a number of classes,

such as Perfluoro carboxylic acids (PFCAs) and perfluoro sulfonic acids (PFSAs) are used as surfactants due to their exceptional surface activities (Han et al., 1992, Kissa, 2001, Boulanger et al., 2005, Sinclair et al., 2007, Liu et al., 2009, Liu et al., 2014, Zhou et al., 2022). Perfluoro carboxylic acids in particular are of importance in this project and are the focus henceforth.

2.3.2.4. Perfluoro Carboxylic Acids in the Environment

Guillette et al. (2020) emphasised that amongst the thousands of PFASs in the CAS registry only PFOA and PFOS have extensive health and exposure data. Information about fate, transport, exposure, toxicology and the extend of exposure of other compounds remains sparse despite a clear indication of the need for more in depth research (Brase et al., 2021, Wang et al., 2017). Foguth et al. (2019) furthermore acknowledged the general lack of neurotoxicological data and potential relevance in neurological disease.

Notably, PFOA in particular, theoretically has 39 isomers of its own of which 11 (1 C_8 chain, 5 C_7 chains, 5 C_6 chains) are documented with CAS. There are limited resources to address physico-chemical properties, environmental risk, or commercial sources for branched isomers, despite having more than 100 commercial sources and extensive data on environmental and human risk for the linear molecule, highlighting further that information is only accumulated for specific compounds and isomers in particular (Ahrens, 2011, Nielsen, 2012).

2.3.2.4.1. Contamination Sources

Studies call attention to the fact that several tonnes of PFCAs are produced annually and while contamination events do occur, concentrations in the environment are also the result of atmospheric oxidation of volatile precursors, such as fluorotelomer compounds and perfluoro sulfonamides (McMurdo et al., 2008, Jahnke and Berger, 2009, Ahrens et al., 2010, Young and Mabury, 2010). Interestingly, the fluorotelomer production is a source for only even-numbered perfluoro carboxylic acids while biodegradation products from fluorotelomer derivatives result in the presence of even and odd-numbered chain lengths if fluorotelomers are released into the environment which can give an indication into the origin of the contamination (Simcik and Dorweiler, 2005, Ahrens et al., 2009, Armitage et al., 2009). The use of fluorotelomers in aqueous film forming foam (AFFF) in firefighting foam formulations in particular have been identified as significant contribution to environmental PFCA contamination, predominantly

in proximity to airports, military bases and training grounds (Backe et al., 2013, D'Agostino and Mabury, 2014, D'Agostino and Mabury, 2017, Mejia-Avendaño et al., 2017b). D'Agostino and Mabury (2017) further highlighted that while the use of perfluoro carboxylic acids and perfluoroalkyl sulfonates was known for AFFF formulations, over 20 additional classes of PFAS have been identified within the same media.

D'Agostino and Mabury (2017) reported temporal profiles of various PFASs (e.g. PFCAs, fluorotelomer betaines) in impacted environmental samples (i.e. river water and sediment) downstream of an airport and note different concentration profiles in different locations over a period of 5 years, attributing decreases in concentration to ongoing transport of substances with environmental fluxes and increases to continuing degradation of precursors following AFFF use. Prevedouros et al. (2006) accentuated that PFCA and fluorotelomer release through wastewater treatment plants, disposal, landfill and incineration is an additional important indirect source for PFCAs to the environment.

Historically, global PFASs production and emissions, were dominated by longer chain lengths of C_8, C_9 and C_{11}, however, in recent years a shift to shorter chains took place, after PFOA and PFOS were phased out due to negative implications of the compounds (Prevedouros et al., 2006, Lorenzo et al., 2015). PFOA is still frequently detected in various environmental matrices globally, despite the phase-out following the 2010/2015 PFOA stewardship and EU ban in 2020 (Yamashita et al., 2008, Armitage et al., 2009, Picó et al., 2011, Wang et al., 2014, Rankin et al., 2016, Cao et al., 2019). Perfluro butanoic acid (PFBA) replaced PFOA in many commercial applications as its believed to hardly accumulate and not induce toxic effects, while retaining similar beneficial properties (Jahnke and Berger, 2009).

2.3.2.4.2. Contaminant Movement in the Environment

McMurdo et al. (2008) stated that direct emission of PFOA occurs as a result of fluoropolymer production and elaborate that both, wet and dry deposition in the environment, are predominantly particle-mediated. However, they investigated the air-water transport of gaseous PFOA and found that the occurring flux to air would not significantly reduce the concentration of perfluoro octanoate and perfluoro octanoic acid in sea water. Aerosol production at the sea surface is continuous, with breaking waves producing air bubbles and upon bursting aerosols

are ejected into the atmosphere leading to atmospheric aerosols of 0.05 to 2 µm with residence times in the order of ten days, allowing the atmospheric transport over 100's to 1000's of kilometres (McMurdo et al., 2008). The effective transport of PFCAs to remote areas is difficult to explain as aerosols are removed from the atmosphere by means of wet and dry deposition within a short time period (Ellis et al., 2004). More volatile fluorotelomer alcohols have been proposed as candidates facilitating long-range atmospheric transport resulting in contamination with PFCA contamination in remote areas, as the precursor telomers are abundant in urbanised air, volatile and have a residence time in the atmosphere of up to 20 days (Ellis et al., 2004, Simcik and Dorweiler, 2005, Benskin et al., 2013).

Ahrens et al. (2010) reported that PFCAs can be volatilised from wastewater following an aqueous aerosol-mediated pathway caused by treatment processes, entering into the wider environment. Notably, concentrations of longer chain PFCAs (>8 carbon) determined in wastewater can underrepresent in-effect concentrations due to adsorption onto solid particles of sewage sludge and enrichment in surface microlayers (Kissa, 2001, Ahrens et al., 2011a). This observation can be made for other matrices (e.g. soil, sediments) considering longer chain PFCAs, whereas short chain PFCAs (C_4 to C_7) follow the flux of water and are transported for longer ranges (Armitage et al., 2009, Ahrens et al., 2010, Sepulvado et al., 2011). The sorption mechanism of PFCA and perfluoro carboxylates to solids is not well characterised but the tendency to bind to particles increases with the chain length (Prevedouros et al., 2006).

Ahrens et al. (2009) established a positive correlation between organic matter and the concentration of PFASs in aquatic sediment, showing that partitioning of longer chain perfluoro carboxylic acids (C >8) is influenced by the overall organic content in the matrix. Similarly, they showed that shorter chain length PFASs are prone to reside in porewater of the investigated sediments. Higgins and Luthy (2006) argued that the organic content of sediments potentially outweighs the binding of perfluoro alkyl substances to the mineral fractions (e.g. clays) of investigated sediments, however, only a small number of sediments was investigated. While sediments do present as an individual matrix with its own merits, behaviour does potentially correlate with findings in soil. Determining the selectivity coefficient using exchange isotherms may allow a better understanding

of the exchange process during soil weathering or cycles of leaching and retention and can aid in the prediction of behaviour of compounds in the environment (Pansu and Gautheyrou, 2006). Adsorption isotherms as such can be determined for individual compounds interacting with soil particles and a liquid fraction, allowing the assessment of binding to soil and potential flux in the environment, however, effects in differing soil properties cannot be extrapolated from the isotherms. The multitude of effects on- and interactions with pollutants in the environment (e.g. movement with water, adsorption and degradation processes) make it difficult to predict compound losses. These effects again are depending on physico-chemical properties of both, matrices and pollutants, including the range of volatility in micropollutants and the possible atmospheric transport associated with it.

PFCAs of varying chain lengths (C_4 to C_{13}) are reported in soil, water and sediments, with the detection of PFBA in 100 % samples collected in Spanish river basins (Lorenzo et al., 2015, Cao et al., 2019). PFBA is most frequently detected and at highest concentrations across sample types, followed by PFOA and PFOS, seemingly confirming that PFBA replaced PFOA as the most manufactured and used PFCA following the PFOA phase-out.

The overall consistent detection of PFASs in waste, wastewater and receiving waters indicate that restriction of some but not all PFASs is potentially not sufficient to prevent exposure routes (Sinclair and Kannan, 2006, van Leeuwen and de Boer, 2007, Ma and Shih, 2010, Nakayama et al., 2019, Lasee et al., 2021).

2.3.2.4.3. Observed Effects of Exposure

Belisle (1981) found that blood level of organic fluorine in humans is dependent on the frequency of exposure and might not be a reliable parameter, as PFOA is only slowly eliminated from the body following exposure. Toxic effects and bioaccumulation of PFCAs have been observed in wildlife and humans, with emphasis on occupational exposure, leading to an increased risk of cancer, immunotoxicity and neurodevelopmental problems (Ahrens et al., 2010, Zhou et al., 2022).

Foguth et al. (2019) found that following developmental exposure of Northern leopard frogs to PFOS and PFOA, both compounds surpass the blood-brain-barrier, accumulate and decreased dopamine levels in the brain. Exposure to different PFOA concentrations did not proportionally increase the body burden beyond 300

ng/L whereas PFOS exposure followed a strong linear correlation up to 3000 ng/L, indicating a significantly higher potential for accumulation (Foguth et al., 2019). Giesy and Kannan (2001) measured concentrations of PFOA and PFOS amongst other compounds across different fish-eating predatory species and found exceeding concentrations of PFOS across different species. The study highlighted increased concentrations in liver tissue, however, plasma samples also presented with a range of concentrations. While PFOS was measured extensively, PFOA was only sporadically detected above the quantification limit in a subset of samples, indicating the risk of bioaccumulation and biomagnification for PFOS rather than PFOA for higher trophic levels within the food chain. Prevedouros et al. (2006) and Ahrens et al. (2010) reported that bioconcentration factors for PFOA to PFDoA (C_8 to C_{12} PFCAs) increased with chain length suggesting a higher risk for bioaccumulation and biomagnification for longer chains however, considering the identical chain length of PFOA and PFOS the influence of functional groups needs to be assessed.

2.3.2.5. Degradation of Perfluoro Carboxylic Acids

Degradation of PFCA precursors like fluorotelomer alcohols is discussed in various contexts however, information for PFCAs themselves is limited (Sinclair and Kannan, 2006, Liu et al., 2007, Plumlee et al., 2009, Liu et al., 2010, Anderson et al., 2016, Anumol et al., 2016, Zhi and Liu, 2018, Abunada et al., 2020). Sinclair and Kannan (2006) observed increases in mass flows during wastewater treatment for multiple PFCAs, attributing this observation to the degradation of precursor compounds (e.g. fluorotelomers (FT)). The biotransformation of 8:2 and 6:2 fluorotelomer alcohols (FTOHs) in pure cultures of soil bacteria (*Pseudomonas*) has been described and highlighted degradation rates depending on solvents involved in the experiment (Liu et al., 2007, Liu et al., 2010). They found that the degradation of PFOA alone was not able to sustain the bacterial cultures, indicating a co-metabolic process (Liu et al., 2007). Schröder (2003) reported that none of the perfluoroalkyl acids (PFAAs) investigated in their study underwent any degradation or mineralisation during treatment under aerobic conditions during a closed loop batch reactor experiment. However, under anaerobic conditions PFOA was metabolised within 25 days (Schröder, 2003). Overall, none of the reviewed studies further elaborated on the degradation of PFCAs. The degradation of fluorinated carboxylic acids through decarboxylation, reductive defluorination

and hydrolytic defluorination has been described, however, significant defluorination has only been observed for monofluorinated carboxylic acids (Key et al., 1997). Prevedouros et al. (2006) and Jahnke and Berger (2009) stated that PFCAs are generally not believed to undergo any metabolic or otherwise degradation in the environment. Key et al. (1997) further elucidated the principle of enzyme inhibition prohibiting biological degradation, stating that perfluorinated agrochemicals and their innate rigidness block non-target molecule-enzyme receptors from appropriately operating, inhibiting the access for non-fluorinated analogues and fluorinated compounds alike. The formed complex also protects the compounds from biological attack which further contributes to the environmental risk of PFCAs (Key et al., 1997).

2.4. Experimental Procedures

Biosolids and soils are complex environmental matrices. Understanding their chemical compositions necessitates appropriate sample preparation, extraction and optionally, purification prior to analysis. Karnjanapiboonwong et al. (2011) discussed that concentrations of micropollutants vary greatly between the two media, with ng/g ranges in soil and possibly µg/g ranges in biosolids. Different orders of magnitudes in expected concentrations need to be considered when selecting approaches for analysis. The available literature shows different approaches to sampling, starting with significant differences in the collection of samples.

2.4.1. Sample Collection and Preparation

Biosolids samples do not necessarily undergo any sample preparation or pre-treatment prior to extraction for analyses. Biosolids, usually already dewatered and thermally dried, occasionally undergo further drying steps to remove residual moisture, and are ground or milled to maximise the available surface area prior to extraction (Fell, 2022, Li et al., 2021a, Zhang et al., 2018, Zuloaga et al., 2012).

Soil sampling depths range vastly depending on publications. The Sewage Sludge Directive 86/278/EEC advises a soil sampling depth of 25 cm, however, it states that a soil core of 10 cm may be sufficient (European Commision, 1986). Most commonly, soil sample cores ranging between 10 and 30 cm depth are utilised for studies concerning micropollutants such as PFASs, often including PFCAs (Anderson et al., 2016, Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Cao et al., 2019,

Jodeh, 2013, Kikuchi et al., 2018, Lorenzo et al., 2015, Sepulvado et al., 2011), occasionally, smaller sample cores (0– 5 cm) are found (Golet et al., 2002, Rhind et al., 2002, Xu et al., 2009). Studies investigating vertical concentration profiles may retrieve soil cores ranging from 75 to 150 cm depth (Karnjanapiboonwong et al., 2011, Biel-Maeso et al., 2019a).

Notably, Sepulvado et al. (2011) and Biel-Maeso et al. (2018) highlighted that hydrophobic compounds can infiltrate deeper layers of soil and discuss transport processes, such as leaching. Sepulvado et al. (2011) further elaborated that PFCA concentrations in deeper layers outweigh concentrations in surface layers and elaborate that the effect is increasing with the chain length of compounds. Correspondingly, it can be concluded that the sampling depth has a significant influence on the study outcome and sampling depths within one study must be consistent. However, with studies utilising varying sampling depths of soil impedes the comparison of chemical loads and results overall.

Soil sample preparation typically includes an initial drying step. Temperatures for this vary from freeze drying at -80 °C (Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Cao et al., 2019, Kikuchi et al., 2018), and air drying at room temperature (Karnjanapiboonwong et al., 2011, Kumirska et al., 2015, Lorenzo et al., 2015, Rhind et al., 2002, Xu et al., 2009) to oven drying at up to 40 °C (Golet et al., 2002). Chemical drying is achieved by the addition of a drying agent, which reduces the overall concentration of the sample while freeze- air and oven drying does not alter the composition of samples. For chemical drying, Berset et al. (1999) identified a lower risk of degradation and a minimised exposure to the atmosphere, potentially resulting in higher concentrations, however, processed samples may present with a reduced reproducibility and the sample itself is diluted as sodium sulphate is added as a drying agent. Freeze-drying and air- or oven-drying were found to be more time-consuming depending on sample size, however, no significant differences in concentrations were found for most compounds (Berset et al., 1999, Wilcke et al., 2003). Elevated temperatures can lead to analyte losses depending on the volatility of the compounds of interest, meaning that suitable temperatures need to be identified (Berset et al., 1999, Wilcke et al., 2003). Dried samples are ground or milled in a subset of publications (Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Cao et al., 2019, Kumirska et al., 2015, Xu et al., 2009) or passed through a sieve or mesh, most commonly with

a 2 mm grain size (Jodeh, 2013, Karnjanapiboonwong et al., 2011, Lorenzo et al., 2015, Sepulvado et al., 2011), however, smaller mesh can also be utilised (Cao et al., 2019, Golet et al., 2002, Kumirska et al., 2015).

The extend of soil characterisation is greatly different between publications, and can include the determination of soil grain particle sizes (Golet et al., 2002, Xu et al., 2009, Jodeh, 2013, Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a), pH (Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Cao et al., 2019, Golet et al., 2002, Kumirska et al., 2015, Rhind et al., 2002, Xu et al., 2009), organic carbon (Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Golet et al., 2002, Kumirska et al., 2015, Sepulvado et al., 2011) or organic matter content (loss-on-ignition method) (Rhind et al., 2002, Xu et al., 2009, Jodeh, 2013, Kikuchi et al., 2018) and cationic exchange capacity (Biel-Maeso et al., 2018, Biel-Maeso et al., 2019a, Kumirska et al., 2015). Other soil parameters, such as phosphorous content and surface area may also be determined.

2.4.2. Sample Extraction

Similarly, extraction and analysis methods employed to recover and quantify pollutants from environmental samples do vary, depending on publication, group of compounds and scope of the respective studies.

Micropollutants in aqueous samples (e.g. river water, wastewater influents and effluents) are subjected to various extraction techniques, including solid phase extraction (SPE) or pressurised liquid extraction (PLE), usually with liquid chromatography – mass spectrometry (LC-MS) detection methods (Karnjanapiboonwong et al., 2011, Li et al., 2021a, Li et al., 2021b, Ruff et al., 2015). Solid sample matrices, soil, raw sludges and biosolids often undergo ultrasound-assisted extraction (UAE) and LC analysis (Biel-Maeso et al., 2018, Gago-Ferrero et al., 2015, Li et al., 2021a, Li et al., 2021b, Niemi et al., 2022) or analysis with gas chromatography (GC) (Kumirska et al., 2019, Maurer et al., 2020). The analysis of selected volatile and semi-volatile PFASs (mainly FTOHs, perfluorooctane sulfonamides (FOSAs) and perfluorooctane sulfonamido ethanols (FOSEs)) in air samples has been shown with sorbent impregnated polyurethane foam (PUF) disk samplers (SIPs) for sampling purposes, soxhlet extraction and gas chromatography hyphenated with mass spectrometry (GC-MS) (Ahrens et al., 2011a, Li et al., 2011, Shoeib et al., 2006). For other environmental samples, such as sediments, soils and biosolids, UAE with sonication baths is most commonly

selected as it presents as a straightforward and affordable extraction technique compared to other more costly or complex extraction methods (Higgins et al., 2005, Lorenzo et al., 2015, Mejia-Avendaño et al., 2017a, Mejia-Avendaño et al., 2017b, Rankin et al., 2016, Tian et al., 2021). However, Sampsonidis (2019) and Fell (2022) utilised an indirect sonication reactor (sonotrode) for the extraction of hydrocarbons from liquid samples and the extraction of pharmaceuticals from biosolids, respectively, highlighting a unique, robust and highly efficient alternative to commonly employed sonication baths. Irrespective of the sonication device, soils and sediments undergoing UAE are introduced to a vessel with a solvent or solvent mixture and exposed to acoustic waves, leading to the mass transfer of pollutants from solid particles into the receiving solvent.

2.4.3. Sample Analysis

Gas and liquid chromatography are both frequently utilised to analyse environmental sample extracts in regard to micropollutant concentrations. Fell (2022) highlighted the advantages of GC, including the sustainability and lower cost compared to LC analysis and addresses limitations, such as the size and polarity restrictions applying to compounds subjected to GC analysis. Compounds analysed by GC often have to undergo derivatisation, substituting labile hydrogen atoms within the compound with more volatile groups (e.g. trimethylsilyl groups (TMS)) and improving volatility of compounds overall.

While GC-MS is applicable to a wide variety of compounds, e.g. PPCPs (Biel-Maeso et al., 2019a, Fell, 2022, Kumirska et al., 2019, Kumirska et al., 2015, Xu et al., 2009), only a selection of PFASs can be analysed using the technique, due to the limited volatility of the majority of compounds in the class. Fluorinated GC target compounds within the class of PFASs include FTOHs, fluorotelomer carboxylic acids (FTCAs), PFCAs, FOSAs, FOSEs and polyfluoroalkyl diphosphate esters (di-PAPs) (Li et al., 2011, Monteleone et al., 2012, Shoeib et al., 2008, Shoeib et al., 2006, Tian et al., 2018, Yao et al., 2018).

LC-MS is often engaged for the analysis of pollutants in complex matrices despite the increased risk of matrix interferences (ion suppression and ion enhancement), making additional steps such as increased sample preparation (e.g. solid phase extraction for purification purposes) and the application internal standards essential for successful analysis (Fell, 2022, Gago-Ferrero et al., 2015, Martín et al., 2010, Yan et al., 2014). Nevertheless, LC provides a powerful and robust

analytical method to detect a wide variety of contaminants with quantification limits as little as 5 ng/g dry weight of sewage sludge products (Fell, 2022, Gago-Ferrero et al., 2015, Martín et al., 2010, Nieto et al., 2010, Ulvi et al., 2022, Yan et al., 2014) Notably, liquid chromatography and mass spectrometric detection are also most commonly employed for the majority of PFASs due to the ease of ionising soluble analytes using electrospray ionisation (Higgins et al., 2005, Lorenzo et al., 2015, Mejia-Avendaño et al., 2017b, Rankin et al., 2016).

2.5. Conclusion

In this chapter, an introduction to sustainability and the governance of chemicals was presented, highlighting the Stockholm Convention as landmark achievement, banning or restricting a number of contaminants known to have adverse effects, and preventing the unintentional production and release of chemicals.

Wastewater treatment processes, sludge treatment and sewage sludge application are highlighted as entry routes for pollutants into the environment, however, the tracing and measurement of pollutants can be difficult due to differing behaviour of compounds, dictated by physico-chemical properties such as partitioning coefficients and hydrophobicity.

Furthermore, emerging contaminants, their entry routes into the environment and adverse effects, were discussed in general, and more specifically for perfluoro alkyl substances, with an extended focus on perfluoro carboxylic acids. Notably, PFCAs are somewhat similar in regard to their persistence in the environment, and are mainly divided into long chain and short chain compounds when assessing the extent of adverse effects.

Sample preparation and extraction and analytical methods employed for the detection of micropollutants in the environment vary depending on sample matrices and the selection of targets, however gas and liquid chromatography in particular in combination with mass spectrometry are of integral importance in the concentration determination.

The following chapter, discusses sampling, instrumental and analytical techniques employed to determine PFASs in complex environmental matrices of importance for this research.

Chapter 3

Technical Background

3.1. Extraction Techniques

Adequate sample extraction is required to allow any form of analytical measurement irrespective of the sample as untreated complex samples cannot enter analytical instruments. As a result, micropollutants of interest need to be extracted from environmental matrices and introduced into a suitable medium, usually analytical grade solvents (e.g. LC-MS grade with >99.9 % purity) prior to analysis. While it is often desirable to use the same extraction techniques between different sample types, settings during the procedure itself may be adjusted to address differences in matrices.

Nieto et al. (2010) and Zuloaga et al. (2012) composed comprehensive reviews of classical and novel extraction techniques. Traditional extraction techniques such as Soxhlet and Soxtet (automated Soxhlet extraction) are described as labour-intensive, time and solvent consuming but still find application today (Ahrens, 2011, Jaffe and Hites, 1985, Li et al., 2011, Shoeib et al., 2008, Shoeib et al., 2006, Tian et al., 2021). Liquid/liquid extraction also requires varying amounts of solvents, however, modern techniques potentially reduce the required time and solvent consumption through the utilisation of ultrasound (ultrasound-assisted extraction, UAE), pressure (pressurised liquid extraction, PLE), or the use of microwaves (microwave-assisted extraction, MAE).

3.1.1. Ultrasound-assisted Extraction

Sonochemistry, the utilisation of acoustic waves to impact chemical processes, is often investigated towards novel method development due to the associated energy conservation and waste minimisation (Mason and Cintas, 2002). Ultrasound utilised in sonochemistry conventionally has a wavelength frequency of 20 kHz to 100 kHz, however, an extended range of up to 2 MHz can be utilised depending on the application. Most commonly used frequencies are 40kHz for sonication baths and 20 kHz for sonication probes (Mason and Cintas, 2002). Irradiating a liquid phase with ultrasound leads to compression (positive pressure) and expansion events (negative pressure) as sound waves pass through the medium, which exceeds the attractive forces of the solvent molecules, leading to the formation

of bubbles (Figure 3-1). Subsequent compression and expansion cycles lead to the growth of the bubbles, and eventually lead to bubble implosion, or cavitation. Within the cavitation bubble high temperatures (1000's °C) and pressures (100's of atm) are achieved, whereas the bubble-liquid interface has less extreme conditions while also experiencing shockwaves and the bulk liquid predominantly experiences intense shear forces (Mason and Cintas, 2002). The continuous formation, growth and collapse of bubbles leads to disruption and agitation throughout the liquid phase, giving rise to the physical and chemical effects. While this might be exploited to increase chemical reaction rates and to influence chemical balance, processes like emulsification, homogenisation, size reduction and extraction may also be achieved (Richards and Loomis, 1927, Mason and Cintas, 2002).

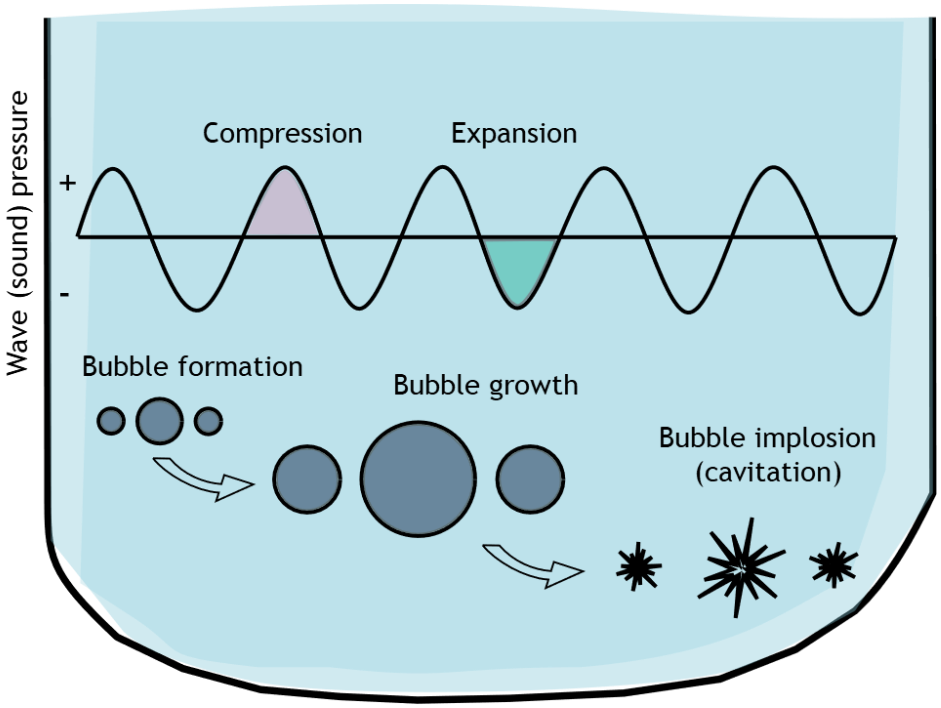


Figure 3-1: Propagation of ultrasound in a liquid phase. As acoustic waves travel through a liquid, compression and expansion events occur, leading to the formation of bubbles. Following the formation and growth, bubbles eventually implode, and cavitation occurs. Cavitation leads to a localised increase in temperature and pressure that can be exploited for extraction purposes.

For ultrasound-assisted extraction (UAE) in particular, Bossio et al. (2008) elaborated how cavitation events cause a considerable outward propagation of the liquid phase (solvent) promoting collisions with sample matrix particles, breaking up the matrix and therefore increasing the surface area that is exposed during the process, aiding the extraction process. Albero et al. (2019) further discussed necessary optimisation steps that are needed for UAE parameters,

including the selection of solvent, sonication time, amplitude (energy), sample/solvent ratio, and pulsing. The use of sonication pulses in particular is of interest, as it allows for the repeated sonication of samples for fractions of seconds (i.e. 40 % pulse – 400 ms out of 1 second), minimising the heating effect of UAE and therefore, minimising the risk of undesired degradation in the process (Mason and Cintas, 2002).

UAE has been described in the literature, usually employing a sonication water bath (Higgins et al., 2005, Bossio et al., 2008, de Sousa et al., 2015, Gago-Ferrero et al., 2015, Lorenzo et al., 2015, Rankin et al., 2016, Mejia-Avendaño et al., 2017a, Mejia-Avendaño et al., 2017b, Tian et al., 2021). However, sonication baths have several shortcomings, including the efficiency dependency on the optimal sample position within the bath (location, immersion angle, depth) as well as the low effective energy reaching the reaction vessel, making it more difficult to reproduce results (Mason and Cintas, 2002). Direct sonication probes by contrast, allow the delivery of the full range of ultrasonic power to samples as probes are directly immersed into the sample vial rather than indirect immersion into sonication baths (Mason and Cintas, 2002). The energy delivery through probes is approximately 100-times higher than in sonication baths, allowing for significantly shortened extraction cycles (i.e. 5 – 10 minutes instead of 10 – 60 minutes) however, main shortcomings of sonication probes is the potential for cross-contamination and probe tip erosion (Albero et al., 2019, Mason and Cintas, 2002).

Furthermore, Mason and Cintas (2002) elaborated on sonication reactors as a third sonication application method. Sonication reactors are utilising ultrasonically vibrating walls and may have the shape of an actual reactor (e.g. block-like enclosing structure) or present as a clamping device allowing indirect sonication, holding reaction vessels in a tight grip delivering ultrasound directly to the glass wall of a vessel. Sonotrodes employing this method combine advantages of both, sonication bath and direct sonication probe, while eliminating disadvantages (Mason and Cintas, 2002). Firstly, the ultrasound is applied indirectly, though the walls of the sample vial, minimising the risk of cross-contamination and probe erosion. Secondly, as water of a bath is not required to deliver the ultrasound to the sample, the energy efficiency is upheld, allowing for short and precise extraction cycles. One disadvantage of sonication reactors is the increased stress

on vessels (e.g. glass vials), restricting the choice of vessel, or shortening the lifespan of used vials, however, the utilisation of reactors for micropollutant extraction purposes presents a highly reproducible and efficient approach for complex environmental matrices (Sampsonidis, 2019, Fell, 2022).

3.2. Separation Techniques

Following successful extractions, the most common approach to analysing environmental samples in regard to micropollutants is the application of targeted analysis. The approach allows the detection of an increasingly high number of compounds utilising different separation techniques (Gago-Ferrero et al., 2015, Ruff et al., 2015, Petrie et al., 2017, Proctor et al., 2019, Ng et al., 2022). The limitation of this approach is that compounds of interest must have been identified beforehand. Novel emerging contaminants of concern, metabolic products and possible transformation products are often not considered or simply missed in the detection. The addition of analytes hence requires additional method development, suspect screening, or compound elucidation in case of unexpectedly detected substances. Contrary to targeted analysis, non-target approaches can be employed to identify novel analytical targets, expanding the current knowledge on complex chemical mixture in the environment.

The separation of compounds based on physico-chemical properties, generally referred to as chromatography, can be achieved by the introduction of mixtures into a two-phase system. A chromatographic setup provides a stationary phase (e.g. a chromatographic column coated or packed with a sorbent) and a mobile phase (e.g. a gas or solvent moving through the stationary phase). Physico-chemical properties of the separated substances (e.g. solubility, polarity, mass, charge, etc.) can have a significant influence on the success of the separation. The combination of chromatography with suitable detection methods allows the conversion of chemical inputs to digital signals, assembling chromatograms detailing the abundance or intensity of separated components depending on their retention time within the separation system.

3.2.1. Liquid Chromatography

In liquid chromatography, the liquid mobile phase carries analytes to be separated through the column while the stationary phase retains substances to a degree. The most common application of LC in analytical laboratories is found in high

performance liquid chromatography (HPLC) employing chromatographic columns filled with porous fine particles creating a large inlet pressure, requiring high-pressure pumps, giving the method its name (Danielson, 2003, Yashin and Yashin, 2012). The principal conditions for the successful separation using LC is the solubility of analytes in the chosen mobile phase as well as the temporary sorption and complete desorption of analytes to the stationary phase in the process (Yamamoto, 2023, Yashin and Yashin, 2012). The time an analyte requires to travel through the chromatographic column, generally referred to as retention time, depends on the interactions between the three partakers – substance/mobile phase, substance/stationary phase and mobile phase/stationary phase (Yashin and Yashin, 2012).

3.2.1.1. Stationary Phases

Sorbents employed as stationary phases for LC can be categorised in silica and modified-silica, polymer types, adsorbents based on oxides and carbon adsorbents (Danielson, 2003, Yashin and Yashin, 2012). Utilised silicas have inherent advantages, such as high surface area and pressure resistance, however, silica-based packings can only be reliably utilised within a pH range of 2 – 7.5, as alkaline conditions can dissolve the silica and exceeding acidic conditions can lead to the cleavage of siloxane bonds holding functional groups (Danielson, 2003).

Silica is the commonly used (polar) stationary phase for normal phase LC (NP-LC) paired with a comparably less polar (unpolar) mobile phase leading to the greater retention of polar compounds (Yashin and Yashin, 2012). NP-LC is customarily dependent on adsorption as principal separation mechanism. Chromatography is achieved through the interaction of functional groups of analytes with the polar groups in the stationary phase (siloxanes) facilitated through hydrogen bonding forces (Danielson, 2003). Throughout the process, solvent and analytes are competing for the available siloxane groups in an adsorption-desorption equilibrium continuously engaging along the flow of the column. The use of more polar solvents (e.g. water or methanol) promotes hydrogen bonding, blocking available siloxane adsorption sites for analytes, hence decreasing the retention of analytes and is therefore not recommended (Danielson, 2003).

Contrary to that, reverse phase LC (RP-LC) utilises polar mobile phases in combination with comparably less polar (unpolar) stationary phases made from chemically-modified silica leading to greater retention of compounds with

increased hydrophobicity (Yashin and Yashin, 2012). Stationary phase modification is achieved by covalently attaching hydrocarbon chains (most commonly C_8 or C_{18}) to the silica base through silanisation reactions (Danielson, 2003). As the transformation of the silica surface is often incomplete leaving hydroxyl groups exposed and therefore available to interact with the mobile phase and analytes, additional treatments with smaller sized carbon chains (e.g. trimethyl chlorosiloxane) are used to "endcap" remaining functional groups of the silica backbone (Danielson, 2003). The chromatographic process in RP-LC is dependent on the phase partitioning coefficients of the analytes, accounting for the solvation of compounds in the mobile phase and the retention by the stationary phase (Danielson, 2003, Yashin and Yashin, 2012). The retention on the stationary phase is mediated by a number of forces, such as hydrogen bonding, dipole – dipole interactions and van-der-Waals forces, however, the latter are the predominant mediator due to interactions between hydrophobic or non-polar groups of analytes and the mobile phase (Danielson, 2003).

While other mechanisms for liquid chromatography, such as ion-exchange (separation of mixtures of anions or cations), size-exclusion (separation of macromolecules) and chiral (sorption of chiral centres to the stationary phase) or the combination of mechanisms do achieve retention, RP-LC is the predominant variant finding application in analytical practice (Yashin and Yashin, 2012).

Danielson (2003) furthermore elaborated on approaches that are used to optimise the separation most commonly beyond the adjustment of flow rates and solvent strengths, including the utilisation of mobile phase gradients, temperature programming and column switching. While it is possible to engage LC separation with an isocratic (unchanged) mobile phase composition, changes to the mixture during chromatography can aid the analysis by decreasing the retention time of later-eluting analytes, while also being able to retain fast-eluting compounds for longer, depending on the setup (Yashin and Yashin, 2012). Overall, Danielson (2003) concluded that the use of gradients during elution improve reproducibility and allow for a higher sample throughput. Temperature programming and column switching can also result in improved separation and sharper peaks with better sensitivity but are less frequently utilised (Danielson, 2003).

3.2.1.2. Mobile Phases

Mobile phase composition utilised for RP-LC most commonly consists of a combination of water and methanol (Higgins et al., 2005, Lorenzo et al., 2015, D'Agostino and Mabury, 2017, Proctor et al., 2019, Skaar et al., 2019), however, combinations with acetonitrile (Santiago et al., 2016, Peng et al., 2018) are also often employed. Generally, an increase in the water fraction of the mobile phase will enhance retention whereas an increase in the organic fraction of the mobile phase leads to a reduced retention during separation (Danielson, 2003). Additives (buffers) frequently used range from formic acid (Santiago et al., 2016, Peng et al., 2018) and ammonium formate (Gago-Ferrero et al., 2015, Lorenzo et al., 2015) to ammonium acetate (Higgins et al., 2005, Gago-Ferrero et al., 2015, Skaar et al., 2019) in concentrations commonly ranging from 1-5 mmol/L. The addition of small amounts of ions results in the improved retention of strongly polar analytes whereas the use of unbuffered mobile phases leads to the fast elution without adequate separation (Danielson, 2003).

RP-LC and its application in the analysis of mixtures (e.g. wastewater extracts, serum extracts) is useful in a multitude of areas (e.g. environmental analysis, food analysis, quality control). The available variety of detectors to work with liquid chromatography (e.g. UV-Vis spectrophotometers or mass spectrometers) is well suited for the separation of non-volatile compounds in moderately complex aqueous samples, however, gas chromatography, and the analysis of volatile compounds in more complex samples can be a valuable addition.

3.2.2. Gas Chromatography

In gas chromatography a gaseous mobile phase carries analytes to be separated through a column while the stationary phase retains substances to a degree, ultimately supplying separated compounds to a detector producing chromatograms. The volatility and resistance to decomposition of analytes are the principal conditions for the successful separation using GC (Yamamoto, 2023). Contrary to LC analysis, the time an analyte requires to travel through the chromatographic column depends solely on the interactions between the analytes and stationary phase, while the mobile phase carries the analyte along, and does not affect selectivity of the separation (Yashin and Yashin, 2012).

3.2.2.1. Stationary Phases

Analytical columns utilised in GC can be divided into packed and capillary columns. Packed columns primarily find application in the measurement of gases, stationary phases of such columns are comparable to LC phases consisting of silica or metal oxides albeit with different pore sizes and dimensions generally (McNair et al., 2019). Capillary columns can be employed to analyse a multitude of compounds and mixtures, and are further subcategorised according to the stationary phase. Wall-coated open tubular (WCOT) columns are extensively used and have a liquid stationary phase bonded to a fused silica backbone; porous-layer open tubular (PLOT) columns employ a thin layer of porous coated particles on the inside of the column whereas support-coated open tubular (SCOT) columns present as a hybrid consisting of a porous layer coated with a thin film of a stationary phase. It is estimated that approximately 80 % of all analysis are performed with WCOT columns (McNair et al., 2019).

Typical inner diameters of capillary columns range from 100 to 530 µm, however, diameters of 250 to 320 µm offer the best agreement of resolution, analysis speed and capacity (McNair et al., 2019). Furthermore, typical column lengths range from 30 – 60 meters but can reach up to 100 meters while conventional film thickness vary between 0.25 to 1.5 µm. Thicker films (>1 µm) offer increased retention capabilities permitting the analysis of larger sample volumes (McNair et al., 2019).

Polysiloxane stationary phases offer extensive chemical and thermal stability and have a wide range of polarities that can be achieved, depending on phase substitutions (Cordero et al., 2012). Low-polarity stationary phases (e.g. 100 % polydimethylsiloxane, 95 % dimethyl – 5 % diphenylpolysiloxane (Figure 3-2)) have versatile applications and cover approximately 80 % of application fields including the analysis of PAHs, brominated flame retardants, lipids and other micropollutants (Berset et al., 1999, Bossio et al., 2008, Portolés et al., 2015b, Maurer et al., 2020). Chromatographic separation using a pure (unipolar) polydimethylsiloxane is exclusively based on dispersion, whereas the introduction of diphenylpolysiloxane subunits lead to the formation of induced dipoles, rendering the stationary phase more polar and hence altering the analyte-stationary phase interactions (Cordero et al., 2012).

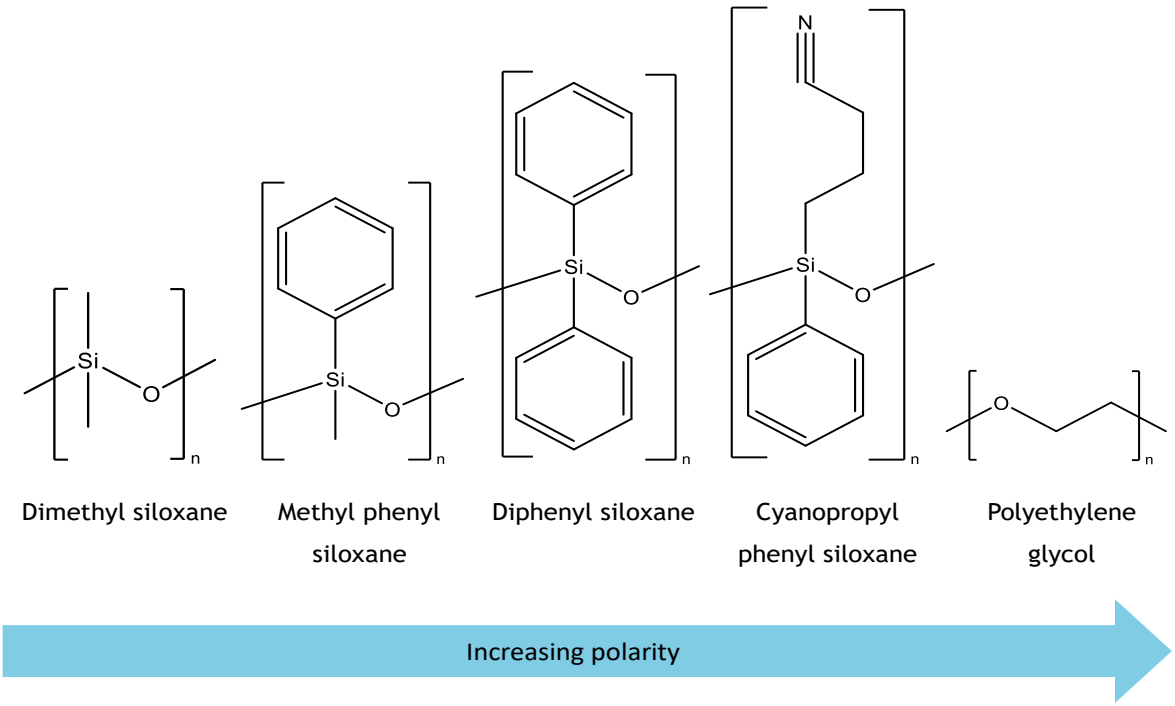


Figure 3-2: Common siloxane groups and polyethylene glycol utilised in capillary (WCOT) GC columns. Polarity increases with the increased utilisation of functional groups attached to the siloxane backbone of the stationary phase.

Further increasing the proportion of diphenylsiloxane, or integrating other monomers such as cyanopropylphenylsilane increases the phase polarity to a mid-range (McNair et al., 2019). Cyanopropylphenylsilane subunits introduce permanent dipole interactions as well as proton sharing, defining the phase retention of mid- to high polarity stationary phases (Cordero et al., 2012). While other substitutions in the polysiloxane polymer are available the described functional groups improve the separation capabilities significantly by introducing additional interactions in addition to the basic dispersion forces found in 100 % dimethylsiloxane (McNair et al., 2019).

In contrast to silicon-based stationary phases, other stationary phases such as polyethylene glycol are able to achieve higher phase polarities (Figure 3-2). The separation of mid- to high-polarity analytes can be achieved on these stationary phases, however, more restrictive operational temperature ranges potentially limit the use (Cordero et al., 2012).

3.2.2.2. Mobile Phases

The commonly utilised GC mobile phases consist of high purity nitrogen, helium or hydrogen. As the mobile phases in GC remain inert, the key liability is to move injected analytes along the chromatographic column to eventually reach the

detector. Directional flow is achieved by applying a carrier gas pressure at the column inlet, towards the column outlet however, most analysis are regulated to achieve a constant flow rate rather than constant pressure, meaning that with temperature changes, the pressure has to be adjusted to maintain flow rates appropriately (Jennings et al., 1997).

The optimum range for column flow rates depends on the column geometry as well as the choice of mobile phase. The choice of helium as a carrier gas balances separation efficiency with an intermediate average flow velocity (1.4 – 2.0 mL/min optimum). While hydrogen does have a better efficiency and allows for faster separations, it is often not selected due to safety concerns of employed gas cylinders of the gas itself. Nitrogen by contrast is less efficient and hence finds less application (Jennings et al., 1997).

Differences in carrier gases can be assessed using the van Deemter equation:

$$H = A + \frac{B}{u} + Cu \qquad (1)$$

With the theoretical plate height H, the average flow velocity u, and contributing factors A (Eddy diffusion), B (longitudinal diffusion) and C (resistance to mass transfer).

A theoretical plate is a hypothetical zone in which both phases, stationary and mobile, and the analytes in question establish an equilibrium with each other. Plate theory describes the occurrence of separation through a series of equilibrium stages. The theoretical plate height is generally considered a measure of efficiency in both gas and liquid chromatography with a small plate height equalling narrow peaks and therefore, better separation.

All contributing factors, A, B and C, account for peak broadening during the chromatographic process. Eddy diffusion occurs in conjunction with packed columns, and is therefore relevant in liquid chromatography while not contributing to peak broadening in WCOT columns commonly employed in modern analytical GC. In LC however, eddy diffusion is depending on the particle size of the stationary phase. Longitudinal diffusion (B) causes the movement of analytes from high-concentration centres to lower-concentration edges of a band, hence broadening detected peaks. Changes in flow rate can address the extend of longitudinal diffusion, however, beyond optimal flow rates benefits may diminish

(Jennings et al., 1997). The resistance to the mass transfer depends on the affinity of analytes to the stationary phase leading to the increased retention of analytes with stronger interactions with the stationary phase, while lesser interacting analytes get moved along with the carrier gas (Jennings et al., 1997).

3.2.2.3. Sample Injection

Split/splittless (SSL) inlets are the most commonly utilised type of inlet in GC, as it allows for two modes of injection. In both cases, a liquid sample is introduced (e.g. manually with a syringe or by autosampler), rapidly volatilised at high temperatures before the gaseous mixture is released to be separated on the chromatographic column. The rapid expansion during vaporisation within the constraint of the inlet and the relatively small amount of stationary phase available in the thin film coating inside the chromatographic column limit injection volumes to usually less than 10 µL (McNair et al., 2019). In splitless mode, the complete volatilised sample is pushed onto the column for separation, whereas split mode allows only a pre-determined fraction of the injected mixture to proceed to analysis, while the larger fraction is removed through a vent valve. Splitless injection can aid with the separation of trace level concentrations while the operation in split mode is advantageous when working with high concentration samples (McNair et al., 2019).

3.2.2.4. Considerations for Optimisation

Chromatographic performance can be optimised beyond the column and carrier gas selection and enables good resolution GC below the optimal flow velocity. However, effects arising from the resistance of the mass transfer will still occur with sharp early eluting peaks, whereas late eluting peaks are broader. GC oven programming is extensively used to control peak retention times while temperature programming in LC remains marginalised (Danielson, 2003). In principle, an increase in oven temperature will promote faster elution of analytes, hence reducing the retention time, sharpening peak shape and improving column efficiency (Danielson, 2003).

Most oven programs include plateaus and ramps increasing temperatures at defined time points and rates, gradually reducing the retention times of late eluting analytes. Routinely oven programs also include a final temperature increase nearing the maximum column temperature in order to ensure the complete stripping of analytes from the stationary phase. McNair et al. (2019)

highlighted the disadvantages of this approach, including noisier signals at higher temperatures and the cooling required to return to starting conditions following an analytical run potentially increasing the run time overall.

GC does present with considerable benefits, including its non-destructiveness, the comparable low cost of analysis and greener credentials as it does not create additional waste streams (Fell, 2022). However, its major drawback, besides the need for spectroscopic confirmation of peak identities is the reliance on the volatility of analytes.

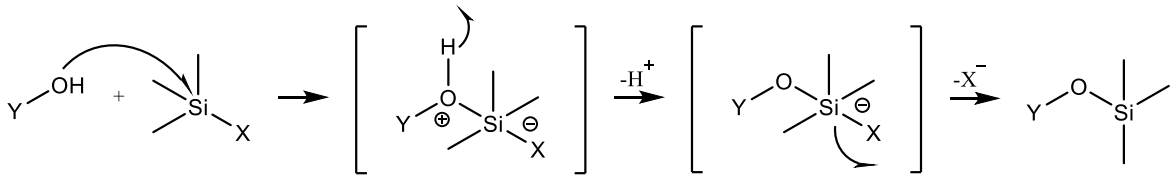


Figure 3-3: Derivatisation principle. Derivatisation with a silylation agent (X-TMS, e.g. N-trimethylsilyl-N-methyltrifluoroacetamide (MSTFA)) follows a second order nucleophilic substitution (S_N2) whereas the nucleophile group (e.g. -OH or -NH group) of a substance (Y-OH) "backside attacks" the electrophile trimethylsilyl group. The formation of a covalent bond between the two reactants causes the release of the labile hydrogen of the nucleophile ultimately also forcing the leaving group (X-) to leave in order to achieve a stable derivate. S_N2 reactions occur in a single step, intermediates (shown in brackets) are shown to illustrate reaction steps occurring simultaneously. Adapted from Fell (2022).

The volatility of a subset of compounds can be altered through derivatisation reactions, replacing labile hydrogen groups with less polar moieties, e.g. trimethyl siloxanes (Figure 3-3) (Fell, 2022). While derivatisation does increase the overall weight of the molecule, the reduced polarity results in better volatilisation and hence, potentially allows the analysis with GC that would not be possible with the non-derivatised counterpart. For example, most PFASs are not volatile enough to be analysed by GC, however, a small selection of compounds, including fluorotelomer alcohols and perfluoro carboxylic acids, can be derivatised forming a methyl ester that is more volatile hence accomplishing GC analysis (Hagen et al., 1981, Alzaga and Bayona, 2004).

3.3. Detection Techniques

Once mixtures of analytes are successfully separated by means of gas or liquid chromatography, a multitude of detectors are available to confirm the compound identity, usually in conjunction with analytical standards aiding the identification (Danielson, 2003, Cordero et al., 2012, Yashin and Yashin, 2012, McNair et al., 2019). Common GC detectors include flame ionisation detectors (FID), thermal conductivity detectors (TCD) or electron capture detectors (ECD) while LC setups can be hyphenated to detectors such as diode array detectors (DAD) or

fluorescence detectors (FLD) amongst others. While some detectors have excellent linear ranges (FID, DAD, FLD) or low-noise performance (FLD), others have conditional functionalities such as the inclusion of a chromophore (DAD), fluorophore (FLD) or similar to FIDs, have several exclusions that cannot be detected (e.g. nitrous oxides, formic acid, etc.) that may be detectable by other means (e.g TCD), however, alternative detectors may have a lower linear range. Overall, the advantages and disadvantages of the varied detection principles needs to be addressed before starting analysis.

3.3.1. Mass Spectrometry

By contrast, mass spectrometry is suitable for application in combination with both, gas and liquid chromatography in a range of matrices, concentrations and setups available. The fundamental principle of MS detection is based on the separation and abundance measurement of charged ions or molecules according to the ratio of mass and charge (m/z) (Kitson et al., 1996, Sleeman and Carter, 2005). Sleeman and Carter (2005) further elaborated, that by convention, the unit of mass used in MS analysis is the unified atomic mass unit (amu or u) which is defined as one-twelfth of the mass of a single ^{12}C carbon isotope. Apart from the extensive detection capabilities of a range of analytes, an inherent advantage of MS analysis is the possibility to gain additional structural information of the detected compounds. The combination of GC or LC with MS detection capabilities has long been a conscious choice due to its high sensitivity and specificity however, the associated technical complexity of MS can present a disadvantage (Pitt, 2009, Sleeman and Carter, 2005).

Detection with MS involves several steps, usually in a low-pressure environment: firstly, the chromatographically separated compounds need be isolated and introduced into the system to undergo ionisation, secondly, analyte ions need to be separated further with a combination of electrical and/or magnetic fields, and finally, detection takes place (Kissa, 2001, Kitson et al., 1996, Sleeman and Carter, 2005, Yamamoto, 2023). Each step of the process can be achieved by different means, with varying ionisation, separation and detection techniques available. Gas chromatography is most commonly available in a setup with an electron impact (EI) ion source, quadrupole ion separation and an electron multiplier for detection. Liquid chromatography often employs an electrospray ionisation (ESI), which can be combined with an orbitrap for separation and

detection purposes. Ionisation techniques address different analytical needs, while separation techniques apply the same principles in different approaches. Ultimately, electron multipliers and orbitraps allow the amplification of an electrical signal and subsequent conversion into a digital output. The resulting mass spectrum is a graphical representation of the abundance of observed ions (y-axis: intensity) depending on a mass-to-charge ratio (x-axis of the plot) (Kitson et al., 1996). The abundancies of ions are normalised to the highest peak of the spectrum (base peak, 100 % intensity or abundance), with all other ion abundancies shown as a corresponding percentage (Mellon, 2003). Sleeman and Carter (2005) highlighted the reproducibility of mass spectra under controlled acquisition conditions, allowing the comparison to standards as well as databases and libraries accumulating spectrometric information.

3.3.1.1. Mass Spectrometric Ionisation Techniques

The formation of gaseous analyte ions is a prerequisite for MS analysis, hence introduction of gaseous analyte from GC separation to an ion source is advantageous, however, techniques for the conversion of liquid sample matrices into gas phase sample ions have been developed. Non-gaseous analytes are transformed into gaseous state of aggregation before or during the ionisation process taking place in the ion source (Sleeman and Carter, 2005).

In GC, the separation depends on a foreline pressure of carrier gas, however, successful detection with MS depends on the maintenance of a high vacuum pressure, accelerating any and all compounds entering the ion source towards the mass analyser and electron multiplier for detection (Eljarrat and Barceló, 2005, Sleeman and Carter, 2005).

3.3.1.1.1. Electron Impact Ionisation

Within an electron impact (EI) ionisation source, a high voltage filament, usually made from tungsten or rhenium, is placed inside a magnetic field. The filament emits electrons, which are repelled towards an electron trap (collector) on the opposite side of the source body (Figure 3-4) (Eljarrat and Barceló, 2005). The vapourised analyte molecules are introduced into the ion source in an orthogonal path to the steady electron beam, to collide with the electrons. The magnets placed on either end of the electron beam induce a spiral motion of the electrons, increasing the probability of collision due to a longer path length (Harvey, 2019). Depending on the energy transfer between the molecule and a colliding or near-

passing electron, radical ions can be formed. The high energy (70 eV) of the bombardment electrons far exceeds the energy required to ionise most compounds (approx. 15 eV) (Eljarrat and Barceló, 2005). Most commonly, the collision with electrons causes the expulsion of a molecular electron, resulting in a positively charged ion radical. Additionally, anion radicals can be formed if an electron is captured by the compound. Millard (1977) stated that it is estimated that only approximately 0.1 % of all introduced analyte molecules are ionised, leaving the majority of molecules neutral and undetected. Once formed, fragments of the ion radical can be lost, either as radical fragment or neutral molecule or both, forming more chemically stable ions (Millard, 1977). Fragmented ions are then accelerated through extraction and focussing lenses, towards a mass analyser (Eljarrat and Barceló, 2005).

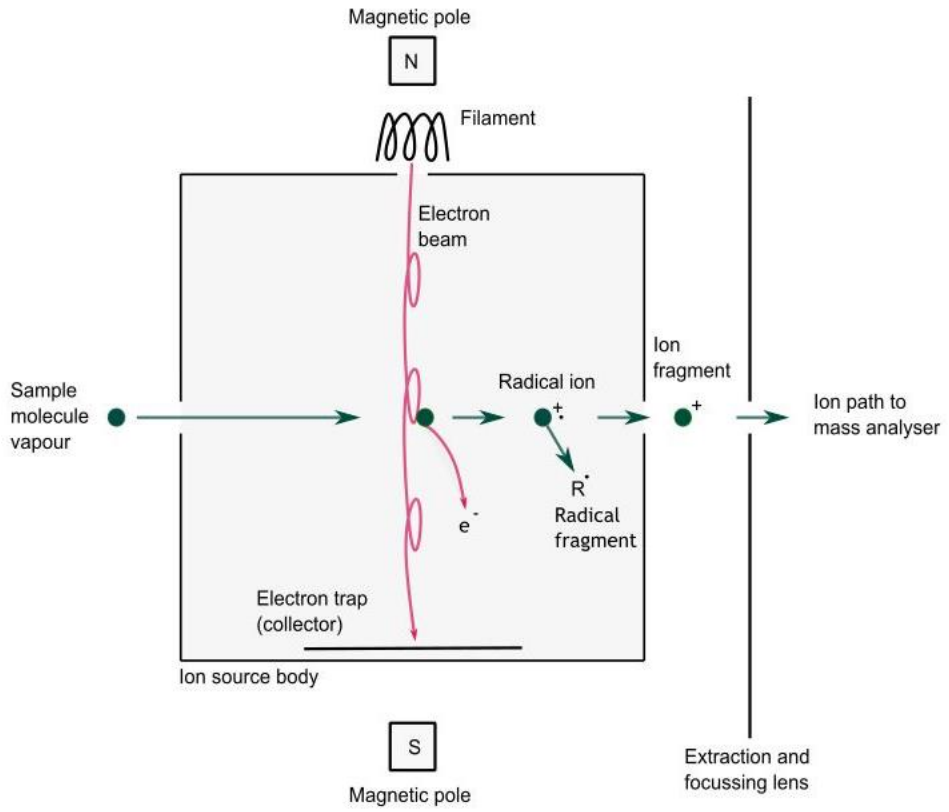


Figure 3-4: Principal structure of an electron impact ionisation source. Sample molecules pass through an electron beam. Collision with or near-passing of electrons leads to the loss of a compound electron, forming a radical ion. The presence of an unpaired electron leads to an unstable state, usually leading to the loss of a radical fragment. The resulting ion fragment is more stable and able to progress to a mass analyser in order to reach a detector subsequently. Modified from Eljarrat and Barceló (2005).

EI is considered a hard ionisation process leading to substantial fragmentation, ultimately limiting the detection of molecule ions (Sleeman and Carter, 2005). The output of fragments generated by EI sources is considered to be fairly constant as this type of ion source is usually operated in a narrow temperature range of 200

to 250 °C and conventionally utilises an electron energy of 70 eV, allowing the use of mass spectral libraries to aid in the identification of compounds. Millard (1977) elaborated that the detection of a greater number of fragments offers a greater amount of information regarding the structure of analytes. However, similarly to GC separation, limitations of this technique arise from the dependency on the volatility, size and polarity of compounds (Eljarrat and Barceló, 2005).

3.3.1.1.2. Electrospray Ionisation

Electrospray ionisation (ESI) is frequently used in combination with LC separations and due to its tendency to cause less fragmentation it is considered a soft ionisation technique (Pitt, 2009, Sleeman and Carter, 2005). As a result, molecule ion masses are more likely to be determined using ESI, however, due to the lack of fragmentation, and the resulting lack of structural detail, the definitive identification of compounds requires additional steps (e.g. tandem mass spectrometry) (Yamamoto, 2023).

ESI is considered an atmospheric pressure ionisation technique since the ionisation occurs at atmospheric pressure rather than within a vacuum system (Sleeman and Carter, 2005). Sample solutions are pumped through a capillary into the atmospheric source chamber, where an applied potential (voltage) and engaged nebuliser gas stream encourage the formation of electrostatically charged droplets, containing analyte ions (Figure 3-5) (Sleeman and Carter, 2005). With the additional exposure to a drying gas stream, droplets are consistently reduced in size, until desolvation is complete, leaving charged gaseous ions of analytes that are propelled towards the extraction and focussing lenses, and the high vacuum region of the ion source (Pitt, 2009, Sleeman and Carter, 2005). Similar to EI, positive and negatively charged ions can result from the ionisation process, but contrary to the EI process, ESI does not depend on electron impact events, making the occurrence more frequent and therefore, easier to monitor subsequently (Pitt, 2009).

The capillary column inlet and the MS entry point beyond the curtain plate are usually off-set by an angle as the introduction of droplets into the ion source increases the risk of contamination of the downstream MS system (Pitt, 2009). ESI is considered well suited for the analysis of moderately polar substances with less restrictive size requirements, allowing the analysis of chemical and biological analytes, e.g. pharmaceuticals, environmental contaminants and peptides (Pitt,

2009). Unlike EI, ESI is able to produce multiply-charged ions, lowering the mass-to-charge ratios, allowing the detection of molecules with masses ranging up to 100 kDa (e.g. proteins) with MS setups limited to 4000 m/z (Harvey, 2019, Pitt, 2009).

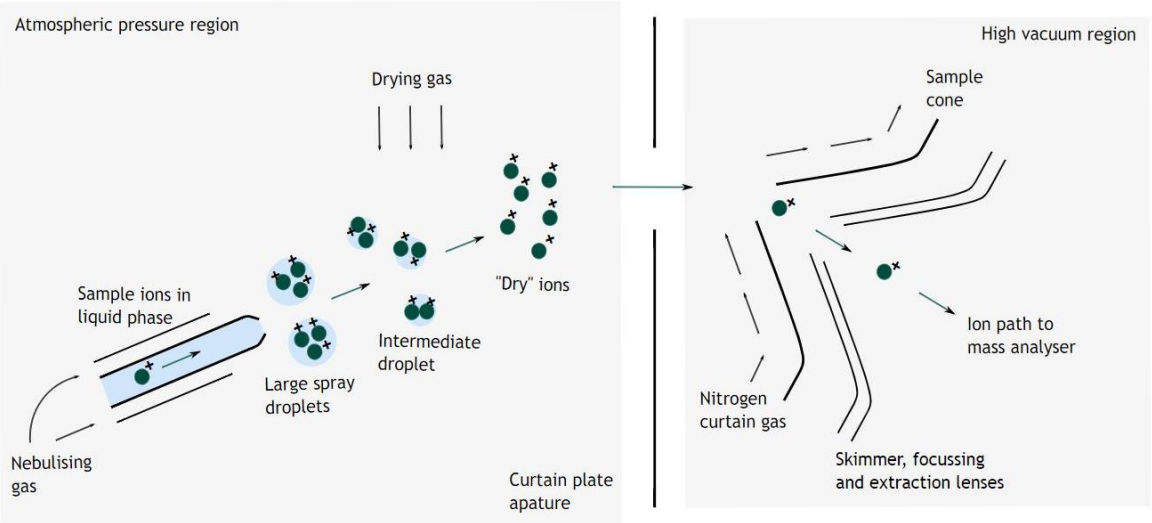


Figure 3-5: Principal structure of an electrospray ionisation source. The analyte solution passes through a capillary column outlet, forming a spray (promoted by nebulising gas and applied potential). Formed droplets are stepwise reduced and dried, leaving charged analyte ions to pass through an apature to the sampling cone. Successful ions progress to a mass analyser in order to reach a detector subsequently. Modified from Mellon (2003) and Harvey (2019).

3.3.1.2. Mass Spectrometric Ion Separation Techniques

The generation of analyte ions in ion sources can be considered the primary step of mass spectrometry, in addition to that, mass separation is required prior to detection. Separation can be achieved by different means, usually involving the exposure of ions to strong magnetic and/or electric fields, as well as the subjection to radiofrequencies and hence, separating masses according to their mass-to-charge ratio. The overall achievable resolution of the MS detection notably depends on the choice of mass analyser as capabilities to distinguish between m/z ratios varies greatly, with quadrupole analysers allowing the differentiation of ± 1 amu, whereas high-resolution MS (e.g. Orbitrap-MS) can determine accurate masses with ± 0.0001 amu (Kitson et al., 1996, Sleeman and Carter, 2005).

3.3.1.2.1. Quadrupole Mass Analyser

In essence, the name quadrupole (Q) accurately describes the principal structure of the mass analyser: four parallel cylindrical rod-shaped electrodes (Figure 3-6) (Kitson et al., 1996). A direct current (DC) voltage is applied to all four rods, with

diagonally located rods having the same polarities and a radiofrequency alternating current (AC) voltage is applied to all rods (Yamamoto, 2023). Ions emitted from the ion source are travelling along the axis of the quadrupole, drawn towards the detector by an applied low potential (Kitson et al., 1996). The motion of ions along the axis can be described as oscillation and is only successful if the quadrupole m/z restriction and actual ion m/z match (Mellon, 2003). Ion separation with quadrupole analysers require the systematic scanning with changing field strengths, as the narrow m/z window granted by the quadrupole only allows a limited number of ions to pass to the detector at one time, depending on their m/z ratio (Mellon, 2003, Sleeman and Carter, 2005).

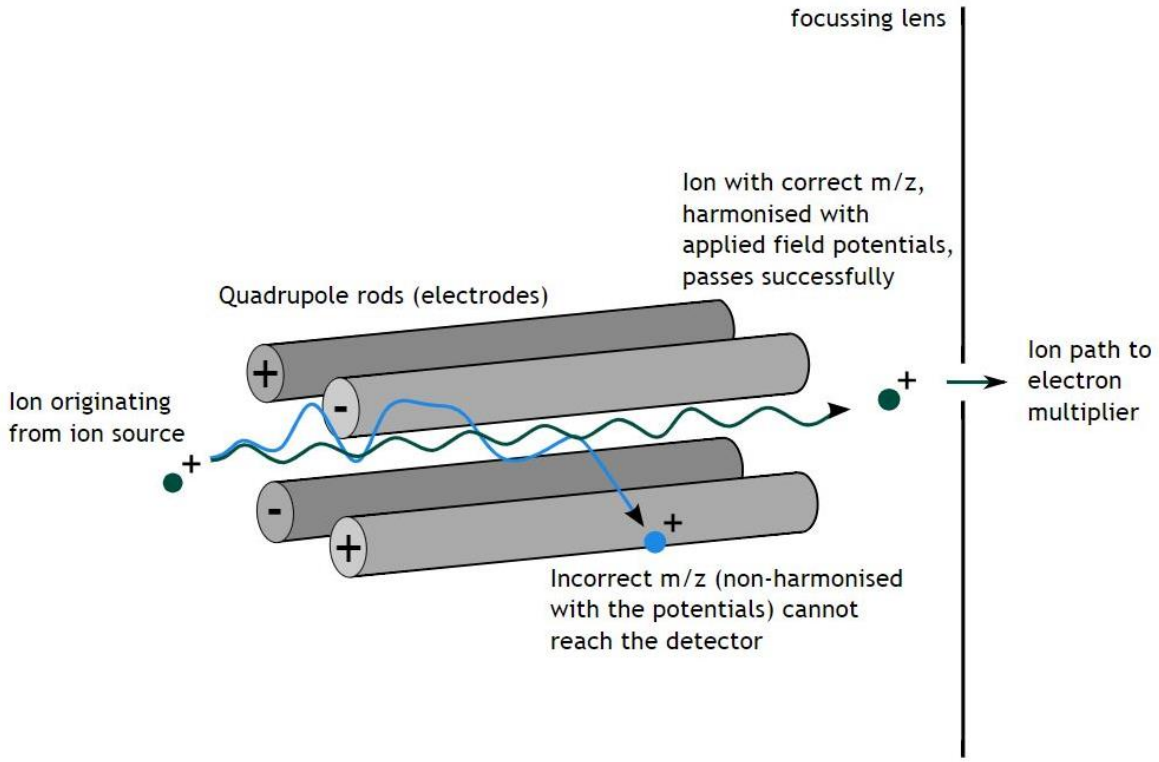


Figure 3-6: Principal structure of a quadrupole mass analyser. Consisting of four rods with varying AC/DC potentials, linking ion source and detector. Ions arriving from the source can only pass through the quadrupole of the m/z ratio and the applied potential match. The path through the quadrupole follows a harmonic oscillation, mismatched ion m/z ratios cannot successfully reach the electron multiplier (detector). Modified from Sleeman and Carter (2005).

The detection of successfully traversing ions using an electron multiplier (EM) in combination with quadrupole mass analysers depends on the conversion of the incoming stream of ions to electrons using a conversion plate (Figure 3-7) (Greaves and Roboz, 2014). For the detection of positive ions, the conversion plate (first dynode) is kept at a negative potential, whereas subsequent discrete dynodes have higher potentials, with the last plate at ground potential. Incoming ions promote the release of electrons from the dynode material upon striking the

surface effectively converting the signal into an electrical current (Mellon, 2003). The arrangement of dynodes within the EM creates a stream with an increasing number of electrons, effectively amplifying the electrical signal by cascading from dynode to dynode (Mellon, 2003). Positively charged ions resulting from EI or positive ESI can be detected following this principle, however, in order to monitor negative ions the potential of the first receiving plate (conversion plate or dynode) needs to be adjusted (Kitson et al., 1996).

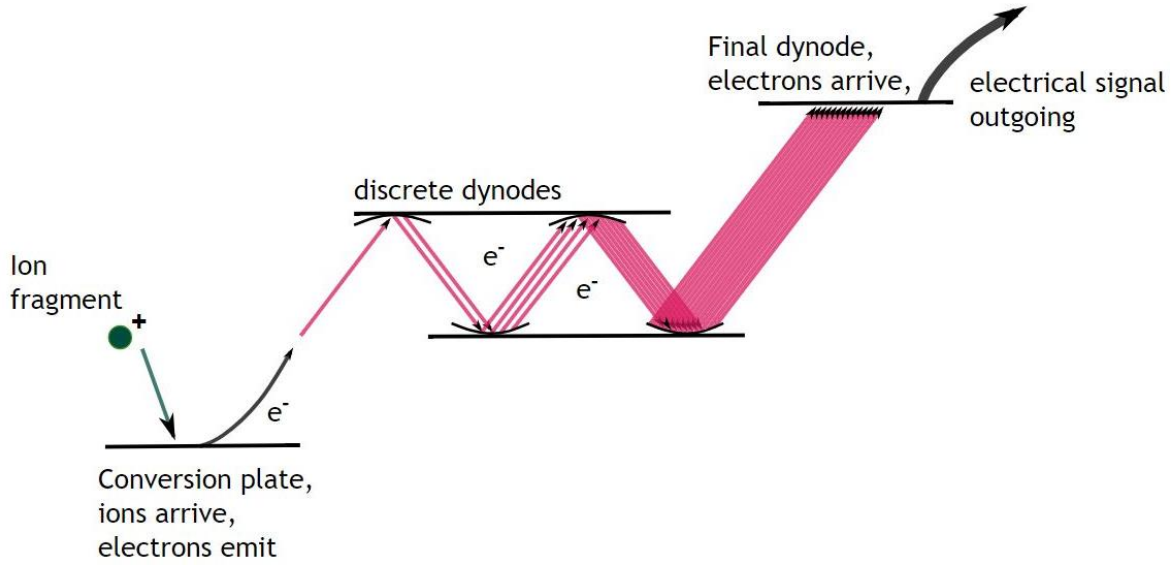


Figure 3-7: Principal structure of an electron multiplier. Analyte ions arriving at the conversion plate are attracted by a potential. The impact of incoming ions is followed by the release of electrons from the dynode material. The sequential use of discrete dynodes allows for the ever increasing number of electrons to amplify the signal, which is subsequently transformed into an electronic read-out. Modified from Greaves and Roboz (2014).

3.3.1.2.2. Orbitrap Mass Analyser

Orbitraps are relatively new (early 2000s') mass analysers requiring an advanced setup with stable ultra-high vacuum in the 10^{-10} Torr range and significant financial commitment, however, an orbitrap analyser offers excellent mass accuracy and high resolution presenting extensive application possibilities (Greaves and Roboz, 2014). The mass analyser consists of three electrodes, firstly, a central, spindle-shaped inner electrode which is held by two end-spacers (Figure 3-8) (Zubarev and Makarov, 2013). Secondly, two cup-shaped outer electrodes, separated by only a small gap, surrounding the central electrode. A voltage is applied between the electrodes, resulting in an electrical field along the linear axis of the trap (Zubarev and Makarov, 2013). Ions originating from the ion source are passed through an ion trap, allowing the high-speed pulsing of ion packets (rather than a continuous stream of ions similar to the quadrupole) to reach the orbitrap (Zubarev and

Makarov, 2013). The injection of ions at high speed in combination with the electrical field between the inner and outer electrodes induces a convoluted movement within the trap, consisting of a rotatory movement around the inner electrode as well as axial oscillation along the linear axis of the central electrode (Greaves and Roboz, 2014). The harmonic oscillating motion of ions within the trap leads to an induced current in the split outer electrodes that is proportional to the m/z of the respective ion and can be analysed with Fourier-transformation analysis, resulting in excellent mass analysing properties (Greaves and Roboz, 2014). Notably, one of the biggest differences to quadrupoles, besides the pulsing of ions, is the measurement of an image current rather than an ion current (Greaves and Roboz, 2014). The extensive dynamic range and resolution possibilities provide substantial benefits of orbitrap analysers, however, limitations of the orbitrap are decreases in resolution depending on the data collection speed and decreases in conjunction with increasing mass (Greaves and Roboz, 2014, Zubarev and Makarov, 2013).

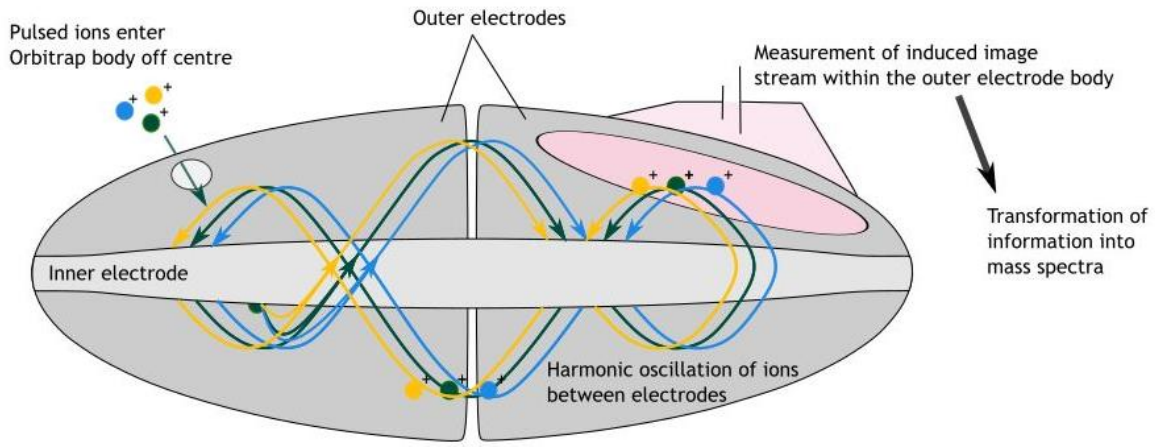


Figure 3-8: Principal structure of an Orbitrap. Pulsed ions are introduced into the space between inner (1) and outer (2) electrodes. The applied potential and overall structure of the mass analyser induce an oscillating movement in the space between electrodes. The kinetic energy and charge of the ions within the applied potentials induce an image stream inside the outer electrodes' material, that can be measured and transformed into mass spectroscopic data. Modified from Greaves and Roboz (2014)

3.3.1.2.3. Further Considerations for Ionisation and Mass Analysis

The presence of undesired components, such as co-extracted compounds from complex environmental samples can impact the ionisation process while solvents or carrier gases used during chromatography have no direct influence during adequate operation (Pitt, 2009). The concurrent presence of additional compounds can cause competition during the ionisation and leads to a reduced signal of the analyte itself. In addition to that, quantitation is considered difficult

with absolute response values as the MS response varies greatly between instruments and depends on factors such as the cleanliness of the ion source, flow rates and applied voltages in the process. To address the aforementioned issues, the use of internal standards (IS) is essential to gain reliable results (Pitt, 2009). Peak area ratios (PAR) of stable isotopic standards added to calibration standards, blanks and samples can easily be distinguished from target analytes, address ion suppression and allow for accurate concentration determination (Pitt, 2009).

The natural occurrence of isotopes can be exploited to gain information about interferences (e.g. co-extracted sample matrix components), however, useful isotope patterns are limited to a discrete collection of elements (e.g. carbon, chlorine, bromine). For example, the approximate naturally occurring ratio of ^{12}C and ^{13}C of 99:1 can be used to estimate the number of carbons within a compound (Kitson et al., 1996).

Full scans (total ion count, TIC) of the available operational m/z range of mass analysers can be a limiting factor, restricting scan rates and therefore, impacting the detection limits of the analysis (Pitt, 2009). Selected ion monitoring (SIM) and multiple reaction monitoring (MRM) allows an alternative approach by increasing the threshold of the applied voltages, to monitor specific m/z values, allowing the detector to allocate more time to monitor relevant (targeted) ions (Pitt, 2009).

Chapter 4

Optimisation of an Ultrasound-assisted Extraction Technique and GC-MS Method Development for the Analysis for Perfluoro Carboxylic Acids

4.1. Introduction

The interest in micropollutants and their fate within the environment first peaked in the 1970s, also leading to the development of first analytical methods for chlorinated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols, and phthalates amongst others. Dioxins for example, occurred mostly as byproducts of industrial processes but also arose from natural processes (e.g. volcano eruptions, forest fires) and persist in the environment. Once the food chain was recognised as a significant exposure route of dioxins it became closely monitored in the 1990's (Fries, 1995, Feil and Ellis, 1998). Other naturally occurring compounds such as PAHs can be found in coal, oil and gas as well as cigarette smoke, fumes from asphalt roads and engine exhausts, and may be most relevant to workers of the respective industries and smokers. While regulating PAHs appears ineffective due to their natural occurrence, monitoring efforts in humans and the environment have been well established for some time (Kuppusamy et al., 2017).

Other emerging contaminants, such as pharmaceuticals and personal care products (PPCPs) and antibiotics in particular, are extensively used in human and veterinary medicine or care (Boxall, 2012, Archer et al., 2017, Ngoc Han et al., 2018, Lee et al., 2021, Ajibola et al., 2022, Hong et al., 2022, Lin et al., 2023, Rapp-Wright et al., 2023) and have been detected in domestic wastewater as early as the 1970s and have continuously gained importance in environmental analysis since then (Biel-Maeso et al., 2019b, Garrison et al., 1976, Karnjanapiboonwong et al., 2011, Kumirska et al., 2015, Maurer et al., 2020).

Poly and perfluoroalkyl substances (PFASs) have been produced, modified and expanded since the early 1940's when the compounds became relevant in various industries, however environmental monitoring long remained a secondary thought (Plunkett, 1941, Benning et al., 1946, Berry, 1951, Dohany, 1982). While all PFASs

contain a minimum of one fully fluorinated carbon moiety, the class of compounds presents as a very broad and diverse group of compounds. The extraordinary strength of C-F bonds, unmatched by any other bond, leads to excellent resistance to degradation and innate properties such as hydrophobicity and lipophobicity enhances surface-active properties of PFASs (Brase et al., 2021). While these properties are worthwhile in consumer products and applications, or as processing aides in the industry, the release into environmental compartments presents unparalleled issues.

4.1.1. Extraction of Micropollutants from Environmental Samples

Commonly employed extraction methods for pollutants from complex environmental sample matrices range from traditional Soxhlet extraction for sediments (Jaffe and Hites, 1985, Ahrens et al., 2011b) to solid/liquid extraction of soils (Kikuchi et al., 2018), SPE for the extraction from water-based samples (Kannan et al., 2005, Karnjanapiboonwong et al., 2011, Portolés et al., 2015a, Ruan et al., 2015, Strynar et al., 2015, Xiao et al., 2021) and MAE from sediment (Munoz et al., 2015) have been investigated alongside sonication-assisted extraction of varying sample types, usually employing a water bath (Higgins et al., 2005, Lorenzo et al., 2015, Mejia-Avendaño et al., 2017a, Mejia-Avendaño et al., 2017b, Rankin et al., 2016, Tian et al., 2021).

The implementation of the ultrasound-assisted extraction relevant to this project has previously been described for the extraction of pharmaceuticals from complex environmental matrices following Sampsonidis (2019) and Fell (2022). Contrary to most publications, the employed ultrasound-assisted extraction is utilising an indirect sonication reactor rather than a sonication bath. In principle, indirect sonication is applied to the outside of an extraction vial to achieve the mass transfer of contaminants from the complex, solid matrix into a solvent, allowing subsequent analysis of samples (Fell, 2022).

Fell (2022) optimised an ultrasound assisted extraction method for the extraction of pharmaceuticals from biosolids using a methanol and water (50 %v/v) mixture at a pH of 2. The study details the statistical evaluation of results using analysis of variance (ANOVA) and design of experiment (DOE) for response optimisation. This approach allowed for the systematic testing of process parameters (e.g. amplitude and pulsing) as well as the comparison of efficiencies for different extraction cycle times. Fell (2022) concluded that overall, an increase in

amplitude increased extraction efficiencies whereas an increase of the pulse beyond 40% and extraction cycle time (>5 minutes) was not advantageous. Extraction parameters previously optimised by Fell (2022) are not changed and therefore, not further discussed in this piece of work. The UAE development performed by Fell (2022) forms the basis further extraction method development performed in this study.

Lorenzo et al. (2015) compared extraction efficiencies for PFASs from soil across different pHs using methanol as an extraction solvent. It was determined that methanol irrespective of the addition of acetic acid showed sufficient recovery rates. Furthermore, they stated that a smaller number of compounds recovered from an alkaline extraction can be explained by the promotion of binding to soil cations during extraction. However, most publications reviewed employed a combination of methanol and water with an addition of sodium hydroxide to achieve an alkaline pH (Anderson et al., 2016, D'Agostino and Mabury, 2017, Mejia-Avendaño et al., 2017a, Munoz et al., 2016, Zhang et al., 2018) or just methanol as the solvent (Li et al., 2011, Lin et al., 2017, Martin et al., 2004, Munoz et al., 2015, Tian et al., 2021). Other extraction solvents, such as alkaline acetonitrile (Guo et al., 2016, Guo et al., 2012, Rankin et al., 2016, Ruan et al., 2015) or a acetonitrile and methanol combination (Shoeib et al., 2008, Tian et al., 2021) were investigated to a lesser extent. Ultimately, considering the previously in-house developed extraction method (Fell, 2022), methanol and water were selected as extraction solvents in this study, however, the optimal pH value for the extraction of PFASs was investigated subsequently.

4.1.2. Gas Chromatography – Mass Spectrometry Method Development

The second objective of this study, following the optimisation of the extraction method for PFASs, was the development of a GC-based analytical method for PFASs, specifically for the analysis of biosolids and soil samples.

The initial literature review focussed on two aspects, the relevance of compounds in soil and biosolids analysis and the application of GC methods for the analysis of samples. Firstly, studies on the quantification of PFASs in biosolids and soil could be divided into two groups, relating to wastewater treatment or agriculture, and contamination with AFFF associated with firefighting foams. Studies informing on agricultural contamination primarily focus on the detection and quantification of perfluoro carboxylic acids (PFCAs) and perfluoro sulfonic acids (PFSAs) (Sepulvado

et al., 2011, Moodie et al., 2021, Pepper et al., 2021, Johnson, 2022, Silva et al., 2022, Zhang and Liang, 2022), the investigation of AFFF included a diverse set of analytes also including fluorotelomer (FT) derivates, perfluorooctane sulfonamides (FOSAs) and perfluorooctane sulfonamido ethanols (FOSEs) amongst others (Backe et al., 2013, D'Agostino and Mabury, 2014, Anderson et al., 2016, D'Agostino and Mabury, 2017, Gobelius et al., 2017, Mejia-Avendaño et al., 2017a, Munoz et al., 2018). Notably, the analysis of the reviewed studies exclusively employed liquid chromatography and tandem mass spectrometry for the analysis of samples.

GC applications described in the literature were mostly used to examine other sample types, such as air samples (Li et al., 2011, Shoeib et al., 2008, Shoeib et al., 2006, Tian et al., 2018, Yao et al., 2018), and PUF foam (Shoeib et al., 2008), dust (Yao et al., 2018), as well as water and leaves (Dufková et al., 2012, Monteleone et al., 2012). The most common target compounds for GC analysis found were FTOHs, fluorotelomer carboxylic acids (FTCAs), PFCAs, FOSAs, FOSEs and polyfluoroalkyl diphosphate esters (di-PAPs) (Figure 4-1). The Literature review narrowed down the possible targets, the primary limiting factors were the volatility and polarity required for GC analysis as successful separation requires analytes to present with limited polarity and intrinsic volatility or semi-volatility in order to be vapourised and carried through the analytical column.

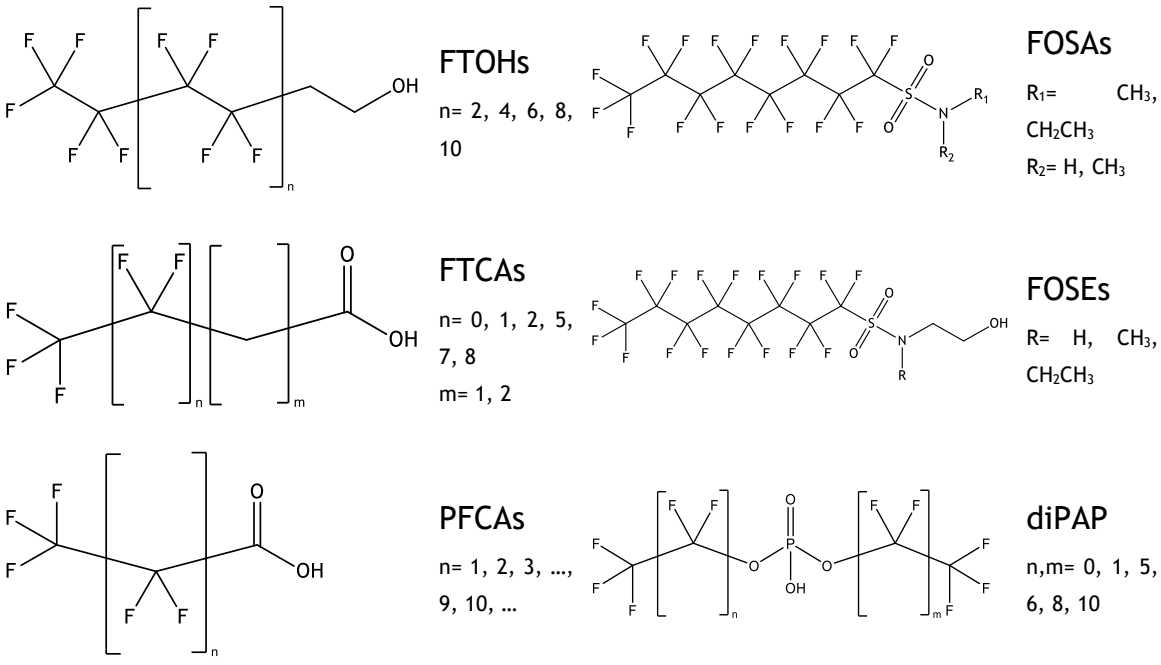


Figure 4-1: Chemical structures of PFAS targets suitable for GC analysis. Fluorotelomers ethanols (FTOHs), fluorotelomer carboxylic acids (FTCAs), perfluoro carboxylic acids (PFCAs), perfluorooctane sulfonamides (FOSAs), perfluorooctane sulfonamido ethanols (FOSEs) and polyfluoroalkyl diphosphate esters (di-PAPs).

Variables n and m indicate the most common compound lengths, variable R indicates different substituents that are possible in the respective positions within the molecule.

Literature concerning GC analysis of PFAS, and PFCAs in particular, entails different polarities in columns, spanning non-polar columns such as 5% diphenyl-95% dimethylsiloxane (DB-5MS) (Dufková et al., 2012, Jaffe and Hites, 1985, Monteleone et al., 2012, Yu et al., 2013), lightly to semi-polar columns such as crossbond trifluoropropylmethyl polysiloxane (RTX-200MS) (Dufková et al., 2012) and highly polar polyethylene glycol columns (DB-WAX) (Ellis et al., 2003, Li et al., 2011, Portolés et al., 2015a, Yao et al., 2018). However, the most compelling difference between studies is the choice of ionisation source used for analysis. Chemical ionisation (CI), positive or negative, appear frequently in the literature (Dufková et al., 2012, Jaffe and Hites, 1985, Li et al., 2011, Monteleone et al., 2012, Portolés et al., 2015a, Shoeib et al., 2006, Yao et al., 2018) while electron impact ionisation (EI) only occupies a nominal part of the literature (Dufková et al., 2012, Jaffe and Hites, 1985, Portolés et al., 2015a).

In this project, the initial method development employs the same biosolids matrix and pharmaceuticals as model compounds as the previous method development performed by Fell (2022). This research chapter aimed to achieve different objectives, firstly, to investigate the chemical composition of soils and biosolids, comparing soil samples from different sampling sides as well as different batches of biosolids and to evaluate results to ensure consistency within the matrix materials to ensure the validity and comparability of results. Secondly, the study investigated the possible increase of the throughput of samples during extraction by increasing the number of samples extracted simultaneously (multiplexing) and ensure that the extraction of biosolids and soils are robust and reproducible between positions of the sonication probe. Additionally, extraction parameters such as the optimal amount of soil samples and pH for the extraction of PFCAs were investigated. Finally, this project aimed to develop a GC-MS analysis method employing electron impact ionisation for the detection of PFCAs.

4.2. Materials and Methods

4.2.1. Reagents

Hexametaphosphate (HMP) was purchased from Merck UK Ltd. (Gillingham, UK). Pharmaceuticals, acetaminophen (AAP), atenolol (ATL), carbamazepine (CBZ), dapsone (DPS) and ibuprofen (IBP) were received from Merck UK Ltd. (Gillingham,

UK). Phenanthrene (P), and triclosan (TCL) were supplied from Merck UK Ltd. (Gillingham, UK). A deuterated anthracene standard (A-D10) was acquired from Merck Ltd. (Gillingham, UK).

Perfluoroalkyl carboxylic acids (PFCAs), perfluoro butanoic acid (PFBA, C_4), perfluoro pentanoic acid (PFPeA, C_5), perfluoro hexanoic acid (PFHxA, C_6), perfluoro heptanoic acid (PFHpA, C_7), perfluoro octanoic acid (PFOA, C_8), perfluoro nonanoic acid (PFNA, C_9), perfluoro decanoic acid (PFDA, C_{10}), perfluoro undecanoic acid (PFUnA, C_{11}) and perfluoro dodecanoic acid (PFDoA, C_{12}) were also obtained from Merck UK Ltd. (Gillingham, UK). ^{13}C isotope standards of PFBA, PFHxA and PFOA were acquired from CK Isotopes Ltd.

Analytical grade solvents, acetonitrile (ACN), methanol (MeOH) and ethyl acetate (EtAc), and formic acid (HCOOH) and ammonia (NH_3) were purchased from Fisher Scientific (Loughborough, UK).

Details concerning the makeup of stock solutions can be found in Appendix B: Chapter 4.

4.2.2. Sample Collection

Soil samples were collected from three fields on Cochno Farm and Research Centre (G81 5QL). Two fields (field 1 and field 2) had been fertilised with biosolids (supplied by Angloscot Biosolids Ltd.) and the third (Control) had received conventional inorganic fertiliser. Soils were retrieved with the help of a soil corer (approx. 3 cm diameter) with a core depth of approximately 10 cm. The initial assessment of soils was performed on samples collected in Septemper 2019 (N= 60, 20 from each respective field), as well as 2 batches of biosolids, collected in September 2019 and April 2020.

4.2.3. Sample Preparation

Biosolids subsamples were ground with mortar and pestle until a homogeneous powder was formed, increasing the surface area and hence, increasing the contact to solvents during extraction (Zhang et al., 2018, Fell, 2022). The powder was stored in a glass jar with lid in the dark, at room temperature. Soil samples were defrosted, dried at 30 °C and passed through a 2 mm sieve subsequently (Yu et al., 2013, Lorenzo et al., 2015). Once remaining sample masses were documented samples were stored in glass jars with lids at 4 °C.

4.2.4. Sample Characterisation

Physico-chemical properties of samples were scrutinised to establish the possible effect of matrix composition on the recovery of micropollutants as Kettler et al. (2001) suggests that the overall textural composition of soils may affect the soil-water retention and organic matter dynamics and can have an influence on the movement of micropollutants through the horizon, therefore, soil particle sizes were also investigated. Water content (WC), and by extension the dry weight of the matrices as well as the organic matter content (loss-on-ignition) were determined for soils and biosolids; additionally, the grain particle size distribution of soils was investigated.

4.2.4.1. Water Content and Organic Matter Content for Biosolids and Soils

Soil and biosolids samples were defrosted overnight at room temperature. 5.0 g ± 0.1 g soil were weighed into clean, labelled glass jars. The subsamples were incubated in an oven at 60 °C for 48 hours, with weighing taking place at room temperature every 24 hours. The loss of mass weighed on subsequent days allows the determination of the water content (WC).

$$WC\ [\%] = \frac{m(soil,25°C) - m(soil,60\,°C,48\,h)}{m(soil,25\,°C)} * 100\ \% \qquad (2)$$

The water content of biosolids was determined according to the principles described for soils. Since the water content in soil did not change significantly when weighed after 24 and 48 hours, a drying time of 24 hours at 60 °C was adapted considering the lower water content in pellets.

Once dried, the organic matter content (OM) was determined using the loss-on-ignition (LOI) method. Davies (1974) showed, the incubation of soil samples in a furnace at 450 °C allowed the determination of the organic matter content without the thermal destruction of clay minerals found in soils. The samples were transferred into tin dishes and weighed before undergoing incubation in a furnace at 450 °C for 3 hours. LOI was determined in relationship to the dry weight (DW) of soil using the equation:

$$LOI\ (OM)\ [\%\ DW] = \frac{m(soil,60\,°C) - m(soil,450\,°C)}{m(soil,60°C)} * 100\ \% \qquad (3)$$

The LOI approach to determine the OM content of biosolids was not sufficient following the procedures for soils. After two hours at 450 °C the ashing in the

furnace was incomplete, leaving a carbon lining in the oven indicating incomplete combustion. The OM determination was therefore extended for further 2 hours at 450 °C to complete the reaction.

20 soil samples were analysed from each of the three fields, whereas five subsamples of every one of the two biosolids samples (biosolids 1 and biosolids 2) were analysed.

4.2.4.2. Particle Size Determination of Soils

Two grams of dried soil sample from the initial sampling cycle (September 2019) were pooled together according to the sampling sites. The resulting three pooled samples were passed through a 2 mm mesh to remove large debris and stones. The remaining sample underwent grain size determination. The particle size of the soil samples was determined as described by Kettler et al. (2001). Briefly, the soil samples were dispersed 1:4 in 3 % HMP and shaken on a reciprocating shaker at 120 rpm for two hours to break up conglomerates of particles. The slurry was poured through a 0.2 mm mesh, the retained sand particles were washed with small amounts of HMP and subsequently dried at 90 °C overnight. The resulting mass corresponds to the sand content of the sample.

The dispersed silt and clay mix was collected in a large beaker. The collected slurry was left undisturbed at room temperature for at least 90 minutes before the solution was decanted, leaving the silt fraction behind. The silt fraction was dried at 90 °C overnight. The particle fractions were calculated as follows:

$$Sand\ [\%] = \frac{m(oven\ dry\ sand)}{m(original\ sample)} * 100\ \% \qquad (4)$$

$$Silt\ [\%] = \frac{m(oven\ dry\ silt\ mass)}{m(original\ sample\ mass)} * 100\ \% \qquad (5)$$

$$Clay\ [\%] = 100 - (Sand\% + Silt\%) \qquad (6)$$

4.2.5. Instrumentation

4.2.5.1. Sonotrode

Extraction was performed with a sonication device purchased from Hielscher Ultrasound Technologies (Figure 4-2). The device consisted of an ultrasonic generator and transducer (UP200St), Vialtweeter and Vialpress. The generator (200 W, 26 kHz) has an adjustable amplitude and pulse range of 20 – 100 % and 10 – 100 %, respectively. The Vialpress clamping device as accessory for the

Vialtweeter-sonotrode can hold up to 5 vials with a diameter of up to 20 mm (Figure 4-2). Since the used vial diameters exceeded 20 mm, only a maximum of 3 samples can be extracted simultaneously.

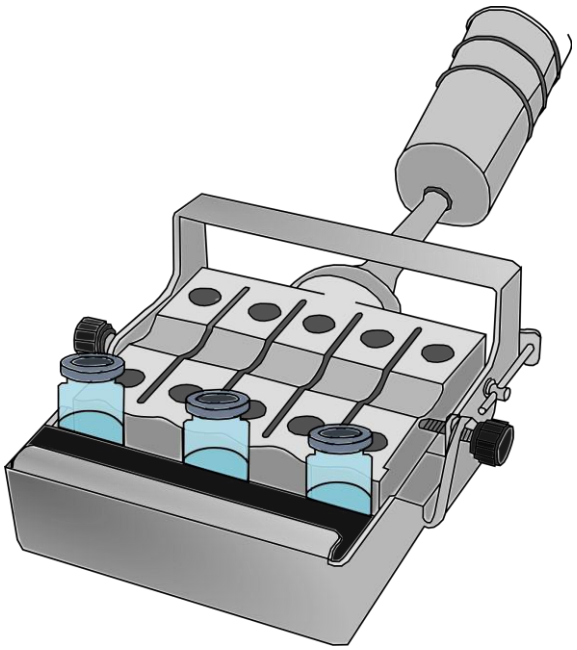


Figure 4-2: Illustration of the Hielscher sonotrode with attachment for indirect sonication, holding 3 vials. Acoustic waves are produced by generator and transducer and transmitted through the probe arm to the vial clamping device. Here, indirect sonication is achieved by the transfer of acoustic waves to the extraction vial containing the sample.

4.2.5.2. Gas Chromatography – Mass Spectrometry

GC-MS Analysis was performed on an Agilent 7890A GC system with Agilent 7693 GC autosampler and Agilent 5975C MS detector. The GC was operated using helium as carrier gas, the split/splitless injection inlet was equipped with a split/splittless injection liner (Agilent 5183-4711). The inlet and transfer line temperatures were set to 250 °C and 280 °C, respectively. Mass spectra generation was adjusted for the respective analyses. Samples were analysed in both, TIC and SIM depending on the stage and objective of the analysis. Respective scan ranges and ion m/z ratios can be found in the results section of this study. MS source and quadrupole were operated at 230 °C and 150 °C, respectively. An injection volume of 1 µL was used across all methods.

4.2.5.2.1. Increasing the Throughput (Multiplexing) of the Ultrasound-assisted Extraction

The GC-MS system was equipped with a DB-5 column (30 m, 0.250 mm inner diameter (ID), 0.25 µm film thickness) for the analysis of pharmaceuticals in spiked

soils and biosolids for the sonication device validation. The study follows the procedure previously described by Fell (2022).

4.2.5.2.2. Gas Chromatography – Mass Spectrometry Method Development for the Detection of Perfluoro Carboxylic Acids Using Electron Impact Ionisation

Method development for the analysis of PFCAs was performed on the same instrument, applying a scouting method to determine optimal chromatographic conditions, and identify suitable MS ion fragments for compound confirmation. The method was trialled with varying low initial plateau temperatures of 30, 35 and 40 °C were investigated as well as different temperature ramps following the initial plateau (3 °C/min, 5 °C/min and 10 °C/min). A variation of flow rates and splits were assessed in the process.

4.2.5.3. Liquid Chromatography – Mass Spectrometry

A Thermo Scientific Dionex Ultimate 3000 UHPLC system with hyphenated Thermo Scientific Q-Exactive Orbitrap mass spectrometer (including a Dionex Ultimate 3000 RS Pump, Dionex Ultimate 3000 RS Autosampler (temperature-controlled at 10 °C) and Dionex Ultimate 3000 RS Column Compartment (temperature controlled at 30 °C) was utilised for the optimisation of soil masses and the extraction pH.

Analysis was performed on a C_{18} Accucore column (Thermo Scientific, 100 mm x 2.1 mm ID, 2.6 µm particle size). The mobile phase was made up of acetonitrile (eluent A) and ammonium formate (eluent B, 10 mmol, pH 3.5).

Samples were analysed with the orbitrap MS using electrospray ionisation (ESI) in negative ion mode (spray voltage -4.5 kV, capillary and auxiliary gas temperatures of 300 °C). The instrument was operated in full MS scan with a range of m/z 80 – 900. The chosen isolation window for parallel reaction monitoring (PRM) was set to m/z 2.0 with a normalised collision energy (NCE) of 35 eV. An injection volume of 10 µL was used throughout the experiment.

4.2.5.4. Software

The GC-MS was operated through GC MSD K Enhanced Chemstation (E.02.01.1177, Windows XP) and initial evaluation of chromatograms was performed on GC MSD K data analysis (E.02.01.1177, Windows XP). The LC-Orbitrap-MS was utilised through Tracefinder (5.1 SP1, Windows 10). GC Chromatograms and mass

spectrums were processed and evaluated with OpenChrom (1.4.0, Windows 10) while LC data was accessed through Masshunter (11.0, Windows 10). Minitab (19.2020.1, Windows 10) was used for statistical analysis. Inkscape (1.1.1, Windows 10) was used to produce schematics and figures. Chemdraw (21.0.0, Windows 10) was utilised for the creation of chemical structures.

4.2.6. Procedure

Soil samples were oven dried at 30 °C for approximately three days prior to weighing out, in line with the literature and previous experiments. Extraction was performed using 10 mL headspace vials from Merck UK Ltd. (Gillingham, UK). Firstly, the respective amount of biosolids (1.0 g) or soil (0.5 g, 1.0 g, 2.0 g) was introduced into the vials. Secondly, samples were spiked with the respectively required standard solutions. Once the samples were dried again, usually overnight, extraction was performed.

The principal procedure was previously described by Fell (2022). Briefly, 2 mL of a methanol and water mixture (50:50 %v/v) was added to each sample vial. The vials were capped with an aluminium cap containing a suitable septum (PTFE/silicone for the extraction of pharmaceuticals, or butyl rubber for the extraction of PFCAs) and inserted into the vialpress of the sonotrode. Sonication was carried out for 5 minutes with an amplitude and pulse of 80 % and 40 %, respectively. Subsequently the vials were moved into the centrifuge for 10 minutes at 5000 rpm to obtain a supernatant without biosolids and soil residues. The supernatant was removed and kept in a labelled, clean vial and the procedure was repeated for two more cycles. Combined extracts were evaporated at 40 °C overnight.

Before GC analysis, samples undergoing derivatisation were re-constituted in 0.950 mL ethyl acetate or acetonitrile and derivatised with 0.050 mL N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA, 40 °C, 30 min) otherwise 1.0 mL of solvent was used (for non-derivatised samples) . For LC analysis samples were reconstituted in 1 mL ACN/water (50 %v/v).

4.2.6.1. Optimisation of Soil Sample Masses for Ultrasound-assisted Extraction

While the extraction of biosolids was previously optimised to an amount of 1 gram per extraction vial (Fell, 2022), the optimal amount of soil samples was not known. For this purpose, 0.5 g, 1.0 g, and 2.0 g of spiked soils were extracted in an effort

to identify an optimum setup for subsequent recovery of the targeted micropollutants from the matrix material. The remaining extraction parameters remained unchanged. Extracts were dried at 40 °C overnight and subsequently reconstituted in EtAc before undergoing derivatisation with MSTFA. Analysis took place on the GC-MS system.

4.2.6.2. Increasing the Throughput (Multiplexing) of the Ultrasound-assisted Extraction

The combination of selected vials and the vialpress attachment of the sonication device allowed for the co-extraction a maximum of 3 vials simultaneously. As the device was previously only employed to extract with the central position of the vialpress, an investigation into the consistency of the extraction across the whole of the press was performed.

Initial experiments were performed following a screening design of experiments with a randomised run order for two and three vials, at concentrations of 100, 250 and 500 ng/mL (data not shown). Consequently, three vials and a spike concentration 500 ng/g for soils and biosolids were selected for the evaluation.

For subsequent GC-MS analysis, 1 µL of MSTFA-derivatised sample was injected into the system. The GC oven was kept at 100 °C for 10 minutes followed by an increase of 10°C/ min to 300 °C with a constant flow rate of 1 mL/min of helium (total run time 30 minutes).

The mass spectrometer was operated in TIC mode, monitoring ions with a size range between m/z of 45 and 550. The solvent delay for ethyl acetate was set to 3.5 minutes. The electron multiplier voltage of 2000 V was correlating with a gain factor of 0.56.

4.2.6.3. Gas Chromatography – Mass Spectrometry Method Development for the Detection of Perfluoro Carboxylic Acids Using Electron Impact Ionisation

The GC-MS analysis of perfluoro carboxylic acids used an initial approach following Dufková et al. (2012). Results were not successfully reproduced, and different columns (DB-5 and DB-17) and conditions were tested for optimal separation. Individual PFCAs ranging from PFBA (C_4) to PFDoA (C_{12}) and a combined standard mixture of PFCAs were injected into the GC system. Variation of the split/splitless injection (splitless, 5:1, 10:1, 20:1) and oven programming was used to identify the optimal GC-MS conditions for analysis. Flow rates of 1.0 mL/min, 1.25 mL/min

and 1.4 ml/min for the helium carrier gas were trialled, along with initial plateaus of 30 °C, 35 °C and 40 °C and holding times of 5 min and 10 min respectively. Furthermore, ramped temperature increases of 3 °C/min, 5 °C/min and 10 °C/min were assessed.

The GC-MS detection proceeds after the solvent delay of 2.9 minutes for acetonitrile (standard solvent). The electron multiplier voltage of 2188 V was correlating with a gain factor of 0.67.

4.2.6.4. Optimisation of pH values for the Ultrasound-assisted Extraction of Perfluoro Carboxylic Acids

Extraction of pharmaceuticals in an acidic pH of 2 has been shown to be efficient (Fell, 2022, Gago-Ferrero et al., 2015). The extraction of PFCAs was assessed at an acidic pH 2, alkaline pH 10 and neutral pH. The remaining extraction parameters remained unchanged. The analysis for this was performed by LC-Orbitrap-MS, the corresponding analysis method has been described by Roberts et al. (2023) and Singer and Roberts (2023) previously. Briefly, the LC gradient eluent profile was as follows: initial composition was 99% of eluent B (ammonium formate) for 5 minutes, decreased to 1 % B over 4 minutes and maintained there for 6 minutes. Following this plateau eluent B is increased back to 99 % with a final equilibrium there for 9 minutes (Figure 4-3). The flow rate was 0.2 mL/min and the total run time was 28 minutes.

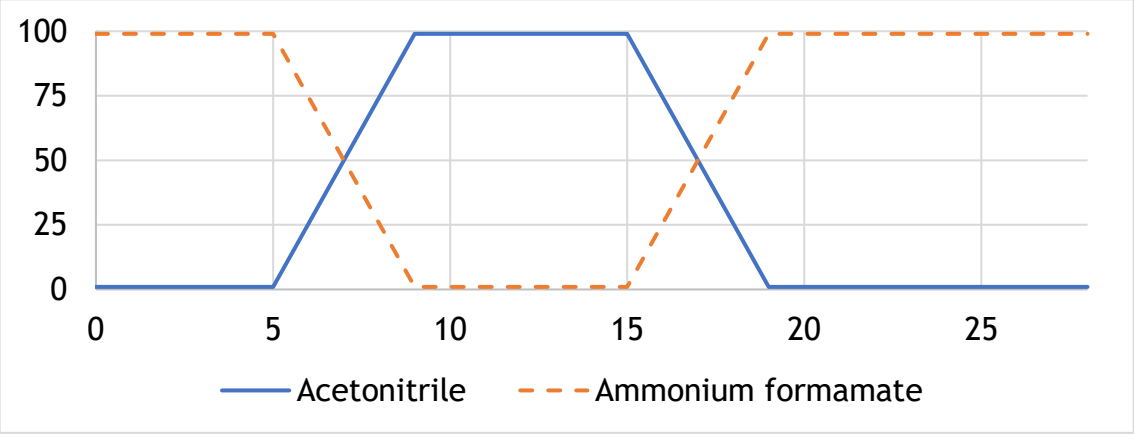


Figure 4-3: Solvent gradient for the determination of PFCAs using LC-Orbitrap-MS. Solvents acetonitrile and ammonium formate alternate between 99 % and 1 % of the respective components. The overall run time for this gradient programming amounted to 28 minutes.

4.3. Results

4.3.1. Sample Characterisation

4.3.1.1. Water and Organic Matter Content for Soils

Summarised data for the soil samples regarding water and organic matter content (WC and OM) can be found in (Table 4-1). Statistical analysis by one-way analysis of variance (ANOVA) showed that there is no significance in WC and OM between fields ($p = 0.179$ (WC) and $p = 0.054$ (OM)).

Table 4-1: Water content and organic matter (loss-on-ignition) of soil samples collected across three fields. Field 1 and 2 received biosolids-amendment whereas field co received inorganic fertiliser. Samples for this part of the study were collected with the initial sampling in September 2019.

	Field 1	**Field 2**	**Field Co**
Water content (WC) / %	49 ± 6	45 ± 6	46 ± 6
Organic Matter Content (OM /LOI) / %	26 ± 6	21 ± 5	22 ± 7

4.3.1.2. Water and Organic Matter Content for Biosolids

The physical appearance of the biosolids samples varied between batches. Biosolids 1 presented as dense, very hard, spherically-uniform pellets of approximately 0.5 – 1.0 cm of diameter, biosolids 2 were irregular discoidal-shaped pellets that appeared less dense. Summarised data of the biosolids analysis considering WC and OM can be found in Table 4-2. While the water contents of the two biosolids samples were proximate, the organic matter content differed by 17 %.

Table 4-2: Water content and organic matter (loss on ignition) of biosolids samples (batches 1 and 2).

	Biosolids 1	**Biosolids 2**
Water content (WC) / %	6.5 ± 0.1	10.1 ± 0.1
Organic matter (OM) / %	55.5± 0.1	72.6 ± 0.1

4.3.1.3. Particle Size Determination of Soils

The summarised data for the soil particle sizes from three fields can be found in (Table 4-3). The soil texture determined is similar between fields, indicating a loamy to silt-loamy soil (Figure 4-4).

Table 4-3: Particle size range and soil characteristics for soils collected on the fields 1 and 2, and Control (Co). Larger particles (e.g. gravel) has been removed prior to the particle size determination. The fractions for sand, silt and clay are given, along with the corresponding particle size ranges.

	Size range	Field 1	Field 2	Field Co
Sand / %	2 mm – 0.02 mm	45	36	34
Silt / %	0.02 mm – 0.002 mm	43	50	55
Clay / %	< 0.002 mm	12	14	11

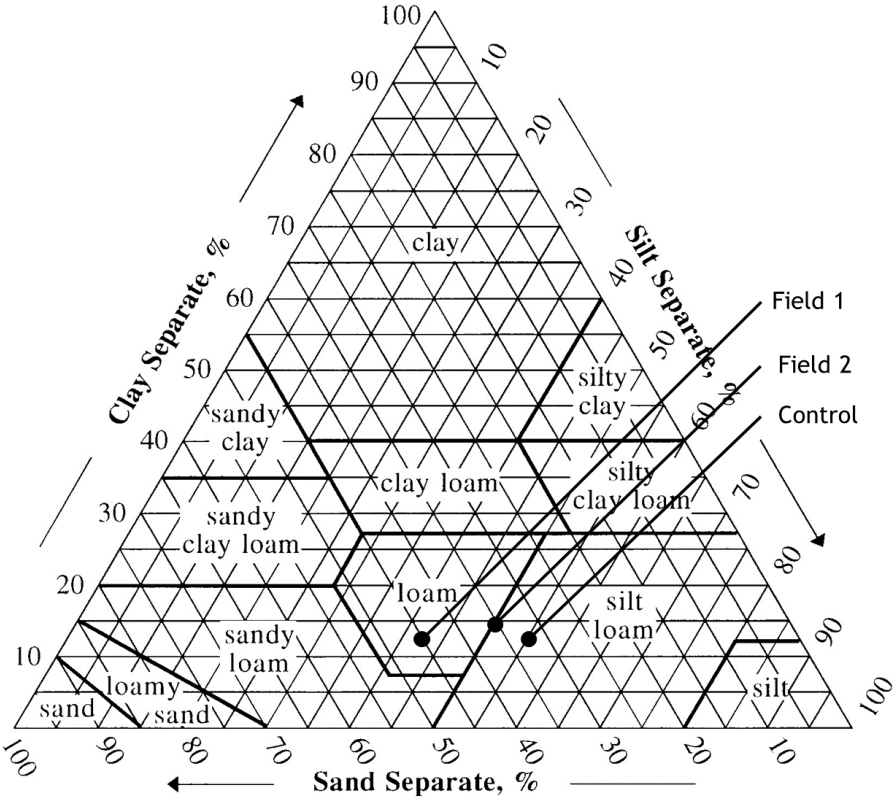


Figure 4-4: Soil texture diagram (USDA, 1993) for soil collected on fields 1, 2 and control. Each side of the triangle represents a main component, clay, sand or silt, and its respective percentage within the soil sample. Within the triangle, every soil is characterised to 100% of its composition. Depending on the composition of soil, different attributes are used to describe samples (e.g. reading 60 % sand + 15 % silt + 25 % clay = 100 % soil corresponding to a sandy clay loam texture). Analysed samples for field 1 and 2 and the control were identified, presenting as loamy or silt loamy soil.

4.3.2. Optimisation of Soil Sample Masses for Ultrasound-assisted Extraction

Notably, soil samples used in this part of the study are all derived from the same pooled soil sample. The analysis of the soil samples was performed with single injections on the GC-MS under the assumption that all sample vials involved in the generation of the data are alike. All samples were spiked with the same amount of 500 ng/mL of the pharmaceutical mixture as well as the 500 ng/mL anthracene-D10 internal standard, irrespective of the weighed in soil amount. This meant that 1 mL of the 500 ng/mL mixed standard solution was added equally, to 0.5 g of soil

or up to two grams of soil respectively. No study was performed to assess the effects of oven-drying prior to extraction, after spiking or after extraction was completed. Following the initial literature research and previous experiments the losses attributed to the drying process were assumed to be minimal (Berset et al., 1999, Fell, 2022, Wilcke et al., 2003).

Pharmaceutical recoveries were corrected with Anthracene-D10 following the calculation:

$$recovery\ \% = \left(\frac{PA_{MAE}}{PA_{MBE}}\right) * \left(\frac{PA_{SBR}}{PA_{SAR}}\right) * 100 \qquad (7)$$

With the mixed pharmaceutical standard solution before extraction (MBE), after extraction (MAE), and Anthracene-D10 before drying (SBR) and after reconstitution (SAR) to account for losses during other stages of the sample processing and analysis.

For data evaluation, PARs between analytes and the internal standard were utilised, PARs are determined across all samples:

$$\mathrm{PAR} = \left(\frac{PA_{analyte}}{PA_{internal\ standard}} * 100\ \%\right) \qquad (8)$$

Chromatographic details corresponding to the target analytes (Figure 4-5) such as the retention times used for the respective compounds and mass-to-charge ratios can be found in

Table 4-4.

Table 4-4: Gas chromatographic and mass spectrometric compound data of selected pharmaceuticals and used internal standard (anthracene-D10) for GC-MS analysis. Retention times (tR) and most abundant product ions for the respective compounds.

Compound	t_R / min	Product ions / m/z				
Anthracene-D10 (A-D10)	9.3	128	89	58	69	-
Acetaminophen (AAP-2TMS)	8.4	206	181	166	223	73
Atenolol (ATL-2TMS)	15.2	72	118	223	-	-
Carbamazepine (CBZ-1TMS)	14.7	193	165	73	-	-
Dapsone (DPS)	19.2	248	108	140	69	92
Ibuprofen (IBP-1TMS)	7.6	73	160	117	263	-
Triclosan (TCL)	12.4	200	345	73	360	-

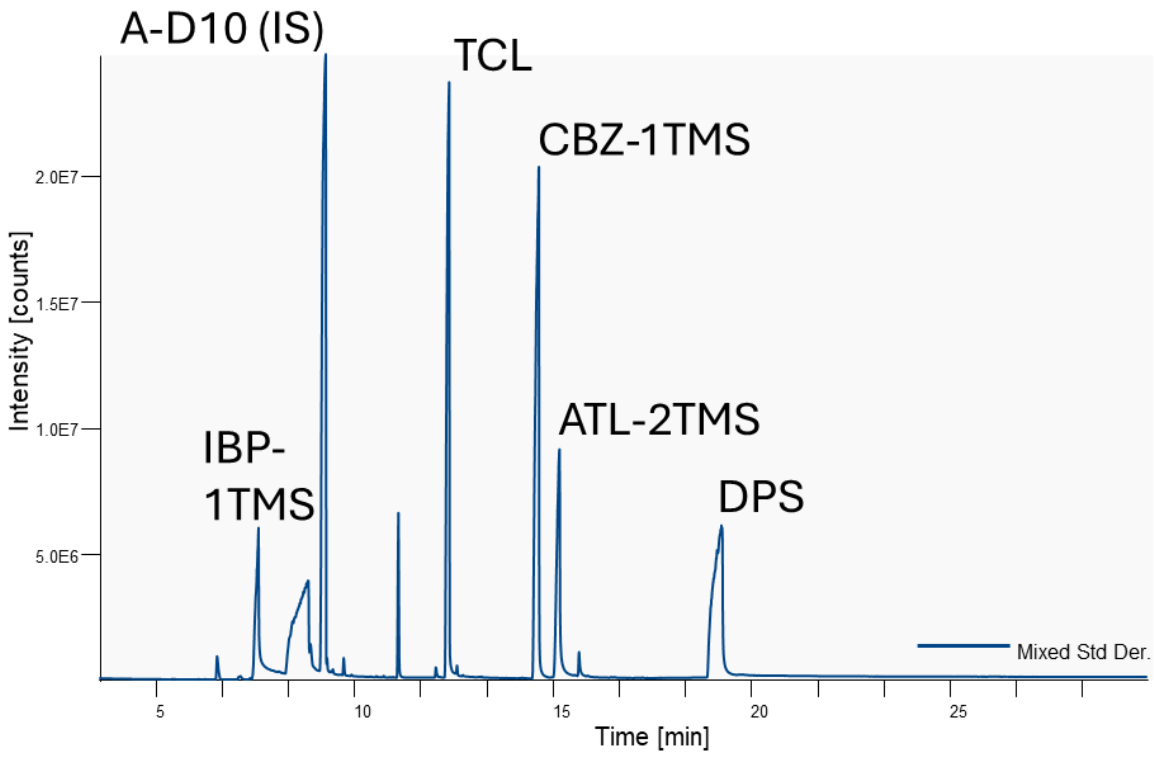


Figure 4-5: Chromatogram of the derivatised mixed pharmaceutical standards used in GC-MS analysis. Anthracene-D10 served as internal standard, ibuprofen-1TMS and carbamazepine-1TMS have an additional trimethylsilyl group following the derivatisation process. The remaining peaks correspond to non-derivatised compounds and compound fractions.

Only acetaminophen, carbamazepine and ibuprofen were recovered across all samples albeit with varying recoveries (Table 4-5 and Figure 4-6). Atenolol and dapsone were not detected in extracts from 2 grams of soil. Generally, the recovery decreases with increasing soil amount, however, results for 1 gram of soil presents with the smallest relative standard deviation (RSD) across samples.

Table 4-5: Relative recovery of MSTFA-derivatised pharmaceuticals from varying amount of soil samples (N=18). Values determined by GC-MS analysis and internal standard correction with Anthracene-D10. ND = not detected.

	Recovery ± RSD / %		
Compounds	**0.5 g**	**1 g**	**2 g**
AAP-2TMS	128 ± 57	88 ± 27	3 ± 2
ATL-2TMS	50 ± 15	27 ± 12	ND
CBZ-1TMS	70 ± 17	63 ± 14	44 ± 53
DPS	15 ± 4	8 ± 3	ND
IBP-1TMS	4 ± 5	2 ± 0.4	2 ± 2
TCL	48 ± 15	28 ± 9	34 ± 68

4.3.3. Increasing the Throughput (Multiplexing) of the Ultrasound-assisted Extraction

Investigation into the consistency of the extraction across the sonication device's vialpress clamping device (Figure 4-2) was performed. Soil extracts and biosolids extracts (biosolids 1), spiked with 500 ng/g of a pharmaceutical mix are analysed by GC-MS. While all soil samples underwent derivatisation, a subset of biosolids samples were analysed in a non-derivatised state.

The peak areas of acetaminophen, atenolol, carbamazepine, dapsone, ibuprofen, triclosan and anthracene-D10 (internal standard) were used to assess the method performance. Retention times and relevant product ion sizes are listed in

Table 4-4.

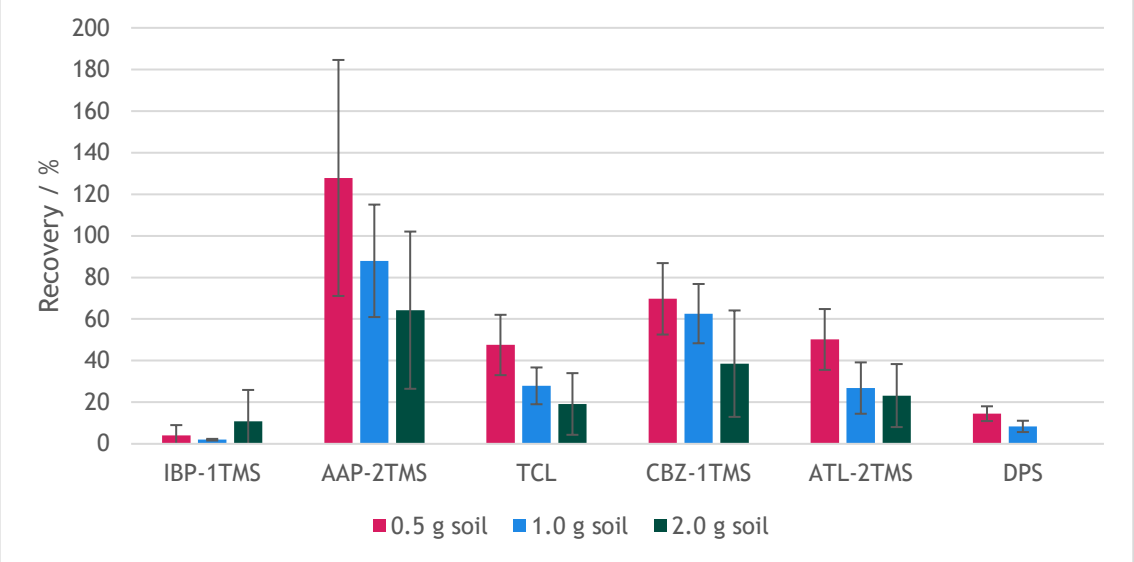


Figure 4-6: Recoveries of selected pharmaceuticals from soil samples using ultrasound-assisted extraction (N=18). All samples (0.5 g, 1.0 g and 2.0 g) were spiked with 500 ng of pharmaceutical standards prior to extraction. Compounds were assessed in the varying amounts of soil samples using GC-MS analysis.

Non-derivatised biosolids samples generally achieved recoveries in the range of 80 – 120 % for ibuprofen (IBP), acetaminophen (AAP) and carbamazepine (CBZ) with relative standard deviations between 15 and 25 % (Figure 4-7). Additionally, atenolol, dapsone and triclosan were recovered with 52 %, 8 % and 19 %, respectively. Derivatised biosolids extracts showed recoveries of only 43 %, 71 % and 59 % respectively, for IBP, AAP and CBZ while achieving RSDs in the range of 10%. As for atenolol, dapsone and triclosan, recoveries also decreased in derivatised samples albeit with improved standard deviations.

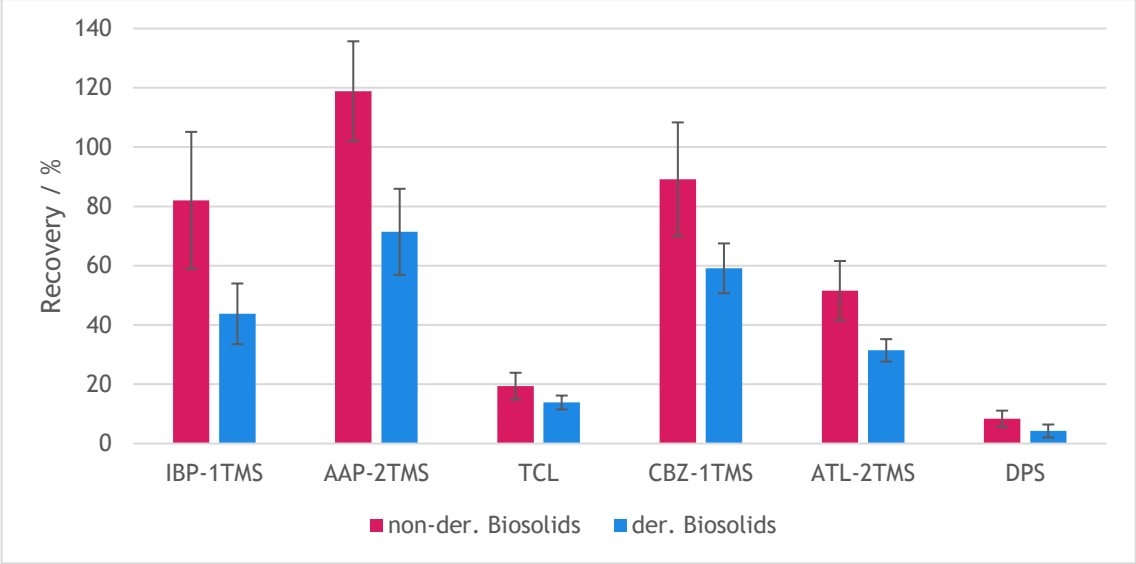


Figure 4-7: Recoveries of selected pharmaceuticals from biosolids samples using ultrasound-assisted extraction (N= 12). All samples (1 gram of sample) were spiked with 500 ng of pharmaceutical standards prior to extraction. Compounds were assessed in non-derivatised and derivatised samples using GC-MS analysis.

The comparison of derivatised and non-derivatised biosolids extracts generally showed higher average recoveries for non-derivatised samples, however, the RSDs were commonly 2-fold higher. As for the analysed derivatised soil samples used in this part of the study, recoveries generally decreased when the amount of soil increased from 0.5 g to 1.0 g of sample. Recoveries of acetaminophen, atenolol and dapsone and were comparable to results achieved in biosolids, however, AAP in particular showed a significant increase in the relative standard deviation. Notably, triclosan showed improved recoveries of 48 % and 28 % in 0.5 g and 1.0 g of soil, respectively, compared to only 19% in non-derivatised biosolids samples. Most strikingly, ibuprofen showed poor recoveries across soil samples (2 – 4 %), whereas results in biosolids ranged between 82 and 44 %, depending on the derivatisation status of samples. Generally, derivatisation seemingly improves the reproducibility of results across biosolids and soil samples but leads to overall lower recoveries.

4.3.4. Gas Chromatography – Mass Spectrometry Method Development for the Detection of Perfluoro Carboxylic Acids Using Electron Impact Ionisation

Separation employing a DB-17 column did not lead to suitable peak shape and separation, and a DB-5 column (30 m, 0.250 mm inner diameter, 0.25 µm film thickness) was selected for method development. Due to the increased concentration (500 ng/mL) of the employed standard mixture a split injection of

20:1 was used for the method development. A helium flow rate of 1.25 mL/min led to the best peak shape.

Initial plateau temperatures below 35 °C did not improve chromatographic results, whereas a temperature of 40°C led to the loss of an analyte due to early elution within the solvent delay (dead volume) (Figure 4-8). Increasing the length of the initial plateau from 5 minutes to 10 minutes did not improve separation, hence a 35 °C plateau was set for 5 minutes.

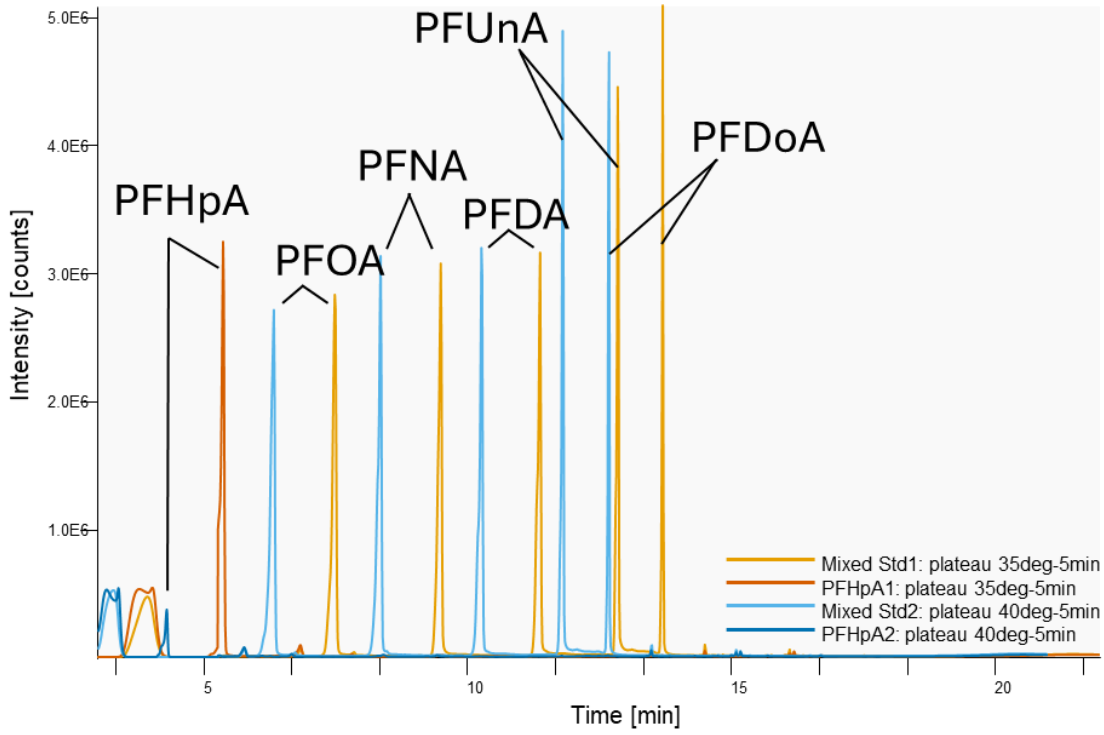


Figure 4-8: Chromatogram of the GC-MS analysis PFCAs (PFHxA - PFDoA). Only PFHpA and bigger produced peaks in the overlayed chromatogram. MS was operated in TIC. Ovenprogramming: initial plateau at 35 and 40 °C for 5 minutes, temperature ramp 5 °C/min to 70 °C, then 20 °C/min to 250 °C for 1 minutes. Helium flow rate of 1,25mL/min, 20:1 split injection.

A subsequent temperature ramp of 5 °C/min was found to be ideal for chromatographic performance with the successful detection of compounds ranging between perfluoro heptanoic acid (C_7) and perfluoro dodecanoic acid (C_{12}). Shorter PFCAs were not retained enough to achieve detection in this setup. PFDoA elutes after 13 minutes at approx. 70 °C, a temperature increase (20 °C/min) is used to adjust the temperature to a maximum of 250 °C (Figure 4-9). The final holding of the increased temperature served as column flush and ensured the complete elution of injected compounds, preparing for the following run. The chosen parameters achieve a short analysis time of 22 minutes whereas the intermediate temperature selected for the initial plateau minimises the time

required for adjustment between analyses compared to lower plateau temperatures investigated. The final oven temperature programming was illustrated in Figure 4-9.

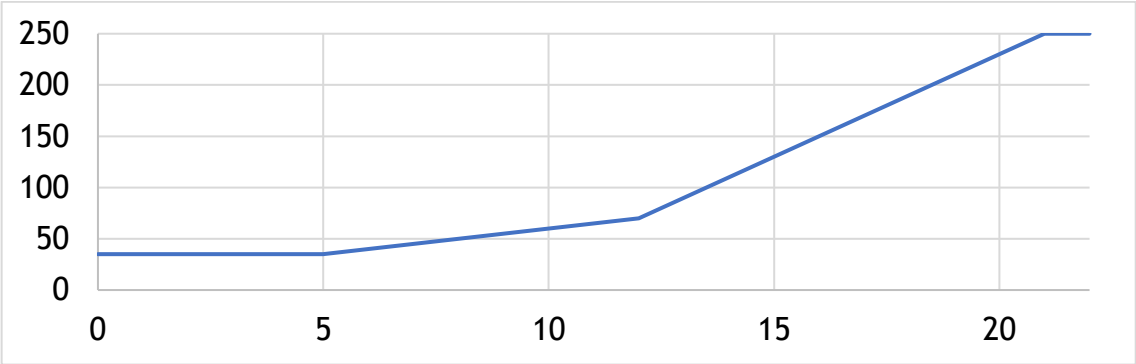


Figure 4-9: Oven temperature program for the determination of PFCAs using GC-MS. Oven programming was optimised with an initial plateau temperature of 35 °C for 5 minutes, followed by a 5 °C/min ramp to 70 °C. PFDoA elutes at approx. 13.7 minutes after which a column flush is performed, by rapidly increasing the oven temperature to 250 °C and holding the temperature for 1 minute.

The initial method development was completed using total ion count (TIC, scan), the compiled analyses were used to identify suitable product fragment sizes for selected ion monitoring. Product ions with m/z 59, 69, 100, 119, 131 and 169 were selected for all detected compounds (Table 4-6). While shared fragment sizes between compounds can be problematic for identification purposes, ample temporal separation still allows for a useful analysis (Figure 4-10).

Table 4-6: Chromatographic compound data of perfluorocarboxylic acids for GC-MS analysis. Retention times (t_R) and most abundand product ions for the respective compounds.

Compound	**t_R / min**	**Product ions / m/z**				
PFHpA	5.3	59	69	131	119	169
PFOA	7.4	59	69	131	119	169
PFNA	9.5	59	69	131	119	169
PFDA	11.4	59	131	69	119	169
PFUnA	12.8	59	131	69	119	169
PFDoA	13.7	59	131	69	100	119

The analysis of standard dilutions resulted in a limit of detection (LOD) of 25 ng/mL (Figure 4-10). A dilution to 10 ng/mL did not result in any measurable peaks, the change in split ratio also did not improve the detection limit.

Additionally, all chromatograms show an unspecified band at the beginning of the run with a maximum at approximately 3.75 min.

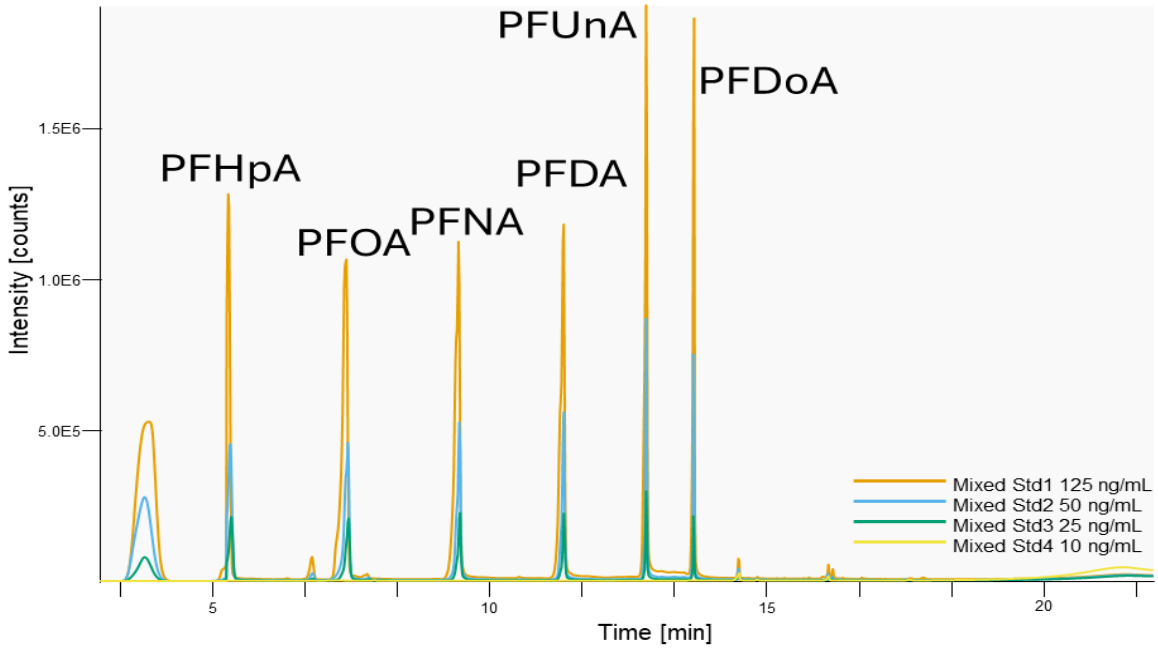


Figure 4-10: Chromatogram of mixed PFCA standards used in GC-EI-MS analysis. The chromatogram shows the absolute intensities achieved depending on the retention time of the analysis. Cumulative chromatogram of 4 analyses covering different concentrations (125 ng/mL, 50 ng/mL, 25 ng/mL and 10 ng/mL). however, the lowest concentration of 10 ng/mL did not lead to the successful detection of any of the target compounds.

4.3.5. Optimisation of pH values for the Ultrasound-assisted Extraction of PFCAs

For this part of the study, calibration standards for the LC-Orbitrap analysis of PFBA (C_4) to PFDoA (C_{12}) covered a range of 0.1 to 500 ng/mL. Calibration sets were run before and after samples. Retention times were confirmed, and calibration standards were checked to be within 30 relative differences following a calibration point weighing of $1/X^2$. The lowest available, within 30% difference standard from either calibration set was selected as limit of detection for the analysis. Generally, the minimum of the calibration range was between 0.5 to 250 ng/mL (Table 4-8). Retention times and product ion sizes used for detection are listed in Table 4-7.

Due to limitations in the setup of this analysis, only three isotope standards were used, matching PFBA, PFHxA and PFOA in structure, while containing a ^{13}C radioisotope backbone. Response ratios (PARs) of samples and standards generally improved with increasing size of the compounds. The response ratio for $^{13}C_4$ PFBA,

$^{13}C_6$ PFHxA and $^{13}C_8$ PFOA during biosolids analysis were 7.1, 13.3 and 36.8 %, respectively.

Table 4-7: Chromatographic compound data of selected perfluoro carboxylic acids (C_4 to C_{12}) for LC-Orbitrap-MS analysis. Retention times (t_R), precursor ion and most abundand product ion for the respective compounds. Similar to the previous GC-MS analysis, PFCAs share common product ions during detection, however, precursor ions are compound-specific.

Compound	t_R / min	Precursor ion / m/z	Product ion / m/z
PFBA	6.4	212.9792	168.9886
PFPeA	7.3	262.9760	218.9862
PFHxA	7.9	312.9728	118.9914
PFHpA	8.3	362.9696	168.9886
PFOA	8.8	412.9664	168.9886
PFNA	9.1	462.9632	168.9886
PFDA	9.4	512.9600	168.9887
PFUnA	9.8	562.9568	168.9886
PFDoA	10.1	612.9537	168.9886
^{13}C PFBA	5.8	218.0090	171.9979
^{13}C PFHxA	7.3	319.0166	120.9979
^{13}C PFOA	8.1	421.0096	171.9979

4.3.5.1. The Effect of Varying pH Values During Extraction in Biosolids Samples

The influence of pH values on the proposed extraction was first assessed within the biosolids matrix, undergoing extraction at pH 7 and 10. Three unspiked biosolids samples were prepared for both respective pHs, and analysed in triplicates. PFPeA, PFNA, PFUnA and PFDoA were not detected in any extracts (Table 4-8). The highest concentrations were measured for PFOA, with 108 ng/mL using a extraction pH 7 and 64 ng/mL at pH 10. The remaining compounds were only measured at lower concentrations, between 1 and 6 ng/mL. Standard deviations between extraction pHs were comparable.

Table 4-8: Concentrations of PFCAs in biosolids samples depending on the extraction pH (N=6). Determination with LC-Orbitrap-MS. The respectively used internal standards are highlighted, along with the calibration ranges of the individual compounds of this analysis.ND = not detected.

Compound	Internal Standard	Calibration range		Amount / ng/g	
		Min. ng/mL	Max ng/mL	pH 7	pH 10
PFBA	^{13}C PFBA	0.25	250	2 ± 1	6 ± 1
PFPeA	^{13}C PFBA	0.1	250	ND	ND
PFHxA	^{13}C PFHxA	0.5	250	0.4 ± 0.2	0.4 ± 0.2

PFHpA	^{13}C PFOA	0.5	250	108 ± 26	64 ± 16
PFOA	^{13}C PFOA	0.25	250	2 ± 1	1 ± 0.1
PFNA	^{13}C PFOA	0.25	250	ND	ND
PFDA	^{13}C PFOA	0.25	250	1 ± 0.1	1 ± 0.3
PFUnA	^{13}C PFOA	0.25	250	ND	ND
PFDoA	^{13}C PFOA	0.5	250	ND	ND

4.3.5.2. The Effect of Varying pH Values During Extraction in Soil Samples

In order to confirm pH selection, a set of three spiked and three unspiked soil samples were extracted at pH values of 2, 7 and 10 and analysed by LC-Orbitrap-MS subsequently. Calibration standards for analysed PFCAs (PFHpA – PFDoA) were run before and after samples. ^{13}C PFOA was used as an internal standard for all analytes. The lowest available, within 30% difference standard from either calibration set was selected as limit of detection for the analysis.

4.3.5.2.1. Extraction From Unspiked Soil Samples

Generally, the minimum of calibration range was between 1 to 250 ng/mL (Table 4-9). Extractions at pH 2 and 7 did not result in any measurable concentrations above the LOD regardless of the target compound in unspiked samples. PFHpA, PFOA and PFDoA showed the highest concentrations with 26, 29 and 17 ng/mL respectively with comparable, low relative standard deviations for all compounds.

Table 4-9: Concentrations of PFCAs in unspiked soil samples at pH 10 (N=3). All analytes share the internal standard of ^{13}C PFOA. Determination with LC-Orbitrap-MS. ND = not detected.

Compounds	**Calibration Range**		**Amount / ng/g**
	Min. ng/mL	**Max ng/mL**	**pH 10**
PFHpA	0.25	250	26 ± 2
PFOA	1	500	29 ± 1
PFNA	-	-	ND
PFDA	0.1	250	7 ± 0.4
PFUnA	5	500	5 ± 0.8
PFDoA	0.5	250	17 ± 0.6

4.3.5.2.2. Extraction From Spiked Soil Samples

Extractions at all pHs did result in a subset of measurable concentrations above the LOD in spiked (50 ng) samples (Table 4-10). Firstly, at pH 2, only PFHpA, PFOA and PFDA were measured at 11, 22 and 4 ng/mL respectively. Standard deviations

are in close agreement. A pH of 7 showed higher recoveries throughout, including PFUnA and PFDoA concentrations of 11 and 13 ng/mL. Finally, at pH 10 concentrations were comparable to extractions at pH 7, however, PFBA was significantly increased to 143 ng/mL. PFBA in particular shows higher relative standard deviations for pH 7 and 10.

Table 4-10: Concentrations of PFCAs in spiked (50 ng) soil samples depending on the extraction pH (N= 9). Determination with LC-Orbitrap-MS. Unspiked and spiked soil samples were run in the same batch and share the previously specified detection limits. ND = not detected.

Compounds	**Amount / ng/g**		
	pH 2	**pH 7**	**pH 10**
PFHpA	11 ± 1	73 ± 12	143 ± 12
PFOA	22 ± 1	31 ± 2	27 ± 1
PFNA	ND	ND	ND
PFDA	4 ± 0.1	18 ± 1	12 ± 1
PFUnA	ND	11 ± 3	4 ± 1
PFDoA	ND	13 ± 2	18 ± 4

4.4. Discussion

4.4.1. Sample Characterisation

4.4.1.1. Water and Organic Matter Content for Soils

The combined data of WC and OM showed a moderate positive correlation between the two parameters (r = 0.521), illustrated by the matrix plot for soil samples (Figure 4-11). One data point was found to be an outlier. Overall, the matrix plot demonstrates that the water content increases with increasing organic matter content.

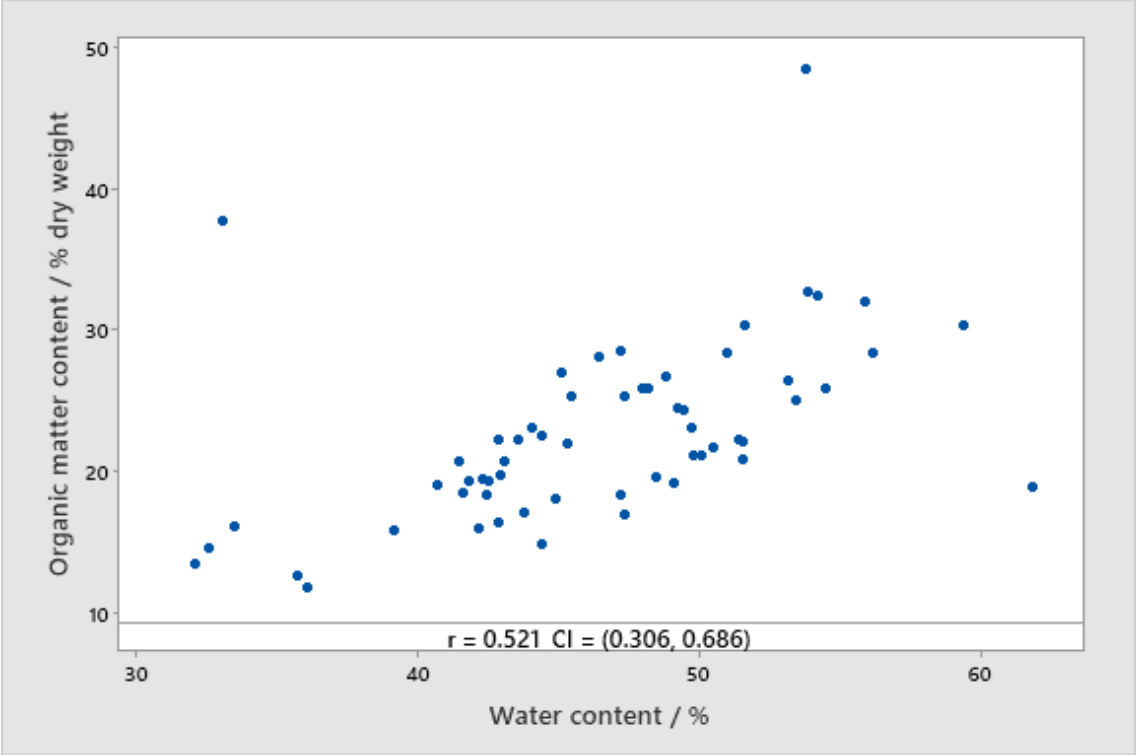


Figure 4-11: Matrix plot of the water and organic matter content using a 95% confidence interval for Pearson Correlation. Correlation of water content (WC) and organic matter content (LOI) in soil samples collected from 3 fields. Every field is represented by 20 subsamples. Generated with Minitab.

4.4.1.2. Water and Organic Matter Content for Biosolids

The processes involved in producing the analysed biosolids samples are not known as the origin of the biosolids is not known hence, differences in appearance and composition cannot be further interpreted.

4.4.1.3. Particle Size Determination of Soils

Overall, the collected data and statistical analysis concludes that firstly, the fields themselves are homogeneous considering water and organic matter content showing no significant differences within groups. Secondly, no significant difference was found between fields, ultimately allowing the comparison of experimental data.

The characterisation of organic matter and water content as well as the particle grading of environmental matrices is essential to allow valid conclusions (Drillia et al., 2005, Higgins and Luthy, 2006, Ahrens et al., 2009, Guo et al., 2016). It can be expected, that with increasing organic matter content the binding of longer chain PFAS to soils increases following the release from applied biosolids. Guo et al. (2016) argues that this phenomenon is caused by the increasing hydrophobicity of an increasing carbon chain length within the homologous sequence of PFCAs.

Contrary to that, as the increase of organic content correlates with increased water content, shorter chain length PFAS likely inhabit the porewater in soil.

4.4.2. Optimisation of Soil Sample Masses for Ultrasound-assisted Extraction

Derivatisation with MSTFA increases the volatility of the compounds of interest by substituting labile hydroxyl groups (hydrogen moiety) with a trimethylsilane ester. The process can occur at multiple labile moieties within the same molecule, resulting in separate reaction products, leading to chromatographic peaks with different masses. Caban and Stepnowski (2018) demonstrate the incomplete conversion of acetaminophen-1TMS to acetaminophen-2TMS, hence highlighting inaccuracies following the derivatisation process with MSTFA in ethyl acetate. However, the increased volatility still proves beneficial for the overall detection of compounds.

Drillia et al. (2005) identified a hysteresis phenomenon in tested soils caused by a combination of matrix and compound properties. As a result they found strong, irreversible interactions between the investigated compounds and particles of the soil leading to the retention of compounds. Results of this study are not directly comparable to results of Drillia et al. (2005), due to the determination of different soil characteristics (total organic matter vs. total organic carbon), varying soil properties (grain particle sizes) and the assessment of different pharmaceutical compounds. The assessment of the individual compound performance, in biosolids and soil samples highlights a significantly different behaviour of ibuprofen depending on the sample matrix involved in the extraction process, potentially indicating the hysteresis phenomenon for ibuprofen in the investigated soils in particular. However, low recoveries for triclosan and atenolol in particular may also be caused by the retention of the compounds caused by binding to soil particles.

The extraction of two grams of soil creates deficits in recovery, likely due to the complete retaining of solvent during the first extraction cycle, not resulting in any supernatant that can be collected. Recoveries in one gram ranging from 2 % and 8 % for ibuprofen and dapsone respectively, to 27 and 28 % for atenolol and triclosan are less than satisfactory while recoveries of 63 and 88 % for carbamazepine and acetaminophen respectively, are more adequate, yet still reduced. 0.5 grams of soil shows higher relative recoveries for all compounds compared to one gram of

soil; however, samples also have higher variation within samples of the same mass. Subsequently, one gram of soil is chosen as sample amount to balance recoveries, variation and low concentrations that are expected in real life samples.

4.4.3. Increasing the Throughput (Multiplexing of the Ultrasound-assisted Extraction

Differences in biosolids and soils highlight the different extend of matrix effects between matrices and potentially needs to be further assessed. However, for the validation of the extraction consistency across the sonication device, both matrices and sample preparation approaches were introduced and assessed by extracting all respective sample combinations in all three investigated sonotrode positions.

Subsequently, the PARs of the investigated compounds were pooled according to the extraction position used in the sonotrode, irrespective of sample type, preparation and analyte. This approach allows the accumulation of 24 data points for every used position of the sonotrode. Following this procedure simplified the statistical analysis by eliminating irrelevant factors (here: the type of compound, derivatisation status and matrix), hence allowing statistical analysis with pooled one-way ANOVA. The resulting dataset can be visualised in the boxplot diagram (Figure 4-12). The minimum, first and third quartiles, median and maximum for all three positions of the vialpress are comparable, considering the pooled peak area ratios of analysed compounds. Position one and five identify outliers in the dataset, yet the comparison amongst positions suggests consistent extraction results. Pooled one-way ANOVA analysis confirmed that there is no significant difference between positions ($p= 0.842$), leading to identical grouping with a 95 % confidence interval following a Tukey pairwise comparison.

Overall, varying sample matrices, sample preparation and sample amounts did not lead to significant differences in the pooled one-way ANOVA employed in this study. This confirms the described ultrasound-assisted extraction as a robust and reliable technique for the extraction of micropollutants from complex environmental matrices employing three sonotrode positions simultaneously. While other classes of compounds may be subjected to different levels of interaction with the complex matrices, the extraction process itself is vigorous and reproducible between extraction positions. Hence, this study confirms the increased efficiency of extraction processes due to the use of three positions

(compared to one position previously) for the ultrasound-assisted extraction of micropollutants.

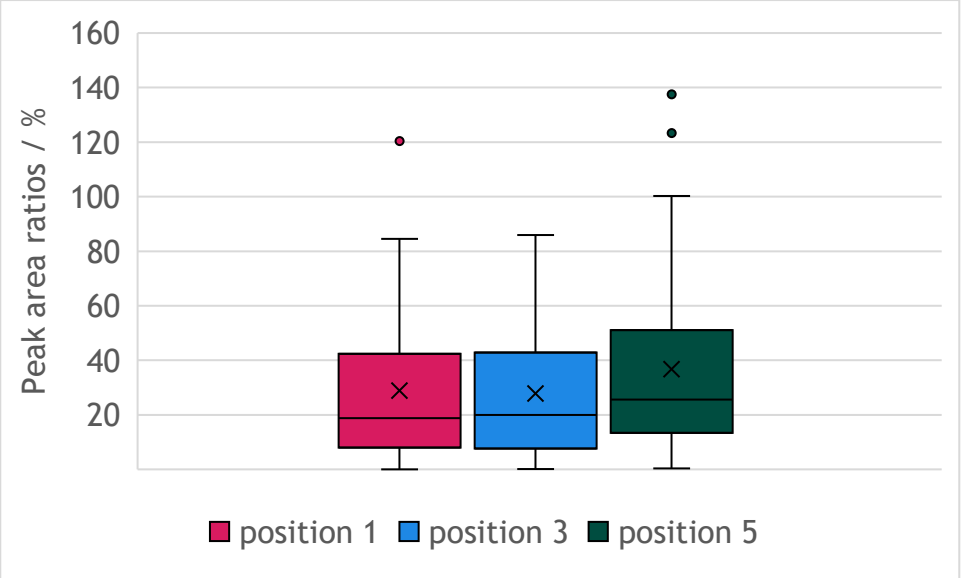
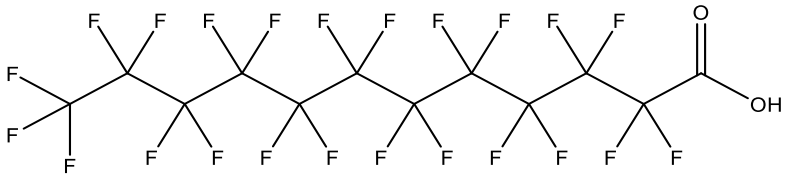


Figure 4-12: Boxplot diagram of pooled PARs of six compounds (acetaminophen, atenolol, carbamazepine, dapsone, ibuprofen and triclosan) and internal standard anthracene-D10 across soil and biosolids extracts depending on the position during the extraction process.

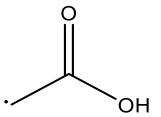
4.4.4. Gas Chromatography – Mass Spectrometry Method Development for the Detection of Perfluoro Carboxylic Acids Using Electron Impact Ionisation

All detected PFCAs shared the same product ions and usually differed by 50 amu, accounting for CF_2 groups: m/z 69 (CF_3) – 119 (CF_3-CF_2) – 169 (CF_3-CF_2-CF_2) – 219 (CF_3-CF_2-CF_2-CF_2). While larger product ions did occur, the smaller components dominated the mass spectra. Proposed corresponding chemical structures can be found in Figure 4-13.

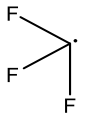
The use of softer ionisation techniques such as CI or atmospheric pressure chemical ionisation (APCI) can result in less fragmentation and therefore improve the overall sensitivity of a method by increasing the abundance of one common molecule ion rather than multiple product ions (Dufková et al., 2012, Portolés et al., 2015a). Dufková et al. (2012) employed a GC-MS method using electron impact ionisation during method development for the detection of perfluoro carboxylic acids, however, they found that the use of negative chemical ionisation achieved LODs at two to three orders of magnitude lower. Portolés et al. (2015a) discussed atmospheric pressure chemical ionisation as an alternative to traditional CI and EI methods, as it generates molecular ions rather than product ions hence being more specific in the subsequent detection.



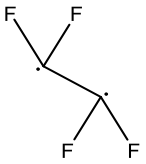
Perfluoro dodecanoic acid /PFDoA
Chemical Formula: $C_{12}HF_{23}O_2$
Exact Mass: 613.96



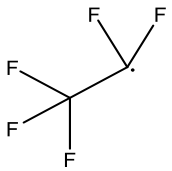
Chemical Formula: $C_2H_3O_2^{\bullet}$
Exact Mass: 59.01



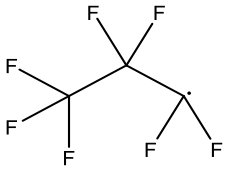
Chemical Formula: $CF_3^{\bullet}$
Exact Mass: 69.00



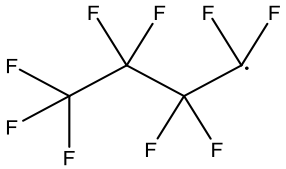
Chemical Formula: $C_2F_4^{2\bullet}$
Exact Mass: 99.99



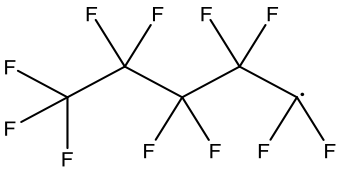
Chemical Formula: $C_2F_5^{\bullet}$
Exact Mass: 118.99



Chemical Formula: $C_3F_7^{\bullet}$
Exact Mass: 168.99



Chemical Formula: $C_4F_9^{\bullet}$
Exact Mass: 218.99



Chemical Formula: $C_5F_{11}^{\bullet}$
Exact Mass: 268.98

Figure 4-13: Proposed observed molecule fragments resulting from electron impact ionisation mass spectrometry (EI-MS). Exemplary precursor molecule PFDoA ($C_{12}HF_{23}O_2$, MW: 613.96 g/mol). Since all PFCAs have a common structure, only differing by the number of CF_2 groups, fragmentation patterns of PFDoA (the largest analysed PFCA of the study) should include all fragments also occurring for smaller acids. 7 Fragments of varying sizes could be identified, corresponding to commonly detected fragments sizes following GC-EI-MS analysis.

The use of derivatisation agents such as diazomethane can lead to the formation of methyl esters and has been discussed in the literature by means of improving volatilisation of compounds (Alzaga and Bayona, 2004, Dufková et al., 2012). The use of MSTFA for the derivatisation of PFCAs was trialled during method development but ultimately not utilised throughout the process. The implementation of derivatisation for the analysis might improve detection by improving peak shape, however, increasing the volatility of smaller PFCAs might amplify problems with the retention of smaller compounds of the homologous series. Alzaga and Bayona (2004) found that carboxylic acids with 6 or less carbons in the backbone would still not be retained enough to be analysed with GC-MS irrespective of derivatisation.

While the minimum detectable concentration of 25 ng/mL is not sufficient for trace analysis of PFCAs in agricultural soils, samples from contaminated environments, such as AFFF-release sights could be investigated. The utilisation of chemical ionisation was outwith the timescale available for this study, hence, for further analysis an LC-MS method is employed.

4.4.5. Optimisation of pH values for the Ultrasound-assisted Extraction of PFCAs

Recoveries between sample matrices and across extraction pHs vary significantly. pH 7 and 10 in spiked soil samples are similar in concentrations and RSD, however, in unspiked samples only pH 10 detected PFCAs. While pooled soil was used across all samples, variations can be caused through random errors, occurring during weighing. Samples are homogenised, however, if one sample by chance contains a higher clay fraction, or an unexpected source of organic carbon (e.g. dried root material) the binding of PFCAs to solid particles can retain compounds (Higgins and Luthy, 2006, Ahrens et al., 2009). Initial experiments were performed using glass GC/LC vials rather than PFAS-specific vials, not explaining the overall low recoveries for spiked concentrations but pose as a potential contributing factor.

Further consideration arose from Washington et al. (2010) analysing samples from biosolids-amended fields and found PFDA and PFDoA at concentrations up to 990 and 530 ng/g respectively. However, produced biosolids within the area potentially included sludge from wastewater treatment facilities handling wastewater streams from industries working with FTOHs. The study found differences in concentrations depending on the soil sampling depth, indicating that longer chain PFAS are more evident in deeper samples than shorter chain lengths. Rankin et al. (2016) detected PFCAs (C_6 – C_{14}) in cumulative concentrations of 0.029 to 24 ng/g dry weight using LC-MS/MS in samples representing all continents. Concentration differences in studies are highlighting the importance of general conditions, such as nearby industrial use or the legacy of AFFF use, compared to moderate concentrations in the wider environment yet low levels of PFCAs is measured in nearly all analysed samples across studies (Washington et al., 2010, Rankin et al., 2016, Zhi and Liu, 2018).

An extraction pH of 10 in line with publications is adopted for PFCA extraction considering overall results in biosolids, unspiked and spiked soils. A calibration range between 0.1 and 250 ng/mL in generally considered sufficient to detect

relevant environmental concentrations. The use of PFAS-specific vials, eliminating glass and PTFE as unnecessary sorbent and contamination risk, is expected to improve recoveries in the process.

4.5. Conclusion

In this chapter, aspects of soil characterisation were evaluated. The identified correlation between organic matter content and water content, as well as the soil particle size distribution are not sufficient to identify contributing factors of pharmaceutical and PFCA distribution in soil. Nevertheless, gathered results in conjunction with the overall literature review suggests that firstly, with increasing organic matter content of soils more hydrophobic compounds are more likely to bind to soil particulate matter. Secondly, with the associated increased water content, smaller and less hydrophobic compounds are likely to reside in the liquid fraction within pores, promoting mobilisation of such compounds. However, more detailed assessment by measuring adsorption/desorption experiments and the profiling of deeper soil cores could be beneficial in the assessment of the movement of compounds through the soil column.

The amount of soil chosen to be used for extraction and the low contaminant concentration levels expected need to be balanced, resulting in the selection of 1 gram of sample over 0.5 gram of soil sample based on better relative standard deviations rather than overall concentrations. Overall, PFCA recoveries from biosolids and soils were most efficient at an alkaline pH of 10.

Gas chromatography – electron impact – mass spectrometry (GC-EI-MS) was investigated to allow an integrated detection of PFCAs in line with other compounds of interest. Ultimately, the combined detection of pharmaceuticals and PFCAs within one GC-EI-MS run would combine previous and current efforts for environmental analysis, towards non-targeted approaches. Notably, the separation of PFCAs using the newly established approach is completed for investigated PFCAs (up to C_{12}) at a temperature of approximately 70 °C, whereas the detection of pharmaceuticals commences from an initial plateau of 100 °C towards a maximum temperature of 300 °C in the approach presented by Fell (2022). Bridging the gap between programs, and introducing a second plateau at 100 °C, potentially enables the separation and subsequent detection of both classes of compounds, within as little as 53 minutes (Figure 4- 14).

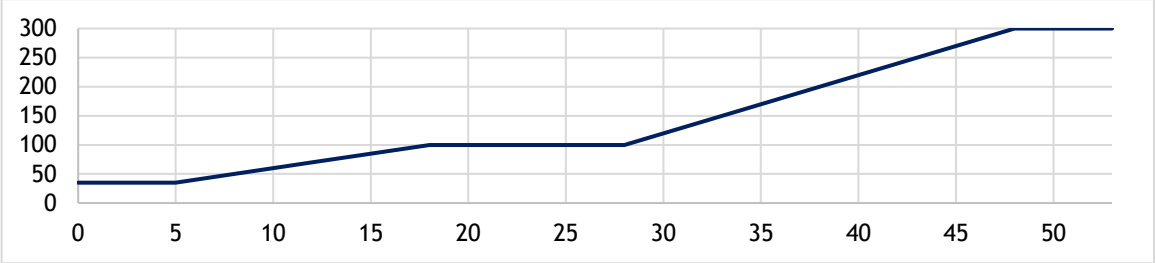


Figure 4- 14: Proposed GC-EI-MS oven programming for the combined separation and detection of PFCAs and pharmaceuticals. Overall run time of the proposed program is 53 minutes. PFCA separation commences at low temperatures, whereas pharmaceuticals are retained for longer.

While achieved limits of detection for PFCAs using GC-EI-MS were not adequate to pursue the detection of PFCAs in agricultural soils, AFFF-release sites may still be investigated with the current method. Further steps, such as adjusting the scale of ultrasound-assisted extraction, e.g. utilising different, less restrictive extraction vials, may allow the extraction of larger amounts of soils to introduce an additional concentration factor, increasing the detectability of compounds, however, such steps were not within the scope of this project. Furthermore, the use of alternative ionisation techniques, such as the utilisation of chemical ionisation, may be beneficial to improve detection limits for PFCAs. Irrespective of the limitations of GC-EI-MS, LC-Orbitrap-MS analysis was adapted for subsequent parts of this project due to its excellent calibration ranges extending to relevant environmental concentration ranges.

Chapter 5

Perfluoro Carboxylic Acid Profiles in Biosolids and Soil using Ultrasound-assisted Extraction and Liquid Chromatography – Orbitrap - Mass Spectrometry Analysis

5.1. Introduction

Per- and polyfluoroalkyl substances (PFASs) and perfluoro carboxylic acids in particular have been of industrial importance due to their excellent performance as surface treatments across industries, however, their persistence in the environment has become scrutinised in research. While there are several classes of PFASs, often only a limited number of compounds, such as perfluoro octanoic acid (PFOA) and perfluoro octane sulfonate (PFOS), are investigated as sentinels. However, production numbers of individual PFASs and their behaviour in the environment, including waterways, sediments and soils can significantly differ from compound to compound.

Varying functional groups and resulting polarities give PFASs a wide range of uses, often exploiting extraordinary surface properties in coatings. However, Kissa (2001) highlighted that adsorption to a solid/liquid interface does not only depend on the PFASs but also on factors such as the nature of the substrate as well as the nature of the liquid phase used. This deduces that in environmental matrices, grain sizes, composition, polarity, or surface area of soils, biosolids, waste and wastewater are contributing to the binding behaviour of PFASs to surfaces, interfaces and potentially promotes the association to undesired substrates. Rankin et al. (2016) elaborated that advantages that are exploited in industrial applications make PFASs persistent in the environment, with limited knowledge about long-term sinks such as soil and other solid matrices such as sediments, nevertheless the sorption mechanisms to environmental matrices is not well understood (Prevedouros et al., 2006).

The continued detection of PFASs in waste, wastewater and receiving bodies shows that the restriction of some but not all PFASs might not be sufficient to protect the environment from exposure (Sinclair and Kannan, 2006, van Leeuwen and de Boer, 2007, Ma and Shih, 2010, Nakayama et al., 2019, Lasee et al., 2021).

Wastewater treatment plants have been identified as a point source for contaminants, while biosolids are proposed as a diffuse source of pollutants although there is currently limited to no monitoring of the concentrations or classes of chemicals found in biosolids.

Modern analytical techniques for liquid samples are now able to identify over 100 compounds in ng/L ranges, using improved extraction techniques and analytical instrumentation such as liquid chromatography hyphenated with mass spectrometry (Peng et al., 2018, Proctor et al., 2019, Ramirez-Morales et al., 2020, Rapp-Wright et al., 2023). The LC analysis of biosolids or receiving soils is more challenging due to the complex nature of the sample matrix, but generally the use of internal standards and adequate sample clean-up are able to address matrix effects and improve the current practice (Mejia-Avendaño et al., 2017a, Kikuchi et al., 2018, Cao et al., 2019, Skaar et al., 2019, Pepper et al., 2021). The extraction method employed in this study was previously developed to extract pharmaceuticals from complex environmental matrices by Sampsonidis (2019) and Fell (2022). Method development towards the efficient extraction of perfluoro carboxylic acids and the successive analysis has been described in the previous Chapter 4.

The objective of this chapter was to determine and compare perfluoro carboxylic acid concentrations and cumulative profiles between biosolids and biosolids-amended soils following continued biannual application of biosolids. This study investigated the level of matrix effect arising from both matrices and assessed whether analysis is possible with minimal sample clean-up following the ultrasound-assisted extraction. Concentrations of nine PFCAs, ranging in size from perfluoro butanoic acid (C_4) to perfluoro dodecanoic acid (C_{12}), were assessed individually to identify presiding compounds. Additionally, cumulative concentration profiles were analysed to determine whether biosolids can be identified as a potential vector for contaminants into agricultural fields. For this purpose, six batches of biosolids and soil samples collected over a time period of up to three years were scrutinised.

5.2. Materials and Methods

5.2.1. Reagents, Stock Solutions and Standards

A complete list of test substances, internal standards and solvents can be found in section 4.2.1. A detailed description of the stock and standard makeup can be found in Appendix C.

5.2.2. Sample Preparation

Soil and biosolids samples were collected over the course of 2 years and 3 years respectively, as detailed in Figure 5-1. Soils were retrieved with the help of a soil corer (approx. 3 cm diameter) with a core depth of approximately 10 cm. Soil sampling took place up to four times a year, for the biosolids treated fields this was before biosolids application in spring and autumn, and approximately three months after application. Biosolids pellets were collected prior to each application. For the conventionally fertilised field (control), soil was collected at the same time as biosolids field soil samples. All samples were kept in glass jars with lid, stored at -80 °C until further use. Soil samples were randomly selected from the sample pool (N = 25 out of 100 for each field, 5 of each sampling time point). Three subsamples were taken from every available biosolids batch (six batches in total). Once collected, samples were stored at -80°C until analysis.

Biosolids and soil samples were defrosted and dried at 30 °C, usually 48 hours were sufficient for the drying process. Soil samples were passed through a 2 mm sieve subsequently. Once remaining sample masses were documented, samples were stored in glass jars with lids at 4 °C. Biosolids samples were ground into a powder using mortar and pestle and stored in glass jars with lids at 4 °C.

1 g ± 0.001 g of soil and biosolids samples were weighed into clean labelled 10 mL headspace vials prior to extraction and analysis. Crimp top seals with butyl rubber septum were used to minimise PFASs contamination in the process.

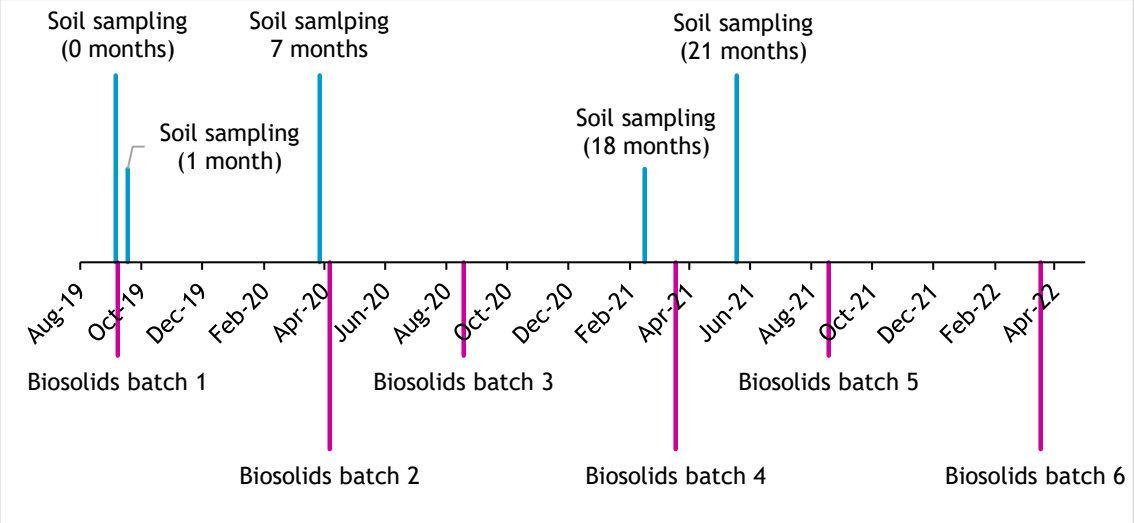


Figure 5-1: Timeline for the collection of soil and biosolids samples. 2019-2022.The sampling campaign began in September 2019 with the collection of the first set of soil and biosolids samples. Soil sampling 2 (after 1 month) was set out to assess the 3 week no-grazing period set out by the Sewage Sludge Directive 86/278/EEC. Successive sampling was partially impacted by COVID-19 restrictions, leaving uneven sampling intervals. Biosolids sampling usually took place on the verge of biosolids application whereas soil collection varied.

5.2.3. Instrumentation

5.2.3.1. Sonotrode

Details concerning the employed sonication device can be found in section 4.2.5.1.

5.2.3.2. Liquid Chromatography – Mass Spectrometry

The analysis of biosolids and soils was performed using a Thermo Scientific Dionex Ultimate 3000 UHPLC system with hyphenated Thermo Scientific Q-Exactive Orbitrap mass spectrometer, detailed in section 4.2.5.3.

Analysis was performed on a C_{18} Accucore column (Thermo Scientific, 100 mm x 2.1 mm, 2.6 µm particle size). Notably, the LC system was also equipped with a PFASs delay column to trap system-related PFASs, such as PFBA that can be found in laboratory components (i.e. tubing). As a consequence, based on the adequate use of the LC system, a system-intern contamination source was less likely. The mobile phase was made up of acetonitrile (eluent A) and ammonium formate (eluent B, 10 mmol, pH 3.5).

5.2.3.3. Software

The LC-Orbitrap-MS was utilised through Tracefinder (5.1 SP1, Windows 10). The LC data was accessed through Masshunter (11.0, Windows 10). Minitab (21.4.1, Windows 10) and Microsoft Excel (16.0.18129.20158, Windows 10) were used for statistical analysis.

5.2.4. Procedure

The extraction process has previously been described in section 4.2.6. Notably, all re-constituted samples, soil and biosolids, were passed through a 20 µm nylon filter to avoid blockages in the LC column. Extracts (10 µL aliquots) were analysed using LC-Orbitrap-MS.

Retention times and precursor and product ion sizes used for detection are listed in Table 5-1. Example chromatograms are shown in Figure 5-2. Calibration standards generally covered a range of 0.1 to 500 ng/mL. Calibration sets (PFBA – PFDoA) were run before and after samples. Retention times were confirmed, and calibration standards were checked to be within 30 relative differences following a calibration point weighing of $1/X^2$. The lowest available, within 30% difference standard from either calibration set was selected as limit of detection for the analysis. A minimum of 4 standards was required for a valid calibration.

Due to limitations in the setup of this analysis, only three isotope standards were used, matching PFBA, PFHxA and PFOA in structure, while containing a ^{13}C radioisotope backbone.

Table 5-1: Chromatographic compound data of selected perfluoro carboxylic acids for LC-Orbitrap-MS analysis. Retention times (t_R), precursor ion and product ion for the respective compounds.

Compound	t_R / min	Precursor ion / m/z	Product ion / m/z
PFBA	6.7	212.9792	168.9886
PFPeA	7.6	262.9760	218.9862
PFHxA	8.1	312.9728	118.9914
PFHpA	8.5	362.9696	168.9886
PFOA	9.0	412.9664	168.9886
PFNA	9.4	462.9632	168.9886
PFDA	9.9	512.9600	168.9887
PFUnA	10.2	562.9568	168.9886
PFDoA	10.6	612.9537	168.9886
^{13}C PFBA	5.8	218.0090	171.9979
^{13}C PFHxA	7.3	319.0166	120.9980
^{13}C PFOA	8.1	421.0096	171.9979

Soil from biosolids-treated pasture, soil from conventionally fertilised pasture (control) and biosolids were analysed on separate calibrations.

Figure 5-2: Chromatograms of the LC-Orbitrap-MS analysis of a mixed perfluoro carboxylic acids (PFBA – PFDoA) standard.MS was operated in multiple reaction monitoring (MRM) with the respective transitions from precursor to product ions.

5.3. Results

5.3.1. Perfluoro Carboxylic Acid Profiles in Biosolids

Reconstituted biosolids extracts were spiked with the mixture of ^{13}C PFCA standards and initially only filtered through a 20 µm nylon filter before sample analysis. However, as illustrated in Figure 5-3, the lack of additional preparation steps led to the co-extraction of matrix components, forming a band in the chromatogram between elution times of approx. 9 and 12.5 minutes, overlapping with the retention times for PFOA to PFDoA (Table 5-1). The chromatographic profile suggests that the band is made up of 3 individual peaks and appears to be an order of magnitude higher than the remaining components of the separation. Noteably, the co-extracted compounds also appear in control soil extracts albeit

at lower concentrations, indicating that they are naturally occurring in the agricultural environment.

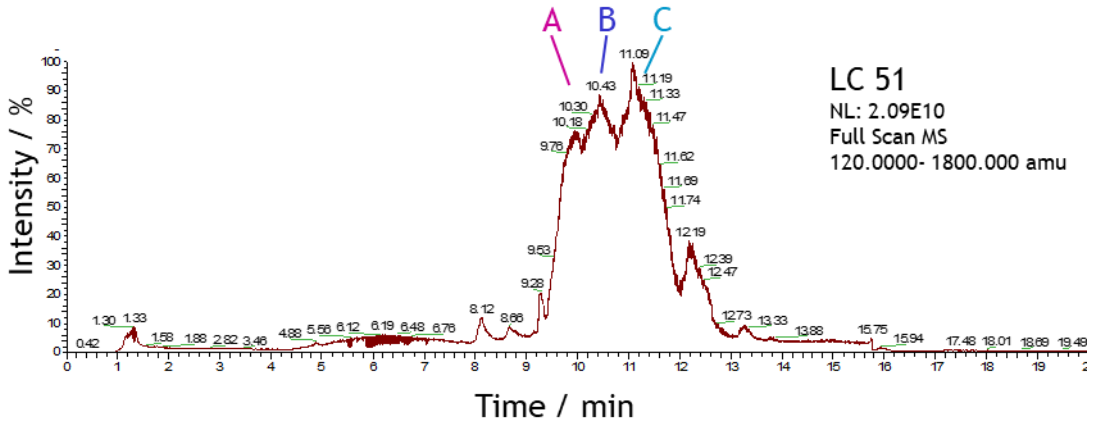


Figure 5-3: Exemplary chromatogram of a biosolids extract (LC 51) following filtration , without additional preparation steps showing a band of three peaks (A, B and C) interfering with the required detection range for target compounds. Chromatogram was captured in full scan mode and ions filtered with an atomic mass unit range between 120.000 and 1800.000. Maximum abundance (100 % intensity) was determined with an absolut abundancy of 2.09E10 counts.

Using the mass spectra gained during analysis, 3 mass spectral peaks (A, B and C) corresponding to the co-extracted compounds making the biosolids dilution necessary could be identified in soil and biosolids samples (Figure 5-4). Subsequently, using the spectrum list, isotope patterns were assessed. The distribution between the main peak (100 % abundance) and the following peaks (+ 1 amu, +2 amu) indicates either ^{13}carbon or nitrogen as the main contributors. This pattern occurs in all three peaks in question (ABC). The presence of an unknown fluorinated compounds was ruled out due to the lack m/z values corresponding to fluorine or fluorinated carbon groups in the mass spectra. Distances between peaks (AB and BC) are equally spaced, indicating a homologous row of compounds. Best fit for the distance between peaks is given by CH_2 groups (14.01510 amu), compared to Nitrogen (14.00253 amu) (Table 5-2).

Potential molecular formulas for the unknown compounds are collated using a monoisotopic mass calculator (https://www.chemcalc.org/) (Table 5-3). The exact mass of the 100% abundance peak is entered and analysed using a range of 0 – 100 carbon, 0 – 200 hydrogen, 0 – 5 nitrogen and 0 – 20 oxygen as potential components with a mass error of 5 amu. The chemical formulas of $C_{20}H_{23}O_3$, $C_{21}H_{25}O_3$ and $C_{22}H_{27}O_3$ were in good agreement between the measured peak data and the theoretical mass, with ± 5 amu difference as tolerance (mass error). The potential formulas were scrutinised by generating theoretical MS fragmentation

spectra, which were found to be in good agreement with the experimental data. Finally, gating the MS spectra with the respective mass ratios, illustrates that the investigated peaks are indeed distinguishable using the mass data of the full range MS scan (Figure 5-5). However, the precise identification of the corresponding structures and the respective molecular formulas was not within the scope of the project.

Ultimately, samples in this experiment originate from complex environmental matrices and the co-extraction of matrix components posed as a drawback that needed to be addressed with additional preparation procedures. The subsequent introduction of a 1:10 dilution factor was sufficient to overcome overloading of the analytical system, making the analysis of all targeted PFCAs possible. Additional washes and equilibration runs were added to further reduce the risk of contamination and carry-over arising from the co-extraction of matrix components.

Samples were expected to contain a range of concentrations of the analytes at or around the limit of quantification (LOQ) and beyond. Due to the nature of the setup, the lowest valid measured calibration standard concentration is defined as the LOQ as well as the limit of detection (LOD) of the analysis. Any sample amount falling below this threshold is is referred to as below limit of detection (<LOD), while the lack of any measured amount is marked as not detected (ND). The high sensitivity and specificity of the LC-Orbitrap-MS analysis allows the differentiation of <LOD and ND while also allowing the bias and uncertenty introduced by equaling LOQ and LOD (Armbruster and Pry, 2008). Armbruster and Pry (2008) further elaborate that the analysis of blanks is useful, defining the highest apparent analyte concentrations that may be expected in samples meant to be containing no analyte.

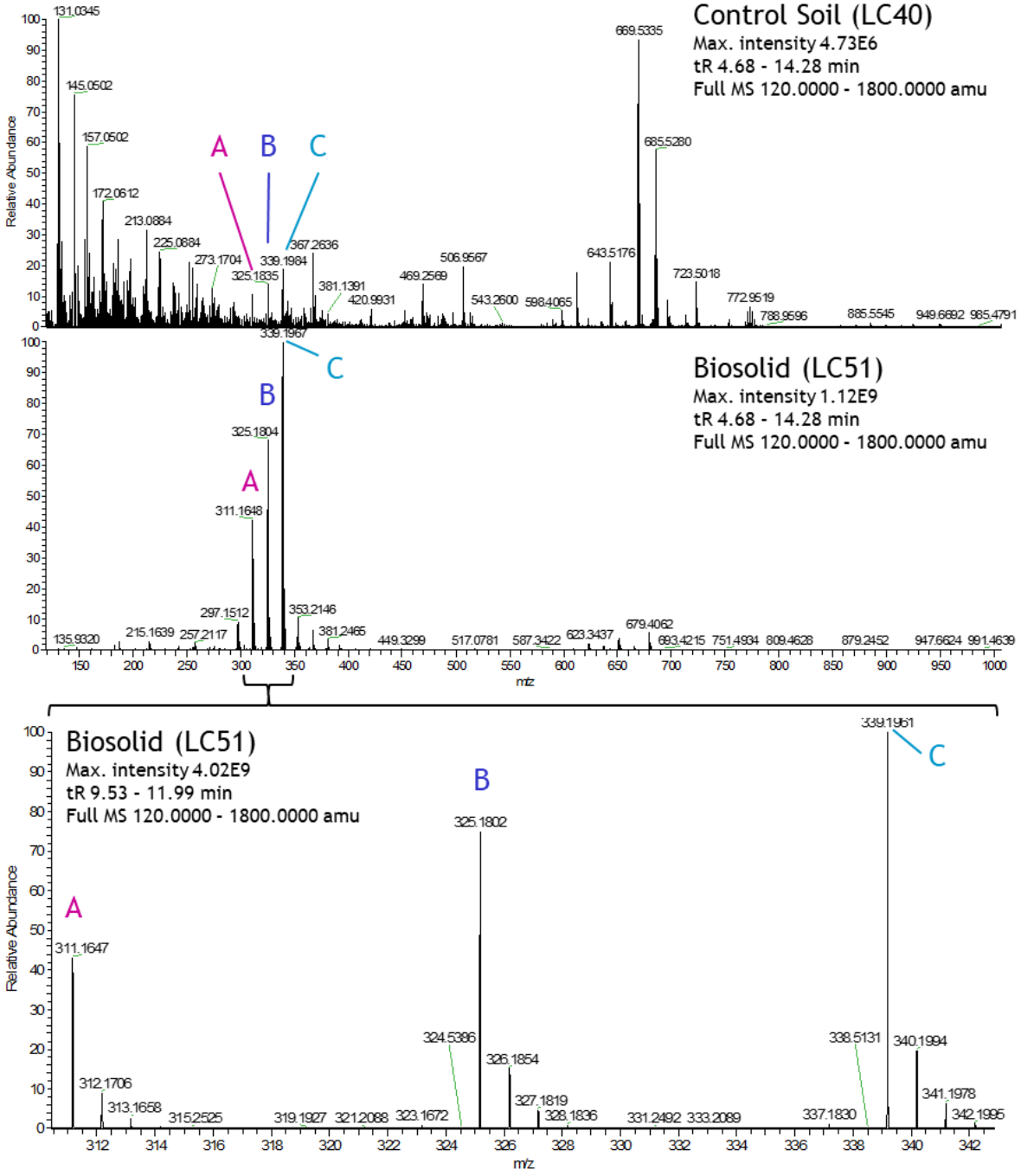


Figure 5-4: Exemplary mass spectra of a control soil (LC40, top) and a biosolids sample (LC51, middle) in full scan mode and zoomed in view on the relevant section of the mass spectra for the biosolids sample (LC 51, bottom). Corresponding retention times (tR) and relative abundancies for the samples are captured in full scan mode, filtered with an atomic mass unit range of 120.000 to 1800.000. Peaks with relevant mass to charge ratios accounting for the respective unknown compounds in the chromatogram are labelled with A, B and C, found in soil samples and biosolids. Zoomed in view of mass spectrum highlights smaller secondary peaks following the highest abundant mass fragment of the unknown compounds. Differences in atomic mass units can be used to assess isotope patterns allowing for an indication of the nature of the unknown compounds.

Table 5-2: Isotope patterns in relevant peaks (A, B and C) for the identification of unknown compounds co-extracted from biosolids and soils. Distances (D) between peaks and precise atomic mass units of relevant constituents.

Peak	m/z	Abundance	
A	311 312 313	100 % 20 % 6 %	D(AB) = 14.0155 amu D(BC) = 14.0159 amu
B	325 326 327	100 % 20 % 6 %	
C	339 340 341	100 % 20 % 6 %	

Table 5-3: Potential molecular formula candidates for the identification of unknown co-extracted compounds in biosolids and soils. Molecular formulas have been generated with the help of a monoisotopic mass calculator. Parameters for the identification were constituents of 0-100 C, 0-200 H, 0-5 N, 0-20 O and a maximal permitted mass error of 5 amu.

#	Exact Mass	Molecular Formula	Monoisotopic Mass	Ppm (mass error)
A1	**311.1648**	**$C_{20}H_{23}O_3$**	**311.1647**	**0.26**
A2		$C_{18}H_{21}N_3O_2$	311.1634	4.57
B1	**325.1804**	**$C_{21}H_{25}O_3$**	**325.1804**	**0.09**
B2		$C_{19}H_{23}N_3O_2$	325.1790	4.22
C1	**339.1967**	**$C_{22}H_{27}O_3$**	**339.1960**	**2.00**

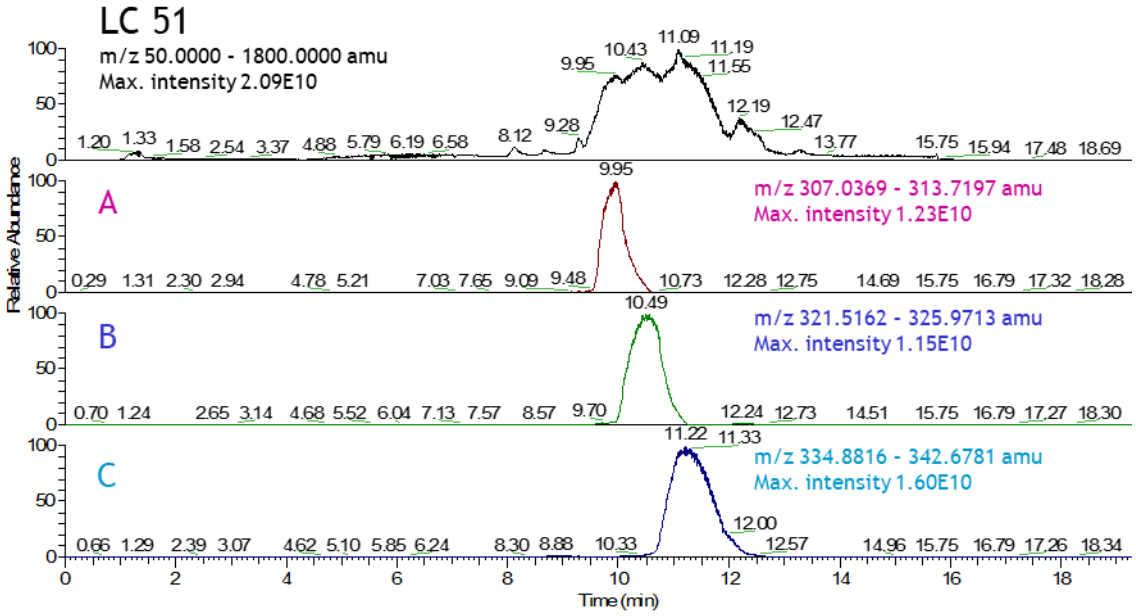


Figure 5-5: Comparison of a full MS range chromatogram of biosolids sample LC 51 and restricted mass gating to identify relevant peaks of the chromatogram. Once corresponding compound masses have been identified and confirmed with theoretical MS fragmentation patterns, the agreement between theoretical and experimental data can be compared by applying narrow mass filters to the chromatogram. In this manner, the individual unknown peaks can be identified by their mass range, and are also detailed with the respective maximum intensities of the chromatographic peaks.

Consequently, for quality control purposes, a blank and a positive control were carried through the extraction and analysis along with the samples. Blanks consisted of the solvents employed, with the methanol/water mixture undergoing sonication without the addition of matrix material. The solvent was subsequently dried in the heating block along with the samples, before being "reconstituted" with the acetonitrile/water utilised for samples. A control soil sample was utilised as positive control since preliminary results of available biosolids samples presented with varying but consistently detected amounts of PFCAs (Chapter 4, Table 4-8). This allowed for a more precise assessment of the employed methods as the spiked concentrations and calculated recoveries were less likely to be affected by intrinsic PFCA contamination. Despite mismatching the matrix, all other aspects of extraction and analysis were kept in line with biosolids samples.

The use of internal standards is compensating for matrix effects in samples and controls but discrepancies in the mismatched control still needed to be considered. Fell (2022) states that the matrix effect can be calculated with the help of the employed isotope standards as:

$$Matrix\ effect\ [\%] = \left(\frac{isotopic\ analyte\ in\ sample}{isotopic\ analyte\ in\ standard\ solution}\right) * 100\ \% \qquad (9)$$

indicating ion enhancement for values greater than 100 % and ion suppression for values smaller than 100%. The matrix effect for ^{13}C PFBA, ^{13}C PFHxA and ^{13}C PFOA in biosolids was determined to be 4%, 7%, and 12%, respectively, indicating a significant amount of ion suppression during analysis of biosolids. In principle, the matrix effect appears to be less with increasing carbon chain length, indicating that larger PFCA analytes are subjected to less matrix effects.

Overall quantification limits were low, with as little as 0.1 ng/g for PFUnA and PFDoA, and 2.5 ng/g for the smaller PFCAs, ranging from C_4 to C_8 (Table 5-4). The upper limit of the calibration for all compounds was 500 ng/g resulting in an excellent range for the determination of PFCAs.

The blank showed varying levels of all PFCAs of interest. PFBA in particular was detected at a high level of 141 ng/g, followed by intermediate levels of PFPeA and PFHxA and low levels of the remaining analytes. The use of untreated (control) soil as positive control introduces a degree of uncertainty as the presence of any trace amounts of the target compounds within the matrix would enhance the

concentration detected, however, effects were assumed to be neglectible. Following the correction with employed internal standards only selected PFCAs in the positive control were detected within the acceptable range (80 – 120 %). Overall, recoveries varied from 41 % for PFDoA to a maximum of 133 % for PFDA, however, PFBA, PFPeA and PFHxA were not included in the setup.

Table 5-4: Chromatographic analysis-depending parameters in the biosolids analyses - limit of quantification (LOQ), blank and control concentrations depending on the analyte.

Compounds	**Internal std.**	**LOQ ng/mL**	**Control ng/mL**	**Blank ng/mL**
PFBA	^{13}C PFBA	2.5	-	141
PFPeA	^{13}C PFBA	2.5	-	26
PFHxA	^{13}C PFHxA	2.5	-	40
PFHpA	^{13}C PFOA	2.5	114 (+14 %)	13
PFOA	^{13}C PFOA	2.5	123 (+23 %)	3
PFNA	^{13}C PFOA	0.5	126 (+26 %)	1
PFDA	^{13}C PFOA	1	133 (+33 %)	2
PFUnA	^{13}C PFOA	0.1	108 (+8 %)	0.1
PFDoA	^{13}C PFOA	0.1	41 (-59 %)	7

PFBA was detected in consistently high levels across biosolids samples (Figure 5-6). Concentrations of PFBA ranged from 143 to 229 ng/g. PFPeA concentrations ranged from 28 to 161 ng/g, the boxplot for this compound identified a low median of 44 ng/g and the highest measured concentration as an outlier within the series. PFHxA presented similar to PFPeA, with concentrations between 22 and 105 ng/g. PFHpA was not detected in 1 sample, however, the remaining sample set ranged from 5 to 33 ng/g. PFOA, PFNA, PFDA and PFUnA reached an average of approx. 4.8 ng/g, only occationally exceeding 10 ng/g within all samples. PFUnA was not detected in a total of 3 samples across the biosolids sample pool. PFDoA showed the least variation considering the larger chain lengths, with only 19 % relative standard deviation for values between 5.6 and 11.4 ng/g.

Noteably, the contaminated blank previously discussed follows a similar profile of concentrations, with PFBA at higher concentrations, followed by PFPeA and PFHxA at an intermediate level and larger PFCAs at low concentrations. This might

indicate overloading of the column due to co-extraction of matrix materials that increase the risk for blockages and carryover was still ongoing during analysis.

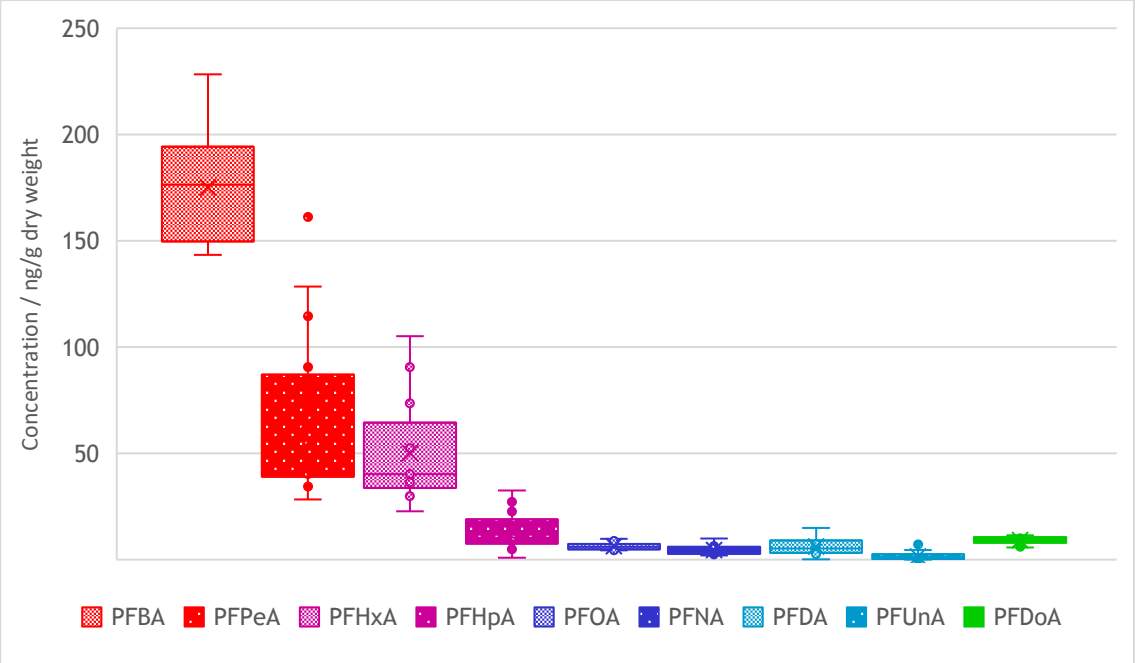


Figure 5-6: Boxplot of PFCA concentrations in biosolids samples depending on the compound across six batches collected between 2019 and 2022 (N= 18). Analysis included PFBA - PFDoA. Boxplots show concentration ranges measured across six biosolids batches, every batch was assessed with 3 sample aliquots.

5.3.2. Perfluoro Carboxylic Acid Profiles in Biosolids-Amended Soil

Reconstituted extracts from biosolids-amended soil were spiked with the mixture of ^{13}C PFCA standards and filtered through a 20 µm nylon filter before analysis. Contrary to the biosolids samples, a dilution step was not necessary to achieve adequate chromatographic performance (Figure 5-7). Similarly, samples were expected to contain low concentrations of the analytes at or around the limit of quantification.

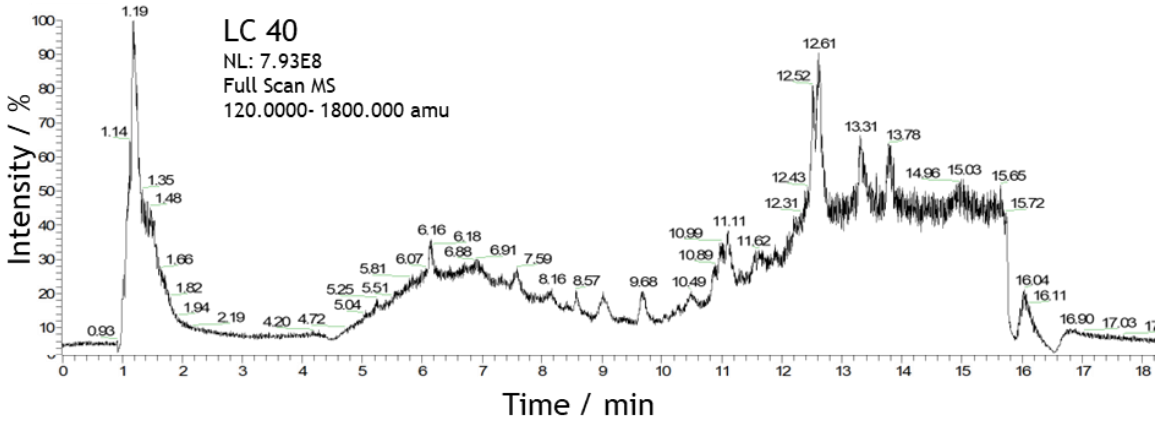


Figure 5-7: Exemplary chromatogram of a soil extract (LC 40) following filtration, without additional preparation steps. Chromatogram was captured in full scan mode and ions filtered with an atomic mass unit

range between 120.000 and 1800.000. Maximum intensity (100 %) was determined with an absolute abundancy of 7.93E8.

The matrix effect for ^{13}C PFBA, ^{13}C PFHxA and ^{13}C PFOA was determined to be 75%, 114%, and 106%, respectively indicating ion suppresion for ^{13}C PFBA and ion enhancement for ^{13}C PFHxA and ^{13}C PFOA during analysis of biosolids-amended soils using LC-Orbitrap-MS. In principle, the matrix effect appears to be best (minimal) for ^{13}C PFOA, indicating that larger PFCA analytes might be subjected to less matrix effects.

Overall quantification limits are low, with as little as 0.1 ng/g for most analytes and 5 ng/g for the PFPeA (Table 5-5). The upper limit of the calibration for all compounds was 500 ng/g resulting in an excellent range for the determination of PFCAs. The blank showed concentrations below the detection limits for all compounds of interest. Similar to the analysis of the biosolids batches, a soil sample originating from the control field was carried through the process as positive control. The summarised chromatographic details can be found in Table 5-5. Recoveries of the control sample varied from 46 % for PFHpA to a maximum of 168 % for PFHxA and PFUnA.

Table 5-5: Chromatographic analysis-depending parameters in the biosolids-amended soil analyses - limit of quantification (LOQ), blank and spiked (50 ng) positive control concentrations depending on the analyte.

Compounds	Internal std.	LOD ng/mL	Control ng/mL	Blank ng/mL
PFBA	^{13}C PFBA	0.1	70 (140 %)	<LOD
PFPeA	^{13}C PFBA	5	70 (140 %)	<LOD
PFHxA	^{13}C PFHxA	2.5	84 (168 %)	<LOD
PFHpA	^{13}C PFOA	0.1	23 (46 %)	<LOD
PFOA	^{13}C PFOA	0.1	77 (144 %)	<LOD
PFNA	^{13}C PFOA	0.25	73 (146 %)	<LOD
PFDA	^{13}C PFOA	0.1	52 (104 %)	<LOD
PFUnA	^{13}C PFOA	0.1	79 (168 %)	<LOD
PFDoA	^{13}C PFOA	0.1	70 (140 %)	<LOD

Across all 25 samples and all 9 analytes, only 18 measurements were found to be above the quantification limit. PFBA, PFPeA, PFHxA and PFUnA did not return any quantifyable values, lying either below the quantification limit or not being detected at all. PFOA and PFDoA were only present in one respective sample at a concentration of 2.7 ng/g and 0.7 ng/g, respectively. PFNA was determined at 0.7

and 0.9 ng/g in two samples, while PFHpA and PFDA were found in seven samples respectively. PFDA was determined at concentrations between 0.1 and 1.8 ng/g while PFHpA was ranging between 1.2 ng/g and 22.2 ng/g (Figure 5-8).

Noteably, for the purpose of statistical evaluation of the dataset, concentrations that were not detected or below LOD were filled in with random values between zero and half-LOD hence appearing as an artificial box (PFBA, PFPeA, PFHxA and PFUnA) within the boxplot. For compounds that were detected in a subset of the samples this leads to a weighing down of boxplots. Altering mean, median, and quartiles in the process.

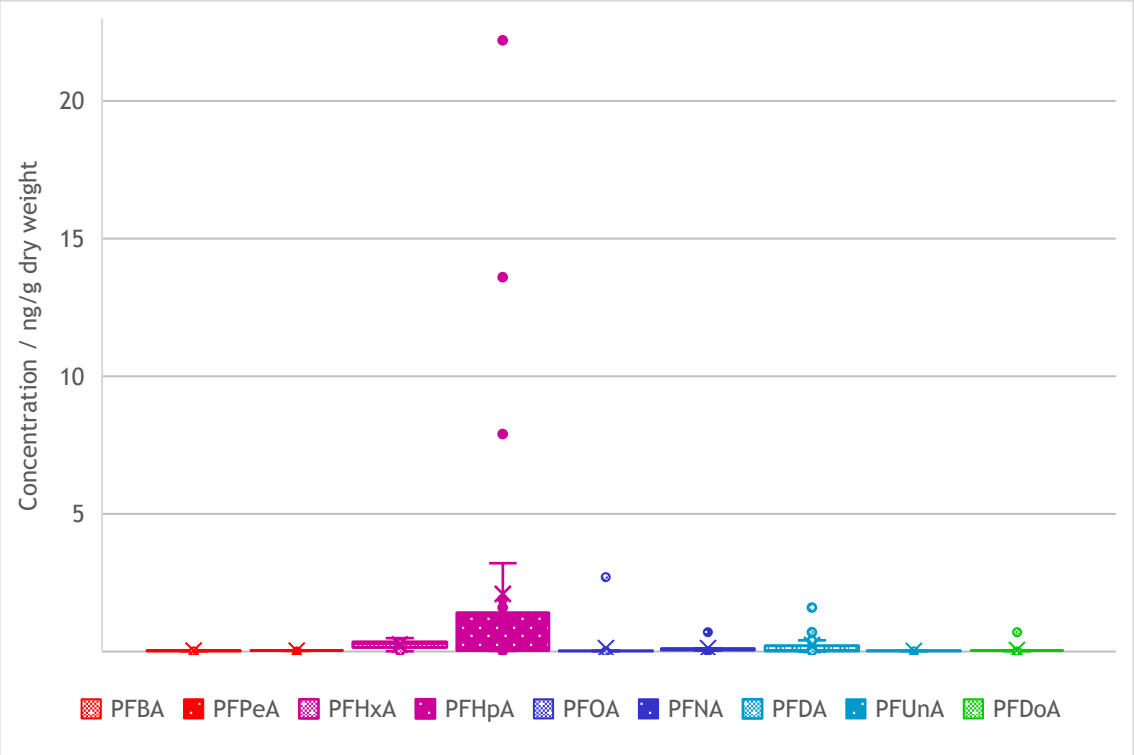


Figure 5-8: Boxplot of PFCA concentrations in biosolids-amended soil depending on the compound. Samples were collected over 2 years between 2019 and 2021 (N= 25).

5.3.3. Perfluoro Carboxylic Acid Profiles in Control Field Soil

Reconstituted extracts of soils from the control site fertilised with inorganic fertiliser were spiked with the mixture of ^{13}C PFCA standards and filtered through a 20 µm nylon filter before analysis. A blank and control were carried through the extraction and analysis. Quantification limits are varied across compounds, with as little as 0.25 ng/g for PFUnA and as much as 5 ng/g and 10 ng/g for PFPeA and PFBA, respectively. The upper limit of the calibration for all compounds was 500 ng/g resulting in an excellent range for the determination of PFCAs. The blank shows concentration below the detection limits for all compounds of interest.

Recoveries of the control sample varied from 95 % for PFNA to a maximum of 192 % for PFUnA. With the exception of PFUnA all PFCA control concentrations were in acceptable concentration range between 80 – 120 %, however, PFBA, PFPeA and PFHxA were not included in the setup of the positive control. The summarised chromatographic details can be found in Table 5-6.

The matrix effect for ^{13}C PFBA, ^{13}C PFHxA and ^{13}C PFOA was determined to be 92%, 98%, and 111 %, respectively indicating ion suppression for PFBA and PFHxA but ion enhancement for PFOA during analysis of control soils using LC-Orbitrap-MS. In principle, the matrix effect appears to be best (smallest) for PFHxA.

Table 5-6: Chromatographic analysis-depending parameters in the control soil analyses - limit of quantification (LOQ), blank and control concentrations depending on the analyte.

Compounds	Internal std.	LOD ng/mL	Control ng/mL	Blank ng/mL
PFBA	^{13}C PFBA	10	-	<LOD
PFPeA	^{13}C PFBA	5	-	ND
PFHxA	^{13}C PFHxA	2.5	-	<LOD
PFHpA	^{13}C PFOA	2.5	103 (+3 %)	<LOD
PFOA	^{13}C PFOA	0.5	105 (+5 %)	<LOD
PFNA	^{13}C PFOA	0.5	95 (-5 %)	<LOD
PFDA	^{13}C PFOA	1	98 (-2 %)	<LOD
PFUnA	^{13}C PFOA	0.25	192(+ 92 %)	<LOD
PFDoA	^{13}C PFOA	1	102(+2 %)	<LOD

Across all 25 samples and all 9 analytes, only 3 measurements were found to be above the quantification limit, one sample containing 1.3 ng/g PFOA, and two samples containing PFDoA at concentrations of 0.5 and 23.2 ng/g. The other analysed compounds did not return any quantifyable values, lying either below the quantification limit or not being detected at all.

5.4. Discussion

5.4.1. Perfluoro Carboxylic Acid Profiles in Biosolids

Sample clean-up with solid phase extraction is often included in workflows in the literature as it also allows for the concentration of extracts. Most commonly, weak anion exchange (WAX) sorbents (Higgins et al., 2005, Munoz et al., 2015, Strynar et al., 2015, Rankin et al., 2016, Kikuchi et al., 2018) or activated carbon based sorbents (Gomez-Canela et al., 2012, Mejia-Avendaño et al., 2017a, Cao et al.,

2019, Skaar et al., 2019, Pepper et al., 2021) are used. Hydrophilic lipophilic balance (HLB) sorbents are also utilised for this purpose, however, publications regarding HLB SPE do usually explore water-based samples (riverwater, wastewater etc.) (Portolés et al., 2015a, Tröger et al., 2018). While SPE does present with multiple advantages it also introduces a degree of selectivity that may interfere with the subsequent advance towards non-targeted approaches and was therefore not included in the methodology.

Statistical analysis found no significant differences between biosolids batches. Analysis of Variance and subsequent principal component analysis (PCA) identified 3 groups within the data set, one containing PFBA, one group for PFPeA and PFHxA and the final group for the remaining PFCAs from PFHpA to PFDoA. ANOVA found that PFPeA and PFHxA are similar to each other, and closer to PFBA rather than the larger PFCAs as indicated in the Tukey pairwise comparison (Figure 5-9-A). The assigned grouping is following the concentration profile of the samples, with PFBA at high levels, PFPeA and PFHxA at intermediate levels, and comparably lower levels of the remaining compounds. The outlier plot (Figure 5-9-B) for this dataset does not identify any outliers exceeding the Mahalanobis distance, the scree plot (Figure 5-9-C) includes two components with eigenvalues greater than 1, accounting for 77 % of the variation in the dataset. The score plot (Figure 5-9-D) is representing an open cluster with relatively even distances, not identifying any grouping. Using the cumulative concentrations of every respective sample to normalise the dataset does not change the grouping or score plot (Figure 5-9-G). However, the distances in the Tukey pairwise comparison increase, and the scree plot identifies 4 components with eigenvalues greater than 1, now accounting for 92 % of the variation in the dataset (Figure 5-9-E and -F).

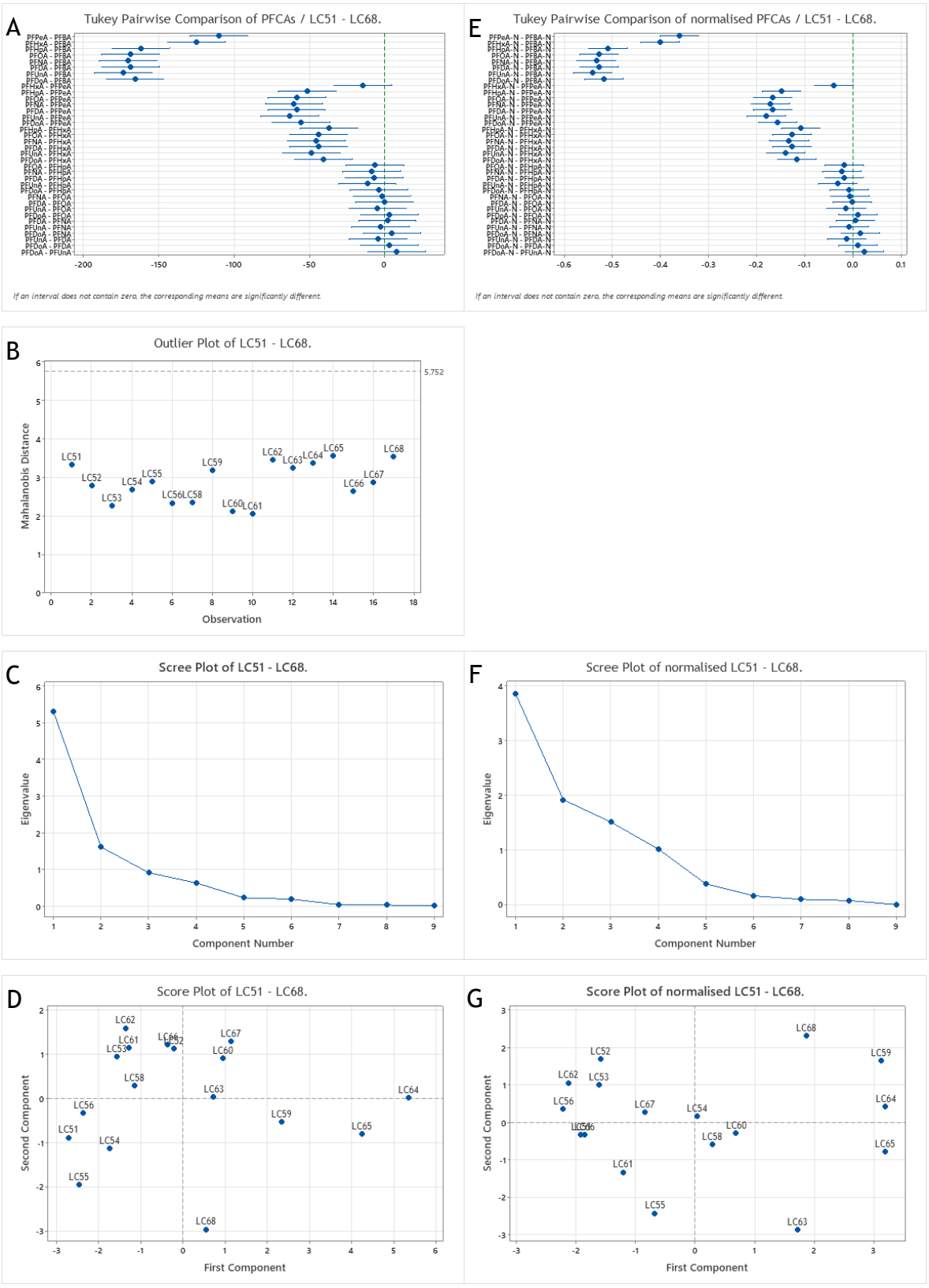


Figure 5-9: Statistical evaluation of biosolids samples across six batches from 2019 to 2022 (N= 18). Before (A-D) and after normalisation (E-G). Graphs generated after ANOVA and PCA include Tukey Pairwise Comparisons (A and E), an outlier plot using Mahalanobis Distances (B), Scree plots identifying principal components required to assess the data set (C and F) and Score plots (D and G) to address the distribution and clustering of sample results.

The evaluation of the generated data by biosolids batch showed that the small-chained PFCAs, from PFBA to PFHxA, make up the main bulk of concentrations (Figure 5-10). Strikingly, PFBA and PFPeA are often not included in studies regarding biosolids and sediments (Higgins et al., 2005, Gomez-Canela et al., 2012, Ruan et al., 2015, Guo et al., 2016, Gallen et al., 2018, Pepper et al., 2021). Moodie et al. (2021) found low levels of PFBA and PFPeA at 0.8 ng/g and 2.0 ng/g, respectively, with higher concentrations for larger PFCA which is in stark contrast to the elevated levels of PFBA, PFPeA and PFHxA detected across all batches of biosolids analysed in this study while the concentrations for PFHpA, PFOA, PFNA, PFDA, PFUnA and PFDoA are in comparable ranges found in the literature (Gallen et al., 2016, Gallen et al., 2018, Moodie et al., 2021, Pepper et al., 2021).

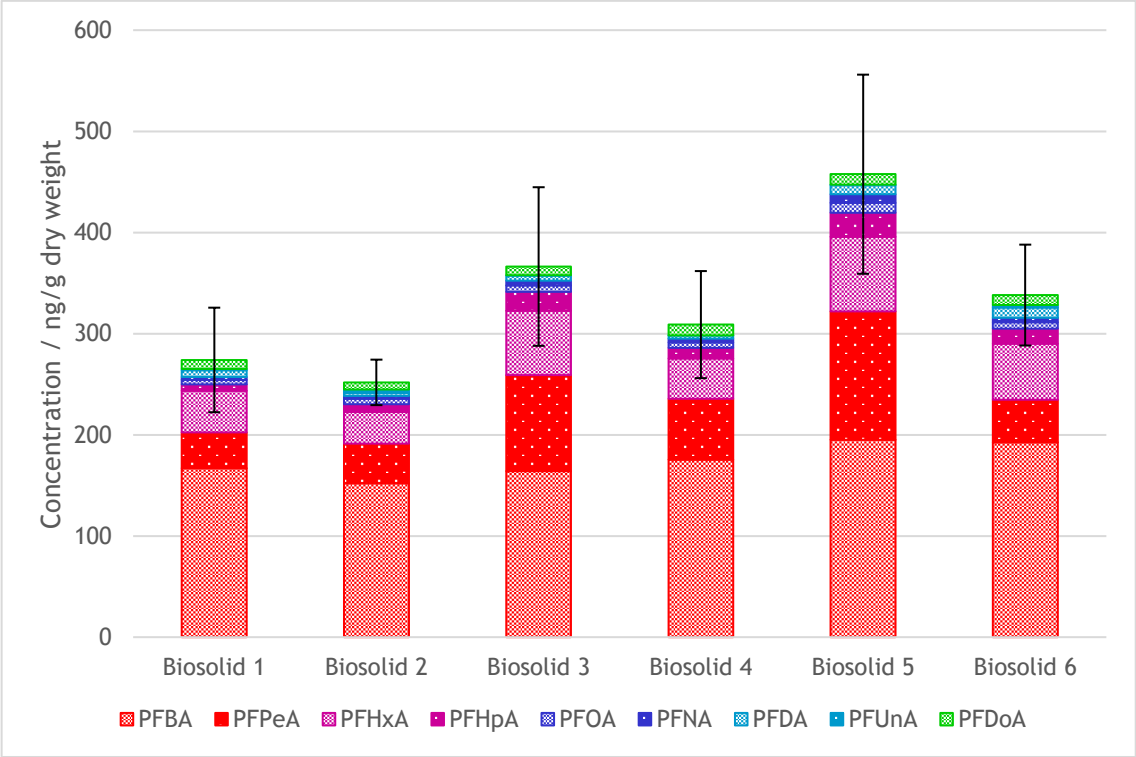


Figure 5-10: Cumulative PFCA concentrations in biosolids samples depending on the batch collected between 2019 and 2022 (N=18). Biosolids are each represented by stacked concentrations of the individual average PFCA concentrations. Error bars corresponding to the bars are also cumulative average errors that were determined individually.

Studies concerning PFCAs in biosolids usually do include the homologous series spanning PFHxA to PFDoA. Amongst those, PFOA, PFDA and PFDoA are the PFCAs measured at higher levels (10s to 20s ng/g) compared to the remaining acids ranging from 0.1 to 5 ng/g (Gallen et al., 2016, Gallen et al., 2018, Moodie et al., 2021, Pepper et al., 2021). Overall, the concentration profile of PFCAs in biosolids in the literature does not conform with the results of this study. The lack of

knowledge about the origin of biosolids is a significant shortcoming when comparing studies from different regions and countries. Factors, such as the proportion of industrial wastewater handled by the wastewater treatment plant the biosolids originate from can have an extensive influence on the biosolids product, its composition as well as the amount of micropollutants found in it.

5.4.2. Perfluoro Carboxylic Acid Profiles in Biosolids-Amended Soil

Statistical analysis found significant differences between analysed compounds in biosolids-amended soil samples. ANOVA and subsequent PCA identified 2 groups within the data set, one containing PFHpA, and the second group for the remaining PFCAs. The differences are indicated in the Tukey pairwise comparison (Figure 5-11-A). While PFDA is following a similar concentration profile across a nearly identical subset of samples, it does not appear to group with PFHpA following the Tukey Comparison. The outlier plot for this dataset does not identify any outliers exceeding the Mahalanobis distance, despite two samples being close to the cut-off (Figure 5-11-B). The scree plot includes three components with eigenvalues greater than 1, accounting for 75 % of the variation in the dataset (Figure 5-11-C). The score plot is representing a close cluster, only identifying two samples with a greater distance to the main cluster (Figure 5-11-D).

Using the cumulative concentrations of every respective sample to normalise the dataset does change the grouping (Figure 5-11-E). PFHxA showed artificially high concentrations due to a comparably higher quantification limit and the use of random values [0, half-LOD] for concentrations below LOD. Only PFHpA is significantly different to the remaining PFCAs and results for PFHxA need to be assessed as data artefact. The scree plot improves in curve shape, with two components with an Eigenvalue of above 1, explaining 63 % of the variation in the dataset (Figure 5-11-F). Due to the artefact formation in the set, the normalisation does not add any value to ANOVA and Eigenanalysis. The score plot however, now identifies a cluster of 7 samples separate to the main sample bulk, all of which show an above LOD measurable concentration of PFHpA (Figure 5-11-G). While the score plot is able to identify this grouping, normalisation of the biosolids-amended soil sample concentrations needs to be evaluated with caution due to the introduced artefact concentrations of PFHxA.

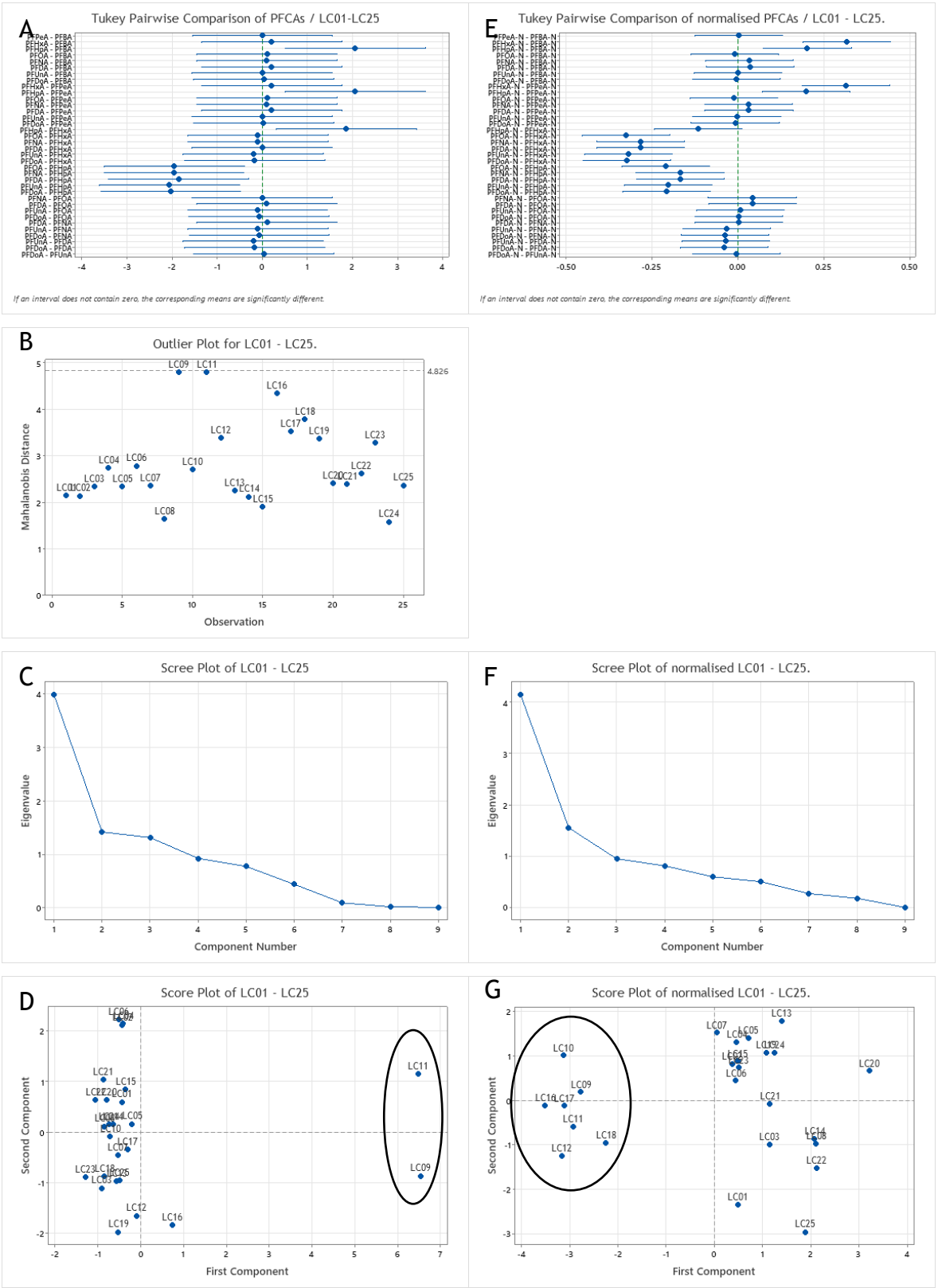


Figure 5-11: Statistical evaluation of biosolids-amended soil samples collected between 2019 to 2021 (N=25). Before (A-D) and after normalisation (E-G). Graphs generated after ANOVA and PCA include Tukey Pairwise Comparisons (A and E), an outlier plot using Mahalanobis Distances (B), Scree plots identifying principal components required to assess the data set (C and F) and Score plots (D and G) to address the distribution and clustering of sample results.

The evaluation of the data according to the sampling time point shows that concentrations peak one month after the biosolids applications and subsequently followed a decreasing trend up to 21 months after application (Figure 5-12). However, the cumulative concentrations for the sampling time points consist primarily of the PFHpA concentration with the remaining compounds only accounting for a minor part.

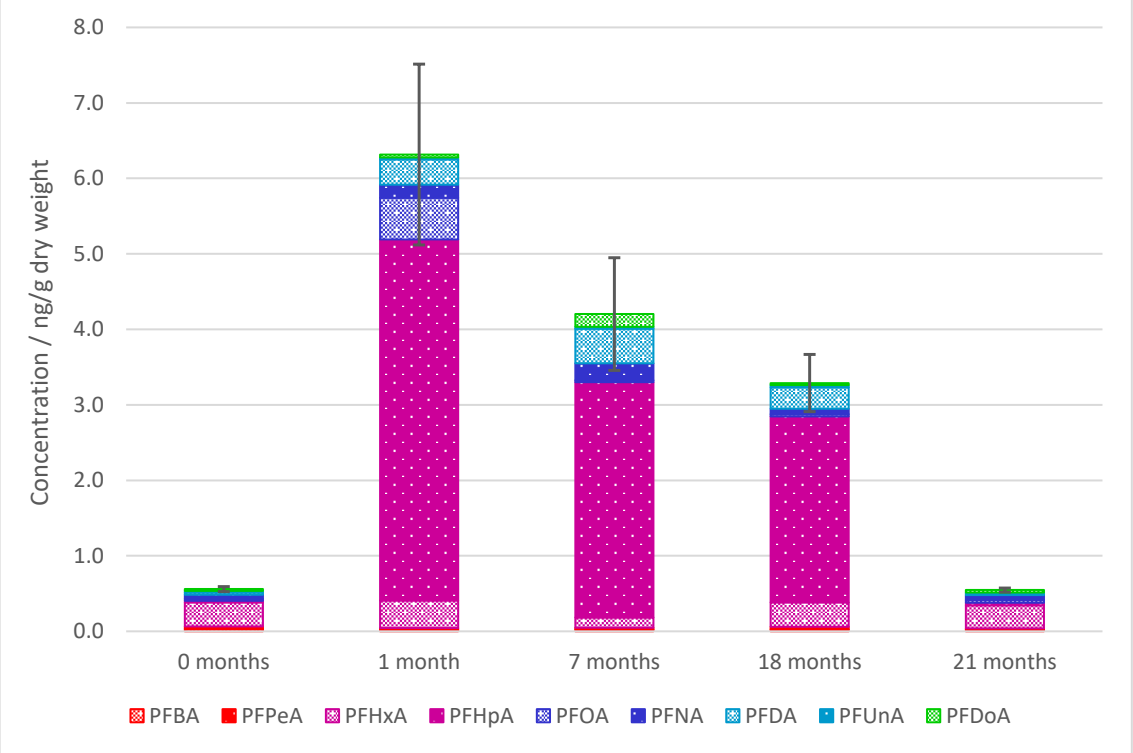


Figure 5-12: Cumulative PFCA concentrations in soil samples depending on the sampling time point (N=25). Soils are each represented by stacked concentrations of the individual average PFCA concentrations. Error bars corresponding to the bars are also cumulative average errors that were initially determined individually.

Contrary to the literature concerning biosolids, the publications investigating soils do occasionally include PFBA and PFPeA (Cao et al., 2019, Kikuchi et al., 2018, Skaar et al., 2019). Cao et al. (2019) analysed soils from a drinking water source area in regard to PFCAs and found low levels of all PFCAs ranging from PFBA to PFDoA. Kikuchi et al. (2018) analysed soils from Swedish background sites not detecting PFBA, PFPeA or PFHxA, however, concentrations of larger PFCAs reached up to 0.8 ng/g for PFUnA. Soil samples from an Arctic study were generally presenting with concentrations below the respective LODs, however, two samples with documented contamination events showed concentrations ranging from 0.7 – 16.8 ng/g (Skaar et al., 2019). In a study of biosolids-amended soil by Pepper et al. (2021) concentrations of PFHxA – PFDoA ordinarily ranged between 0.1 ng/g

(PFUnA) and 0.8 ng/g (PFOA), PFPeA and PFBA were not included in the analysis. Similarly, Rankin et al. (2016) analysed surface soils from all continents, detecting PFHxA with up to 7.5 ng/g, 9.9 ng/g of PFOA and up to 4 ng/g of PFHpA. Additionally, long-chain PFCAs were also measured at 0.8 ng/g to 1.5 ng/g. In contrast, Washington et al. (2010) analysed sludge-applied soils near a wastewater treatment plant and found maximum concentrations of 986 ng/g, 300 ng/g and 500 ng/g, respectively, while also detecting PFHxA, PFHpA, PFOA and PFNA at concentrations of up to 35 ng/g, 80 ng/g, 312 ng/g and 137 ng/g, respectively.

Cao et al. (2019) found that PFBA and PFHpA are most abundant during the analysis, while Rankin et al. (2016) and Skaar et al. (2019) highlighted PFHxA and PFOA as most prolific. Results from other studies highlight the abundance of long-chained PFCAs such as PFDA, PFUnA and PFDoA (Kikuchi et al., 2018, Washington et al., 2010). Ultimately, the profile present in soils, biosolids-amended or not, are of great variation, potentially depending on nearby contamination sources such as wastewater treatment plants or manufacturing locations, or the introduction of contaminants through particle-dependent and -independent atmospheric transport.

5.4.3. Perfluoro Carboxylic Acid Profiles in Control Field Soil

Since only three results are reported in control soil, the statistical analysis using ANOVA and PCA does not appear to be a useful tool. Both ANOVA and PCA were driven by the high-LOD-dependent random concentration values used to fill the dataset that was previously discussed for biosolids-amended soil samples. The score plot shows an even cluster with slightly higher distances for the samples containing measured PFDoA concentrations (data not shown). The direct comparison and statistical analysis of all soil samples, biosolids-amended and control, is missleading due to the differences in detection limits for compounds, as well as the resulting use of artificial values for below-LOD concentrations.

Lorenzo et al. (2015) and Mejia-Avendaño et al. (2017a) emphasised that limits of detection can be considerably higher, with less accuracy for compounds with mismatched isotope-labelled internal standards. However, highest LODs were gained for PFBA, PFPeA, PFHxA and PFOA, of which only PFPeA was mismatched during analysis. While the matrix effects in soil were overall notably less compared to biosolids, matrix-dependent extraction efficiencies, ion suppresion and the lack of sample cleanup may complicate the quantification (Lorenzo et al., 2015).

McMurdo et al. (2008) detailed that PFOA deposition in the environment, irrespective of wet or dry deposition, appeared to be particle-mediated in variable ranges. However, no literature concerning other PFCAs were found to that regard, indicating that the detection of high levels of PFDoA in control soil was an anomaly. However, the near-zero presence of PFCAs in control soil along with the increased number of detection events in biosolids-amended soil did suggest that firstly, the use of biosolids can be associated to the disparity, introducing the PFCA contamination into soil as no other source, such as a nearby wastewater treatment plant or industrial production site could be identified. Secondly, since elevated concentrations of PFHpA and PFDA are not found in control soil, despite geographical proximity, atmospheric deposition was ruled out as a source for the elevated concentrations detected in biosolids-amended soil (Sepulvado et al., 2011).

Sepulvado et al. (2011) reported results following batch experiments as well as the analysis of soil cores demonstrating a significantly higher leaching potential for short chained PFCAs compared to longer-chained counterparts. Additionally, the studies highlighted that PFCA levels decrease with soil sampling depth, regardless of biosolids loading rates, with longer chained PFCAs being the dominant detected PFCAs (Sepulvado et al., 2011, Pepper et al., 2021). Ahrens et al. (2010) proposed that chain lengths of 7 or less are dominant in the dissolved phase, intermediate chain lengths of 7 to 11 can be found in dissolved and particle bound state, while chain lengths of 11 and above are only found in a particle-bound state in sediments. Following these assumptions in sediments, and observations in other studies discussed similar processes can be assumed in soil horizons. Mejia-Avendaño et al. (2017a) and Zhi and Liu (2018) underlined that the organic matter content and clay fractions are crucial factors for the retention of PFCAs in soil. Mejia-Avendaño et al. (2017a) concluded that the soil type is the most important factor when assessing retention potential.

Considering the nature of the process of biosolids being applied to agricultural land and eventually decomposing, a dilution effect is expected, showing the distribution of relatively high concentrations in biosolids to lower, consistent concentrations in soil. Comparing concentration profiles between biosolids and biosolids-amended soil highlight that short-chained PFCAs, PFBA, PFPeA and PFHxA are found at elevated levels in biosolids yet they do not appear to be

retained in soils (Figure 5-13). Differences in concentration profiles between the two matrices can be attributed to different processes occurring: firstly, as aforementioned, the mobilisation of compounds likely differs depending on the chain length. Short-chained PFCAs likely reside in porewater and are potentially transported with runoff, rain and leachage once biosolids undergo breakdown and hence, do not dominate the soil profiles after the biosolids-amendment. Secondly, longer PFCAs, ranging from PFHpA to PFDoA, are expected to bind to solid matrices and are less likely to be found in liquid fractions. However, particle-dependent transport, may still occur for longer chains, horizontally as well as vertically down the soil column beyond surface soil (Ahrens et al., 2010, Sepulvado et al., 2011, Lorenzo et al., 2015, Mejia-Avendaño et al., 2017a, Zhi and Liu, 2018, Pepper et al., 2021). Thirdly, the occurence of a hysteresis phenomenon linked to the sample matrix cannot be fully ruled out, potentially leading to reduced extraction of environmental concentrations, requiring further experimental data to assess the adsorption and desorption of the individual PFCAs to relevant compositions of the soil matrix (Drillia et al., 2005).

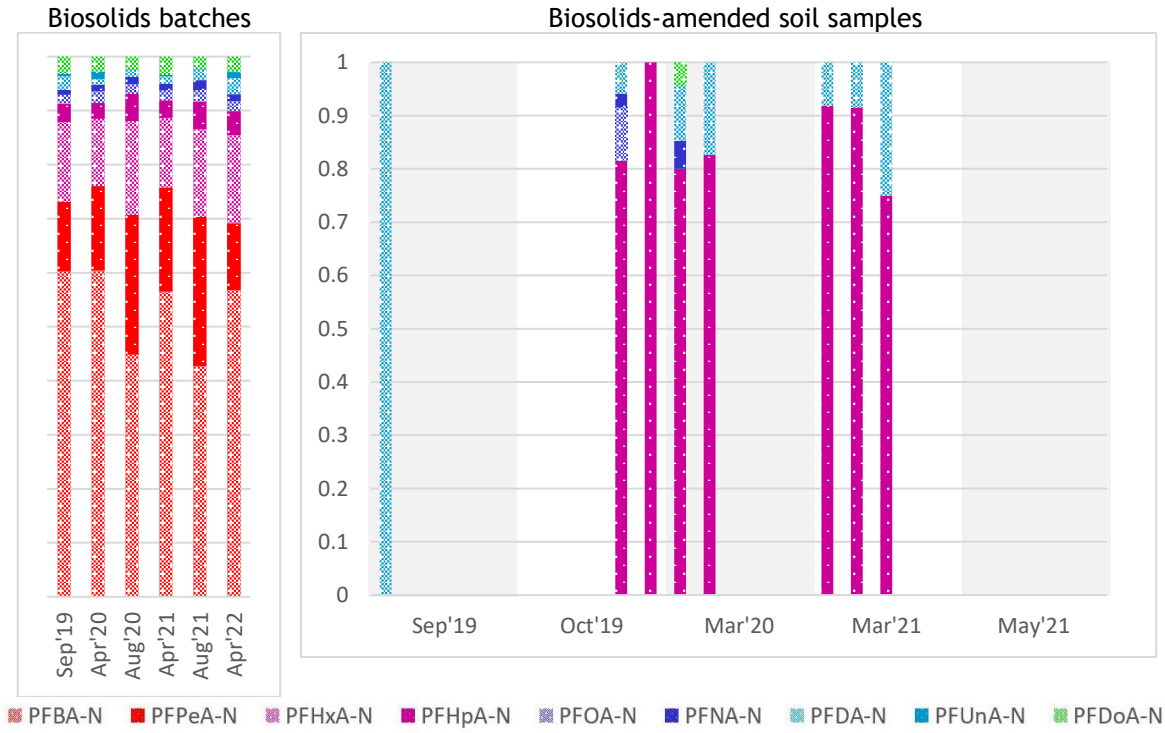


Figure 5-13: Direct comparison of normalised PFCA profiles in biosolids batches (N=18 in 6 batches, left) and biosolids-amended soils samples (N=25 in 5 sampling points, right) collected over a time period of up to 3 years (2019 – 2022). The cumulative concentrations of PFCAs within one sample were used to normalise the respective data set. Short-chain PFCAs (PFBA, PFPeA and PFHxA) dominate the profile in biosolids. Notably, PFHpA and PFDA dominate the profiles in biosolids-amended soil with detection in seven samples each, respectively. However, the majority of soil samples did not return measurable concentrations of PFCAs above the limit of detection of the analysis. It can be seen that profiles between the two analysed matrices do not comform to one another.

PFHpA was found across biosolids batches with an average concentration of 13.2 ng/g while the soil samples peak with an average concentration of 4.8 ng/g across samples taken one month after the initial biosolids batch application took place. The sudden increase and subsequent decrease of concentrations over 21 months in amended soil that followed the initial sampling point and biosolids application (Figure 5-12) cannot be explained in regard to the continued treatment of the field with biosolids in the course of the monitoring period (Figure 5-10). Following the sampling timeline, the continued application of biosolids twice a year would be expected to have an influence on the concentrations measured, however, PFDA also shows the same spiked concentration one month after application and a subsequent reduction of the concentrations up to 21 months of monitoring.

Noteably, only the 1 month (3 weeks) soil sampling point took place in closely timed proximity to the biosolids application, while all other soil sampling points had a minimum lag phase of two months (but usually 5-6 months) post-application (Figure 5-1). The European Commision (1986) issued the Sewage Sludge Directive 86/278/EEC specifying a three week no-grazing period for livestock following biosolids application to minimise harmful effects on soil, vegetation, animals and man and defines maximum allowed concentrations for heavy metal contaminations in soil and sludge. However, there is no literature available regarding the selection of this time frame, and there are also no amendments to the document to include other potentially harmful contaminants, such as PFCAs. The increased concentrations indicated in the sampling point closely following the biosolids application may imply that the environmental risk follwing the application outlasts the 3 week no-grazing period. In order to understand the full extent of environmental ramifications of biosolids application, an extended investigation into the breakdown of biosolids may improve the understanding and overall assessment of the environmental risk of this practice.

5.5. Conclusion

In this chapter, PFCA concentration profiles in biosolids and soils were investigated. Initially, the co-extraction of matrix components had to be addressed. The study identified a homologous row of compounds that are in high abundance in biosolids and also naturally occur in soil samples, as shown in a control soil sample. The molecular formulas, $C_{20}H_{23}O_3$, $C_{21}H_{25}O_3$ and $C_{22}H_{27}O_3$, provided the best fit for the gained chromatographic and mass spectrometric data.

The ratio of carbon and hydrogen indicates a unsaturated, cyclic chemical structure, as aliphatic compounds would require more hydrogen atoms within the molecule.

The matrix effect in biosolids was found to be significantly higher than in soils, causing a significant amount of ion suppression, also leading to the need of a dilution step for biosolids samples. Despite improving the outcome of the analysis, the presence of all PFCAs in the carried blank indicates the overloading of the LC column as an additional consideration. Issues arising from that fact might make additional clean-up steps relevant.

PFBA, PFPeA and PFHxA were the dominant compounds found in biosolids. The PFCA profiles were consistent across batches over a sampling cycle of 3 years. Contrary to that, PFHpA and PFDA had the highest detection frequencies in biosolids-amended soils while most remaining compounds were falling below detection limits. However, only PFHpA was found to be in statistically significant concentrations within the biosolids-treated soil samples. The discrepancies of PFCA-profiles between biosolids and biosolids-amended soils were not fully explained due to the complexity of processes occurring after biosolids application and subsequent breakdown of pellets. However, different behaviour for short- and long-chained compounds are expected in regard to mobilisation within the environment. Short-chained PFCAs are more likely to move with liquid fractions, whereas larger PFCAs remain localised and particle-bound. Additionally, the potential of hysteresis phenomenon needs to be further investigated.

Control soil, that never received any biosolids did not show significant amounts of PFCAs within the monitored time period of two years, indicating that firstly, the deposition of fluorotelomer precursors is unlikely to have occurred and secondly, implying that the application of biosolids does indeed introduce contamination into the soil, as no other source of contamination could not be identified.

Chapter 6

Conclusions and Recommendations

6.1. Introduction

The intend of this project was two-fold, the development of a high-throughput ultrasound-assisted extraction (UAE) technique for the analysis of perfluoro carboxylic acids (PFCAs) and the demonstration of prevalent PFCA-contamination levels across biosolids and biosolids-amended soils. To achieve the aims of this project, the following objectives were set:

I. To increase extraction capabilities of a sonication probe to minimise cost and labour arising from UAE.
II. To evaluate and optimise an UAE method for the extraction of PFCAs from biosolids and soils.
III. To investigate the use of gas chromatography – mass spectrometry (GC-MS) for the detection of PFCAs in complex samples at environmental concentration levels.
IV. To assess concentration ranges between biosolids and soils, to evaluate the mass transfer from one to the other, and the environmental burden associated with PFCAs overall.

6.2. Summarised Details and Key Findings of Chapter 4

Chapter 4 initially introduced per- and polyfluoroalkyl substances (PFASs) as a whole, within the context of anthropogenic use and environmental contamination. The literature review followed a two-sided approach towards GC-MS method development, firstly, aiming to identify PFASs relevant to the biosolids and soil matrices in an agricultural setting and secondly, investigating applications of GC-MS within published studies irrespective of the analysed sample types and matrices. Ultimately, PFCAs were selected as targets for this project.

To begin with, matrix materials were characterised, highlighting differences between biosolids batches regarding their water and organic matter content whereas soil samples from different agricultural fields were found to match closely in composition. While determined parameters were not sufficient to

identify factors contributing to the binding of pollutants to matrix materials, it simplified the comparison of results from samples of different origins.

Furthermore, a previously developed UAE method was extended, firstly, to be used for the extraction of contaminants from soil samples as a novel sample matrix engaged in the process, secondly, to increase the number of samples to be extracted simultaneously, maximising efficiency of the process, and thirdly, to target PFCAs as a novel targeted group of compounds. The soil sample amount of 1 gram was selected despite marginally higher recoveries from a smaller (0.5 g) sample amount (albeit with higher relative standard deviations) in an attempt to balance recoveries of targeted compounds and the extractability of trace amounts from soils. Recoveries across the employed sonication device were found to be consistent, increasing capabilities from single extraction vials to triplets of vials undergoing extraction simultaneously, allowing for the processing of up to 48 samples within a day following the described procedures. The extraction of PFCAs from biosolids and soil samples was optimised in regard to the employed pH values of the extraction process, with significantly improved recoveries at pH 10, whereas neutral and acidic pHs did only return a subset of detectable concentrations in spiked and unspiked biosolids and soil samples using LC-MS.

Finally, chapter 4 engaged in the GC-MS method development for the detection of PFCAs engaging an electron impact ionisation source. Despite attempts of method optimisation, it was not possible to enable the analysis of smaller PFCAs ranging from C_4 to C_6 while separation for the remaining compounds was achieved. The attained limit of detection of 25 ng/mL was not suitable for the analysis of low environmental concentrations expected within agricultural settings using GC-EI-MS. Increasing sample amounts during extraction was not possible due to the volume restraints of the employed 10 mL vials. The introduction of concentration factors during re-constitution was also not a viable option, as the employed PFAS-specific PP GC vials required a minimum volume of approximately 600 µL for successful sample injection into the instrument. Considering these restrictions, a switch to the analysis with liquid chromatography (LC) took place.

6.3. Summarised Details and Key Findings of Chapter 5

Chapter 5 introduced structural differences between PFASs that potentially influence the behaviour in environmental settings. The main focus of this study

was the characterisation of PFCAs in biosolids and soils through advanced analytical techniques. Liquid chromatography coupled to an Orbitrap mass analyser overcame the concentration limitations of a previously developed GC-MS method, allowing the detection of all nine investigated PFCAs at environmental concentrations across matrices.

Initial analysis of biosolids identified co-extracted matrix components prohibiting the successful analysis of target compounds. The introduction of a dilution step prior to analysis overcame the limitation of the observed co-elution, however, column overloading remained a problem, as indicated by the detection of carry-over in the analysed blank. The observed matrix effect indicated significant ion suppression within biosolids, however, PFCAs were consistently detected across all analysed samples and batches of biosolids. PFBA, was detected at elevated levels (144 – 228 ng/g), PFPeA and PFHxA presented with intermediate concentrations (22 – 161 ng/g), whereas larger PFCAs (PFHpA – PFDoA) were detected with 0.3 – 33 ng/g.

The matrix effect in soil samples was found to be minimal and did not require the introduction of additional steps prior to analysis. Control soil samples showed only 3 measurable concentrations across all compounds, within the sample set. For biosolids-amended soil samples, small PFCAs (C_4 to C_6) were not detected within any soil samples. Generally, PFHpA (1 – 22 ng/g) and PFDA (0.3 – 2 ng/g) were the most frequently detected compounds, found in seven samples each, respectively.

Statistical analysis of the biosolids dataset found significant differences between compounds, with 3 groups within the dataset following the apparent concentration profile. The assessment of cumulative concentration profiles of biosolids batches found no significant differences between batches, indicating the continuous contamination with PFCAs in proportionate concentration profiles across three years of sampling.

The statistical evaluation of biosolids-amended soil samples identified PFHpA as significantly different within the assessed sample set. PFDA did not group with PFHpA, despite a similar concentration profile across a near-identical subset of samples. Cumulative profiles across sampling time points show a spike in concentration, primarily attributed to the observed PFHpA concentration, after 1 month and subsiding over the remaining monitoring period. Notably, the 1-month

sampling point was the only sampling point in close proximity to the biosolids application whereas other soil sampling times had an apparent lag-phase of approx. 3 months following the biosolids application.

Concentration profiles of biosolids and biosolids-amended soils do not present the same distribution of concentrations across compounds. Short-chained PFCAs do not appear to be retained by soil following the amendment, however, larger PFCAs were found across sampling time points. The near-zero detection of PFCAs in control soil indicates that the biosolids-amendment can be linked to the presence of contamination in the analysed biosolids-amended soils.

6.4. Recommendations for Future Work

This thesis introduced the use of a sonication device as means to employ ultrasound for the purpose of PFCA contamination extraction from complex environmental matrices. The application of indirect sonication allowed for a minimised risk of cross contamination and the delivery of uniform high-energy ultrasound for more time-efficient protocols compared to traditional sonication setups. The performed method development extended the capabilities towards the extraction of PFCAs from soil matrices by adjusting the extraction parameters, keeping the selection of extraction solvents in line with a previously developed extraction method of pharmaceuticals from biosolids. Therefore, it is recommended that sequential extraction of pharmaceuticals and PFCAs is investigated in order to combine analysis efforts in environmental matrices working towards a holistic assessment of environmental pollution. Notably, the apparent differences in temperature programming during GC-MS for the analysis of PFCAs and pharmaceuticals, give rise to the opportunity to unify both analysis within one program, with the separation of PFCAs at lower temperatures, followed by the elution of pharmaceuticals beyond a temperature of 100 °C.

One shortcoming of GC-MS analysis for PFCAs, such as the lack of retention of smaller PFCA compounds was highlighted. In order to address this deficiency, the adaptation of two-dimensional gas chromatography (GCxGC) could be investigated. Overall, GCxGC in combination with time-of-flight mass spectrometry (TOF-MS) presents as a highly sensitive analytical technique suitable for the analysis of complex environmental matrices. Notably, the application of gas chromatographic separation is limited to a select group of volatile and semi-

volatile PFASs, however, the extension of analysis towards PFCA precursors, such as fluorotelomer alcohols, could increase the impact of analysis by providing a more comprehensive perspective on the environmental burden of PFASs in the environment.

Finally, the analytical limitation arising from the comparably high limit of detection due to intense fragmentation of the employed electron impact source has been discussed. The instrumental switch to chemical ionisation may provide a solution to this issue, as the softer ionisation technique is expected to increase sensitivity of the method. However, chemical ionisation requires a separate ionisation source and the use of a reagent gas in addition to the conventional GC-MS setup, implying the rise of additional cost associated with this approach. While chemical ionisation principally can be applied to any micropollutants to be analysed, the deviation from electron impact ionisation also limits the use of available spectral libraries, adding onto the analytical method development.

6.5. Conclusion

The research efforts leading up to the Stockholm convention and the signing of the convention itself presented a major achievement towards environmental safety in the 1990s. Since then, little has changed in regard to legislative documentation to restrict production, use and release or monitoring of contaminants in the environment. Irrespective of that, continued and increased research efforts are upheld investigating the multitude of pollutants in environmental compartments and their potential effects on the environment and its biota.

This study, in line with other publications, shows a strong link between biosolids-based amendment of agricultural land and the occurrence of pollution. Despite this the application of biosolids remains a valid practice globally. While the ban on the application of untreated sludge to agriculture was a significant step in the EU, within Europe only the Netherlands and Switzerland proceeded to outright ban the use of any sludge-derived products due to concerns regarding human and environmental health.

The ultrasound-assisted extraction method employed in this project presents a superior approach to UAE by utilising an indirect sonication reactor, overcoming limitations associated to conventional extraction methods using sonication baths

or direct probes. The introduction of multiplexing during the extraction significantly improved the economic aspect of the extraction, in addition to the benefits of sustainability innate to ultrasound-assisted techniques and excellent reproducibility of results. The described studies of this project further elaborate the steps taken to reliably analyse perfluoro carboxylic acids in biosolids and soils with both, liquid and gas chromatography hyphenated to mass spectrometers. The current level of method development of GC-EI-MS was not sufficient to be carried on for the determination of trace concentrations of PFCAs in environmental matrices. However, the presented method can provide the basis towards the analysis in conjunction of chemical ionisation, potentially achieving improved sufficient detection limits after adaptation of the method. By contrast, liquid chromatography was able to determine environmental concentrations as low as 0.1 ng/g despite increased matrix effects occurring in biosolids.

Irrespective of outlined advances made in this project, improvements in environmental risk assessment are still needed to address potential ecotoxicological effects of the application of biosolids to agricultural land considering the implications to livestock and wildlife seen in other studies. To date, no official predicted no effect concentrations or predicted environmental concentrations of PFCAs are available to assess the impact of PFCA contamination in the wider environment.

Appendix A: Chapter 2

Persistent Organic Pollutants listed under the Stockholm Convention

Table A-1: All persistent organic pollutants (POPs) listed under the Stockholm Convention. Chemicals are grouped into 3 annexes: A - elimination, B - restriction and C - unintentional production. Originally listed POPs known as "the dirty dozen" are indicated (*) (United Nations, 2019)

Annex A - Elimination	*Pesticide*	*Pesticice and industrial use*	*Industrial chemical*
Aldrin*	Chlordane*	Chlordecone	
Decabromodiphenyl ether	Dechlorane Plus	Dicofol	
Dieldrin*	Endrin*	Heptachlor*	
Hexabromobiphenyl	Hexabromocyclododecane	Hexabromodephenyl ether and heptabromodiphenyl ether	
Hexachlorobenzene*	Hexachlorobutadiene	Alpha hexachlorocyclohexane	
Beta hexachlorocyclohexane	Lindane	Methoxychlor	
Mirex*	Pentachlorbenzene	Pentachlorophenol and its salts and esters	
Polychlorinated biphenyls*	Polychlorinated naphthalenes	Perfluorooctanoic acid, its salts and PFOA-related compounds	
Perfluorohexane sulfonic acid its salts and PFHxS-related compounds	Short-chain chlorinated paraffins	Technical endosulfan and its related isomers	
Tetrabromodiphenyl ether	Toxaphene*	UV-328	

Annex B – Restriction		
Dichlorodiphenyltrichloroethane*	Perfluorooctane sulfonic acid its salts and perfluorooctane sulfonyl fluoride	

Annex C – Unintentional Production		**Unintended production**
Hexachlorobenzene*	Hexachlorobutadiene	Pentachlorobenzene
Polychlorinated dibenzo-p-dioxins*	Polychlorinated dibenzofurans*	Polychlorinated naphthalenes

Sustainable Development Goals

Table A-2: Sustainable development goals detailed in the 2030 agenda for sustainable development (United Nations, 2015)

Goal 1	End poverty in all its forms everywhere
Goal 2	End hunger, achieve food security and improved nutrition and promote sustainable agriculture
Goal 3	Ensure healthy lives and promote well-being for all at all ages
Goal 4	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
Goal 5	Achieve gender equality and empower all women and girls
Goal 6	Ensure availability and sustainable management of water and sanitation for all
Goal 7	Ensure access to affordable, reliable, sustainable and modern energy for all
Goal 8	Promote sustained, inclusive and sustainable economic growth, full and protective employment and decent work for all
Goal 9	Build resilient infrastructure, promote inclusive and sustainable industrialisation and foster innovation
Goal 10	Reduce inequality within and among countries
Goal 11	Make cities and human settlements inclusive, safe, resilient and sustainable
Goal 12	Ensure sustainable consumption and production patterns
Goal 13	Take urgent action to combat climate change and its impacts
Goal 14	Conserve and sustainably use the ocean, seas and marine resources for sustainable development
Goal 15	Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss
Goal 16	Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels

Appendix B: Chapter 4

Chemical Structures

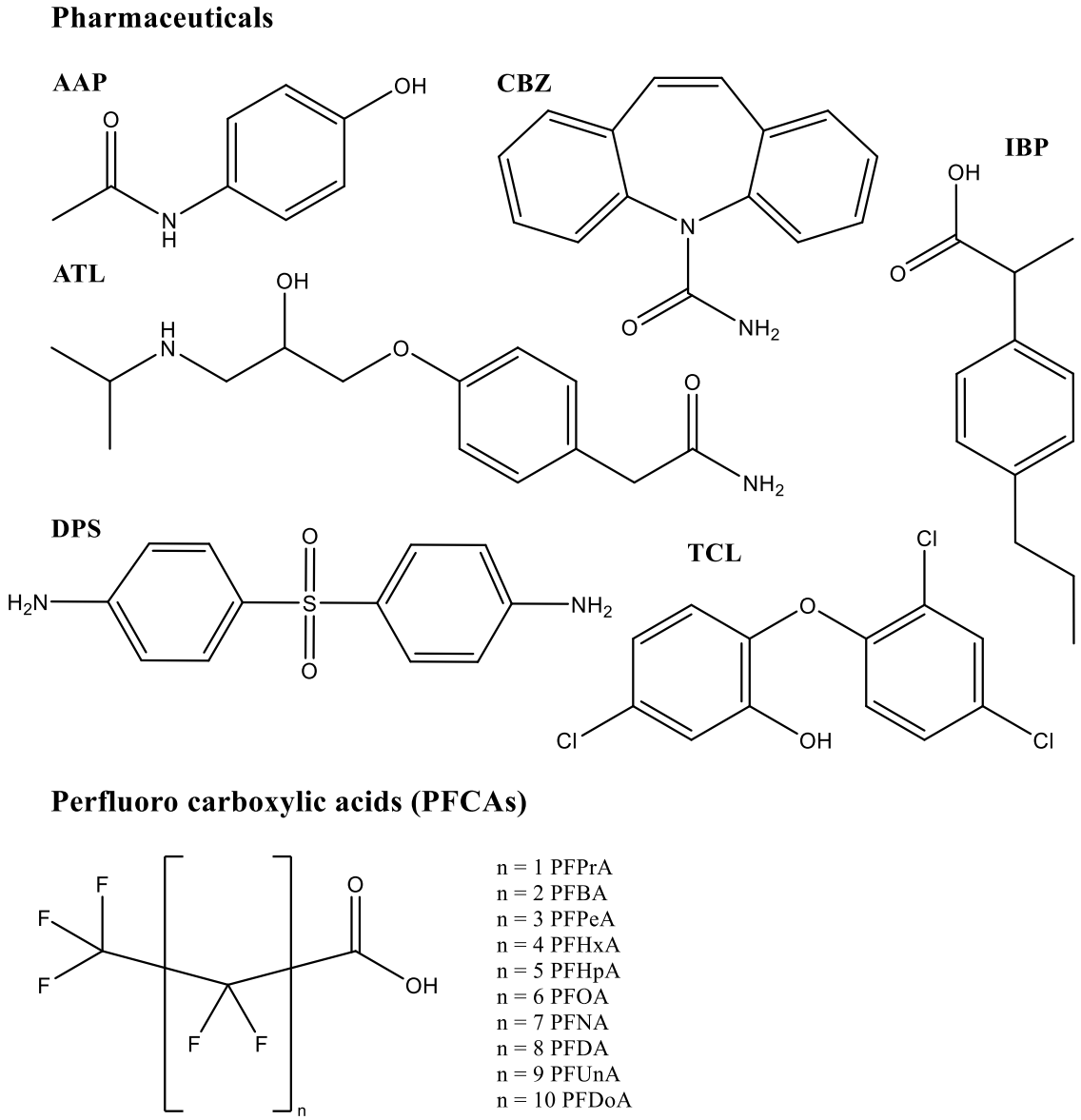


Figure B-1: Chemical structures of selected pharmaceutical and personal care products and perfluoro carboxylic acids. Acetaminophen (AAP), atenolol (ATL), carbamazepine (CBZ), dapsone (DPS), ibuprofen (IBP) and triclosan (TCL).

Overview of Chemicals and Solvents Employed in This Project

Table B-1: Names, identifier, CAS number, molecular weight, boiling points, octanol/water partitioning coefficient and acid dissociation constant of utilised standards and compounds.

	Name and identifier		CAS	MW / g/mol	Bp / °C	logP	pKa
PPCPs	Acetaminophen	AAP	103-90-2	151.16	>500	0.46	9.38
	Atenolol	ATL	29122-68-7	266.3	-	0.16	9.60
	Carbamazepine	CBZ	298-46-4	236.27	399	2.45	13.90
	Dapsone	DPS	80-08-0	248.3	-	0.97	2.41
	Ibuprofen	IBP	15689-27-1	206.28	157	3.97	5.30
	Triclosan	TCL	3380-34-5	289.5	120	4.76	7.90
PFCAs	Perfluorobutanoic acid	PFBA	422-64-0	214.04	-	-	-
	Perfluoropentanoic acid	PFPeA	2706-90-3	264.05	-	-	-
	Perfluorohexanoic acid	PFHxA	307-24-4	314.05	157	3.48	-0.16
	Perfluoroheptanoic acid	PFHpA	375-85-9	364.06	175	4.15	-2.29
	Perfluorooctanoic acid	PFOA	335-67-1	414.07	189	4.81	-0.5
	Perfluorononanoic acid	PFNA	375-95-1	464.08	218	5.48	-0.21
	Perfluorodecanoic acid	PFDA	335-76-2	514.08	218	-	-
	Perfluoroundecanoic acid	PFUnA	2058-94-8	564.09	160	-	-
	Perfluorododecanoic acid	PFDoA	307-55-1	614.1	245	-	-
Others	Anthracene-D10	A-D10	1719-06-8	188.29	341	4.45	-
	N-Methyl-N-(trimethylsilyl) trifluoroacetamide	MSTFA	24589-78-4	199.25	-	-	-
	13C Perfluorobutanoic acid	13C PFBA			-	-	-
	13C Perfluorohexanoic acid	13C PFHxA			157	3.48	-0.16
	13C Perfluorooctanoic acid	13C PFOA			189	4.81	-0.5
	Phenanthrene	P	85-01-8	178.23	338	4.46	
	Sodium hexametaphosphate	HMP	68915-31-1	221.94	-	-	-
Solvents and additives	Acetonitrile	ACN	75-05-8	41.05	76	-0.34	-
	Ammonia	NH3	7664-41-7	17.03	-33	-2.66	32.5
	Ammonium formate	HCOONH4	540-69-2	63.06	180	-	3.8
	Ethyl acetate	EtAc	141-78-6	88.11	77	0.73	-
	Formic acid	HCOOH	64-18-6	46.03	101	-0.54	3.77
	Methanol	MeOH	67-56-1	32.04	65	-0.77	-
	Water	H2O	7732-18-5	18.02	100	-1.38	7

Chapter 4: Stock Solutions

3% HMP for the grain particle size determination of soils was made up by dissolving 15 g of the compound in 500 mL ultrapure water (Elga Purelab Chorus, 18 MΩ). A mixed standard solution for pharmaceuticals was made by adding 25 mg of each compound listed above in 50 mL methanol, the 500 µg/mL solution was then subsequently diluted as required. A 500 µg/mL stock solution of anthracene-D10 was made up in methanol.

2500 µg/mL stock solutions of individual PFCAs were made by weighing 12.5 mg of compound into 5 mL volumetric flasks. The stock solutions were subsequently diluted to 500 µg/mL. Combining 200 µL of the PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA and PFDoA standard allow the use of a mixed standard of PFCAs for method development.

A Methanol/water (ultrapure, Elga, 18 MΩ) (50:50 %v/v) mixture was used for extraction of pharmaceuticals and PFCAs from biosolids and soil. While the pH was adjusted to 2 for pharmaceuticals (previously optimised by Fell (2022)), a neutral pH and pH 10 were also investigated for the extraction of PFCAs. Formic acid was used to adjust to an acidic pH and ammonia allows the adjustment to alkaline pH.

Soil characterisation (Analysis of Variance)

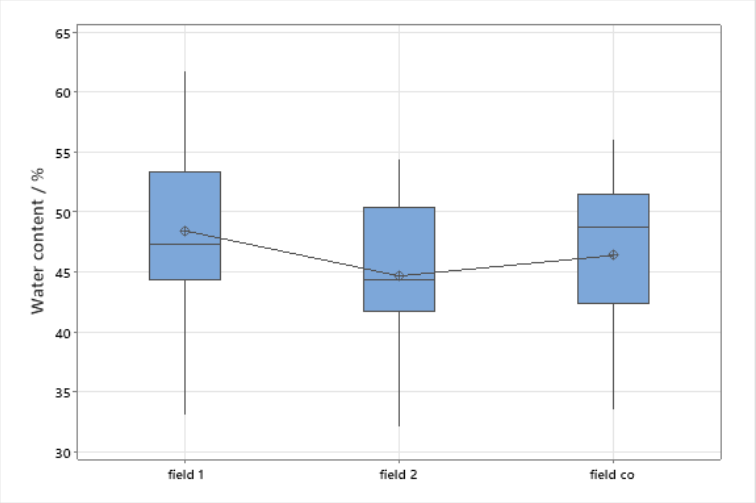


Figure B-2: Boxplot diagram of the water content (%) in analysed soil samples from three fields. Each respective soil was analysed in 20 subsamples. Graph generated through ANOVA analysis using Minitab

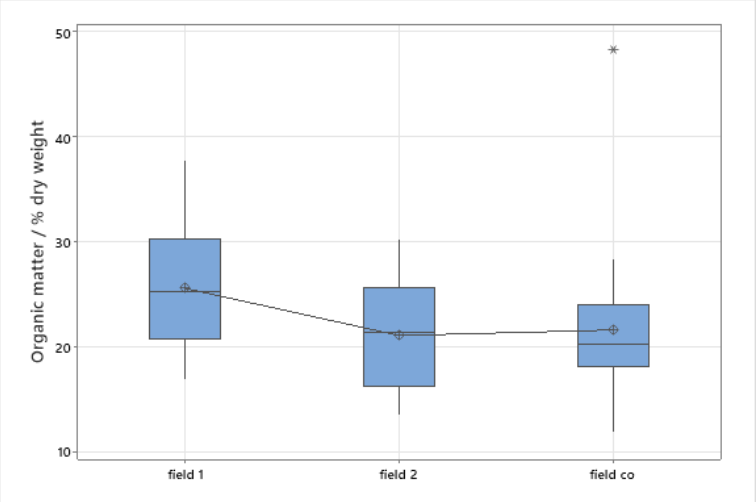


Figure B-3: Boxplot diagram of the organic matter content (% dry weight) in analysed soil samples from three fields. Field one and two previously received biosolids amendment, field co received inorganic fertiliser. Each respective soil was analysed in 20 subsamples. Graph generated through ANOVA analysis using Minitab

Optimisation of Soil Sample Masses and Increasing the Throughput (Multiplexing) for Ultrasound-assisted Extraction

Table B-2: Sample extraction order for the optimisation of soil masses and increasing the throughput (multiplexing) for ultrasound-assisted extraction. 3 samples were extracted simultaneously, using the outer two and middle position of the vial clamping attachment. Samples 13 – 30 were used to investigate the ideal amount of soil to use for extraction, samples 1 - 24 were used for the investigation into increasing the throughput of the extraction method.

#	Extraction run	sonotrode position	sample type	Derivatised / non-derivatised
1	1	1	biosolids 1	non-der.
2	1	3	biosolids 1	non-der.
3	1	5	biosolids 1	non-der.
4	2	1	biosolids 1	non-der.
5	2	3	biosolids 1	non-der.
6	2	5	biosolids 1	non-der.
7	3	1	biosolids 1	derivatised
8	3	3	biosolids 1	derivatised
9	3	5	biosolids 1	derivatised
10	4	1	biosolids 1	derivatised
11	4	3	biosolids 1	derivatised
12	4	5	biosolids 1	derivatised
13	5	1	0.5 g soil (pooled)	derivatised
14	5	3	0.5 g soil (pooled)	derivatised
15	5	5	0.5 g soil (pooled)	derivatised
16	6	1	0.5 g soil (pooled)	derivatised
17	6	3	0.5 g soil (pooled)	derivatised
18	6	5	0.5 g soil (pooled)	derivatised
19	7	1	1.0 g soil (pooled)	derivatised
20	7	3	1.0 g soil (pooled)	derivatised
21	7	5	1.0 g soil (pooled)	derivatised
22	8	1	1.0 g soil (pooled)	derivatised
23	8	3	1.0 g soil (pooled)	derivatised
24	8	5	1.0 g soil (pooled)	derivatised
25	9	1	2.0 g soil (pooled)	derivatised
26	9	3	2.0 g soil (pooled)	derivatised
27	9	5	2.0 g soil (pooled)	derivatised
28	10	1	2.0 g soil (pooled)	derivatised
29	10	3	2.0 g soil (pooled)	derivatised
30	10	5	2.0 g soil (pooled)	derivatised

Gas Chromatography – Mass Spectrometry Method Development for the Detection of Perfluoro Carboxylic Acids Using Electron Impact Ionisation

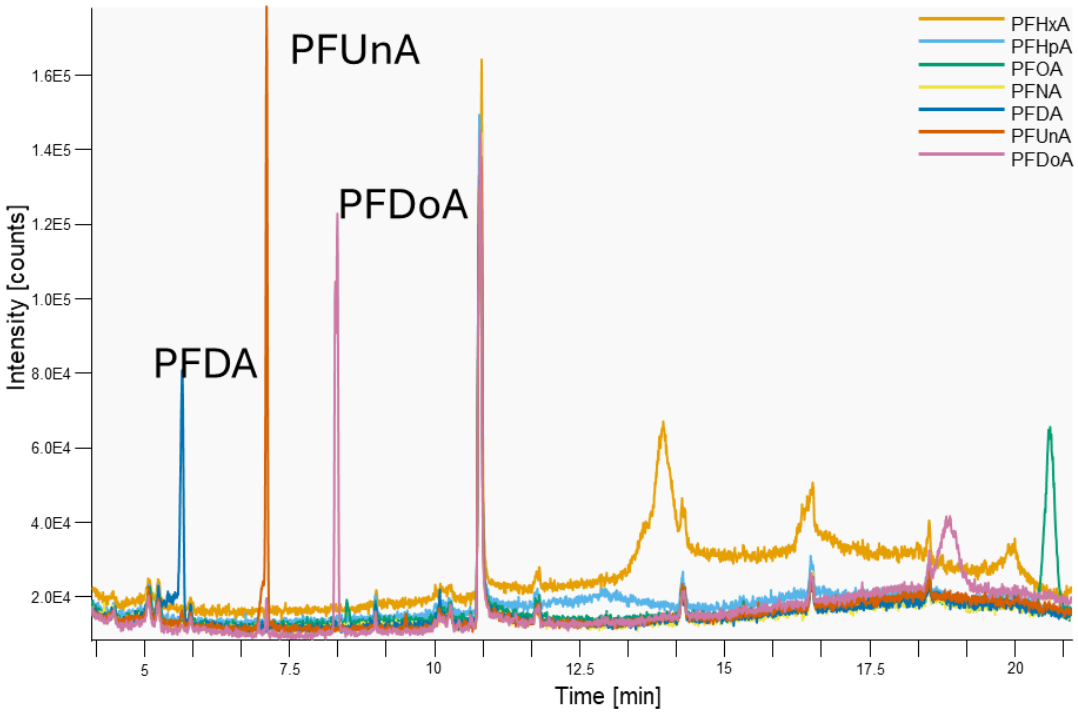


Figure B-4: Chromatogram of initial GC-MS runs of PFCAs (PFHxA - PFDoA). Only PFDoA, PFUnA and PFDA produced peaks in the chromatogram. MS was operated in total ion count (TIC, scan). Unknown compound (tR approx. 10.8 min) found in all standards. Ovenprogramming: initial plateau at 40 °C for 5 minutes, temperature ramp 10 °C/min to 170 °C for 3 minutes. Helium flow rate of 1.25mL/min, splitless injection.

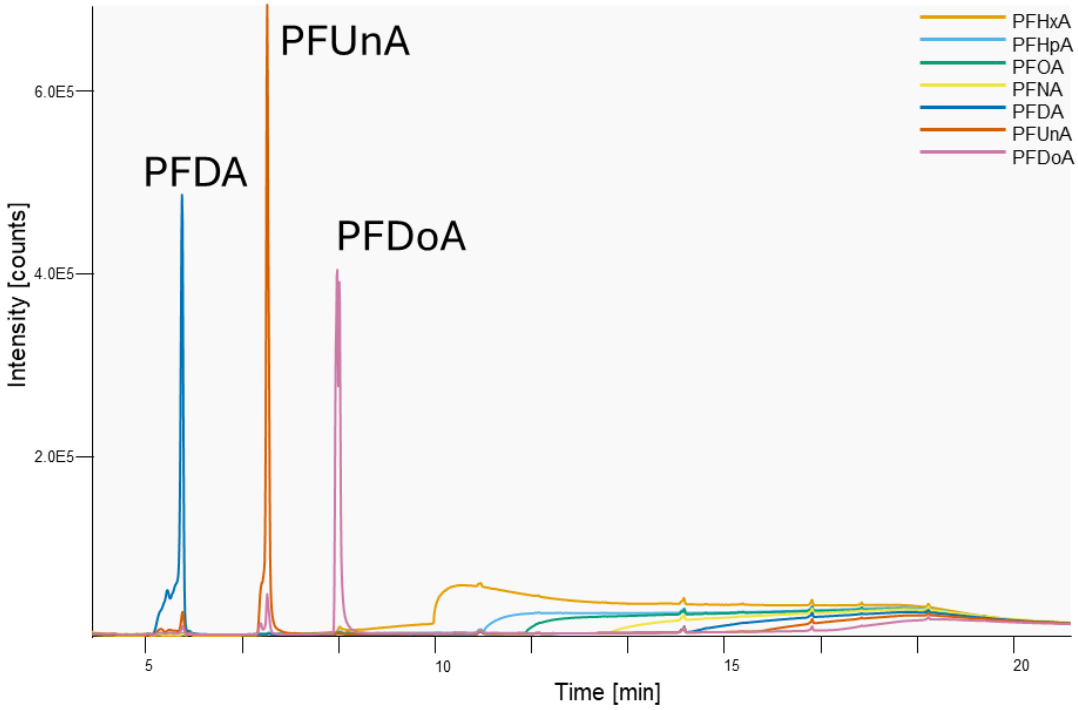


Figure B-5: Chromatogram of initial GC-MS runs of PFCAs (PFHxA - PFDoA). Only PFDoA, PFUnA and PFDA produced peaks in the chromatogram. MS was operated in selected ion monitoring (SIM, m/z's: 59, 69, 100,

119, 131, 169). Ovenprogramming: initial plateau at 40 °C for 5 minutes, temperature ramp 10 °C/min to 170 °C for 3 minutes. Helium flow rate of 1,25mL/min, splitless injection.

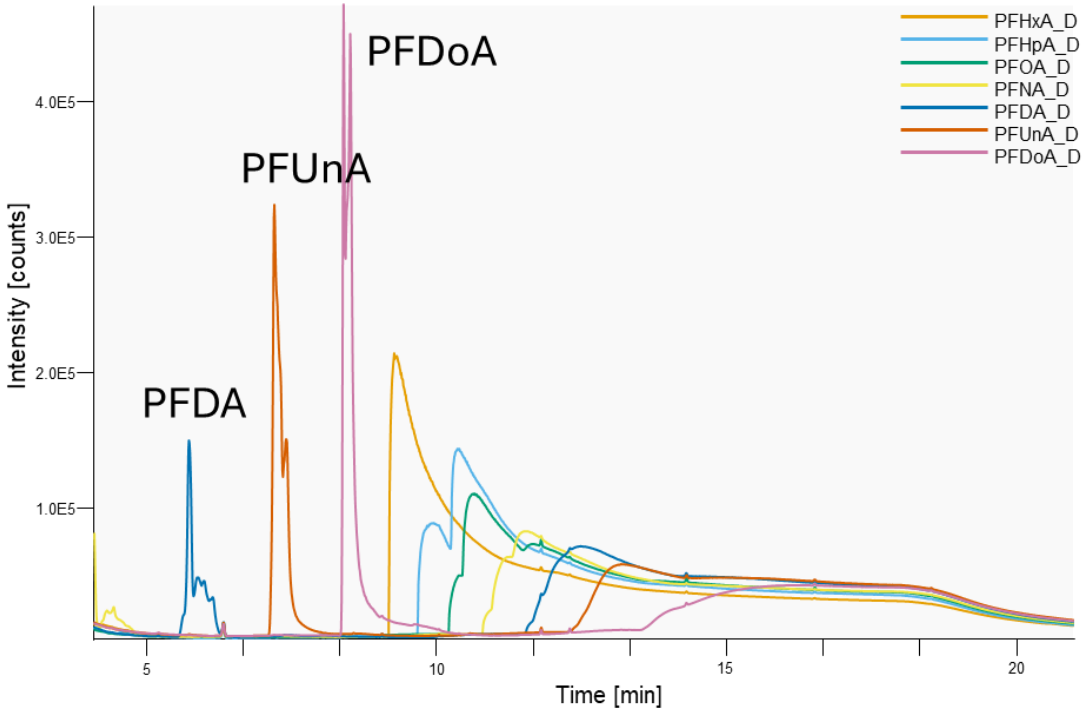


Figure B-6: Chromatogram of initial GC-MS runs of MSTFA-derivatised PFCAs (PFHxA - PFDoA). Only PFDoA, PFUnA and PFDA produced peaks in the chromatogram. MS was operated in selected ion monitoring (SIM, m/z's: 59, 69, 100, 119, 131, 169). Ovenprogramming: initial plateau at 40 °C for 5 minutes, temperature ramp 10 °C/min to 170 °C for 3 minutes. Helium flow rate of 1,25mL/min, splitless injection.

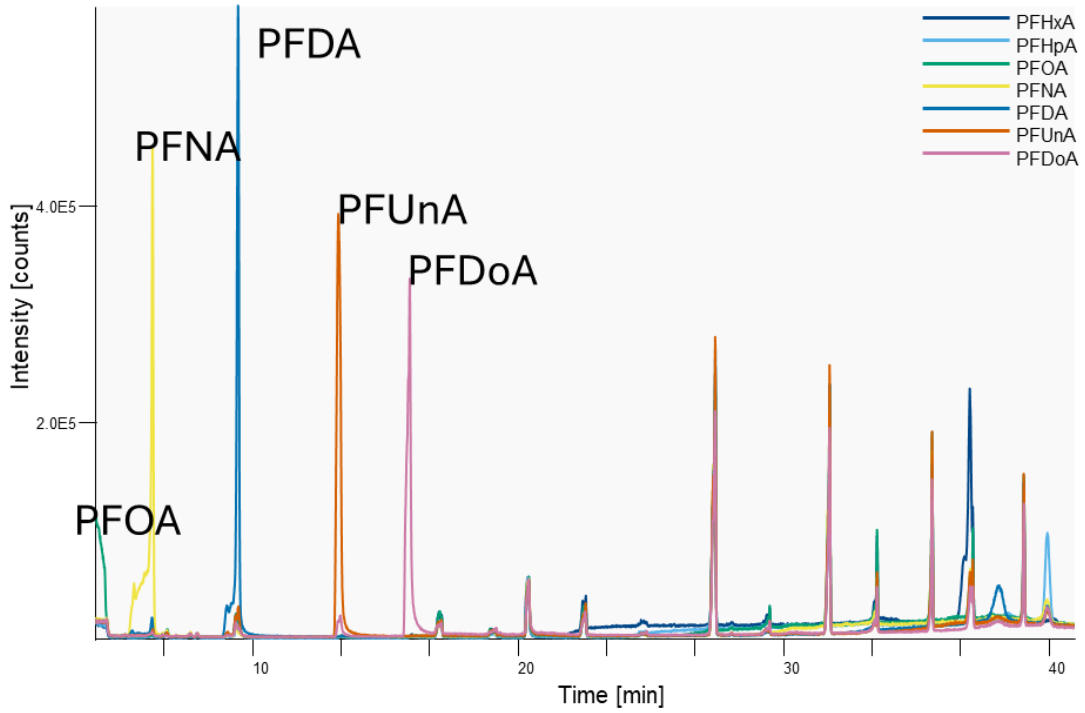


Figure B-7: Chromatogram of the GC-MS analysis PFCAs (PFHxA - PFDoA). Only PFOA and bigger produced peaks in the chromatogram. MS was operated in TIC. Ovenprogramming: initial plateau at 30 °C for 10 minutes, temperature ramp 5 °C/min to 170 °C for 3 minutes. Helium flow rate of 1,25mL/min, splitless injection.

The Effect of Varying pH Values During the Extraction of Perfluoro Carboxylic Acids from Soil Samples

Table B-3: Sample extraction order for the pH value optimisation for ultrasound-assisted extraction of PFCAs. 3 samples were extracted simultaneously, using the outer two and middle position of the vial clamping attachment. Soil samples were extracted at pH values of 2, 7 and 10. Soil samples were spiked with a PFCA-standard mixture, negative controls did not receive any spike prior to extraction.

Extraction run	sonotrode position	sample type	Extraction pH
1	1	biosolids 1	7
1	3	biosolids 1	7
1	5	biosolids 1	7
2	1	biosolids 1	10
2	3	biosolids 1	10
2	5	biosolids 1	10
3	1	biosolids 1 +ve Co	10
3	3	biosolids 1 +ve Co	10
3	5	biosolids 1 +ve Co	10
4	1	soil (pooled)	2
4	3	soil (pooled)	2
4	5	soil (pooled) -ve Co	2
5	1	soil (pooled)	10
5	3	soil (pooled)	7
5	5	soil (pooled)	7
6	1	soil (pooled)	7
6	3	soil (pooled)	10
6	5	soil (pooled) -ve Co	10
7	1	soil (pooled) -ve Co	7
7	3	soil (pooled)	10
7	5	soil (pooled)	2

Appendix C: Chapter 5

Chapter 5: Stock Solutions and Standards

2 mg/mL stock solutions of individual PFCAs were made by weighing 2-5 mg of compound into clean, labelled 14 mL falcon tube (polypropylene) and adding the respectively required amount of acetonitrile and water (50 %v/v) to achieve the desired concentration. 50 µL of each stock solution was combined in a 10 mL volumetric flask and filled to the vessel mark with acetonitrile to gain a mixed standard solution at 10,000 ng/mL. The mixed standard solution was used for the setup of a nested serial dilution prior to analysis (**Error! Reference source not found.Error! Reference source not found.**). It was kept in the fridge at 4°C for up to 3 months.

A methanol and water (50:50 %v/v) mixture was used for extraction of PFCAs from soil. Ammonia allows the adjustment to an alkaline pH of 10.

1 mL of each 50 µg/mL ^{13}C standard solutions (3) are combined to make up 10 mL of a 5000 ng/mL stock solutions in methanol. 10 µL of the mixed standard is added to every sample vial during reconstitution with acetonitrile and water (50 %v/v).

Table C-1: Nested serial dilution profile used for mixed PFCA standard setup. Initially, the prepared 10,000 ng/mL mixed standard solution is diluted 1:2, 1:4 and 1:10. Subsequently a nested 1:10 serial dilution creates a set of 12 calibration standards, spanning concentrations from 0.1 ng/mL to 500 ng/mL.

	Dilutions	1:10	1:10	1:10	1:10	
10,000 ng/mL	1:2	5,000	500	50	5	0.5
	1:4	2,500	250	25	2.5	0.25
	1:10	1,000	100	10	1	0.1

Resulting standard series

500	250	100	50	25	10	5	2.5	1	0.5	0.25	0.1

1.5 mL polypropylene LC vials and caps with a polyimide/silicone septum are used for all standards, samples, blanks and controls, minimising the binding of PFCA analytes to the wall of the vessel compared to commonly used glass vials while avoiding contamination arising from frequently employed septas made from PTFE/silicone.

Perfluoro Carboxylic Acid Profiles in Biosolids and Soil using Ultrasound-assisted Extraction and Liquid Chromatography – Orbitrap - Mass Spectrometry Analysis

Table C-1: Sample label re-assignment and ultrasound-assisted extraction order for LC-Orbitrap-MS analysis. Field 1 (F1) and Field 2 (F2) as well as biosolids batches 1-6. Controls were randomly selected control soils, blanks were the respective solvents used in the process. Extraction of 3 vials simultaneously with the vial clamping device of the sonotrode. Due to size restrictions the two outer positions (1 and 5) and the central position (3) were engaged in the process. Field 1 was extracted independently (runs 1-9), whereas field 2 and biosolids were extracted at the same time (runs 10-25 over 2 days).

Sample name	New label	Extraction run	Extraction position
F1 09/2019 #01	LC 01	4	1
F1 09/2019 #09	LC 02	5	3
F1 09/2019 #11	LC 03	2	5
F1 09/2019 #14	LC 04	3	3
F1 09/2019 #17	LC 05	9	5
F1 10/2019 #01	LC 06	1	1
F1 10/2019 #13	LC 07	6	5
F1 10/2019 #17	LC 08	1	3
F1 10/2019 #19	LC 09	7	5
F1 10/2019 #20	LC 10	9	3
F1 04/2020 #01	LC 11	7	1
F1 04/2020 #06	LC 12	8	5
F1 04/2020 #08	LC 13	6	5
F1 04/2020 #09	LC 14	1	5
F1 04/2020 #15	LC 15	2	1
F1 03/2021 #04	LC 16	3	5
F1 03/2021 #05	LC 17	4	3
F1 03/2021 #07	LC 18	9	1
F1 03/2021 #15	LC 19	7	3
F1 03/2021 #17	LC 20	3	1
F1 06/2021 #09	LC 21	8	3
F1 06/2021 #14	LC 22	6	3
F1 06/2021 #15	LC 23	2	3
F1 06/2021 #17	LC 24	8	1
F1 06/2021 #19	LC 25	4	5
Field 1 Control	LC Co	6	1
Field 1 Blank	LC Bl	7	1
F2 09/2019 #01	LC 26	14	1
F2 09/2019 #07	LC 27	20	3
F2 09/2019 #11	LC 28	17	5
F2 09/2019 #18	LC 29	12	3
F2 09/2019 #19	LC 30	17	3

F2 10/2019 #01	LC 31	11	1
F2 10/2019 #04	LC 32	20	1
F2 10/2019 #09	LC 33	22	5
F2 10/2019 #11	LC 34	23	1
F2 10/2019 #12	LC 35	25	3
F2 04/2020 #01	LC 36	10	5
F2 04/2020 #03	LC 37	19	5
F2 04/2020 #06	LC 38	10	3
F2 04/2020 #08	LC 39	11	5
F2 04/2020 #13	LC 40	12	5
F2 03/2021 #03	LC 41	13	5
F2 03/2021 #04	LC 42	21	3
F2 03/2021 #07	LC 43	10	1
F2 03/2021 #09	LC 44	25	1
F2 03/2021 #13	LC 45	21	5
F2 06/2021 #04	LC 46	13	1
F2 06/2021 #09	LC 47	24	3
F2 06/2021 #12	LC 48	11	3
F2 06/2021 #17	LC 49	16	1
F2 06/2021 #30	LC 50	22	1
Field 2 Control	LC Co 2	15	1
Field 2 Blank	LC Bl 2	15	5
Biosolids 1 #1	LC 51	18	3
Biosolids 1 #2	LC 52	12	1
Biosolids 1 #3	LC 53	19	3
Biosolids 2 #1	LC 54	15	3
Biosolids 2 #2	LC 55	17	1
Biosolids 2 #3	LC 56	18	5
Biosolids 3 #1	LC 57	19	1
Biosolids 3 #2	LC 58	16	3
Biosolids 3 #3	LC 59	14	5
Biosolids 4 #1	LC 60	21	1
Biosolids 4 #2	LC 61	24	1
Biosolids 4 #3	LC 62	23	5
Biosolids 5 #1	LC 63	14	3
Biosolids 5 #2	LC 64	18	1
Biosolids 5 #3	LC 65	13	3
Biosolids 6 #1	LC 66	23	3
Biosolids 6 #2	LC 67	16	5
Biosolids 6 #3	LC 68	24	5
Biosolids Control	LC Co 3	20	5
Biosolids Blank	LC Bl 3	22	3

Table C-2: PFCA (PFBA-PFDoA) concentrations in biosolids-amended soil collected from field 1 2019-2021.

ng/g	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA
LC01	ND	ND	< LOD	< LOD	< LOD	< LOD	0.1	< LOD	< LOD
LC02	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC03	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC04	ND	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC05	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC06	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC07	<LOD	ND	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC08	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC09	<LOD	< LOD	< LOD	22.2	2.7	0.7	1.6	< LOD	< LOD
LC10	<LOD	ND	< LOD	1.6	< LOD	< LOD	< LOD	< LOD	< LOD
LC11	<LOD	ND	< LOD	13.6	< LOD	0.9	1.8	< LOD	0.7
LC12	<LOD	< LOD	< LOD	1.9	< LOD	< LOD	0.4	< LOD	< LOD
LC13	ND	ND	ND	ND	ND	ND	ND	ND	ND
LC14	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC15	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC16	<LOD	ND	< LOD	7.9	< LOD	< LOD	0.7	< LOD	< LOD
LC17	<LOD	ND	< LOD	3.2	< LOD	< LOD	0.3	< LOD	< LOD
LC18	<LOD	ND	< LOD	1.2	< LOD	< LOD	0.4	< LOD	< LOD
LC19	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC20	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC21	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC22	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC23	<LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC24	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
LC25	<LOD	ND	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

Table C-3: PFCA (PFBA-PFDoA) concentrations in control soil collected from field 2 2019-2021.

ng/g	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA
LC26	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC27	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD
LC28	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC29	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC30	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC31	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC32	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC33	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD
LC34	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC35	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC36	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD
LC37	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.5
LC38	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC39	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC40	<LOD	ND	<LOD	<LOD	1.3	<LOD	<LOD	<LOD	<LOD
LC41	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC42	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC43	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23.2
LC44	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC45	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC46	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC47	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC48	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD
LC49	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LC50	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table C-4: PFCA (PFBA-PFDoA) concentrations in biosolids batches collected beetween 2019-2022.

Sample	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA
LC51	143.5	28.2	29.8	<LOD	4.3	2.6	8.4	1.9	6.0
LC52	201.7	44.3	55.3	8.6	5.1	2.6	8.0	1.1	10.7
LC53	155.4	34.4	37.7	10.2	4.3	2.6	4.3	0.5	10.1
LC54	147.4	40.9	40.2	12.4	5.6	3.0	2.9	3.1	7.2
LC55	146.8	40.2	22.7	4.8	5.9	3.8	2.7	4.4	5.6
LC56	162.1	36.9	31.1	5.5	4.5	1.8	2.6	3.1	8.1
LC58	144.5	75.4	36.5	9.2	5.4	3.6	4.5	0.3	8.6
LC59	183.6	114.6	90.5	28.0	7.3	6.3	5.3	0.5	8.6
LC60	181.5	83.5	52.5	15.0	6.8	5.5	5.3	0.7	11.4
LC61	152.1	54.4	30.0	6.5	5.5	3.6	3.4	ND	10.3
LC62	193.1	42.3	37.5	9.1	4.3	2.5	<LOD	ND	9.9
LC63	161.5	90.6	43.0	11.1	8.7	7.0	4.5	0.4	9.2
LC64	228.4	161.2	105.1	32.5	11.1	8.4	7.2	0.6	10.9
LC65	195.7	128.4	73.6	27.2	9.8	10.0	14.9	1.6	10.4
LC66	184.0	37.8	36.3	8.4	6.0	3.2	10.7	ND	10.4
LC67	217.8	43.9	54.0	13.7	7.2	4.5	10.0	0.3	11.1
LC68	176.4	44.1	76.1	22.7	5.9	5.7	9.6	7.1	7.8

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