Sources, pathways, exposures and hazards of perfluorinated chemicals in the Orange River Catchment

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Seeds are dropped in dirt, covered in darkness and struggle to reach the light – Sandra King.

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Sources, pathways, exposures, and hazards of perfluorinated chemicals in the Orange River Catchment

Summary

Persistent organic pollutants (POPs) can be classified as widely distributed organic compounds, sharing a suite of physical and chemical properties, occurring in all environmental compartments with serious toxicological potential. Due to the properties and potential danger associated with POPs, they have come under scientific scrutiny and have commanded attention from governmental and non-governmental groups alike. The global recognition of the inherent risk of POPs culminated in the development of the Stockholm Convention (SC). The main aim of the SC is to protect humans and the environment from chemicals that are persistent, bio-accumulate and tend to become widely geographically distributed. South Africa, as a signatory of the SC has the responsibility to undertake appropriate research, development, monitoring and cooperation pertaining to persistent organic pollutants. There is growing concern over the toxicity, environmental distribution and bio-accumulation of a group of POPs, namely perfluorinated compounds (PFCs). These compounds are widespread toxic POPs that are used extensively in various industrial applications. Contrary to the bio-accumulative pattern of most POPs that partition into fatty tissues, PFCs bind to proteins. Although data concerning PFCs is limited for the South African environment, these compounds have been detected in human and wildlife populations. This study focussed on the analysis of selected PFCs from multiple environmental matrices in the Orange-Senqu River basin (OSRB), the largest river systems in South Africa. Matrices analysed included fresh water, sediment and waste streams, fresh water fish, as well as birds nesting in aquatic environments. The aim of the project was to determine the levels of twelve PFCs [perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), perflurooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA) and perfluototetradecanoic acid (PFTA)] in the Orange River Basin where high concentrations were found previously, and to establish possible sources, pathways, exposures and hazards of the PFCs identified and quantified. Water samples contained quantifiable concentrations of PFBS (0.24 ng/L) and PFUnA (0.17 ng/L). The presence of these compounds is likely from use in crop farming, or as surfactants in mining or in the textiles and upholstery industries. In the case of sediments and tailings, only six samples contained PFCs. In sediment PFOS (2 – 4 ng/g) and PFHxA (5 ng/g) was detected, and in tailing I found PFHxA (4 ng/g) and PFOA (8 ng/g). Possible sources identified include aviation, mining and/or wastewater treatment plants. None of the fish samples analysed had detectable concentrations, whereas all PFCs analysed for were detected in eggs. However, the detection frequency varied from one compound

in a single sample to PFOS with a 95% detection rate in eggs. The PFOS concentrations ranged from 0.3 – 2800 ng/g wm, with the highest median PFOS concentration detected in African Darter eggs (1100 ng/g wm). The lowest concentrations were in African Sacred Ibis eggs (10 ng/g wm). As reported in literature, I found that long-chain PFSAs were predominant in wild bird eggs followed by long-chain PFCAs, short-chain PFCAs and short-chain PFSAs. This pattern is likely due to differences in the bio-accumulation potential of PFCs based on their chain length. To further investigate the concentrations and patterns of PFCs in wild bird eggs, multivariate statistical analysis was performed. A cluster analysis indicated a grouping where PFOS was separated from all the other congeners. This coincides with literature, as PFOS is the main congener found in biota. The congener profile of PFCs in individual species were compared. PFOS was the dominant congener detected in all bird species. However, PFNA, PFDA and PFUnA concentrations were statistically significantly different between the bird species. The significance of congeners could be contributed to the foraging method and/or type of diet. The African Darter had the highest concentrations of PFCs compared to any other species, followed by the Reed Cormorant and White-breasted Cormorant. The congener profiles of PFCs in bird eggs were further investigated using principle component analysis (PCA) indicating that PFCs varied depending on species, feeding habitat, and collection site. Additionally, the concentrations of PFCs differed significantly between species. These differences could be attributed to multiple factors such as exposure routes (diet, feeding habitat, and area of sampling) and differences in toxicokinetics (absorption, distribution, transformation, and elimination) of PFCs. The distribution of congeners at different sites were further investigated. Welverdiend had the highest frequency of detection of PFC congeners. The possible sources associated with these congeners in the surrounding area are WWTP, farmlands, active and abandoned mines. However, ratios of PFHpA, PFOA, and PFNA indicated precursor breakdown and precipitation as a contributing source. Schoemansdrift had higher concentrations of PFNA and PFDA than any other site. The high concentrations of PFOS were found in Orkney and could be due to mining utilised in the surrounding area. Upington had the highest levels of PFTrDA that seems to be associated with a WWTP. Toxicological data is dependent on which no-observed-adverse-effect level (NOEL) is used from literature. If the least conservative option is chosen, with maximum PFOS levels: African Darter, Reed Cormorant, White-breasted Cormorant, Grey Heron, Cattle Egret, Glossy Ibis and Black-headed Heron will all have levels above the NOEL. In conclusion; PFCs are present in the South African environment, with high concentration levels found in bird eggs. Although there is not yet consensus on the toxicological no-observed-adverse-effect level (NOELs) for PFCs in birds, the PFC exposure in conjunction with exposure to other POPs and organic toxicants may have detrimental effects on the South African aquatic bird population. The combined toxicological effect of these chemical loadings on bird populations may be a cause for concern.

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Keywords: POPs, Perfluorinated compounds, PFOS, water, sediment, fish, wild bird eggs, South Africa.

ABBREVIATIONS

ACOX	Acyl-co-enzyme A oxidase
AD	African Darter
BHH	Black-headed Heron
CE	Cattle Egret
CoA	Co-enzyme A
CRM	Certified reference material
DEA	Department of Environmental Affairs
dd.H_2O	Double deionised water
DDT	Dichlorodiphenyltrichloroethane
DWA	Department of Water and Sanitation
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
ESI	Electron spray ionisation
ESM	Environmental sound management
FABP	Fatty acid binding proteins
FASAAs	Perfluoroalkyl sulfonamidoacetic acids
FASAs	Perfluroalkyl sulfonamides
FASEs	Perfluroalkyl sulfonamidoethanols
FTALs	Fluorotelomer saturated aldehydes
FTCA	Fluorotelomer carboxylates
FTOHs	Fluorotelomer alcohols
FTSAs	Fluorotelomer sulfonates
FTUALs	Fluorotelomer unsaturated aldehydes
FTUCA	Fluorotelomer unsaturated carboxylates
GC-MS/MS	Gas chromatography coupled to tandem mass spectrometry
GDP	Gross domestic product
GI	Glossy Ibis
GH	Grey Heron
HCl	Hydrochloric acid
HDPP	High density polypropylene
HPLC-MS/MS	High pressure liquid chromatography coupled to tandem mass spectrometry
IUCN	International Union for Conservation of Nature
K_{oa}	Octanol/ air partition coefficient
LHWP	Lesotho Highlands Water Project
LOD	Limit of detection
LOEC	Lowest-observed-effect concentration
LOQ	Limit of quantification
M8PFOA	Labelled Perfluorooctanoic acid
M8PFOS	Labelled Perfluorooctanesulfonic acid
MeOH	Methanol
MRM	Multiple reaction monitoring
MTBE	Methyl tert-butyl ether
MS	Mass spectrometer
NaOH	Sodium hydroxide
ND	No detects
NEMA	National Environmental Management Act
NECSA	South African Nuclear Energy Corporation

ABBREVIATIONS

NH_4OH	Ammonium hydroxide
NIPS	National Implementation Plans
NOELs	No-observed-effects levels
OAT	Organic anion transporter
OECD	Organization for Economic Cooperation and Development
ORASECOM	Orange-Senqu River Commission
PAHs	Polycyclic aromatic hydrocarbons
PAPs	Polyfluoroalkyl phosphoric acid esters
PCA	Principle component analysis
PCB	Polychlorinated Biphenyl
PE	Polyethylene
PEEK	Polyetheretherketone
PerFASs	Perfluoroalkyl substances
PFASs	Polyfluoroalkyl and perfluoroalkyl substances
PFBS	Perfluorobutanesulfonic acid
PFCAs	Perfluoroalkyl caboxylates
PFCs	Perfluorinated compounds
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPAs	Perfluoroalkyl phosphonates
PFSAs	Perfluoroalkyl sulfonated
PFTA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUnA	Perfluoroundecanoic acid
pKa	Acid dissociation constant
PolyFASs	Polyfluoroalkyl substances
POPS	Persistent organic pollutants
POSF	Perfluorooctylsulfonyl fluoride
PP	Polypropylene
PPAR	Peroxisome proliferator-activated receptors
RBCs	Red blood cells
RC	Reed Cormorant
SI	Sacred Ibis
SC	Stockholm Convention
SSC	Secretariat of the Stockholm Convention
SPE	Solid phase extraction
TBA	Tetrabutylammonia
TOF	Time of flight
TOF-HRMS	Time of flight high resolution mass spectrometry
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
USA	United States of America

ABBREVIATIONS

VLDLs	Very low density lipoproteins
WBC	White-breasted cormorant
WMA	Water management area
WWTP	Waste water treatment plant

CONTENTS

Chapter 1: Introduction

"We forget that the water cycle and the life cycle are one"
Jacques Cousteau

INTRODUCTION

In the modern world, a vast array of organic chemicals is produced through anthropogenic activity that enter the environment. Among these chemicals are persistent organic pollutants (POPs). They are either intentionally produced as pesticides and industrial chemicals or as by-products in industrial or thermal processes (the last include both natural and anthropogenic). POPs as a group have similar physical and chemical properties they are resistant to photolytic, chemical and biological degradation (Bouwman, 2004; Jones & de Voogt, 1999). Therefore, POPs are recalcitrant and are distributed between various environmental compartments. POPs tend to accumulate in the tissue of organisms (bioaccumulation) and are toxic. Their physical and chemical characteristics and resistance to decomposition allows transportation over long distances, leading them to be found globally (Scheringer, 2009).

Due to the lipophilic nature of most POPs, these compounds are bio-accumulative and tend to bio-magnify in the food web (Scheringer, 2009). This is of particular concern as POPs have been associated with negative health effects including cancers, dysfunctional immune and reproductive systems, birth defects and greater susceptibility to diseases (Porta, 2014; Post *et al.*, 2012). Due to the above mentioned concerns, the United Nations Environment Programme (UNEP) initiated the Stockholm Convention (SC) on POPs to regulate, eliminate or reduce the use of these compounds (Bouwman, 2004).

1.1. Stockholm Convention

The SC on POPs protects human health and the environment through the restriction of use and production (intentional and unintentional) of POPs (Lallas, 2001). The SC was adopted on 22 May 2001, and came into force on 17 May 2004 (Stockholm Convention, 2016). The five objectives of the SC are to: (1) eliminate the release and use of 23 POPs listed in the convention, (2) support the transition towards safer alternatives, (3) consider other chemicals which can be added to the POPs list, (4) remediate or safely destroy old stockpiles and equipment containing POPs and (5) increase public awareness towards a POPs-free future.

Over the last decade, the following advances in controlling the use and production of POPs have been made: (Secretariat of the Stockholm Convention (SSC), 2012)

- Currently, 180 countries are parties to the SC, thereby committing to decreasing the amount of POPs present in the environment while regulating these compounds.
- To date, National implementation plans (NIPs) of 163 Parties have been submitted. NIPs, which have to be submitted every four years, will assist in indicating the status and effectiveness of implementation of the SC. This data is used to evaluate if parties are fulfilling their obligation to the SC, thereby protecting global human health and the environment from their harmful effects.

- Support in the form of guidance and capacity building was provided to the signatories to assist with implementation of SC.
- No further registration for exemption of aldrin, chlordane, dieldrin, heptachlor, hexachlorobenzene and mirex can be made.
- Eleven new POPs were added to the SC, increasing the scope of SC, and more are under consideration.
- Baseline levels of POPs in ambient air, human milk and blood was determined through the submission of regional and global reports (UNEP, 2009; UNEP 2013).
- Many developing countries as well as countries with economies in transition do not currently have the capacity or technology to monitor POPs in their region. Therefore, both regional and sub-regional centres for capacity building and transfer of technology were developed.
- The Polychlorinated Biphenyl (PCB) Elimination Network was established to facilitate information exchange ensuring environmentally sound management (ESM) of PCBs. The target is to ensure ESM of PCBs waste by 2028.
- The Global Alliance was established to investigate alternatives to dichlorodiphenyltrichloroethane (DDT) for disease vector control. Five groups have been established under the following topics: reduce barriers for new chemicals and products as well as non-chemical methods, integrated vector management, cost effective alternatives to DDT, malaria vector resistance patterns and mechanisms.
- Coordination and cooperation between the Basel, Rotterdam and Stockholm conventions was implemented.

These ten major achievements mentioned above form part of a bigger picture, which together highlights the success of the SC thus far to reduce and eliminate the threats posed by POPs. In South Africa, the SC assisted to establish a co-operative strategy to promote sustainable development and to preserve and enhance a safe human environment of South Africa through the National Environmental Management Act (NEMA).

South Africa as a party (signed 4 September 2002) is legally bound to abide to the obligations listed in the SC (Bouwman, 2004). One of these obligations is to ensure that the NIP shows continual implementation of the SC, including providing baseline data for POPs in the South African environment. It is therefore important to investigate and evaluate the status of POPs in the country. With the increasing number and volume of organic chemicals produced and released, it is important to generate background data on the levels of potentially toxic compounds in the environment. It will greatly assist South Africa to meet the SC requirements if the analytical capabilities for these analyses are developed locally, while establishing a database with information on the presence and levels of current and candidate POPs. One of the challenges is

the increasing list of POPs such as perfluorinated compounds (PFCs) are particularly challenging, where limited data is available on the levels and effects in the environment and human health.

1.2. Environmental fate of PFCs

PFCs have useful properties as they are oil and water repellent, resistant to heat and chemical reactions, persistent and stable (due to their carbon-fluorine bonds) which makes them popular to use in a variety of products such as water-, soil-, and stain resistant coatings for clothing, leather and carpets, aviation hydraulic fluids, firefighting foams, adhesives, surfactants, emulsifiers and coatings (Corsini *et al.*, 2014; Houde *et al.*, 2006). Due to the chemical characteristics and multiple uses, they are found globally in a variety of environmental and human matrices. PFCs do not follow the classic bio-accumulation pattern of POPs by partitioning into fatty tissues, but rather have high affinity for proteins, causing concern regarding their toxicological implications as described in **Chapter 2 Section 2.1.4** (Jones *et al.*, 2003). The precise mechanism of partitioning and sources of PFCs introduced into the environment is not known and remains under investigation. Direct (manufacturing and use) and indirect (substances degrade to form PFCs) sources are responsible for PFCs found in environmental and human matrices (Martin *et al.*, 2006). One of the main routes of exposure for humans is through intake of contaminated food, although metabolites of precursor compounds and exposure from various consumer products might also play a role (Domingo, 2012; Martin *et al.*, 2006; Trudel *et al.*, 2008; Washburn *et al.*, 2005). Currently, more data is required to evaluate the precise route of exposure of PFCs (Gomis *et al.*, 2015). Compared to well-known POPs such as DDT, the environmental fate and risk associated with PFCs are not well understood making it important to investigate PFCs and generate data to fill knowledge gaps. However, multiple factors contribute to the uncertainty associated with the analysis of PFCs, making it difficult to ensure the quality of analytical data (Martin *et al.*, 2004). Therefore, adequate extraction and analysis techniques are crucial in evaluating the presence and concentrations of PFC residues.

1.3. Analysis of PFCs

The preferred method for the analysis of PFCs is high pressure liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) with negative electron spray ionisation (-ESI; Jahnke & Berger, 2009). Increased selectivity and sensitivity can be achieved by time of flight high resolution mass spectrometry (TOF-HRMS), but these instruments are not widely available (Trojanowicz & Koc, 2013). After selection of the instrumentation, an optimized extraction method is required to ensure reliable analytical results.

Sample preparation impacts on all the steps in an analysis as each can interfere with the identification, confirmation, and quantification of analytes (Chen *et al.*, 2008). An entire analysis

process can be invalidated due to poor sample treatment and badly prepared sample extracts. Initially, an ion-pair extraction method using tetrabutylammonia (TBA) was employed, but the limit of detection (LOD) was not adequate in highly contaminated samples and will not be further discussed. The method was amended by replacing the TBA with methyl tert-butyl ether (MTBE), although LODs were within a suitable range, the co-extraction of lipids made this method unsuitable for biological matrices (Hansen *et al.*, 2001; Jahnke & Berger, 2009; Ylinen *et al.*, 1985). Currently, the general method used for the extraction of PFCs is solid-liquid extraction using solvent mixtures corresponding with mobile phases or by employing a potassium hydroxide digestion (Taniyasu *et al.*, 2005; Trojanowicz & Koc, 2013). Regarding the clean-up of the extracts, Powley *et al*. (2008) developed an efficient method using ENVI-Carb™ for dispersive clean-up. This has become a very popular last step for almost any type of sample extract (Jahnke & Berger, 2009). The use of solid phase extraction as a clean-up is also becoming popular by using weak anion exchange or polymeric cartridges (Taniyasu *et al.*, 2005). Therefore, it is crucial to evaluate analytical strategies and special attention is required for quality control measures to ensure reliable data is generated.

The presence of contamination, lack of commercially available analytical standards, the physical-chemical characteristics of PFCs, matrix effects resulting in ionisation problems, and lack of matrix certified reference material (CRMs), and co-elution of interfering compounds are a few of the challenges to be faced during the analysis of PFCs (van Leeuwen *et al.*, 2006).

1.4. Studies in South Africa

Currently, only limited data on PFCs are available from South Africa, and the analysis thereof was not done locally, emphasizing the need to develop these capabilities in South Africa. Data concerning these compounds is limited for the South African environment; to date only five studies have been performed targeting PFCs in the South Africa environment. These studies have identified PFCs in maternal serum and cord blood (max: 1.6 ng/mL), in bird and crocodile eggs (max: 2300 ng/g), as well as in crocodile plasma (max: 50 ng/g) (Bouwman *et al.*, 2014, 2015; Bouwman & Pieters, 2013; Christie *et al.*, 2016; Hanssen *et al.*, 2010).

The Orange-Senqu River Commission (ORASECOM) which is the institution responsible for managing the Orange-Senqu River basin performed a study on POPs in this basin. The first assessment of POPs for ORASECOM found the presence of various POPs and polycyclic aromatic hydrocarbons (PAHs) in sediments, fish, and bird eggs. In general, the concentrations for most POPs were low, but high for certain PAHs in certain sediments. For perfluorooctanesulfonic acid (PFOS), varying levels were found: the maximum level found was in fish was 1.7 ng/g wm, and in the bird eggs 2300 ng/g wm, while high levels from other bird eggs from other regions of the world are 300 - 1300 ng/g. The levels found in bird eggs from the Orange River were some of the highest ever found in biota anywhere in the world.

Further investigation to identify the presence of PFCs and identify the possible sources responsible for these PFCs is of great importance. The current study will investigate water, sediment, fish, and wild bird eggs for 12 PFCs in the Orange River basin.

1.5. Aims and objectives of the current study

The aim of the project is to determine the levels of 12 PFCs in the Orange River basin where high levels were found previously, and to establish possible sources, pathways, exposures, and hazard of the PFCs identified. This will be achieved by completing the following objectives of the study:

- Determining the presence and levels of PFCs in water, sediment, fish, and wild bird eggs and to evaluate the possible sources that could contribute to the levels found.
- Investigate the distribution and congener profiles of PFCs and identify possible routes of exposure.
- Perform a comparison between obtained and global data and contribute to data that can be used for the Stockholm Convention and other international conventions and treaties concerning POPs.

Chapter 2: Literature review

"Water is the driving force of all nature"
Leonardo de Vinci

The environment is continuously exposed to pollutants released by anthropogenic activity, highlighting the need for continued environmental and biological monitoring. Monitoring is one of the intrinsic steps used in risk assessments when determining the possible threat associated with a given pollutant to humans as well as wildlife (Kocagöz *et al.*, 2014; Van der Oost *et al.*, 2003). One group of organic pollutants commonly found in the environment is polyfluoroalkyl and perfluoroalkyl substances (PFASs), also known as perfluorinated compounds (PFCs).

2.1. Perfluorinated compounds

PFCs have been used extensively as lubricants and additives in polymers due to their favourable chemical characteristics. This group is divided into three classes **(Figure 2.1)**: perfluoroalkyl substances with a fully fluorinated alkyl chain (PerFASs), polyfluoroalkyl substances (PolyFASs) with a partially fluorinated alkyl chain, and fluorinated polymers (Ahrens & Bundschuh, 2014; Buck *et al.*, 2011; Organization for Economic Cooperation and Development (OECD, 2013). PolyFASs and fluorinated polymers are known precursors of PerFASs as they can degrade to form PFSAs as well as PFCAs (See **Section 2.1.3**).

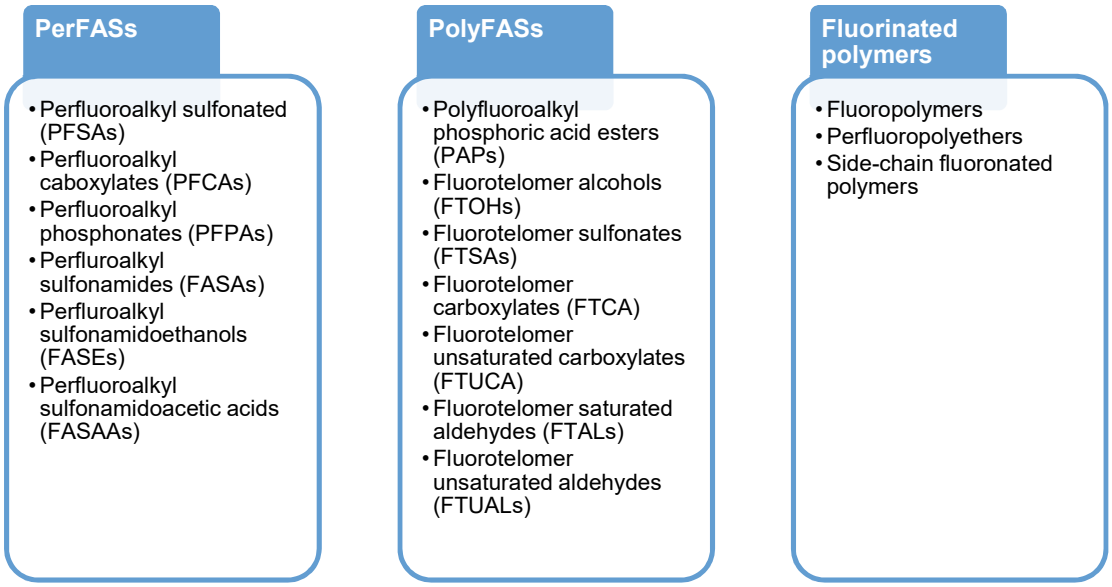


Figure 2.1: Environmentally relevant groups of polyfluoroalkyl and perfluoroalkyl substances (OECD, 2013)

The global distribution and ubiquitous detection of these synthetic compounds in environmental media has raised concerns. PFCs have unique physiochemical properties that varies with chain length and functional group. Long-chain PFCs, PFSAs and PFCAs with a perfluorocarbon chain length of ≥ C7 and ≥ C6, respectively, have a greater tenacity to bio-accumulate than short-chain

(< C6) PFCs (Ahrens & Bundschuh, 2014; Buck *et al.*, 2011). Therefore, the current study focussed specifically on the PFSAs and PFCAs (**Table2.1**).

2.1.1. Physical and chemical characteristics

PFCs are aliphatic compounds consisting of a fluorinated hydrocarbon backbone to which various functional groups (R) are attached. PFCs are amphiphilic as they have a hydrophobic alkyl chain and hydrophilic functional group. The strength of the C-F bond makes these PFCs heat resistant, chemically inert, and imparts an amphiphilic characteristic that provides PFCs with desired surfactant properties. Combined, these properties make PFCs highly versatile and useful for industrial and manufacturing applications. The perfluoroalkyl moiety $F(CF_2)_xR$ is depicted in **Figure 2.2** and represents the basic structure of PFCs (Buck *et al.*, 2011; De Voogt & Sáez, 2006; de Vos *et al.*, 2008; Kim *et al.*, 2014). PFSAs have a sulfonate moiety that makes them ideal surfactants due to their lower surface tension, whereas, PFCAs have a carboxylate group and are used as emulsifiers, surfactants and in chemical synthesis processes (Buck *et al.*, 2011).

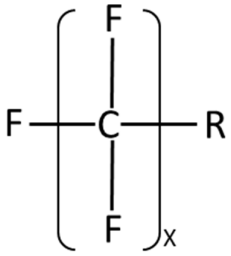


Figure 2.2: Perfluoroalkyl moiety (R: Neutral: CH_2CH_2OH; $-SO_3NH_2$ or anionic end groups: -COO-; $-SO_3^-$;$-OPO_3$)

Limited experimental data is currently available on the basic physiochemical properties (**Table 2.1**), such as vapour pressure, acid dissociation constant (pKa), and octanol/ air partition coefficient (K_{oa}). These properties are crucial in understanding the environmental fate including accumulation and partitioning behaviour. Current estimations of pKa values for PFCs range from 0 – 4, indicating that these compounds will exist in an anion form when in contact with fresh water at typical environmental pH (6.5 – 9) (Houde *et al.*, 2011; Möller *et al.*, 2010). Although some data is available, it is controversial with most of the data originating from various models rather than being calculated experimentally (Ding & Peijnenburg, 2013). Experimentally determining these values are difficult due to the purity of chemicals, lack of appropriate analytical methods, solubility, aggregation of chemicals, dissociation in water, and sorption to the wall of containers (Ding & Peijnenburg, 2013). Only once these problems are resolved can the behaviour and fate of PFCs in the environment be elucidated. Nevertheless, it is clear that PFCs are ubiquitous in the

environment and identification of possible sources and routes of exposure are of utmost importance (Ahrens & Bundschuh, 2014).

Table 2.1: Physical and chemical properties of selected PFCs at 25°C (Ding & Peijnenburg, 2013; PerkinElmer (Chem ACX database), 2016; Royal Society of Chemistry (Chemspider database), 2015)

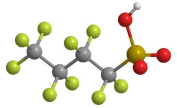
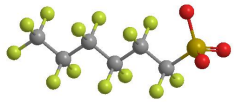
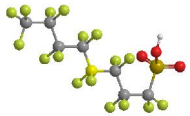
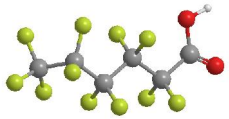
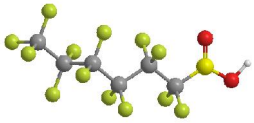
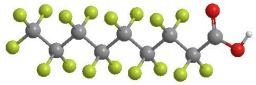
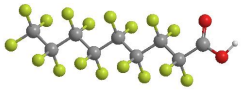
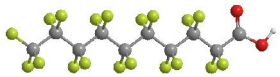
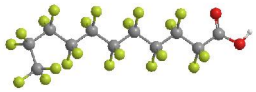
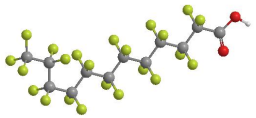
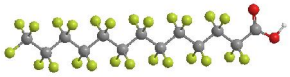
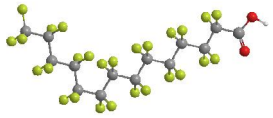
Type	Compound	3D conformation	MW	Empirical formula	Vapour pressure (Pa)	Log Kow	Solubility (mg/L)	Henry's constant	Melting point (°C)	Boiling point (°C)
PFSAs	Perfluorobutanesulfonic acid (PFBS; CL: 4)		300.10	$C_4HF_9O_3S$	5.18×10^{-1}	2.41	107	1.912×10^{-4}	36.86	214
	Perfluorohexanesulfonic acid (PFHxS; CL:6)		422.10	$C_6F_{13}SO_3NA$	3.12	4.34	7.59	4.0×10^{-3}	41.25	221.92
	Perfluorooctanesulfonic acid (PFOS, CL: 8)		500.13	$C_8HF_{17}O_3S$	3.2×10^{-1}	*2.45	2.1×10^{-1}	4.0×10^{-3}	90	145
PFCAs	Perfluorohexanoic acid (PFHxA; CL:6)		314.05	$CF_3(CF_2)_4COOH$	1.14×10^{2}	*0.07	2.95×10^{-1}	9.1×10^{-4}	23	*157
	Perfluoroheptanoic acid (PFHpA; CL: 7)		364.06	$C_7HF_{13}O_2$	2.07 x 10	*1.31	6.61	2.2×10^{-3}	*30	185
	Perfluorooctanoic acid (PFOA; CL: 8)		414.07	$C_8HF_{15}O_2$	4.19	*1.92	$*3.4 \times 10^{3}$	$*1.02 \times 10^{-3}$	*60	*189
	Perfluorononanoic acid (PFNA; CL: 9)		464.08	$C_9HF_{17}O_2$	1.27	*2.57	1.8×10^{-1}	9.3×10^{-3}	*72	*218

Table 2.1 continued: Physical and chemical properties of selected PFCs at 25°C (Ding & Peijnenburg, 2013; PerkinElmer (Chem ACX database), 2016; Royal Society of Chemistry (Chemspider database), 2015)

Type	Compound	3D conformation	MW	Empirical formula	Vapour pressure (Pa)	Log Kow	Solubility (mg/L)	Henry's constant	Melting point (°C)	Boiling point (°C)
PFCAs	Perfluorodecanoic acid (PFDA; CL: 10)		514.08	$CF_3(CF_2)_8COOH$	2.3×10^{-1}	*2.90	2.8×10^{-2}	1.6×10^{-2}	*85	*218
	Perfluoroundecanoic acid (PFUnA; CL: 11)		564.09	$C_{11}HF_{21}O_2$	1.0×10^{-1}	5.76	1.5×10^{-3}	3.0×10^{-2}	*103	194
	Perfluorododecanoic acid (PFDoA; CL: 12)		614.10	$C_{12}HF23O2$	8.6×10^{-2}	6.41	7.59×10^{-5}	8.7×10^{-2}	*108	210
	Perfluorotridecanoic acid (PFTrDA, CL: 13)		664.11	$C_{13}HF_{25}O_2$	1.6×10^{-2}	8.25	2.51×10^{-6}	6.88×10^{-4}	130	235
	Perfluorotetradecanoic acid (PFTA; CL: 14)		714.11	$C_{14}HF_{27}O_2$	1.56×10^{-7}	4.35	8.39×10^{-3}	5.09×10^{-3}	*130	240

* Calculated experimentally; CL: Chain length

2.1.2. Production, sources and use

Although PFCs do not occur naturally, they have been manufactured for more than 60 years for industrial applications. PFCs are synthesised by electrochemical fluorination or telomerisation of tetrafluoroethylene units (Lau, 2012; Posner, 2012). Electrochemical fluorination is where a raw organic material undergoes electrolysis leading to the replacement of all H atoms by F atoms, resulting in a mixture of perfluorinated isomers and homologues of the raw material. Telomerisation is the process of manufacturing PFCs where a perfluoroalkyl iodide is reacted with tetrafluoroethylene, to form longer perfluorinated chains (Buck *et al.*, 2011). Due to the chemical characteristics of PFCs, they are utilized in a variety of industrial and manufacturing applications. As the physical chemical characteristics of PFCs vary according to chain length and functional group, specific PFCs are associated with particular industrial applications (**Table 2.2**). An inventory of discharges, emissions and losses of these hazardous substances is needed to assess the efficacy of the measures adopted for the reduction of PFCs as stated in the Stockholm Convention (Castiglioni *et al.*, 2014).

Although scientific gaps still exist in determining the exact source of PFCs, they enter the environment through point and non-point sources (**Figure 2.3**). PFCs are released into the environment throughout their life cycle: during production, along the supply chains, product use, and disposal. Direct sources refer to PFC emissions from their product life cycle (manufacture, use, disposal), while indirect sources refer to formation of PFCs from degradation of precursors (Buck *et al.*, 2011; Butt *et al.*, 2014). From the PFCs found in environmental compartments, only PFBS, PFOS, PFOA and PFNA and fluortelomers are known to be directly used or produced (Eschauzier, 2013; So *et al.*, 2004). However, other homologues (C4 – C13) are present as impurities, and can contribute significantly to the PFCs found in the environment (Armitage *et al.*, 2009). One of the main contributing sources identified is industrial and municipal waste treatment plants that do not have the adequate technology to remove PFCs. Additionally, PFC precursors can be degraded in waste water treatment plants (WWTP) and lead to release of the resultant PFSAs and PFCAs into the aquatic environment (Ahrens & Bundschuh, 2014; Ahrens *et al.*, 2010; Schultz *et al.*, 2006). Therefore, the majority of these emissions (95%) are released directly into the aquatic environment through industrial and household waste and runoff while emissions through the atmosphere are limited (5%). However, a reliable environmental inventory is lacking, and information is required on the total amount of production and direct/indirect emissions.

Table 2.2: Summary of uses and sources of PFCs (Castiglioni *et al.*, 2014; Chen *et al.*, 2016; Conder *et al.*, 2010; Guo *et al.*, 2009; Luthy, 2006; OECD, 2013; Paul *et al.*, 2009; Simcik & Dorweiler, 2005; Sinclair & Kannan, 2006; Zafeiraki *et al.*, 2014; Zushi & Masunaga, 2009).

Compound	Uses and sources	Industry branch
Perfluorobutanesulfonic acid (PFBS; CL: 4)	• Additives in aviation hydraulic fluids and insulators • Wetting agent used in metal plating • Surfactants used in oil and mining productions • Raw materials in automotive components • Active ingredient in biocides • Utilized as an insulator and flame retardant in electronics • Process chemical as a catalyst • Waste water treatment plants	Aviation and airspace; metal plating; fire-fighting; oil production and mining; electronics; biocides; automotive construction; medical consumables, food processing, polymerization; household products; textiles and leather tanning
Perfluorohexanesulfonic acid (PFHxS; CL:6)	• Firefighting foams • Carpet treatments • Additives in aviation hydraulic fluids and insulators • Wetting agent used in metal plating • Surfactants used in oil and mining productions • Active ingredient in biocides • Used as flame retardants in protective clothing • Aqueous film forming foams • Process chemical as a catalyst	Aviation and airspace; metal plating; fire-fighting; oil production and mining; biocides; household products; textiles and leather tanning
Perfluorooctanesulfonic acid (PFOS, CL: 8)	• Storm water runoff from industrial areas • Additives in aviation hydraulic fluids and insulators • Wetting agent used in metal plating • Surfactants used in oil and mining productions • Atmospheric degradation of precursors • Used as flame retardants in protective clothing • Aqueous film forming foams • Additives in hydraulic fluid • Leachate from landfill sites.	Aviation and airspace; metal plating; fire-fighting; oil production and mining production; household products; textiles and leather tanning

Table 2.2 continued : Summary of uses and sources of PFCs (Castiglioni *et al.*, 2014; Chen *et al.*, 2016; Conder *et al.*, 2010; Guo *et al.*, 2009; Luthy, 2006; OECD, 2013; Paul *et al.*, 2009; Simcik & Dorweiler, 2005; Sinclair & Kannan, 2006; Zafeiraki *et al.*, 2014; Zushi & Masunaga, 2009).

Compound	• **Uses and sources**	**Industry branch**
Perfluorohexanoic acid (PFHxA; CL:6)	• Popcorn packaging • Pre-treated carpeting and carpet care liquids • Home textile and upholstery • Floor waxes and sealant • Waste water treatment plants • Used in raw materials in automotive components • Active ingredient in biocides • Aqueous film forming foams • Degradation of precursors • Fast food wrappers • Process chemical during fluorination,	Automotive construction; medical; food processing; polymerization; biocides; household products; textiles and leather tanning
Perfluoroheptanoic acid (PFHpA; CL: 7)	• Popcorn packaging • Pre-treated carpeting and carpet care liquids • Home textile and upholstery • Floor waxes and sealant • Thread seal tapes and pastes • Non-stick cookware • Dental floss and plaque removers • Storm water runoff • Used in raw materials in automotive components • Active ingredient in biocides • Fast food wrappers • Process chemical during fluorination	Automotive construction; medical consumables; food processing; polymerization; biocides; household products; textiles and leather tanning
Perfluorooctanoic acid (PFOA; CL: 8)	• Process aid in the manufacture of fluoropolymers such as PTFE • Aqueous film forming foams • Pre-treated carpeting and carpet care liquids • Non-stick cookware • Food contact paper • Used in thread sealant tape and pastes • Dental floss and plaque remover	Automotive construction, medical consumables, food processing, polymerisation, household products, textiles and leather tanning

Table 2.2 continued : Summary of uses and sources of PFCs (Castiglioni *et al.*, 2014; Chen *et al.*, 2016; Conder *et al.*, 2010; Guo *et al.*, 2009; Luthy, 2006; OECD, 2013; Paul *et al.*, 2009; Simcik & Dorweiler, 2005; Sinclair & Kannan, 2006; Zafeiraki *et al.*, 2014; Zushi & Masunaga, 2009).

Compound	• **Uses and sources**	**Industry branch**
	• Impregnation sprays • Popcorn packaging • PTFE cookware • Home textile and upholstery • Floor waxes and sealant • Storm water runoff • Atmospheric degradation of precursors • Used in raw materials in automotive components • Cosmetics,	
Perfluorononanoic acid (PFNA; CL: 9)	• Popcorn packaging • Pre-treated carpeting and carpet care liquid • Home textile and upholstery • Floor waxes and sealant, • Thread seal tapes and pastes • Non-stick cookware • Dental floss and plaque removers • Waste water treatment plant sludge • Storm water runoff • Atmospheric degradation of precursors • Used in raw materials in automotive components • Fast food wrappers • Emulsifying agent in production of fluoropolymers	Automotive construction, medical consumables, food processing and polymerization
Perfluorodecanoic acid (PFDA; CL: 10)	• Popcorn packaging • Pre-treated carpeting and carpet care liquids • Home textile and upholstery • Floor waxes and sealant, • Waste water treatment plant sludge • Storm water runoff • Fast food wrappers • Emulsifying agent in production of fluoropolymers,	Household products, textiles, leather tanning and food processing

Table 2.2 continued : Summary of uses and sources of PFCs (Castiglioni *et al.*, 2014; Chen *et al.*, 2016; Conder *et al.*, 2010; Guo *et al.*, 2009; Luthy, 2006; OECD, 2013; Paul *et al.*, 2009; Simcik & Dorweiler, 2005; Sinclair & Kannan, 2006; Zafeiraki *et al.*, 2014; Zushi & Masunaga, 2009).

Compound	• **Uses and sources**	**Industry branch**
Perfluoroundecanoic acid (PFUnA; CL: 11)	• Popcorn packaging • Pre-treated carpeting and carpet care liquids • Home textile and upholstery • Floor waxes and sealant • Thread seal tapes and pastes • Non-stick cookware • Dental floss and plaque removers • Waste treatment plant sludge • Storm water runoff • Atmospheric degradation of precursors • Emulsifying agent in production of fluoropolymers	Food processing, household products, textiles and leather tanning and construction
Perfluorododecanoic acid (PFDoA; CL: 12)	• Popcorn packaging • Pre-treated carpeting and carpet care liquids • Home textile and upholstery • Floor waxes and sealant • Thread seal tapes and pastes • Non-stick cookware • Dental floss and plaque removers • Waste water treatment plant sludge • Atmospheric degradation of precursors • Fast food wrappers • Emulsifying agent in production of fluoropolymers	Food processing, household products, textiles, leather tanning and construction
Perfluorotridecanoic acid (PFTrDA, CL: 13)	• Atmospheric degradation of precursors • Paper of fast food boxes • Waste water treatment plants • Emulsifying agent in production of fluoropolymers	Food processing, household products, textiles and leather tanning
Perfluorotetradecanoic acid (PFTA; CL: 14)	• Atmospheric degradation of precursors	Household products, textiles and leather tanning

The total direct and indirect historic emissions of PFOS and its precursors (perfluorooctylsulfonyl fluoride (POSF) based substances), were estimated to be 6800 – 45 300 tons during 1972 – 2002 and the total emissions of PFCAs were estimated to be 3200 – 7300 tons during 1951 – 2004 (Ahrens & Bundschuh, 2014; Paul *et al.*, 2009). It is hypothesised that the POSF-based substances will decrease due to voluntary phase-out and regulations prohibiting production of these substances. Although some countries are still producing these substances, industrial focus has moved towards the production of short-chain PFCs (Wang *et al.*, 2015).

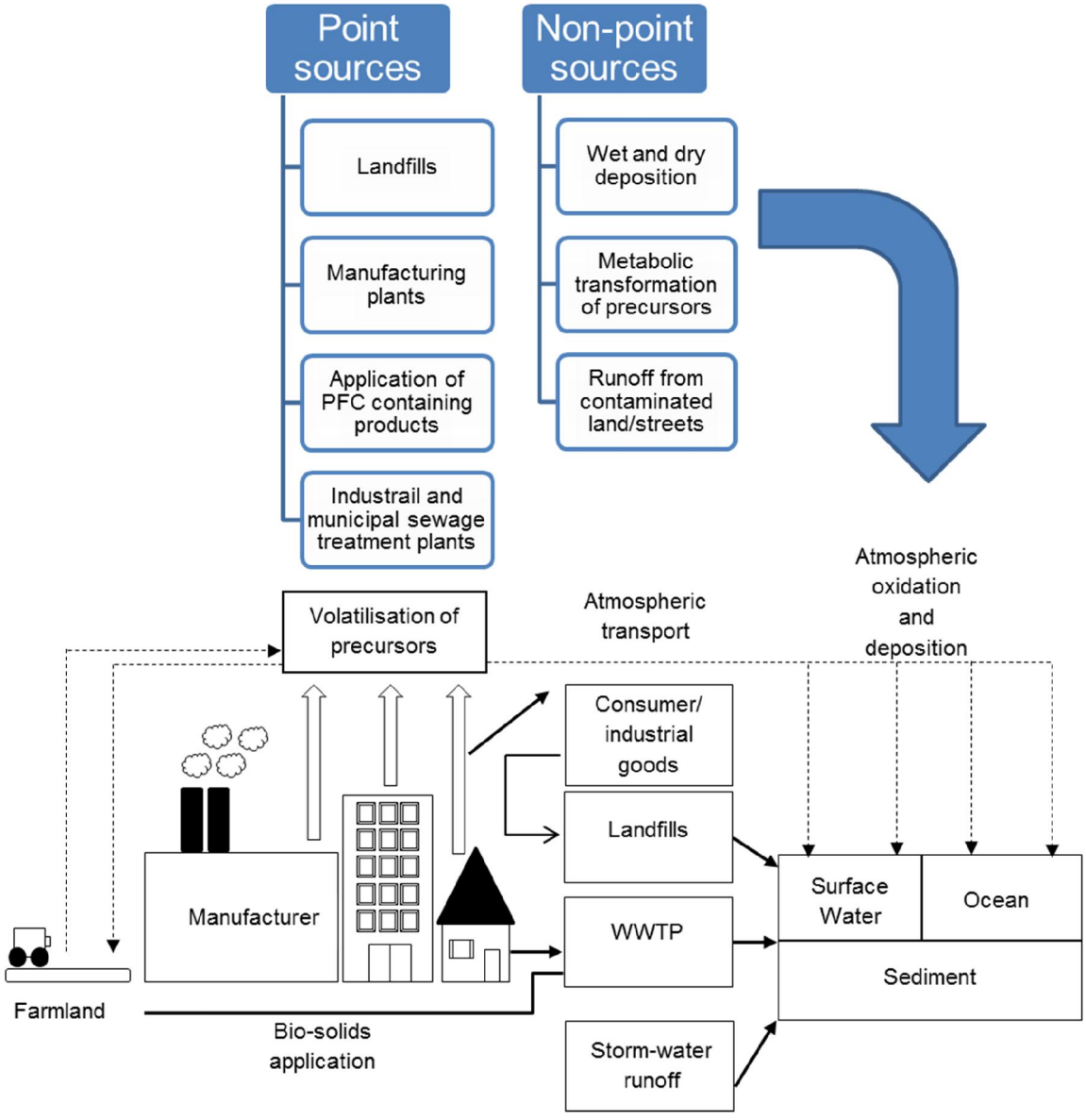


Figure 2.3: Summary of point and non-point sources and the environmental pathway contributing to PFCs present in the environment (adapted from Lee, 2013).

PFC levels in the environment are influenced by precipitation through storm water runoff and atmospheric inputs where PFCs are solubilised in rain and transported. Due to the PFCs potential

for long-range transport, factors such as runoff and precipitation can contribute significantly to quantifiable presence in remote areas (Xiao *et al.*, 2012). The ratios of certain PFC congeners in abiotic matrices can be used to determine the probable source of contamination (Armitage *et al.*, 2009). A ratio of PFHpA/ PFOA greater than one, indicates atmospheric precipitation sources as this generally leads to higher PFHpA concentrations in the environment (Simcik & Dorweiler, 2005). Ratios of PFOS/ PFOA, greater than one, can indicate fluoropolymer sources and WWTP processes in the surrounding environment. Additionally, ratios of PFOA/ PFNA between 7 – 15 are indicative of direct emissions from manufacturing processes, while ratios less than one indicate secondary sources such as degradation of precursors (Armitage *et al.*, 2009; Guo *et al.*, 2015). Precursor degradation can also be indicated through the distribution between even number carbon PFCAs compared to odd number carbon PFCAs. Fluoropolymers are manufactured as molecules with even number carbon chains. However, as fluoropolymers degrade, both even and odd PFCs are formed, with even carbon chained molecules being more prevalent than the odd chains (Guo *et al.*, 2015; Martin, *et al.*, 2004). Predominant levels of C8, C9, C11 and C13 reflect atmospheric and oceanic transport of direct sources, while C10 and C12 reflect indirect sources (Armitage *et al.*, 2009). The variety of sources from which PFCs are released reflects the complex and continuous nature of environmental exposure to complex PFC mixtures. This makes it difficult to definitively determine the sources responsible for PFC contamination.

2.1.3. Environmental fate and transport

The environmental fate of PFCs describes their transport, partitioning, and transformation processes after release into the environment. Release of PFCs to the environment occurs through non-point and point sources as discussed in **Section 2.1.2.** PFCs and their precursors are subject to transformation (**Table 2.3**) and transport into the aquatic and atmospheric compartments (**Figure 2.4**). Precursor compounds represent chemicals that can degrade through reactions such as atmospheric oxidation, biological metabolism (**Table 2.3**), and hydrolysis to form PFSAs and PFCAs.

Table 2.3: Summary of biotransformation of precursors via microbial, rats and mice, and fish (Butt *et al.*, 2014).

Precursor	Microbial	Rats and mice	Fish
PAPs	PFHxA, PFHpA	PFHxA, PFHpA, PFOA, PFNA, PFDA	PFOA
FTOHs	PFOA, PFHxA, PFHpA, PFNA	PFOA, PFNA, PFHxA, PFHpA	PFOA, PFDA, PFHxA, PFNA
FTCAs	PFOA, PFHxA, PFDA	PFOA, PFNA	PFOA, PFNA, PFDA
FTUCAs	PFOA, PFHpA, PFHxA	PFOA, PFHpA, PFNA	PFOA
FTALs		PFOA, PFNA	

Two major transport mechanisms of PFCs are atmospheric and aquatic transport, but the relative contribution of each pathway remains unresolved (Butt *et al.*, 2010). The atmospheric transport of volatile precursor chemicals such as FTOHs are likely the main source of PFCs in remote regions such as the artic (Butt *et al.*, 2010; Ellis *et al.*, 2004; Prevedouros *et al.*, 2006). Studies indicate that PFOA is directly released into the atmosphere from fluoropolymer manufacturing facilities, and can contribute to the global loading of this compound (Barton *et al.*, 2006). The second pathway is through aquatic transport whereby the PFCs are directly released into the water supply by industrial and manufacturing processes or through the lifecycle of products. As PFCs are water soluble and persistent, it is hypothesized that they have a high potential for long-range aquatic transport (Prevedouros *et al.*, 2006).

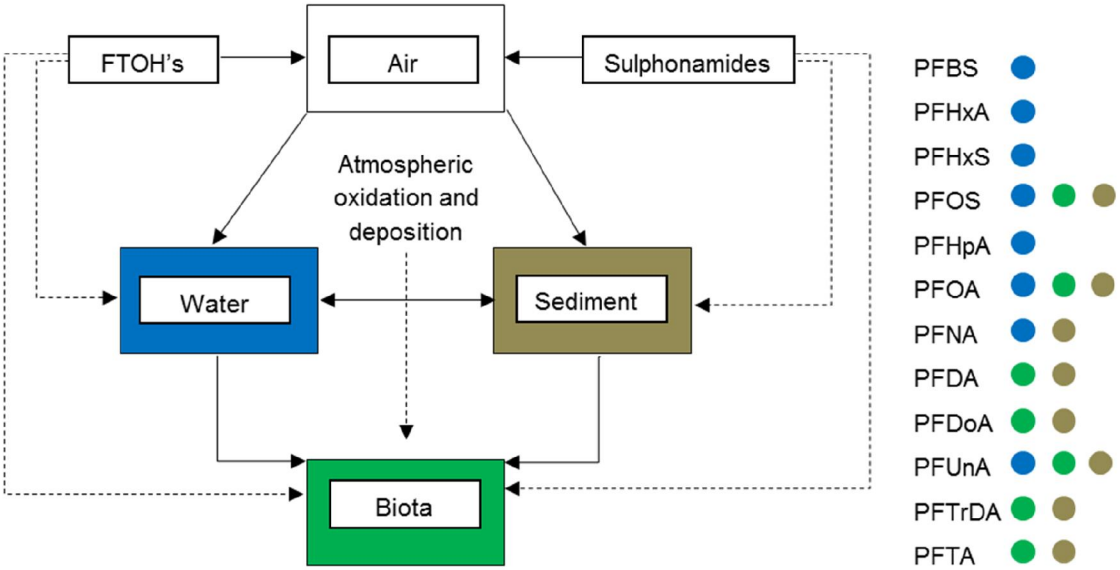


Figure 2.4: Partitioning of PFCs between different compartments. Each colour represent a different matrix and associated PFCs are colour-coded accordingly (adapted from: Bertin *et al.*, 2014; Simcik & Dorweiler, 2005)

It is difficult to compare the contribution of each pathway as the effect is a combination since both contribute to levels of PFCs found globally. Aquatic transport together with the discharges and atmospheric loadings of PFCs to surface waters, as well as the discharge of precursor compounds, all contribute to the levels found in the aquatic system. The atmospheric transport is the combination of discharge of PFCs into the atmosphere, discharge of precursors, transformation to PFCs, and surface-air transport, that, together with aquatic transport, occur multiple times, leading to global distribution (Buck *et al.*, 2011; Butt *et al.*, 2010; Prevedouros *et al.*, 2006; Wania & Mackay, 1996). This emphasizes the importance of understanding the mechanisms and environmental fate of PFCs to evaluate the impact of these compounds on the ecosystem.

The environmental cycling pathways of PFCs are dependent on environmental conditions such as salinity, temperature and physical and chemical properties (**Table 2.1**) of the compounds, which are mainly dependent on the chain length and functional groups (Ahrens & Bundschuh, 2014; OECD, 2013; Prevedouros *et al.*, 2006). Short-chain PFCs are dominantly hydrophilic and generally more mobile in aquatic systems. Long-chain PFCs on the other hand have a higher hydrophobicity and tend to bind to particles. Therefore, long-chain PFCs have the potential to bio-accumulate within the environment. Due to these physiochemical characteristics, short-chain PFSAs and PFCAs tend to leach into the aquatic system, and long-chain PFSAs and PFCAs accumulate in sediments and biota (**Figure 2.4**) (Conder *et al.*, 2010; Martin *et al.*, 2004; Sharpe *et al.*, 2010). Additionally, the functionality of the group impacts the sorption of PFCs as it has an effect on the hydrophobicity of the molecule (Higgins & Luthy, 2006). For example, the slightly larger size of the sulfonate moiety compared to the carboxylate leads to increased hydrophobicity resulting in stronger sorption to sediment (Higgins & Luthy, 2006).

Environmental conditions can also influence the partitioning of PFCs between different environmental compartments. Sorption of PFCs to sediment is influenced by sediment-specific and solution-specific parameters. Organic carbon content is the main sediment parameter influencing sorption, indicating the importance of hydrophobic interaction. However, sorption increases with increasing solution of Ca^{2+} and Mg^{2+} and decreasing pH, likely due to the importance of electrostatic interactions (Higgins & Luthy, 2006; Kostianoy *et al.*, 2012). Additionally, the variability of detection of PFCs from sediment and water may be influenced by meteorological conditions such as seasonal changes in temperature, precipitation, the corresponding changes in flow rates of rivers, and the occurrence of floods (Lasier *et al.*, 2011; Liu *et al.*, 2015a). These factors can cause exponential changes in the concentration of PFOS as reported in literature (Lasier *et al.*, 2011).

The repositories of PFCs within organisms are not lipids as with most classical POPs. Although the hydrophobic part of the chain may interact with lipids, the main mode of accumulation is through proteins. The proteinophilic nature of PFCs increase with chain length, where the short-chain PFCs were 1 – 2 orders of magnitude less proteinophilic than their long-chain counter parts (Conder *et al.*, 2010; Martin *et al.*, 2004). Studies suggest trophic transfer of PFCs between food webs, although field studies have suggested that invertebrates may accumulate PFCs to a higher concentration than fish (Conder *et al.*, 2010; Tomy *et al.*, 2004). Midge larvae (*Chironomus riparius*) exposed to spiked sediment indicated bio-accumulation of long-chain PFCAs (PFUnA, PFDoA, PFTrDA and PFTA) and PFOS, with no accumulation of short-chain PFCs (Bertin *et al.*, 2014). Another study investigated bio-accumulation in aquatic oligochaete *Lumbriculus variegatus* with accumulation of the following pattern: PFOS > PFDA > PFUnA > PFDoA > PFTrDA > PFTA (Lasier *et al.*, 2011). Homeotherms are more likely to be exposed to PFCs through their food consumption in a similar fashion as higher trophic fish. However, bio-magnification in homeotherms is more than a partitioning process between water

and organism, and is influenced by diets, reproductive processes, growth, and volatilization of compounds to the air via gaseous respiratory mechanism, only to name a few (MacKay & Fraser, 2000).

There are varieties of ways in which humans can be exposed to PFCs including ingestion, inhalation, and dermal exposure. Exposure sources other than food include food contact materials, consumer goods, personal care items, and air. However, as with other classical POPs, PFC contaminated food is likely the main intake route, specifically through protein rich foods such as fish and meat (Stahl *et al.*, 2011).

Human exposure to PFCs is divided into three groups': (1) occupational, (2) general and (3) foetal. Studies indicate that people subject to occupational exposure have higher levels of PFCs than the general population (Olsen *et al.*, 2010). General exposure occurs through indoor and outdoor air, aerosols, drinking water, dust and food. Although food consumption is the main exposure route for adults, dust, soil and sand may be a significant source of exposure to infants and toddlers (Shoeib *et al.*, 2011). During foetal and early infant development, there is a transfer of pollutant from the mother to the child through maternal blood (from the placenta) and breastfeeding. Therefore, PFC levels in infants may be elevated. This is of particular concern as children are particularly susceptible to the deleterious health effects posed by pollutants. PFOA and PFOS, which are the most predominant PFCs found in the environment, have been linked to an array of negative health effects including: hepatomegaly, hepatic peroxisome proliferation, liver, testicular and pancreatic tumours, reproductive and developmental deficiencies reduced foetal mass, skeletal and cardiac malformations, neurotoxicity, and immunotoxicity (Fàbrega *et al.*, 2014; Pereiro *et al.*, 2014; Stahl *et al.*, 2014).

2.1.4. Toxicity

As PFCs do not partition into fatty tissues, but bind to proteins (liver, blood and kidney), there is increased concern regarding their toxicological implications (Jones *et al.*, 2003; Longnecker *et al.*, 2008). As previously discussed, the bio-accumulation potential of PFCs is dependent on chain length and functional groups. Bio-accumulation varies between organisms and is impacted by species specific considerations (metabolism, feeding behaviour and trophic position), gender and reproductive status (Ahrens & Bundschuh, 2014).

Various studies have shown that PFCs can bio-magnify into higher trophic levels. In biota, PFOS is generally the predominant PFC, and concentrations increase with an organism's trophic level, through the food chain, indicating a high bio-accumulation potential. In contrast, PFOA has a low bio-accumulation potential, and has similar concentrations among species from different trophic levels (Ng & Hungerbuhler, 2014). PFOS concentration, on average, are threefold higher in biota than PFOA, the lower bio-accumulation potential may be attributed to differences in physiochemical properties of the functional groups (Martin *et al.*, 2003a). Although the phase-out

of POSF has corresponded with a decreasing concentration of PFOS in biota, the concentrations of other PFCs, especially long-chain PFCAs show no clear trend. There has even been increases in certain congeners depending on trophic level and location (Gebbink *et al.*, 2011; Haukås *et al.*, 2007; Johansson *et al.*, 2014; Paul *et al.*, 2009). This indicates the possibility of a continued long-term exposure to PFCs within the environment, raising concern on the long-term toxicological implications to wildlife and humans.

The toxicokinetics of PFCs in mammals has been extensively studied and three important parameters for PFC uptake and deposition have been identified (Ng & Hungerbühler, 2013):

1. PFCs are strongly bound to albumin in plasma, making blood an important accumulation medium;
2. PFCs transport into cells is likely controlled by a combination of passive diffusion and facilitation by transporter proteins such as organic anion transporter (OAT) proteins, which are important in facilitating re-absorption of organic anions to blood;
3. PFCs bind to cytosolic fatty acid binding proteins (FABPs), which are present in a number of cell types.

Albumin, OATs and FABPs all participate in fatty acid metabolism and in turn influencing receptors present in the process (Ng & Hungerbühler, 2013). Currently, it is proposed that the peroxisome proliferator-activated receptors (PPAR) play a large role in the toxicity of PFCs (Corsini *et al.*, 2014). The PPAR belong to the nuclear hormone receptor superfamily that contains three sub-types: PPAR α, β/ δ and γ. These receptors regulate physiological processes that impacts lipid homeostasis, reproduction, inflammation, wound healing, and carcinogenesis. There are important species differences in receptor specificity, activity and related ligand binding/activation, which all contribute to the resulting sensitivity towards PFC toxicity (Chinetti *et al.*, 2000; Corsini *et al.*, 2014; Vanden Heuvel, 2006).

The antagonism of PPARα has been linked to tumour formation in the liver, altered expressions of genes involved in peroxisome proliferation, and cell cycle control leading to apoptosis (Lau *et al.*, 2007). Studies have been conducted to evaluate if PPARα agonistic mode of action is involved in the hepatic toxicity and hepatocellular adenomas observed in PFC exposed rat bioassays (Klaunig *et al.*, 2003). Results from these studies indicate PFOA and PFOS are capable of inducing peroxisome proliferation in mouse, rat, and humans. However, it is also possible that they may induce peroxisome proliferation, further affecting lipid metabolism and transport (Lau *et al.*, 2007; Luebker *et al.*, 2002).

The effect of short and long-chain PFCs to induce peroxisome proliferation via PPARα has also been explored, which in turn could induce altered expression and damage to liver tissue. Rats treated with PFBS, PFHxS or PFOS all had significantly increased acyl-co-enzyme A (CoA) oxidase (ACOX) activity. Acyl-CoA is a group of coenzymes involved in the metabolism of fatty acids, where CoA forms a complex by attaching to the end of a long-chain fatty acid and undergoes β-oxidation. ACOX is transcriptionally regulated by PPARα. All three PFCs increased

the ACOX activities; however, the PFBS dose required was 50 times higher than that of PFOS, possibly indicating faster elimination due to shorter chain length (Lau *et al.*, 2007). An additional study evaluated PFHxA, PFOA, PFNA, and PFDA in male and female rats, indicating all compounds except PFHxA increased β-oxidation in males. However, for females, only PFNA and PFDA indicate an increased β-oxidation, possibly demonstrating differences in bio-accumulation between sexes (Kudo *et al.*, 2000; Lau *et al.*, 2007).

2.2. Bio-indicators and bio-monitors

Previous studies conducted on the presence of organic pollutants within the South African environment identified PFCs in wild bird eggs **(Chapter 1, Section 1.4)**. As mentioned above, the concentrations of PFCs are highly variable and can be affected through various parameters and exposure routes. Because living organisms bio-accumulate organic pollutants, they are often used as indicators of pollution within an ecosystem, as biotic matrix concentrations are less variable and susceptible to change than concentrations in abiotic matrices. To evaluate the occurrence and levels of these pollutants, environmental monitoring is required to assess and classify the environmental quality of ecosystems (Van der Oost *et al.*, 2003). In this study, water, sediment, wild bird eggs, and fish were selected as bio-indicators and bio-monitors.

2.2.1. Water

Without water, life could not exist. However, it is not only the amount of water available, but also its quality (Chaplin, 2001) that is crucial in maintaining a healthy ecosystem. Therefore, it is essential to evaluate the presence and concentrations of pollutants in aquatic ecosystems. Additionally, humans consume water and the pollutants present can negatively impact human health. Atmospheric PFCs can undergo wet and dry deposition to various aquatic environments, but the majority of contamination originates from wastewater discharges to rivers. Source of PFC contaminated discharge include fluorochemical manufacturing facilities, discharge of firefighting foams, surface run-off water, landfill leachates and degradation of precursor compounds present in consumer goods (Ahrens, 2011; Möller *et al.*, 2010). Along with this global transportation, the presence of PFCs in water provides the chemicals a pathway into drinking water and consequently the food web (Ahrens, 2011).

The physiochemical properties of PFCs suggest that water is the main environmental compartment to which these compounds partition (Conder *et al.*, 2010). However, studies indicate that concentrations of PFCs in water can be orders of magnitude lower than in biological samples (Benskin *et al.*, 2010). When high concentrations of PFCs are found in water the main source is generally related to industrial or municipal waste streams in industrialised or highly urbanised areas (Taniyasu *et al.*, 2003). The predominant PFCs found in water are PFOS and PFOA.

However, due to the restrictions or elimination of production, short-chain PFCs are increasingly being used as a substitute resulting in increasing environmental levels of short-chain PFCs (Möller *et al.*, 2010). PFCs have been identified in a wide array of aquatic environments from various regions around the world (**Table 2.4**). This suggests that contamination of waterways and oceans occurs even in regions where manufacturing is not present. No data is currently available on the presence of PFCs in South African waters.

Table 2.4: Global summary of comparable values of PFCs in water (ng/L), reported in scientific literature

Water source	Area	PFOS	PFHxA	PFBS	PFHpA	PFDA	PFUnA	PFDoA	PFOA	PFNA	PFHxS	Reference
Channel	Spain	0.05		0.05					0.08	0.07		(Gómez *et al.*, 2011)
Channel	Spain	0.05		0.05					0.08	0.07		
River	France	17	11	4	5	1	0.1	0.1	9	1	14	(Labadie & Chevreuil, 2011)
River	France	17	11	4	5	1	0.1	0.1	9	1	14	
River	Germany	4	3	45	0.5				3.57		2	(Möller *et al.*, 2010)
River	China	11		16	2	2	0.4		23	12	1	(Pan *et al.*, 2011)
Lake	United states of America (USA)	30							34		3	(Sinclair *et al.*, 2006)
Lake	China	26										(Taniyasu *et al.*, 2003)
River	China	262									NA	(Wang *et al.*, 2013)
River	China	7			23				27.9		95	(Yang *et al.*, 2011)
Lake	China	394			18			3	36.7		7	(Yang *et al.*, 2011)

2.2.2. Sediment

PFCs can be accumulated in sediment and soil through multiple routes, including: atmospheric deposition, surface runoff, the use of pesticides and insecticides containing PFCs, and the application of waste sludge for fertilisation of agricultural land. Studies suggest that short-chain PFCs tend to accumulate in the water compartments, whereas long-chain PFCs are distributed in surface sediments (Ahrens *et al.*, 2010). PFCs have been detected in sediment from various locations around the globe (**Table 2.5**).

Table 2.5: Global summary of some comparable concentrations (ng/g dm) of PFCs in sediment reported in scientific literature

Area	PFOS	PFOA	PFBS	PFHxS	PFHpA	PFHxA	PFNA	PFDA	PFUnA	PFDoA	PFTA	Reference
France	0.13	0.02										(Gómez *et al.*, 2011)
China	0.13	0.02										
China	3.76	0.63		0.07			0.24	0.34	0.34	0.58	0.44	(Higgins *et al.*, 2005)
China	3.76	0.63		0.07			0.24	0.34	0.34	0.58	0.44	
China	4.3			0.1	0.03	0.06	0.05	0.30	0.29	1.7		(Labadie & Chevreuil, 2011)
China	0.79	0.94	0.08	1.34	1.68		4.82	0.38	0.48	0.28		(Pan *et al.*, 2011)
USA	457											(Wang *et al.*, 2013)
USA	0.48	0.18	0.13	0.10	0.13		0.07	0.50	0.03	0.04		(Yang *et al.*, 2011)
France	0.31	0.52	0.27	0.03	0.33		0.51	0.29	0.18	0.07		

A variety of factors play an intrinsic role on the extent to which PFCs can undergo absorption to and desorption from sediments and soils (type of sediment/ soil, pH, organic content and moisture) that in turn influences the movement and transportation of PFCs within the environment (Du *et al.*, 2014; Pan *et al.*, 2009; You *et al.*, 2010). In the case of PFOS, there is a positive correlation between the concentration of PFOS and conductivity as well as chemical oxygen demand (Pan *et al.*, 2014). Studies suggest that PFOS can undergo aquatic transport over long distances before absorption occurs in estuaries due to changes in salinity (Pan & You, 2010). PFCs appear to reside mainly in upper sediment layers and in top soil, primarily because of the high organic carbon and protein content in the top layers (Fromme *et al.*, 2009).

2.2.3. Fish

Fish are present in almost all aquatic environments and play a fundamental role in sustaining aquatic food webs through energy transfer from lower to higher trophic levels. Despite limitations such as their mobility, fish are considered a feasible organism for pollution monitoring (Austin, 1998; Van der Oost *et al.*, 2003). Pollutants may accumulate in fish though, direct uptake from the water by the gills, ingestion of suspended particles, or via consumption of contaminated food (Van der Oost *et al.*, 2003). Limited information is available on the accumulation of PFCs in fresh water fish as most studies have focused on marine organisms. As in water and sediment, PFOS and PFOA are the PFCs most frequently detected in fish tissue (**Table 2.6;** Fernández-Sanjuan *et al.*, 2010).

Table 2.6: Summary of comparable concentrations of PFCs in fish (ng/g wm) species from other parts of the world reported in scientific literature

Fish Species	Scientific name	Area	PFOS	PFOA	PFHxS	PFHpA	PFNA	PFDA	PFUnA	PFDoA	PFTA	PFHpA	PFTrDA	References
Sharptooth catfish	*Clarias gariepinus*	South Africa	1.7											(Bouwman & Pieters, 2013)
Common Carp	*Cyprinus carpio*	USA	124											(Kannan *et al.*, 2005)
European chub	*Leuciscus cephalus*	France	45		0.1	0.1		3.2	2.4	10		0.1		(Labadie & Chevreuil, 2011)
Bluegill	*Lepomismacrochirus*	USA	180											(Malinsky *et al.*, 2011)
Black Crappie	*Pomoxis nigromaculatus*	USA	55											
Smallmouth bass	*Micropterus dolomieu*	USA	165											
Walleye	*Sander vitreus*	USA	50.8											
Sauger	*Sander canadensis*	USA	41.7											
Northen pike	*Esox lucius*	USA	50.5											
Barbel	*Barbus barbus*	France	100.7				7	5.6	211	16				(Miège *et al.*, 2012)
Commen beam	*Abramis brama*	France	121.1	0.2			15	5	48	8				
White beam	*Blicca bjoerkna*	France	73	0.1			26	3.5	92	8				
Chub	*Squalius cephalus*	France	44.3				6.1	4.8	166	10				
Silver carp	*Hypophthalmichthys molitrix*	China	3.21	0.16			0.3	1.6	0.9	0.7	0.7		0.2	(Pan *et al.*, 2011)
Crucian carp	*Carassius auratus*	China	9.04	0.19	0.07	0.16	0.78	4.07	1.39	0.83	0.83	0.16	0.3	
Common carp	*Cyprinus carpio*	China	4.98				0.1	0.8	0.4	0.1	0.1		0.1	
Small mouth bass,	*Micropterus dolomieu*	USA	29	4										(Sinclair *et al.*, 2006)
Largemouth bass	*Micropterus salmoides*	USA	71	6										
European whitefis	*Coregonus lavaretus*	Italy	19.1											(Squadrone *et al.*, 2014)
European perch	*Perca fluviatili*	Italy	22.4											

To date, there is still uncertainty on PFCs bio-accumulation, bio-magnification, and bio-concentration within fish. Literature indicates that dietary exposure to PFC does not lead to bio-magnification in trout (Martin *et al.*, 2003a), whereas waterborne PFOS was bio-concentrated in bluegill fish leading to increased mortality (Giesy *et al.*, 2010). Uptake and disposition of PFCs in fish probably follow a similar mechanism as in mammals, with albumin, OATs and FABPs as they main carrier proteins. Many of these proteins or structurally similar proteins have been identified in fish (Manera & Britti, 2006; Popovic *et al.*, 2010). Albumin-like proteins are found in salmonids including rainbow trout (Manera & Britti, 2006). In carp, high density lipoproteins perform may of the same transport functions (De Smet *et al.*, 1998). Accumulation of PFCs in fish mostly occur in the plasma, liver, and kidney, whereas adipose and muscle have lower levels (Ng & Hungerbühler, 2013). PFCs accumulate into fish in a time- and concentration-dependant matter, with the main route of exposure through water and diet (Peng *et al.*, 2010). Studies conducted globally (**Table 2.6**) found measurable levels of PFCs in fish emphasising the importance of continued monitoring and evaluation.

2.2.4. Birds

Due to increased agriculture, urbanisation, and industrialisation, birds are in contact to an environment impacted by pollution from a variety of sources such as agricultural, industrial, and commercial products. This causes exposure to pollutants through ingestion, breathing and food/ water consumption which can affect their behaviour, body condition and reproductive success (Greenwood, 2004). Because birds are diverse with a wide range of feeding habits, long-living (pollutants burdens increase) and have a complex physiology, makes them favourable bio-indicators for an extensive range of environments and pollutant classes (Furness, 1993). Birds reacts to change in the environment, and the presence or absence of birds may indicate ecological conditions of habitats and form an important link between the food web and pollutant distribution (Zakaria & Rajpar, 2010). Birds have been used for more than 60 years as qualitative indicators and quantitative monitors of pesticides in food webs (Furness, 1993). This research has identified top predators as ideal organisms for monitoring pollutants as information regarding bio-accumulation and bio-magnification through trophic levels can be obtained (Furness, 1993; Zakaria & Rajpar, 2010).

Water birds are suitable for monitoring aquatic pollution as they are highly mobile, widely distributed, and occupy high trophic levels (Kocagöz *et al.*, 2014; Zamani *et al.*, 2015). Bird's eggs have been used in many studies as a monitoring tool with little adverse effects to the bird population to evaluate contaminant levels in the environment (Kocagöz *et al.*, 2014). The removal of a single egg from a clutch does not affect abundant species negatively, and can provide information for multiple contaminants within a species and associated food and water. The concentration levels found can be indicative of uptake of pollutants from foraging near the colony

several days before egg-laying, or accumulated body burdens of the female over time, depending on the species (species-specific differences in metabolic efficiencies as well as physiological differences in the egg formation process) and contaminants (Zamani *et al.*, 2015). Sampling of bird feathers is also being reviewed as possible monitoring tool. Due to the non-invasive nature of collecting feathers, there are virtually no effects on the live bird, especially if body feathers are removed or collected from the nests/preening area. Feathers can be stored without freezing, museums have large collections (could assist with historical level of pollutants) and feathers have been successfully used as a monitoring unit for inorganic pollutants such as mercury. Therefore, feathers can be investigated as possible monitoring matrix can be investigated for pollutant accumulation (Furness, 1993).

Limited information on the toxicity of PFCs in birds is available. Most studies have focused on single compounds such as PFOS and PFOA rather than combined effects. Studies performed to evaluate acute and chronic exposure to PFOS in Mallards and Bobwhite quails found results confirming decreased body weight and increased liver mass, with PFOS levels of 111 and 119 ug/g associated with mortality (Newsted *et al.*, 2006). Reproductive toxicity, however, was observed at relatively low concentrations (100 ng/g PFOS) compared to the acute and chronic studies. Reduced hatching success of domestic chicken eggs injected with PFOS/PFOA were found with pathological changes in the liver (bile duct hyperplasia, peri-portal inflammation and necrosis) with similar levels found in wild bird eggs (Molina *et al.*, 2006; Yanai *et al.*, 2008).

It is hypothesised that chicken embryos are considerably more sensitive to PFOS exposure than species exposed naturally to the contaminant (Molina *et al.*, 2006). In contrast, other studies indicate developmental toxicity for leghorn chickens at 1000 ng/g, and hatchability decrease lowest-observed-effect concentration (LOEC) of 5000 ng/g (Peden-Adams *et al.*, 2009; Yanai *et al.*, 2008). However, PFOS concentrations as low as 150 ng/g indicated decreased hatching success for tree swallows (Custer *et al.*, 2012). The variation in proposed LOECs are concerning, as varying concentrations are found in the environment (**Table 2.7**). It is also suggested that PFOS and PFOA are developmental neuro-toxicants that affect post-hatch cognitive performances, causing impaired imprinting behaviour in the hatchlings (Pinkas *et al.*, 2010).

Body-compartment specific patterns have been investigated in literature to evaluate if eggs are adequate in representing PFC concentrations in the adult bird population. Liver, plasma, red blood cells (RBCs), whole brain, muscle, adipose tissue, and eggs of female herring gulls were collected for the study. The mean concentration of PFSAs in tissue were the highest in adipose tissue, followed by liver > plasma > muscle > RBCs > brain. For PFCAs the highest mean concentration was in the brain, followed by plasma > liver > RBCs > adipose > muscle. Long-chain PFCs are the dominant compounds, but detected infrequently in muscle and adipose tissue. In limited tissue distribution studies of birds, the liver was the compartment with the highest PFSA and PFCA concentration. For the eggs, the albumin and the yolk was separated for analysis,

PFCs were below detection limit in the albumin. Concentrations of ΣPFSA and ΣPFCAs in the yolks were higher (3-fold and 17-fold respectively) than levels in the liver. Although both albumin and the yolk contain proteins, absolute accumulation in the yolk might be due to a function of carrier proteins transferred from the ovum into the yolk. The PFCA pattern between the liver and yolk was different, showing preferential accumulation of the long-chain PFCAs in the yolk. It is possible that transfer proteins (vitellogenin and very low density lipoproteins; VLDLs) which are produced in the liver and transported from the liver to the ovaries via the blood will play a role in transferring the PFCs to the yolk (Newsted *et al.*, 2007).

Although there is a significant increase in PFC levels measured in the egg compared to the adult bird, the PFC pattern in the combined tissue compartments compared to the pattern in the yolk is similar. Therefore, birds eggs are adequate representatives of PFC concentrations in birds (Gebbink & Letcher, 2012). However, as with most classical POPs, maternal transfer from the female to the eggs is present. This results in a decrease in the total body burden of the female birds and could explain the discrepancy between levels found in eggs and adult females. Therefore, in ova transfer is a substantial elimination route for PFCs (Gebbink & Letcher, 2012; Holmström & Berger, 2008; Verreault *et al.*, 2006).

PFOS is the dominant PFSA found in bird eggs. However, long-chain PFCAs, PFUnA and PFTrDA with odd number of carbons have been reported with increasing regularity in since 2008 (Gebbink & Letcher, 2012; Gebbink *et al.*, 2009; Wang *et al.*, 2008a). There is no clear trend to establish the reason for the increase in these specific long-chain PFCAs. It is hypothesised that loadings of PFCs are influenced by urban and industrial activities as well as the diets and feeding ecology, PFC specific *in ovo* enrichment and accumulation of precursors and biodegradation thereof in the bird species analysed (Letcher *et al.*, 2015). However, diet is a major factor influencing the exposure of PFCs, and studies suggest that aquatic prey may the main source of accumulation (Gebbink *et al.*, 2009; Gebbink *et al* 2011).

The present study explored levels of PFCs within the Orange River basin, the largest freshwater catchment in South Africa. It examined the concentration, congener patterns, and possible sources of PFCs in water, sediment, fish, and wild bird eggs. Additionally, mine tailings and bird feathers were assessed for the presence of PFCs as possible source indicators. The following section will discuss the species selection, collection, processing, extraction, and analysis of the selected samples.

Table 2.7: Global summary of PFC concentrations (ng/g wm) in bird eggs, reported in scientific literature

Country	Area	Bird species	Scientific name	Feeding guild	Feeding habitat	N	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnA	PFTrDA	PFDoA	PFTA	Reference
Norway	Hornøya	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	0.22	0.13	0.16	21	NA	1.0	0.77	0.23	NA	(Verreault *et al.*, 2007)
Norway	Røst	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	0.31	0.17	0.23	22	NA	1.3	1.1	0.26	NA	
Norway	Hornøya	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	0.31	<0.09	0.30	40	NA	3.4	2.3	0.64	NA	
Norway	Røst	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	0.80	0.64	0.94	42	NA	1.6	2.8	0.58	NA	
Norway	Hornøya	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	1.07	0.15	1.05	37	NA	4.2	2.5	0.71	NA	
Norway	Røst	Herring gull	*Larus argentatus*	Generalist	Aquatic	5	<0.009	<0.22	<0.17	0.97	0.65	1.11	42	NA	2.6	1.9	0.78	NA	
Baltic sea	Heuwiese	Cormorant *spp*	*Not specified*	Piscivore	Aquatic	13	NA	NA	NA	0.7	1.1	1.4	90	1.5	1.4	NA	0.5	NA	(Rüdel *et al.*, 2011)
Germany	Haseldorf	Cormorant *spp*	*Not specified*	Piscivore	Aquatic	13	NA	NA	NA	4.9	1.4	3.8	540	17.4	8.7	NA	2.6	NA	
Germany	Saarlouis	Rook	*Corvus frugilegus*	Scavenger	Terrestrial	14	NA	1	1	0.3	0.5	3	6.2	0.8	1.5	NA	0.5	NA	
China	Quanzhhou	Little egret	*Egretta garzetta*	Generalist	Terrestrial / Aquatic	2	<0.01	<0.01	<0.01	0.23	1.19	3.1	46	7.0	27	NA	4.03	NA	(Wang *et al.*, 2008b)
China	Quanzhhou	Night heron	*Nycticorax nycticorax*	Generalist	Aquatic	2	<0.01	<0.01	<0.01	0.29	0.05	0.60	143	6.4	6.9	NA	2.49	NA	
China	Xiamen	Little egret	*Egretta garzetta*	Generalist	Terrestrial / Aquatic	2	<0.01	<0.01	<0.01	<0.01	0.77	1.33	70	3.8	5.8	NA	1.03	NA	
China	Xiamen	Night heron	*Nycticorax nycticorax*	Generalist	Aquatic	3	<0.01	<0.01	<0.01	0.31	0.50	1.37	123	6.8	11.1	NA	1.37	NA	
China	Hong Kong	Night heron	*Nycticorax nycticorax*	Generalist	Aquatic	9	<0.01	<0.01	<0.01	0.08	0.03	0.45	115	6.8	13.4	NA	13	NA	
China	Hong Kong	Great white egret	*Ardea alba*	Generalist	Aquatic	10	<0.01	<0.01	<0.01	0.07	0.07	0.31	20	0.94	2.91	NA	2.41	NA	
Canada	Granite Islands	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	0.98	9	NR	7.7	13	10	3.9	1.1	(Gebbink *et al.*, 2009)
Canada	Agawa Rocks	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	0.51	9.6	NR	8.6	20	23	6.5	2.9	
Canada	Big Sister Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	1.2	3.1	NR	12	14	15	12	5.4	
Canada	Gull Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	2.1	NR	4.1	7.9	10	3.2	1.5	

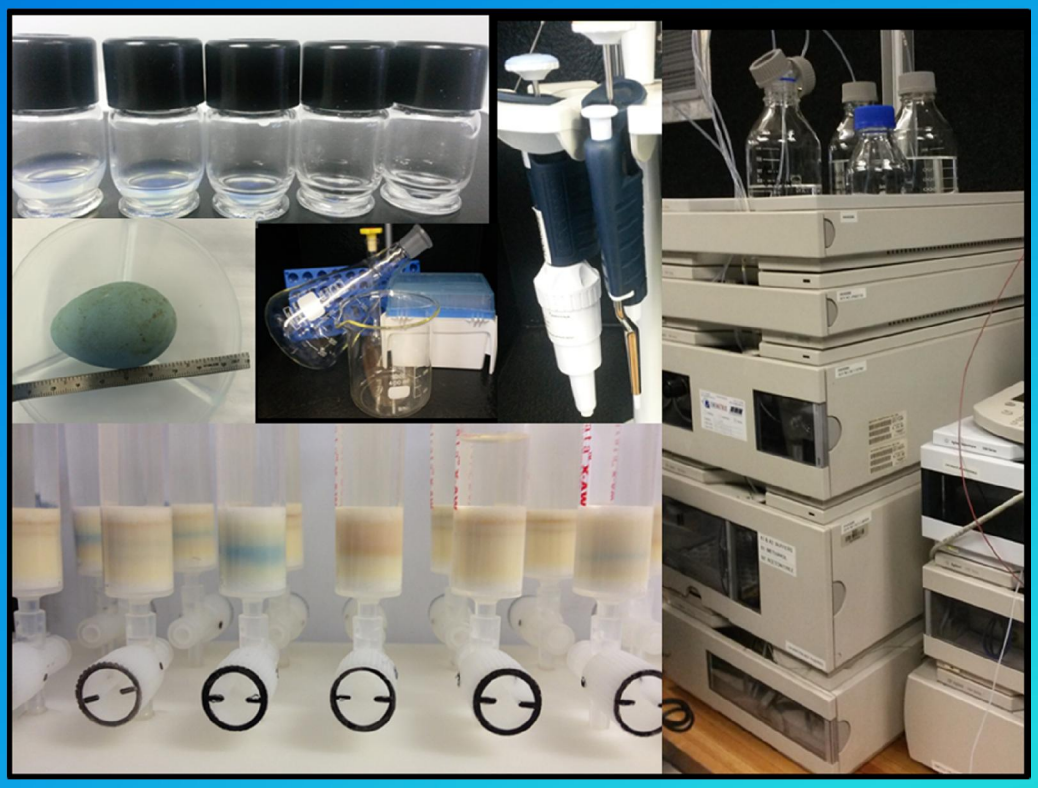
Table 2.7 continued: Global summary of PFC concentrations (ng/g wm) in bird eggs, reported in scientific literature

Country	Area	Bird species	Scientific name	Feeding guild	Feeding habitat	N	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnA	PFTrDA	PFDoA	PFTA	Reference
Canada	Channel-Shelter Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	0.71	NR	6.5	13	12	4.7	2.8	(Gebbink *et al.*, 2009)
Canada	Double Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	2.6	16	NR	20	28	29	12	4.6	
Canada	Chantry Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	0.45	2.1	NR	4	6.1	13	3.5	2.6	
Canada	Fighting Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	0.64	NR	7.8	12	17	11	7.4	
Canada	Middle Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	1.6	NR	22	25	20	16	6.5	
Canada	Port Colborne	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	1.3	NR	10	15	13	6.1	3.6	
Canada	Niagara River	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	2.6	NR	13	18	19	7.5	3.7	
Canada	Hamilton Harbour	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	1.1	NR	7.6	11	15	12	7.2	
Canada	Toronto Harbour	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	0.4	4.4	NR	11	16	16	13	7.9	
Canada	Snake Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	1.4	NR	14	24	19	11	3.9	
Canada	Strachan Island	Herring gull	*Larus argentatus*	Generalist	Aquatic	13	NA	NA	NA	NR	<0.10	2.1	NR	12	22	17	8.9	2.9	
Iceland	Vestmann-seyjar Iceland	Common murre	*Uria aalge*	Piscivore	Aquatic	10	NA	NA	NA	NA	<5.8	<3.9	16	38	30	NA	28	NA	(Löfstrand *et al.*, 2008)
Faroe Island	Sandøy	Common murre	*Uria aalge*	Piscivore	Aquatic	10	NA	NA	NA	NA	<5.8	<3.9	15	<8.2	57	NA	19	NA	
Norway	Sklinna	Common murre	*Uria aalge*	Piscivore	Aquatic	10	NA	NA	NA	NA	<5.8	<3.9	85	42	26	NA	4.6	NA	
Norway	Hjelmsøya	Common murre	*Uria aalge*	Piscivore	Aquatic	10	NA	NA	NA	NA	<5.8	<3.9	85	<8.2	18	NA	2.7	NA	
Sweden	Stora Karlsö	Common Murre	*Uria aalge*	Piscivore	Aquatic	10	NA	NA	NA	NA	<5.8	48	400	<8.2	82	NA	18	NA	
Australia	Mount Annan	Australian white ibis	*Threskiornis molucca*	Scavenger	Terrestrial / Aquatic	10	<0.3	<0.4	<0.4	2.4	0.4	0.5	53	1.8	0.65	0.29	3.1	0.22	(Thompson *et al.*, 2011)
Australia	Homebush	Australian white ibis	*Threskiornis molucca*	Scavenger	Terrestrial / Aquatic	6	<0.3	<0.4	<0.4	1.1	<0.8	<0.9	30	<1.1	0.24	0.15	0.53	<0.6	

Table 2.7 continued: Global summary of PFC concentrations (ng/g wm) in bird eggs, reported in scientific literature

Country	Area	Bird species	Scientific name	Feeding guild	Feeding habitat	N	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFUnA	PFTrDA	PFDoA	PFTA	Reference
Australia	Sydney Harbour	Silver gull	*Larus novaehollandiae*	Generalist	Aquatic	8	<0.3	<0.4	<0.4	3.2	1.1	0.88	39	2.5	2.2	2.4	6.6	1.2	(Thompson *et al.*, 2011)
USA	San Francisco Bay - South Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA	10.4	7.91	13.4	1253	22	8.91	NA	12.8	NA	(Sedlak & Greig, 2012)
USA	San Francisco Bay -Central Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA	2.84		5.19	280	7.97	4.84	NA	5.9	NA	
USA	San Francisco Bay -North Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA		2.7	7.04	104	8.4	7.15	NA	6.1	NA	
USA	San Francisco Bay - South Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA	25	24	31	1244	22	7.8	NA	7.6	NA	
USA	San Francisco Bay -Central Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA	4.4	5.9	3.8	123	8.9	7.2	NA	9.6	NA	
USA	San Francisco Bay -North Bay	Double-crested cormorant	*Phalacrocorax auritus*	Piscivore	Aquatic	3	NA	NA	NA			5.9	84	9.5	4	NA	4.1	NA	
Sweden	South-West Sweden	Swedish peregrin falcon	*Falco peregrinus*	Carnivore - other birds	Terrestrial / Aquatic	10	NA	NA	NA	NA	NA	1.6	83	3.1	4.2	7.3	3.2	2.7	(Holmström *et al.*, 2010)

Chapter 3:
Materials and methods



"You cannot achieve an aim unless you have a method"
W. Edwards Deming

During this study, water, sediment, mine tailings, freshwater fish and wild bird eggs were collected in the Orange River basin. The study was approved by the North-West University Ethics Committee (NWU-00044-07-S3) with the required permits obtained from the Gauteng, Free State, Northern Cape provinces and the Lesotho Department of Environmental and Nature Conservation (FAUNA 1035/2015). All of the species collected were listed as "Least Concern" by the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2015).

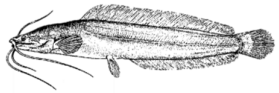
3.1. Species sampled

A bio-indicator is a group or an individual organism that is easily monitored and used to describe the quality of an ecosystem they are found in (Siddig *et al*., 2016). Fish serve as a well-suited bio-indicator for assessing water quality including chemical pollution (Jia & Chen, 2013; Siddig *et al*., 2016). The fish species selected in this study form part of the Order Siluformes, one of the world's largest groups of fish, consisting of 31 families, 400 genera and over 2200 described species (Skelton, 2001). The majority are freshwater species. Siluformes are most abundant in South America, but also occur in North America, Africa, and Asia. The majority have three or four pairs of barbels around the mouth, and do not have scales, although many species from South America have bony plates along their body. Several species have a sharp spine in the dorsal and pectoral fins, some also have a large adipose fin behind the rayed dorsal fin (Hecht *et al*., 1988; Skelton, 2001). Catfish generally prefer slow-flowing reaches of rivers and lakes, and vary in size from small parasitic species, to giant species that weigh in excess of 30 kg. They are very hardy and adaptable, and can survive out of water for considerable time if they remain moist (Hecht *et al*., 1988). This makes catfish suitable for intensive aquaculture without aeration or high water exchange rates, making them a valuable food source. One species, the barbel or sharptooth catfish (*Clarias gariepinus*) is a valued aquaculture species in South Africa (Skelton, 2001).

The sharptooth catfish was selected as the indicator species for the study (**Table 3.1**) due to its abundance in the Orange River system and use of food source to the local communities. As an omnivore, and its preference for bottom-dwelling in the river system, makes it well suited to study bio-accumulation and bio-magnification.

As discussed in **Section 2.2.4**, birds are one of the best bio-monitoring organisms for monitoring organic pollutants. Birds are specifically useful for emphasizing bioaccumulation and bio-magnification through trophic levels (Furness, 1993; Zakaria & Rajpar, 2010). Aquatic birds were selected due to their known sensitivity to contamination (Guruge *et al*., 1997) as well as their non-migratory or locally nomadic status (**Table 3.2**). Biological information, including classification, distribution, habitat, diet and breeding habitats are summarised in **Table 3.2**; additionally the birds were divided into groups according to feeding habitat and guild. A guild is defined as a group of species that exploit the same class of environmental resources in a similar way.

Table 3.1: Biological information on fish species sampled, *Clarias gariepinus* (Hecht *et al.*, 1988; Skelton, 2001).

Common name	Basic appearance	Classification and scientific name	Physical traits	Distribution	Habitat and general information	Diet	Breeding habits
Sharptooth catfish		**Order:** Siluriformes **Family:** Clariidae **Species & genus:** *Clarias gariepinus*	Helmet like bony head and dorsal-ventrally flattened bodies with long anal – and dorsal fins along the length of their bodies. Eyes small, lateral, large mouth, sub-terminal. Colour varies from black to light brown, often marbled in shades of olive green or grey, underparts of head and abdomen white. Red flush to extremities of fins when spawning.	Widely distributed in Africa found as far north as the Nile River and as far south as the Orange- and Umtamvuna systems. Translocated to Eastern – and Western Cape	Occurs in mostly any habitat, but favours floodplains, large slow rivers, lakes and dams. Can endure harsh conditions, and usually is the last inhabitant of drying river sources, where it may form burrows. Moves overland under moist conditions by crawling.	Omnivorous, preys, scavenges, or grubs on any available organic food source. May hunt in packs, herding smaller fish **Main diet:** fish, birds, frogs, small mammals, reptiles, crabs, insects, plant matter and plankton	Breeds in summer after rains, eggs are laid on vegetation and hatch within 25 – 40 hours. Growth is rapid, but dependant on local conditions. Lives for 8 or more years.

The environmental resources can be food, shelter, habitat, or nest sites (Korňan & Kropil, 2014; Simberloff & Dayan, 1991). For this study, the birds were divided according to similar food sources, thus resulting in feeding guilds. Additionally, this will assist in assessing the influence of trophic level on the concentration of PFCs. Furthermore, species were divided into three distinct feeding habitats namely aquatic, terrestrial and combined.

Table 3.2: Biological information and images on bird species sampled (Hockey *et al.*, 2011; Tarboton, 2011).

Common name	Basic appearance	Classification and scientific name	Physical traits	Distribution in South Africa	Habitat and general information	Diet	Breeding habits	Egg description
African Darter		**Order:** Suliformes **Family:** Anhingidae **Species & genus:** *Anhinga rufa*	Length: 85 cm. Yellow sharp pointed bill with long slender neck. Brown, black and white colouring	Widely distributed in Southern Africa where there are suitable wetlands	Opportunistic local movement linked to wetland conditions. Found mainly in still and slow moving water bodies with open water. **Feeding habitat:** Aquatic	**Feeding method:** Dives for food in mid-water or along bottom (1-6 m) and spears fish. **Main diet:** Fish and frogs. **Guild:** Piscivore	Colonial breeder. **Nests:** Untidy platform of sticks or dead reeds, shallow cup lined with grass. In tree forks over water or on islands. **Breeding season:** Throughout the year. **Clutch size**: 2 - 7	**Appearance:** Elongated sub-elliptical and chalky white in colour. Can have brown marks. **Size:** 54 x 35 mm
Black-headed Heron		**Order:** Pelecaniformes **Family:** Ardeidae **Species & genus**: *Ardea melanocephala*	Length: 92 cm. Head and hind neck black and dark to grey plumage, with yellow eyes	Common and widespread. Largely absent from arid regions	Mostly resident, but moves locally in response to food availability. Mostly in open grasslands and transformed landscapes. Often near, but not dependent on wetlands, partial to flooded fields and marshes. **Feeding habitat:** Combined	**Feeding method:** Solitary hunter. **Main diet:** Mainly terrestrial invertebrates, but also reptiles, small mammals and birds. **Guild:** Generalist	Monogamous colonial or mono-colonial breeder. **Nests:** Loose platform of twigs, lining mostly leaves, but variety of softer materials (some of human origin) also used. Placed high in trees/reed beds normally over water. **Breeding season:** Throughout the year. **Clutch size:** 2 - 4	**Appearance:** Oval, pale blue to bluish green. **Size:** 62 x 44 mm

Table 3.2 continued: Biological information and images on bird species sampled (Hockey *et al.*, 2011; Tarboton, 2011).

Common name	Basic appearance	Classification and scientific name	Physical traits	Distribution in South Africa	Habitat and general information	Diet	Breeding habits	Egg description
Cattle Egret		**Order:** Pelecaniformes **Family:** Ardeidae **Species & genus**: *Bubulcus ibis*	Length: 54 cm. White plumage with yellow bill and legs. The legs turn red during breeding season	Common throughout South Africa but sparse in the dry regions	Undertakes local nomadic movements tracking rainfall, but also disperses over large distances. Found in grasslands and grassy savanna, man-made pastures and agricultural land. **Feeding habitat:** Terrestrial	**Feeding method:** Hunts by walking steadily or stands still and stabs prey**. Main diet:** Insects, frogs, reptiles, small rodents and fish. **Guild:** Insectivore	Monogamous colonial breeder. **Nests:** Untidy platform made of dry sticks, weed stems and reeds, lined with grass. Found in trees or reed beds above water**. Breeding season:** September – January. **Clutch size**: 2 - 7	**Appearance:** Elliptical pale green or greenish blue and unmarked. **Size:** 45 x 34 mm
Glossy Ibis		**Order:** Pelecaniformes **Family:** Threskiornithidae **Species & genus:** *Plegadis falcinellus*	Length: 49 – 66 cm. Reddish brown plumage and shiny green wings. Brownish bill and pale olive-grey to dark brown legs	Centred on South African highveld in Gauteng and Free State	Locally common, some sedentary, others dispersive and nomadic. Present in shallow freshwater inland lakes and floodplains, marshes, sewage works, irrigated farmlands and open grasslands in parks and farms. **Feeding habitat:** Terrestrial	**Feeding method:** Foraging in flocks, walking slowly, taking live prey by pecking and probing in mud **Main diet:** Insects, fish, frogs and lizards. **Guild:** Insectivore	Monogamous colonial breeder. **Nests:** Small platform of reeds, stick and branches lined with leaves and grass. Found in most dense patches of reeds or on ground on a large reed bed island. **Breeding season:** September – February **Clutch size**: 2 - 4	**Appearance**: Oval, deep greenish blue or pale blue. **Size:** 51 x 36 mm

Table 3.2 continued: Biological information and images on bird species sampled (Hockey *et al*., 2011; Tarboton, 2011).

Common name	Basic appearance	Classification and scientific name	Physical traits	Distribution in South Africa	Habitat and general information	Diet	Breeding habits	Egg description
Grey Heron		**Order:** Pelecaniformes **Family:** Ardeidae **Species & genus:** *Ardea cinerea*	Length: 98 cm. White neck with grey colouring. Black stripe from bill to top of nape and extends into 3 long, black plumes	Widespread, absent from arid regions	Tends to move in response to habitat availability. Prefers shallow water bodies, rarely founds in dry grasslands. **Feeding habitat:** Terrestrial	**Feeding method:** Diurnal feeder, strikes, spears or catches prey in bill. **Main diet:** Fish, frogs, rodents and small birds. **Guild:** Piscivore	Monogamous colonial breeder. **Nests:** Nest with shallow central basin of small sticks or reeds, lined with grass. Found in tree for or above water in reed beds. Breeding season: July – January. **Clutch size:** 2 - 4	**Appearance:** Oval, blue or greenish blue and unmarked. **Size:** 60 x 43 mm
Reed Cormorant		**Order:** Suliformes **Family:** Phalacrocoracidae **Species & genus:** *Phalacrocorax africanus /Microcarbo africanus)*	Length: 60 cm. Black glossy plumage with yellow bill and ruby red eyes. Bare yellow-orange skin above and in front of eyes turn red during courtship	Widespread in South Africa, but sparse in dry regions	Resident and partial migrant, subject to local movement. Coastal numbers increase during winter and inland numbers in summer. Patterns might be reversed at other sites (eg Baberspan). Prefers all fresh water habitats except fast flowing streams. **Feeding habitat:** Aquatic	**Feeding method:** Typically forages singly, but may hunt cooperatively with others. Usual forages in water from 2 - 10 m deep. Hunts by pursuit diving. **Main diet:** Fish and frogs, occasionally small bird. **Guild:** Piscivore	Monogamous colonial breeder. **Nests:** Untidy platform of sticks or dead reeds, with shallow or deep cup lined with grass. In fork of a tree over water or on an island. Also in large reed beds or on the ground. **Breeding season**: Year round with a peak in summer (October – January) **Clutch size**: 3 - 4	**Appearance:** Elliptical chalky greenish white. **Size:** 44 x 30 mm

Table 3.2 continued: Biological information and images on bird species sampled (Hockey *et al*., 2011; Tarboton, 2011).

Common name	Basic appearance	Classification and scientific name	Physical traits	Distribution in South Africa	Habitat and general information	Diet	Breeding habits	Egg description
Sacred Ibis		**Order:** Pelecaniformes **Family:** Threskiornithidae **Species & genus:** *Threskiornis aethiopicus*	Length: 89 cm. Head and neck bare, with white body feathers and tail. Distinctive metallic blue-black plumes across back, closed wings and tail	Common throughout South Africa, but highest densities in moist, high-altitude plateau, and winter rainfall regions	Mostly resident, but some nomadic. Mostly found at inland freshwater wetlands, but also in grasslands, open habitats and agricultural lands. Highly adapted to man-modified habitats such as farm dams, sewage works, cultivated fields, abattoirs and waste dump sites. **Feeding habitat:** Combined	**Feeding method:** Forages in groups. Walks slowly, taking live prey by pecking and probing in mud. Also scavenges at refuge tips and abattoirs. **Main diet:** Waste, insects, fish, frogs, vegetable matter and young birds. **Guild:** Scavenger	Monogamous colonial breeder. **Nests:** Large platform of sticks and branches, or reeds lined with softer material such as grass or leaves. Mostly in reed beds or trees, but on protected island may nest on the ground. **Breeding season:** August – November **Clutch size:** 2 - 5	**Appearance:** Oval or slightly rounded oval dull white, with a faint greenish tinge and red/brown spots. **Size:** 66 x 44 mm
White-breasted Cormorant		**Order:** Suliformes **Family:** Phalacrocoracidae **Species & genus:** *Phalacrocorax carbo/ Phalacrocorax lucidus*	Length: 90 cm. Mostly glossy brown/black plumage with lower face, chin, throat and breast white to pinkish brick	Common along entire coast line and widely inland with patchily distribution in arid regions	Sedentary, but nomadic in response to changing water levels. Mostly found on dams, streams and rivers. **Feeding habitat:** Aquatic	**Feeding method**: Forages singly or in groups by diving for food. Jaws adapted to catch slow moving benthic fish**. Main diet:** Fish, frogs and crabs. **Guild:** Piscivore	Monogamous colonial breeder. **Nests:** Inland population makes a flat platform of sticks, twigs, plant material and human debris (plastic). Found in trees, protected islands and cliffs close to rivers or dams. **Breeding season:** August – November **Clutch size**: 2 - 5	**Appearance:** Sub-elliptical, pale bluish white with chalky white layer. **Size:** 63 x 40 mm

3.2. Site selection

Results from previous studies conducted on the Orange and Vaal River showed that POPs and other pollutants are present in the South African environment (Bouwman & Pieters, 2013; Davies & Mundalamo, 2010; Lusilao-Makiese *et al.*, 2013; Mccarthy *et al.*, 2007; McCarthy & Venter, 2006; Munyika *et al.*, 2014; Naicker *et al.*, 2003; Nieuwoudt *et al.*, 2009; Pieters, 2007; Wepener *et al.*, 2011). The presence of PFCs in the Orange and Vaal River basin specifically has only been reported in one study, where high levels were found in bird eggs (2300 ng/g) (Bouwman & Pieters, 2013). The motivations for the selection of sites is stated and elaborated in **Tables 3.4 – 3.5.**

The Orange River basin (called the Senqu River in Lesotho), is the largest basin in Southern Africa covering an area in the order of 1 000 000 km^2 including the entire Lesotho, large parts of South Africa (600 000 km^2), and southern regions of Botswana and Namibia (**Figure 3.1**). The Orange-Senqu originates in Lesotho, becomes the Orange River at the Lesotho border, traverses central and western South Africa, forms the southern border of Namibia, and joins the Atlantic Ocean near Alexander Bay (ORASECOM, 2015). The Orange River is divided into three sub-basins, namely the Upper-, Middle- and Lower Orange and consists of perennial- (river with continues flow all year round), and ephemeral (only flow during certain seasons or during rainfall) rivers.

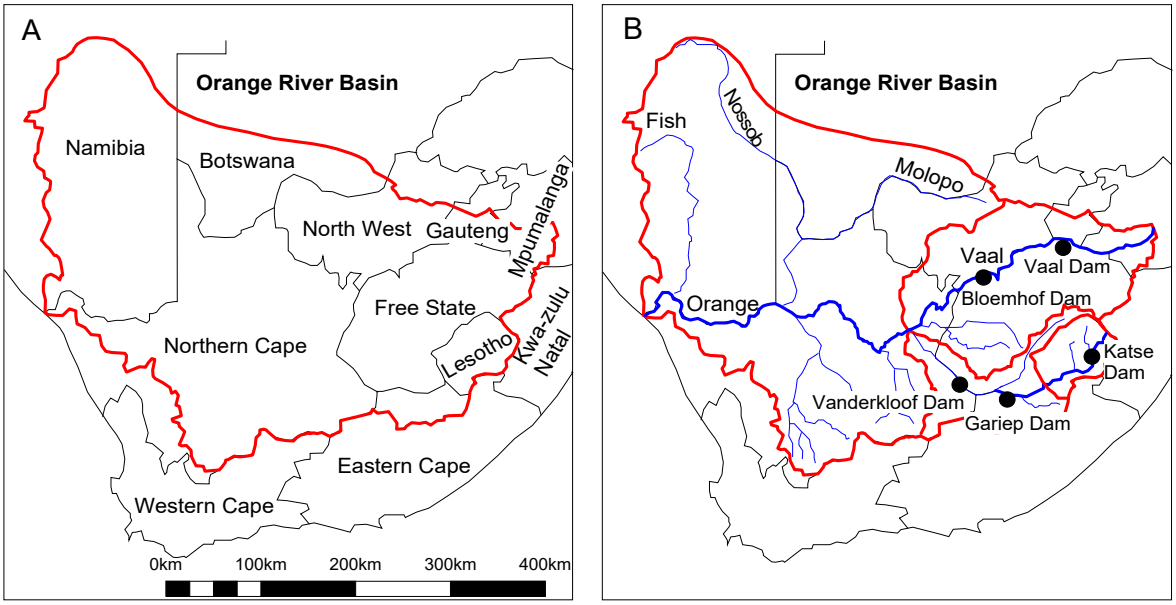


Figure 3.1: Map of South Africa showing the Orange-Senqu River basin (A) and major rivers and dams (B) (MapViewer v8)

The Upper Orange refers to the section from Lesotho Highlands to Gariep Dam, and drains most of the Lesotho Highlands (**Figure 3.2**). To the east, this section is dominated by mountains and is characterised by higher rainfall (600 mm – 1000 mm) than the rest of the basin. Dominance of

higher temperatures in the summer leads to higher levels of evapotranspiration (ORASECOM, 2015; United Nations Development Programme (UNDP), 2014). The Katse Dam and the Mohale Dam are the two major dams in the Senqu catchment. These dams together with the Matsoku Weir with its interlinking tunnels and transfer tunnels to the Upper Vaal Catchment forms part of the Lesotho Highlands Water Project (LHWP). The purposes of the LHWP is to transfer water to the Vaal River System and to generate electricity for Lesotho. This sub-basin also includes the two largest dams in South Africa, namely the Gariep Dam and the Vanderkloof Dam. This region experiences minimal impacts from industry and mining activities, and is mostly dominated by rural communities rather than urban communities (ORASECOM, 2015).

The Middle Orange is the part of the river from the Gariep Dam to the confluence with the Vaal River. The Vaal River (largest tributary of the Orange River, originates from the Drakensberg) with its tributaries draining most of the north-eastern parts of the basin, this includes the central Highveld of South Africa (ORASECOM, 2015). The tributaries of the Vaal River are the Klip-, Wilge-, Liebenbergsvlei Mooi-, Schoonspruit-, Harts- and Riet Rivers. This section is characterised by relative intermediate rainfall (400 mm – 800 mm), and high temperatures in the summer. The major dams in this section are the Vaal Barrage, Groot Draai, and Bloemhof. The area is impacted by gold and coal mining, significant dryland and irrigation agriculture and large settlements (ORASECOM, 2015).

The Lower Orange is from the Orange-Vaal confluence to where the river mouths into the ocean (Department of Water affairs and Forestry (DWAF), 2004). The main tributaries are the Ongers and Sak rivers from the northern Karoo, the Kuruman and Molopo rivers from the Northern Cape Province, north of the Orange and southern parts of Botswana, and the Fish River from the southern part of Namibia (ORASECOM, 2015). These rivers drain from semi-arid and arid areas, where water is mostly used for irrigation and to a lesser extent for urban use and stock watering purposes (DWAF, 2004). This section is characterised by lower rainfall (100 mm – 400 mm) compared to the rest of the basin, generating little usable surface runoff. If runoff occurs, it is highly variable and intermittent due to low and infrequent rainfall. The area is mainly impacted by erosion and agriculture along the river. Salinity in the Lower Orange is elevated due to the transfer of water out of the basin, which reduces the dilution of saline irrigation return flow to the river (ORASECOM, 2015).

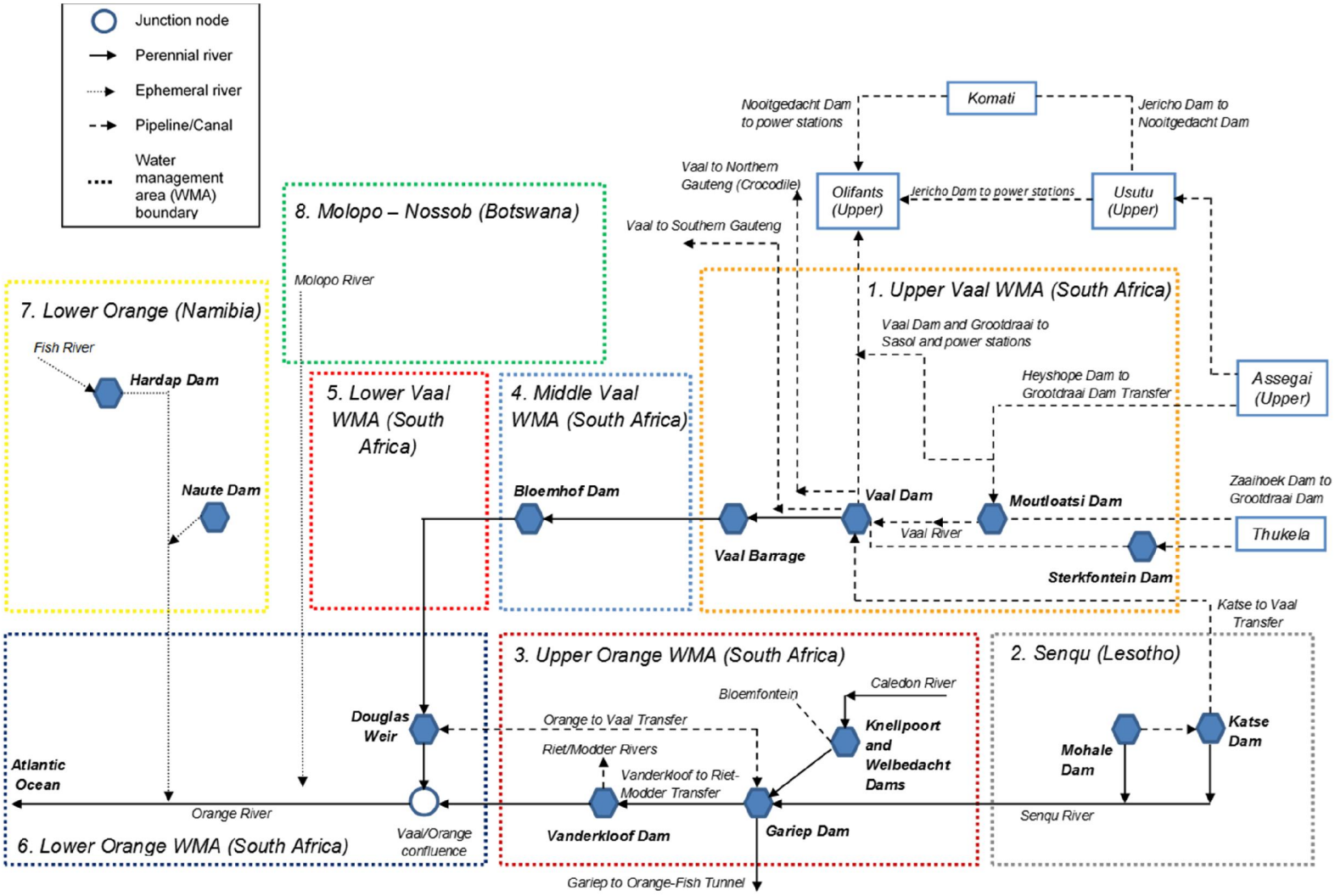


Figure 3.2: Integrated schematic of the Orange River basin (adapted from ORASECOM, 2013) (Water management area (WMA)

The main uses of the Orange River are irrigation, mining and production of hydro-electricity, and are seldom used for domestic or industrial water, with the exception of the water used in the Vaal River system (ORASECOM, 2015). The main sources of pollution in the Orange River basin is erosion, mining and mineral processing, not sufficiently treated industrial effluent, inappropriate disposal of waste, domestic effluent and impact of agricultural activities (ORASECOM, 2015). This leads to nutrient enrichment in the basin, one of the main water quality issues in the Orange River basin (UNDP, 2014). Mining in the Orange River basin is well developed and contribute significantly to the economies of Botswana, Namibia, and South Africa (**Table 3.3**). The major minerals found in the basin include, diamonds, semi-precious stones, gold, uranium, base metals (copper, manganese and iron) and industrial minerals.

Table 3.3: Main mining sectors produced within the Orange River basin (UNDP, 2014)

Country	Mining sectors	Contribution to GDP*	Other mining activities
Botswana	Diamond Copper, soda-ash and nickel	35%	Asbestos, chromite, manganese, coal
Lesotho	Mining not a dominant contributor	<1%	None
Namibia	Diamond	9%	Uranium
South Africa	Gold	7%	Coal, antimony, chromite, fluorite, gems, industrial diamonds, manganese, platinum, vanadium, vermiculite, coal, limestone

*Gross domestic product (GDP)

Although mining contributes significantly to the economies of these countries, the impact on water quality has detrimental effects (ORASECOM, 2015). Some mining activities have exposed metal containing sulphide ores that produce sulphuric acid when it encounters water. This, in turn results in extremely acid water with high concentrations of salts and metals. Additionally, when mines close down, they are no longer dewatered, and recharge of groundwater in subsurface mine workings continue, causing the water table of acid mine drainage to rise, leading to decanting into surface waters (UNDP, 2008, UNDP, 2014). PFOS was utilized as a wetting agent in mining and oil surfactants, as well as enhancing the amount of metal recovery in copper and gold mines (Brooke *et al.*, 2004) . Due to high levels of PFOS found in bird eggs in a previous study (Bouwman & Pieters, 2013), the current study was undertaken to expand the site selection and type of samples collected. Water, sediment, fish, bird eggs, and bird feathers were collected from sites along the Orange River basin. Additionally, mine tailing were collected to establish if this could be a source of the high levels found in the previous study. The sampling sites with site descriptions are summarised in table format (**Table 3.4 –3.5**). If similar sites occurred from one sample type to the next, the general description of the site was not repeated. A sampling area map for each sample type is supplied before each summary table (**Figure 3.3 –3.4**). .

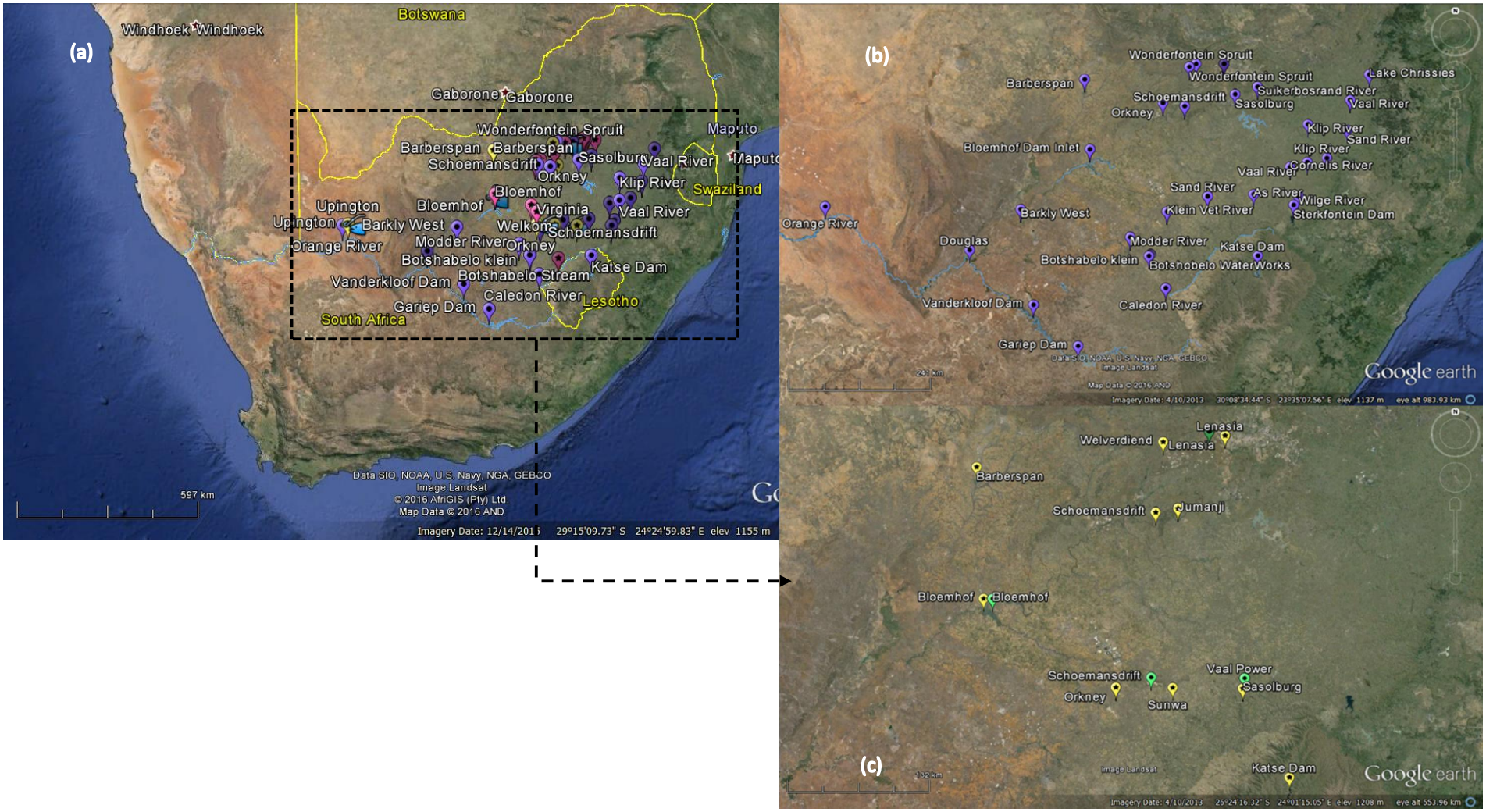


Figure 3.3: Sampling areas and sites (a) for water (b), fish (green) and wild bird eggs (yellow; c) collected (adapted from Google Earth)

Table 3.4: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

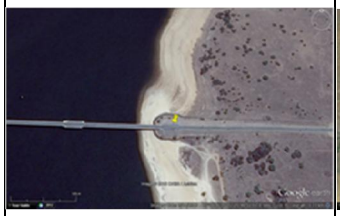
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/04/30	27°38'18.31"S 25°40'51.35"E	Bloemhof Dam Inlet			Located at the confluence of the Vaal River and the Vet River, on the border between the North West and the Free State. Protected area with two separate nature reserves. Bloemhof Dam nature reserve and Sandveld Nature Reserve. Fed with the outflow from the Vaal dam, as well as rain collected in the Vaal-, Vet-, Vals- and Sand River catchment areas. Bloemhof is an agricultural town.	Sample collected in Bloemhof dam, close to the road (R) 34. Wild bird eggs collected on island in Dam.	Cattle Egret, Reed Cormorant, African Darter, Sacred Ibis, Black-headed Heron and Grey Heron. *Clarias gariepinus*
2013/05/28	28°34'2.53"S 26°57'25.34"E	Klein Vet River			The Vet River is a west-ward flowing tributary of the Vaal River. Vet River flows north-westwards to meet the Vaal at the Bloemhof Dam. The Vet river is formed at the confluence of the Klein Vet and the Groot Vet upstream from Erfenis Dam. Mostly used for the irrigation of crops such as maize, groundnuts, sorghum sunflowers as well as domestic use.	Sample collected in the Klein Vet River, close to the National road (N1. Farmlands in the surrounding area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

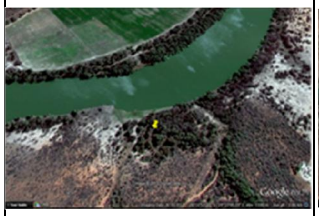
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/04/30	28°32'57.80"S 24°33'4.00"E	Barkly West			Barkly West is a town in the Northern Cape, situated on the north bank of the Vaal River, west of Kimberley. The area is known for diamond mining. Cattle and maize farming in neighbouring area.	Sample collected in Vaal River, close to surrounding farmlands.	None
2014/04/30	29° 9'50.29"S 23°41'34.66"E	Douglas			Douglas is a town situated near the confluence of the Orange and Vaal Rivers in the Northern Cape. Stock farming and agriculture (Groundnuts, potatoes, cotton and grapes) predominates in surrounding area.	Sample collected in River, close to R357, with surrounding farmlands.	None
2014/05/01	29°59'57.66"S 24°45'23.33"E	Vander-kloof Dam			Between Northern Cape and Free State. Situated 130 km downstream from Gariep Dam on the Orange River. Second largest dam in SA. Hydropower generation, storage of water for urban and irrigation use. Small residential area - Vanderkloof. Maize, wheat, sorghum, groundnuts, potatoes, onions and stock farming in surrounding area.	Sample collected from dam, close to Vanderkloof residential area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

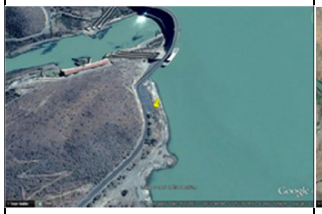
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/01	30°37'41.52"S 25°30'22.82"E	Gariep Dam			Gariep dam nature reserve situated between the border of Eastern cape and Free state. Largest storage reservoir in South Africa. Supplies water to parts of the Vaal, Fish and Sundays catchments. Water is released to Vanderkloof Dam for power generation.	Sample collected in dam, small farmlands in surrounding area.	None
2014/05/01	29°43'27.78"S 26°57'45.31"E	Caledon River			Tributary of the Orange River, originates in the Drakensberg Mountains in Lesotho. Flows south-west and enters South Africa in the Free State north of Wepener. Flow west-wardly before joining the Orange River, just before the Gariep Dam. Primary source of water for Maseru. Close to Wepener, with wheat, maize and stock farming in the area.	Sample collected in river, close to R26.	None
2014/05/02	29°13'5.94"S 26°41'52.82"E	Botshabelo Stream			Big informal settlement. Main River is the Modder River, Klein Modder is in the middle of Botshabelo, a tributary of Modder River. Modder river drains central region of Free State province. Modder can be dry for long periods, particular during winter months.	Sample taken in the Klein Modder River. Sewage outflows are discharged into Klein Modder.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
014/0502	29°14'19.61"S 26°41'5.10"E	Botsha-belo water works			Discharges 5 mega litres of treated effluent into the Klein Modder river per day.	Sample taken from discharge into river.	None
2014/05/02	28°57'44.17"S 26°21'27.68"E	Modder River			Modder is a tributary of Riet River (westward flowing tributary of the Vaal River). Maize, wheat, sorghum, grapes, potatoes, tomatoes, and stock faming in surrounding area.	Sample collected in river, close to N1. Farmlands in surrounding area.	None
2014/05/02	28°19'19.16"S 27°37'2.35"E	Sand River (Senekal)			Sand River is a tributary of Vet River (tributary of Vaal river). Located close to Welkom and Virginia in the gold mining centre of the Free State. Thabang (Welkom) waste water treatment works discharges treated waste into the Sand River. Maize, sunflower, wheat and stock farming in neighbouring area.	Sample taken at Sand Spruit - confluence with Sand river. In a residential area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/02	28°16'19.51"S 28°22'20.96"E	As River			As River is a tributary of Liebenbergsvlei River (tributary of Wilge River) situated in the Eastern Free State, one of the discharge points for the Lesotho Highlands. Maize, sunflower and wheat and stock farming.	Sample collected from river, close to N5. Farmlands in surrounding area.	None
2014/05/02	28°16'56.46"S 29° 7'8.29"E	Wilge River (Harrismith)			Wilge River is tributary of the Vaal River. Forms part of the Tugela-Vaal water transfer scheme, where water is transferred from the Tugela River basin to the Vaal river basin. Tributaries of the Wilge River are Nuwejaarspruit, Elands River, Liebenbergsvlei, Meul- and Cornelis River.	Sample taken outside Harrismith. Residential and industrial sites in surrounding area.	None
2014/05/02	28°24'56.38"S 29° 2'38.58"E	Sterk-fontein Dam			Located just outside Harrismith, in the Free State,and located on the Nuwejaarspruit (tributary of Wilge River). Water is received from KwaZula-Natal and stored in the Sterkfontein Dam, and released to the Vaal river via the Wilge River as needed. Mainly serves domestic and industrial uses. Dam is surrounded by the Sterkfontein Dam nature reserve.	Sample collected in the nature reserve.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/03	27°50'29.83"S 28°57'39.67"E	Cornelis River			Tributary of Wilge river.	Sample collected in wetland area, close to N3, surrounded by residential area	None
2014/05/03	26°38'17.77"S 28°23'19.50"E	Suikerbos-rand River			Blesbokspruit which is a tributary of the Vaal River, has Suikerbosrand as atributary, and of poor quality due to mine water deviants, diffuse- and urban runoff and point source discharges. This includes the industrial centres of Nigel and Heidelberg. The catchment is sparsely populated with commercial agriculture as the main land use. Multiple Sewage works on the river discharging effluent into it. Maize and sunflower faming in surrounding area.	Sample collected from river, close to N3. Farmlands in surrounding area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

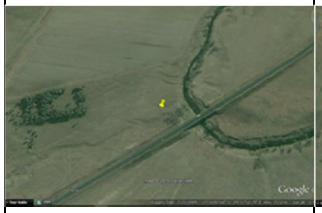
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/04	26°57'31.00"S 29°14'39.01"E	Vaal River (Stan-derton)			The Vaal River is the largest tributary of the Orange River. Main tributaries of the Vaal include Harts River, Vals River, Waterval River, Bamboes spruit, Blesbokspruit, Mooi River, Vet River, Renoster River, Riet River, Klip River and Wilge River. Standerton is a large commercial and agricultural town consisting of stock and maize farming.	Sample collected in close to residential and commercial area.	None
2014/05/04	27°10'57.58"S 29°14'2.69"E	Klip River			Tributary of the Vaal River, main river draining the portion of Johannesburg, south of Witwatersrand, and includes JHB CBD and Soweto. This includes multiple industrial-, commercial and residential sectors. There is significant runoff from the paved urbanised areas, and discharges from mines in the surrounding areas.	Sample collected in an unpopulated area, with maize farmlands in the surrounding area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

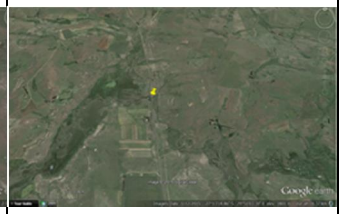
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/04	27°41'1.82"S 29°34'3.73"E	Klip River (Memel)			Memel is a small town, with a large informal settlement (Zumani). Stock and maize farming occur on the outskirts of the town	Sample collected in the residential area.	None
2014/05/02	29°14'33.76"S 26°40'22.80"E	Botsha-belo Klein (Klein Modder River)			Big informal settlement. Main River - Modder River, Klein Modder is in the middle of Botshabelo, a tributary of Modder River. Modder river drains central region of Free State province. Modder can be dry for long periods, particular during winter months.	Sample collected on the outskirts of the town, near the informal settlement.	None
2014/05/05	27°14'27.60"S 29°53'21.44"E	Sand River			Sand River is a tributary of Vet River (tributary of Vaal river). Located close to Welkom and Virginia in the gold mining centre of the Free State. Thabang (Welkom) waste water treatment works discharges treated waste into the Sand River. Farming in the area consists of maize, sunflower, wheat and stock farming.	Sample collected close to the wetland with farms in the surrounding area.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/05	26°46'41.12"S 29°55'14.59"E	Vaal River			A town close to the sampling site, Standerton is a large commercial and agricultural town consisting of stock and maize farming.	Sample collected in a minimal impacted area, close to N11, with farmlands.	None
2014/05/05	26°22'18.01"S 30°13'33.92"E	Lake Chrissie			The Lake Chrissie pan veld area is a high lying plateau interspersed with more than 300 lakes and pans in a wetland and grassland environment. Four river systems originate from this sandy plateau - the Komati (to the north) the Umpuluzi and Usutu (to the south east) and the Vaal (to the west). Lake in Mpumalanga, shallow and large lake, with a maximum depth of 6m. Trout farming, timber industry, and coal mining in surrounding areas.	Sample collected from one of the pans.	None

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2014/05/10	26°18'58.97"S 27°22'58.08"E	Wonder-fontein spruit Carletonville			Wonderfontein spruit is a tributary of the Mooi River, which is a tributary of the Vaal River. The headwaters of the Wonderfontein Spruit originated around mine residue deposits of several old and abandoned mines. The mine tailing dams, sand dumps and rock dumps are potential contributors to diffuse contamination. Multiple active gold mines are discharging fissure and process water into water environment.	Sample collected from spruit, close to R500. Farmlands in surrounding area.	*Clarias gariepinus*
2014/05/16	28°27'50.18"S 21°14'29.87"E	Orange river (Upington)			Town in the Northern Cape, and closest large centre to the Augrabies Falls. Landscapes are very arid, but with fertile soil. Cotton, sunflower seeds, potatoes, grapes and stock farming in surrounding area.	Sample collected close to farm and residential area.	African Darter, Cattle Egret and Sacred Ibis. *Clarias gariepinus*
2013/01/04	26°34'33.66"S 25°35'4.48"E	Barbers-pan			On the Harts River a northern tributary of the Vaal River. Rises near Lichtenburg, and flows in a south-westerly direction through the North West and Northern Cape provinces. Diamond mining occurs along the river. Barberspan is a declared nature reserve.	Sample collected in nature reserve. Surrounded by farming land.	Cattle Egret

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

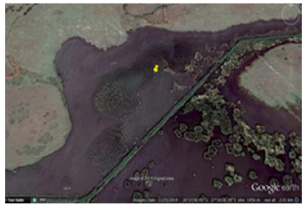
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2013/01/13	26°45'48.24"S 28° 1'38.25"E	Sasolburg			Sasolburg is a large industrial town.	Sample collected close to a residential zoning area. New Vaal coal mine and a power station in the surrounding area.	Cattle Egret and Black-headed Heron. *Clarias gariepinus*
2013/01/13	26°21'49.90"S 27°16'29.22"E	Wonderfontein Spruit (Welverdiend)			Wonderfontein spruit is a tributary of the Mooi River, which is a tributary of the Vaal River. The headwaters of the Wonderfontein Spruit originated around mine residue deposits of several old and abandoned mines. The mine tailing dams, sand dumps and rock dumps are potential contributors to diffuse contamination. Multiple active gold mines are discharging fissure and process water into water environment.	Sample collected from a wetland. , gold mine and farming in the surrounding area.	Cattle Egret and Reed Cormorant

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

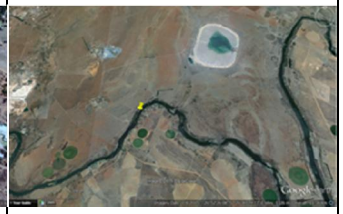
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2013/01/13	26°57'52.70"S 27°12'40.51"E	Schoe-mansdrift			Situated on the Vaal River. The Vaal River is the largest tributary of the Orange River. Main tributaries of the Vaal include Harts River, Vals River, Waterval River, Bamboes spruit, Blesbokspruit, Mooi River, Vet River, Renoster River, Riet River, Klip River and Wilge River.	Sample collected close to farming areas.	African Darter, Cattle Egret and White-breasted Cormorant. *Clarias gariepinus*
2013/01/13	26°55'23.10"S 26°51'26.37"E	Orkney			Situated on Koekemoerspruit, tributary of the Vaal River. The middle vaal WMA is located downstream of the confluence of the Vaal and the Rietspruit Rivers and upstream of Bloemhof dam. Mostly rural population present of the Schoonspruit areas. Water utilized for irrigation and urban use. Pollution of spruit experienced due to diamond digging on the banks of the river.	Sample collected river, close to Buffelsfontein gold mine.	Reed Cormorant and African Darter.

Table 3.4 continued: Description of sites where water, fish and wild bird egg samples collected for the study (DWA, 2015; Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Sampling site description	Species sampled
2013/02/02	26°18'13.00"S 27°50'6.62"E	Lenasia			The Klip River is a wetland south of Johannesburg, initially a water source, and later a purifier of polluted water from the Witwatersrand urban-industrial-mining complex.	Sample collected in wetland area. Surrounded by informal settlements.	Glossy Ibis, Cattle Egret, Sacred Ibis and Black-headed Heron *Clarias gariepinus*
2013/01/13	29°12'10.43"S 28°28'43.61"E	Katse Dam			Second largest dam in Africa. Forms part of the Lesotho Highlands water project. Has minimal human impact in adjacent area.	Sample collected in dam.	White-breasted Cormorant

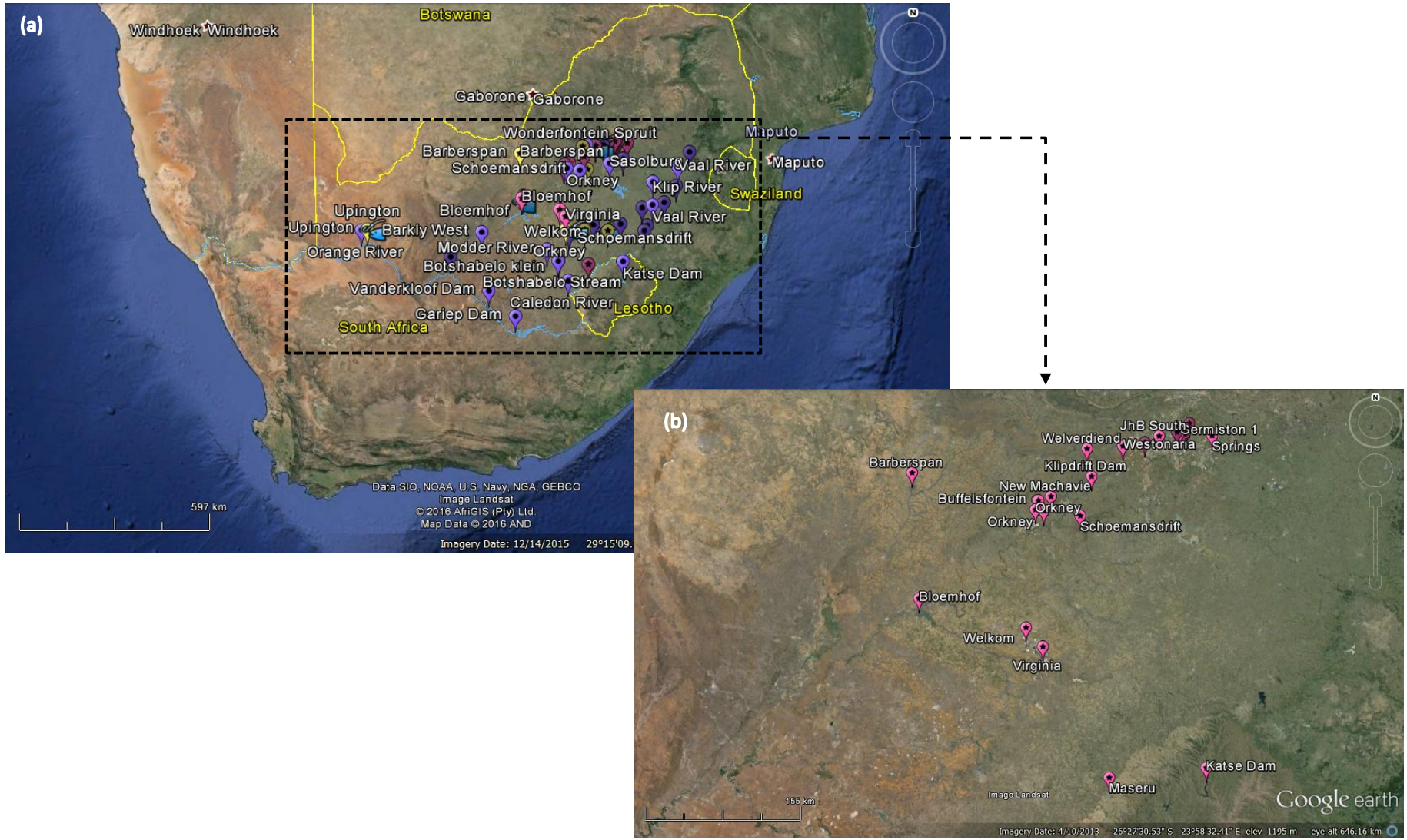


Figure 3.4: Sampling areas and sites (a) for sediment and tailing samples (b; adapted from Google Earth)

Table 3.5: Description of sediment and tailing samples collected for the study (DWA, 2015; Google Earth 2015)

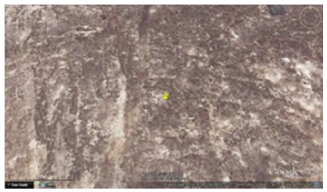
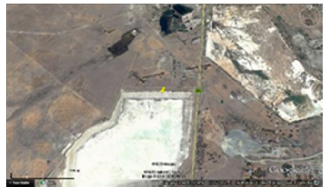
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/03/07	26°49'37.34"S 26°47'38.45"E	Stilfonten			Gold mine established in 1949, operations stopped in 1992, rehabilitation and waste treatment underway. Area where sampled is a mine waste solutions footprint area.	Tailing
2013/03/07	26°54'38.33"S 26°46'36.34"E	Orkney			Mine established in early 1940s and active to this day. Sampled at the East Complex tailing storage facility.	Tailing
2013/05/28	28° 7'49.00"S 26°50'59.95"E	Virginia			First mining in 1949. Area where sampled is the Merriespruit 4A - tailings storage facility. Operations ceased in April 2010. Virginia is mostly dominated by mining, gold-extraction plants and the manufacture of sulfuric acid from gold ore. Has the deepest pipe mine. Commercial farms surrounding the area primarily grow maize and stock farming.	Tailing
2013/05/28	27°57'38.79"S 26°41'6.25"E	Welkom			First mining in 1948. Forms part of Welkom gold mine, does not have any active producing shafts at the moment. Mining of gold and uranium. Welkom also produces goods such as steel, lumber, dairy products and beef.	Tailing

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/05/29	26°10'7.05"S 28°13'27.22"E	Jet Park			Sample collected at Witkoppies Dam. Dam is surrounded with an industrial area, manufacturing companies - C&N Petroleum Equipment (couplers, fuel and oil pumps), Electric Systems SA (design and build of LV Electrical distribution systems, and variable speed drive systems in the mining, sugar, materials handling and steel industries), Fuel Flex Hose and Fittings (Manufacture composite hoses, fuel and chemical transfer equipment). Roto-Plastics - Manufacturing of a variety of plastic products. Close to aviation field	Sediment
2013/05/29	26° 7'23.63"S 28°15'59.45"E	Kempton Park			Close to OR Tambo International and a brick quarry. Sampled in Pumula Nature reserve, close to Blaawpan Dam	Sediment
2013/05/29	26°13'50.18"S 28°29'26.15"E	Springs			Coal and gold mines. Coal - First mine 1988. First gold mining in 1899. Grootvlei Proprietary mines (1904). Industrial centres of Gauteng: Maize processing, platinum's precious and base metal refineries, glass producer of float glass paper mill, industrial diamonds and beverage cans, foods packaging, bricks, swimming pool equipment, hose and coupling manufacturer. Sample taken from Grootvlei Proprietary gold mine, open pit and underground shafts. Still producing.	Tailing

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

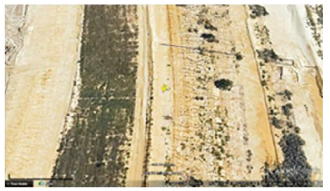
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/05/31	26°11'56.88"S 28°10'41.22"E	Germiston 1			Close to the Germiston railway junction. Part of Knight Gold plant - Crown gold recovery project - re-treat old mine waste and lime dumps, to recover gold left behind by original mining operations. Sample collected from one of the tailing disposal facilities.	Tailing
2013/05/31	26°12'19.25"S 28° 8'49.56"E	Germiston 2			Central Rand Mineral Tenure - Simmer & Jack. Surface tailings retreatment facility. Sample collected from one of the treatment areas.	Tailing.
2013/05/03	26°14'32.45"S 27°58'23.78"E	Johannesburg South			Central Rand Mineral Tenure - Crown Mines Ferreira. Surface tailings retreatment facility. Using modern milling and carbon-in-pulp technology. Land uncovered is reclaimed and developed for light industry.	Tailing
2013/05/31	26°20'12.78"S 27°37'21.32"E	Westonaria			Kloof Gold mine established 1934, intermediate to ultra-deep gold mine, still in use. - Libanon division Shaft nr 10. Sample collected at the tailing storage facility. Underground and rock dump mining.	Tailing

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

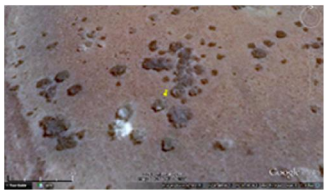
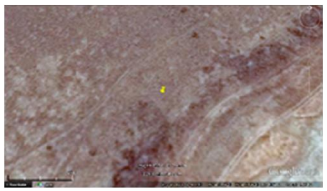
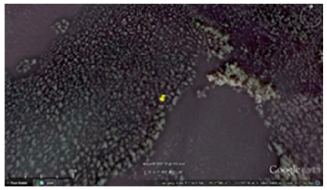
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/01/23	27°41'49.03"S 25°38'28.07"E	Bloemhof			Sample collected from Bloemhof Dam, farmlands in surrounding area.	Sediment
2013/01/04	26°34'33.66"S 25°34'53.68"E	Barberspan			Sample collected from Barberspan, farmlands in surrounding area.	Sediment
2013/01/04	26°21'49.90"S 27°16'29.22"E	Welverdiend			Sample collected from wetland.	Sediment

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

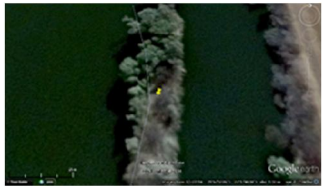
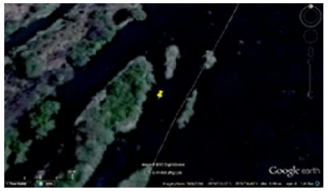
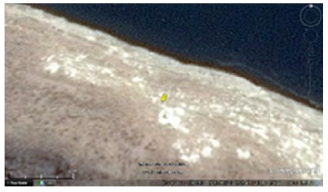
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/01/08	26°57'52.70"S 27°12'40.51"E	Schoemans-drift			Sample collected on riverbank.	Sediment
2013/01/13	26°55'23.10"S 26°51'26.37"E	Orkney			Sample collected close to riverbank..	Sediment
2013/02/02	26°18'13.00"S 27°50'6.62"E	Lenasia			Sample collected in wetland.	Sediment
2013/01/04	29°12'10.43"S 28°28'43.61"E	Katse Dam			Sample collected from dam.	Sediment

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

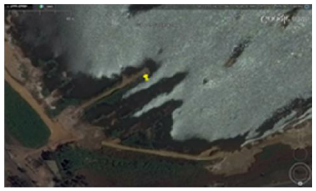
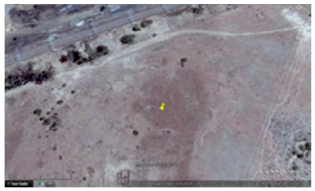
Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2013/01/04	29°17'53.48"S 27°30'45.95"E	Maseru			Maseru is the capital and largest city of Lesotho, and located on the Caledon River. Population of approx. 227,880. Large industries (flour mills, textile and footwear companies) and urban and rural settlements. Sample collected from Maqalika Dam, water is pumped into this reservoir from the Caledon River.	Sediment
2013/06/01	26°49'46.23"S 26°48'18.54"E	Buffelsfontein			Simmer & Jack Mines Ltd consists of former Hartebeesfontein and Buffelsfontein gold mines, 11 shafts and two gold plants. Opened in 1955 and 1954. Uranium was also mined until the mid-1990. Currently only gold is produced. Sample collected from Mine waste solutions footprint.	Tailing
2013/06/01	26°13'11.05"S 28°13'17.14"E	East Rand			ERPM gold plant Operated by DRD Gold, this plant is part of the Crown gold recovery project (Crown Gold Recoveries (Pty) Ltd)) which operates three gold plants that re-treat old mine waste and slime dumps to recover gold left behind by the original mining operations.	Tailing
2013/06/01	26°47'37.00"S 26°55'32.36"E	New Machavie			New Machavie was actively mined between the 1930s and early 1940s. The reopening of the mine was scheduled for 2003, but this has been delayed due to problems regarding surface mining rights. Sample collected from one of the tailing disposal facilities.	Tailing.

Table 3.5 continued: Description of sediment and tailing samples collected for the study (DWA, 2015, Google Earth 2015)

Sampling date	Coordinates	Location	Sampling area	Surrounding area	General site description	Type of sample
2014/01/17	26°36'44.14"S 27°19'10.52"E	Klipdrift Dam			Earth-fill type dam established in 1990 located on Ensel Spruit near Potchefstroom. Water supplied by Loopspruit which is a tributary of Mooi River. Loopspruit runs through mining areas. The Loopspruit flows into the Mooi River on the southern side of Potchefstroom, upstream from the Thlokwe sewage works. Primary purpose of irrigation. Sample collected from Klipdrift dam. Surrounding area are farmlands.	Sediment

3.3. Sampling methods

All equipment used during sampling was made of stainless steel, polyethylene (PE) or polypropylene (PP) unless stated otherwise. No glass containers or equipment came into contact with the samples, as PFCs are known to adsorb to these surfaces (United States Environmental Protection Agency (US EPA), 2009). Sample contamination or cross-contamination was prevented by rinsing every utensil or collection vessel before collection of samples with soap, deionised water, high pressure liquid chromatography (HPLC) grade methanol (MeOH, Romil), acetone and hexane (Honeywell: Burdick and Jackson®) (US EPA, 1994, US EPA, 2009).

3.3.1. Sediment and Tailings

Sediment and tailing samples (**Figure 3.4, Table 3.5**) were prepared by collecting the top 1 - 10 cm of five collection points within a 10 m radius per site, and stirring the mixture thoroughly to obtain a homogenous sample. Sub-samples were stored in pre-cleaned high-density polypropylene (HDPP) bottles at -20°C until further processing. The samples were thawed, air dried, ground and sieved (mesh size 0.5 mm), before extraction (**Section 3.5.1.2**).

3.3.2. Water

Water was collected (**Figure 3.3**; **Table 3.4**) by submerging a pre-cleaned PP bottle, pre-rinsed with river/dam water, into the river/dam and filled to the brim to eliminate air. The containers were labelled, covered with a brown paper bag, and stored at -4°C until further analyses.

3.3.3. Fish

Sharptooth catfish (*Clarias gariepinus*) were sampled (**Figure 3.3**; **Table 3.4**) from six sites, with ten fish per site, using gill- (73-, 93-, 118- and 150 mm), seine-, and fyke nets. Fish were captured and temporarily held in a keep net until they were measured, weighed, and euthanized by severing the spinal cord. The fillets of the fish were dissected, transferred to a pre-cleaned PP bottle or PP bag, and stored at -20°C until further sample preparation in the laboratory. The fish samples required processing in the form of milling to achieve a homogenised sample size for the extraction. This was achieved by processing each sample individually with a knife mill (Retsch Knife Mill GM 300, Haan, Germany). Thereafter, the samples were bottled in pre-cleaned PP containers and frozen at -20°C until extraction and analysis if these samples (**Section 3.5.1.3**).

3.3.4. Wild bird eggs

Eggs of wild birds were collected (**Figure 3.3; Table 3.4**) from 12 sites along the Orange-Vaal river basin. Breeding colonies were identified during a low-flying aerial survey and by visiting previous collection sites. Eggs were hand-collected, under permits, from the nests, wrapped in aluminium foil that had been pre-cleaned and clearly marked. The eggs were transported in a portable freezer and stored in egg carton containers at -20°C in the laboratory freezer until further processing (**Section 3.4**).

3.3.5. Feathers

It proved remarkably difficult to collect feather samples, and only five samples were collected. Water birds hardly ever use their feathers to line the nests, and the parents already moulted prior to the start of the breeding season (**Table 3.2**).

3.4. Homogenisation and egg parameter measurement

All equipment was pre-cleaned with soap and warm tap water, rinsed three times with double deionised water (dd.H_2O, 18.2 MΩ) and allowed to dry. Thereafter, the equipment was rinsed in triplicate with MeOH to remove any fluorinated contaminants. The eggs were removed from the freezer, photographed and the mass, width, and length measured. Each measurement was repeated in triplicate and the means were used for further statistical analysis. After completion of the measurements, the eggs were placed in individual watch glasses and allowed to thaw. Once thawed, the eggs contents were carefully transferred from the shell into pre-cleaned PP centrifuge tubes using PP funnels and stainless steel utensils. Eggshells were gently rinsed using tap water to remove the inner membrane and allowed to air dry (Laporte, 1982). The thickness of the eggshells was measured to the nearest 0.01 mm with a digital calliper (Kroeplin B2R20S, Schlüchtern, Germany) (Custer *et al.*, 1983; Jaspers *et al.*, 2005). Triplicate measurements were taken at the apexes and middle of each egg and the means were used in further statistical analysis (Bouwman *et al.*, 2008; Jaspers *et al.*, 2005).

Each egg was homogenised individually to evaluate the distribution of pollutants between the same species. The contents of the eggs (yolk and albumin) were homogenised with an ultrasonic homogeniser (Misonix sonicator 3000, Farmingdale, New York, USA). To prevent contamination, the homogeniser was cleaned with soap and water, followed by dd.H_2O, acetone, hexane, and MeOH between each sample. After homogenisation, the samples were stored at -20°C until chemical analysis (**Section 3.5**). The blank controls were two store-brought chicken eggs, which were pre-screened for any PFC contamination, and were exposed to the same procedure followed with the samples.

3.5. Chemical analysis

The preferred method for the analysis of PFCs is high pressure liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) together with negative electron spray ionisation (-ESI; Jahnke & Berger, 2009). Other methods include single stage MS, time of flight (TOF) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). High selectivity and sensitivity can be achieved by time of flight high resolution mass spectrometry (TOF-HRMS), but these instruments are not widely available.

After selection of instrumentation, other problems such as contaminated blanks from polymeric parts may occur. To prevent this, all Teflon tubing was removed and replaced by stainless steel and/or polyetheretherketone (PEEK) tubing. If contamination persisted, the inclusion of a column at the exit of the HPLC pump but before the injector was proposed (Jahnke & Berger, 2009; Trojanowicz & Koc, 2013). Most methods use C18 or C8 column phases for separation of PFCs, but use of fluorinated stationary phases are increasing due to prevention of co-elution of biological mass interferences (Ballesteros-Gómez *et al.*, 2010).

Sample preparation impacts on all the steps in an analysis; it can interfere with the identification, confirmation and quantification of analytes (Chen *et al.*, 2008). An entire analysis process can be invalidated due to poor sample treatment and badly prepared sample extracts. An optimized extraction method is required to ensure reliable analytical results. The general method used for the extraction of PFCs is solid liquid extraction using solvent mixtures corresponding with mobile phases (Trojanowicz & Koc, 2013). Regarding the clean-up of the extracts, Powley *et al.* (2008) developed an efficient method using ENVI-Carb™ for dispersive clean-up. This has become a very popular last step for almost any type of sample extract (Jahnke & Berger, 2009).

3.5.1. Extraction and clean-up

Before commencing with sample preparation, extraction, clean-up, and analysis all the laboratory equipment used were washed with phosphate free soap, and rinsed with warm tap water followed by dd.H_2O (18.2 MΩ) and allowed to dry. Thereafter, the equipment was rinsed in triplicate with HPLC grade MeOH, acetone, and hexane. All the solvents used were HPLC grade, unless stated otherwise. Standards used were from Wellington Laboratories, unless stated otherwise, and were between 97 and 99% pure. During and after extraction, samples were evaporated under a gentle flow of nitrogen gas at 40°C in a Turbo-vap®II (Caliper Life sciences, Massachusetts, United States) or a Reacti-Vap™ (Thermo Scientific, Massachusetts, United States). After the final concentration step, the extracts were reconstituted to 250 µL 60:40 ammonium formate (5 mM,

pH 6.2, Fluka, Sigma-Aldrich) and filtered using 0.22 µm nylon syringe filters (Membrane Solutions) prior to instrumental analysis.

3.5.1.1 Water

Water samples were processed on the day that they were received, and extracted according to the method described by Labadie & Chevreuil (2011). After thorough mixing by inversion, a 100 mL aliquot was transferred into pre-cleaned HDPP tubes and spiked with carbon-labelled PFOA (M8PFOA, Wellington Laboratories, Guelph, Canada). The samples were subsequently mixed and the pH was adjusted to approximately three using 200 mM hydrochloric acid (HCl, purity 32%: Merck, Darmstadt, Germany). Samples were centrifuged (1350 x g) before solid phase extraction (SPE) using Strata™ X–AW™ weak anion exchange cartridges (500 mg/12 mL, Phenomenex, Torrance, USA).

The SPE cartridge was conditioned with 8 mL MeOH: 0.2% Ammonium hydroxide (NH_4OH, Fluka, Sigma Aldrich, Merck, Darmstadt, Germany) and 6 mL MeOH, after which the SPE was equilibrated with 6 mL water before the sample was loaded. Before the cartridge was vacuum dried for 10 minutes, it was washed with 4 mL 25 mM sodium acetate (pH 4.5, Fluka, Sigma-Aldrich, Merck, Darmstadt, Germany) and 6 mL MeOH. The SPE cartridge was eluted by gravity flow with 7 mL MeOH: 0.2% NH_4OH and the eluates were dried at no higher than 40°C under a gentle stream of nitrogen and reconstituted.

3.5.1.2 Sediment and mine tailings

Sediment and tailing samples were extracted and analysed using a method adapted from Yang *et al.* (2011) and Labadie & Chevreuil,(2011). The samples were hand mixed in their original containers and 5 g subsamples weighed into pre-cleaned HDPP centrifuge tubes and spiked with labelled PFOA (M8PFOA). Thereafter, 5 mL of 100 mM sodium hydroxide (NaOH, CC Imelmann, Promark Chemicals, Robertsham, South Africa) in 20% (v/v) MeOH: dd.H_2O was added and the sample was extracted for one hour in an ultrasonic bath (Bransonic® 8800, Branson, Danbury, USA). After sonication, 10 mL of MeOH was added and the samples were extracted for an additional 30 minutes in the ultrasonic bath. Following ultrasonication, the extracts were acidified to pH 3 using 200 mM of HCl, and centrifuged. The supernatant was removed and diluted to a final volume of 50 mL using dd.H_20. Strata™ X–AW™ weak anion exchange SPEs were used for sample clean-up (similar method as in **Section 3.5.1.1**).

3.5.1.3 Wild bird eggs and fish

The extraction protocol was based on methods developed for the analysis of blood by Bytingsvik *et al.* (2012) and Powley *et al.* (2008). Homogenised egg samples were thawed overnight at 6°C and vortexed. A 1 mL aliquot (approximately 1 g) was transferred into a pre-cleaned HDPP centrifuge tube and samples were spiked with labelled PFOS (M8PFOS, Wellington Laboratories, Guelph, Canada) and M8PFOA. A 5 mL volume of MeOH was added and the samples were mixed for 30 minutes at ambient temperature. The samples were subsequently centrifuged and the supernatant removed. The extraction was repeated using 3 mL of MeOH. The supernatants were pooled and evaporated at no more than 40°C under a gentle stream of nitrogen until a 2 mL volume remained. Approximately 3 – 5 g of activated carbon (ENVI-Carb™, Supelclean™, Sigma-Aldrich, Merck, Darmstadt, Germany) was added to the 2 mL extract and mixed vigorously for 1 minute. The activated carbon was pelleted by centrifugation. If the sample still had a yellow hue, or if fat was visible, the clean-up process with the ENVI-Carb™ was repeated. Thereafter, the extracts were dried at no higher than 40°C under a gentle stream of nitrogen and reconstituted.

3.5.1.4 Feathers

The presence of PFCs in bird feathers was investigated using a screening approach. The feathers provided were heavily contaminated with bird guano. To remove the guano, feathers were gently rinsed with dd.H_2O after which feathers were cut into fine shreds. Rather than extract on mass basis, a single feather including the rachis, and calamus was seen as a single sample and the mass noted. The feather was spiked with M8PFOS and M8PFOA and extracted using 10 mL of MeOH for one hour in an ultrasonic bath. This extract was then dried at no higher than 40°C under a gentle stream of nitrogen and reconstituted.

3.5.2. Analysis

The chromatographic system used was an Agilent 1100 Series HPLC coupled to a Waters Micromass Quattro Micro electrospray ionization tandem mass spectrometer (ESI-MS). The column used was a FluoroSep RP-Octyl (5 µm x 150 mm x 2.0 mm). This column separates known interferences and prevents misidentification of PFC congeners. The instrumental conditions were adapted and optimised from literature (Ballesteros-Gómez *et al.*, 2010; US EPA, 2009) using commercially obtained authentic PFC standards listed in **Table 3.6** (Wellington Laboratories).

Table 3.6: General information of the PFCs selected for analysis

Compound	Acronym	MW	Empirical formula
Perfluorobutanesulfonic acid	PFBS	300.10	$C_4HF_9O_3S$
Perfluorohexanoic acid	PFHxA	314.05	$CF_3(CF_2)_4COOH$
Perfluoroheptanoic acid	PFHpA	364.06	$C_7HF_{13}O_2$
Perfluorohexanesulfonic acid	PFHxS	422.10	$C_6F_{13}SO_3NA$
Perfluorooctanoic acid	PFOA	414.07	$C_8HF_{15}O_2$
Perfluorononanoic acid	PFNA	464.08	$C_9HF_{17}O_2$
Perfluoroocatanesulfonic acid	PFOS	500.13	$C_8HF_{17}O_3S$
Perfluorodecanoic acid	PFDA	514.08	$CF_3(CF_2)_8COOH$
Perfluoroundecanoic acid	PFUnA	564.09	$C_{11}HF_{21}O_2$
Perfluoridodecanoic acid	PFDoA	614.1	$C_{12}HF23O2$
Perfluorotridecanoic acid	PFTrDA	664.11	$C_{13}HF_{25}O_2$
Perfluototetradecanoic acid	PFTA	714.11	$C_{14}HF_{27}O_2$

The HPLC mobile phase selected was 5 mM ammonium formate (pH 6.2) in water (mobile phase A) and 100% MeOH (mobile phase B) with a flow rate of 0.400 mL/min. The column temperature was set at 30°C and 10 µL sample volume was injected. A total run time of 45 minutes with the gradient conditions listed in **Table 3.7** achieved desired separation of the compounds.

Table 3.7: Gradient conditions used for the separation of selected PFCs

Time	Flow (mL/min)	% Solvent A	% Solvent B
Initial	0.400	60	40
1.00	0.400	60	40
25.00	0.400	10	90
30.00	0.400	10	90
35.00	0.400	0	100
35.10	0.400	60	40
45.00	0.400	60	40

The MS conditions were optimised with authentic PFC standards (Wellington Laboratories), and two multiple reaction monitoring (MRM) transitions were selected for each compound (**Table 3.8**). The polarity of the MS was set for ESI negative ionization with the source temperature at 100°C and desolvation temperature of 350°C. The cone gas and desolvation gas flow was 50 L/Hr and 700 L/Hr respectively. The MS has an inter-scan delay of 0.2 s and a dwell time of 0.05 s.

Table 3.8: MRM transitions used for the separation of the selected PFCs

Compound	MW	Ion	CV	Transition 1	CE	Transition 2	CE
PFBS	300.10	$[M-H]^-$	40	299>79.9	30	299>98.9	30
PFHxA	314.05	$[M-H]^-$	10	312.9>269.0	10	312.9>118.9	20
PFHpA	364.06	$[M-H]^-$	15	364>320.1	10	364>169	15
PFHxS	422.10	$[M-Na]^-$	40	399.1>79.9	40	399.1>99	35
PFOA	414.07	$[M-H]^-$	15	413>369	10	413>169	20
PFNA	464.08	$[M-H]^-$	10	463>419	10	463>219	15
PFOS	500.13	$[M-H]^-$	35	499>80	45	499>99	40
PFDA	514.08	$[M-H]^-$	15	513>469	10	513>269	15
PFUnA	564.09	$[M-H]^-$	15	563>519	10	563>269	20
PFDoA	614.10	$[M-H]^-$	15	613>569	15	613>319	20
PFTrDA	664.11	$[M-H]^-$	15	663>619	15	N/A	N/A
PFTA	714.11	$[M-H]^-$	15	713.2>669.1	15	713.2>219	30
M8PFOA	422.00	$[M-H]^-$	20	421>172	20	421>376.30	10
M8PFOS	507.00	$[M-H]^-$	35	507>79.90	40	507>99	40

3.5.3. Analytical quality control

A six- to eight-point calibration curve was constructed for quantification using matrix-matched as calibration blends prepared in solvent. Matrix-matched calibration curves were constructed using extracted blank matrix samples spiked with a known amount of native and carbon labelled PFCs. These calibration curves were combined as both provided similar regression equations that when combined gave a R_2 value greater than 0.9. A blank matrix sample, method blank and spiked matrix matched recovery control samples were included in each extraction series to ensure the quality of analytical runs. Results reported have not been corrected for recoveries as the use of labelled standards compensated for losses during the extraction procedure. The limit of detection (LOD) and limit of quantification (LOQ) was determined using linear regression (Miller & Miller, 2000). In short, a calibration curve was prepared by spiking extracted matrix with native and labelled PFCs. A linear regression was applied, where S_a (uncertainty in the intercept) was defined as the intercept and the LOD was defined as three times the standard deviation of $S_{Y/X}$ (random calibration uncertainty) and the LOQ was defined as ten times the standard deviation of $S_{Y/X}$.

3.6. Data Analysis

Obtained concentrations are reported in ng/g wm for the fish and bird eggs, and ng/g dm for the sediment. For statistical analysis and comparisons between sites, species and ecological parameters no-detects (ND) were assigned a value of ½ LOD. Statistical analyses were performed on log-transformed data (ng/g wm), unless specified otherwise, utilizing Microsoft

Excel, STATISTICA (StatSoft; version 10) and XLSTAT (Addinsoft; version 2015). Parametric statistical analysis was performed, assuming normal distribution, as the dataset was larger than 30 samples (n=105). To compare sites, species and compounds a one-way ANOVA with a post-hoc Tukey analyses were performed using STATISTICA. A p-value smaller than 0.05 ($p < 0.05$) was considered statistically significant.

Exploratory statistics was done using cluster analyses as well as principal component analysis (PCA). A joining tree cluster analysis, single linkage rule, Euclidean distance, was conducted to evaluate the groupings formed by the PFC congeners within the data set (Fowler *et al.*, 1998). PCAs were performed with XLSTAT2015 to determine the distribution patters of pollutants within the wild bird egg dataset. Major ions and environmental factors were plotted on the PCA as additional data. Where no detection of compounds were present, the value was replaced by a ½ LOD. As patterns of the compounds were evaluated, zero-values would not be environmentally representative. Therefore, the substitution with ½ LODs were done to ensure comparability (Fowler *et al.*, 1998). The goal of a PCA is to identify which variables explain the largest amount of variance within a data. Variables further from the axis of the PCA bi-plot contribute the most, and variables closer to the axis contribute less to the variation of the data set. To minimise the effect of individual concentrations of compounds and to facilitate better distribution within a dataset to evaluate patterns and relationships between compounds, the data was transformed according to a method by Howel,(2007). Data was log-transformed, and the centred log ratio of each proportion (p) was determined by dividing the geometric mean across the sample: $\log(p_{ij}/g(p_j))$ where $g(p_j) = (p_{1j}/p_{2j}\ldots\ldots,p_{dj})_{1/d}$ where d pollutants are measured in n samples so that p_{ij} is the proportion of pollutant i in sample j (Howel, 2007).

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Chapter 4: Results

"The first law of ecology: everything is connected to' everything else"
Barry Commoner

This chapter will report on the results of PFCs in the various matrices analysed, including concentrations and congener patterns. As wild bird eggs was the only matrix with appreciable levels of PFCs, this matrix is further investigated using multivariate statistical analysis as described in Chapter 3. The bird egg results are reported in ng/g wm, and the abbreviations for sites and species are summarised in **Table 4.1.**

Table 4.1: Abbreviation list for species, sites and PFC congeners

Species	Abbreviation	Sites	Abbreviation	PFC congeners
African Darter	AD	Barberspan	P	**Short-chain PFSAs**
Black-headed Heron	BHH	Bloemhof	H	PFBS, PFHxS
Cattle Egret	CE	Katse Dam	K	**Long-chain PFSA**
Glossy Ibis	GI	Lenasia	N	PFOS
Grey Heron	GH	Orkney	O	**Short-chain PFCAs**
Reed Cormorant	RC	Sasolburg	A	PFHxA, PFHpA, PFOA
Sacred Ibis	SI	Schoemansdrift	M	
White-breasted cormorant	WBC	Upington	U	**Long-chain PFCAs**
		Welverdiend	W	PFNA, PFDA, PFUnA, PFDoA, PFTA, PFTrDA

4.1 Comparison between matrices

Sediment, mine tailings, fish, feathers and bird eggs were analysed for PFCs. Of the 62 fish samples analysed, none contained detectable levels of PFCs (**Figure 4.1a**). Two (6%) of the 33 water samples analysed had detectable levels of PFCs, these were water samples collected at Barkley West (0.24 ng/L PFBS; **Figure 4.1b**) and Caledon (1.2 ng/L PFUnA; **Figure 4.1b**). Of the 11 sediment samples analysed, three (27%) had measurable levels of PFCs (**Figure 4.1c**); sediment collected from Jet Park (4.4 ng/g PFOS, Figure 4.1c), Welverdiend (11 ng/g PFOS, Figure 4.1c) and Orkney (4.6 ng/g PFHxA, **Figure 4.1c**). As previously discussed, mining activity was identified as a possible source of PFCs. Of the 13 mine tailing, three contained PFCs; Buffelsfontein (4.44 ng/g PFHxA), Orkney (4.79 ng/g PFHxA) and Germiston (8.08 ng/g PFOA). Eggs from eight bird species were sampled (n=105) from nine sites. Of the eggs analysed, 95% had measurable levels of PFCs (Figure 4.1a). The main contributing congener was PFOS (95%), followed by PFTrDA (47%), PFOA (40%), PFNA (36%), PFTA (35%), PFDA (34%), PFUnA (28%), PFDoA (25%), PFBS (19%), PFHxS (5%), PFHpA (4%) and PFHxA (3%) (**Figure 4.1d**). To assess the suitability of feathers as an alternative monitoring matrix and to investigate preening behaviour as a source of PFCs, a screening method was applied to bird feathers collected from Upington. Two feathers had detectable levels of PFOS and PFUnA respectively. Due to the limited data obtained from other matrices, the rest of the chapter will focus on bird eggs.

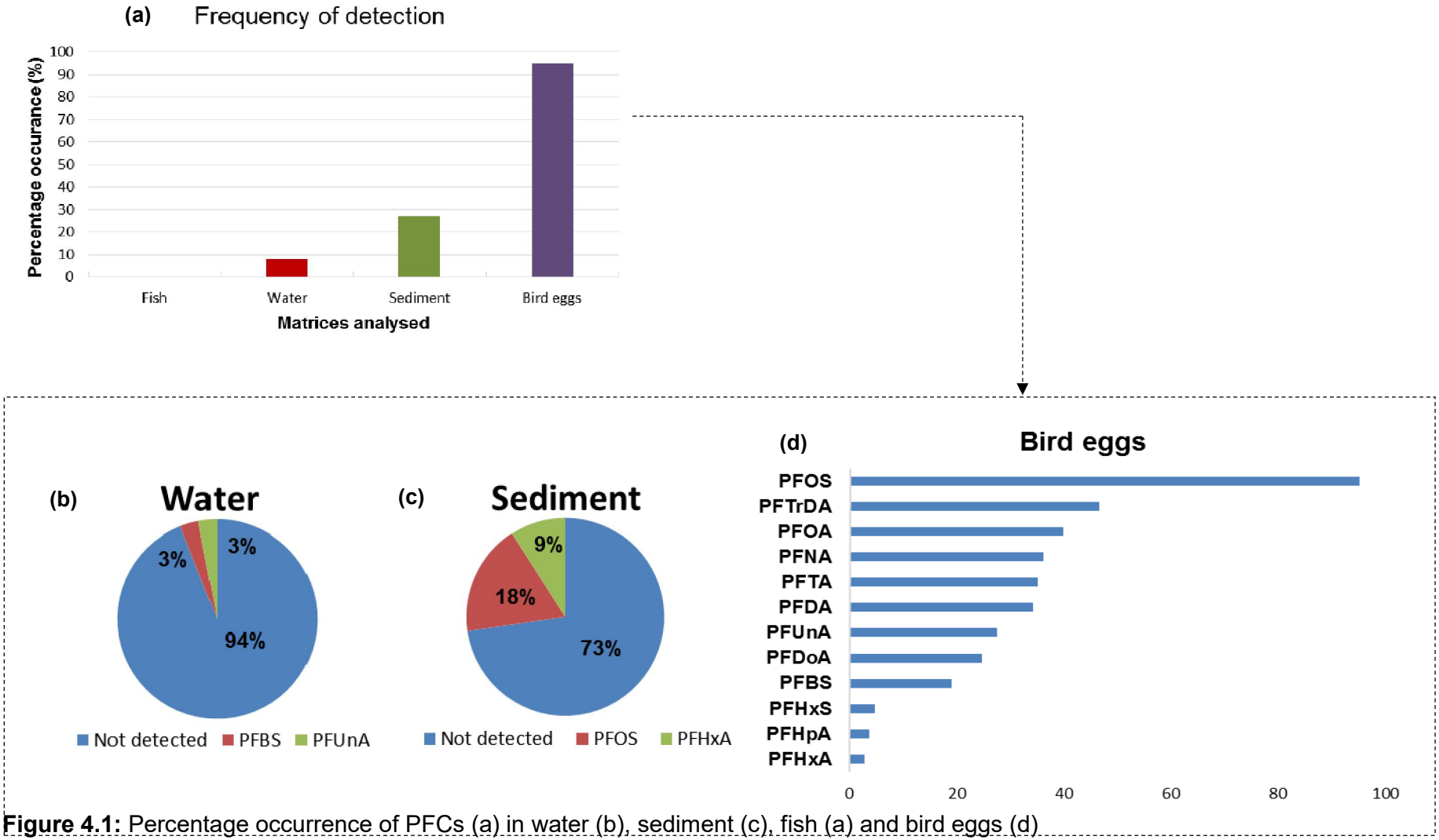


Figure 4.1: Percentage occurrence of PFCs (a) in water (b), sediment (c), fish (a) and bird eggs (d)

4.2. PFCs in wild bird eggs

4.2.1. Concentrations of PFCs

PFCs were measured in 95% of the eggs analysed, with PFOS as the most prevalent congener (**Figure 4.1a; Table 4.2**). The ΣPFCs ranged from 60 – 1200 ng/g wm (**Table 4.2**), with the highest concentration found in African Darter eggs, and the lowest in the Glossy Ibis eggs (**Table 4.2**). The highest median concentration of ΣPFCs was found in African Darter eggs (1100 ng/g), followed by White-breasted Cormorant eggs (490 ng/g), Reed Cormorant eggs (240 ng/g), Grey Heron eggs (70 ng/g), and the lowest in the Black-headed Heron eggs (20 ng/g). The highest concentration of PFOS was found in an African Darter egg collected from Bloemhof (2817 ng/g) and the lowest concentration was found from a Cattle Egret egg collected from Barberspan (2 ng/g).

When comparing the distribution PFCs according to chain length; the sum of long-chained PFSA (PFOS) was the highest (430 ng/g), followed by the long-chain PFCAs (PFDA, PFUnA, PFDoA, PFTrDA and PFTA; 20 ng/g). The short-chain PFSAs (PFBS and PFHxS; 3 ng/g) and PFCAs (PFHxA, PFHpA and PFOA; 2 ng/g) were the lowest (**Figure 4.2**).

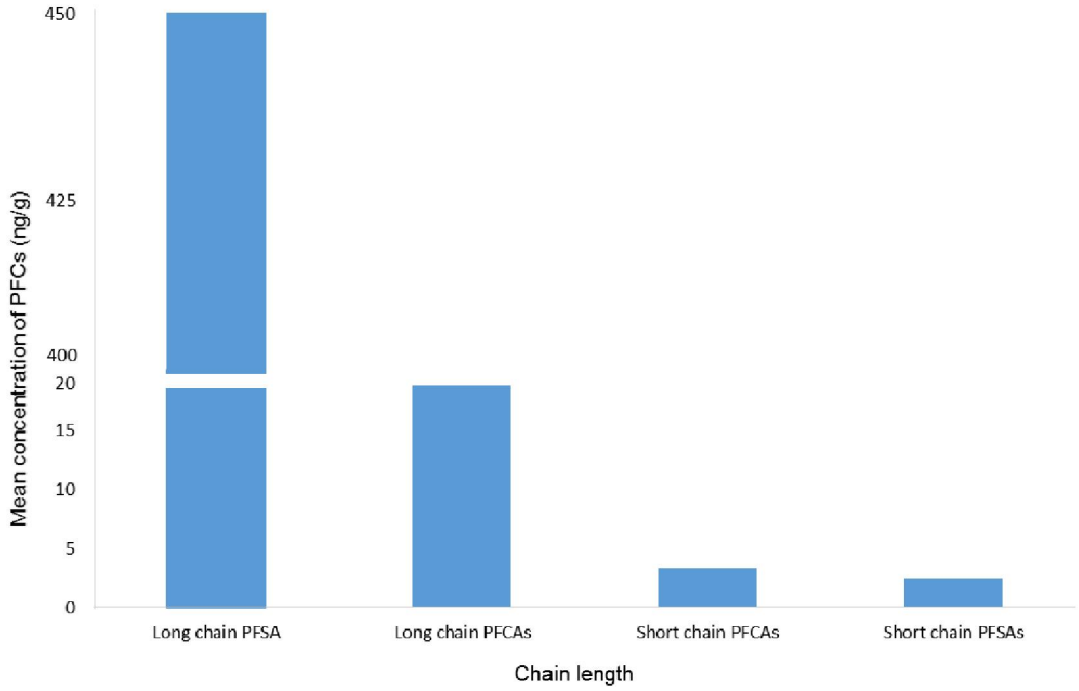


Figure 4.2: The ΣPFCs distribution according to chain length

Table 4.2: PFC concentrations (ng/g wm) in eggs of selected species

Congeners	Min	Max	Mean	Median	Congeners	Min	Max	Mean	Median
Piscivorous species									
African Darter (n = 26)					**Grey *Heron (n = 4)***				
PFBS	ND	20	2	0.1	PFBS	ND	ND	ND	ND
PFHxA	ND	5	0.3	0.1	PFHxA	ND	ND	ND	ND
PFHxS	ND	30	1	0.07	PFHxS	ND	ND	ND	ND
PFHpA	ND	80	5	0.2	PFHpA	ND	ND	ND	ND
PFOS	30	2800	1100	1200	PFOS	80	950	550	590
PFOA	ND	9	2	0.1	PFOA	ND	ND	ND	ND
PFNA	ND	20	10	10	PFNA	ND	ND	ND	ND
PFDA	ND	120	20	10	PFDA	ND	3	1	1
PFUnA	ND	9	4	4	PFUnA	ND	ND	ND	ND
PFDoA	ND	10	3	0.1	PFDoA	ND	ND	ND	ND
PFTrDA	ND	4	2	3	PFTrDA	ND	ND	ND	ND
PFTA	ND	8	2	0.01	PFTA	ND	ND	ND	ND
ΣPFCs	50	2900	1200	1200	ΣPFCs	50	260	120	70
Reed Cormorant (n = 11)					***White-breasted Cormorant (n = 9)***				
PFBS	ND	7	2	ND	PFBS	ND	7	1	0.1
PFHxA	ND	ND	ND	ND	PFHxA	ND	0.4	0.2	0.07
PFHxS	ND	ND	ND	ND	PFHxS	ND	ND	ND	ND
PFHpA	ND	ND	ND	ND	PFHpA	ND	ND	ND	ND
PFOS	40	1950	1100	200	PFOS	7	670	370	470
PFOA	ND	13	2	0.07	PFOA	ND	ND	ND	ND
PFNA	ND	22	6	6	PFNA	ND	15	4	4
PFDA	ND	25	6	2	PFDA	ND	70	20	20
PFUnA	ND	9	3	0.2	PFUnA	ND	12	5	4
PFDoA	ND	13	5	0.1	PFDoA	ND	17	3	0.1
PFTrDA	ND	4	2	3	PFTrDA	ND	5	1	0.05
PFTA	ND	7	3	4	PFTA	ND	7	1	0.01
ΣPFCs	80	2000	580	240	ΣPFCs	8	740	400	490
Insectivore species									
Cattle Egret (n = 30)					**Glossy Ibis *(n = 4)***				
PFBS	ND	9	2	0.1	PFBS	ND	ND	ND	ND
PFHxA	ND	1	0.2	0.1	PFHxA	ND	ND	ND	ND
PFHxS	ND	9	0.4	0.2	PFHxS	ND	8	2	0.07
PFHpA	ND	ND	ND	ND	PFHpA	ND	ND	ND	ND
PFOS	0.3	1000	76	9	PFOS	0.3	260	80	50
PFOA	ND	5	1	0.07	PFOA	ND	2	0.6	0.07
PFNA	ND	10	0.9	0.1	PFNA	ND	0.4	0.2	0.1
PFDA	ND	4	0.4	0.4	PFDA	ND	0.1	0.06	0.04
PFUnA	ND	12	1	0.2	PFUnA	ND	ND	ND	ND
PFDoA	ND	12	2	0.1	PFDoA	ND	5	1	0.1
Insectivore species									
Cattle Egret (n = 30)					**Glossy Ibis *(n = 4)***				
PFTrDA	ND	5	1	0.05	PFTrDA	ND	4	1	0.05
PFTA	ND	6	2	0.1	PFTA	ND	0.9	0.2	0.01
ΣPFCs	6	1000	90	20	ΣPFCs	1	260	80	50
Scavenger species					**Generalist species**				
Sacred Ibis (n = 15)					**Black-headed Heron *(n = 6)***				
PFBS	ND	7	0.5	0.1	PFBS	ND	ND	ND	ND
PFHxA	ND	10	0.8	0.1	PFHxA	ND	ND	ND	ND
PFHxS	ND	40	2	0.07	PFHxS	ND	ND	ND	ND
PFHpA	ND	ND	ND	ND	PFHpA	ND	ND	ND	ND
PFOS	0.3	30	10	10	PFOS	3	730	130	6
PFOA	ND	9	3	2	PFOA	ND	3	1	0.07
PFNA	ND	20	2	0.1	PFNA	ND	0.4	0.2	0.12
PFDA	ND	ND	ND	ND	PFDA	ND	7	1	0.04
PFUnA	ND	ND	ND	ND	PFUnA	ND	8	2	0.2
PFDoA	ND	5	1	0.1	PFDoA	ND	6	1	0.1
PFTrDA	ND	4	3	3	PFTrDA	ND	4	2	2
PFTA	ND	5	1	1	PFTA	ND	1	0.3	0.01
ΣPFCs	10	80	30	30	ΣPFCs	3	730	160	20

ND: Not detected

4.2.2. Congener profile of PFCs in wild bird eggs

A cluster analyses was conducted with log-transformed data to evaluate the groupings formed by the PFC congeners within the data set. A joining tree cluster analysis with single linkage rule, Euclidean distance was performed (**Figure 4.3**). The cluster analysis grouped the PFC congeners into three groupings (1 - 3). The cluster separated PFOS (**Figure 4.3**; **1A**) from all other congeners (**Figure 4.3**; **1B**). The second grouping is formed between long-chain PFCAs and PFOA (**Figure 4.3**; **2A**) against a mixture of long- and short-chain PFCAs and short-chain PFSAs (**Figure 4.3**; **2B**). The final group consisted of short-chain PFCs (**Figure 4.3**; **3**).

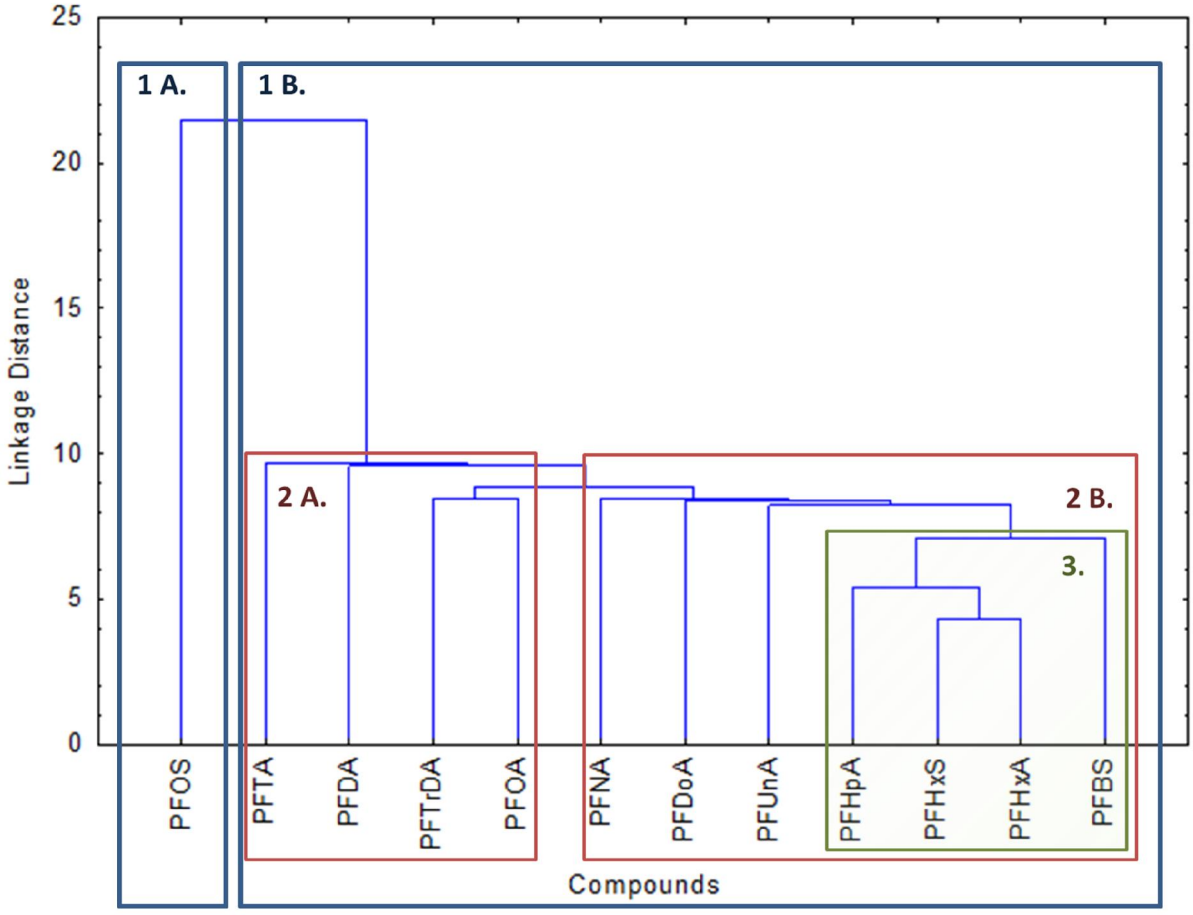


Figure 4.3: Cluster analysis of compounds in bird egg data with grouping indicators (1A, 1B; 2A, 2B and 3)

The congener profiles of PFCs in individual species were compared (**Figure 4.4**). PFOS was the dominant congener detected, contributing more than 90% in the African Darter, Grey Heron, Reed Cormorant, White-breasted Cormorant, Glossy Ibis and Black-headed Heron eggs. Whereas, only 87% in the Cattle Egret, and 48% in the Sacred Ibis eggs. For the Sacred Ibis eggs, the other

congeners contributed, such as PFHxS (9%), PFOA (11%), PFTrDA (9%), PFNA (7%) and PFTA (6%).

The levels of PFOS, PFNA, PFDA, and PFUnA differed statistically significantly between the bird species ($p < 0.05$, one-way ANOVA of log-transformed data; **Figure 4.4**). PFOS levels were significantly higher in the African Darter eggs compared to the Cattle Egret, Reed Cormorant and Sacred Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test) whereas the Reed Cormorant eggs had significantly higher levels than the Cattle Egret and Sacred Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test). PFDA levels were significantly higher in the African Darter eggs compared to the Cattle Egret, Black-headed Heron and Sacred Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test). The White-breasted Cormorant eggs had statistically significantly higher levels of PDFA than the Cattle Egret, Black-headed Heron, Sacred Ibis and Glossy Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test) and the Reed Cormorant eggs had significantly higher levels compared to the Sacred Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test). The White-breasted Cormorant eggs had higher levels for PFDA and PFUnA than the Cattle Egret, Black-headed Heron, Grey Heron, Sacred Ibis and Glossy Ibis eggs ($p < 0.05$, one-way ANOVA, post-hoc Tukey test).

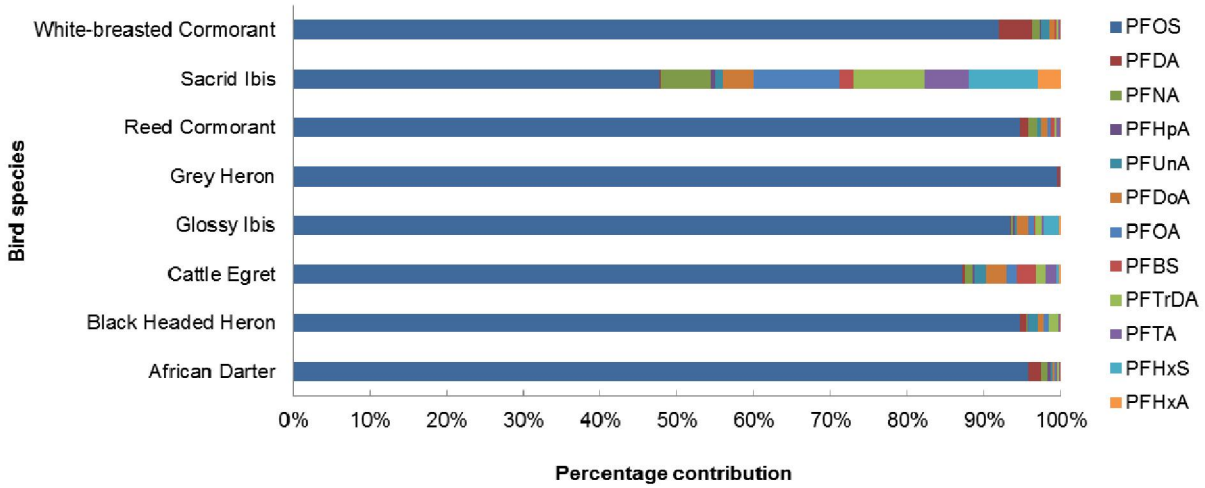


Figure 4.4: Proportional contribution of congeners to Σ PFCs in bird eggs

When PFCs were grouped according to their chain length categories, there were statistically significant differences between the bird species analysed as well as the sites where the bird eggs were collected from (one-way ANOVA, $p < 0.05$, log-transformed data). The post-hoc Tukey analysis indicated a statistically significant difference between the long-chain PFSA and PFCAs between the bird species. For the long-chained PFSAs, the African Darter eggs had higher levels than the Cattle Egret, Black-headed Heron, and the Sacred Ibis eggs. For the long-chained PFCAs, the African Darter eggs had higher levels than the Cattle Egret, White-breasted Cormorant, Black-headed Heron, and Sacred Ibis eggs. However, the Glossy Ibis eggs had higher

concentrations than the African Darter eggs. Comparing the variation of PFCs (as per chain length) per species per site, the following was seen:

- For the African Darter eggs, the proportional contribution of long-chain PFCAs at Upington was higher than at any other site (**Figure 4.5**).
- Welverdiend had a higher proportional contribution of long-chain PFCAs for the Reed Cormorant eggs than at any other site. However, Orkney had the highest PFSA contribution (**Figure 4.6**).
- The White-breasted Cormorant eggs from Schoemansdrift had a higher proportional contribution of long-chain PFCAs than Katse Dam (**Figure 4.7**).
- For the Cattle Egret eggs, long-chain PFCAs and short-chain PFSAs had a higher proportional contribution at Upington than any other site. Bloemhof had the highest contribution of short-chain PFCAs (**Figure 4.8**).
- Bloemhof had the largest proportional contribution of long-chain and short-chain PFCAs for the Glossy Ibis eggs (**Figure 4.9**).
- For the Black-headed Heron eggs, Sasolburg had the highest proportional contribution of long-chain PFCAs, and Lenasia the highest contribution of short-chain PFCAs. Bloemhof had the highest proportional contribution for long-chain PFSA (**Figure 4.10**).
- Bloemhof had the lowest proportional contribution of long-chain PFSA, and the highest short-chain PFSA proportional contribution for the Sacred Ibis (**Figure 4.11**).

To investigate the variations occurring between sites, a post-hoc Tukey analysis was performed with the significant differences summarised in **Table 4.3**.

Table 4.3: Summary of statistical significant differences between congeners and sites

Short-chain PFSAs	Long-chain PFSA	Long-chain PFCAs
Welverdiend had higher levels than all other sites.	Schoemansdrift had higher levels than Sasolburg, Bloemhof, Lenasia and Katse Dam. Welverdiend had higher levels than Barberspan, Sasolburg, Lenasia and Katse Dam.	Welverdiend had higher levels than all other sites. Schoemansdrift had higher levels than Barberspan, Sasolburg, Bloemhof, Lenasia, Upington and Katse Dam. Orkney had higher levels than Barberspan, Sasolburg, Lenasia and Upington.

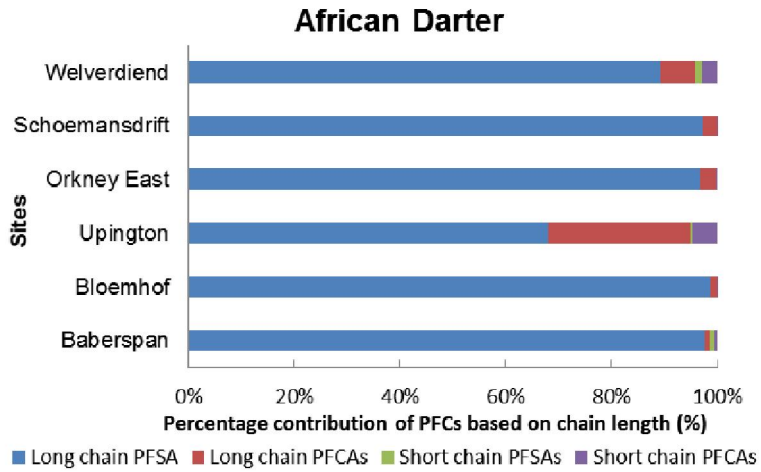


Figure 4.5: Chain length proportions of African Darter species per site

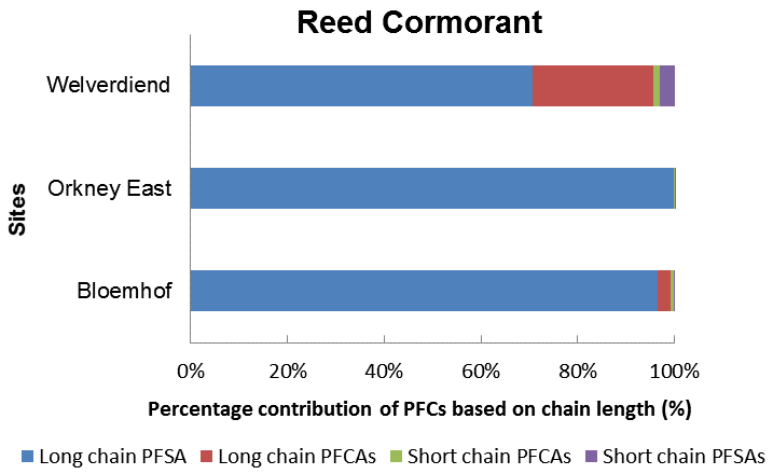


Figure 4.6: Chain length proportions of Red Cormorant species eggs per eggs site

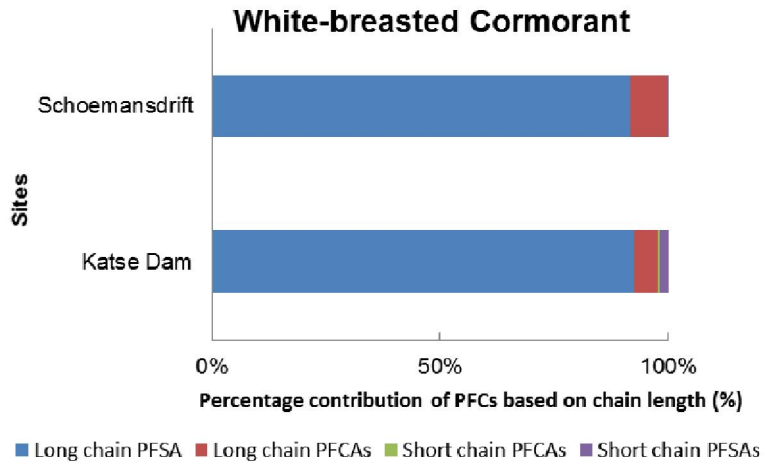


Figure 4.7: Chain length proportions of White-breasted Cormorant species eggs per site

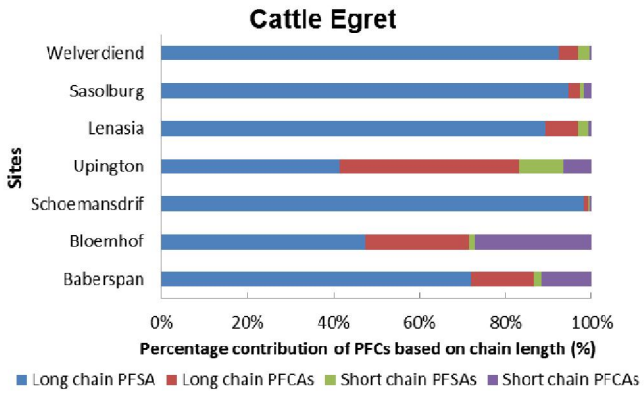


Figure 4.8: Chain length proportions of Cattle Egret species eggs per site

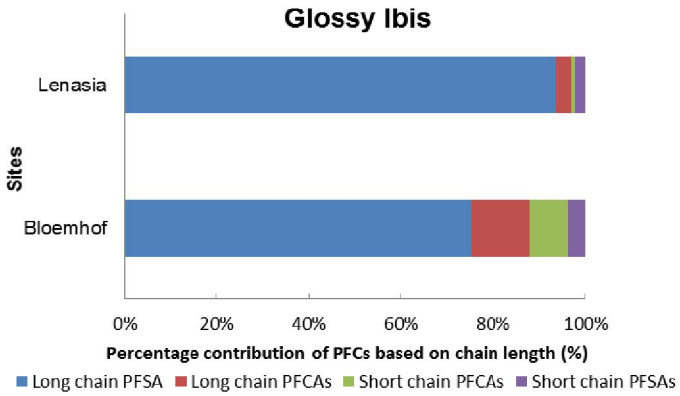


Figure 4.9: Chain length proportions of Glossy Ibis species eggs per site

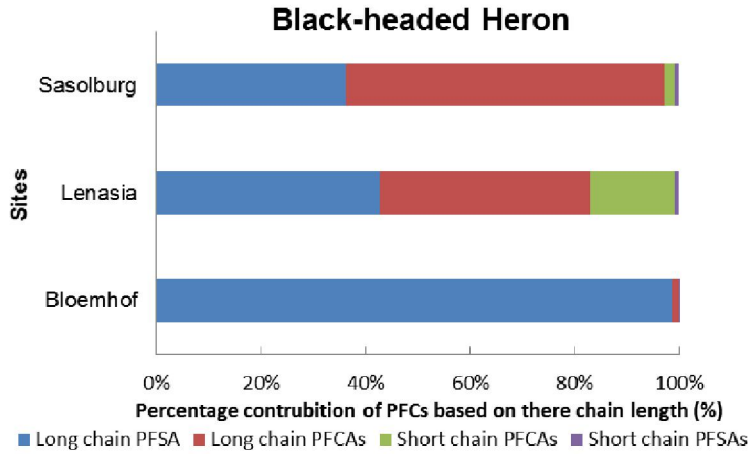


Figure 4.10: Chain length proportions of Black-headed Heron species eggs per site

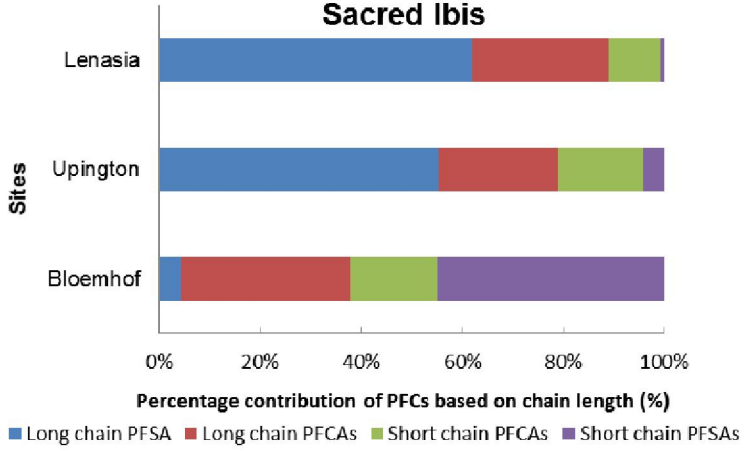


Figure 4.11: Chain length proportions of Sacred Ibis species eggs per site

4.2.3 The impact of trophic guild on the distribution of PFCs

A statistically significant difference was found between trophic guilds and concentrations of PFOS, PFOA, PFNA, PFDA and PFUnA (one-way ANOVA, $p < 0.05$, log-transformed data). The ΣPFCs in the trophic guilds were highest in the piscivores (860 ng/g), followed by generalist (160 ng/g), insectivores (90 ng/g), and scavengers (30 ng/g). The post-hoc Tukey test ($p < 0.05$) indicated that piscivores had a significantly higher proportional contribution of PFOS, PFNA, PFDA and PFUnA. Additionally, for PFOA, scavengers had a higher proportion than insectivores (**Figure 4.12**).

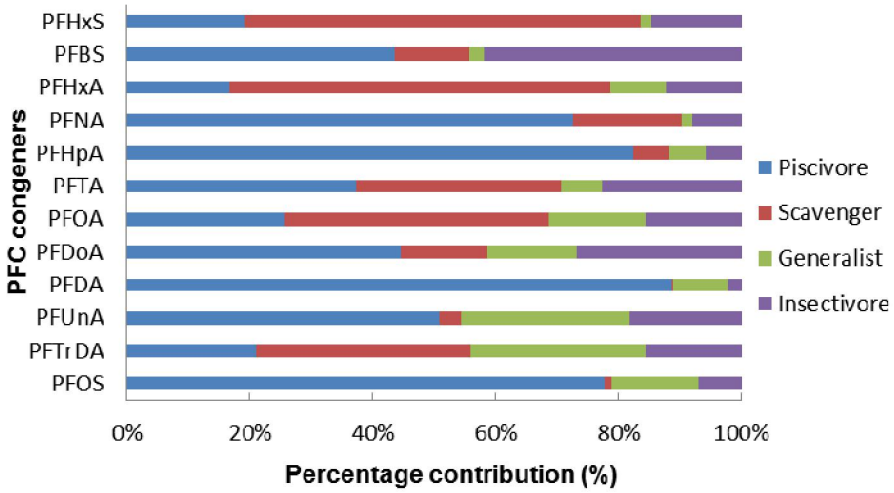


Figure 4.12: Percentage distribution of congeners between bird eggs from feeding guilds

4.2.4 Investigating the effect of feeding habitat on the distribution of PFCs

The species selected were divided into feeding habitats as described in **Chapter 3, Table 3.2**; aquatic (White-breasted Cormorant, Reed Cormorant and African Darter), terrestrial (Glossy Ibis, Grey Heron and Cattle Egret) and combined (Sacred Ibis and Black-headed Heron). A one-way ANOVA (log-transformed data) was preformed to evaluate the effect of feeding habitat on the congener profiles of PFCs. The analysis indicated statistically significant differences ($p < 0.05$) for PFOS, PFOA, PFNA, PFDA, PFUnA, PFTrDA and PFTA. The post-hoc Tukey indicated that aquatic feeding birds had statistically significantly higher levels of PFCs with the exception of PFTrDA and PFTA. The levels of PFTrDA and PFTA were similar in all feeding habitats (**Figure 4.13**).

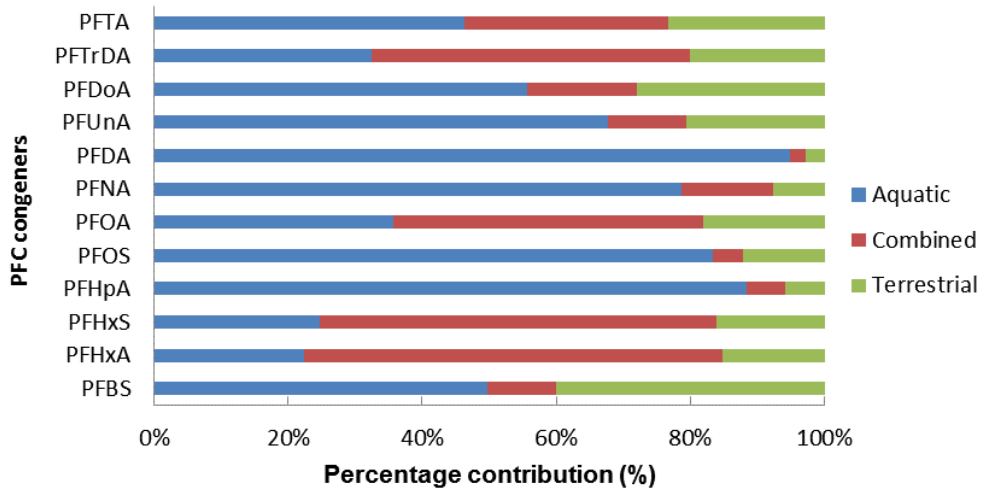


Figure 4.13: Proportional contribution of PFC congeners to feeding habitat

4.2.5 Contribution of site selection to PFC congener profile

To investigate the possible effect of the collection sites and possible source contributions on concentrations and profiles of PFCs, a one-way ANOVA and post-hoc Tukey test was performed using log-transformed data. The site selection had a significant impact on the concentrations of PFCs ($p < 0.05$). The statistically significant differences are described below:

- PFBS: Welverdiend had higher levels than Barberspan, Schoemansdrift, Bloemhof, Lenasia, and Upington
- PFHpA: Welverdiend had higher levels than Schoemansdrift, Bloemhof, Lenasia, and Upington
- PFOS: Schoemansdrift had higher levels than Barberspan, Lenasia, and Upington. Orkney had higher levels than Upington, Barberspan and Lenasia
- PFNA: Schoemansdrift had higher levels than Lenasia
- PFDA: Schoemansdrift had higher levels than Barberspan, Sasolburg, Bloemhof, Lenasia, and Upington. Welverdiend had higher levels than Lenasia. Barberspan, and Sasolburg had the lowest levels
- PFUnA: Welverdiend and Schoemansdrift had higher levels than Bloemhof, Lenasia, and Upington
- PFDoA: Welverdiend had higher levels than all other sites
- PFTrDA: Upington had higher levels than Barberspan, Schoemansdrift, Orkney, Bloemhof, and Lenasia. Welverdiend was higher than Schoemansdrift and Bloemhof
- PFTA: Welverdiend had higher levels than Barberspan, Schoemansdrift, Bloemhof, and Orkney. Orkney and Sasolburg had the lowest levels

Comparing the mean concentrations of PFOS (**Figure 4.14**), Orkney had the highest concentration (1600 ng/g) followed by Schoemansdrift (1100 ng/g), Bloemhof (520 ng/g), Barberspan (420 ng/g), Welverdiend (400 ng/g), Katse Dam (100 ng/g), Lenasia (50 ng/g), Upington (24 ng/g), and Sasolburg (16 ng/g). The maximum and minimum concentrations of congeners at each collection site is summarised in **Table 4.4** and the mean is plotted in **Figure 4.15**.

Table 4.4: Minimum and maximum concentrations of PFCs measured at each collection site

Congeners	Min (ng/g)	Max (ng/g)
PFBS	Sasolburg, Orkney, Schoemansdrift, Bloemhof, Barberspan, Lenasia (LOD)	Welverdiend (7)
PFHxA	Sasolburg, Orkney, Schoemansdrift, Welverdiend, Barberspan, Lenasia, Upington (LOD)	Barberspan (1)
PFHxS	Sasolburg, Orkney, Schoemansdrift, Katse Dam (LOD)	Barberspan (5)
PFHpA	All sites LOD except Welverdiend	Welverdiend (7)
PFOS	Sasolburg (16)	Orkney (1600)
PFOA	Sasolburg and Katse Dam (LOD)	Upington (3)
PFNA	Sasolburg (LOD)	Schoemansdrift (7)
PFDA	Sasolburg and Barberspan (LOD)	Schoemansdrift (30)
PFUnA	Barberspan (LOD)	Welverdiend (5)
PFDoA	Orkney, Sasolburg and Katse Dam (LOD)	Welverdiend (9)
PFTrDA	Bloemhof (0.3)	Welverdiend (3)
PFTA	Sasolburg and Orkney (LOD)	Welverdiend (5)

PFTrDA and PFOS were found in eggs collected at all the collection sites (**Figures 4.16 and 4.17; Table 4.5**). Among the PFCs analysed, PFOS was the most prevalent congener detected at each site (82 – 100%), followed by PFTrDA (15 – 94%), PFOA (8 – 71) and PFDA (6 – 69%). Sasolburg and Barberspan had the lowest occurrence of PFC congeners, while Welverdiend had the highest with only PFHxA not detected in any of the eggs collected at this site. PFBS was only detected at Katse Dam (25%) and Welverdiend (88%), whereas PFHxA was only detected at Barberspan and Bloemhof, and PFHxS at Lenasia and Welverdiend.

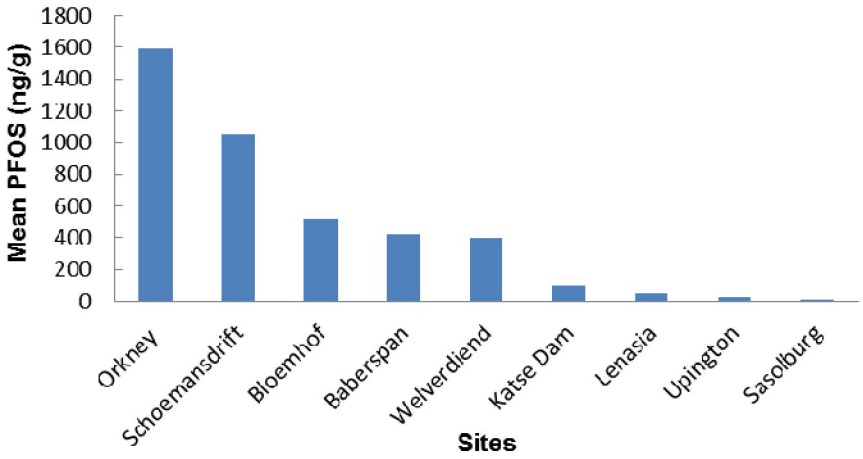


Figure 4.14: Mean concentrations of PFOS at each collection site

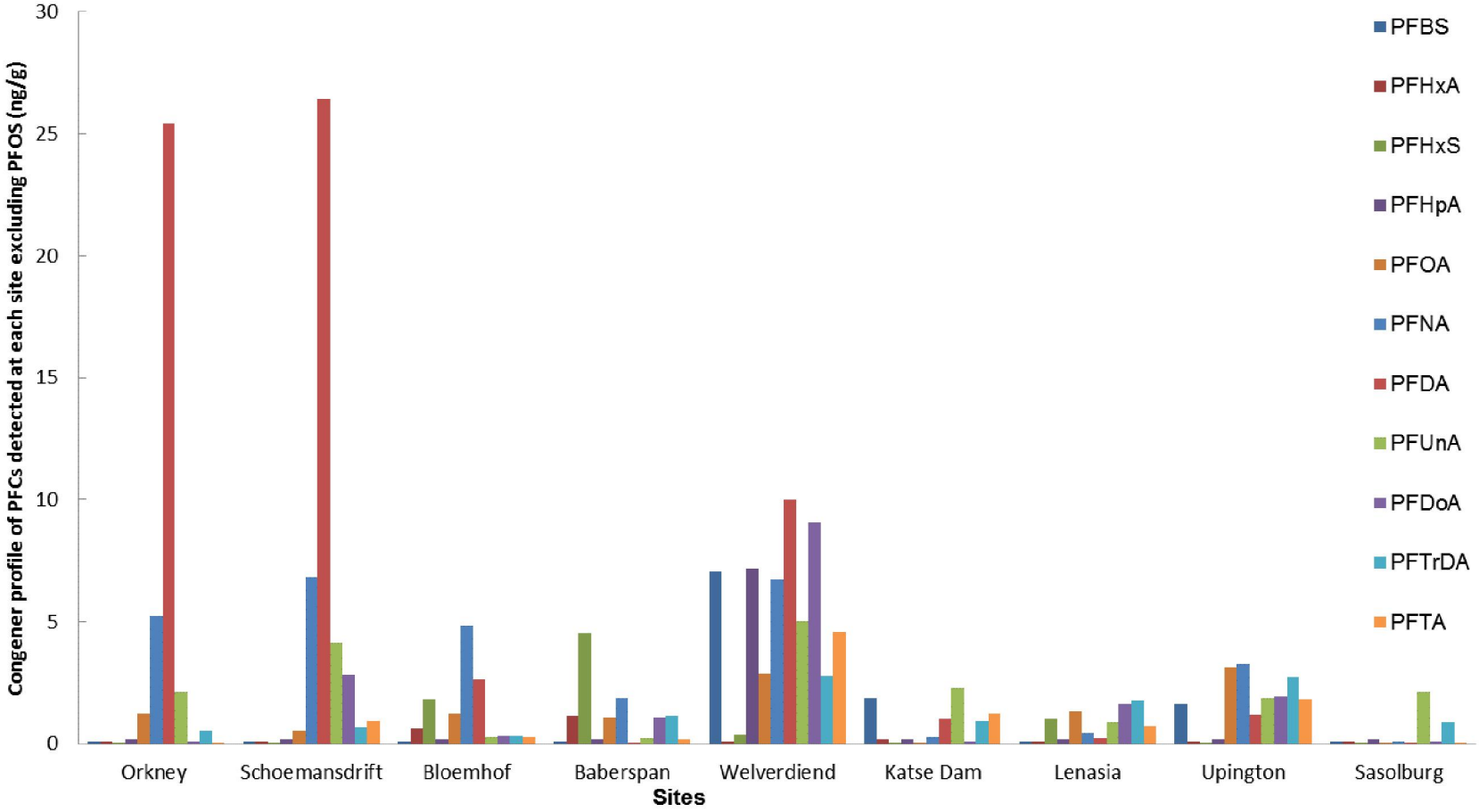


Figure 4.15: Mean concentrations of PFC congeners, excluding PFOS, detected in the wild bird eggs collected at each site

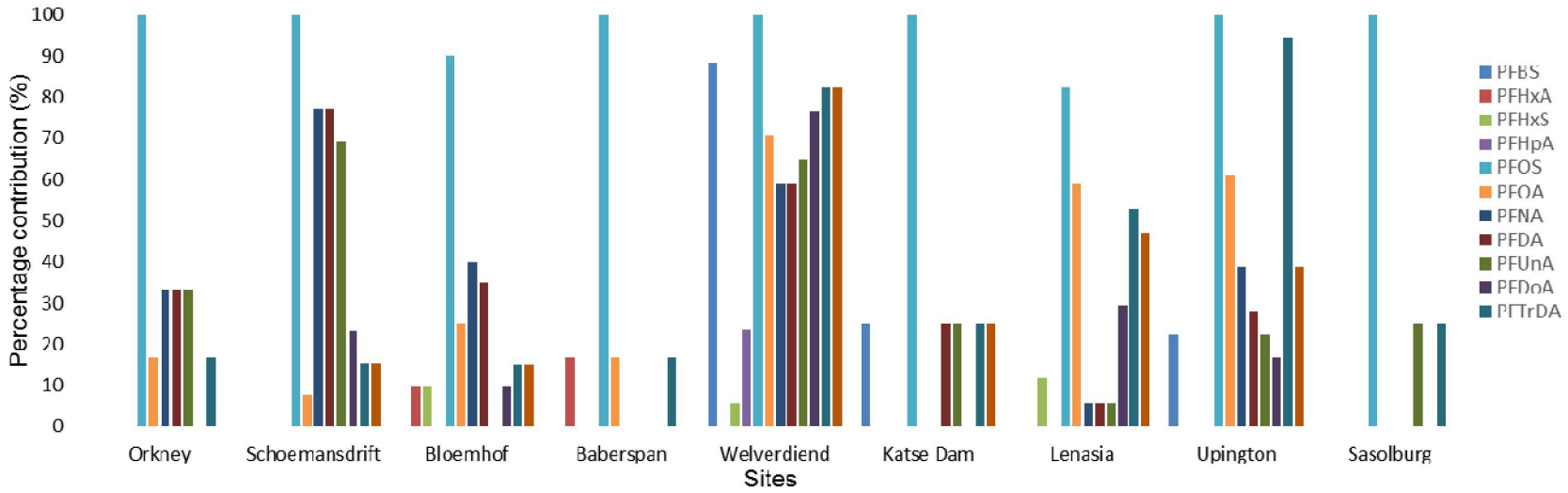


Figure 4.16: Composition of PFCs at each collection site.

Table 4.5: Percentage occurrence of PFCs in collection sites

Collection site	PFBS	PFHxA	PFHxS	PFHpA	PFOS	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTA
Barberspan	0	17	0	0	100	17	0	0	0	0	17	0
Bloemhof	0	10	10	0	90	25	40	35	0	10	15	15
Katse Dam	25	0	0	0	100	0	0	25	25	0	25	25
Upington	22	0	0	0	100	61	39	28	22	17	94	39
Lenasia	0	0	12	0	82	59	6	6	6	29	53	47
Orkney	0	0	0	0	100	17	33	33	33	0	17	0
Sasolburg	0	0	0	0	100	0	0	0	25	0	25	0
Schoemansdrift	0	0	0	0	100	8	77	77	69	23	15	15
Welverdiend	88	0	6	24	100	71	59	59	65	76	82	82

4.2.6 PCA analysis of PFCs

A principle component analysis (PCA) was conducted to investigate the congener profiles of PFCs in bird eggs, as well as the possible contribution of environmental factors (pH, hardness, TDS and EC), major contributing ions (Na, K, Mg, NH_4, CO_3, Ca, HCO_3, F, Cl, SO_4, SI, Tal, PO_4, NO_3,) and geographical distribution. As discussed previously in **Chapter 2, Section 2.1.3** environmental conditions can also influence the partitioning of PFCs between different environmental compartments (Higgins & Luthy, 2006; Kostianoy *et al.*, 2012). These factors can cause exponential changes in the concentration of PFOS as reported in literature (Lasier *et al.*, 2011). Historical inorganic water chemistry data was utilized for the comparison in the PCA bi-plot (**Figure 4.18 – 4.19**; Huizenga *et al.*, 2013)

The same PCA analysis is described in **Figure 4.18 – 4.22**. For clarity, **Figure 4.18** indicates only the PCA congeners and the distribution of ions used in water quality determination while **Figure 4.19** indicates PCA congeners and the distribution of environmental factors. The distribution of wild bird eggs is present in **Figure 4.20**, with focus on the groupings of bird species highlighted in **Figure 4.21**, and sites in **Figure 4.22**.

In the PCA bi-plot (**Figure 4.18**), factor 1 explained 42% of the variance within the data set and was a contrast between PFDA, PFNA and PFOS with positive scores, and PFTA, PFTrDA, PFDoA and PFOA with negative scores. Ions grouped along factor 1 and was a contrast between CO_3, SO_4 and Mg with positive scores, and NO_3, PO_4, NH_4 and SI with negative scores. Environmental factors is grouped along factor 1 (**Figure 4.19**), with a contrast between pH with positive scores, and hardness with negative scores. Bird species was separated along Factor 1 (**Figure 4.21**) and was a contrast between African Darter and White-breasted Cormorants eggs with positive loadings, and Sacred Ibis eggs with negative loadings. Factor 2 explained 18% of the variance, and was a contrast between PFTA and PFDA with positive scores and PFHxS, and PFHxA with negative scores. Ions grouped along factor 2 (**Figure 4.18**) and was a contrast between NO3, Na and PO4 with positive scores, and NH4, Mg and CO3 with negative scores. Additionally, a Cattle Egret eggs grouping is seen in quadrant 1 and 2. Groupings of sites were also formed in the following quadrants (**Figure 4.22**): Quadrant 1 formed a grouping of birds from Upington, Quadrant 3 formed a combined grouping of birds from Schoemansdrift and Bloemhof, in Quadrant 3 and 4 a grouping from Welverdiend, and in 4 a grouping from Lenasia.

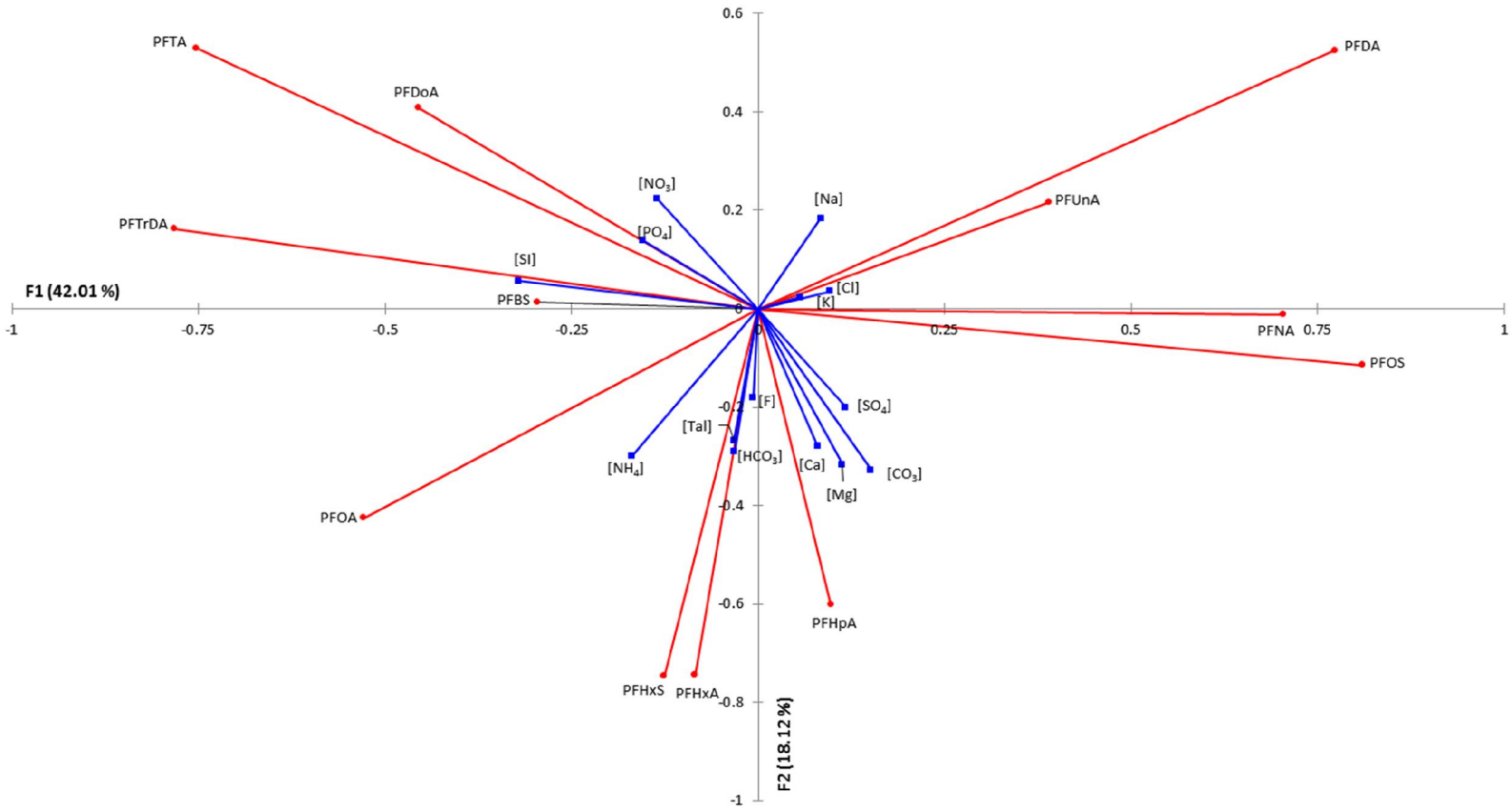


Figure 4.18: Bi-plot of PCA for PFCs in bird eggs together with major ions

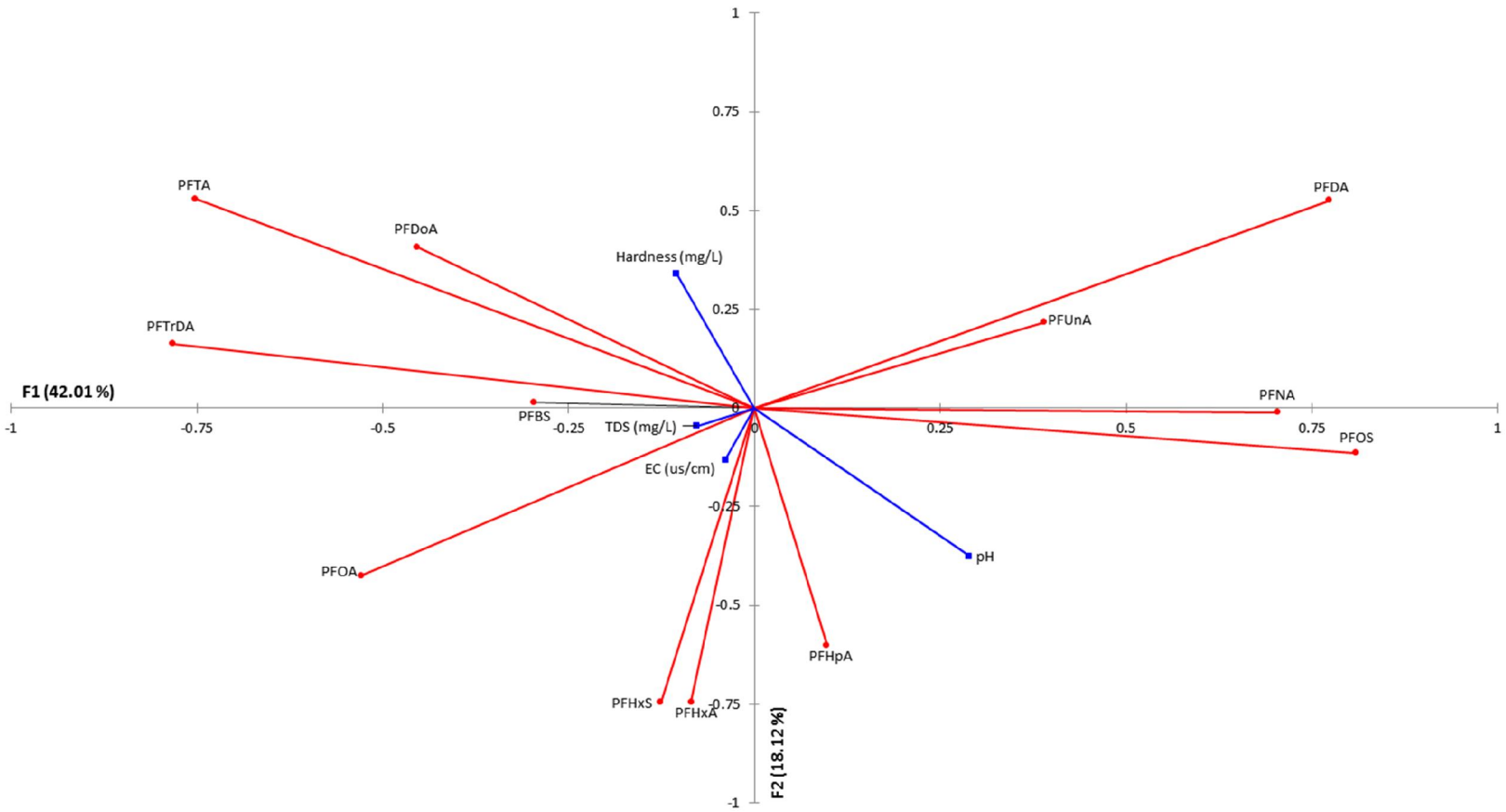


Figure 4.19: Bi-plot of PCA for PFCs in bird eggs together with environmental factors

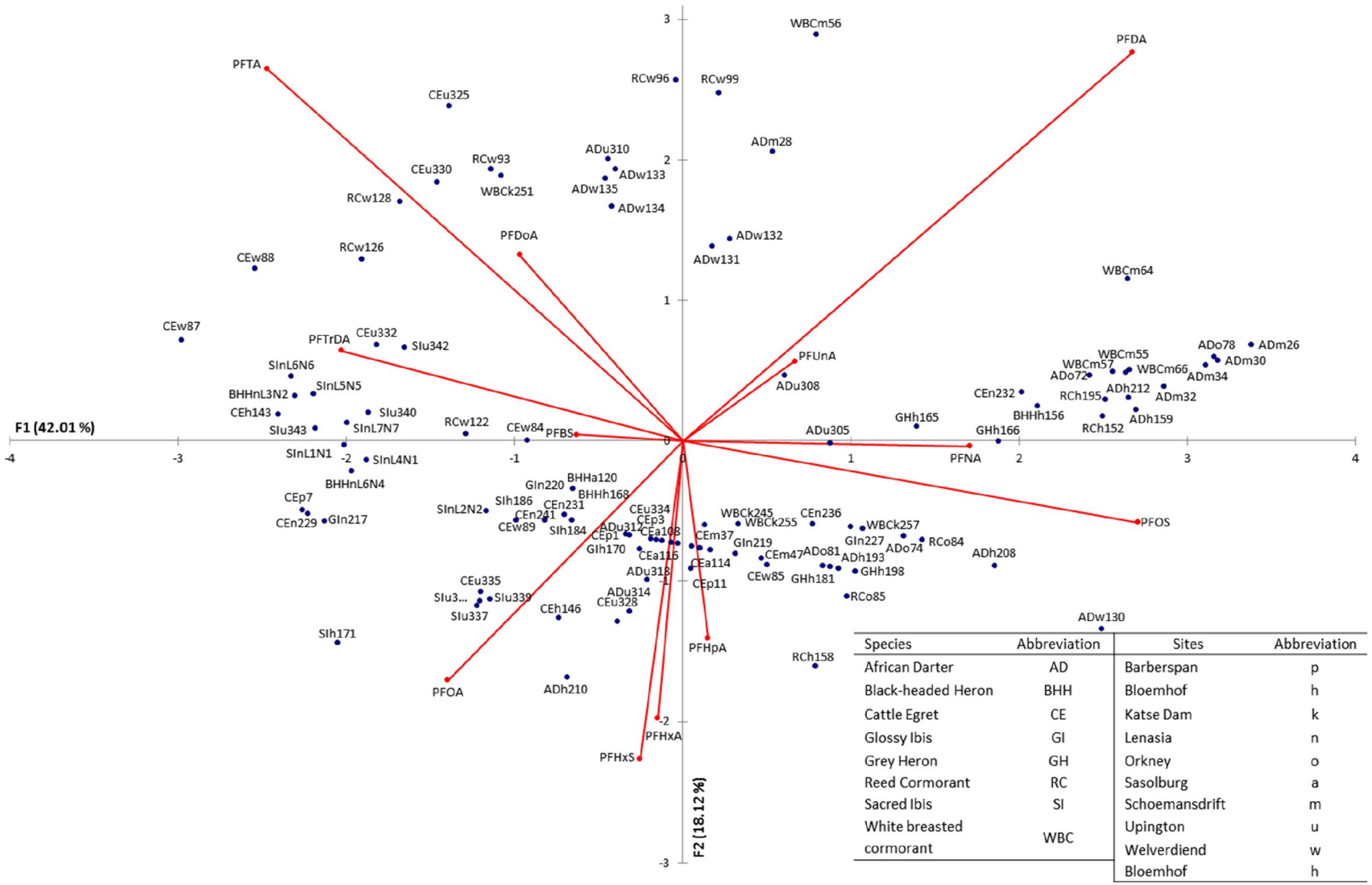
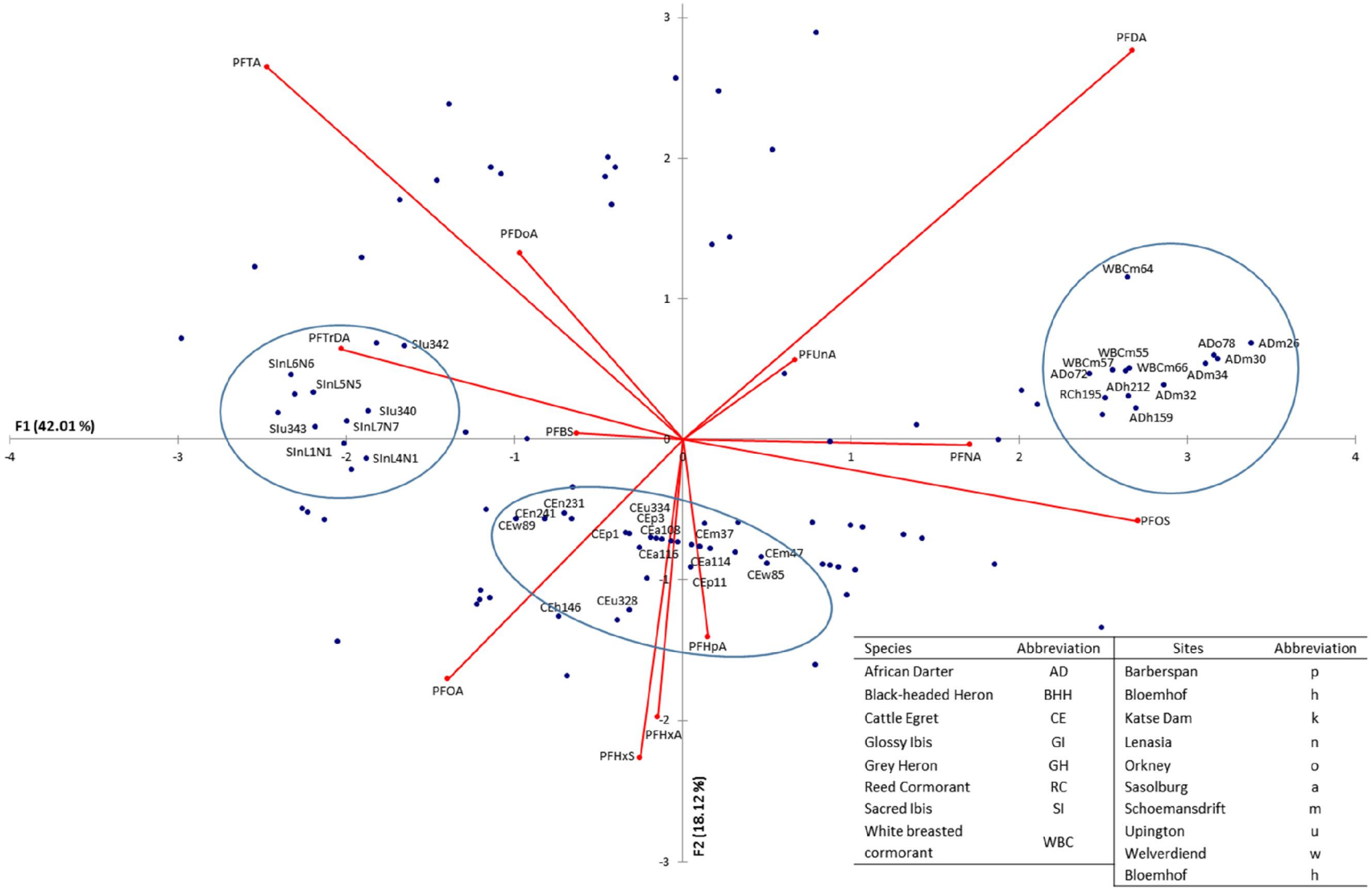


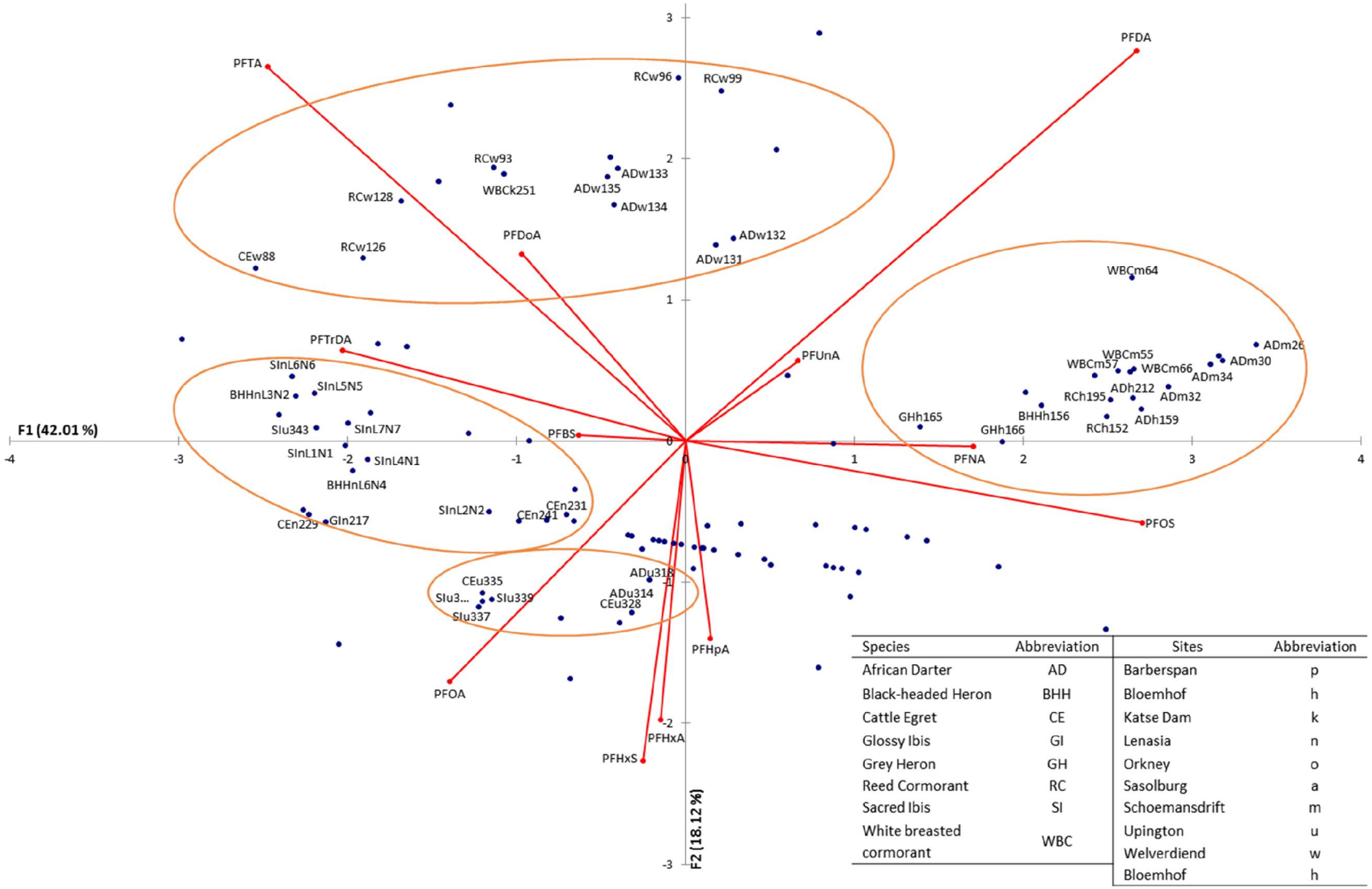
Figure 20: Bi-plot of PCA for PFCs in bird eggs together with bird eggs



Species	Abbreviation
African Darter	AD
Black-headed Heron	BHH
Cattle Egret	CE
Glossy Ibis	GI
Grey Heron	GH
Reed Cormorant	RC
Sacred Ibis	SI
White breasted cormorant	WBC

Sites	Abbreviation
Barberspan	p
Bloemhof	h
Katse Dam	k
Lenasia	n
Orkney	o
Sasolburg	a
Schoemansdrift	m
Upington	u
Welverdiend	w
Bloemhof	h

Figure 4.21: Bi-plot of PCA for PFCs in bird eggs together with bird species grouping



Species	Abbreviation
African Darter	AD
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Glossy Ibis	GI
Grey Heron	GH
Reed Cormorant	RC
Sacred Ibis	SI
White breasted cormorant	WBC

Sites	Abbreviation
Barberspan	p
Bloemhof	h
Katse Dam	k
Lenasia	n
Orkney	o
Sasolburg	a
Schoemansdrift	m
Upington	u
Welverdiend	w
Bloemhof	h

Figure 22: Bi-plot of PCA for PFCs in bird eggs together with site groupings

Chapter 5: Discussion

"For if one link in nature's chain might be lost, another might be lost, until the whole of things will vanish by piecemeal"
Thomas Jefferson

As discussed in Chapter 2, PFCs have been manufactured for over 60 years in various commercial products and industrial processes. PFCs, first detected in 1968 in human blood, are now detected globally in a variety of environmental and biological matrices (Benskin, 2011). Their physiochemical properties lead to persistence, bio-accumulation, long range transport potential and significant toxicological effects to both wildlife and humans (Ahrens & Bundschuh, 2014; Armitage *et al.*, 2009; Benskin, 2011; Giesy & Kannan, 2002). Due to the hazardous nature of these compounds, continuous monitoring and management of PFCs in the environment is crucial, especially in elucidating possible sources of exposure. To this end, the aim of this project was to determine the levels of 12 PFCs in various matrices from within the Orange River basin. The study focused on the distribution and congener profiles of PFCs to identify possible sources and to compare South African data with global patterns.

The current chapter has been divided into the following sections: (1) comparison of PFC detected in the various matrices, and (2), the occurrences, concentrations and patterns of PFCs in wild bird eggs. PFCs in wild bird eggs will be discussed considering the congener profile of PFCs in wild bird eggs between: species, trophic guild, feeding habitat and the geographic area in which eggs were collected. A global comparison of PFCs found in wild bird eggs is discussed, as well as the potential toxicological impact of PFCs on the South African environment.

5.1 Comparison between matrices

To assess the distribution of PFCs within the environment, various matrices were analysed. These matrices were chosen to indicate possible sources of PFCs as well as to assess if bio-accumulation or bio-magnification occurred. Therefore, matrices included the abiotic matrices of water and sediment as well as biotic matrices fish, specifically *Clarias gariepinus,* and various bird species (**Chapter 4, Figure 4.1a**).

As stated previously the physiochemical characteristics of PFCs suggest that water is the main environmental compartment to which these compounds partition (Conder *et al.*, 2010). However, due to bio-accumulation and bio-magnification, concentrations of PFCs in water can be orders of magnitude lower than in biological samples (Benskin *et al.*, 2010). Additionally, seasonal changes in meteorological conditions such as temperature, flow rates of rivers, and precipitation may play a role in the concentration of PFCs detected in water (Lasier *et al.*, 2011; Liu *et al.*, 2015). This resulted in the following, where concentrations of PFCs are lower during dry seasons and higher during wet seasons, due to increased runoff resulting from precipitation. PFOS and PFOA are the predominant congeners found in water, although, probably due to restrictions of production of long-chain PFCs, the environmental concentrations of short-chain PFCs are increasing (Moller *et al.*, 2010). The general pattern of detection found in literature is PFOS > PFOA > PFHxS > PFHpA > PFBS > PFUnA. In the current study, only 6% of water samples

analysed had measurable concentrations (**Chapter 4, Figure 4.1b**), namely Barkley West (0.24 ng/L PFBS) and Caledon (1.17 ng/L PFUnA). The samples from these sites were collected during the dry season with 300 mm average rainfall in the Northern Cape, and 260 mm in the Free State (rainfall per month; DWS, 2015). In literature (**Chapter 2; Table 2.4**), concentrations of PFBS and PFUnA range from 0.05 – 45 ng/L. The Barkley West area is impacted by diamond mining and maize farming (**Chapter 3; Table 3.4;** DWA, 2015). Therefore, the concentrations of PFBS can be attributed to its use in crop biocides or as a surfactant in the mining industry. The Caledon River originates in Lesotho, which is known for textile industries (Bahta & Groenewald, 2015). PFUnA sources include home textiles and upholstery that could contribute to the occurrence of PFUnA in water. As discussed in **Chapter 2; Section 2.1.3**, sorption of PFCs is influenced by sediment-specific and solution-specific parameters. For the selected rivers and dams, only historical inorganic water chemistry datasets were available for 1972 – 2011 (Huizenga *et al.*, 2013). PFC sorption increases with increasing solution of Ca^{2+} and Mg^{2+} and decreasing pH (Higgins & Luthy, 2006; Kostianoy *et al.*, 2012). The historical data for the sites were within normal ranges of natural water for major ions, as well as environmental pH concentrations. However, as this is historical data, there is a possibility that the current concentrations might be different.

In the previous study conducted on the Orange River basin, no PFOS was detected in the 61 sediment samples analysed by Oekometrics (a commercial and accredited laboratory in Germany) (Bouwman & Pieters, 2013). In the current study on the basin, PFCs were detected in 27% of sediment samples analysed (**Chapter 4, Figure 4.1c**). Three sediment samples contained measurable concentrations of PFCs; these were collected from Jet Park (4 ng/g PFOS), Welverdiend (2 ng/g PFOS) and Orkney (5 ng/g PFHxA).

In literature, concentrations of PFOS in sediments range from 0.13 – 457 ng/g, and 0.06 – 3.2 ng/g for PFHxA (**Chapter 2, Table 2.5**). Jet Park is a highly industrialized area, with a variety of manufacturing companies, and close to Africa's largest commercial airport (**Chapter 3; Table 3.5**). Aviation is a recognised source of PFCs, as PFOS is a surfactant in aviation hydraulic fluids, petroleum products and firefighting foams (OECD, 2013). Welverdiend is a historically rich gold mining area since the 1940s; although many of the mines are closed, legacy and current activity can still affect the area regarding perfluorinated chemicals. This area contains large mine tailing dams and as PFOS is used as a cyanide wetting agent, gold mining activity could contribute to PFOS measured in the aquatic environment. Although Orkney is also a large gold and uranium mining area, the PFC found, PFHxA is associated with wastewater treatment plants. Therefore, the PFHxA detected in the area may be associated with wastewater discharged into the river from the rural population in the surrounding areas. The current findings agree with previously reported literature, as, apart from PFOS, even-chain length PFCAs (PFHxA) are detected more frequently in sediments (Beškoski *et al.*, 2013; Higgins *et al.*, 2005).

In the previous study conducted (Bouwman & Pieters, 2013), one of the possible sources of PFCs identified was active or abandoned mines, where mine tailing dams and sand dumps could potentially contributes to diffusion pollution of PFCs. Therefore, mine-tailing samples were analysed from mines situated within and around the Orange River basin. The age, activity level and mining processes involved differed between the mines. Three of the 13 collected mine tailings had quantifiable concentrations of PFCs: Buffelsfontein (4.44 ng/g PFHxA), Orkney (4.79 ng/g PFHxA) and Germiston (8.08 ng/g PFOA). Of the three mines, two are currently active; Buffelsfontein and Orkney, whereas the Germiston site forms part of the remediation of old mine tailings. PFHxA and PFOA are not known compounds used during mining production. However, the degradation of precursors used in this industry can result in the formation/ release of PFHxA and PFOA. The analysis of tailing samples was particularly challenging due to extremes in pH, as well as large differences in particle size of the particulate matter. These parameters can affect the extraction efficiency, as well as detectability of PFCs due to the effect of matrix constituents. Additionally, the effect of the tailing environment on PFCs is unknown. Therefore, the tailings results are indicative only, as further development work on the matrix is required.

Clarias gariepinus is an omnivorous benthopelagic fish and known to have a relatively high fat content; it is therefore suited for the monitoring of organic pollutants that are associated with sediment and accumulate within the food web (Wepener *et al.*, 2011). The diet of these fish includes phytoplankton, macrophytes, insects, zooplankton, fish, and detritus (Deribe *et al.*, 2011). Additionally, this fish species is caught and eaten by recreational and subsistence anglers and there is a substantial aquaculture industry for fresh fish consumption in South Africa (Food and Agriculture Organization of the United Nations; (FOA) Fishery Statistics, 2006). Although PFOS was detected during this study at relatively high concentrations in bird eggs, and is the predominant PFC detected in fish together with PFOA, none of the fish samples analysed contained detectable concentrations of PFCs. In a previous study conducted by ORASECOM (Bouwman & Pieters, 2013), eight pooled fish fillet samples (n = 10) of *Clarias gariepinus* were analysed for PFOS. Two pools had detectable concentrations of PFOS; from Aliwal North (Lesotho; 2.6 ng/g) and from Rooipoort (Northern Cape; 1.7 ng/g). However, these concentrations were below the LOQ (26 ng/g) achieved for the current study.

In general, PFCs are linked with protein rather than lipids. However, the type and function of the protein strongly influences the binding of PFCs. Although the uptake and deposition route of PFCs is still unknown, it is likely similar to the mechanism in mammals. As PFCs are proteinophilic, deposition is associated with carrier proteins such as albumin, OATs and FABPs (**Chapter 2, Section 2.1.4**). Many of these proteins or functionally similar proteins have been identified in fish (De Smet *et al.*, 1998; Manera & Britti, 2006; Popovic *et al.*, 2010). Therefore, accumulation of PFCs in fish occur primarily in plasma, liver, and kidney, with minimal concentrations found in adipose and muscle (Ng & Hungerbühler, 2013). For this study, fish muscle tissue was analysed. Fish muscle mainly consists of myosin. Myosin is an actin motor

protein which function is converting ATP into kinetic energy. This protein structure is therefore not ideally suited to binding organic chemicals as is the case for albumin or lipoproteins. Therefore, a more suitable matrix for analysis, which is known to accumulate PFCs would be the liver, kidney, or plasma. Due to these shortcomings in the matrix analysed, it is difficult to draw conclusions on the contribution of fish to the concentrations of PFCs detected in wild bird eggs or as an indicator for PFCs within the aquatic environment.

Previous studies conducted globally have identified PFOS as the predominant PFC detected in wild bird eggs. During the 2013 study conducted by Bouwman & Pieters (2013), the same pattern was observed where all eggs analysed had detectable concentrations of PFOS. In the current study, 105 single eggs were analysed for the presence of 14 PFC congeners. All PFCs analysed for were detected in eggs. However, the detection ranged between one compound in a single sample, to PFOS with a 95% detection rate in eggs (**Chapter 4, Figure 4.1d**). The main contributing congeners other than PFOS was PFOA (40%) and the long-chain ΣPFCAs (25 - 47%). The proportional contribution of the short-chain ΣPFCs varied from 3 - 19%, with PFBS as the main contributing short-chain congener. Other research found that long-chain PFCs predominated in biota; PFOS > PFUnA > PFTrDA (Gebbink *et al.*, 2009; Gebbink & Letcher, 2012; Wang, *et al.*, 2008a). Long-chain PFCs have higher bio-accumulation potential, than short-chain PFCs, and therefore found more frequently than other PFC congeners. When high concentrations of organic pollutants are detected in biota, there are correspondingly high concentrations in abiotic matrices as well as in other members of the food web. However, this was not apparent in the current study.

Various factors can influence the concentrations of PFCs within abiotic environmental compartments as described in **Chapter 2; Section 2.1.3**. Factors include, *inter alia*, changes in metrological conditions such as precipitation, seasonal variation in anthropogenic activity, as well as the physical and chemical properties of the PFCs themselves. Differences between species are even more complex, as there is often difference in feeding ranges, metabolic efficiencies, longevity, reproduction rates, as well as possible exposure pathways. Additionally, concurrent sampling is crucial when comparing different matrices within a sample set (Conder *et al.*, 2010). However, when sampling a river basin with as large an area as the Orange River basin, this is logistically difficult. For example, it is not always possible to catch fish and collect wild bird eggs at the same time or at the same site due to the specific behaviour, habitat preferences, and breeding seasons of the species involved. With wild birds, determining association with a specific site or prey species is further complicated due to their feeding ranges and natal dispersal. Bird species selected for this study varied between residents and birds that are locally nomadic (**Chapter 3, Table 3.2**). Locally nomadic birds will move between areas in response to, *inter alia*, meteorological conditions such as rainfall, as well as food availability. Therefore, a bird can travel over long distances and exposure due to differences in region-specific anthropogenic activity can be very diverse. Furthermore, PFC concentrations are influenced by urban and industrial

activities, diet, feeding ecology, *ovo* enrichment, accumulation of precursors, and the biodegradation thereof (Letcher *et al.*, 2015).

Studies conducted on PFOS accumulation in eggs have found preferential accumulation in the yolk (**Chapter 2, Section 2.2.4**). The accumulation of PFOS in the yolk is likely due to in *ovo* transfer of specific proteins from the liver to the yolk during egg formation. A secondary accumulation pathway is the transfer of PFCs to the yolk from the female bird to the ovaries via blood (Nordén *et al.*, 2013). Although PFCs are proteinophilic, PFOS has been associated with very low density lipoproteins (VLDL) followed by phosvitin and lipovitellin (Newsted *et al.*, 2007) all elements of egg yolk. Additionally, the mixture of water, proteins, and lipids found in the egg yolk provides the ideal environment for PFCs suiting both their hydrophilic heads and hydrophobic tails of the molecule. The low concentrations found in the other matrices, compared to the high levels in the bird eggs could be due to the preferential accumulation due to carrier proteins present, as well as the type of protein the PFCs bind. Additionally, the metrological differences, species feeding range, natal dispersal, and breeding season can influence the concentrations found in in the matrices.

The use of non-invasive techniques that do not cause the loss of individuals within a population are of growing interest to environmental scientists. One of the proposed techniques is the collecting of moulted/shed feathers. Feathers have been used successfully as a monitoring tool for mercury, as well as for PFCs specifically in other studies (Alava *et al.*, 2015; Calle *et al.*, 2015; Meyer *et al.*, 2009). Therefore, the use of feathers as a monitoring tool for PFCs was investigated. Feathers were surprisingly difficult to collect. Although many species use feathers as nest lining, these are not suitable for analysis due to large quantities of debris and guano sticking to the feathers making them unsuitable for analysis. Additionally, the selected species nest in high trees (on islands within the river or on the riverbank; **Figure 5.1 A**) or on reed beds within the river (**Figure 5.1 B**). These sites are difficult to access. Initially, roosts were investigated as birds preen when roosting. However, virtually no feathers were present at these sites and only five feathers were eventually collected at the Upington site. To assess the presence of PFCs in wild bird feathers, a screening technique was used. Of the five feathers analysed, two had detectable concentrations of PFOS and PFUnA, respectively. Due to the limited number of samples, little sample could be sacrificed for method optimisation and no quantification was performed. However, if the logistic limitation can be resolved, the use of bird feathers as a bio-monitoring tool for PFCs requires further investigation.

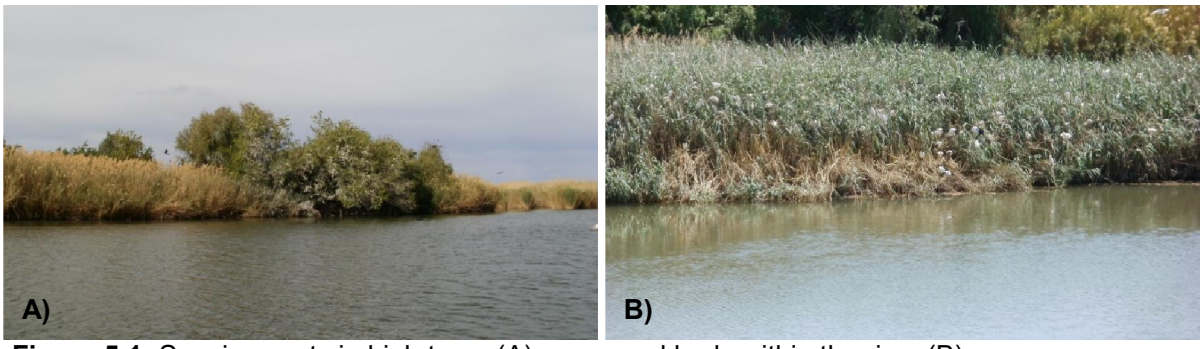

Figure 5.1: Species nests in high trees (A) or on reed beds within the river (B)

5.2. PFCs in wild bird eggs

5.2.1. Concentrations of PFCs

As stated earlier, PFOS was detected in all samples in the study conducted by Bouwman and Pieters, 2013. Samples from that study were pooled with five wild bird egg samples per species grouping, per site. Only PFOS was analysed. The concentrations of the species pools were the following (**Table 5.1**):

Table 5.1: Result from previous study conducted in the Orange River basin

Species	PFOS (ng/g wm)	Site	ΣPFOS (ng/g wm)
African Darter	1600	Bloemhof	800
Reed Cormorant	660	Barberspan	290
Little Egret	510	Eldorado Park	70
Grey Heron	370	Potchefstroom	60
Great White Egret	350		
Cattle Egret	300		
Sacred Ibis	40		
Black-headed Heron	10		
Glossy Ibis	5		

PFOS ranged from 5 – 1600 ng/g wm, with the highest concentration detected in the African Darter eggs and the lowest concentration measured in Glossy Ibis eggs (Bouwman & Pieters, 2013). In the current study, 105 single eggs were analysed. The PFOS concentrations ranged from 0.3 – 2800 ng/g wm, with the highest median PFOS concentration detected in the African Darter eggs (1100 ng/g wm), and the lowest concentrations in the Sacred Ibis eggs (10 ng/g wm) (**Chapter 4, Table 4.2**). The mean concentration detected in the Glossy Ibis eggs was 70 ng/g wm. The African Darter eggs had PFOS concentrations double that of any other bird species collected in both South African studies.

Comparing the distribution pattern in the bird eggs, the following was seen for Bouwman & Pieters (2013): African Darter > Reed Cormorant > Grey Heron > Cattle Egret > Sacred Ibis >

Black-headed Heron > Glossy Ibis. For the current study: African Darter > Grey heron > Reed Cormorant > White-breasted cormorant > Black-headed heron > Cattle egret > Glossy Ibis > Sacred Ibis. In both studies conducted within the South African environment, piscivores had the highest concentrations of PFCs, followed by insectivores and scavengers. The Glossy Ibis and Black-headed Heron eggs had higher concentrations of PFOS. However, this was mainly due to high concentrations detected in a single eggs (Glossy Ibis from Lenasia 256 ng/g wm; Black-headed Heron (725 ng/g wm) from Bloemhof). Comparing the ΣPFCs, the Sacred Ibis eggs had higher concentrations than the Glossy Ibis eggs (**Chapter 4, Table 4.2**). Although ΣPFOS was higher in the Glossy Ibis eggs, the Sacred Ibis eggs had a higher distribution of the other PFCs. When looking at single eggs, the highest concentration of PFOS was found in an African Darter egg collected from Schoemansdrift (2800 ng/g wm), while Sacred Ibis eggs from Bloemhof, Glossy Ibis and Cattle Egrets eggs from Lenasia had concentrations below the detection limit.

As reported in literature, I found that long-chain PFSAs were predominant in wild bird eggs followed by long-chain PFCAs, short-chain PFCAs and short-chain PFSAs (**Chapter 4, Figure 4.2**). This observed pattern is likely due to variation in the bio-accumulation potential of PFCs based on their chain length. The bio-accumulation potential of short-chain PFCs are lower, therefore these compounds are generally found at lower concentrations within the environment. However, with increased regulation of long-chain PFCs, there has been an increase in the use of short-chain PFCs as industrial replacements. Therefore, the concentrations of these compounds are increasing within the environment (Ahrens & Bundschuh, 2014; Buck *et al.*, 2011). This change is not yet prevalent in the South African environment. To further investigate the concentrations and patterns of PFCs in wild bird eggs, multivariate statistical analysis was performed and will be discussed in the following sections.

5.2.2. Congener profiles of PFCs in wild bird eggs

One of the aims of the current project was to investigate the distribution and congener profiles of PFCs, and to identify possible routes of exposure. This will be discussed in the following section as well as **Section 4.2.5**.

A cluster analysis was performed to identify homogenous clusters within a dataset (Fowler *et al.*, 1998). Three groupings of PFC congeners are formed, likely due to source contribution (**Chapter 4**; **Figure 4.2**).

- Congener grouping 1 (**Figure 4.3, 1A and 1B**) was PFOS separated from all the other congeners. This coincides with literature, as PFOS is the main congener found in biota (Gebbink *et al.*, 2011; Martin *et al.*, 2003b). PFOS was also the congener detected in 95% of the wild bird eggs and the highest concentrations quantified. PFOS uses and sources (**Chapter 2; Table 2.2**) are generally associated with the following industries: aviation and aerospace, metal plating, oil and mining, household products, and textiles. Identification

of the main industries responsible for PFOS sources is difficult, due to the widespread collection sites and different source inputs on the Orange River basin from industrial, urban and commercial activities. Further investigation of proportional contributions and site-specific congener profiles may assist in identifying possible sources. This will be discussed in **Section 5.2.5**.

- Congener group 2 (**Figure 4.3; 2A and 2B**) was a grouping between PFTA, PFDA, PFTrDA and PFOA (**2A**) against PFNA, PFDoA, PFUnA, PFHpA, PFHxS, PFHpA and PFBS (**2B**). Grouping 2A congener's sources are mainly associated with atmospheric degradation and food processing industries, whereas grouping 2B (PFNA, PFDoA, PFUnA) is related to WWTPs, food processing, atmospheric degradation and textile sources. The short-chain PFCs that form part of group 2B are active ingredients in biocides. However, the low levels of these congeners present in the bird eggs may also contribute to this grouping association.
- Congener group 3 (**Figure 4.3**) contained PFHpA, PFHxS, PFHpA and PFBS, which as mentioned previously is related to biocide sources. Additionally, short-chain PFCs are not known to bio-accumulate. However, they are increasingly found due to phase out of PFOS and PFOA (Haukås *et al.*, 2007; Johansson *et al.*, 2014).

The congener profile of PFCs in individual species were compared (**Chapter 4, Figure 4.4**). PFOS was the dominant congener detected in all bird species. However, PFNA, PFDA and PFUnA also had statistically significance between the bird species. The significance of congeners could be contributed to the foraging method and/or type of diet. As stated in literature, diet seems to be the major factor influencing exposure to PFCs, and that aquatic prey may be the main source (Gebbink *et al.*, 2009; Gebbink *et al.*, 2011). Additionally, fish is also known to have exponentially higher concentrations than the surrounding area, resulting in highlighting the significance of dietary fish in PFC accumulation in the food web (Sinclair *et al.*, 2006).

The African Darter had the highest concentrations of PFCs compared to any other species, followed by the Reed Cormorant and White-breasted Cormorant. Their diet is predominantly fish, from the Cyprinidae and Cichlidae family. However, species such as the yellow fish (*Labeobarbus kimberleyensis*) are listed as near threatened and vulnerable in the IUCN red list of threatened species (International Union for Conservation of Nature (IUCN), 2015). Therefore, we did not obtain permits to sample for these species. Evaluating the movement and method of foraging can indicate the possible source of PFC concentrations detected in the bird species.

The African Darter and Reed Cormorant are opportunistic feeders, with local movement, whereas the White-breasted Cormorant is sedentary, but nomadic in response to changing water concentrations (**Chapter 3; Table 3.2**). The White-breasted Cormorant tends to not forage over long distances, and build their nests adjacent to food sources. The local movement with

opportunistic feeding of the African Darter and the Reed Cormorant could introduce these species to a larger variety of sites where they could be exposed to PFCs. However, the White-breasted Cormorant is mostly sedentary, and will generally be exposed to the single area of nesting and feeding. The manner of foraging could also contribute to the differences in concentrations for PFCs between the different bird species.

The African Darter (1 – 6 m) and Reed Cormorant (< 2 m) forage in the water and dive down to catch prey. The difference observed in concentration between the African Darter and Reed Cormorant could be due to the different fish consumed due to the depth of foraging in the water (Hockey *et al*., 2011; Tarboton, 2011). Additionally, this could explain the differences in concentrations between the White-breasted Cormorant and the Grey Heron. The White-breasted Cormorant tends to catch mid to shallow dwelling slow-moving prey and the Grey Heron tends to forage in shallow water or flies over water and waits to catch prey. Furthermore, the Grey Heron forage near the colony site, or as far as 35 km away.

The Glossy Ibis also had PFOS as the main contributing congener (94%), with low proportional contributions from the other congeners. The main diet of the Glossy Ibis is insects, fish, frogs and lizards from shallow freshwater, grasslands, parks and farmlands (Hockey *et al*., 2011; Tarboton, 2011). Due to this diet, one would expect a similar congener distribution profile as the Cattle Egret. However, only four Glossy Ibis eggs were analysed in the current study (Cattle Egret n= 30). This could explain the inconsistency of congener profile between these species. One should also consider the feeding range of these species, as the Cattle Egret feed up to 15 km from breeding sites.

Lower dominance of PFOS are found in the Cattle Egret (87%) and Sacred Ibis (48%). The variation of other PFC congeners in the Cattle Egret was not as large as in the Sacred Ibis. The foraging and diet differs for these species compared to the others. The Cattle Egret consumes insects, frogs, reptiles, small rodents and fish and are generally found in grasslands, man-made pastures and agricultural lands. Whereas the Sacred Ibis is a known scavenger, species that has adapted to foraging food in refuge tips and abattoirs. Feeding from these areas could expose the Sacred Ibis to other possible sources of PFCs (**Chapter 2; Table 2.2**). Additionally, the Sacred Ibis tend to feed long distances from breeding sites. In the current study, a statistical significant difference ($p < 0.05$) between congeners and species were found for the long-chain PFCs, namely PFOS, PFNA, PFDA and PFUnA. This statistical significance can be ascribed to the diet and foraging techniques of the bird species. Although diet is reported as the main influence, the environment where the eggs were collected may also play a role and contribute to the loading of PFCs.

When PFCs were grouped according to their chain length categories, there were statistically significant differences between the chain length and bird species, as well as the sites where the bird eggs were collected ($p < 0.05$, **Section 4.2.2**). The post-hoc Tukey analysis indicated a

statistical significant difference between long-chain PFSA and PFCAs. In literature, generally patterns of long-chain PFCAs are described, with minimal patterns of short-chain PFCs. Although short-chain PFCs are found in the environment, it is rare and inconsistent per site/ species. A study conducted by Schiavone *et al* (2009) on Gentoo Penguin (*Pygoscelis papua*) eggs had a pattern of PFUnA > PFOS > PFDoA > PFHpA and PFHpA >PFUnA > PFDA > PFDoA in Adelie penguin (*Pygoscelis adeliae*) eggs. Here short-chain PFCs were present in high concentrations. However, this is not always the case. In most studies the following pattern is seen and consists mostly of long-chain PFCs: PFOS > PFUnA > PFDA > PFTrDA > PFDoA (herring gull and great cormorant (Nordén *et al.*, 2013). And PFOS > PFTrDA > PFDoA > PFUnA > PFDA (gull species, (Gebbink, *et al.*, 2011). In the current study, the following distribution of short, and long-chain PFCs were observed between the species and sites:

The African Darter and Cattle Egret had the highest long-chain PFCA proportional contribution and the Cattle Egret had highest proportional contribution of the short-chain PFSAs from Upington (**Figure 4.5 and4.8**). The surrounding area of Upington is associated with farmlands and a WWTP that can account for the sources contributing to the birds.

Welverdiend had higher contribution of long-chain PFCAs for the Reed Cormorant (**Figure 4.6**). Multiple sources occur, which could contribute to the Reed Cormorant distribution such as farmlands, abandoned and active mines and WWTPs. However, Orkney had the highest contribution of PFSAs, with gold mines as the main contributing source in the environment.

The White-breasted Cormorant eggs from Schoemansdrift had a higher proportional contribution of long-chain PFCAs than bird eggs from Katse Dam (**Figure 4.7**). The Katse Dam is a pristine environment, where Schoemansdrift is situated adjacent to a commercial and agricultural town.

Bloemhof had multiple bird species with the following main contributions: Cattle Egret and Glossy Ibis had the highest contribution of short-chain PFCAs, and long-chain PFCAs for the Glossy Ibis (**Figure 4.8 and 4.9**). Long-chain PFSA was the largest contributor for the Black-headed Heron (**Figure 4.10**). The Sacred Ibis had the lowest long-chain PFSA and highest short-chain PFSA contribution (**Figure 4.11**). Bloemhof is located at the confluence of the Vaal and Vet River. This is a protected area, with minimal surrounding impact. However, Bloemhof is an agricultural town, therefore sources from stormwater runoff can impact the site. Additionally, water from the Vaal River which drains multiple industrial, commercial and urban areas, can contribute to the variation of PFCs found with different bird species (**Chapter 3, Table 3.4**).

5.2.3 Trophic guild and PFCs

Dietary intake of PFCs is the most important factor in the accumulation of these compounds within an organism. As PFCs are known to bio-magnify along the food web, it is likely that higher concentration of PFCs are expected at higher trophic levels. A statistically significant difference

was found between trophic guilds and concentrations of PFOS, PFOA, PFNA, PFDA and PFUnA (one-way ANOVA, $p < 0.05$, log-transformed data). Long-chain PFCs are increasing, depending on compound, trophic level and location (Gebbink *et al.*, 2011; Haukås *et al.*, 2007; Johansson *et al.*, 2014; Paul *et al.*, 2009). Increasing body burdens of PFCs according to trophic level has been seen in different bird species (Houde *et al.*, 2006). The pattern observed in the current study is piscivores > generalist > insectivores > scavengers. Piscivores had higher concentrations of PFOS, PFNA, PFDA and PFUnA than any other guild, emphasising the consumption of aquatic organisms as the main contributor to the loading of PFCs in piscivorous bird species (**Section 5.2.2**). The generalist species had higher concentrations of PFCs than the insectivores and the scavengers. The generalist species mainly consume terrestrial invertebrates, but also small mammals, reptiles and birds (**Chapter 3, Table 3.2**). The contribution of higher trophic feeding than the insectivore could contribute to the higher concentrations of PFCs. Scavengers had a higher concentration of PFOA than insectivores. PFOA has a low bioaccumulation potential, and commonly similar concentrations between species from different trophic concentrations are seen (Ng & Hungerbuhler, 2014). Scavengers adapt to available food sources in the surrounding areas. This includes places such as sewage works and refuge tips (**Chapter 2; Table 2.2**). Known sources of PFOA include household products and food packaging, which are generally found in refuge tips. PFOA can leach from these products into the surrounding environment, where scavenger species can be exposed to it when foraging for food.

5.2.4 Feeding habitat and PFCs

In the current study the chain length pattern was the same for aquatic, terrestrial habitats and combined habitats (Long-chain PFCs > Short-chain PFCs). Long-chain PFCs are more bio-accumulative than short-chain PFCs, and although short-chain PFCs were not as prevalent, it is possible that they are present in the environment, but at lower concentrations. Exposure patterns and differences in toxicokinetics in PFC profiles, such as elimination half-lives, can differ widely between congeners and across species. Additionally, precursor compounds can be present in bird species, and toxicokinetics for the compounds per species can be different for absorption, distribution, transformation and elimination (Gebbink & Letcher, 2011; Newsted *et al.*, 2006; Yoo *et al.*, 2009).

In the current study, the eggs from the aquatic habitat had higher levels of PFCs, followed by the terrestrial and combined habitats. ANOVA analysis was statistically significant ($p > 0.001$) for congeners PFOS, PFOA, PFNA, PFDA, PFUnA, PFTrDA and PFTA. The post-hoc Tukey indicated that the main contribution was from the aquatic habitat that had significant higher concentrations. This coincides with literature where lower concentrations of PFOS is generally found in terrestrial birds than aquatic birds (Gebbink *et al.*, 2011; Houde *et al.*, 2006; Thompson *et al.*, 2002; Wang, *et al.*, 2008).

However, there is an exception with congeners PFTrDA and PFTA, where terrestrial and aquatic habitats contain similar concentrations. In other studies, PFTrDA have been found to dominate among the PFCs detected (Gebbink, *et al.*, 2011; Holmström & Berger, 2008; Nordén *et al.*, 2013; Rüdel *et al.*, 2011; Verreault *et al.*, 2007). Atmospheric degradation of precursors contribute to PFTA and PFTrDA (**Chapter 2, Table 2.2**), which could deposit in both aquatic and terrestrial environment.

5.2.5 Sites and PFC congener profile

A statistical significance was found after an ANOVA analysis between the sites and concentration profiles of PFCs. All the PFC congeners except PFHxA were detected at Welverdiend (**Figure 4.15**), with the highest concentrations of the following congeners: PFBS, PFHpA, PFUnA, PFDoA and PFTA compared to any other site (**Figure 4.14**). Welverdiend is situated on the Wonderfontein Spruit, which is a tributary of the Mooi River, which in turn is a tributary of the Vaal River. Multiple abandoned and active mines are in the surrounding areas, discharging fissures and process water into the environment. Additionally, farmlands are present in the surrounding area, as well as a WWTP (**Chapter 3; Table 3.4**). The WWTP exceeds design capacity, which may lead to untreated effluent discharged into the Wonderfontein Spruit (DWA, 2009).

Comparing the surrounding environmental sources to known sources of congener profiles, might lead to better understanding where these congeners may come from. PFBS known uses and sources are: as surfactants used in mining, active ingredient in biocides, and from in WWTPs. PFHpA is used as an active ingredient in biocides. PFUnA and PFDoA is present in WWTPs and due to breakdown of precursor compounds. PFTA is generally due to breakdown of precursor compounds.

To establish if direct or indirect sources (**Chapter 2, Section 2.1.2**) are responsible for the congeners found, ratios between PFHpA/PFOA, PFOS/PFOA and PFOA/PFNA can be evaluated (Armitage *et al.*, 2009; Guo *et al.*, 2015). Please note, this has only been utilized with water and sediment samples, and metabolic and toxicokinetics can play a role between different species (Newsted *et al.*, 2006). The ratios were the following: PFHpA/PFOA (7.4), PFOS/PFOA (99) and PFOA/PFNA (0.36). Ratios of PFHpA/PFOA > 1 could indicate atmospheric precipitation of the compounds after degradation of precursor compounds. PFOS/PFOA ratios > 1 could indicate direct fluoropolymer sources, or possibly sources from a WWTP. Ratios of PFOA/PFNA < 1 indicate secondary sources such as wet and dry deposition, metabolic transformation of precursors or runoff from contaminated land/streets (**Chapter 2, Figure 2.2**). Therefore, is seems the congener profile found in Welverdiend are due to the variety of sources found on the Wonderfontein Spruit (abandoned and active mines, farmland and WWTP) as well as precursor breakdown and precipitation.

The Schoemansdrift site had higher PFNA and PFDA congeners than any other site (**Table 4.4, Figure 4.14 and 4.15**). Additionally, PFUnA were detected in more than 68% of the samples from there. Lower frequency of PFDoA, PFTrDA, PFTA and PFOA was detected (8 – 23%). Schoemansdrift is situated on the Vaal River, which is the main tributary of the Orange River. The town close to the sampling site (Standerton) is a large commercial and agricultural (stock and maize) town (**Chapter 3, Table 3.4**). The sample was collected close to farmlands. Ratios of PFOS/PFOA (164) and PFOA/PFNA (0.73) could indicate the type of sources contributing to the high concentrations found. The high PFOS/PFOA ratio could indicate a fluoropolymer source or waste from a WWTP. To our knowledge, no direct fluoropolymer sources are present in the surrounding area, as well as no immediate WWTP. However, a rural informal settlement is present close to Schoemansdrift, where domestic and human waste could be discharged directly into the river. The low PFOA/PFNA ratio indicates secondary sources such as wet and dry deposition, metabolic transformation of precursors, or runoff from contaminated land/streets. Human and domestic waste could be sources of PFNA, PFUnA, PFDoA, PFTrDA, PFDA and PFOA (**Chapter 2, Table 2.2**). Main sources of PFTA on the other hand is mostly from atmospheric degradation of precursors (**Chapter 2, Table 2.2**).

Upington had higher concentrations of PFTrDA than any other site (**Section 4.2.5; Table 4.4, Figure 4.15**). PFTrDA is associated with atmospheric degradation, food processing, WWTPs and emulsifying agents in production of fluoropolymers (**Chapter 2, Table 2.2**). A WWTP is in the surrounding area, which could possibly contribute as the source of PFTrDA. Other congeners detected were PFOA, PFNA, PFTA, PFDA, PFUnA, PFBS and PFDoA (in decreasing order; 61 – 17%). Upington mainly consists of farmlands and a small residential area. The ratios of PFOS/PFOA (4.76) and PFOA/PFNA (0.6) were calculated to establish the possible sources. The ratio of PFOS/PFOA were much lower than the other sites. This could be attributed to the concentration of PFOA. Upington had the highest concentration of PFOA. Sources responsible for PFOA is generally from medical consumables, food processing, household products and textiles (**Chapter 2; Table 2.3**). The source(s) predominant in Upington might be from storm water runoff from farmlands and the residential area. However, a low ratio of PFOA/PFNA was calculated, indicating possible degradation of precursors that could contribute to the congeners present.

Orkney had the highest PFOS concentration compared to any other site (**Table 4.4, Figure 4.14**). PFOS was detected in 100% of the samples with less frequency of PFNA, PFOA and PFTrDA (33 – 17%). Orkney is situated on Schoonspruit, which is a tributary of the Vaal River (**Chapter 2, Table 3.4**), mostly surrounded by a rural population and water is utilized for irrigation and urban use. Additionally, pollution from small-scale diamond digging is present on the riverbanks, and mines established since the 1940s, some of which are still active in the surrounding area (**Chapter**

2, **Table 3.5**). Ratios of PFOS/PFOA (230) and PFOA/PFNA (0.46) were calculated for Orkney. No known direct fluoropolymer sources or WWTP is located in the immediate area. The sources of PFOS could be attributed to the mining in the area, where PFOS was used as a surfactant in mining productions. PFNA, PFOA and PFTrDA could be detected due to atmospheric degradation of precursor compounds as indicated by the calculated ratio (< 1) (**Chapter 2, Table 2.2**).

Short-chain PFCs are known not to bio-accumulate and is sporadically detected in biota (Conder *et al.*, 2010; Gebbink, *et al.*, 2011). Barberspan and Bloemhof were the only sites with quantifiable occurrence in eggs of PFHxA (**Chapter 4, Table 4.5, Figure 4.16**), although in low frequency of 17% and 10%, respectively. The highest concentration was 11 ng/g (Bloemhof). PFHxA is used as an active ingredient in biocides and Bloemhof is an agricultural town, which could explain the presence of PFHxA. On the other hand, PFHxS was only detected in Lenasia (12%), Bloemhof (10%), and Welverdiend (6%) eggs, with 35 ng/g the highest concentration (Bloemhof). PFHxS is also used as an active ingredient in biocides. This could indicate that farmers in the surrounding area is utilizing a biocide containing these compounds.

Results from the previous study conducted (Bouwman & Pieters, 2013) on the Orange River basin had the following site pattern of mean PFOS in eggs: Bloemhof (800 ng/g) > Barberspan (290 ng/g) > Eldorado Park (Lenasia 70 ng/g) > Potchefstroom (60 ng/g) (**Table 5.1**). Comparing the same sites, the patterns were the same for the current study. However, more sites were selected in the current study. Orkney had the highest concentrations, and Sasolburg the lowest. Comparing the ratios of PFOS/PFOA, the following pattern per site was observed: Orkney (230) > Schoemansdrift (164) > Bloemhof (130) > Welverdiend (99) > Lenasia (27) > Upington (5) > Barberspan (3). Ratios could not be calculated for Sasolburg or Katse Dam. All of the calculated ratios were > 1, indicating a fluoropolymer source, or waste from a WWTP. However, to our knowledge, only one company (Pelchem) is a fluorine based industry focussing on the method development and production of fluorochemicals for commercial purposes (South African Nuclear Energy Corportation (NECSA), 2012). This company is not in the vicinity of any site in the current study. Additionally, in a report by the Department of Environmental Affairs (DEA) the following is listed: PFOS imports to South Africa increased by 20 000 kg from 2010 – 2013, without any indication on the applications of PFOS in the industrial products. Furthermore, a survey conducted indicated that fire extinguishers in South Africa containing PFOS were phased out 10 years ago (DEA, 2013). This is evidence that PFOS is utilised in the South African industry, but no documentation on the uses is currently available.

5.2.6 PCA analysis of PFC concentrations in wild bird eggs

A principle component analysis (PCA) was conducted to investigate the congener profiles of PFCs in bird eggs, as well as the possible contribution of environmental factors, major contributing ions,

and geographical distribution. On the PCA bi-plot (**Chapter, Figure 4.18**), Factor 1 explained 42% of the variance, with a contrast between PFDA (long-chain PFCA; C10), PFNA (long-chain PFCA; C9) and PFOS (long-chain PFSA; C8) with positive scores against PFTA (long-chain PFCA; C14), PFTrDA (log-chain PFCA; C13) and PFOA (short-chain PFCA; C8) with negative scores.

Groupings according to chain length occurred in the bi-plot. Long-chain PFCAs with carbon chain length C12 – 14, C9 – C11 and short-chain PFCs C6 and C7 grouped together. In the past, long-chain PFCAs C9 - C11 were detected more frequently than C12 - 14. However, this seems to be changing, and PFUnA and PFTrDA are increasingly being detected and reported (Gebbink & Letcher, 2012; Wang, *et al.*, 2008).

PFDoA, PFTrDA and PFTA were associated with phosphates (PO_4) and nitrates (NO_3). These ions are generally associated with agricultural sites and municipal waste (partially treated and untreated) (Lenntech, 2016). Known sources of PFDoA and PFTrDA are WWTPs, and this grouping could indicate WWTPs as the main source contribution of these compounds **(Chapter 2, Table 2.2)**.

PFDA, PFUnA, and PFNA were associated with sodium (Na), chloride (Cl) and potassium (K) ions. Sources related with these ions are agricultural waste (Na and K), industrial waste (Na), landfills (Na and K), WWTPs (Na and Cl), textile and leather industries (Na), oil and base drilling (Cl) (Lenntech, 2016). None of the known sources for these congeners are associated with agriculture, oil and base drilling or road salt storage. However, landfills, WWTPs and industrial waste are known sources. The textile and leather industries do use PFDA and PFUnA in pre-treatment **(Chapter 2, Table 2.2)**.

Ammonia (NH_4) sources of pollution could be agricultural sites, human and livestock waste, atmospheric deposition of combustion processes, strip mining, oil refineries, food processing plants and waste gas treatment (Lenntech, 2016). Of all these possible sources of NH_4 pollution for South Africa, PFOA could linked with the food processing industry branch.

PFHpA was associated with a grouping of ions (sulphates, carbonates, calcium and magnesium). All of these ions are known sources of mining and industrial waste (Lenntech, 2016). Although PFHpA has no connotation with these sources, it is a possibility that it might be due to atmospheric deposition, rather than a direct source.

A PCA bi-plot investigating the contribution of environmental factors was also investigated (**Figure 4.19**). Hardness was grouped together with PFDoA, pH with PFOS and PFHpA, and TDS and EC with PFOA. Studies have shown that sorption of PFCs increased with an increase in hardness (Ca^{2+} and Mg^{2+}) and decreasing pH (Higgins & Luthy, 2006; Kostianoy *et al.*, 2012). Additionally, chain length also had an impact on the sorption properties of PFCs. PFDoA might be stronger associated with fluctuating concentrations of hardness. PFOS and PFHpA is associated with pH. Studies have indicated the influence of pH on PFOS, but limited studies are available regarding PFHpA (Higgins & Luthy, 2006; Kostianoy *et al.*, 2012).

To evaluate the congener profiles of PFCs in birds as well as the impact of geological distribution, a PCA bi-plot was constructed (**Figure 4.20 - 22**). On factor 1, a contrast was seen between African Darter and White-breasted Cormorant with positive scores, against Sacred Ibis with negative scores. This pattern seems to be influenced by the diet of the birds. The African Darter and White-breasted Cormorants are piscivores, and the Sacred Ibis a scavenger. A strong association between piscivores and PFOS is seen in literature where piscivores generally have higher concentrations than other guilds (Houde *et al.*, 2006). Along factor 2, a grouping of Cattle Egrets (insectivores) is formed, and associated with the short-chain PFCs (PFHxS, PFHxA, PFHpA and PFOA). Terrestrial animals tend to have a different distribution of compounds due to the variety in the diet (Houde *et al.*, 2006), which may be the possible reason for the grouping.

Groupings between sites are also seen on the bi-plot. This could possible indicate similar sources related with the sites. Lenasia was associated with PFTrDA and PFOA. Lenasia is situated on the Klip River and acts as a purifier of polluted water from the Witwatersrand urban-industrial-mining complex. PFOA is used in a variety of urban and industrial products, which may attribute to the grouping with Lenasia **(Chapter 2, Table 2.2)**. A big informal settlement surrounds Lenasia, and waste water entering the catchment might attribute to the PFTrDA connotation.

Welverdiend was grouped with PFTA, PFDoA and PFTrDA. The surrounding area of Welverdiend is exposed to sources, which could be attributed to the presence of PFCs. These include abandoned and active mines, farmlands, and a WWTP. PFTrDA and PFDoA have been found in effluent from WWTPs. PFTAs main source is from atmospheric degradation of precursors (**Chapter 2, Table 2.2**).

Schoemansdrift and Bloemhof are associated with PFOS. As discussed earlier (**Section 5.2.5**), it is hypothesised that the concentrations of PFOS in Schoemansdrift might be due to the surrounding human and animal waste entering the system from the informal settlement close to Schoemansdrift. Bloemhof, on the other hand, is an agricultural area, in the lower reaches of the Vaal River. Although no immediate source of PFOS can be identified from the surrounding area, the contribution of PFOS to the area might be downstream transport of water via the Vaal River.

5.2.7 Comparison of concentrations and patterns with published data

One of the aims of the current project was to compare the obtained data with reports from around the globe. I only used literature that analysed whole single bird egg contents. From these, I only report the mean concentrations on a wet mass basis for comparison. The eggs from the United States of America (USA) had the highest concentrations (514 ng/g), followed by the current study in South Africa (398 ng/g), and the lowest concentration was found in the Faroe Island (15 ng/g) (**Figure 5.2**). The samples from the USA were collected in a highly urbanized area where storm water runoff and wastewater effluent was the main source (Sedlak & Greig, 2012). In South Africa, samples were collected from remote regions, agricultural, industrialised, and urbanised areas,

where the main contributing source seems to be WWTPs and atmospheric degradation The Faroe Island had the lowest concentrations and is seen as a remote area with less population and industry sources (Löfstrand *et al.*, 2008).

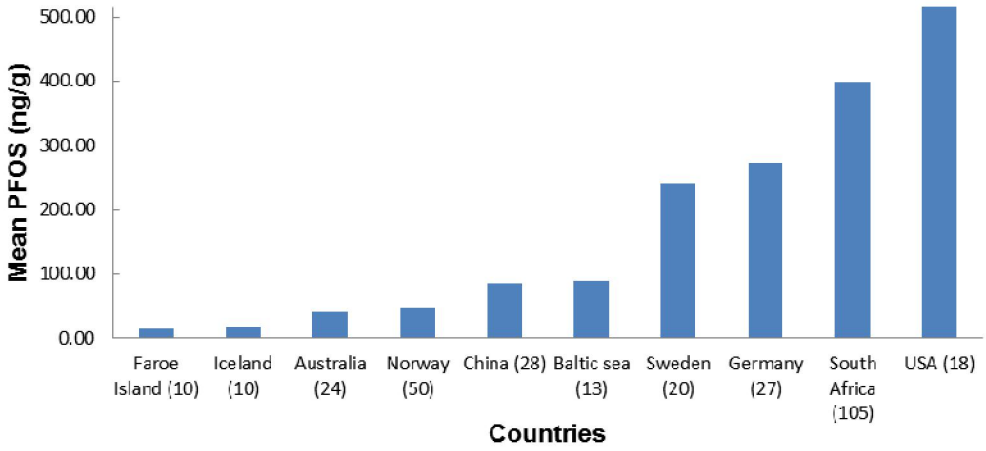

Figure 5.2: Global comparison of mean PFOS concentrations (ng/g) (Gebbink *et al.*, 2009; Holmström *et al.*, 2010; Löfstrand *et al.*, 2008; Rüdel *et al.*, 2011; Sedlak & Greig, 2012; Thompson *et al.*, 2011; Verreault *et al.*, 2007; Wang, *et al.*, 2008b)

However, when comparing the countries by feeding guild (**Figure 5.3**), South African piscivore eggs (827 ng/g) had the highest concentrations, followed by German piscivore eggs (540 ng/g), and USA piscivore eggs (515 ng/g). The piscivore eggs from South Africa had 1.5 times higher concentrations than any other country. Literature also indicates that piscivore bird eggs generally have higher concentrations than any other guild (Houde *et al.*, 2006). The lowest concentrations were found in scavenger birds from Germany (6.2 ng/g). Scavengers feed on any available food; therefore, concentrations are highly dependent on the surrounding area and sources.

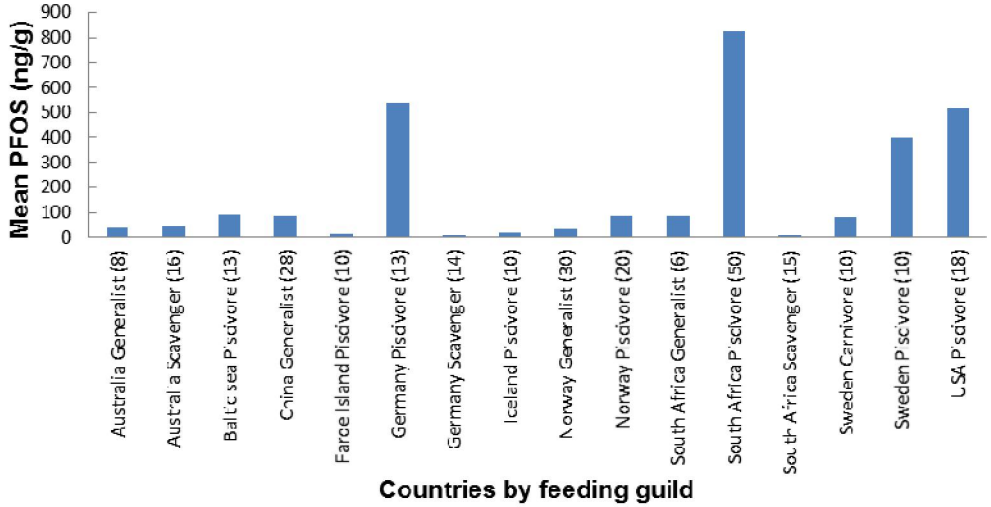

Figure 5.3: Comparison between feeding guild in selected studies (Gebbink *et al.*, 2009; Holmström *et al.*, 2010; Löfstrand *et al.*, 2008; Rüdel *et al.*, 2011; Sedlak & Greig, 2012; Thompson *et al.*, 2011; Verreault *et al.*, 2007; Wang, *et al.*, 2008b)

The PFC congeners PFOA, PFNA, PFUnA and PFDoA were compared globally (**Figure 5.4**). The studies selected analysed and detected only these congeners in all bird eggs analysed. Sweden had the highest concentrations of PFUnA (82 ng/g) and PFNA (48 ng/g), where Iceland had the highest PFDoA (28 ng/g) and USA the highest PFOA (6.8 ng/g). Concentrations for the current study were in the same range as Germany for these congeners.

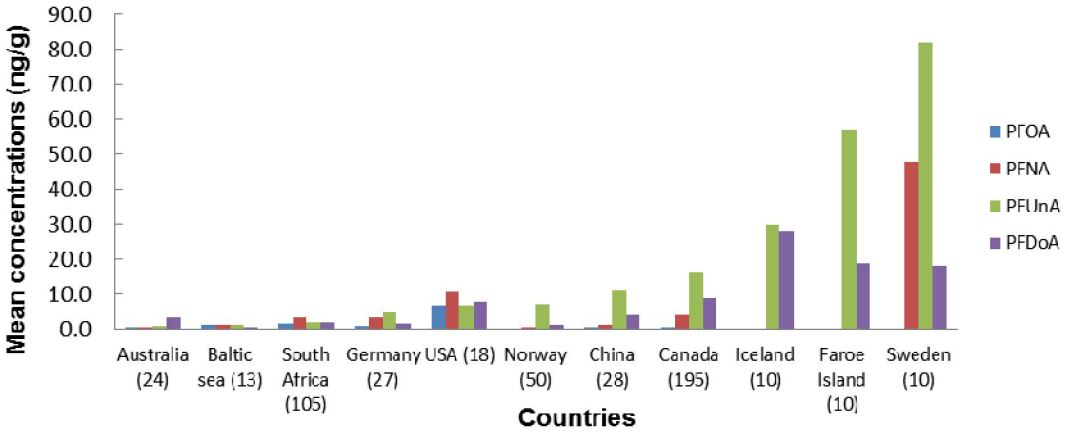

Figure 5.4: Comparison between countries for PFOA, PFNA, PFUnA and PFDoA (Gebbink *et al.*, 2009; Holmström *et al.*, 2010; Löfstrand *et al.*, 2008; Rüdel *et al.*, 2011; Sedlak & Greig, 2012; Thompson *et al.*, 2011; Verreault *et al.*, 2007, *et al.*, 2008)

Frequency of detection for each country for PFCs (PFHxS, PFOA, PFNA, PFOS, PFUnA and PFDoA) were evaluated (**Figure 5.5**). South Africa had a higher relative frequency of detection (51%), followed by China, USA, and Norway (12%). Australia (6%), Germany (4%) and Baltic Sea (2%). The current study is the biggest study done thus far for single egg analysis of PFCs (n=105), and is contributing a significant amount to data gaps of PFCs in Southern Africa.

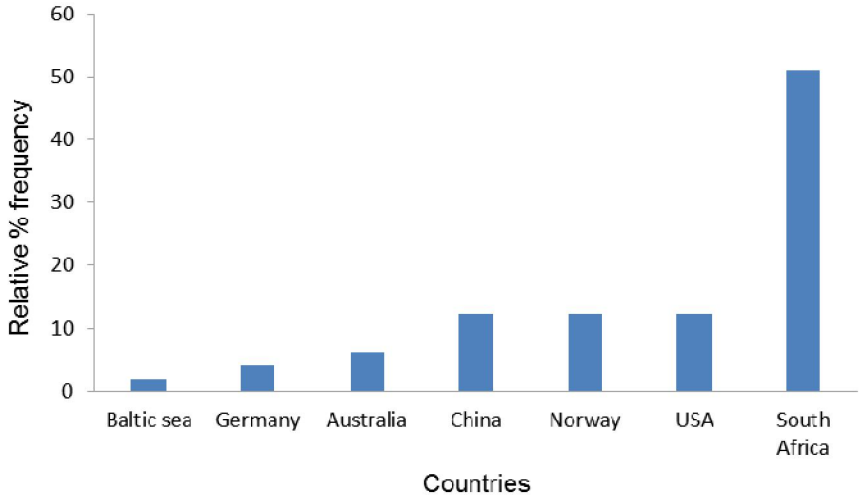

Figure 5.5: Relative frequency of detection for PFCs (PFHxS, PFOA, PFNA, PFOS, PFUnA and PFDoA) (Rüdel *et al.*, 2011; Sedlak & Greig, 2012; Thompson *et al.*, 2011; Verreault *et al.*, 2007; Wang, *et al.*, 2008b)

5.2.8 Hazard assessment

Based on the weight of evidence currently available, PFCs have a negative impact on the health of birds (Custer *et al.*, 2012; Molina *et al.*, 2006; Newsted *et al.*, 2006b; O'Brien, *et al.*, 2009b; Peden-Adams *et al.*, 2009; Yanai *et al.*, 2008). However, no consensus effects level has been proposed. One of the reasons for this is the variation in the concentrations linked to negative health impacts between laboratory and field studies. From field and laboratory studies, the following concentration dependant effects can be summarised:

- Pathological changes in the liver (bile duct hyperplasia, peri-portal inflammation and hepatic cell necrosis) were associated with concentrations of 1000 ng/g PFOS while a median lethal dose of 4900 ng/g was reported for seven day old White Leghorn chicken (*Gallus domesticus*) (Molina *et al.*, 2006).
- PFOS concentrations of 111 000 ng/g and 119 000 ng/g in the liver was associated with mortality in ten day old mallards (*Anas platyrhynchos*) and quail (*Colinus virginianus)* respectively (Newsted *et al.*, 2006).
- Studies conducted with White Leghorn chicken (*Gallus domesticus*) embryos indicated that concentrations of up to 10 000 ng/g for PFOA and PFUnA had no observable effect on hatching success. A decrease in hatching success was observed after exposures greater than 93 000 ng/g (O'Brien*et al.*, 2009a).
- A decrease of hatching success for White Leghorn chicken (*Gallus domesticus*) embryos was observer for PFOS concentrations of 93 000 ng/g (O'Brien *et al.*, 2009b).

- A field study conducted indicated PFOS concentrations as low as 150 – 200 ng/g decreased hatching success of Tree swallows (*Tachycineta bicolor*) (Custer *et al.*, 2012, Custer *et al.*, 2014).
- Concentrations of 5000 ng/g PFOA led to splayed legs for White Leghorn chickens (*Gallus domesticus*) (Yanai *et al.*, 2008). Additionally, a high degree of embryo death and decrease in hatching success was observed at 5000 ng/g.
- Significant immunological and neurological effects were observed at 1 000 ng/g PFOS for 14 day old White Leghorn chickens (*Gallus domesticus*) (Peden-Adams *et al.*, 2009). Increased spleen mass, lysozyme activity, shorter right wings, increased frequency and severity of brain asymmetry were some of the effects reported.

The range of concentrations PFCs (150 – 119 000 ng/g) causing toxicological effects and mortality from literature is large, with no definite benchmarked values. This variation is concerning, as concentrations measured in the environment also fluctuate (**Chapter 2; Table 2.7**). Comparing concentrations from the current study (**Chapter 4, Table 4.2**) with these concentrations, the following deductions are made:

- If the concentrations (93 000 – 119 000 ng/g) from Newsted *et al.*(2006); O'Brien *et al.*, (2009a,b) are compared, none of the eggs analysed for this study contained concentrations close to the effects concentrations.
- However, if the concentrations (1000 – 5000 ng/g) from Molina *et al.*(2006); Peden-Adams *et al.*(2009); Yanai *et al.*(2008) are used, the African Darter, Reed Cormorant mean and maximum concentrations are above 1000 ng/g. The maximum concentration measured in Cattle Egret eggs also exceeds this concentration. Therefore, although the concentration is below the suggested lethal dose (5000 ng/g), possible immunological, neurological and pathological changes may occur within these bird population.
- In contrast, the field study conducted by Custer *et al.*(2012, 2014) indicated concentrations as low as 150 ng/g can cause lower hatchling success. The African Darter, Reed Cormorant, White-breasted Cormorant and Grey Heron have mean concentrations higher than this. The maximum concentrations measured in the Cattle Egret, Glossy Ibis and Black-headed Heron were above this range. The Sacred Ibis was the only species analysed with concentration below concentrations reported to have a toxicological effect.

One should note however, that exposure patterns and differences in toxicokinetics between species could explain the differences in PFC profiles (Nordén *et al.*, 2013). Elimination half-lives can differ widely between homologues and species (Newsted *et al.*, 2006; Yoo *et al.*, 2009). Hatchability success or deformities of chicks were not investigated during the current study. However, many of the eggs had developing embryos, so there seems to be no acute embryo toxicity.

Table 5.2: Summary of mean and maximum concentrations per bird species

Species	Mean PFOS (ng/g)	Maximum PFOS (ng/g)
African Darter	1100	2800
Reed Cormorant	550	2000
White-breasted Cormorant	370	670
Grey Heron	550	950
Cattle Egret	75	1000
Glossy Ibis	80	260
Black-headed Heron	130	730
Sacred Ibis	10	30

Utilizing data from laboratory experiments such as egg injection studies does not always reflect scenarios in field studies, as various other factors play a role in the environment. Extrinsic factors and exact amounts of PFOS biologically incorporated into the whole egg is not reported, and might skew data when compared to field studies (Custer *et al.*, 2014). Additionally, laboratory studies generally evaluate only single compounds, whereas birds in the environment are exposed to a mixture of PFCs (Custer *et al.*, 2012), as well as a plethora of other organic pollutants. Studies conducted on South African birds indicate exposure to a variety of compounds including brominated flame retardants, polychlorinated biphenyls and pesticides (Bouwman, 2004; Bouwman *et al.*, 2008; Bouwman *et al.*, 2013; Bouwman *et al.*, 2014; Polder *et al.*, 2008; Quinn *et al.*, 2013). The combined toxicological effect of these chemical loadings on bird population success could have detrimental effects. The decrease in breeding colonies found in South Africa may be an indication of the effects of these chemical loadings on South African wild birds (Bouwman & Pieters, 2013).

Chapter 6: Conclusions & recommendations

"Where the quality of life goes down for the environment, the quality of life goes down for humans" George Holland

PFCs are persistent contaminants that are widely distributed in the global environment. Due to continual identification of these compounds in the environment, it is of key importance to determine the sources responsible. This study contributed in identifying the current levels of PFCs as well as the possible sources associated along the Orange River basin. Water, sediment, mine tailings, fish, feathers and wild bird eggs were analysed. Currently data concerning PFCs in Africa is scarce, and this study may fill these data gaps.

No PFCs were detected in the fish analysed. This could be due to bioaccumulation of PFCs in fish occur in plasma, liver and kidney, rather than muscle tissue (Ng & Hungerbuhler, 2014). Only 6% of water and 27% of sediment analysed had detectable levels. The minimal detection of PFCs could be ascribed to seasonal variation and precipitation which may influence levels of PFCs in water and sediment (Lasier *et al.*, 2011; Liu *et al.*, 2015a). Minimal detection of PFCs in mine tailings could be indicative that mining might not be the source of PFCs in the environment. However, the pH variation within tailings could influence the sorption of PFCs, and mining should not be discounted due to minimal levels found in mine tailings in the present study. There may be ways that PFCs might be released to the environment other than from tailings.

The identification of PFCs in the bird feathers could indicate the use of bird feathers as non-invasive bio-monitoring tools. PFCs were detected in all wild bird eggs analysed, with PFOS as the main contributor. Significant differences in concentrations of PFCS between bird species were seen. These differences could be attributed to multiple factors such as exposure routes (diet, feeding habitat, natal dispersal, and area of sampling) and differences in toxicokinetics (absorption, distribution, transformation and elimination) of PFCS per bird species.

Outcomes from the study:

- As far as we could determine, this is the largest study conducted thus far for single egg analysis (n=105) of PFCs.
- PFOS was the main congener detected in 95% of the eggs analysed. This is in line with a previous study conducted on the Orange River basin, as well as with current literature.
- The African Darter had higher concentrations of PFCs than any other species. The Sacred Ibis had the lowest concentration of PFOS, but the Glossy Ibis had the lowest concentration of PFCs. The variation in concentrations was attributed to differences in foraging and type of diet between bird species.
- A different congener pattern was seen for the Sacred Ibis compared to the other bird species. The contribution of PFOS was lower than in other species, with more of the other congeners detected. This could be due to the association with Sacred Ibis with human waste in the form of refuge tips and abattoirs.

- Welverdiend had the highest frequency of detection of PFC congeners. The possible sources associated with these congeners in the surrounding area are WWTP, farmlands, active and abandoned mines. However, ratios of PFHpA, PFOA, and PFNA indicated precursor breakdown and precipitation as a contributing source.
- Schoemansdrift had higher levels of PFNA and PFDA than any other site. The high ratio of PFOS/PFOA could indicate a direct fluoropolymer source or contribution from a WWTP. However, none is present in the area and the high levels of PFOS could be due to domestic and human waste discharged into the river. Main sources of PFDA is from atmospheric degradation
- The high level of PFOS were found in Orkney and could be due to mining utilised in the surrounding area. Orkney had the highest ratio of PFOS/PFOA, indicating a direct fluoropolymer source or waste from a WWTP. However, no such source is known in the immediate area, and further investigation is required.
- Upington had the highest levels of PFTrDA that seems to be associated with a WWTP. However, lower ratios of PFOS/PFOA and PFOA/PFNA were found, indicating the degradation of precursors as the source.
- Lowest levels of PFCs were detected at Sasolburg. However, the amount of samples were low (n=4). Barberspan had the lowest ratio of PFOS/PFOA, indicating detected PFOS and PFOA are from atmospheric degradation.
- Toxicological data is dependent on which no observed adverse effect level (NOEL) is used from literature. If the least conservative option is chosen, with maximum PFOS levels: African Darter, Reed Cormorant, White-breasted Cormorant, Grey Heron, Cattle Egret, Glossy Ibis and Black-headed Heron will all have levels above the NOEL. However, as variations of these levels are present in literature, further investigation is required.

Recommendations:

- Due to distance and time constraints during the sampling of the current project, samples were frozen after collection. Additionally, contemporaneous collection of samples were not possible. Therefore, the following is recommended for future studies:
    - Collection of water samples must be performed at the same time as gathering of the sediment and biota samples. To evaluate the effect of seasonal change and precipitation on the concentrations of PFCs, water and sediment should be collected in Summer, Spring, Winter and Autumn.
    - Water samples must not be frozen, and analysed within 14 days after collection.
    - Wild bird eggs and fish must be sampled at the same time, preferable from the same collection site.

- Due to the significance of the diet and foraging of bird species, different food source can be sampled to evaluate the contribution of the differences in diet to the levels of PFCs. Different fish species and insects can be collected for the evaluation.
- During the sampling of the fish, only muscle was collected. For future studies, plasma, liver and kidney should be considered to evaluate the differences in PFC concentration between these compartments.
- Although evidence of the amount of PFOS imported to South Africa is available, no indication is given on its applications or use. Therefore, monitoring programs evaluating the uses of PFOS in South Africa is advised. This could assist in identifying possible sources responsible for PFCs in the environment.
- Further research evaluating the toxicity of PFCs on South African birds are required. Hatchability success as well as chick development at environmental concentrations and mixtures should be evaluated to understand the effect thereof on the birds.
- Due to evidence of degradation of precursors as one of the sources of PFCs, an air monitoring study is proposed to identify which precursors might be responsible.
- Additionally, as degradation of precursors in biota could be a supplementary source of PFCs, precursors should be evaluated in fish and wild bird eggs.
- Current studies of PFCs in bird eggs separate the yolk from the albumen, and analyse them separately. This is recommended for future studies to evaluate the accumulation in each and establish if this coincides with trends found in literature.

Future studies:

The mechanism of transfer of PFCs from the adult female bird to the egg has not yet been elucidated. However, it is currently accepted that PFCs are transferred from the liver of the female bird to the yolk of the egg through very-low-density lipoproteins, phosvitin and lipovitellin (Newsted *et al.*, 2007). Additionally, the yolk composition that is a combination of both lipids and protein, may play a role in the preferential accumulation of PFCs as this environment is ideally suited to their amphiphilic nature (Nordén *et al.*, 2013). Studies in humans have indicated PFCs have the ability to bind with serum albumin (Jones *et al.*, 2003) which is the main carrier protein in human blood. Albumin can bind up to ten molecules of fatty acids and release them through passive diffusion. The transfer of fatty acids to cells are mainly driven by concentration differences between membranes (Mathews *et al.*, 2000). During the formation of the egg white (albumen) of the egg, the uterine tissue (except for those that are transferred from the blood such as transferrin), secretes albumin. However, studies have shown that PFCs accumulate in the yolk rather than the protein rich egg white. Apart from the transfer of PFCs to the yolk with the very low-density lipoproteins, phosvitin and lipovitellin, the proteins forming the albumen of the egg can also contribute as a source to the PFC concentration. As the albumin is formed in the oviduct

of the bird, it can bind to PFCs from die surrounding tissue and blood. As the albumin reaches the yolk, a diffusion gradient can form and the PFCs are released to the yolk. Therefore, PFCs can accumulate into the yolk from the liver of the female bird, as well as through the albumin formed around the egg. This explains the higher levels of PFCs in the birds eggs compared to other compartments analysed in the female bird (Gebbink & Letcher, 2012; Holmström & Berger, 2008; Verreault *et al.*, 2007). However, future research is required to evaluate if the albumin secreted from the uterine tissue contains PFCs before forming around the developing yolk.

In conclusion, PFCs are present in the South African environment, with high concentrations found in bird species. Although contradiction exist in the toxicological no-observed-effects levels (NOELs) for PFCs in birds, the concurrent exposure to these compounds with other POPs may have detrimental effects on the South African bird population. Therefore, monitoring of these compounds in biotic and abiotic samples and the identification of possible contributing sources are of importance. Identifying the fish and invertebrate species consumed by the birds can assist in elucidating the main contributing sources of PFCs.

References

"Literature adds to reality, it does not simply describe it." C. S. Lewis

Ahrens, L. 2011. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. *Journal of Environmental Monitoring*. 13(1):20–31.

Ahrens, L. & Bundschuh, M. 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environmental Toxicology and Chemistry*. 33(9):1921–1929.

Ahrens, L., Taniyasu, S., Yeung, L.W.Y., Yamashita, N., Lam, P.K.S., & Ebinghaus, R. 2010. Distribution of polyfluoroalkyl compounds in water, suspended particulate matter and sediment from Tokyo Bay, Japan. *Chemosphere*. 79(3):266–272.

Alava, J.J., McDougall, M.R.R., Borbor-Córdova, M.J., Calle, K.P., Riofrio, M., Calle, N., Ikonomou, M.G., & Gobas, F.A.P.C. 2015. Perfluorinated Chemicals in Sediments, Lichens, and Seabirds from the Antarctic Peninsula — Environmental Assessment and Management Perspectives. In: Emerging Pollutants in the Environment - Current and Further Implications. 51–73.

Armitage, J.M., Macleod, M., & Cousins, I.T. 2009. Comparative assessment of the global fate and transport pathways of long-chain perfluorocarboxylic acids (PFCAs) and perfluorocarboxylates (PFCs) emitted from direct sources. *Environmental Science & Technology*. 43(15):5830–6.

Austin, B. 1998. The effects of pollution on fish health. *Journal of Applied Microbiology*. 85 Suppl 1(DECEMBER 1998):234S–242S.

Bahta, Y.T. & Groenewald, J.A. 2015. The potential impact of a Southern African common External Tariffs Regime on the Economy of Lesotho. *International Journal of Food and Agricultural Economics*. 3(2):75–89.

Ballesteros-Gómez, A., Rubio, S., & van Leeuwen, S. 2010. Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level. *Journal of Chromatography. A*. 1217(38):5913–21.

Barton, C. A, Butler, L.E., Zarzecki, C.J., Flaherty, J., & Kaiser, M. 2006. Characterizing perfluorooctanoate in ambient air near the fence line of a manufacturing facility: comparing modeled and monitored values. *Journal of the Air & Waste Management Association (1995)*. 56:48–55.

Benskin, J.P. 2011. Application of perfluorinated acid isomer profiles for manufacturing and exposure source determination. University of Alberta Libraries.

Benskin, J.P., Yeung, L.W.Y., Yamashita, N., Taniyasu, S., Lam, P.K.S., & Martin, J.W. 2010. Perfluorinated acid isomer profiling in water and quantitative assessment of manufacturing

source. *Environmental Science & Technology*. 44(23):9049–9054.

Bertin, D., Ferrari, B.J.D., Labadie, P., Sapin, A., Garric, J., Budzinski, H., Houde, M., & Babut, M. 2014. Bioaccumulation of perfluoroalkyl compounds in midge (*Chironomus riparius*) larvae exposed to sediment. *Environmental Pollution*. 189:27–34.

Beškoski, V.P., Takemine, S., Nakano, T., Slavković Beškoski, L., Gojgić-Cvijović, G., Ilić, M., Miletić, S., & Vrvić, M.M. 2013. Perfluorinated compounds in sediment samples from the wastewater canal of Pančevo (Serbia) industrial area. *Chemosphere*. 91(10):1408–1415.

Bouwman, H. 2004. South Africa and the Stockholm Convention on Persistent Organic Pollutants. *South African Journal of Science* 100:323–329.

Bouwman, H., Booyens, P., Govender, D., Pienaar, D., & Polder, A. 2014. Chlorinated, brominated, and fluorinated organic pollutants in Nile crocodile eggs from the Kruger National Park, South Africa. *Ecotoxicology and Environmental Safety*. 104:393–402.

Bouwman, H., Govender, D., Underhill, L., & Polder, A. 2015. Chlorinated , brominated and fluorinated organic pollutants in African Penguin eggs : 30 years since the previous assessment. *Chemosphere*. 126:1–10.

Bouwman, H. & Pieters, R. 2013. POPs , PAHs and elemental levels in sediment , fish and wild bird eggs in the Orange – Senqu River basin. Report 002/2013 for ORASECOM, Pretoria.

Bouwman, H., Polder, A., Venter, B., & Skaare, J.U. 2008. Organochlorine contaminants in cormorant, darter, egret, and ibis eggs from South Africa. *Chemosphere*. 71(2):227–41.

Bouwman, H., Viljoen, I.M., Quinn, L.P., & Polder, A. 2013. Halogenated pollutants in terrestrial and aquatic bird eggs: Converging patterns of pollutant profiles, and impacts and risks from high levels. *Environmental Research*. 126:240–253.

Brooke, D., Footitt, A., & Nwaogu, T.A. 2004. Environmental risk evaluation report : Perflurorooctanesuphonate (PFOS). Environmental Agency. OCTANESULPHONATE (PFOS).

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., Voogt, P. De, Jensen, A.A., Kannan, K., Mabury, S. A., & van Leeuwen, S.P.J. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*. 7(4):513–541.

Butt, C.M., Berger, U., Bossi, R., & Tomy, G.T. 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Science of the Total Environment*. 408(15):2936–2965.

Butt, C.M., Muir, D.C.G., & Mabury, S. A. 2014. Biotransformation pathways of fluorotelomer-

based polyfluoroalkyl substances: A review. *Environmental Toxicology and Chemistry*. 33(2):243–267.

Bytingsvik, J., van Leeuwen, S.P.J., Hamers, T., Swart, K., Aars, J., Lie, E., Nilsen, E.M.E., Wiig, O., Derocher, A.E., & Jenssen, B.M. 2012. Perfluoroalkyl substances in polar bear mother-cub pairs: a comparative study based on plasma levels from 1998 and 2008. *Environment International*. 49:92–9.

Calle, P., Alvarado, O., Monserrate, L., Cevallos, J.M., Calle, N., & Alava, J.J. 2015. Mercury accumulation in sediments and seabird feathers from the Antarctic Peninsula. *Marine Pollution Bulletin*. 91(2):410–417.

Castiglioni, S., Valsecchi, S., Polesello, S., Rusconi, M., Melis, M., Palmiotto, M., Manenti, A., Davoli, E., & Zuccato, E. 2014. Sources and fate of perfluorinated compounds in the aqueous environment and in drinking water of a highly urbanized and industrialized area in Italy. *Journal of Hazardous Materials*. 282:51–60.

Chaplin, M.F. 2001. Water: Its importance to life. *Biochemistry and Molecular Biology Education*. 29(2):54–59.

Chen, S., Jiao, X., Gai, N., Li, X., Wang, X., Lu, G., Piao, H., Rao, Z., & Yang, Y. 2016. Perfluorinated compounds in soil, surface water, and groundwater from rural areas in eastern China. *Environmental Pollution*. 211:124–131.

Chen, Y., Guo, Z., Wang, X., & Qiu, C. 2008. Sample preparation. *Journal of Chromatography A*. 1184(1–2):191–219.

Chinetti, G., Fruchart, J., & Staels, B. 2000. Peroxisome proliferator-activated receptors (PPARs): Nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflammation Research*. 49:497–505.

Christie, I., Reiner, J.L., Bowden, J.A., Botha, H., Cantu, T.M., Govender, D., Guillette, M.P., Lowers, R.H., Luus-Powell, W.J., Pienaar, D., Smit, W.J., & Guillette, L.J. 2016. Perfluorinated alkyl acids in the plasma of South African crocodiles (*Crocodylus niloticus*). *Chemosphere*. *Elsevier Ltd*. 154:72–78.

Conder, J.M., Hoke, R. A, Wolf, W. De, Russell, M.H., & Buck, R.C. 2010. Are PFCAs Bioaccumulative ? – A Critical Review and Comparison with Persistent Lipophilic Compounds. Environmental Science & Technology. 42(4):1–31.

Corsini, E., Luebke, R.W., Germolec, D.R., & DeWitt, J.C. 2014. Perfluorinated compounds: Emerging POPs with potential immunotoxicity. *Toxicology Letters*. 230(2):263–70.

Custer, C.M., Custer, T.W., Dummer, P.M., Etterson, M. A., Thogmartin, W.E., Wu, Q., Kannan, K., Trowbridge, A., & McKann, P.C. 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Archives of Environmental Contamination and Toxicology*. 66(1):120–138.

Custer, C.M., Custer, T.W., Schoenfuss, H.L., Poganski, B.H., & Solem, L. 2012. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reproductive Toxicology*. 33:556–562.

Custer, T.W., Erwin, R.M., & Stafford, C. 1983. Residues in Common Tern Eggs from Organochlorine Nine Atlantic Coast Colonies , 1980. *Waterbird Society*. 6:197–204.

Davies, T.C. & Mundalamo, H.R. 2010. Environmental health impacts of dispersed mineralisation in South Africa. *Journal of African Earth Sciences*. 58(4):652–666.

De Smet, H., Blust, R., & Moens, L. 1998. Absence of albumin in the plasma of the common carp *Cyprinus carpio*: Binding of fatty acids to high density lipoprotein. *Fish Physiology and Biochemistry*. 19(1):71–81.

De Voogt, P. & Sáez, M. 2006. Analytical chemistry of perfluoroalkylated substances. *Trends in Analytical Chemistry*. 25(4):326–342.

de Vos, M.G., Huijbregts, M. A. J., van den Heuvel-Greve, M.J., Vethaak, A. D., Van de Vijver, K.I., Leonards, P.E.G., van Leeuwen, S.P.J., de Voogt, P., & Hendriks, A. J. 2008. Accumulation of perfluorooctane sulfonate (PFOS) in the food chain of the Western Scheldt estuary: Comparing field measurements with kinetic modeling. *Chemosphere*. 70(10):1766–73.

DEA (Department of Environmental Affairs). 2013. Development of national inventory for ten new persistent organic pollutants (POPs) in South Africa. First Intrim Report. South Africa.

Ding, G. & Peijnenburg, W.J.G.M. 2013. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Critical Reviews in Environmental Science and Technology*. 43(6):598–678.

Domingo, J.L. 2012. Health risks of dietary exposure to perfluorinated compounds. *Environment International*. 40:187–95.

Du, Z., Deng, S., Bei, Y., Huang, Q., Wang, B., Huang, J., & Yu, G. 2014. Adsorption behavior and mechanism of perfluorinated compounds on various adsorbents-A review. *Journal of Hazardous Materials*. 274:443–454.

DWA (Department of Water and Sanitation). 2009. Executive summary for Minicipal waste water treatment: North West Province. South Africa.

DWA (Department of Water and Sanitation). 2015. Department of water and sanitation [Date accessed: 12 November 2015]. URL https://www.dwa.gov.za/default.aspx.

DWAF (Department of Water Affairs and Forestry). 2004. Internal strategic perspective: Lower Orange River water management area DWAF Report nr 14/000/00/0304.

Ellis, D. A., Martin, J.W., De Silva, A.O., Mabury, S. A., Hurley, M.D., Sulbaek Andersen, M.P., & Wallington, T.J. 2004. Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. *Environmental Science & Technology*. 38(12):3316–3321.

Eschauzier, C. 2013. Perfluoroalkyl acids in drinking water : Sources, fate and removal. University of Amsterdam.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., & Nadal, M. 2014. PBPK modeling for PFOS and PFOA: Validation with human experimental data. *Toxicology Letters*. 230(2):244–51.

Fernández-Sanjuan, M., Meyer, J., Damásio, J., Faria, M., Barata, C., & Lacorte, S. 2010. Screening of perfluorinated chemicals (PFCs) in various aquatic organisms. *Analytical and Bioanalytical Chemistry*. 398:1447–1456.

Fowler, J., Cohen, L., & Jarwes, P. 1998. Practical Statistics for Field Biology. 2nd ed. John Wiley & Sons: New York.

Fromme, H., Tittlemier, S. A, Völkel, W., Wilhelm, M., & Twardella, D. 2009. Perfluorinated compounds--exposure assessment for the general population in Western countries. *International Journal of Hygiene and Environmental Health*. 212(3):239–70.

Furness, R.W. 1993. Birds as monitors of pollutants. Birds as monitors of environmental change. Chapman & Hall: London.

Gebbink, W. A., Hebert, C.E., & Letcher, R.J. 2009. Perfluorinated carboxylates and sulfonates and precursor compounds in herring gull eggs from colonies spanning the Laurentian Great Lakes of North America. *Environmental Science & Technology*. 43(19):7443–7449.

Gebbink, W. A. & Letcher, R.J. 2011. Body compartment accumulation and burden and transfer to eggs of perfluoroalkyl sulfonates in Herring gulls as avian model. *Organohalogen Compounds*. 73:1957–1960.

Gebbink, W. A. & Letcher, R.J. 2012. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environmental Pollution*. 162:40–47.

Gebbink, W. A., Letcher, R.J., Burgess, N.M., Champoux, L., Elliott, J.E., Hebert, C.E., Martin,

P., Wayland, M., Weseloh, D.V.C., & Wilson, L. 2011. Perfluoroalkyl carboxylates and sulfonates and precursors in relation to dietary source tracers in the eggs of four species of gulls (Larids) from breeding sites spanning Atlantic to Pacific Canada. *Environment International*. 37(7):1175–1182.

Gebbink, W. A., Letcher, R.J., Hebert, C.E., & Chip Weseloh, D.V. 2011. Twenty years of temporal change in perfluoroalkyl sulfonate and carboxylate contaminants in herring gull eggs from the Laurentian Great Lakes. *Journal of Environmental Monitoring*. 13(12):3365.

Giesy, J.P. & Kannan, K. 2002. Perfluorochemical surfactants in the environment. *Environmental Science & Technology*. 36(7):146A–152A.

Giesy, J.P., Naile, J.E., Khim, J.S., Jones, P.D., & Newsted, J.L. 2010. Aquatic toxicology of perfluorinated chemicals. *Reviews of Environmental Contamination and Toxicology*. 202:1-52

Gómez, C., Vicente, J., Echavarri-Erasun, B., Porte, C., & Lacorte, S. 2011. Occurrence of perfluorinated compounds in water, sediment and mussels from the Cantabrian Sea (North Spain). *Marine Pollution Bulletin*. 62(5):948–55.

Gomis, M.I., Wang, Z., Scheringer, M., & Cousins, I.T. 2015. A modeling assessment of the physicochemical properties and environmental fate of emerging and novel per- and polyfluoroalkyl substances. *Science of The Total Environment*. 505:981–991.

Greenwood, J.J.D. 2004. Birds As Biomonitors : Principles and Practice. In: Anseling, A. (ed.) Bird Numbers 1995, Proceedings of the International Conference and 13th meeting of the European Bird Census Council, Parnu, Estonia. *Bird Census News.* 13(2000):1–10.

Guo, C., Zhang, Y., Zhao, X., Du, P., Liu, S., Lv, J., Xu, F., Meng, W., & Xu, J. 2015. Distribution, source characterization and inventory of perfluoroalkyl substances in Taihu Lake, China. *Chemosphere*. 127:201–207.

Guo, Z., Liu, X., & Krebs, K.A. 2009. Perfluorocarboxylic Acid Content in 116 Articles of Commerce. EPA/600?R-09/033.

Guruge, K.S., Tanabe, S., Fukuda, M., Yamagishi, S., & Tatsukawa, R. 1997. Accumulation pattern of persistent organochlorine residues in common cormorants (*Phalacrocorax carbo*) from Japan. *Marine Pollution Bulletin*. 34(3):186–193.

Hansen, K.J., Clemen, L. A., Ellefson, M.E., & Johnson, H.O. 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environmental Science & Technology*. 35(4):766–770.

Hanssen, L., Halina, R., Odland, Ø., Moe, K., & Sandanger, T.M. 2010. Perfluorinated

compounds in maternal serum and cord blood from selected areas of South Africa : results of a pilot study. *Journal of Environmental Monitoring* 12:1355–1361.

Haukås, M., Berger, U., Hop, H., Gulliksen, B., & Gabrielsen, G.W. 2007. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution*. 148:360–371.

Hecht, T., Uys, W., & Britz, P.. 1988. The culture of sharptooth catfish, *Clarias gariepinus* in southern Africa. South Africa.

Higgins, C.P., Field, J. A, Criddle, C.S., & Luthy, R.G. 2005. Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environmental Science & Technology*. 39(11):3946–3956.

Higgins, C.P. & Luthy, R.G. 2006. Sorption of perfluorinated surfactants on sediments. *Environmental Science & Technology*. 40(23):7251–7256.

Hockey, P.A.R., Dean, W.R.J., & Ryan, P.G. 2011. Roberts Birds of Southern Africa. 7th ed. Trustees of the John Voelcker Bird Book Fund.

Holmström, K.E. & Berger, U. 2008. Tissue distribution of perfluorinated surfactants in common guillemot (Uria aalge) from the Baltic Sea. *Environmental Science & Technology*. 42(16):5879–84.

Holmström, K.E., Johansson, A.K., Bignert, A., Lindberg, P., & Berger, U. 2010. Temporal trends of perfluorinated surfactants in Swedish peregrine falcon eggs (*Falco peregrinus*), 1974-2007. *Environmental Science & Technology*. 44:4083–4088.

Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R., & Muir, D.C.G. 2006. Biological monitoring of polyfluoroalkyl substances: A review. *Environmental Science & Technology*. 40(11):3463–3473.

Houde, M., Silva, A.O. De, Muir, D.C.G., Letcher, R.J., Canada, E., Saint-laurent, C., & Street, M. 2011. Monitoring of Perfluorinated Compounds in Aquatic Biota : An updated review. *Environmental Science & Technology*. 45:7962–7973.

Howel, D. 2007. Multivariate data analysis of pollutant profiles: PCB levels across Europe. *Chemosphere*. 67(7):1300–7.

Huizenga, J.M., Silberbauer, M., Dennis, R., & Dennis, I. 2013. An inorganic water chemistry dataset (1972-2011) of rivers, dams and lakes in South Africa. *Water SA*. 39(2):335–339.

IUCN (International Union for Concervation of Nature). 2015. The IUCN Red List of Threatened Species [Date accessed: 25 October 2015]. URL http://www.iucnredlist.org/.

Jahnke, A. & Berger, U. 2009. Trace analysis of per- and polyfluorinated alkyl substances in various matrices-how do current methods perform? *Journal of Chromatography. A*. 1216(3):410–21.

Jarnburg, U., Holstrom, K., Bavel, V.B., & Kärrman, A. 2006. Perfluoroalkylated acids and related compounds (PFAS) in the Swedish environment. *Chemistry, Sources, Exposure*.

Jaspers, V., Covaci, A., Maervoet, J., Dauwe, T., Voorspoels, S., Schepens, P., & Eens, M. 2005. Brominated flame retardants and organochlorine pollutants in eggs of little owls (*Athene noctua*) from Belgium. *Environmental Pollution*. 136(1):81–88.

Jia, Y.T. & Chen, Y.F. 2013. River health assessment in a large river: Bioindicators of fish population. *Ecological Indicators*. 26:24–32.

Johansson, J.H., Berger, U., Vestergren, R., Cousins, I.T., Bignert, A., Glynn, A., & Darnerud, P.O. 2014. Temporal trends (1999–2010) of perfluoroalkyl acids in commonly consumed food items. *Environmental Pollution*. 188:102–108.

Jones, K.C. & de Voogt, P. 1999. Persistent organic pollutants (POPs): state of the science. *Environmental Pollution*. 100(1–3):209–221.

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., & Giesy, J.P. 2003. Binding of Perfluorinated Fatty Acids To Serum Proteins Paul. *Environmental Toxicology and Chemistry / SETAC*. 22(11):2639–49.

Kannan, K., Tao, L., Sinclair, E., Pastva, S.D., Jude, D.J., & Giesy, J.P. 2005. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. *Archives of Environmental Contamination and Toxicology*. 48(4):559–66.

Kim, M., Li, L.Y., Grace, J.R., & Yue, C. 2014. Selecting reliable physicochemical properties of perfluoroalkyl and polyfluoroalkyl substances (PFASs) based on molecular descriptors. *Environmental Pollution*. 196:462-472

Klaunig, J.E., Babich, M. A, Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R. A, & Fenner-Crisp, P. A. 2003. PPAR α agonist-induced rodent tumors: modes of action and human relevance. *Critical Reviews in Toxicology*. 33(6):655-780

Kocagöz, R., Onmuş, O., Onat, I., Cağdaş, B., Sıkı, M., & Orhan, H. 2014. Environmental and biological monitoring of persistent organic pollutants in waterbirds by non-invasive versus invasive sampling. *Toxicology Letters*. 230(2):208–17.

Korňan, M. & Kropil, R. 2014. What are ecological guilds? Dilemma of guild concepts. *Russian*

Journal of Ecology. 45(5):445–447.

Kostianoy, A.G., Boer, J. De, Garrigues, P., Gu, J., Jones, K.C., Knepper, T.P., Newton, A., & Sparks, D.L. 2012. The Handbook of Environmental Chemistry: Polyfluorinated chemicals and transformation products. Springer.

Kudo, N., Bandai, N., Suzuki, E., Katakura, M., & Kawashima, Y. 2000. Induction by perfluorinated fatty acids with different carbon chain length of peroxisomal beta-oxidation in the liver of rats. *Chemico-biological Interactions*. 124(2):119–32.

Labadie, P. & Chevreuil, M. 2011. Partitioning behaviour of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). *Environmental Pollution*. 159(2):391–397.

Lallas, P.L. 2001. The Stockholm Convention on Persistent Organic Pollutants. *The American Journal of International Law*. 95(3):692–708.

Laporte, P. 1982. Organochlorine Residues and Eggshell Measurements of Great Blue Heron Eggs from Quebec. *Colonial Waterbirds*. 5:95–103.

Lasier, P.J., Washington, J.W., Hassan, S.M., & Jenkins, T.M. 2011. Perfluorinated chemicals in surface waters and sediments from northwest Georgia, USA, and their bioaccumulation in *Lumbriculus variegatus*. *Environmental Toxicology and Chemistry*. 30(10):2194–2201.

Lau, C. 2012. Perfluorinated compounds. *Molecular, Clinical and Environmental Toxicology*. 101:165-217

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., & Seed, J. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicological Sciences*. 99(2):366–394.

Lee, H. 2013. Environmental chemistry of commercial fluorinated surfactants: Transport, Fate, and sources of Perfluoroalkyl acid contamination in the environment. University of Toronto.

Lenntech. 2016. Water Treatment and Purification [Date accessed: 17 January 2016]. URL http://www.lenntech.com/.

Letcher, R.J., Su, G., Moore, J.N., Williams, L.L., Martin, P. A., de Solla, S.R., & Bowerman, W.W. 2015. Perfluorinated sulfonate and carboxylate compounds and precursors in herring gull eggs from across the Laurentian Great Lakes of North America: Temporal and recent spatial comparisons and exposure implications. *Science of The Total Environment*. 538:468–477.

Liu, B., Zhang, H., Yao, D., Li, J., Xie, L., Wang, X., Wang, Y., Liu, G., & Yang, B. 2015a. Chemosphere Perfluorinated compounds (PFCs) in the atmosphere of Shenzhen , China : Spatial distribution , sources and health risk assessment. *Chemosphere*. 138:511–518.

Liu, S., Lu, Y., Xie, S., Wang, T., Jones, K.C., & Sweetman, A.J. 2015b. Exploring the fate, transport and risk of Perfluorooctane Sulfonate (PFOS) in a coastal region of China using a multimedia model. *Environment International*. 85:15–26.

Löfstrand, K., Jörundsdóttir, H., Tomy, G., Svavarsson, J., Weihe, P., Nygård, T., & Bergman, Å. 2008. Spatial trends of polyfluorinated compounds in guillemot (Uria aalge) eggs from North-Western Europe. *Chemosphere*. 72(10):1475–1480.

Longnecker, M.P., Smith, C.S., Kissling, G.E., Hoppin, J.A, Butenhoff, J.L., Decker, E., Ehresman, D.J., Ellefson, M.E., Flaherty, J., Gardner, M.S., Langlois, E., Leblanc, A., Lindstrom, A.B., Reagen, W.K., Strynar, M.J., & Studabaker, W.B. 2008. An interlaboratory study of perfluorinated alkyl compound levels in human plasma. *Environmental Research*. 107(2):152–9.

Luebker, D.J., Hansen, K.J., Bass, N.M., Butenhoff, J.L., & Seacat, A.M. 2002. Interactions of flurochemicals with rat liver fatty acid-binding protein. *Toxicology*. 176(3):175–185.

Lusilao-Makiese, J.G., Cukrowska, E.M., Tessier, E., Amouroux, D., & Weiersbye, I. 2013. The impact of post gold mining on mercury pollution in the West Rand region, Gauteng, South Africa. *Journal of Geochemical Exploration*. 134:111–119.

Luthy, R.G. 2006. Sorption of Perfluorinated Surfactants on Sediments. *Environmental Science & Technology*. 40: 7251–7256.

MacKay, D. & Fraser, A. 2000. Bioaccumulation of persistent organic chemicals: Mechanisms and models. *Environmental Pollution*. 110(3):375–391.

Malinsky, M.D., Jacoby, C.B., & Reagen, W.K. 2011. Determination of perfluorinated compounds in fish fillet homogenates: method validation and application to fillet homogenates from the Mississippi River. *Analytica Chimica Acta*. 683(2):248–57.

Manera, M. & Britti, D. 2006. Assessment of blood chemistry normal ranges in rainbow trout. *Journal of Fish Biology*. 69(5):1427–1434.

Martin, J.W., Ellis, D. A., Mabury, S. A., Hurley, M.D., & Wallington, T.J. 2006. Atmospheric chemistry of perfluoroalkanesulfonamides: Kinetic and product studies of the OH radical and Cl atom initiated oxidation of N-ethyl perfluorobutanesulfonamide. *Environmental Science & Technology*. 40(3):864–872.

Martin, J.W., Kannan, K., Berger, U., de Voogt, P., Field, J., Franklin, J., Giesy, J.P., Harner, T., Muir, D.C.G., Scott, B., Kaiser, M., Järnberg, U., Jones, K.C., Mabury, S. A, Schroeder, H., Simcik, M., Sottani, C., van Bavel, B., Kärrman, A., Lindström, G., & van Leeuwen, S. 2004. Analytical challenges hamper perfluoroalkyl research. *Environmental Science & Technology*. 38(13):248A–255A.

Martin, J.W., Mabury, S. A, Solomon, K.R., & Muir, D.C.G. 2003a. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry / SETAC*. 22(1):196–204.

Martin, J.W., Mabury, S. A, Solomon, K.R., & Muir, D.C.G. 2003b. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental toxicology and chemistry / SETAC*. 22(1):189–195.

Martin, J.W., Smithwick, M.M., Braune, B.M., Hoekstra, P.F., Muir, D.C.G., & Mabury, S. A. 2004. Identification of Long-Chain Perfluorinated Acids in Biota from the Canadian Arctic. *Environmental Science & Technology*. 38:373–380.

Mathews, C.K., Holde, K.E. Van, & Ahern, K.G. 2000. Biochemistry. Benjamin Cummings.

Mccarthy, T.S., Arnold, V., Venter, J., & Ellery, W.N. 2007. The collapse of Johannesburg's Klip River wetland. *South African Journal of Science*. 103:391–397.

McCarthy, T.S. & Venter, J.S. 2006. Increasing pollution levels on the Witwatersrand recorded in the peat deposits of the Klip River wetland. *South African Journal of Science*. 102(1–2):27–34.

Meyer, J., Jaspers, V.L.B., Eens, M., & de Coen, W. 2009. The relationship between perfluorinated chemical levels in the feathers and livers of birds from different trophic levels. *The Science of the Total Environment*. 407(22):5894–900.

Miège, C., Peretti, A., Labadie, P., Budzinski, H., Le Bizec, B., Vorkamp, K., Tronczyński, J., Persat, H., Coquery, M., & Babut, M. 2012. Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France). *Analytical and Bioanalytical Chemistry*. 404(9):2721–2735.

Miller, J.N. & Miller, J.C. 2000. Statistics and Chemometrics for Analytical Chemistry. Prentice Hall.

Molina, E.D., Balander, R., Fitzgerald, S.D., Giesy, J.P., Kannan, K., Mitchell, R., & Bursian, S.J. 2006. Effects of air cell injection of perfluorooctane sulfonate before incubation on development of the white leghorn chicken (*Gallus domesticus*) embryo. *Environmental Toxicology and Chemistry / SETAC*. 25(1):227–232.

Möller, A., Ahrens, L., Surm, R., Westerveld, J., van der Wielen, F., Ebinghaus, R., & de Voogt, P. 2010. Distribution and sources of polyfluoroalkyl substances (PFAS) in the River Rhine watershed. *Environmental Pollution*. 158(10):3243–3250.

Munyika, S., Kongo, V., & Kimwaga, R. 2014. River health assessment using macroinvertebrates

and water quality parameters: A case of the Orange River in Namibia. *Physics and Chemistry of the Earth, Parts A/B/C*. 76-78:140–148.

Naicker, K., Cukrowska, E., & McCarthy, T.S. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental Pollution*. 122(1):29–40.

NECSA. 2012. Research & Development Annual Report. South Africa.

Newsted, J.L., Beach, S. A., Gallagher, S.P., & Giesy, J.P. 2006. Pharmacokinetics and acute lethality of perfluorooctanesulfonate (PFOS) to juvenile mallard and northern bobwhite. *Archives of Environmental Contamination and Toxicology*. 50(3):411–420.

Newsted, J.L., Coady, K.K., Beach, S. A., Butenhoff, J.L., Gallagher, S., & Giesy, J.P. 2007. Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. *Environmental Toxicology and Pharmacology*. 23:1–9.

Ng, C. A & Hungerbuhler, K. 2014. Bioaccumulation of Perfl uorinated Alkyl Acids : Observations and Models. *Environmental Science & Technology*. 48:4637-48

Ng, C. A & Hungerbühler, K. 2013. Bioconcentration of perfluorinated alkyl acids: How important is specific binding? *Environmental Science & Technology*. 47(13):7214–7223.

Nieuwoudt, C., Quinn, L.P., Pieters, R., Jordaan, I., Visser, M., Kylin, H., Borgen, A.R., Giesy, J.P., & Bouwman, H. 2009. Dioxin-like chemicals in soil and sediment from residential and industrial areas in central South Africa. *Chemosphere*. 76(6):774–783.

Nordén, M., Berger, U., & Engwall, M. 2013. High levels of perfluoroalkyl acids in eggs and embryo livers of great cormorant (*Phalacrocorax carbo sinensis*) and herring gull (*Larus argentatus*) from Lake Vänern, Sweden. *Environmental Science and Pollution Research International*. 20(11):8021–30.

O'Brien, J.M., Carew, A.C., Chu, S., Letcher, R.J., & Kennedy, S.W. 2009a. Perfluorooctane sulfonate (PFOS) toxicity in domestic chicken (*Gallus gallus domesticus*) embryos in the absence of effects on peroxisome proliferator activated receptor alpha (PPARα)-regulated genes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 149(4):524–530.

O'Brien, J.M., Crump, D., Mundy, L.J., Chu, S., McLaren, K.K., Vongphachan, V., Letcher, R.J., & Kennedy, S.W. 2009b. Pipping success and liver mRNA expression in chicken embryos exposed in ovo to C8 and C11 perfluorinated carboxylic acids and C10 perfluorinated sulfonate. *Toxicology Letters*. 190(2):134–139.

OECD (Organization for Economic Cooperation and Development). 2013. OECD / UNEP Global PFC Group Synthesis paper on per- and polyfluorinated chemicals (PFCs), Environment, Health

and Safety, Environment Directorate, OECD.

Olsen, G.W., Logan, P.W., Hansen, K.J., Simpson, C. A, Burris, J.M., Burlew, M.M., Vorarath, P.P., Venkateswarlu, P., Schumpert, J.C., & Mandel, J.H. 2010. An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. *AIHA journal : a journal for the science of occupational and environmental health and safety*. 64(5):651–659.

ORASECOM. 2013. The Orange – Senqu river basin Infrastructure catalogue. Report 001/2013.

ORASECOM. 2015. Orange-Senqu River Awareness Kit [Date accessed: 12 July 2015]. URL http://www.orangesenqurak.org/river/Geography/Geomorphology/Mineral+Reserves.aspx.

Pan, C. G., Ying, G. G., Liu, Y.-S., Zhang, Q. Q., Chen, Z. F., Peng, F. J., & Huang, G. Y. 2014. Contamination profiles of perfluoroalkyl substances in five typical rivers of the Pearl River Delta region, South China. *Chemosphere*. 114:16–25.

Pan, G., Jia, C., Zhao, D., You, C., Chen, H., & Jiang, G. 2009. Effect of cationic and anionic surfactants on the sorption and desorption of perfluorooctane sulfonate (PFOS) on natural sediments. *Environmental Pollution*. 157(1):325–330.

Pan, G. & You, C. 2010. Sediment-water distribution of perfluorooctane sulfonate (PFOS) in Yangtze River Estuary. *Environmental Pollution*. 158(5):1363–1367.

Pan, Y., Shi, Y., Wang, J., Jin, X., & Cai, Y. 2011. Pilot investigation of perfluorinated compounds in river water, sediment, soil and fish in Tianjin, China. *Bulletin of Environmental Contamination and Toxicology*. 87(2):152–7.

Paul, A.G., Jones, K.C., & Sweetman, A.J. 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environmental Science & Technology*. 43(2):386–392.

Peden-Adams, M.M., Stuckey, J.E., Gaworecki, K.M., Berger-Ritchie, J., Bryant, K., Jodice, P.G., Scott, T.R., Ferrario, J.B., Guan, B., Vigo, C., Boone, J.S., McGuinn, W.D., DeWitt, J.C., & Keil, D.E. 2009. Developmental toxicity in white leghorn chickens following in ovo exposure to perfluorooctane sulfonate (PFOS). *Reproductive Toxicology*. 27(3–4):307–318.

Peng, H., Wei, Q., Wan, Y., Giesy, J.P., Li, L., & Hu, J. 2010. Tissue distribution and maternal transfer of poly- and perfluorinated compounds in Chinese sturgeon (Acipenser sinensis): implications for reproductive risk. *Environmental science & technology*. 44(5):1868–74.

Pereiro, N., Moyano, R., Blanco, A, & Lafuente, A. 2014. Regulation of corticosterone secretion is modified by PFOS exposure at different levels of the hypothalamic-pituitary-adrenal axis in adult male rats. *Toxicology Letters*. 230(2):252–62.

PerkinElmer. 2016. ChemACX Database [Date accessed: 18 September 2015]. URL http://www.chemacx.com/chemacx/Forms/Home/ContentArea/Home.aspx.

Pieters, R. 2007. An assessment of dioxins , dibenzofurans and PCBs in the sediments of selected freshwater bodies and estuaries in South Africa. North-West University.

Pinkas, A., Slotkin, T. A., Brick-Turin, Y., Van der Zee, E. A., & Yanai, J. 2010. Neurobehavioral teratogenicity of perfluorinated alkyls in an avian model. *Neurotoxicology and Teratology*. 32(2):182–186.

Polder, A., Venter, B., Skaare, J.U., & Bouwman, H. 2008. Polybrominated diphenyl ethers and HBCD in bird eggs of South Africa. *Chemosphere*. 73(2):148–54.

Popovic, M., Zaja, R., & Smital, T. 2010. Organic anion transporting polypeptides (OATP) in zebrafish (Danio rerio): Phylogenetic analysis and tissue distribution. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 155(3):327–335.

Porta, M. 2014. Human contamination by persistent toxic substances: the rationale to improve exposure assessment. *Environmental Science and Pollution Research International*. 22(19):1456-1456

Posner, S. 2012. Polyfluorinated Chemicals and Transformation Products. The Handbook of Environmental Chemistry. *Springer Berlin Heidelberg*. 17(2012):25–39.

Post, G.B., Cohn, P.D., & Cooper, K.R. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. *Environmental Research*. 116:93–117.

Powley, C.R., George, S.W., Russell, M.H., Hoke, R. A., & Buck, R.C. 2008. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. *Chemosphere*. 70(4):664–672.

Prevedouros, K., Cousins, I.T.., Buck, R.C., & Korzeniowski, S.H. 2006. Critical Review Sources, Fate and Transport of Fate. *Environmental Science & Technology*. 40(1):32–44.

Quinn, L.P., Roos, C., Pieters, R., Loken, K., Polder, A., Skaare, J.U., & Bouwman, H. 2013. Levels of PCBs in wild bird eggs: Considering toxicity through enzyme induction potential and molecular structure. *Chemosphere*. 90(3):1109–1116.

Royal Society of Chemistry. 2015. ChemSpider [Date accessed: 24 January 2016]. URL http://www.chemspider.com/.

Rüdel, H., Müller, J., Jürling, H., Bartel-Steinbach, M., & Koschorreck, J. 2011. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from

representative German ecosystems. *Environmental Science and Pollution Research*. 18(9):1457–1470.

Scheringer, M. 2009. Long-range transport of organic chemicals in the environment. *Environmental Toxicology and Chemistry*. 28(4):677–690.

Schiavone, A., Corsolini, S., Kannan, K., Tao, L., Trivelpiece, W., Torres, D., & Focardi, S. 2009. Perfluorinated contaminants in fur seal pups and penguin eggs from South Shetland, Antarctica. *The Science of the Total Environment*. 407(12):3899–904.

Schultz, M.M., Higgins, C.P., Huset, C. A., Luthy, R.G., Barofsky, D.F., & Field, J.A. 2006. Fluorochemical mass flows in a municipal wastewater treatment facility. *Environmental Science & Technology*. 40(23):7350–7357.

Secretariat of the Stockholm Convention SSC. 2012. Success Stories Stockholm Convention 2001 - 2011. 1-169.

Sedlak, M.D. & Greig, D.J. 2012. Perfluoroalkyl compounds (PFCs) in wildlife from an urban estuary. *Journal of Environmental Monitoring*. 14(1):146.

Sharpe, R.L., Benskin, J.P., Laarman, A.H., MacLeod, S.L., Martin, J.W., Wong, C.S., & Goss, G.G. 2010. Perfluorooctane sulfonate toxicity, isomer-specific accumulation, and maternal transfer in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*. 29(9):1957-1966

Shoeib, M., Harner, T., M. Webster, G., & Lee, S.C. 2011. Indoor sources of poly- and perfluorinated compounds (PFCS) in Vancouver, Canada: Implications for human exposure. *Environmental Science & Technology*. 45(19):7999–8005.

Siddig, A. A. H., Ellison, A.M., Ochs, A., Villar-Leeman, C., & Lau, M.K. 2016. How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in Ecological Indicators. *Ecological Indicators*. 60:223–230.

Simberloff, D. & Dayan, T. 1991. The Guild Concept and the Structure of Ecological Communities. *Annual Review of Ecology and Systematics*. 22(1):115–143.

Simcik, M.F. & Dorweiler, K.J. 2005. Ratio of perfluorochemical concentrations as a tracer of atmospheric deposition to surface waters. *Environmental science & technology*. 39(22):8678–83.

Sinclair, E. & Kannan, K. 2006. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environmental Science & Technology*. 40(5):1408–1414.

Sinclair, E., Mayack, D.T., Roblee, K., Yamashita, N., & Kannan, K. 2006. Occurrence of

perfluoroalkyl surfactants in water, fish, and birds from New York State. *Archives of Environmental Contamination and Toxicology*. 50(3):398–410.

Skelton, P.H. 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struik.

So, M.K., Taniyasu, S., Yamashita, N., Giesy, J.P., Zheng, J., Fang, Z., Im, S.H., & Lam, P.K.S. 2004. Perfluorinated compounds in coastal waters of Hong Kong, South China, and Korea. *Environmental Science & Technology*. 38(15):4056–4063.

Squadrone, S., Ciccotelli, V., Favaro, L., Scanzio, T., Prearo, M., & Abete, M.C. 2014. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Italy: analysis of two edible fish from Lake Maggiore. *Chemosphere*. 114:181–6.

Stahl, L.L., Snyder, B.D., Olsen, A.R., Kincaid, T.M., Wathen, J.B., & Mccarty, H.B. 2014. Perfluorinated compounds in fish from U.S. urban rivers and the Great Lakes. *Science of the Total Environment.* 499:185–195.

Stahl, T., Mattern, D., & Brunn, H. 2011. Toxicology of perfluorinated compounds. *Environmental Sciences Europe*. 23(1):38.

Stockholm Convention. 2016. Stockholm Convention [Date accessed: 17 March 2014]. URL http://chm.pops.int/.

Taniyasu, S., Kannan, K., Horii, Y., Hanari, N., & Yamashita, N. 2003. A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. *Environmental Science & Technology*. 37(12):2634–2639.

Taniyasu, S., Kannan, K., So, M.K., Gulkowska, A., Sinclair, E., Okazawa, T., & Yamashita, N. 2005. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *Journal of Chromatography A*. 1093(1–2):89–97.

Tarboton, W. 2011. Roberts Guide to the Nests and Eggs of southern African Birds. Trustees of the John Voelcker Bird Book Fund: South Africa.

Thompson, J., Roach, A., Eaglesham, G., Bartkow, M.E., Edge, K., & Mueller, J.F. 2011. Perfluorinated alkyl acids in water, sediment and wildlife from Sydney Harbour and surroundings. *Marine Pollution Bulletin*. 62(12):2869–75.

Thompson, M., Ellison, S.L.R., & Wood, R. 2002. Quality assurance schemes for analytical laboratories, harmonized guidelines for single laboratory validation of methods of analysis. (IUPAC Technical Report). *Pure and Applied Chemistry*. 74(5):835–855.

Tomy, G.T., Budakowski, W., Halldorson, T., Helm, P.A., Stern, G.A., Friesen, K., Pepper, K., Tittlemier, S.A., & Fisk, A.T. 2004. Fluorinated Organic Compounds in an Eastern Arctic Marine

Food Web. *Environmental Science & Technology*. 38(24):6475–6481.

Trojanowicz, M. & Koc, M. 2013. Recent developments in methods for analysis of perfluorinated persistent pollutants. *Microchimica Acta*. 180(11–12):957–971.

Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., & Hungerbühler, K. 2008. Erratum to "Estimating consumer exposure to PFOS and PFOA" (Risk Analysis (2008) 28, 2, (251-269)). *Risk Analysis*. 28(3):807.

UNDP (United Nations Development Programme). 2008. Orange-Senqu River basin preliminary transboundary diagnostic analysis.

UNDP (United Nations Development Programme). 2014. Orange – Senqu River Basin Transboundary Diagnostic Analysis ORASECOM Report 002/2014.

UNEP (United Nations Environment Programme). 2009. Global monitoring plan for persistent organic pollutants. (March).

UNEP. (United Nations Environment Programme) 2013. Human Exposure To POPs Across the Globe: POPs levels and human health implications. (October 2012).

US EPA (United States Environmental Protection Agency). 1994. Method 1613B: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC / HRMS [Date accessed: 18 February 2015]. URL http://www.epa.gov/cwa-methods/approved-cwa-test-methods-organic-compounds.

US EPA (United States Environmental Protection Agency). 2009. Method 537: Determination of selected perfluorinated alkyl acids in drinking water by solid phase extraction and liquid chromatography/tandem mass sepctrometry (LC/MS/MS) [Date accessed: 18 February 2015]. URL http://www.epa.gov/water-research/epa-drinking-water-research-methods.

Van der Oost, R., Beyer, J., & Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*. 13(2):57–149.

van Leeuwen, S.P.J., Karrman, A., van Bavel, B., de Boer, J., & Lindstrom, G. 2006. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples. *Environmental Science & Technology*. 40(24):7854–60.

Vanden Heuvel, J.P. 2006. Differential activation of nuclear receptors by Perfluorinated fatty acid analogs and natural fatty acids: A comparison of human, mouse, and rat peroxisome proliferator-activated receptor α, β, and γ, Liver X Receptor-β, and Retinoid X Receptor-α. *Toxicological Sciences*. 92(2):476–489.

Verreault, J., Berger, U., & Gabrielsen, G.W. 2007. Trends of perfluorinated alkyl substances in herring gull eggs from two coastal colonies in northern Norway: 1983-2003. *Environmental Science & Technology*. 41:6671–6677.

Verreault, J., Villa, R. A., Gabrielsen, G.W., Skaare, J.U., & Letcher, R.J. 2006. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environmental Pollution*. 144(3):1053–1060.

Wang, S., Wang, H., & Deng, W. 2013. Perfluorooctane sulfonate (PFOS) distribution and effect factors in the water and sediment of the Yellow River Estuary, China. *Environmental monitoring and assessment*. 185(10):8517–24.

Wang, Y., Yeung, L.W.Y., Taniyasu, S., Yamashita, N., Lam, J.C.W., & Lam, P.K.S. 2008a. Perfluorooctane sulfonate and other fluorochemicals in waterbird eggs from South China. *Environmental Science & Technology*. 42(21):8146–8151.

Wang, Y., Yeung, L.W.Y., Yamashita, N., Taniyasu, S., So, M.K., Murphy, M.B., & Lam, P.K.S. 2008b. Perfluorooctane sulfonate (PFOS) and related fluorochemicals in chicken egg in China. *Chinese Science Bulletin*. 53:501–507.

Wang, F., Shih, K & Leckie, J.O. 2015. Effect of humic acid on the sorption of perfluorooctacne sulfonate (PFOS) and Perfluorobutane sulfonate (PFBS) on Boehmite. *Chemosphere*. 118:213-218

Wania, F. & Mackay, D. 1996. Peer reviewed: tracking the distribution of persistent organic pollutants. *Environmental Science & Technology*. 30:390A–6A.

Washburn, S.T., Bingman, T.S., Braithwaite, S.K., Buck, R.C., Buxton, L.W., Clewell, H.J., Haroun, L. A., Kester, J.E., Rickard, R.W., & Shipp, A.M. 2005. Exposure Assessment and Risk Characterization for Perfluorooctanoate in Selected Consumer Articles. *Environmental Science & Technology*. 39(11):3904–3910.

Wepener, V., van Dyk, C., Bervoets, L., O'Brien, G., Covaci, A., & Cloete, Y. 2011. An assessment of the influence of multiple stressors on the Vaal River, South Africa. *Physics and Chemistry of the Earth, Parts A/B/C*. 36(14–15):949–962.

Xiao, F., Simick, M.F & Gulliver, J.S. 2012. Partitioning characteristics of Perfluorooctane sulfonate between water and foods. *Archives of Environmental Contamination and Toxicology*. 63:42-48.

Yanai, J., Dotan, S., Goz, R., Pinkas, A., Seidler, F.J., Slotkin, T. A, & Zimmerman, F. 2008. Exposure of developing chicks to perfluorooctanoic acid induces defects in prehatch and early posthatch development. *Journal of Toxicology and Environmental Health. Part A*. 71(2):131–

133.

Yang, L., Zhu, L., & Liu, Z. 2011. Occurrence and partition of perfluorinated compounds in water and sediment from Liao River and Taihu Lake, China. *Chemosphere*. 83(6):806–14.

Ylinen, M., Hanhijärvi, H., Peura, P., & Rämö, O. 1985. Quantitative gas chromatographic determination of perfluorooctanoic acid as the benzyl ester in plasma and urine. *Archives of Environmental Contamination and Toxicology*. 14(6):713–717.

Yoo, H., Guruge, K.S., Yamanaka, N., Sato, C., Mikami, O., Miyazaki, S., Yamashita, N., & Giesy, J.P. 2009. Depuration kinetics and tissue disposition of PFOA and PFOS in white leghorn chickens (*Gallus gallus*) administered by subcutaneous implantation. *Ecotoxicology and Environmental Safety*. 72(1):26–36.

You, C., Jia, C., & Pan, G. 2010. Effect of salinity and sediment characteristics on the sorption and desorption of perfluorooctane sulfonate at sediment-water interface. *Environmental Pollution*. 158(5):1343–1347.

Zafeiraki, E., Costopoulou, D., Vassiliadou, I., Bakeas, E., & Leondiadis, L. 2014. Determination of perfluorinated compounds (PFCs) in various foodstuff packaging materials used in the Greek market. *Chemosphere*. 94:169–176.

Zakaria, M. & Rajpar, M.N. 2010. Bird species composition and feeding guilds based on point count and mist netting methods at the Paya Indah Wetland Reserve, Peninsular Malaysia. *Tropical Life Sciences Research*. 21(2):7–26.

Zamani, M., Khorasani, N., Bakhtiari, A.R., & Rezaei, K. 2015. Source identification of perylene in surface sediments and waterbird eggs in the Anzali Wetland, Iran. *Environmental Pollution*. 205:23–32.

Zushi, Y. & Masunaga, S. 2009. Identifying the nonpoint source of perfluorinated compounds using a geographic information system based approach. *Environmental Toxicology and Chemistry*. 28(4):691–700.