# Persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River basin

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Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Science with Zoology* at the North-West University

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| Promoter: | Prof H Bouwman |
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Graduation May 2023

23505257

**Declarations**

The samples were collected by the North-West University and analysed by Oekometrics in Germany.

I conducted the data analyses, interpreted the data, and drafted all the chapters.

I consulted co-authors for clarification purposes only.

All four manuscript chapters are ready for submission.

This work is my own and has been checked for plagiarism.

I received permission from my supervisor and co-authors to submit these manuscripts as part of my dissertation for my degree, *Doctor of Philosophy in Science with Zoology* at North-West University.

# Persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River basin

**Velesia Lesch**

## Preface

My thesis concerns the of persistent organic pollutants in an abiotic and biotic matrix from the Orange-Senqu River Basin. The two matrixes used are wild bird eggs and sediment. The thesis is presented in an article format.

Outline of the thesis: This thesis is presented in six chapters

**Chapter 1:** Background information and an introduction to the two matrixes as well as the persistent organic pollutants. A brief overview of the Orange-Senqu River Basin and the threat it faces is also provided.

**Chapter 2:** A review on the Cattle Egret as a near-global terrestrial indicator of pollution

**Chapter 3:** Persistent organic pollutants in eight species of wild bird eggs from four sites in the Vaal River

**Chapter 4:** The first country-wide survey on polychlorinated biphenyls and polychlorinated dibenzo-*p*-dioxins and dibenzofurans concentrations in sediment from 22 sites across South Africa

**Chapter 5:** The largest multi-pollutant survey on persistent organic pollutants in sediment from 61 sites across four countries

**Chapter 6:** Discussion, conclusions, and recommendations

**Acknowledgements**

I would like to thank my mentor, supervisor, and rolemodel Professor Henk Bouwman.

And my co-supervisor Prof Rialet Pieters

I would also like to thank my dear friends and family, especially my loving mother and father.

To the love of my life Nicolaas, I cannot express in words my appreciation for your support, compassion and love.

# Persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River basin


## Abstract

The Orange-Senqu River Basin (OSRB) stretches over four southern Africa countries that are all Parties to the Stockholm Convention on Persistent Organic Pollutants (SCPOPs). The main tributary, the Vaal River on the eastern side of the basin drains a large industrial region before it confluences with the Orange-Senqu River. The Orange-Senqu River with its origin in Lesotho flows west providing irrigation water for agricultural activities. It confluences with the Vaal River near Douglas before continuing westwards, flowing past mining, rural, and agricultural activities, discharging into the Atlantic Ocean. Persistent organic pollutants (POPs) are omnipresent in the environment and concerns about the pollution in the OSRB prompted several surveys. Two matrices, an abiotic and a biotic matrix were selected to investigate the pollution in the basin.

Due to their physical and chemical properties, POPs bioaccumulate in higher trophic level species. Bird eggs were selected as a biotic matrix since birds inhabit many niches and trophic levels. In Chapter 2, I review the Cattle Egret (*Bubulcus ibis*) as a suitable near-global indicator of terrestrial pollution. It is the most wide-spread Ardeid with an extensive natural expansion, occurring in high numbers and breed in colonies with other Ardeids. In Chapter 3 I evaluate the effectiveness of eggs as indicators of POPs pollution of nine Ardeids species collected from four locations and analysed for 21 pesticides, five polybrominated diphenyl ethers (PBDEs), 18 polychlorinated biphenyls (PCBs) including 6 non dioxin-like PCBs (NDL-PCBs) and 12 dioxin-like PCBs (DL-PCBs), 17 polychlorinated dibenzo-p-dioxins and dibenzo-p-furans (PCDD/Fs), and perfluorooctane sulfonate (PFOS). The evaluation showed that aquatic predators had higher PFOS and PCB concentrations than terrestrial predators and scavengers, while PCDD/F dominated in eggs of terrestrial species. The highest organochlorine pesticide (OCP) concentrations were in eggs from the industrial regions of Gauteng. PFOS concentrations (2300 ng/g wm) in eggs collected at Bloemhof Dam pose a severe risk and I identified this location as a PFOS hotspot. The evaluation of the data showed the importance of multi-species studies sampling from multiple locations to assess the risk that POPs pose to avian populations as hotspots and species at risk may be missed by species- and site-restricted studies.

In Chapter 4, sediment as the abiotic matrix were collected in 2002 from 22 sites across South Africa and analysed for DL-PCBs and PCDD/Fs. The concentrations quantified in sediment ranged considerably between sites (41–25 000 ng/kg dm) with the highest toxic equivalency quotient (ΣTEQ) of 22 ngTEQ/kg dm quantified from the highly industrialised Gauteng province. The ΣTEQ concentrations from seven sites, all

close to industrial activities, exceeded international sediment quality guidelines (ISQG). In Chapter 5, sediment collected in 2009, from 61 sites across the OSRB were analysed for all POPs listed in the SCPOPs in 2010. Nine sites near industrial regions had quantifiable ΣOCP concentrations, while four of the sites exceeded ISQG for dichlorodiphenyltrichloroethanes (DDTs; max 9 ng/g dm). No quantifiable concentrations of PFOS were detected in sediment from hotspots identified from the bird egg study. Lindane was quantified at three sites in the upper Orange-Senqu River that all exceeded SQGs (1 ng/g dm). PBDE concentrations (15 ng/g dm) were highest near the confluence of the Orange-Senqu River and Vaal River than in the industrial region. However, PCB concentrations (Sum DL-PCB and NDL-PCB) were highest in industrial regions (340 ng/kg dm) and even higher near mining activities (1053 ng/kg dm). Most of the sediment samples had quantifiable concentrations of PCDD/F most notably on the eastern side of the basin near industry. Industry is therefore likely the main contributor to POPs pollution in the basin. The temporal trend suggests that PCB and PCDD/F concentrations in Gauteng are decreasing. Future studies should therefore concentrate on POP concentrations in sediment on the eastern side of the basin near industry. However, bird eggs are more sensitive matrixes and should therefore be selected over sediment. Furthermore, POPs added to the SCPOPs since 2010 would need a wider survey. Studies along freshwater systems should prioritise biota rather than sediments to enable better assessments of current pollution trends.

**Key words:** Sediment, wild bird egg, Vaal River, South Africa, Africa, POP, PCB, PCDD/F, DDT.

# Persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River basin

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**Chapter 5**

Figure 1: Site numbers of the 61 sediment sampling sites in the Orange-Senqu River Basin. Coordinates are provided in Table S1.

**Chapter 6**

## List of acronyms and abbreviations

| | |
|---|---|
| AD | African Darter |
| ANOVA | Analysis of variance |
| ASI | African Sacred Ibis |
| BFM | Bloemfontein |
| BHH | Black-headed Heron |
| CE | Cattle Egret |
| DDD | Dichlorodiphenyldichloroethane |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| dm | dry mass |
| EET | embryo induced eggshell thinning |
| EPA | Environmental Protection Agency |
| EPA | Environmental protections agency |
| g | gram |
| GC/MS | Gas Chromatography Mass Spectrometry |
| GH | Grey Heron |
| GI | Glossy Ibis |
| GWE | Great White Egret |
| HCB | Hexachlorobenzene |
| JHB | Johannesburg |
| km | kilometer |
| KNP | Kruger National Park |
| KZN | KwaZulu-Natal |
| LC/MS-MS | Liquid chromatography–mass spectrometry |
| LE | Little Egret |
| LRAT | Long range atmospheric transport |
| m | meter |
| ng/g | nanogram per gram |
| ng/kg | nanogram per kilogram |
| OCP | Organochlorine Pesticide |

| | |
|---|---|
| OHP | Organohalogen pesticides |
| OSRB | Orange-Senqu River Basin |
| PBDE | Polybrominated biphenyl ether |
| PCB | Polychlorinated biphenyls |
| PCDD | Polychlorinated dibenzodioxins |
| PCDD/F | Polychlorinated dibenzofurans and dibenzo-p-dioxins |
| PCDF | Polychlorinated dibenzo-p-dioxins |
| PFAS | Per- and Polyfluorinated Substances |
| PFC | Perfluorinated compound |
| PFOS | Perfluorooctane sulfonic acid |
| POP | Persistent organic pollutant |
| PTA | Pretoria |
| p-value | Probability value |
| RC | Reed Cormorant |
| RSA | Republic of South Africa |
| SCPOPS | Stockholm Convention on Persistent Organic Pollutants |
| TDA | Transboundary diagnostic analysis |
| TEQ | Toxic equivalency quotients |
| TOC | Total Organic Content |
| US | United States |
| WHO | World Health Organization |
| wm | wet mass |
| α | alpha |
| β | beta |
| γ | gamma |
| Σ | sigma |

# Persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River basin

## Chapter 1

## 1. Introduction

A rapid increase in industrialization, urbanization, and globalization started at the turn of the 19th century. New discoveries in chemistry and manufacturing were driven by supply and demand (e.g. better, more efficient, cheaper) products by an ever increasing human population. In addition, efficient pest control measures in agriculture and disease vector control have benefited from these developments and demands, but also caused environmental impacts.

Rachel Carson, in her iconic book 'Silent spring' (Carson, 1962), forcefully and eloquently described the severe impacts that pesticides such as DDT had on biota.

> *'There was a strange stillness. The birds for example — where had they gone? Many people spoke about them, puzzled and disturbed. The feeding stations in the backyards were deserted. The few birds seen anywhere were moribund: they trembled violently and could not fly. It was a spring without voices ... only silence lay over the fields and woods and marsh."*

The social conscience awakened by this book 50 years ago resulted in a range of interventions and actions to address the insidious threat from some chemicals. Dichlorodiphenyltrichloroethane (DDT) was banned and the United States Environmental Protection Agency was created as a result of this book and other actions. The sad history of the effects of the use of Agent Orange in chemical warfare in Vietnam added to the global consciousness of the dangers that such compounds pose to the environment and human health (Young et al., 2004). Many other such dangerous chemicals were identified with similar persistent and toxic effects and were banned by countries. However, the banning of compounds did not address global pollution of compounds that are toxic, travel long distances, and break down slowly in the environment and biota. To combat the effects of such chemicals on a global scale, an international treaty, the Stockholm Convention on Persistent Organic Pollutants (SCPOPs) was negotiated in 2000 and became international law in 2004. Currently, there are 186 states that are party to the SCPOPs (Stockholm Convention, 2016a).

Persistent organic pollutants (POPs) are lipophilic compounds that are environmentally persistent, can be conveyed over long distances, and are toxic (Newman., 2015). In general, there are three groups or classes of POPs: organochlorine pesticides (OCPs), industrial compounds such as polybrominated diphenyl ethers (PBDE), and unintentionally produced compounds such as polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibensofurans (PCDFs). Some of them can be placed in more than one of these classes. The subject of my thesis concerns the presence, concentrations, and threats these compounds may pose in the Southern African environment.

### 1.1 Overview

In this chapter, I will briefly review the three POPs categories and summarize the international action that has been taken on POPs. South Africa is a Party to the SCPOPs, therefore subject to the obligations and requirements thereof. Part of the SCPOPs obliges Parties to support

and report studies on POPs and the effectiveness of the implementation of the SCPOPs. Focussing on environmental concerns, I will discuss two frequently-used matrixes to measure environmental concentrations (sediment and wild bird eggs) in pursuit of South Africa's obligations to assess the effectiveness of interventions to reduce releases to the environment. For this purpose, establishment of historical trends and distribution would be crucial. In this thesis, I present studies in support of a basis to assess progress in achieving the objective of the SCPOPs, "…to protect human health and the environment from persistent organic pollutants". Based on this overview, I will formulate the aims and objectives of this thesis.

## 1.2 Persistent organic pollutant classes

### 1.2.1 Organochlorine pesticides

Organochlorine pesticides are a group of chemicals designed, produced, and formulated to control pests such as insects, fungi, and weeds. Organochlorine pesticides are effective due to their persistent nature and ability to disrupt physiological activities. Aldrin, DDT, dieldrin, heptachlor, chlordane, endrin, hexachlorobenzene (HCB), mirex, lindane, and toxaphene are all OCPs that had been used for pest control (Stockholm Convention, 2016b). The application of such chemicals also affected non-target organisms and humans (Carson 1962; Damalas et al., 2011; Khan et al., 2005). A complete list of such effects falls outside the scope of this thesis, and I will only refer to a few here to illustrate. DDTs have been linked to endocrine disruption, carcinogenesis, and birth defects (Bornman et al., 2022; Horak et al., 2021; Soto and Sonnenschein, 2010; Soto and Sonnenschein, 2015). In animals, thyroid follicular cell tumours have been found in rodents (Hurley, 1998), testicular oocytes in fish (Pieterse et al., 2010) and thinner egg shells in birds exposed to pesticides (Bouwman et al., 2008; Bouwman et al., 2019). Pesticides ultimately have the ability to reduce populations and destroy ecosystems (Carson, 1962; Peakall, 1970; Shore et al., 2019; Sparling et al., 2001)

Most of the OCPs are listed under Annex A of the SCPOPs requiring Parties to eliminate the production and use of these compounds. DDT, however, is listed under Annex B with Parties obliged to restrict the production and use of this compound for malaria control (Stockholm Convention, 2016b). Human breast milk from lactating woman in malaria plagued regions were shown to contain DDT concentrations exceeding the maximum residue limit (Bouwman et al., 1990; Bouwman et al., 2012). Perfluorooctane sulfonic acid (PFOS), in Annex B, has an "acceptable purpose" as a pesticide to control leaf-cutting ants (Stockholm Convention, 2016b).

### 1.2.2 Industrial compounds

Industrial compounds are used in industrial processes and manufacture. Some will be discussed here, briefly. The industrial POPs include PBDE and perfluorinated chemicals (PFCs) such as PFOS. PBDEs are used as additives in flame retardants in synthetic materials such as plastics, paints and textiles (de Wit, 2002). The PBDE compounds like all other POPs are persistent, capable of long-range transport, and can bioaccumulate and biomagnify (Segev et al., 2009; Stockholm Convention, 2016b). PBDEs have been associated with carcinogenicity in rodents (National Toxicology Program, 1986), effects on neurodevelopment (Eriksson et al., 2001), and thyroid dysfunction (Morse et al., 1993; Rosiak et al., 1997). PFOS is also very persistent, however unlike many other POPs that tend to accumulate in lipid tissue, PFOS has a strong affiliation with proteins. This compound is used in firefighting foams, metal plating, electric and electronic components, and in aviation hydraulic fluids (Stockholm

Convention, 2016b). It should be noted that PFOS is also a degradation product of many other PFCs. Exposure to PFOS can result in immune suppression (Pachkowski et al., 2019) and endocrine disruption (White et al., 2011). In fish, embryonic and maternal exposure in elevated concentrations of PFOS can lead to deformities and mortality in offspring (Wang et al., 2011). PBDEs are listed under Annex A, while PFOS is listed in Annexes A and B of the SCPOPs.

Polychlorinated biphenyls (PCBs) are listed in Annexes A and C as they are still actively used (although being phased out), but are also a by-product of combustion. PCBs are used in industry as heat exchange fluids and additives in paint and plastics, to list a few applications/uses (Stockholm Convention, 2016b). Some PCB congeners are referred to as dioxin-like compounds (depending on the number of chlorine atoms and their positions). Due to their continued use, leakage from storage, and unintentional formation in combustion processes, PCBs enter the environment where they become available for uptake by fauna and flora (Collins and Fryer, 2006; Odabasi et al., 2015). Genotoxicity, neurotoxicity, carcinogenesis, and acute lethality were observed in animals exposed to PCBs, while respiratory problems, chloracne, and hepatic damage were observed in humans (ATSDR, 2014), to name but a few. In Asia, the Yusho and Yusheng rice bran oil incidents occurred when rice oil was accidentally contaminated by technical formulations of PCB. This resulted in countless health problems including teratogenic effects and increased occurrences of still births (Ikeda, 1996; Yoshimura, 2003).

### 1.2.3 Unintentionally produced compounds

Unintentionally produced compounds are by-products of industrial processes and combustion. These include, *inter alia*, PCDDs, PCDFs and PCBs. The former two are collectively referred to as PCDD/Fs. PCBs can be divided into two groups, dioxin-like PCBs (DL-PCBs) that have similar toxicity to PCDD/F, and non dioxin-like PCBs (NDL-PCBs) that are mainly unintentionally produced combustion processes (Van den Berg et al., 2006). There are over 200 congeners of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic and has a toxic equivalency factor (TEF) value of one (1). All other dioxin like compounds have TEF values in relation to the toxicity of 2,3,7,8-TCDD. 2,3,7,8, TCDD caused great harm as a production pollutant in chlorinated herbicides that were used in chemical warfare in Vietnam (Young et al., 2004). One of the most consequential incidents of PCDD/F exposure is the “Seveso incident” in 1976 in Italy when a chemical reactor exploded, resulting in numerous adverse health effects and the intentional (via euthanasia) and unintentional death of many wild and domesticated animals (Fiedler, 1996). The toxicity of mixtures of dioxin like compounds are expressed as toxic equivalency (TEQ; Van den Berg et al., 2006).

PCDDs have different binding affinities, although they, *inter alia*, react with the aryl hydrocarbon receptor (AhR; Mandal, 2005) through which they mediate most of their harmful effects. Prolonged exposure and elevated levels of PCDDs can cause reproductive and developmental problems (Eleni et al., 2013; Kobayashi et al., 2017; Papadopoulou et al., 2013; Patandin et al., 1998). Moreover, PCDDs have been linked to immune and enzyme disorders and are human carcinogens (Newman., 2015; Şahin and Saçan., 2018; Stockholm Convention, 2016b). PCDD/Fs and PCBs are listed under Annex C with the aim of reducing the unintentional releases of such compounds. It should be mentioned however, that there are also natural sources for some POPs (Bouwman, 2003), that include natural burning such as vegetation fires and volcanic eruptions (Fiedler, 1996). In addition, there are some indications

that PCDDs can be formed biologically from chlorinated phenols under the right environmental conditions (Fiedler, 2003).

Regular monitoring is needed to assess South Africa's effectiveness of interventions in support of the SCPOPs obligations and aims to reduce and/or eliminate the release of POPs. South Africa ranked first in African countries in number of published literature focused on POPs, the majority of which comes from the North-West University (Olisha et al., 2022). Both abiotic and biotic monitoring matrixes are frequently used to assess POP pollution (Horak et al., 2021). However, more research is needed for South Africa to monitor its successful implementation of its SCPOPs obligations (Bouwman, 2003). PCDD/F concentrations, in particular have been greatly underrepresented in literature (Olisah et al., 2022).

## 1.3 Matrices

### 1.3.1 Sediment

Sediment is a useful abiotic sample matrix since it is easy and cost effective to collect and does not require lengthy and complicated ethical applications (Nieuwoudt et al., 2009; Olukunle et al., 2012; Riedo et al., 2021; Singovszka et al., 2017). Due to the hydrophobic nature of POPs, sediment acts as a sink, trapping compounds into the organic component of sediments (Ustaoğlu and Tepe., 2019). Although studies have shown that microorganisms have a high tolerance to POPs (Girones et al., 2021), it is the biomagnification of POPs in higher trophic level species that pose a risk. Sediment core samples can be used to investigate historical pollution trends (Bigus et al., 2014). Sediment cores sampled in the Selenga River located in Siberia, Russia, showed an increase in PCB concentrations from the 1930s, probably due to combustion processes, while organochlorines such as DDTs and PCBs peaked between the 1960s and 1980s (Adams et al., 2018).

The concentrations of different POPs in sediment are attributed to the physical and chemical properties of the matrix and the compound, and proximity to pollution sources (Dueri et al., 2008). Sediment are good indicators of point source pollution since higher concentrations of POPs have been quantified in sediments near pollution sources such as industry (Fiedler, 2003). POPs are also capable of long range atmospheric transport (LRAT; Wania and Mackay, 1995), and as a result, POPs have been quantified in sediment and soil from remote regions such as Antarctica (Klánová et al., 2008) and the Arctic (Wang et al., 2019).

### 1.3.2 Wild bird eggs

Bird eggs are a regularly-used biotic matrix to monitor POP concentrations since they decompose slowly, have similar organic compositions, and are easy and cost effective to collect while not hindering population dynamics (Keller et al., 2013: Nieuwoudt et al., 2009; Swackhamer et al., 2009). Bird eggs represent the pollution uptake of the female bird prior to breeding (Braune et al., 2007; Gao et al., 2009; Jaspers et al., 2005). Bird species occupy different habitats, and feeding guilds providing the opportunity to monitor different exposure routes on different trophic levels. The most common route of human exposure to POPs is through diet, especially animal products (Djien Liem et al., 2000). Gull eggs from arctic regions have been found to contain elevated concentrations of PCBs and the consumtion of these eggs have been discourage (Braune et al., 2007). Eggs can also be used as biomonitoring tools and biomarkers (Van den Steen et al., 2009) as eggshell thinning has been linked to increased DDE concentrations (Lundholm, 1997), while discolouration of eggshell pigments may be useful as none-invasive biomarkers (de Vargas et al., 2010). Large-scale monitoring

studies focused on one species in multiple countries may aid in assessing exposure patterns and POP environmental distributions and behaviour. Common Starling and Unicolour Starling eggs (*Sturnus vulgaris* and *Sturnus unicolor*, respectively) from three continents (Eens et al., 2013) have been investigated, while blue tit eggs (*Cyanistes caeruleus*) across Europe may prove effective (Van den Steen et al., 2010). However, no near-global indicator species of terrestrial POPs has been identified and promoted.

### 1.4 South Africa

Freshwater is a scarce and important resource in Southern Africa. Due to regular droughts and limited rainfall, humans depend on large rivers and water reservoirs such as dams for water needs, emphasizing the importance of good water quality. According to the Council for Scientific and Industrial Research (CSIR, 2011), only 35% of South Africa's freshwater rivers are in good condition compared with 57% of freshwater tributaries. Furthermore, 57% of rivers and 65% of wetland ecosystems are threatened (CSIR, 2011). This is concerning, considering South Africa has 28 Ramsar sites (Figure 1) that contain countless fauna and flora and play host to numerus migratory birds (Ramsar Sites Information Service, 1991). Some of the biggest contributors to water pollution in South Africa are raw sewage, industrial waste, agricultural runoff, and mining activities (Musingafi, 2014; Oberholster et al., 2008).

According to the South African Human Rights Commission (SAHRC, 2021) raw sewage has been leaking from municipalities into the Vaal River and its tributaries polluting the Vaal River beyond acceptable conditions. The Vaal River is one of the most important rivers in South Africa (Braune, 1986). Originating near Breyten in Mpumalanga, the Vaal River flows past several towns, through a number of large impoundments before flowing past Gauteng, the industrial heartland of South Africa. Several tributaries, including Klip River, Blesbok Spruit and Riet Spruit drain large industrial regions of Gauteng before its confluence with the Vaal River. Both biotic and abiotic samples from these rivers had elevated concentrations of POPs (Groffen et al., 2018; Nieuwoudt et al., 2009; Polder et al., 2008; Rimayi et al., 2016; Wepener et al., 2011). Indeed, a large portion of the available South African literature on water pollution have focused on mid- to lower regions of the Vaal River where it flows through Gauteng and into the North West Province. The Vaal River continues west towards the Atlantic Ocean, passing along mining, rural, and agricultural communitiesbefore its confluence with the Orange-Senqu River near the town of Douglas in the Northern Cape Province. The Orange-Senqu River is one of the largest rivers in Southern Africa, its source is in the Maloti Mountains of eastern Lesotho. It flows past rural and agricultural communities before its confluence with the Vaal River. The Vaal River and Orange-Senqu River catchments form the Orange-Senqu River Basin (OSRB). The basin covers an area of 1 000 000 km² and is shared between Botswana, Lesotho, Namibia, and South Africa whom are all Parties to the SCPOPs (Lange et al., 2007).

South Africa has a large agricultural sector, with approximately 15 million hectares of land used for cultivation, while a large section of this land is still treated with pesticides (Van der Laan et al., 2017; Quinn et al., 2011). The use of DDT as an agriculture pesticide in South Africa was banned in the 1970s (Bouwman, 2003). However, there is an exemption for the use of DDT in malaria endemic regions such as Limpopo and KwaZulu-Natal (KZN). Large quantities of DDT were given to African countries for malaria vector control. DDT concentrations quantified in wild bird eggs from Limpopo exceeded a "critical level for reproductive success" (Bouwman et al., 2013) while sediment quantified from KZN were found

to exceed sediment quality guidelines (Humphries, 2013). In addition, eggshell thinning was observed in eggs from both treated regions (Bouwman et al., 2013; Bouwman et al., 2019).

Organochlorine pesticides have been quantified in environmental samples outside malaria treated regions (Barnhoorn et al., 2015; Bouwman et al., 2021; Pheiffer et al., 2018; Quinn et al., 2009). A number of OCPs have been quantified in water and sediment samples from South African rivers, including aldrin, a pesticide used to control insect pests, endrin, an insecticide used on grains, and mirex, an insecticide used for termites and ants (Birungi et al., 2018; Quinn et al., 2009). Mirex however, has never been registered as pesticide in South Africa.

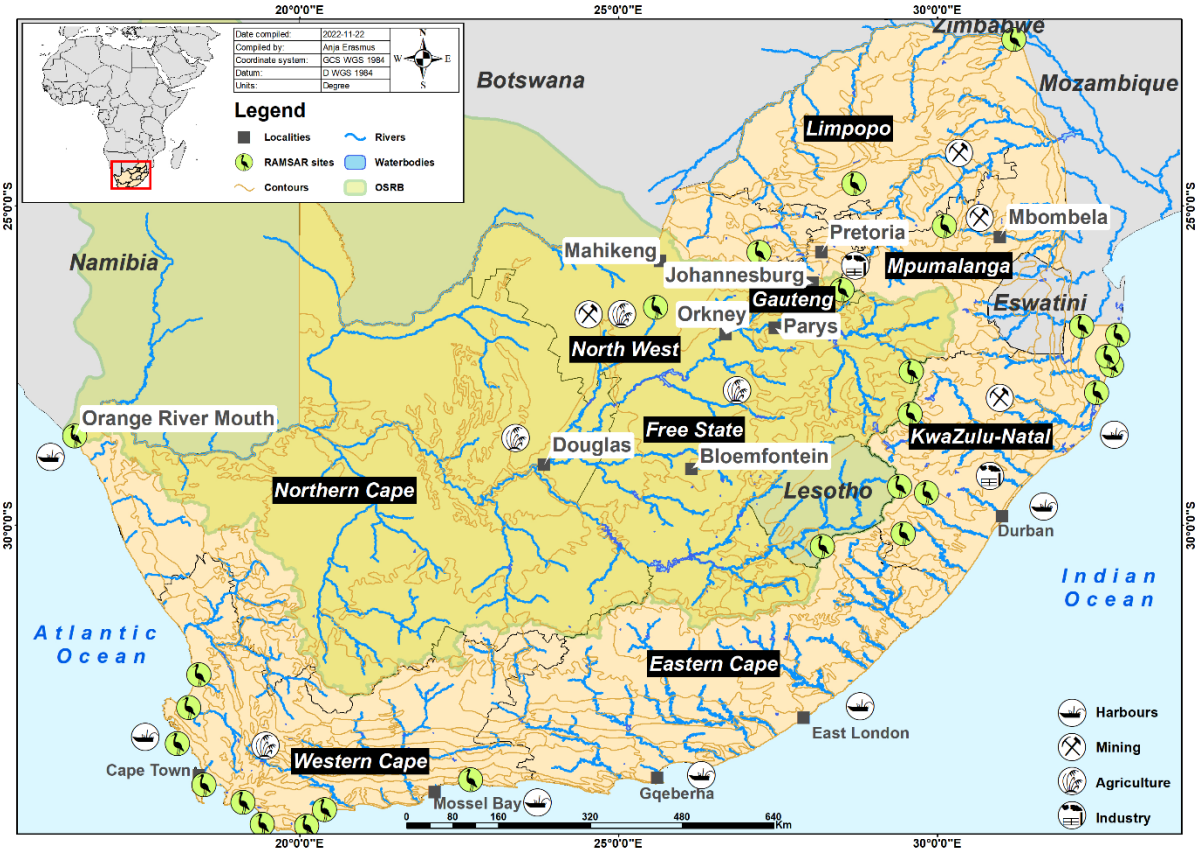


**Figure 1:** Map of Southern Africa showing the major rivers, the Orange-Senqu River Basin boundaries RAMSAR sites, towns and cities mentioned in text, major industrial and mining sites, and harbours.

Perfluorinated alkyl substances (PFASs) are less frequently investigated in South Africa. PFOS in particular, are usually found in greater quantities than other PFASs in biota (Groffen et al., 2018; Lesch et al., 2017). However, PFOS was quantified in river water (Mudumbi et al., 2014), and sediment (Fagbayigbo et al., 2022) from the Western Cape while invertebrates, fish, water, and sediment had quantifiable concentrations of PFOS in the Vaal River catchment (Groffen et al., 2018; Lesch et al., 2017). PCBs have been quantified in most types of biotic and abiotic samples when analysed for the compound (Nieuwoudt et al., 2009; Polder et al., 2008; Rimayi et al., 2016; Wepener et al., 2011), demonstrating its widespread distribution within the environment.

Reports on PCDD/F concentrations in particular, are greatly underrepresented in literature (Olisah et al., 2022), but have been reported in quantifiable concentrations in soils, sediment, and air (Nieuwoudt et al., 2009; Rimayi et al., 2016: Rimayi et al., 2022) and human blood

(Pieters and Focant, 2014). Slightly more research has been conducted on the fate of PBDE in South Africa. PBDEs have been quantified in sediment, fish, water, and bird eggs (Chokwe et al., 2015; Daso et al., 2013; Odusanya et al., 2009; Polder et al., 2008; Quinn et al., 2020).

## 2. Aims, objectives, and hypotheses

Based on the above, the overall aim of this thesis is to assess persistent organic pollutants in biotic and abiotic components of the Orange-Senqu River Basin.

Pursuant to this aim, the following objectives and hypotheses will be addressed:

1. To identify, review, and promote the use of eggs of a near-global terrestrial bird as indicator of environmental pollution.
    - Hypothesis: The Cattle Egret *Bubulcus ibis* is the only near-global terrestrial bird whose eggs can be used to uniformly and comparably measure and interpret POPs and other pollutants.

2. To investigate POP concentrations in wild bird eggs of the Vaal River, evaluate the effectiveness of a multi-species-multiple location-survey and to assess effectiveness in identifying hotspots, risks, and possible impacts.
    - Hypothesis 1: The evaluation of the concentrations quantified in eggs of each species supports and promotes the effectiveness of multi-species-multiple-location-survey in identifying hotspots, risks, and possible impacts.
    - Hypothesis 2: Concentrations of selected POPs at identified hotspots pose a risk to wild birds.

3. To report PCB and PCDD/F concentrations in sediment collected in 2002 from across South Africa, evaluate temporal trends based on data published after 2002, and compare quantified concentrations with international sediment quality guidelines.
    - Hypothesis 1: The concentrations quantified in sediment from 2002 reflect and support trends observed in more recently published data.
    - Hypothesis 2: PCBs and PCDD/Fs are omnipresent, while concentrations quantified in sediment from industrial regions exceed international sediment quality guidelines.

4. To quantify and assess all POPs listed in the SCPOPs in 2010 in sediment from 61 localities in South Africa, compare the results with other reports, identify hotspots, trends, and patterns, and compare with international sediment quality guidelines.
    - Hypothesis 1: The concentrations quantified in sediment will reflect patterns and trends observed in previous reports, existing hotspots will be reconfirmed, and previously unknown hotspots will be identified.
    - Hypothesis 2: Sediment from South African freshwater bodies does not exceed PCB and PCDD/F international sediment quality guidelines
    - Hypothesis 3: Legacy pesticides remain omnipresent and continue to pose a risk to the environment.

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# Chapter 2

# The Cattle Egret *Bubulcus ibis* as a near-global indicator of terrestrial pollution

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## Abstract

Persistent organic pollutants (POPs) are omnipresent in the environment. Due to their physical and chemical properties, POPs bioaccumulate in higher trophic level species. Birds inhabit many niches and trophic levels and share physiological characteristics with humans. Due to their mobility, and breeding and feeding habits, birds are exposed to POPs. Wild bird eggs reflect the pollution of the environment and exposure experienced by the female prior to egg formation. Bird eggs are relatively easy to collect, easy to handle and store, and decompose slowly. In addition, bird eggs can also be used as biomarkers by examining the eggshell pigmentation and thickness. Aquatic bird eggs have been used most often as indicators of POPs pollution, with less published literature on terrestrial birds that also suffer consequences of pollution. Here, we review candidate species whose eggs can be used as near-global indicators. We found the Cattle Egret (*Bubulcus ibis*) as a suitable near-global indicator of terrestrial pollution. It is the most wide-spread Ardeid with extensive natural expansion, not threatened, occur in high numbers, breed in colonies with other Ardeids, has a high trophic status, and eggs are relatively large and easy to collect. Eggs of Cattle Egrets from eight countries and four continents have been analysed for pollutants such as PCBs, DDTs, HCH, HCB, dicofol, aldrin, dieldrin, endrin, heptachlor, mirex and endosulfan. Trans-continental and multi-country studies using Cattle Egret eggs would therefore be possible.

**Key words:** DDT, POP, bird egg, PCB, avian, eggshell thickness, range expansion

## 1. Introduction

Birds are taxonomically well described, occupy many niches and trophic levels, and are often associated with humans with whom they share many foods such as grains and fish. Birds are also endothermic, a physiological characteristic only shared with mammals, both therefore facing parallel threats from pollution based on common exposure and endothermic biology (Bouwman et al., 2019; Burger and Elbin, 2015a, 2015b; Lopez-Antia et al., 2017). The mobility of birds allows them to discriminate and select between advantageous and non-supporting areas to breed, feed, shelter, and drink (Chase and Walsh, 2006). This combination of characteristics is why birds are often used as environmental indicators, from small to regional, and global scales (Machange et al., 2005; Nath et al., 2019; Pereira and Cooper 2006). Due to their mobility, birds encounter a wide range of agricultural and industrial pollutants such as persistent organic pollutants (POPs) and metals via water, air, and food. The differences in the bird's exposure are then due to behaviour, niches, trophic levels, and life histories, further influenced by the wide range of habitats they occupy. It therefore stands to reason and has been confirmed, often determined from analyses of their eggs, that bird populations differ in their pollutant profiles because of these factors (Bouwman et al., 2021, Elliott et al., 2015; Fremlin et al., 2020; Reindl and Falkowska, 2019; Schmitt et al., 2018).

Wild bird eggs reflect contamination in the environment wherein the female bird feeds prior to and during egg formation (Aurigi et al., 2000; Boncompagni et al., 2003; Reindl and Falkowska, 2019). Eggs of wild birds are suitable and useful to measure POPs concentrations reflecting those of the adult females as well as the exposure experienced by the embryo (Fängström et al., 2005). Compared with other vertebrates, bird eggs are relatively easy to collect, and the removal of eggs from nests will not have lasting effects except for endangered species. Several authors therefore promote the use of bird eggs as indicator of pollution of the environments in which they live (Bustnes et al., 2007; Custer et al., 1990; Furness and Greenwood, 2013; Padayachee et al., 2022).

Birds experience deleterious effects of pollutants. We list several studies here. Endocrine disrupting pollutants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) in birds and other animals alter thyroid hormone function and neuro-endocrine systems, alter physiological stress responses (Dawson, 2000; Fry, 1995; Langer et al., 1998), affects gonadal steroid hormones (Beard et al., 2000), delay and impair sexual maturation and mating behaviour (Ottinger et al., 2005), cause thinner eggshells, affect embryological development, and may lead to embryo mortality (Lundholm, 1997; Zimmermann et al., 1997). Eggshell pigmentation may be indicators of the female bird's health and habitat condition (Jagannath et al., 2008; Moreno and Osorno, 2003). Therefore, much information can be drawn from eggs about the health of bird populations of the same or similar species when compared across regions.

Most published studies concentrate on aquatic birds and their eggs. This is to be expected as pollutants concentrate through more trophic levels in aquatic than in terrestrial environments (Bouwman et al., 2013; Erikson et al., 2016; Yohannes et al., 2017). Osprey eggs (*Pandion haliaetus*) had five to ten times higher PFA concentrations than Tawny Owl (*Strix aluco*) and Common Kestrel (*Falco tinnunculus*) eggs from Sweden (Erikson et al., 2016). While all three are raptors, the Osprey as aquatic predator had much higher concentrations. Bouwman et al. (2013) found concentrations of ΣDDT three orders of magnitude higher in aquatic-feeding heron eggs than terrestrial-feeding Cattle Egret eggs from the same region. The higher levels

in aquatic birds does not mean that terrestrial birds do not suffer consequences. The decline and recovery of the Eurasian Sparrowhawk (*Accipiter nisus*; Crosse et al., 2012; Newton and Wyllie, 1992) is a good example. The terrestrial Cattle Egret had significantly thinner eggs with increasing ΣDDT concentrations in their eggs from regions where DDT was used (Malik et al., 2011; Mora,1991), and is still used for malaria control (Bouwman et al., 2013).

Examples of terrestrial birds as indicators include the Little Owl (*Athene noctua*; Jaspers, 2005), Snow Bunting (*Plectrophenax nivealis*; Warner et al., 2019), and the Eurasian Sparrowhawk (Crosse et al., 2012; Newton and Wyllie, 1992; Padayachee et al., 2022). Padayachee et al. (2022) reviewed POPs in raptors globally. The only raptor used as terrestrial indicator in both hemispheres was the Peregrine Falcon (*Falco pereginus*; Padayachee et al., 2022). However, the Peregrine Falcon is scarce, sensitive to multiple stressors, and nests are widely distributed (Gainzarin et al., 2010) with much effort needed to collect eggs. There are other terrestrial bird species with a near-global distribution that could serve as a pan-continental indicator of pollution. The Barn Owl (*Tyto alba*) is a candidate (Eulaers et al., 2014; Sheffield, 1997). Like all owls, they breed solitary and far between. This allows higher spatial resolution of pollutant patterns (Eulaers et al., 2014). The number of eggs that can be sampled will be limited due to distances between nests. Although the House Sparrow (*Passer domesticus*) is fairly common where they occur, breeding pairs occupy small ranges, and the eggs are small (ca. 0.6 – 2.9 g depending on locality; Dhananjayan et al., 2011; Steyn et al., 2018) requiring pooling for analyses. Probably because of these factors, little work on pollutants in House Sparrow eggs have been published (Bouwman et al., 2013; Dhananjayan et al., 2011; Nossen et al., 2016), indicating its lack of monitoring utility. The Rock (Feral) Pigeon (Dove) *Colomba livia* has a near-global distribution (All about Birds, 2022a) with very large numbers (260 million globally; BirdLife International, 2022a). It is granivorous but also feeds on berries, shoots, green leaves, and food scraps in cities and surrounding farmlands (Harris et al., 2016; Hockey et al., 2005; Moon and Zeigler, 1979). This bird breeds solitary, mostly on buildings and rocky ledges. Obtaining enough eggs though, would be problematic given the difficulty of reaching solitary nests.

Terrestrial, trans-equatorial migrants breed mostly in the Northern Hemisphere and some in the Southern hemispheres (Maclean, 1990; Salewski and Bruderer, 2007; Somveille et al., 2013), so hardly any corresponding conspecific eggs from the south or north, reducing the utility of migratory bird eggs as global indicators of terrestrial pollution. It may be argued that eggs from hemispheric migratory birds should indicate pollution from the non-breeding hemisphere, and up to a point they do. However, they come in contact with pollutants during both legs of migration, at the non-breeding sites, and after their return before breeding, confounding geographic assessments. There are very few terrestrial bird species that migrate across all continents. There are the migratory races of the Peregrine Falcon (*F. pereginus calidus* between Eurasia and Africa, and *F. pergrinus tundrius* in the America's), all of whom also breed in the Northern Hemisphere. Birds such as Barn Swallows (*Hirundo rustica*), House Martins (*Delichon urbicum*), European Bee-eaters (*Merops apiaster*), and White Storks (*Ciconia ciconia*) breed in the Southern Hemisphere, but in small numbers in small areas. Therefore, these birds have little utility as a global indicator.

There is, however, one terrestrial bird that is predatorial, with a near-global breeding distribution that would be especially useful, the Cattle Egret (*Bubulcus ibis*, with three subspecies *B. ibis ibis*; *B. ibis coromandus*, and *B. ibis seychellarum*) (Figure 1). Cattle Egrets are the most abundant and omnipresent heron species globally (Kushlan and Hancock, 2005)

(Figure 2), with an estimated global population of 4 to 9.85 million birds (Birdlife international 2022b). It feeds almost exclusively on terrestrial prey, therefore at a higher trophic level than the Rock Pigeon *C. livia*. The aim of this article is to review and assess the Cattle Egret as an avian indicator of terrestrial pollution. We review the biology of the bird, distribution, and concentrations of organochlorine in eggs worldwide. We discuss some of the implications such pollutants may have on birds and assess the utility and advantages and disadvantages of their eggs as a near-global indicator of terrestrial POPs pollution.

## 2. Materials and methods

### 2.1 Literature

A literature search was conducted to find studies that measured the concentrations of POPs in eggs of all *Bubulcus ibis* subspecies. Google scholar was used as the primary search engine. Additional studies were traced using the references of already identified publications.

Key words used were; Cattle Egret, *Bubulcus ibis*, Cattle Egrets and DDT, Cattle Egrets and organochlorines, Cattle Egrets and POPs, *Bubulcus ibis* and DDT, *Bubulcus ibis* and organochlorines, *Bubulcus ibis* and POPs, Cattle Egret distribution, Cattle Egret range expansion, Heron eggs and DDT, DDT, and bird eggs, heron eggs and organochlorines

### 2.2 Criteria to select data articles

Literature used were restricted to publications in peer reviewed journals. Only concentrations presented in table format with clear concentrations and units were considered; data represented only graphically were excluded. Concentration data in Cattle Egret eggshells, feathers, carcasses, and droppings were excluded from this review, only data on egg contents were used.

### 2.3 Measuring unit conversions and groupings

Values reported in parts per million (ppm), parts per billion (ppb), milligram per kilogram (mg/kg) and nano-gram per gram (ng/g) were compiled in a table and converted to microgram per kilogram (µg/kg). Concentrations were converted from dry mass (dm) to wet mass (wm) (Clatterbuck et al., 2018).

### 2.4 Statistics

As far as possible, we report summed (Σ) concentrations with the number of congeners analysed in subscript, such as $\Sigma PCB_6$. In case where this information was not apparent, we report it as $\Sigma PCB_?$. In studies where more than one set of samples were reported from a country, the arithmetic mean was calculated from the reported means as individual data were normally not supplied. Descriptive statistics for the residue concentrations of the organochlorines in eggs are listed in Tables S1-10 in the supplementary material. The year and location of samples collected are listed with concentrations in the supplementary material. Only where relevant, are locations and year of sampling mentioned in text. The year of sample collection is also indicated in the graphs.

## 3. Cattle Egret biography

### 3.1 Description

The Cattle Egret is a medium sized heron (Ardeidae) with white feathers and a sharp yellow bill (Kushlan and Hancock, 2005). They measure on average 51 cm in length with a wingspan

between 24 to 27 cm for males and 24 to 26 cm for females. Their mass can vary between 270-510 g, depending on sex and feeding status (Kushlan and Hancock, 2005). During the breeding season, adults develop orange/gold breeding plumage (Figure 1) that differ slightly between the subspecies. Cattle Egrets are provisionally divided into three subspecies; *Bubulcus ibis ibis*, the nominate race, which refer to Cattle Egrets originating from Africa and spread to western Asia, southern Europe, and the Americas; *B. ibis coromandus*, the eastern race, which refers to Cattle Egrets located in the remaining regions of Asia, Philippines, Australia, New Zealand, and Oceania; and *B. ibis seychellarum*, that are Cattle Egrets endemic to the Seychelles in the Indian Ocean (Figure 2). However, the status of *B. ibis seychellarum* as a subspecies is questioned (Kushlan and Hafner, 2000; Rasmussen and Anderton, 2005). According to the IUCN (2002), *Bubulcus ibis* is categorised as Least Concern.

### 3.2 Reproduction

Cattle Egrets breed during various times of the year depending on local climate, location, and availability of food (Kushlan and Hancock, 2005). However, they do not breed everywhere where they occur, especially in the colder regions. The nests are constructed over a period four to five days, using reeds, twigs, and branches. They build nests in reed beds, bushes, shrubs, trees, or on the ground near water (Kushlan and Hancock, 2005). Cattle Egrets roost and breed in small to large colonies, in many cases alongside other egret, heron, and similar colonially breeding water bird species (Kushlan and Hafner, 2000). Males partake in courting rituals and may display aggressive and territorial behaviour towards rival males. Females pair with different males each season and extra-pair copulations occur (Kushlan and Hancock, 2005). Once paired, the females lay two to five medium oval eggs that have a pale blue tint. Eggs become faded with time (Abdullah et al., 2017), and eggshells shift to whitish-green when dried (Kobayashi, 1948). Both parents incubate and feed the chicks (Kushlan and Hancock, 2005). Siblicide is common in this species, especially under restricted food availability (Creighton and Schnell, 1996).

### 3.3 Cattle Egret range expansion

Since the early 1900s, the Cattle Egret has undergone a rapid natural and human-facilitated near-global range expansion (Figure 2). It is one of the fastest range-expanding bird species (Del Hoyo et al., 1992). It occurs on all continents and on many large and small islands. Cattle Egrets can fly long distances, even crossing the Atlantic Ocean (Kushlan and Hancock, 2005). The range expansion of both the nominate and the eastern races are summarised by Maddock and Geering (1994) and elaborated in Figure 2.

Not only is the Cattle Egret now a cosmopolitan species, but the number of breeding colonies have also increased dramatically (Kushlan and Hafner, 2000). For instance, breeding colonies in Algeria increased from 51 to 87 between 1999 and 2007 (Bachir et al., 2011). Most of this expansion was unaided by humans. However, Cattle Egrets were intentionally released on the Hawaiian Islands in 1959 (Paton et al., 1986) and in Australia (Glover, 1965) as a biological control agent of pests. In addition, humans have aided in creating favourable habitats for these birds by habitat alteration, deforestation, and redirecting of waterways (Blaker, 1971; Kushlan and Hancock, 2005). The current global population is estimated at 4 to 9.85 million birds (Birdlife international 2022b).

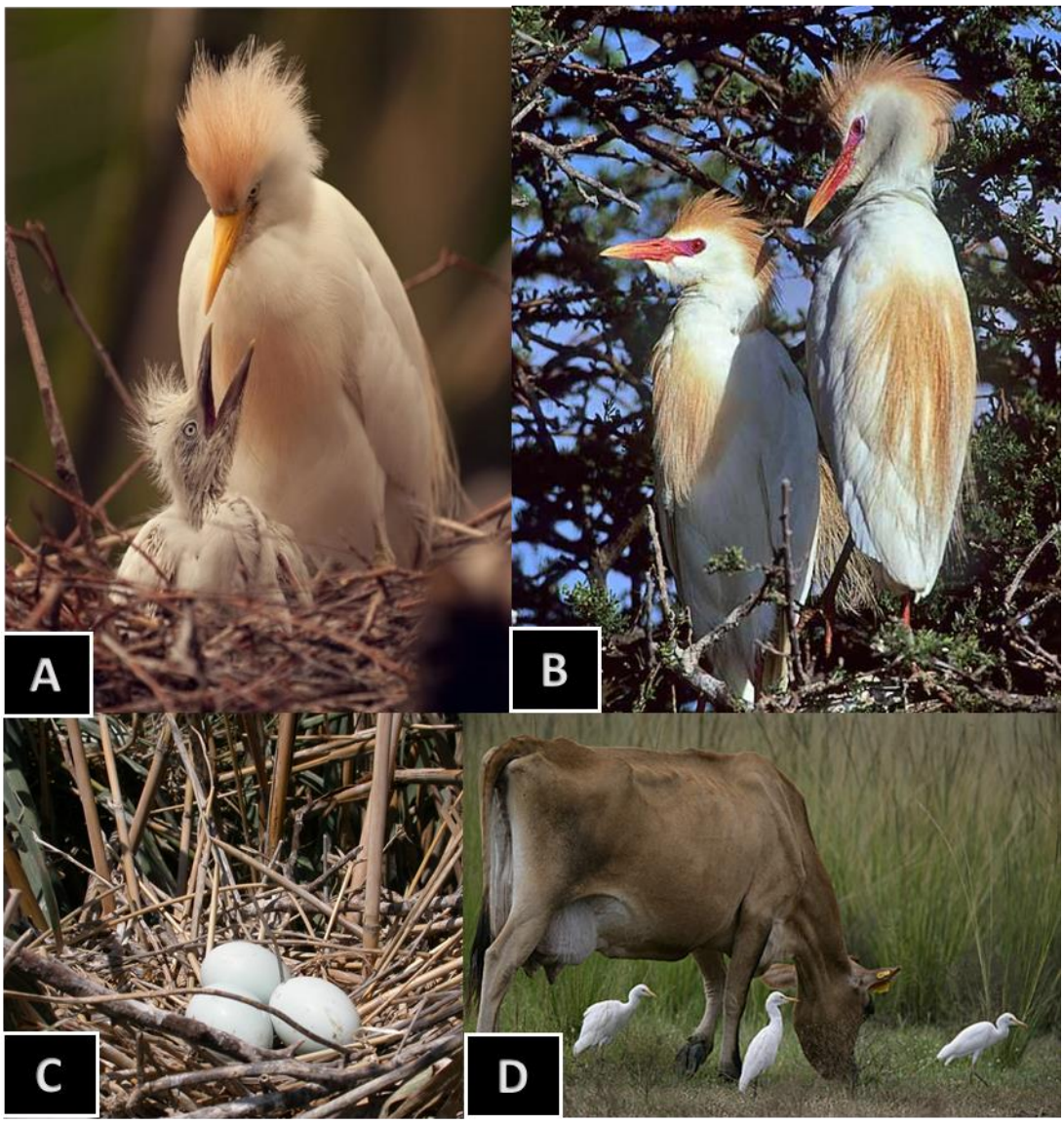



**Figure 1:** A) Adult Cattle Egret along with a nestling. B) Cattle Egrets in breeding plumage. C) Cattle Egret nest with eggs. D) Cattle Egrets foraging alongside cattle. Images from Warwick Tarboton.

3.3.1 Nominate race

The nominate race has its historic range in tropical central Africa, whereafter it quickly spread northwards towards Spain and Portugal (Figure 2). The first record we could find for Europe was an 1856 bird list (Bonaparte, 1856). Since then, it has spread across Europe and Great Britain. Cattle Egrets gradually expanded southwards, with breeding in South Africa recorded by the 1920s (Kushlan and Hancock, 2005; Siegfried, 1965). Today, this species occupies most of the African continent except some deserts. Cattle Egret also expanded westwards, towards South America and later to North America (Browder, 1973) spreading as far north as Alaska by 1981 (Maddock and Geering, 1994) and as far South as King George Islands in the Antarctic (Trivelpiece et al., 1987). Since their invasion of North America, they have spread over most of the southern half of the continent, breeding in all but four contiguous USA states, and in Manitoba, Ontario, and Saskatchewan in Canada (Fogarty and Hetrick,1973; The

Texas Breeding Bird Atlas, 2022). All About Birds (2022b) has an observation map online, although many historic records are not shown.

#### 3.3.2 Eastern race

The distribution and range expansion of the eastern race of the *B. ibis coromandus* is less well documented than the nominate. The eastern race has a historical range in southern Asia, extending from India all the way east towards southern Japan and the Philippines (Figure 2). It expanded to Australia (Hewitt, 1960) and New Zealand (Maddock and Geering, 1994; Turbott et al., 1963). The rage expansions summarised here can be attributed to the bird's ability to utilise rice fields and nest in cities (Kushlan and Hafner, 2000).

### 3.4 Habitat and diet

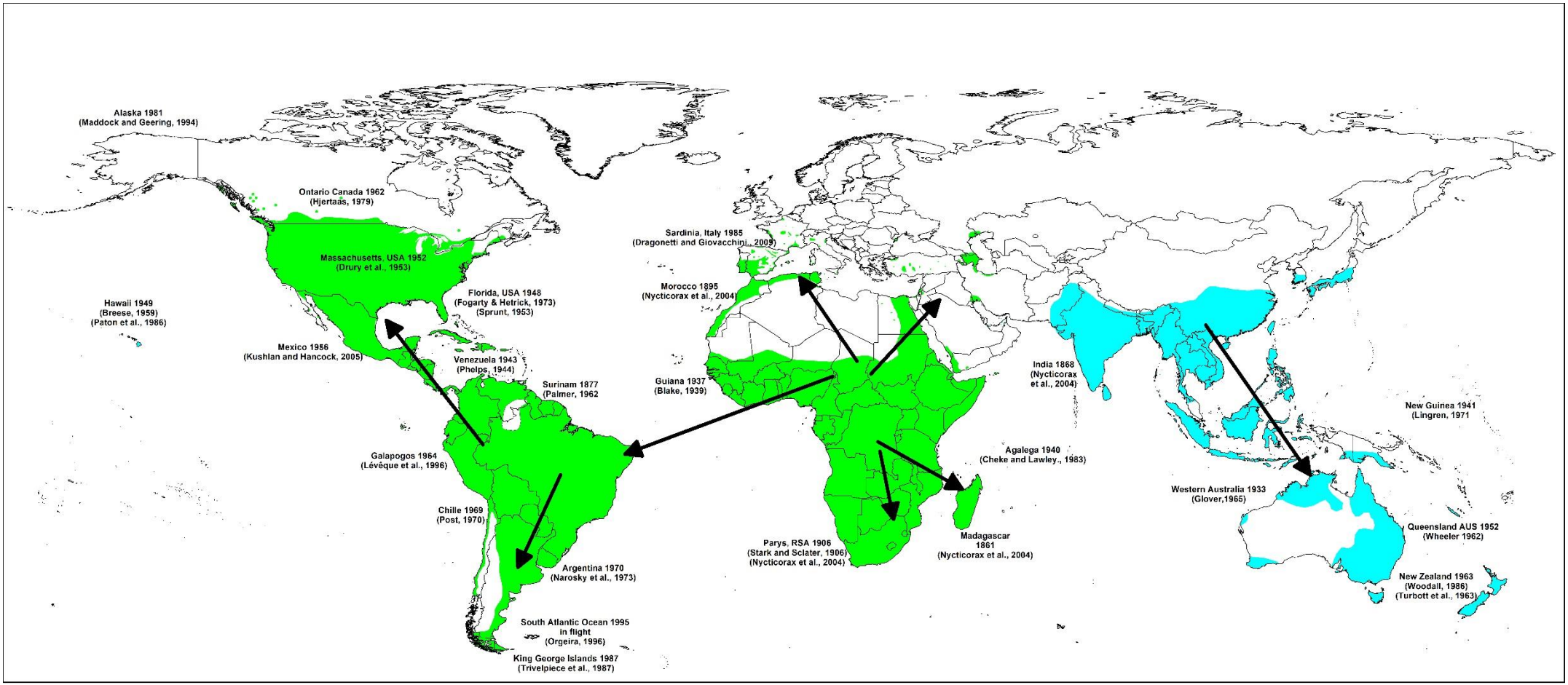
Cattle Egrets occur in a variety of habitats, including grasslands, wetlands, pastures, waste and sewage dumps, and in agricultural landscapes (Kushlan and Hancock, 2005). Cattle Egrets occupy a high trophic level as they primarily prey on insects such as grasshoppers, crickets, flies and their maggots, beetles, moths, and spiders. They occasionally also catch and eat frogs, fish, crayfish, small snakes, small mammals, lizards, and earthworms (Kushlan and Hancock, 2005).

Cattle Egrets display highly opportunistic feeding behaviours (Kopij, 1999) and have symbiotic relationships with large herbivorous mammals (Figure 1) such as cattle (Family: Bovidae) and rhinoceroses (Family: Rhinocerotidae). Evidence supports that this symbiotic relationship developed in Africa when the birds followed slow-moving African Buffalo herds (Genus: Syncerus) during grazing (Butler and Kok, 2007; Kushlan and Hancock, 2005). Cattle Egrets are not only twice as successful in obtaining prey when they feed in close association with large mammals, but this strategy also requires two-thirds less energy expenditure (Grubb, 1976; Heatwole, 1965). As a result of the high trophic level, it is possible that Cattle Egrets bio-accumulate contaminants (Pascoe et al.,1996). Breeding Cattle Egrets feed within 15 km (maximum 28 km) of their breeding colonies, restricting the area where they can take up pollutants (Butler and Kok, 2007).

### 3.5 Cattle Egrets as bio-indicators of water quality

Although the Cattle Egret is essentially a terrestrial bird based on its diet, they breed and roost near water. The presence or absence of these birds near aquatic roosting and breeding locations can be used as a bio-indicator of the associated water and environmental quality (Kushlan and Hafner, 2000). During the 1970s, Cattle Egrets, among other species, started to disappear as breeding birds along parts of the Nile Delta in Egypt. One of the possible explanations was the use of DDT on cotton plantations that may have caused unfavourable conditions and a decline in insect populations. However, due to a lack of chemical analyses at the time, this was never proven. Only in the 1980s when organochlorine use was restricted, did some of the bird populations recover (Mullié et al., 1992)

**Figure 2:** Range expansion of the Cattle Egret and the first confirmed sighing at new locations (NatureServe and IUCN, 2021). This map is not up to date. For instance, Cattle Egrets are now breeding in the UK in small numbers. Two additional distribution maps are available at Birdlife International, and All About Birds.



## 4. Results and Discussion

We report on arithmetic concentrations as published by various authors or calculated by ourselves (Figs 3 and 4). It should be kept in mind though, that different extraction and analytical methods were used. We also had to apply conversion factors to obtain ng/g wet-mass based concentrations. Therefore, comparisons should be read in context of factor- or order-of-magnitude patterns, not small-scale differences. The data are available in Table S1 in Supplementary Materials.

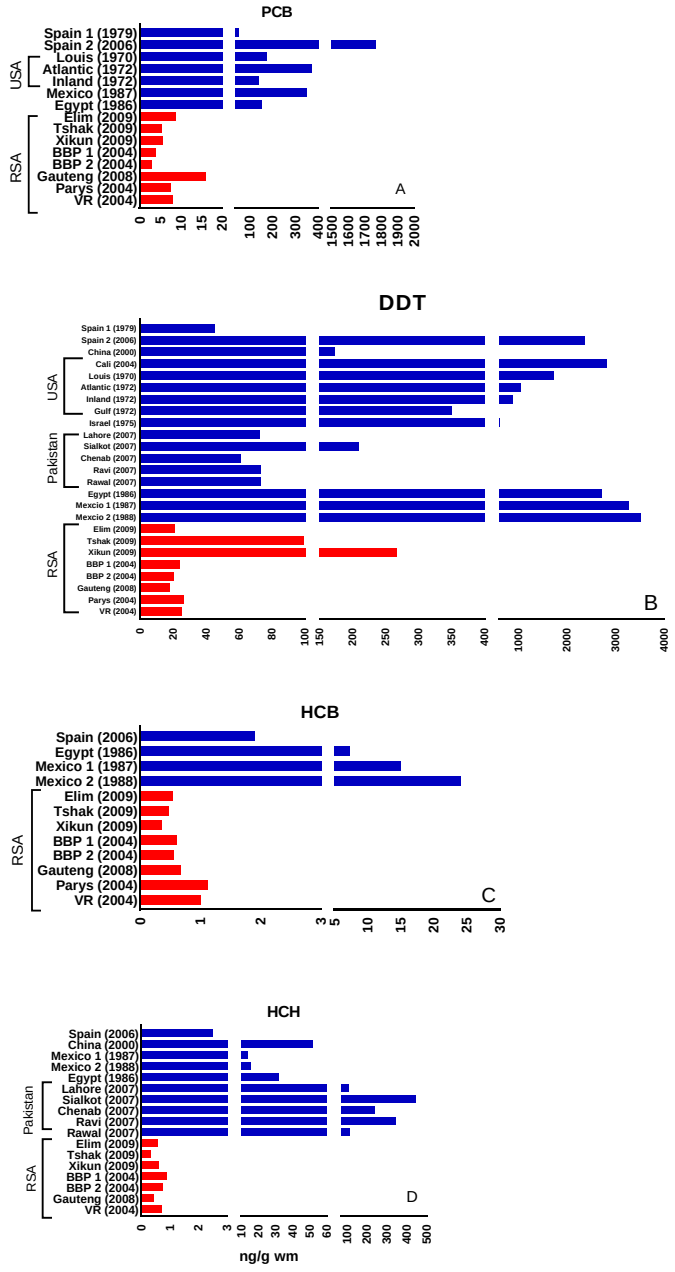



**Figure 3:** Bar graphs representing A) PCB, B) DDT, C) HCB, and D) HCH concentrations in Cattle Egret eggs reported by various authors (Table S1). Blue bars are from the Northern Hemisphere, and red from the Southern Hemisphere. RSA – Republic of South Africa.

### 4.1 Compounds

The literature review shows that quantifiable residues of POPs occur in Cattle Egret eggs when measured. PCBs were detected in samples from five countries of which Spain reported the highest mean $\Sigma PCB_?$ concentration (Figure 3A). The eggs used in that particular study (Ruiz et al., 1982) were collected in 1979 from Ebro Delta; a wetland in the north-eastern part of Spain that houses over 60 000 bird breeding pairs and hosts an additional 250 000 birds during winter months (Mañosa et al., 2001). This area is used for agriculture, specifically rice cultivation that may make use of pesticides (Mañosa et al., 2001; Martínez-Vilalta, 1989). Lower mean $\Sigma PCB_7$ concentrations were detected in eggs collected almost 30 years later in Aiguabarreig Spain; an ecologically important wetland that eventually flows via the Ebro River into the Ebro Delta.

All reported mean ΣPCB concentrations from the USA, Egypt, and Mexico were within the same magnitude (Faber and Hickey, 1973; Mullie et al., 1992; Mora, 1991; Ohlendorf et al., 1979). These reports date back to the 1970s and 80s when PCBs was still used extensively. No more recent reports on Cattle Egrets are available from those countries. The mean ΣPCB concentrations reported from countries in the Northern Hemisphere were higher than from the Southern Hemisphere (Figure 3A).

The same pattern can be observed for compounds such as DDT, hexachlorobenzene (HCB) and hexachlorocyclohexane (HCH), with some exceptions (Figure 3C-D). The highest ΣDDT concentrations were detected in Mexicali Valley, Mexico (Mora, 1991) where bird carcasses had ΣDDE concentrations of up to 20 000 ng/g wm (Mora, 1991). The reported ΣDDT concentrations from the USA (Cali), Spain, Egypt, and Israel were of the same order of magnitude as those from Mexico. The USA banned the use of DDT in 1972 (Beard, 2006), while Mexico still made use of DDT until 2001 (Giácoman-Vallejos et al., 2018; Stockholm Convention, 2001).

Eggs from the Southern hemisphere had lower ΣDDT concentrations than those of the Northern hemisphere except for sites from Limpopo province in South Africa (Figure 4B). Eggs collected from heronries at Tshakhuma Dam and Xikundu Dam had ΣDDT concentrations in the same order of magnitude than those from China and Pakistan. This was to be expected since parts of Limpopo (a malaria endemic region) is sprayed with DDT to control malaria, to this day (Bornman et al., 2022). It should be noted that DDT is also a by-product of dicofol production and a formulation contaminant (Blus and Henny., 1997; Kallenborn et al., 2013) and was only reported in studies from Pakistan (Figure 4A). The compound dicofol has a half-life of about two years in the environment and only few studies report on dicofol concentrations. Cattle Egret eggs also had higher concentrations of DDE than wading birds with an omnivorous diet (Perry et al., 1990).

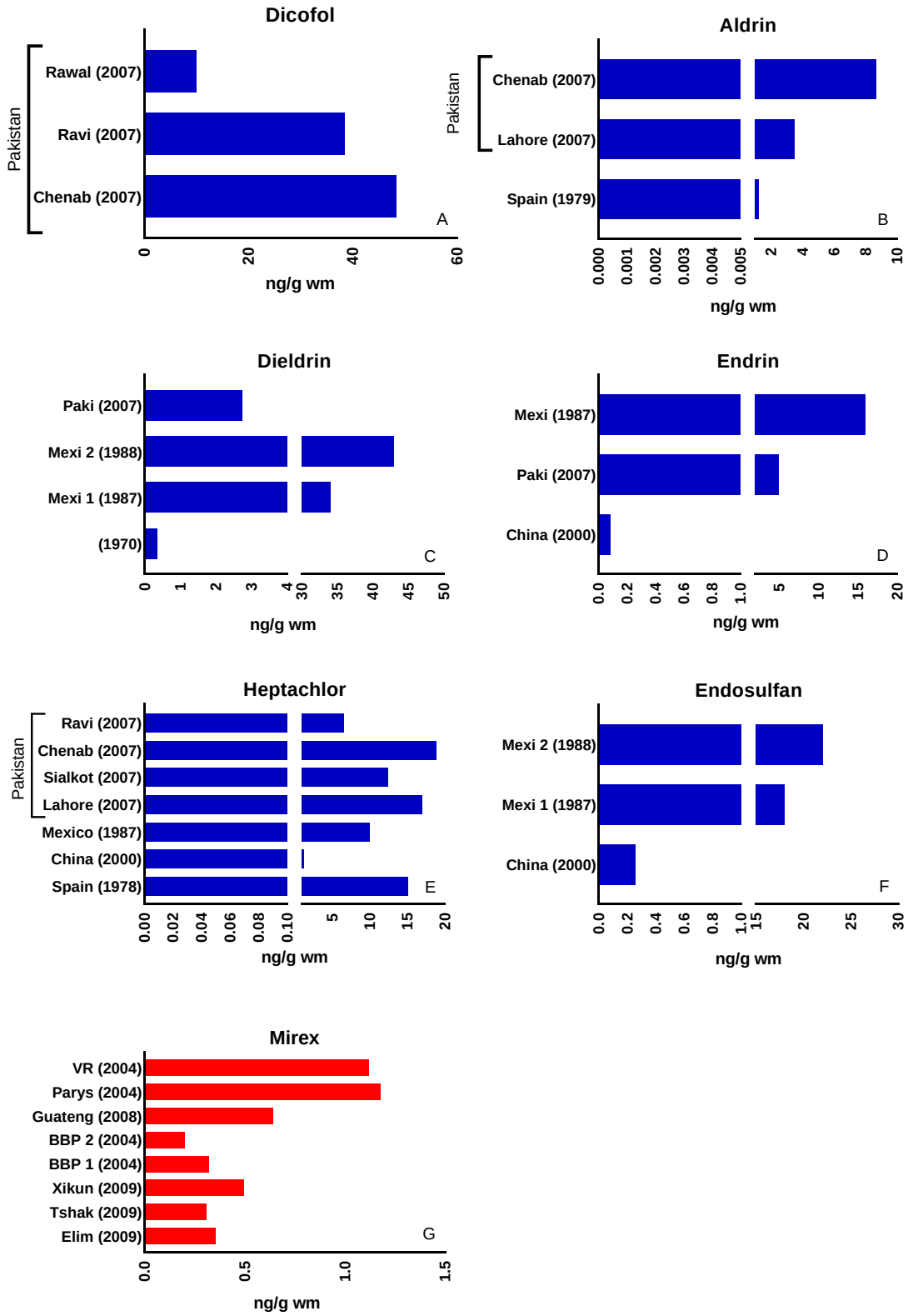



**Figure 4:** Bar graphs representing A) Dicofol, B) Aldrin, C) Dieldrin, D) Endrin, E) Heptachlor, F) Endosulfan and G) Mirex concentrations in Cattle Egret eggs reported by various authors (Table S1). Blue and red bars are from the Northern and Southern hemispheres, respectively.

Aside from dicofol, other compounds such as HCH, aldrin, dieldrin, DDT, endrin and heptachlor were also quantified in eggs from Pakistan (Figures 3 and 4). Malik et al. (2011) not only investigated the egg contents of Cattle Egrets for POP residues but also in their prey and sediments associated with their heronries. They found biomagnification of POPs through the food web as higher levels of pollutants were found in the eggs. This claim was later strengthened by Khan et al. (2014) with similar results. Both studies attributed the organochlorine residues detected to current and historical use of such compounds in agriculture and to current industrial practices.

Carcasses of adult herons from the USA were found with lethal concentrations of organochlorines, especially dieldrin, PCB and DDE, in their brain tissues (Ohlendorf et al., 1981). However, samples collected in Louisiana USA, prior to those collected in Mexico, had the lowest dieldrin concentration. It should be kept in mind that aldrin can break down to dieldrin, both being used as pesticides (Purnomo, 2017).

HCH concentrations from Pakistan were higher than concentrations reported by other countries (Figure 4D). The report found β-HCH and γ-HCH (lindane) in greater quantities (Khan et al., 2014). The HCH residues detected, specifically γ-HCH, was suggested to derive from its use in crop protection and sanitation by the authors. Eggs collected from Tai Lake, China, reported the second highest HCH concentration, followed by Egypt. Only samples from Spain collected in 2006 had HCH concentrations of the same order of magnitude than those reported from the Southern hemisphere. Endrin and endosulfan concentrations were the highest in samples from Pakistan with the lowest concentrations of both compounds detected in eggs from China (Figures 4 D and F).

Only studies from South Africa analysed Cattle Egret eggs for mirex (Figure 4G). The highest mean mirex concentrations were found in eggs collected along the Vaal River, specifically near Parys, with slightly lower concentrations found upstream in Gauteng. Mirex has not been registered in South Africa as a pesticide. The authors attributed the presence of Mirex to background contamination, especially from its use as a flame retardant (Bouwman et al., 2008).

Although not the main aim of this assessment, Cattle Egret eggs, feathers, and organs have been used to monitor metals such as lead, copper, mercury, and cadmium (Boncompagni et al., 2003; Bostan et al., 2007; Mullie et al., 1992; Shahbaz et al., 2013; Yasmeen et al., 2019). Perry et al. (1990) reported DDE residues in organs such as liver, heart, brain, muscle, skin, and feathers. DDE residues were higher in the skin and feathers compared with other organs, while these concentrations were all lower than in eggs. In addition, HCH, HCB, and DDTs were detected in Cattle Egret carcasses in Mexico (Mora and Anderson, 1991).

### 4.2 Implications of exposure

#### 4.2.1 Monitoring populations

DDT exposure can lead to breeding impairment, embryo defects, and mortality (Aurigi et al., 2000), causing population declines in some Ardeid populations (Blus and Henny 1997; Bouwman et al., 2013; Connel et al., 2003; Cooke 1973; Laporte 1982). One of the best ways to assess the impact pollutants have on Cattle Egret is to monitor breeding, breeding success, hatching success, population size, and growth. The Cattle Egret's population growth shows that this species can colonise new habitats and that their numbers grow rapidly once established. Furthermore, Cattle Egrets demonstrated rapid recovery when faced with

declining numbers due to biological control measures imposed by some countries (Nunes et al., 2010). Moreover, Cattle Egrets produce larger clutch sizes when occupying new areas (Ranglack et al., 1991), suggesting faster population growth compared with natal African colonies. This change in clutch size is temporary as the population over time stabilizes.

### 4.2.2 Eggshell thinning

Eggshell thinning is the process by which the eggshell does not develop to the thickness it should be during egg formation and is a principal cause of reproductive failure, explaining the decline in some bird populations (Graveland and Drent, 1997). Eggshell thinning during embryo development does occur; calcium and other minerals are mobilised from the shell to subsidize embryo development (Castilla et al., 2010: Karlsson and Lilja, 2008). The thinning of the eggshell induced by the developing embryo is referred to as embryo-induced eggshell thinning, or EET. Orłowski and Hałupka (2015) found that altricial bird species, which include Cattle Egrets, displayed the highest thinning (12%) EET. Several factors can influence the eggshell thickness other than EET, including the size, colour, and maculation of the eggs. Furthermore, the genetics, heath, egg incubation time, and the diet of the female bird may also influence the eggshell thickness (Castilla et al., 2009; Castilla et al., 2010; Snyder and Meretsky, 2003).

Increased concentrations of organochlorine compounds may lead to reproductive failure and mortality for many bird species, especially due to eggshell thinning associated with *p,p'*-DDE but also PCBs (Lundholm, 1997; Mora, 1991; Peakall and Lincer, 1996; Peakall et al., 1973). Aurigi et al. (2000) reported that *p,p'*-DDE can thin eggshells by as much as 20% and can lead to embryo mortality and defects. A study conducted in 1964 in the USA found DDE concentrations of 202 000 ng/g wm along with an 18% decline in eggshell thickness to cause embryonic mortality and lead to a population decline in gull colonies. A reduction in eggshell thickness of 11% was associated with shell flaking and egg breakage (Hickey and Anderson, 1968). Concentrations as low as 2 ng/g DDT wm have been reported to cause a reduction in eggshell thickness in predatory birds (Pain et al., 1999). Cattle Egret eggs, collected in 1953, had a 0.2 mm thicker mean eggshell thickness than eggs laid in 1986 (Mora, 1991). Cattle Egret eggshells from an area in South Africa where DDT is still used for malaria control were up to 33% thinner with increased DDT concentrations (Bouwman et al., 2013). Studies examining DDT concentrations in eggshells pre-, during-, and after DDT exposure reveal the same pattern (Elliott et al., 1988). Eggshell thickness drastically declined during times of DDT exposure and eggshells in general were thicker pre-exposure (Elliott et al., 1988).

4.2.3 Cattle Egret eggs as terrestrial indicator of POPs

The wide distribution, colonial breeding, high and expending numbers, well known biology, trophic position, and previous use of the eggs suggests that this bird is the best candidate as a near-global bird to research and monitor terrestrial pollution when weighed against other candidates, notably the omnivorous Rock Pigeon *C. livia* that has a similar distribution but occur in higher numbers. We list the reasons and advantages of using the Cattle Egret.

- Cosmopolitan, near-global bird species
- With climate change, further range expansion may be expected
- Easy to identify
- Cattle Egrets live up to 23 years
- Cattle Egret eggs are consumed in some parts of the world

- Residues of organochlorines as well as metals have been quantified in Cattle Egret eggs and organs
- Much data exists on other Ardeids that are aquatic, allowing comparisons between aquatic and terrestrial pollution in closely related birds
- Effects such as eggshell thinning has been detected
- Terrestrial feeders that are likely to accumulate POPs and other pollutants

- Mostly insectivorous but also small vertebrates
- Frequently forage in agricultural areas
- Cattle Egrets feed within 15 km of their breeding colonies, restricting the areas where they are exposed to pollutants in their food during breeding

- Colonial nesting, also with other Ardeids and aquatic birds
- Breeding colonies are easy to locate, also from the air
- Cattle Egret eggs are relatively easy to collect in sufficient numbers from colonies
- Because of colonial breeding, single eggs can be collected from multiple nests, leaving eggs behind in the nest for incubation
- Collection of eggs have no long-term effects on population growth and can be done with minimal stress and disturbance
- With care, minimal disturbance during collection can be achieved, and displaced chicks find their way back to their nests (personal observations, H Bouwman)
- Because multiple Ardeids breed colonially together, eggs from both terrestrial and aquatic birds can be collected from the same locations and at the same time
- Eggs can be stored frozen for very long periods to investigate legacy pollution or trends at a later stage.
- Other sample mediums include adult and juvenile feathers, whole carcasses, organs, blood, and droppings

Drawbacks in using Cattle Egret eggs are the ethical implications and permits required for the collection, transporting, and storage of samples, but this is the case for all birds in most countries. Collection at colonies must be done carefully so not to disturb other breeding colonies. Mora (1991) observed nestling mortality and destroyed nests after repeated visits.

Although we list collection of multiple Ardeid species at the same colony at the same time as an advantage, breeding may not always be synchronous.

## 5. Conclusions

We conclude that organochlorine residues were detected in various concentrations in Cattle Egret eggs even long after the application of such pollutants have terminated in the particular area. Cattle Egrets have undergone one of the most impressive natural expansions of the twentieth century and continue to spread and populate newly cultivated areas. Cattle Egrets are one of the few terrestrial feeding heron species and the use of this species as indicator of terrestrial pollution may aid in understanding why certain species of bird populations are in decline due to reproductive failure as a result of increasing environmental pollution. Using the same species across multiple countries, catchments, and continents will provide highly comparable data to assess terrestrial pollution, allowing to monitor the effectiveness of the Stockholm Convention combatting terrestrial pollution.

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# The Cattle Egret *Bubulcus ibis* as a near-global indicator of terrestrial pollution

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## Supplementary material

Table S1: All data points used in review

| Compound | Country | City / Region | Year sampled | Original reported value | New mean (ng/g wm) | Reference |
|---|---|---|---|---|---|---|
| Heptachlor epoxide | China | Tai Lake | 2000 | 5.02 ng/g dw | 1,255 | Dong et al., 2003 |
| Hept. Epoxide | Mexico | Mexicali valley | 1987 | 0.010 ppm ww | 10 | Mora., 1991 |
| Heptachlor | Pakistan | Lahore | 2007 | 68 ppb dw | 17 | Khan, et al. 2014 |
| Heptachlor | Pakistan | Sialkot | 2007 | 50 ppb dw | 12,5 | Khan, et al. 2014 |
| Heptachlor | Pakistan | Chenab | 2007 | 18.9 ng/g ww | 18,9 | Malik et al., 2011 |
| Heptachlor | Pakistan | Ravi River | 2007 | 6.6 ng/g ww | 6,6 | Malik et al., 2011 |
| Heptachlore | Spain | Delta de l'Ebre | 1979 | 0.2429 ppm lm | 15 | Ruiz et al, 1982 |
| α-Endosulfan | China | Tai Lake | 2000 | 0.5 ng/g dw | 0,125 | Dong et al., 2003 |
| β-Endosulfan | China | Tai Lake | 2000 | 0.52 ng/g dw | 0,13 | Dong et al., 2003 |
| Endosulfan | Mexico | Mexicali valley | 1987 | 0.018 ppm ww | 18 | Mora., 1991 |
| Endosulfan | Mexico | Mexicali valley | 1988 | 0.022 ppm ww | 22 | Mora., 1991 |
| Mirex | South Africa | Gauteng | 2008/9 | 0.64 ng/g wm | 0,64 | Bouwman et al., 2021 |
| Mirex | South Africa | Elim | 2009/10 | 5.7 ng/g lm | 0,3534 | Bouwman et al., 2013 |
| Mirex | South Africa | Tshakhuma dam | 2009/10 | 5 ng/g lm | 0,31 | Bouwman et al., 2013 |
| Mirex | South Africa | Xikundu dam | 2009/10 | 8 ng/g lm | 0,496 | Bouwman et al., 2013 |
| Mirex | South Africa | Baberspan | 2004/5 | 0.32 ng/g ww | 0,32 | Bouwman et al., 2008 |
| Mirex | South Africa | Vaal Rivier | 2004/5 | 1.12 ng/g ww | 1,12 | Bouwman et al., 2008 |
| Mirex | South Africa | Baberspan | 2004 | 4 ng/g lw | 0,248 | Polder et al., 2008 |
| Mirex | South Africa | Parys | 2004 | 19 ng/g lw | 1,178 | Polder et al., 2008 |
| HCB | Egypt | Giza, Egypt | 1986 | 0.029 mg/kg dw | 7,25 | Mullie et al., 1992 |
| HCB | Mexico | Mexicali valley | 1987 | 0.015 ppm ww | 15 | Mora., 1991 |
| HCB | Mexico | Mexicali valley | 1988 | 0.024 ppm ww | 24 | Mora., 1991 |
| HCB | South Africa | Gauteng | 2008/9 | 0.67 ng/g wm | 0,67 | Bouwman et al., 2021 |
| HCB | RSA | Baberspan | 2004/5 | 0.61 ng/g wm | 0,61 | Bouwman et al., 2008 |
| HCB | RSA | Vaal Rivier | 2004/5 | 1 ng/g wm | 1 | Bouwman et al., 2008 |
| HCB | South Africa | Elim | 2009/10 | 8.8 ng/g lm | 0,5456 | Bouwman et al., 2013 |
| HCB | South Africa | Tshakhuma dam | 2009/10 | 7.6 ng/g lm | 0,4712 | Bouwman et al., 2013 |
| HCB | South Africa | Xikundu dam | 2009/10 | 5.7 ng/g lm | 0,3534 | Bouwman et al., 2013 |
| HCB | South Africa | Baberspan | 2004 | 9 ng/g lw | 0,558 | Polder et al., 2008 |
| HCB | South Africa | Parys | 2004 | 18 ng/g lw | 1,116 | Polder et al., 2008 |
| HCB | Spain | Aiguabarreig, Catalan | 2006 | 1.9 ng/g wm | 1,9 | Huertas et al., 2016 |
| ∑PCB | Egypt | Giza, Egypt | 1986 | 0.609 mg/kg dw | 152,25 | Mullie et al., 1992 |
| Aroclor 1260 (PCB) | Mexico | Mexicali valley | 1987 | 0.348 ppm ww | 348 | Mora., 1991 |
| ∑PCB | RSA | Gauteng | 2008/9 | 16 ng/g wm | 16 | Bouwman et al., 2021 |
| ∑PCB | RSA | Elim | 2009/10 | 140 ng/g lm | 8,68 | Bouwman et al., 2013 |
| ∑PCB | RSA | Tshakhuma dam | 2009/10 | 87 ng/g lm | 5,394 | Bouwman et al., 2013 |
| ∑PCB | RSA | Xikundu dam | 2009/10 | 89 ng/g lm | 5,518 | Bouwman et al., 2013 |
| ∑PCB | RSA | Baberspan | 2004/5 | 3.8 ng/g ww | 3,8 | Bouwman et al., 2008 |
| ∑PCB | RSA | Vaal Rivier | 2004/5 | 8 ng/g ww | 8 | Bouwman et al., 2008 |
| Sum PCB | RSA | Baberspan | 2004 | 46 ng/g lw | 2,852 | Polder et al., 2008 |
| Sum PCB | RSA | Parys | 2004 | 122 ng/g lw | 7,564 | Polder et al., 2008 |
| Sum PCB | Spain | Aiguabarreig, Catalan | 2006 | 51 ng/g wm | 51 | Huertas et al., 2016 |
| PCB | Spain | Delta de l'Ebre | 1979 | 28.524 ppm lm | 1769 | Ruiz et al, 1982 |
| PCB | USA | Louisiana | 1970 | 28 mg/kg lw | 173,6 | Faber and Hickey., 1973 |
| PCB | USA | Southern atlantic | 1972 | 0.37 ppm wm | 370 | Ohlendorf et al., 1979 |
| PCB | USA | Inland | 1972 | 0.14 ppm wm | 140 | Ohlendorf et al., 1979 |
| α-HCH | China | Tai Lake | 2000 | 0.76 ng/g dw | 0,19 | Dong et al., 2003 |
| β-HCH | China | Tai Lake | 2000 | 206.8 ng/g dw | 51,7 | Dong et al., 2003 |
| ʒ-HCH | China | Tai Lake | 2000 | 0.08 ng/g dw | 0,02 | Dong et al., 2003 |
| HCH | Mexico | Mexicali valley | 1987 | 0.014 ppm ww | 14 | Mora., 1991 |
| HCH | Mexico | Mexicali valley | 1988 | 0.016 ppm ww | 16 | Mora., 1991 |
| B-HCH | Pakistan | Lahore | 2007 | 281 ppb | 70,25 | Khan, et al. 2014 |
| y-HCH | Pakistan | Lahore | 2007 | 158 ppb | 39,5 | Khan, et al. 2014 |
| B-HCH | Pakistan | Sialkot | 2007 | 1380 ppb | 345 | Khan, et al. 2014 |
| y-HCH | Pakistan | Sialkot | 2007 | 419 ppb | 104,75 | Khan, et al. 2014 |
| B-HCH | Pakistan | Chenab | 2007 | 17.9 ng/g | 17,9 | Malik et al., 2011 |
| y-HCH | Pakistan | Chenab | 2007 | 29.9 ng/g | 29,9 | Malik et al., 2011 |
| ∑HCH | Pakistan | Chenab | 2007 | 239.1 ng/g | 239,1 | Malik et al., 2011 |
| B-HCH | Pakistan | Ravi River | 2007 | 47.1 ng/g | 47,1 | Malik et al., 2011 |
| y-HCH | Pakistan | Ravi River | 2007 | 21.8 ng/g | 21,8 | Malik et al., 2011 |
| ∑HCH | Pakistan | Ravi River | 2007 | 344.3 ng/g | 344,3 | Malik et al., 2011 |

| Compound | Country | City / Region | Year sampled | Original reported value | New mean (ng/g wm) | Reference |
|---|---|---|---|---|---|---|
| B-HCH | Pakistan | Rawal Lake | 2007 | 10.1 ng/g | 10,1 | Malik et al., 2011 |
| y-HCH | Pakistan | Rawal Lake | 2007 | 12.9 ng/g | 12,9 | Malik et al., 2011 |
| ∑HCH | Pakistan | Rawal Lake | 2007 | 114.7 ng/g | 114,7 | Malik et al., 2011 |
| ∑HCH | South Africa | Gauteng | 2008/9 | 0.43 ng/g wm | 0,43 | Bouwman et al., 2021 |
| ∑HCH | South Africa | Elim | 2009/10 | 9.6 ng/g lm | 0,5952 | Bouwman et al., 2013 |
| ∑HCH | South Africa | Tshakhuma dam | 2009/10 | 5.4 ng/g lm | 0,3348 | Bouwman et al., 2013 |
| ∑HCH | South Africa | Xikundu dam | 2009/10 | 10 ng/g lm | 0,62 | Bouwman et al., 2013 |
| Sum HCH | South Africa | Vaal Rivier | 2004/5 | 0.73 ng/g-1 ww | 0,73 | Bouwman et al., 2008 |
| Sum HCH | South Africa | Baberspan | 2004/5 | 0.9 ng/g-1 ww | 0,9 | Bouwman et al., 2008 |
| Sum HCH | South Africa | Baberspan | 2004 | 12 ng/g lw | 0,744 | Polder et al., 2008 |
| Sum HCH | South Africa | Parys | 2004 | 12 ng/g lw | 0,744 | Polder et al., 2008 |
| Sum HCH | Spain | Aiguabarreig, Catalan | 2006 | 2.5 ng/g wm | 2,5 | Huertas et al., 2016 |
| y-HCH | Egypt | Giza, Egypt | 1986 | 0.129 mg/kg dw | 32,25 | Mullie et al., 1992 |
| p,p'-DDE | China | Tai Lake | 2000 | 670.24 ng/g dw | 167,56 | Dong et al., 2003 |
| p,p'-DDD | China | Tai Lake | 2000 | 8.56 ng/g dw | 2,14 | Dong et al., 2003 |
| p,p'-DDT | China | Tai Lake | 2000 | 12.56 ng/g dw | 3,14 | Dong et al., 2003 |
| p,p' DDE | Egypt | Giza, Egypt | 1986 | 10.895 mg/kg dw | 2723,75 | Mullie et al., 1992 |
| DDE | Israel | | 1975 | 0.62 ppm wm | 620 | Perry et al., 1990 |
| p,p'-DDE | Mexico | Mexicali valley | 1987 | 3.22 ppm ww | 3220 | Mora., 1991 |
| p,p'-DDD | Mexico | Mexicali valley | 1987 | 0.017 ppm ww | 17 | Mora., 1991 |
| p,p'-DDT | Mexico | Mexicali valley | 1987 | 0.033 ppm ww | 33 | Mora., 1991 |
| p,p'-DDE | Mexico | Mexicali valley | 1988 | 3.49 ppm ww | 3490 | Mora., 1991 |
| p,p'-DDT | Mexico | Mexicali valley | 1988 | 0.017 ppm ww | 17 | Mora., 1991 |
| p, p'-DDD | Pakistan | Lahore | 2007 | 213 ppb | 53,25 | Khan, et al. 2014 |
| p, p'-DDE | Pakistan | Lahore | 2007 | 23 ppb | 5,75 | Khan, et al. 2014 |
| p,p'-DDT | Pakistan | Lahore | 2007 | 18.3 ppb | 4,575 | Khan, et al. 2014 |
| o,p'-DDT | Pakistan | Lahore | 2007 | 35 ppb | 8,75 | Khan, et al. 2014 |
| p, p'-DDD | Pakistan | Sialkot | 2007 | 466 ppb | 116,5 | Khan, et al. 2014 |
| p, p'-DDE | Pakistan | Sialkot | 2007 | 73 ppb | 18,25 | Khan, et al. 2014 |
| p,p'-DDT | Pakistan | Sialkot | 2007 | 231 ppb | 57,75 | Khan, et al. 2014 |
| o,p'-DDT | Pakistan | Sialkot | 2007 | 65 ppb | 16,25 | Khan, et al. 2014 |
| DDD | Pakistan | Chenab | 2007 | 126.2 ng/g | 126,2 | Malik et al., 2011 |
| DDE | Pakistan | Chenab | 2007 | 64.2 ng/g | 64,2 | Malik et al., 2011 |
| o,p'-DDT | Pakistan | Chenab | 2007 | 36.3 ng/g | 36,3 | Malik et al., 2011 |
| p,p'-DDT | Pakistan | Chenab | 2007 | 27 ng/g | 27 | Malik et al., 2011 |
| ∑DDT | Pakistan | Chenab | 2007 | 60.7 ng/g | 60,7 | Malik et al., 2011 |
| DDD | Pakistan | Ravi River | 2007 | 165.6 ng/g | 165,6 | Malik et al., 2011 |
| DDE | Pakistan | Ravi River | 2007 | 58.2 ng/g | 58,2 | Malik et al., 2011 |
| o,p'-DDT | Pakistan | Ravi River | 2007 | 28.7 ng/g | 28,7 | Malik et al., 2011 |
| p,p'-DDT | Pakistan | Ravi River | 2007 | 41.4 ng/g | 41,4 | Malik et al., 2011 |
| ∑DDT | Pakistan | Ravi River | 2007 | 73.4 ng/g | 73,4 | Malik et al., 2011 |
| DDD | Pakistan | Rawal Lake | 2007 | 152.9 ng/g | 152,9 | Malik et al., 2011 |
| DDE | Pakistan | Rawal Lake | 2007 | 53.25 ng/g | 53,25 | Malik et al., 2011 |
| o,p'-DDT | Pakistan | Rawal Lake | 2007 | 28.8 ng/g | 28,8 | Malik et al., 2011 |
| p,p'-DDT | Pakistan | Rawal Lake | 2007 | 57.7 ng/g | 57,7 | Malik et al., 2011 |
| ∑DDT | Pakistan | Rawal Lake | 2007 | 73.1 ng/g | 73,1 | Malik et al., 2011 |
| ∑DDT | South Africa | Gauteng | 2008/9 | 18 ng/g wm | 18 | Bouwman et al., 2021 |
| ∑DDT | South Africa | Elim | 2009/10 | 340 ng/g lm | 21,08 | Bouwman et al., 2013 |
| ∑DDT | South Africa | Tshakhuma dam | 2009/10 | 1600 ng/g lm | 99,2 | Bouwman et al., 2013 |
| ∑DDT | South Africa | Xikundu dam | 2009/10 | 4300 ng/g lm | 266,6 | Bouwman et al., 2013 |
| ∑DDT | South Africa | Baberspan | 2004/5 | 24 ng/g-1 ww | 24 | Bouwman et al., 2008 |
| ∑DDT | South Africa | Vaal Rivier | 2004/5 | 25 ng/g-1 ww | 25 | Bouwman et al., 2008 |
| Sum DDT | South Africa | Baberspan | 2004 | 331ng/g lw | 20,522 | Polder et al., 2008 |
| Sum DDT | South Africa | Parys | 2004 | 425 ng/g lw | 26,35 | Polder et al., 2008 |
| Sum DDT | Spain | Aiguabarreig, Catalan | 2006 | 45 ng/g wm | 45 | Huertas et al., 2016 |
| DDT | Spain | Delta de l'Ebre | 1979 | 38.0594 ppm lm | 2359,7 | Ruiz et al, 1982 |
| p,p'-DDE | USA | Salton Sea, California | 2004 | 2.81 ug/g dw | 2810 | Henny et al., 2008 |
| DDE | USA | louisiana | 1970 | 26 mg/kg lw | 1612 | Faber and Hickey., 1973 |
| DDT | USA | louisiana | 1970 | 1.86 mg/kg lw | 115,32 | Faber and Hickey., 1973 |
| DDE | USA | Southern atlantic | | 1.04 ppm wm | 1040 | Ohlendorf et al., 1979 |
| DDE | USA | Inland | | 0.88 ppm wm | 880 | Ohlendorf et al., 1979 |
| DDE | USA | gulf coast | | 0.35 ppm wm | 350 | Ohlendorf et al., 1979 |

Table S2: Descriptive statistics of DDT residues in Cattle Egret eggs.

| Country | China | Egypt | Mexico | Pakistan | South Africa | Israel | Spain | USA |
|---|---|---|---|---|---|---|---|---|
| Number of values | 1 | 1 | 2 | 5 | 8 | 1 | 2 | 5 |
| Minimum | 172.8 | 2724 | 3270 | 60.7 | 18 | 620 | 45 | 0.003 |
| 25% Percentile | 172.8 | 2724 | 3270 | 66.51 | 21 | 620 | 45 | 0.2015 |
| Median | 172.8 | 2724 | 3389 | 73.1 | 24.5 | 620 | 5951 | 0.7025 |
| 75% Percentile | 172.8 | 2724 | 3507 | 141.1 | 80.75 | 620 | 11858 | 0.95 |
| Maximum | 172.8 | 2724 | 3507 | 208.8 | 266 | 620 | 11858 | 1 |
| Range | 0 | 0 | 237 | 148.1 | 248 | 0 | 11813 | 0.997 |
| Mean | 172.8 | 2724 | 3389 | 97.66 | 62.5 | 620 | 5951 | 0.6011 |
| Std. Deviation | 0 | 0 | 167.6 | 62.33 | 86.51 | 0 | 8353 | 0.4052 |
| Std. Error of Mean | 0 | 0 | 118.5 | 27.88 | 30.59 | 0 | 5906 | 0.1812 |
| Coefficient of variation | 0.00% | 0.00% | 4.95% | 63.83% | 138.40% | 0.00% | 140.40% | 67.42% |
| Geometric mean | 172.8 | 2724 | 3386 | 86.76 | 36.66 | 620 | 730.5 | 0.2377 |
| Geometric SD factor | 1 | 1 | 1.051 | 1.644 | 2.618 | 1 | 51.49 | 11.82 |

Table S3: Descriptive statistics of Endosulfan residues in Cattle Egret eggs.

| Country | China | Mexico |
|---|---|---|
| Number of values | 1 | 2 |
| Minimum | 0.255 | 18 |
| 25% Percentile | 0.255 | 18 |
| Median | 0.255 | 20 |
| 75% Percentile | 0.255 | 22 |
| Maximum | 0.255 | 22 |
| Range | 0 | 4 |
| Mean | 0.255 | 20 |
| Std. Deviation | 0 | 2.828 |
| Std. Error of Mean | 0 | 2 |
| Coefficient of variation | 0.00% | 14.14% |
| Geometric mean | 0.255 | 19.9 |
| Geometric SD factor | 1 | 1.152 |

Table S4: Descriptive statistics of HCB residues in Cattle Egret eggs.

| Country | Mexico | South Africa | Egypt | Spain |
|---|---|---|---|---|
| Number of values | 2 | 6 | 1 | 1 |
| Minimum | 15 | 0.4 | 7.25 | 2 |
| 25% Percentile | 15 | 0.475 | 7.25 | 2 |
| Median | 19.5 | 0.5 | 7.25 | 2 |
| 75% Percentile | 24 | 0.7775 | 7.25 | 2 |
| Maximum | 24 | 1.1 | 7.25 | 2 |
| Range | 9 | 0.7 | 0 | 0 |
| Mean | 19.5 | 0.6117 | 7.25 | 2 |
| Std. Deviation | 6.364 | 0.2546 | 0 | 0 |
| Std. Error of Mean | 4.5 | 0.1039 | 0 | 0 |
| Coefficient of variation | 32.64% | 41.62% | 0.00% | 0.00% |
| Geometric mean | 18.97 | 0.5769 | 7.25 | 2 |
| Geometric SD factor | 1.394 | 1.428 | 1 | 1 |

Table S5: Descriptive statistics of Heptachlor residues in Cattle Egret eggs.

| Country | China | Mexico | Pakistan | Spain |
|---|---|---|---|---|
| Number of values | 1 | 1 | 4 | 1 |
| Minimum | 1.25 | 10 | 6.6 | 7.6 |
| 25% Percentile | 1.25 | 10 | 8.075 | 7.6 |
| Median | 1.25 | 10 | 14.75 | 7.6 |
| 75% Percentile | 1.25 | 10 | 18.43 | 7.6 |
| Maximum | 1.25 | 10 | 18.9 | 7.6 |
| Range | 0 | 0 | 12.3 | 0 |
| Mean | 1.25 | 10 | 13.75 | 7.6 |
| Std. Deviation | 0 | 0 | 5.47 | 0 |
| Std. Error of Mean | 0 | 0 | 2.735 | 0 |
| Coefficient of variation | 0.00% | 0.00% | 39.78% | 0.00% |
| Geometric mean | 1.25 | 10 | 12.76 | 7.6 |
| Geometric SD factor | 1 | 1 | 1.605 | 1 |

Table S6: Descriptive statistics of Mirex residues in Cattle Egret eggs.

| Country | Gauteng | Elim | Tshakhuma Dam | Xikundu Dam | Baberspan one | Vaal River | Baberspan two | Parys |
|---|---|---|---|---|---|---|---|---|
| Number of values | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Minimum | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| 25% Percentile | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| Median | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| 75% Percentile | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| Maximum | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| Range | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| Std. Deviation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Std. Error of Mean | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Coefficient of variation | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| Geometric mean | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 1.1 | 0.3 | 1.2 |
| Geometric SD factor | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Table S7: Descriptive statistics of PCB residues in Cattle Egret eggs.

| Country | Egypt | Mexico | South Africa | Spain | USA |
|---|---|---|---|---|---|
| Number of values | 1 | 1 | 8 | 2 | 3 |
| Minimum | 152.3 | 348 | 2.9 | 51 | 0.1 |
| 25% Percentile | 152.3 | 348 | 4.2 | 51 | 0.1 |
| Median | 152.3 | 348 | 6.55 | 909.5 | 0.4 |
| 75% Percentile | 152.3 | 348 | 8.51 | 1768 | 1.7 |
| Maximum | 152.3 | 348 | 16 | 1768 | 1.7 |
| Range | 0 | 0 | 13.1 | 1717 | 1.6 |
| Mean | 152.3 | 348 | 7.235 | 909.5 | 0.7333 |
| Std. Deviation | 0 | 0 | 4.081 | 1214 | 0.8505 |
| Std. Error of Mean | 0 | 0 | 1.443 | 858.5 | 0.491 |
| Coefficient of variation | 0.00% | 0.00% | 56.41% | 133.50% | 116.00% |
| Geometric mean | 152.3 | 348 | 6.385 | 300.3 | 0.4082 |
| Geometric SD factor | 1 | 1 | 1.698 | 12.27 | 4.124 |

Table S8: Descriptive statistics of HCH residues in Cattle Egret eggs.

| Country | China | Mexico | Pakistan | South Africa | Egypt | Spain |
|---|---|---|---|---|---|---|
| Number of values | 1 | 2 | 5 | 6 | 1 | 1 |
| Minimum | 51.91 | 14 | 109.8 | 0.3 | 32.25 | 2.5 |
| 25% Percentile | 51.91 | 14 | 112.2 | 0.4725 | 32.25 | 2.5 |
| Median | 51.91 | 15 | 239.1 | 0.65 | 32.25 | 2.5 |
| 75% Percentile | 51.91 | 16 | 397 | 0.7225 | 32.25 | 2.5 |
| Maximum | 51.91 | 16 | 449.8 | 0.9 | 32.25 | 2.5 |
| Range | 0 | 2 | 340 | 0.6 | 0 | 0 |
| Mean | 51.91 | 15 | 251.5 | 0.62 | 32.25 | 2.5 |
| Std. Deviation | 0 | 1.414 | 147.4 | 0.1862 | 0 | 0 |
| Std. Error of Mean | 0 | 1 | 65.91 | 0.06582 | 0 | 0 |
| Coefficient of variation | 0.00% | 9.43% | 58.59% | 30.03% | 0.00% | 0.00% |
| Geometric mean | 51.91 | 14.97 | 215.6 | 0.5913 | 32.25 | 2.5 |
| Geometric SD factor | 1 | 1.099 | 1.891 | 1.414 | 1 | 1 |

Table S9: Descriptive statistics of Endrin residues in Cattle Egret eggs.

| Country | China | Mexico | Pakistan |
|---|---|---|---|
| Number of values | 1 | 1 | 1 |
| Minimum | 0.0875 | 16 | 5 |
| 25% Percentile | 0.0875 | 16 | 5 |
| Median | 0.0875 | 16 | 5 |
| 75% Percentile | 0.0875 | 16 | 5 |
| Maximum | 0.0875 | 16 | 5 |
| Range | 0 | 0 | 0 |
| Mean | 0.0875 | 16 | 5 |
| Std. Deviation | 0 | 0 | 0 |
| Std. Error of Mean | 0 | 0 | 0 |
| Coefficient of variation | 0.00% | 0.00% | 0.00% |
| Geometric mean | 0.0875 | 16 | 5 |
| Geometric SD factor | 1 | 1 | 1 |

Table S10: Descriptive statistics of Aldrin residues in Cattle Egret eggs.

| Country | Pakistan | Spain |
|---|---|---|
| Number of values | 2 | 1 |
| Minimum | 34 | 2.75 |
| 25% Percentile | 34 | 2.75 |
| Median | 38.5 | 2.75 |
| 75% Percentile | 43 | 2.75 |
| Maximum | 43 | 2.75 |
| Range | 9 | 0 |
| Mean | 38.5 | 2.75 |
| Std. Deviation | 6.364 | 0 |
| Std. Error of Mean | 4.5 | 0 |
| Coefficient of variation | 16.53% | 0.00% |
| Geometric mean | 38.24 | 2.75 |
| Geometric SD factor | 1.181 | 1 |

Table S11: Descriptive statistics of Dieldrin residues in Cattle Egret eggs.

| Country | USA | Mexico 1 | Mexico 2 | Pakistan |
|---|---|---|---|---|
| Number of values | 1 | 1 | 1 | 1 |
| Minimum | 0.3509 | 34 | 43 | 2.75 |
| 25% Percentile | 0.3509 | 34 | 43 | 2.75 |
| Median | 0.3509 | 34 | 43 | 2.75 |
| 75% Percentile | 0.3509 | 34 | 43 | 2.75 |
| Maximum | 0.3509 | 34 | 43 | 2.75 |
| Range | 0 | 0 | 0 | 0 |
| Mean | 0.3509 | 34 | 43 | 2.75 |
| Std. Deviation | 0 | 0 | 0 | 0 |
| Std. Error of Mean | 0 | 0 | 0 | 0 |
| Coefficient of variation | 0.000% | 0.000% | 0.000% | 0.000% |
| Geometric mean | 0.3509 | 34 | 43 | 2.75 |
| Geometric SD factor | 1 | 1 | 1 | 1 |

Table S12: Descriptive statistics of Dicofol residues in Cattle Egret eggs.

| **Country** | **Pakistan** |
|---|---|
| **Number of values** | **3** |
| | |
| **Minimum** | 10 |
| **25% Percentile** | 10 |
| **Median** | 38.4 |
| **75% Percentile** | 48.3 |
| **Maximum** | 48.3 |
| **Range** | 38.3 |
| | |
| **Mean** | 32.23 |
| **Std. Deviation** | 19.88 |
| **Std. Error of Mean** | 11.48 |
| | |
| **Coefficient of variation** | 61.68% |
| | |
| **Geometric mean** | 26.47 |
| **Geometric SD factor** | 2.341 |

Table S13: Concentrations of compounds detected in Cattle Egret eggs, not discussed or mentioned in the article.

| Compound | n | Concentration | Unit | ng/g wm | Location | Country | Reference |
|---|---|---|---|---|---|---|---|
| **∑BDE** | 5 | 0.7 | ng/g wm | 0.7 | Xikundu dam | RSA | Bouwman et al., 2013 |
| **∑BDE (153)** | 4 | 0.03 | ng/g wm | 0.03 | Elim | RSA | Bouwman et al., 2013 |
| **∑chlordanes** | 11 | 1.1 | ng/g wm | 1.1 | Barberspan | RSA | Bouwman et al., 2008 |
| **∑chlordanes** | 9 | 0.4 | ng/g wm | 0.4 | Vaal River | RSA | Bouwman et al., 2008 |
| **∑chlordanes** | 4 | 3 | ng/g wm | 3 | Elim | RSA | Bouwman et al., 2013 |
| **∑chlordanes** | 6 | 3.1 | ng/g wm | 3.1 | Tshakhuma | RSA | Bouwman et al., 2013 |
| **∑chlordanes** | 5 | 42 | ng/g wm | 42 | Xikundu dam | RSA | Bouwman et al., 2013 |
| **∑chlordanes** | 6 | 0.62 | ng/g wm | 0.62 | Gauteng | RSA | Bouwman et al., 2021 |
| **∑PBDE** | 6 | 4.3 | ng/g wm | 4.3 | Gauteng | RSA | Bouwman et al., 2021 |
| **∑PBDE (8)** | | 2 | ng/g lm | 0.124 | Baberspan | RSA | Polder et al., 2008 |
| **∑PBDE (8)** | | 4 | ng/g lm | 0.248 | Parys | RSA | Polder et al., 2008 |
| **BHC** | 5 | 0.17 | | 0.17 | Louisiana | USA | Faber and Hickey., 1973 |
| **cis-Chlordane** | 4 | 0.085 | ng/g wm | 0.085 | Elim | RSA | Bouwman et al., 2013 |
| **cis-Chlordane** | 6 | 0.18 | ng/g wm | 0.18 | Tshakhuma | RSA | Bouwman et al., 2013 |
| **cis-Chlordane** | 5 | 2.2 | ng/g wm | 2.2 | Xikundu dam | RSA | Bouwman et al., 2013 |
| **HBCD** | 1 | 1.2 | ng/g wm | 1.2 | Gauteng | RSA | Bouwman et al., 2021 |
| **Oxychlordane** | 4 | 1.5 | ng/g wm | 1.5 | Elim | RSA | Bouwman et al., 2013 |
| **Oxychlordane** | 6 | 1.6 | ng/g wm | 1.6 | Tshakhuma | RSA | Bouwman et al., 2013 |
| **Oxychlordane** | 5 | 7.3 | ng/g wm | 7.3 | Xikundu dam | RSA | Bouwman et al., 2013 |
| **Oxychlordane** | | 11 | ng/g lm | 0.682 | Baberspan | RSA | Polder et al., 2008 |
| **Oxychlordane** | | 7 | ng/g lm | 0.434 | Parys | RSA | Polder et al., 2008 |
| **Oxychlordane** | 40 | 0.01 | ppm wm | 0.01 | Mexicali Valley | Mexico | Mora., 1991 |
| **Oxychlordane** | 10 | 0.02 | ppm wm | 0.02 | Mexicali Valley | Mexico | Mora., 1991 |
| **Oxy-Chlordane** | 11 | 0.84 | ppm wm | 0.84 | Barberspan | RSA | Bouwman et al., 2008 |
| **Oxy-Chlordane** | 9 | 0.4 | ppm wm | 0.4 | Vaal River | RSA | Bouwman et al., 2008 |
| **PeCB** | 9 | 0.2 | ppm wm | 0.2 | Aiguabarreig | Spain | Huertas et al., 2015 |
| **Trans-Nonachlor** | 11 | 3.3 | ppm wm | 3.3 | Barberspan | RSA | Bouwman et al., 2008 |
| **Trans-Nonachlor** | 4 | 1.2 | ppm wm | 1.2 | Elim | RSA | Bouwman et al., 2013 |
| **Trans-Nonachlor** | 6 | 0.98 | ppm wm | 0.98 | Tshakhuma | RSA | Bouwman et al., 2013 |
| **Trans-Nonachlor** | 5 | 40 | ppm wm | 40 | Xikundu dam | RSA | Bouwman et al., 2013 |

**Chapter 3**

# Dioxins, PFOS, and 20 other persistent organic pollutants in nine species of wild bird eggs from the Vaal River, South Africa

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## Abstract

The Vaal River and its tributaries drain the largest and most populated industrial and mining region in Southern Africa. Eggs of nine wild bird species, including herons, ibis, cormorant, egrets and darters, representing three habitats and four feedings guilds, were collected at four locations in 2009. The eggs were analysed for POPs including 21 organochlorine pesticides (OCPs), five polybrominated diphenyl ether (PBDE) classes, 18 polychlorinated biphenyls (PCBs) that include six non dioxin-like PCBs (NDL-PCB) and 12 dioxin-like PCBs (DL-PCB) , 17 polychlorinated dibenzo-p-dioxins and dibenzo-p-furans (PCDD/Fs), and perfluorooctane sulfonate (PFOS). Aquatic predators had higher PFOS and PCDD/F concentrations, while PCBs dominated in eggs of terrestrial species. Organochlorine pesticides, PBDEs, and PCBs were strongly associated with eggs from the industrial regions of Gauteng, while PCDD/F concentrations were evenly distributed between eggs. PCDD/F and PCB toxic equivalency quotient concentrations were low with no adverse effects expected. PFOS peaked towards the west at Bloemhof Dam where high concentrations (2300 ng/g wm) pose a severe risk. Bloemhof Dam was therefore identified as an unexpected hotspot, the source of the PFOS is unknown. This study highlighted the importance of multi-species studies sampling from multiple locations to assess the risk that POPs pose to avian populations as hotspots and species at risk may be missed by species- and site-restricted studies. Since 2009, more POPs were added to the Stockholm Convention on Persistent Organic Pollutants that should be investigated.

**Key words:** PCB, PBDE, PCDD/F, DDT, pesticide, heron, Ardeidae

## 1. Introduction

One of Southern Africa's largest rivers, the Vaal River, flows westwards from Mpumalanga province to the Atlantic (Figure 1). It flows through South Africa's most industrialised regions before passing through rural and agricultural areas. The Vaal River merges with the Orange-Senqu River near the town of Douglas, forming the Orange-Senqu River Basin (OSRB), that stretches over four countries (Botswana, Lesotho, Namibia, and South Africa) covering approximately 1 000 000 $km^2$ (Lange et al., 2007). The mouth of the river at the South Atlantic Ocean, was once a flourishing wetland with over 20 000 resident water birds and attracting many migrant birds. However, the number of resident birds have drastically decreased (Anderson et al., 2003). This wetland is a Ramsar site but on the Montreux Record (Ramsar Sites Information Service, 1991a).

There are seven Ramsar Sites located in the OSRB (Lets'eng-la-Letsie, Barbers Pan, Blesbok Spruit, Kgaswane Mountain Reserve, Seekoeivlei Nature Reserve and Ingula Nature Reserve; Ramsar Sites Information Service, 1991b). Southern Africa is, however, a water-scarce region; many rural households, agriculture, mining, and industry directly make use of the OSRB's surface and groundwater. The influx of agricultural and industrial products (including persistent organic pollutants, POPs) is a major cause of concern (Chokwe et al., 2019; Groffen et al., 2021; Gilbert et al., 2016; Quinn et al., 2009). POPs investigated include organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and metals in bird eggs (Bouwman et al., 2008; Chokwe et al., 2015; Polder et al., 2008; Van der Schyff et al., 2016).

Organochlorine pesticides can bio-accumulate in lipid tissue and are resistant to degradation (Newman 2015). In many African countries, the current and historical use of dichlorodiphenyltrichloroethane (DDT) in controlling disease and pests led to unintentional consequences such as eggshell thinning in many bird species (Bitman et al., 1970; Holm et al., 2006; Lundholm, 1997), and human health effects (Bornman et al., 2010). However, less literature is available on other major groups of POPs in bird eggs from South Africa including perfluorooctane sulfonate (PFOS), polychlorinated dibenzofurans and dibenzo-p-dioxins (PCDD/Fs) and PCBs (none dioxin –like PCBs (NDL-PCBs) and dioxin-like PCB (DL-PCBs))

POPs are capable of long-range atmospheric disposition due to their volatility and regularly end up in oceans and rivers (Wania and Mackay, 1995). Many of these compounds are banned or severely restricted (Stockholm Convention, 2016a). PFOS for example, a per- and polyfluorinated substance (PFAS), has been used as water repellent, in firefighting foams, and as an adjuvant in certain insecticides (Newman 2015: Stockholm Convention, 2016a). In addition, PFOS emerged as a common degradation product of a number of PFASs. Residues in the environment reflect current and historical production of such compounds.

Dioxin-like PCBs were commercially produced for the use in electrical transformers for a number of applications and as additives in lubricants and plasticizers (Newman 2015; Stockholm Convention, 2016a). In addition, the destruction of the materials and equipment containing these compounds have been a cause for concern (Orisakwe et al., 2019). PCDD/Fs are released as contaminants in the production of commercial products such as DL-PCBs. In addition, PCDD/Fs are by-products in combustion and bleaching processes associated with kraft pulp mills (Newman 2015). PBDEs are used in polyurethane foams and in fabrics, furniture, and electronic goods (Newman 2015: Stockholm Convention, 2016a).

Bird eggs are good monitoring tools (Medvedev and Markove, 1995; Lebedev et al., 1998). They have a fairly consistent composition, decompose slowly, are easy to handle, and can be randomly sampled in a cost-effective manner. Futhermore, eggs represent the pollutant uptake by the female bird in a period before the egg is laid, while giving insight into the effects these compounds have in both the female bird and in the developing egg (Braune, 2007; van den Steen et al., 2006). In addition, embryos and foetuses are more sensitive to POPs than adults. The embryonic exposure to compounds prior to organ development results in greater consequences than exposed after organ development (Caralson and Duby, 1973).

Both aquatic and terrestrial birds have been used as pollution indicators (Aurigi et al., 2000; Bouwman et al., 2019; Bouwman et al., 2021; Eljarrat et al., 2019). Elevated PFOS concentrations can lead to endocrine disruption (Jensen and Leffers., 2008) and organ dysfunction, especially in the liver (Hoff et al., 2005). PCBs can cause reproductive abnormalities and lead to developmental effects (Barron et al., 1995). At elevated concentrations, PBDEs effected behavioural and growth abnormalities in the American kestrel (*Falco sparverius*; Fernie et al., 2006 and 2008).

Knowledge on POPs of the Stockholm Convention of Persistent Organic Pollutants (SCPOPs) in Southern Africa is restricted to only a few compounds. The current study was done under the auspices the Orange-Senqu River Commission's (ORASECOM) 2010 Joint Basin Survey on POPs in the OSRB as part of the transboundary diagnostic analysis of the OSRB that also assessed all POPs listed at that time. The aims of this study were therefore to investigate the concentrations of 22 POPs, listed in the SCPOPs in 2010, in wild bird eggs in the Vaal River, to identify hotspots, and to assess possible impacts. As far as we are aware, this is the only study that analysed all POPs listed in the SCPOPs in 2010 in birds on a large geographic scale.

## 2. Materials and methods

### 2.1 Aquatic bird egg sampling

2.1.1 Bird egg sampling locations and descriptions

The necessary provincial permits and the appropriate ethical clearances (NWU-00055-07-S3 and NWU-00594-19-A9) were obtained. Wild aquatic bird eggs were collected from four breeding colonies in the OSRB in 2010/11 (Figure 1). Efforts so far have recorded 154 heronries in South Africa (Harebottle, 2019), although this number is believed to be severely underestimated. The four breeding colonies selected were located during aerial surveys. The Potchefstroom colony location is next to the Mooi River and is close to residential property and a golf course. The colony at Barbers Pan (a Ramsar Site) is in a bird sanctuary with no town or city close by. The Bloemhof Dam colony is the farthest west on Snake Island in the impoundment. The colony at Eldorado Park is within a suburb in a highly industrialised region of Gauteng province. Eggs from nine species were sampled: Grey Heron (*Ardea cinerea*), African Darter (*Anhinga rufa*), Glossy Ibis (*Ardea melanocephala*), Great White Egret (*Ardea alba*), Reed Cormorant (*Microcarbo africanus*), African Sacred Ibis (*Threskiornis aethiopicus*), Little Egret (*Egretta garzetta*), Cattle Egret (*Bubulcus ibis*), and Glossy Ibis (*Plegadis falcinellus*). General distributions and descriptions, habitat preferences, breeding behaviour, diet, and egg descriptions are summarised in Table S1.

### 2.1.2 Egg sampling effort

Eggs were sampled from nests by either climbing trees using rock-climbing gear or using ladders on smaller trees. Although efforts were made to collect eggs of the same species at all sites, this was not possible. Eggs were labelled, carefully stored, and transported to the laboratory where they were photographed before being frozen until sample preparation. On the day of sample preparation, selected eggs were measured and pooled per species and locations as presented in Table 1. Egg contents were ultrasonically homogenised. Samples of the 16 pools were sent with the necessary permits to Oëkometric GmbH - The Bayreuth Institute of Environmental Research, in Germany. This is an accredited POPs laboratory. Coordinates of sampling locations, the closest water source, and analytical pool numbers are presented in Table 1.

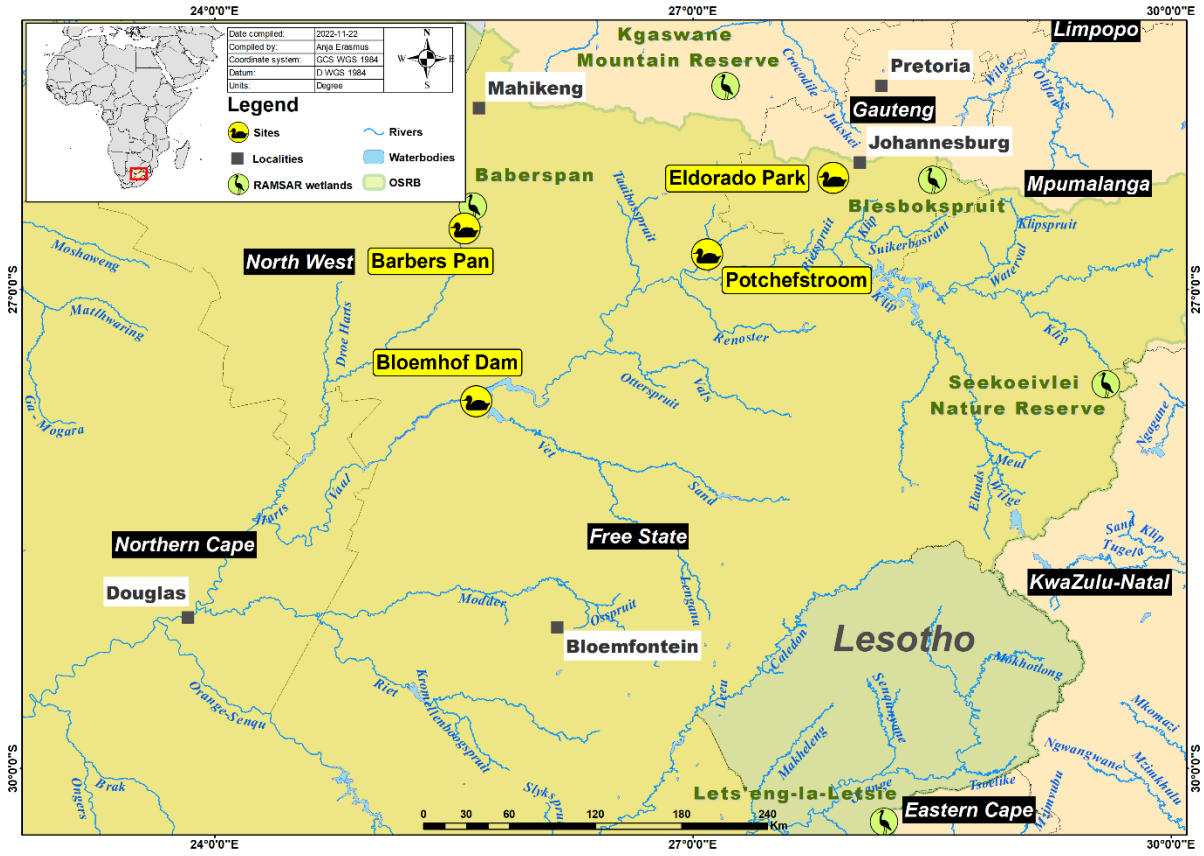


**Figure 1:** Map illustration the wild bird egg sampling locations along the Vaal River catchment.

**Table 1:** Summary of the wild bird species from which eggs were collected at each location, along with the GPS coordinates, closest river, pool number, number of eggs per pool (n), habitat and feeding guilds according to Hockey et al. (2005), as well as average egg mass (g) of species sampled.

| Location | Longitude | Latitude | River | Pool no | n | Common name | Scientific name | Habitat guild | Feeding guild | Egg mass |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 6 | Grey Heron | *Ardea cinerea* | Aquatic | Large aquatic predator | 61 |
| Barbers Pan | 25.57 | -26.6 | Harts River | 5 | 5 | African Darter | *Anhinga rufa* | Aquatic | Large aquatic predator | 37 |
| | | | | 14 | 5 | Black-headed Heron | *Ardea melanocephala* | Terrestrial | Terrestrial insectivore | 60 |
| | | | | 2 | 3 | Great White Egret | *Ardea alba* | Aquatic | Large aquatic predator | 61 |
| | | | | 3 | 5 | Grey Heron | *Ardea cinerea* | Aquatic | Large aquatic predator | 61 |
| | | | | 6 | 3 | African Darter | *Anhinga rufa* | Aquatic | Large aquatic predator | 37 |
| Bloemhof Dam | 25.64 | -27.7 | Vaal River | 8 | 5 | Reed Cormorant | *Microcarbo africanus* | Aquatic | Large aquatic predator | 21 |
| | | | | 10 | 6 | African Sacred Ibis | *Threskiornis aethiopicus* | Wetland | Scavenger | 62 |
| | | | | 11 | 4 | Little Egret | *Egretta garzetta* | Aquatic | Small aquatic predator | 28 |
| | | | | 16 | 6 | Cattle Egret | *Bubulcus ibis* | Terrestrial | Terrestrial insectivore | 27 |
| Eldorado Park | 27.88 | -26.3 | Klip River | 4 | 5 | African Sacred Ibis | *Threskiornis aethiopicus* | Wetland | Scavenger | 62 |
| | | | | 7 | 5 | Reed Cormorant | *Microcarbo africanus* | Aquatic | Large aquatic predator | 21 |
| | | | | 9 | 5 | Glossy Ibis | *Plegadis falcinellus* | Wetland | Small aquatic predator | 34 |
| Potchefstroom | 27.09 | -26.78 | Mooi River | 12 | 5 | Black-headed Heron | *Ardea melanocephala* | Terrestrial | Terrestrial insectivore | 60 |
| | | | | 13 | 4 | Black-headed Heron | *Ardea melanocephala* | Terrestrial | Terrestrial insectivore | 60 |
| | | | | 15 | 5 | Cattle Egret | *Bubulcus ibis* | Terrestrial | Terrestrial insectivore | 27 |

### 2.3 Chemical analyses

Eggs were analysed for PCDD/F (Regulation EC 1883/2006 and EPA 1613 B with high resolution GC/MS, LOQ TCDD/F–HxCDD/F = 0.00005 ng/g wet mass (wm), HpCDD/F = 0.00015 ng/g wm, OCDD/F = 0.0005 ng/g wm), 18 PCBs, that include DL-PCB -77, -81, -105, -114, -118, -123, -126, -156, -157, -167, -169, and -189 and NDL-PCBs -28, -52., -101., -138., -153., and 180 (Regulation (EC) 1883/2006, ASU L 00.00-12 and ASU L 00.00-38 with high resolution GC/MS, LOQ DL-PCB 81, 126, 169 = 0.0005 ng/g wm, DL-PCB 77, 105, 114, 123, 156, 157, 167,169 = 0.005 ng/g wm, DL-PCB 118 = 0.050 ng/g wm, NDL-PCB 28, 52, 101, 138, 180 = 0.1 ng/g wm), five PBDE classes reported as tetra-, penta-, hexa- and hepta-BDE, as well as hexabromobiphenyl HBB (proprietary method and EPA 1614 with high resolution GC/MS, LOQ 0.05–0.1 ng/g wm), PFOS (proprietary method with LC/MS-MS, LOQ = 1 ng/g wm), and 21 pesticides including α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachlorocyclohexane (lindane), HCB, heptachlor, aldrin, dieldrin, endrin, heptachloroepoxide, chlordane (trans- and cis-), *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p*-DDD, *o,p'*-DDT, *p,p'*-DDT, mirex, pentachlorobenzene, chlordecone and toxaphene (ASU L 00.00-12, ASU L 00.00-38 and a proprietary method (based on S19 multimethod) with high resolution GC/MS, LOQ = 0.1–2 ng/g wm). POPs analyses were executed observing quality assurance and quality control protocols as per ISO/IEC 17025:2005 accreditation that covered sample logistics, preparation, calibration, extraction, clean-up, measurement, quantification, quality control, concentration calculations, and reporting. Toxic equivalency quotients (TEQs) were calculated according to WHO (2005), and all are reported as exclusive (van den Berg et al., 2006).

### 2.4 Statistical analyses and measuring unit conversions

Descriptive and comparative statistics were done using GraphPad Prism version 9.0.2. To compare published data with the current data, certain concentration unit conversions were done. Values reported in parts per million (ppm), parts per billion (ppb), milligram per kilogram (mg/kg), and microgram per kilogram (µg/kg) were converted to nano-gram per gram (ng/g). Concentrations values in lipid mass (lm) were converted to wet mass (wm) (Clatterbuck et al.,

2018). The ΣPCB value is the total concentration of both DL-PCBs and NDL-PCBs. The PCB TEQ value consists of only DL-PCBs.

## 3. Results

Summary results are given in Table 2 and presented in several ways in Figures 2 to 7. The concentration quantified of individual congeners can be viewed in the supplementary material in Table S2.

### 3.1 Bird egg concentrations

OCP compounds such as α-HCH, lindane, heptachlor, aldrin, endrin, heptachloroepoxide, chlordane (trans- and cis -), mirex, pentachlorobenzene, chlordecone, toxaphene, *o,p'*-DDE, *o,p'*-DDD, and *o,p'*-DDT had no quantifiable concentrations in any egg.

The highest ΣOCP concentration were quantified in eggs of Great White Egret eggs (423 ng/g wm) from Bloemhof Dam, primarily as a result of the high *p,p'*-DDE (400 ng/g wm) (Figure 2A and B; Table 2). This egg pool had double the ΣOCP concentration than Reed Cormorant eggs from Bloemhof Dam (180 ng/g wm), Potchefstroom (150 ng/g wm), and an order of magnitude greater than the African Sacred Ibis egg pool from Eldorado Park (19 ng/g wm) (Table 2). The majority of the ΣOCP concentrations were comprised of *p,p'*-DDE. However, other OCPs were also quantified in some eggs (Table 2). The highest β-HCH concentration was in Black-headed Heron eggs (6 ng/g wm) from Barbers Pan (Figure 2C; Table 2). The highest HCB (2 ng/g wm) (Figure 2D) and dieldrin (9 ng/g wm) (Figure 2E) concentrations were in African Sacred Ibis eggs from Eldorado Park.

The highest PFOS concentrations were quantified in African Darter eggs (2300 ng/g wm) and Reed Cormorant eggs (1100 ng/g wm) from Bloemhof Dam (Figure 2E). PFOS was also the dominant compound in most species, except Great White Egret, African Sacred Ibis, Black-headed Heron, and Glossy Ibis where ΣOCPs dominated (Table 2). African Sacred Ibis eggs from Eldorado Park had the highest ΣPBDE concentrations (19 ng/g wm), followed by Reed Cormorant eggs from Potchefstroom (Figure 2G; Table 2). The highest ΣPCB concentration in any pool was in African Darter eggs (100 ng/g wm) from Bloemhof Dam followed by Reed Cormorant eggs (54 ng/g wm) from Potchefstroom (Figure H; Table 2). ΣPCDD/F concentrations were highest in Black-headed Heron eggs (9 ng/g wm) from Barbers Pan, followed by African Sacred Ibis eggs from Eldorado Park (7 ng/g wm) (Figure 2I).

Irrespective of species, the highest mean ΣPCB, ΣPCDD/F, ΣPBDE, ΣOCP, and ΣDDT were in Eldorado Park eggs (Figure 3A-E). In addition, all OCPs were dominant at Eldorado Park, except β-HCH which was dominant at Barbers Pan (Figure 3F-I). Bloemhof Dam had the highest mean PFOS concentration, followed by Barbers Pan (Figure 3J).

**Table 2:** Summary of all quantified concentrations detected in wild bird eggs. All concentrations are expressed in ng/g wm except for the TEQ values which are expressed in ng/kg wm.

| | | | | | | | | | | ng/g wm | | | | | | | WHO-TE 2005: Exclusive | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Site** | **Pool no** | **PFOS** | **ΣPBDE** | **β-HCH** | **HCB** | **Dieldrin** | ***p,p′*-DDD** | ***p,p′*-DDT** | ***p,p′*-DDE** | **ΣDDT** | **ΣOCP** | **Dl-PCB*** | **NDL-PCB**** | **ΣPCB** | **∑PCDD/F** | **WHO PCB TEQ (ng/kg wm)** | **WHO PCDD/F TEQ (ng/kg wm)** |
| Grey Heron | Barbers Pan | Pool 1 | 7 | 2 | 3 | 1 | 4 | | | 38 | 38 | 46 | 1 | 8 | 9 | 1 | 1 | 0.2 |
| Great White Egret | Bloemhof Dam | Pool 2 | 350 | 0.2 | 1 | | 2 | 8 | 12 | 400 | 420 | 420 | 1 | 5 | 6 | 0.2 | 0.7 | 0.1 |
| Grey Heron | Bloemhof Dam | Pool 3 | 720 | 0.4 | 1 | 1 | | | | 54 | 54 | 56 | 1 | 9 | 10 | 0.2 | 2 | 0.1 |
| African Sacred Ibis | Eldorado Park | Pool 4 | 69 | 20 | 2 | 2 | 9 | | 10 | 130 | 140 | 160 | 3 | 33 | 35 | 7 | 2 | 0.4 |
| African Darter | Barbers Pan | Pool 5 | 850 | 0.3 | 2 | 1 | 2 | 3 | | 86 | 86 | 91 | 1 | 6 | 7 | 0.3 | 1 | 0.1 |
| African Darter | Bloemhof Dam | Pool 6 | 2300 | 0.4 | 2 | 1 | 2 | | | 90 | 90 | 95 | 14 | 88 | 100 | 5 | 12 | 2 |
| Reed Cormorant | Potchefstroom | Pool 7 | 200 | 6 | 1 | 1 | 2 | | | 150 | 150 | 150 | 9 | 45 | 54 | 1 | 5 | 0.2 |
| Reed Cormorant | Bloemhof Dam | Pool 8 | 1100 | 0.2 | 1 | 1 | | | | 180 | 180 | 180 | 4 | 18 | 22 | 1 | 2 | 0.3 |
| Glossy Ibis | Potchefstroom | Pool 9 | 5 | 1 | | | 3 | 1 | 3 | 55 | 58 | 61 | 1 | 6 | 7 | 1 | 1 | 0.2 |
| African Sacred Ibis | Bloemhof Dam | Pool 10 | 17 | 0.4 | 1 | | 2 | | 1 | 70 | 71 | 74 | 0.3 | 2 | 3 | 0.1 | 0.3 | 0.01 |
| Little Egret | Bloemhof Dam | Pool 11 | 500 | 1 | | | | | | 19 | 19 | 19 | 1 | 8 | 9 | 1 | 1 | 0.2 |
| Black-headed Heron | Potchefstroom | Pool 12 | 6 | 1 | 1 | 1 | 4 | | | 27 | 27 | 33 | 0.2 | 2 | 2 | 0.2 | 0.4 | 0.04 |
| Black-headed Heron | Potchefstroom | Pool 13 | 17 | 3 | 1 | 1 | 6 | | | 24 | 24 | 32 | 4 | 43 | 47 | 6 | 7 | 2 |
| Black-headed Heron | Barbers Pan | Pool 14 | 6 | 0.5 | 6 | 1 | 7 | | | 27 | 27 | 41 | 2 | 14 | 16 | 9 | 3 | 1 |
| Cattle Egret | Potchefstroom | Pool 15 | 7 | 0.2 | 1 | | 1 | | | 18 | 18 | 20 | 1 | 6 | 7 | 1 | 1 | 0.2 |
| Cattle Egret | Bloemhof Dam | Pool 16 | 580 | | | | | | 1 | 27 | 28 | 28 | 0.3 | 2 | 2 | 0.3 | 0.3 | 0.03 |

*Dl- Dioxin like

** NDL-Non dioxin like

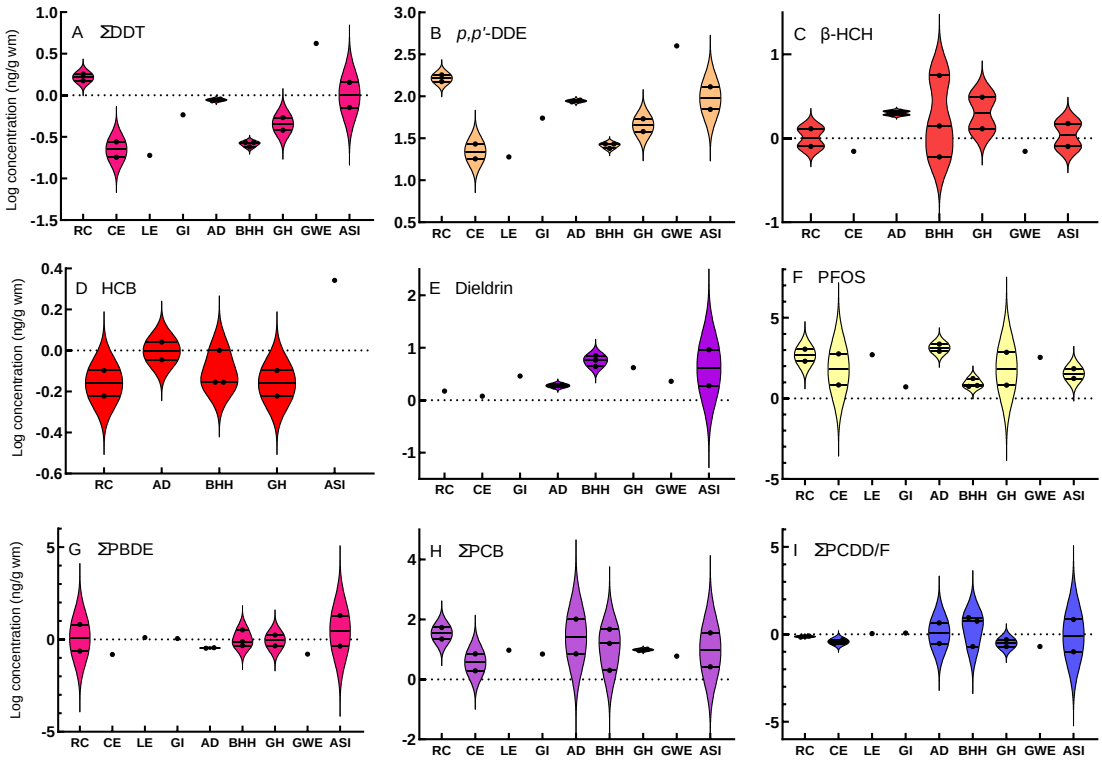


**Figure 2**: Violin plots (frequency distributions) of concentrations of selected compounds quantified in bird eggs regardless of sampling location. Horizontal lines are medians and 25 and 75% quartiles. Species are arranged according to increasing reported mean egg mass. RE = Reed Cormorant, CE = Cattle Egret, LE = Little Egret, GI = Glossy Ibis, AD = African Darter, BHH = Black Headed Heron, GH = Grey Heron, GWE = Great White Egret, and ASI = African Sacred Ibis.

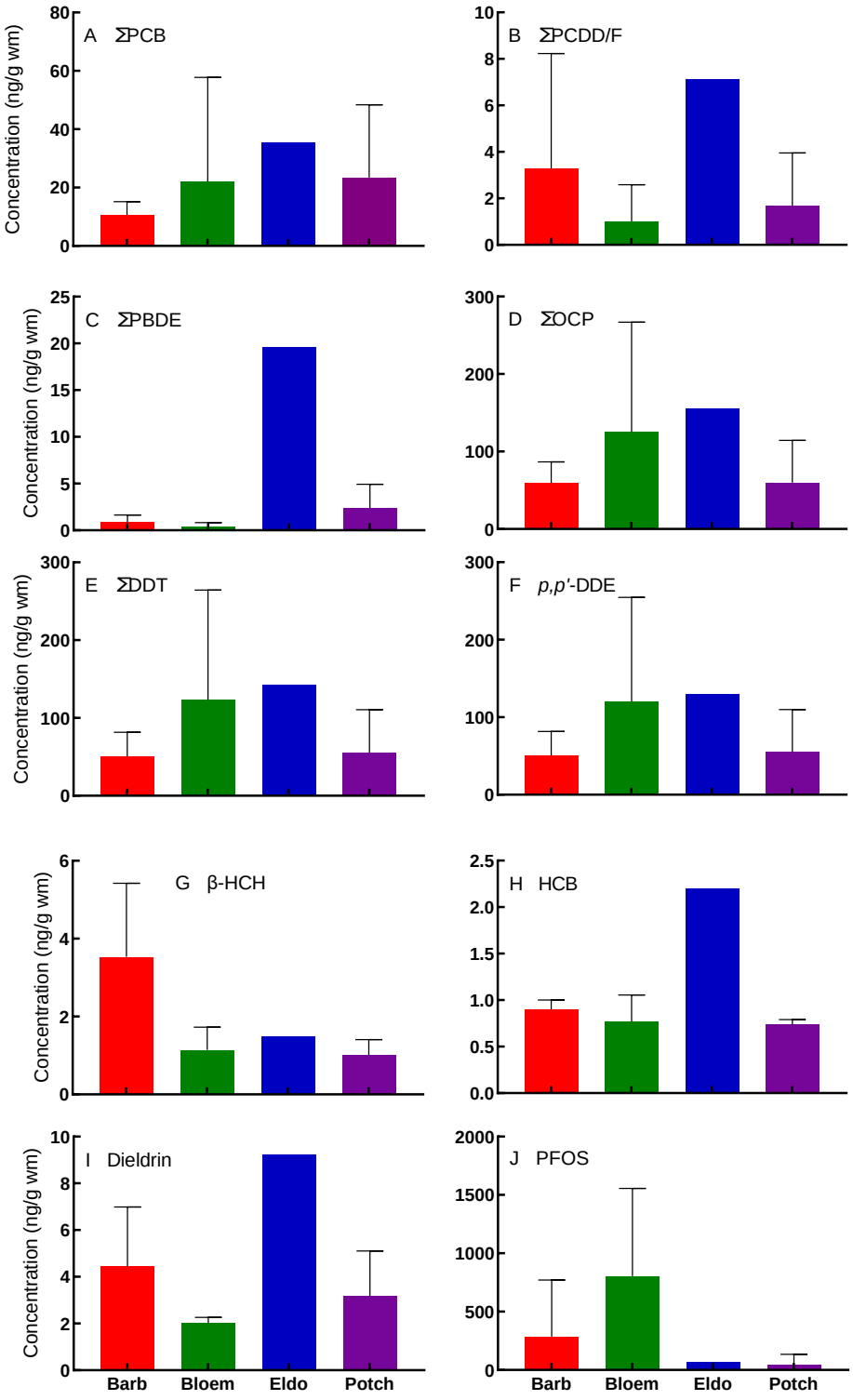


**Figure 3**: Mean concentrations and standard deviations of selected compounds quantified at each sampling location regardless of species. Barb = Barbers Pan, Bloem = Bloemhof Dam, Eldo = Eldorado Park, and Potch = Potchefstroom.

### 3.2 Guilds

The species were grouped according to habitat guilds: aquatic, terrestrial, and wetland (Table 1; Figure 4). There were no significant differences between habitat guilds (one-way ANOVA, Tukey's multiple comparisons) for ΣPCDD/F, ΣPBDE, β-HCH, and dieldrin (Figure 4A-D). There were statistically significant differences between aquatic and terrestrial habitat guilds for PFOS, ΣOCP, and *p,p'*-DDE (Figure 4E-G), and between terrestrial and wetland habitat guilds for ΣPCB (Figure 4I). Since only one data point was available for HCB in the wetland guild, we performed a two-way, unpaired t-test between terrestrial and aquatic eggs which was not significantly different (Figure 4H).

We grouped all species according to their feeding guilds: large aquatic predators (LAP), small aquatic predators (SAP), scavengers (S), and terrestrial insectivores (TI) (Table 1). There were no significant differences between feeding guilds (one-way ANOVA, Tukey's multiple comparison) for PFOS, ΣPCDD/F, ΣPCB, ΣPBDE, dieldrin, and β-HCH (Figure 5A-F). A statistically significant difference was found between large aquatic predators and terrestrial insectivores for ΣOCP and *p,p'*-DDE (Figure 5G and H). We performed two-way, unpaired, t-tests for HCB and found no statistically significant difference (Figure 5I).

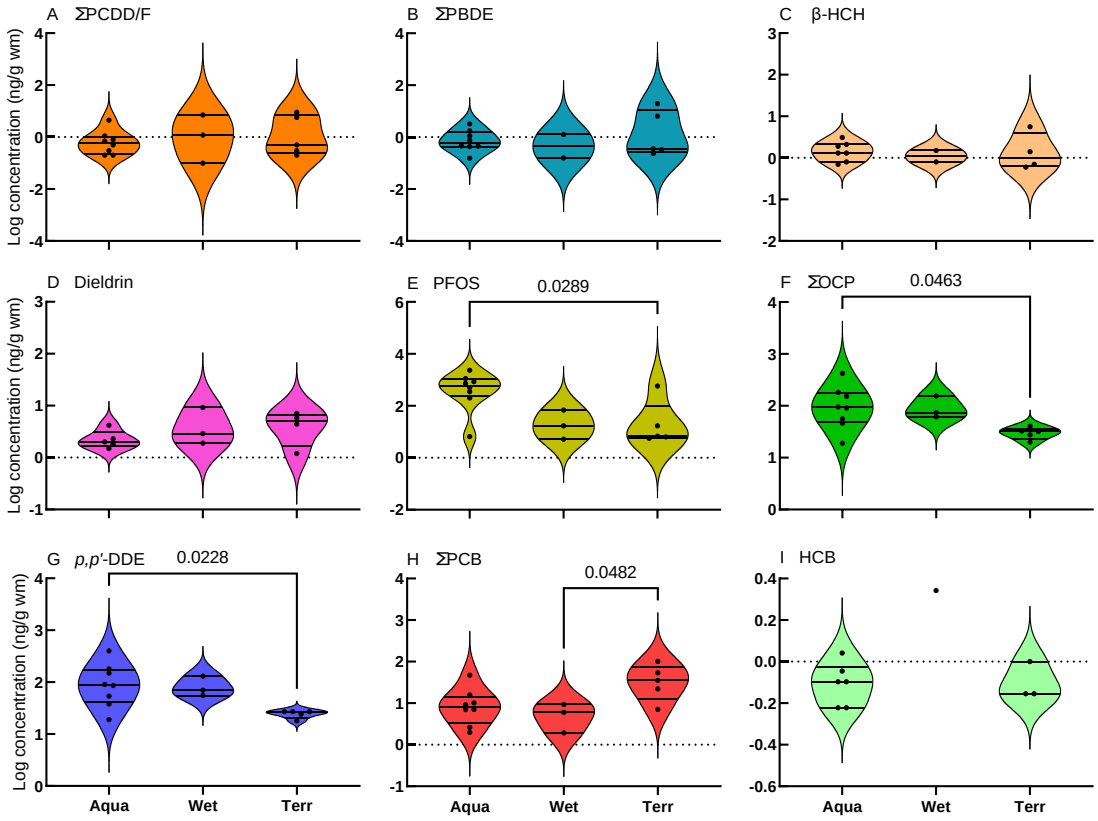


**Figure 4:** Violin plots (frequency distributions) of concentrations of selected compounds quantified in bird eggs according to habitat guilds. Horizontal lines are medians and 25 and 75% quartiles. Habitat guilds are expressed as aquatic, wetland and terrestrial. Aqua = aquatic, Wet = wetland, and Terr = terrestrial. ANOVA p-values of guilds that were found to be statistically significant different are indicated with brackets. Two-way unpaired t-test was performed for HCB.

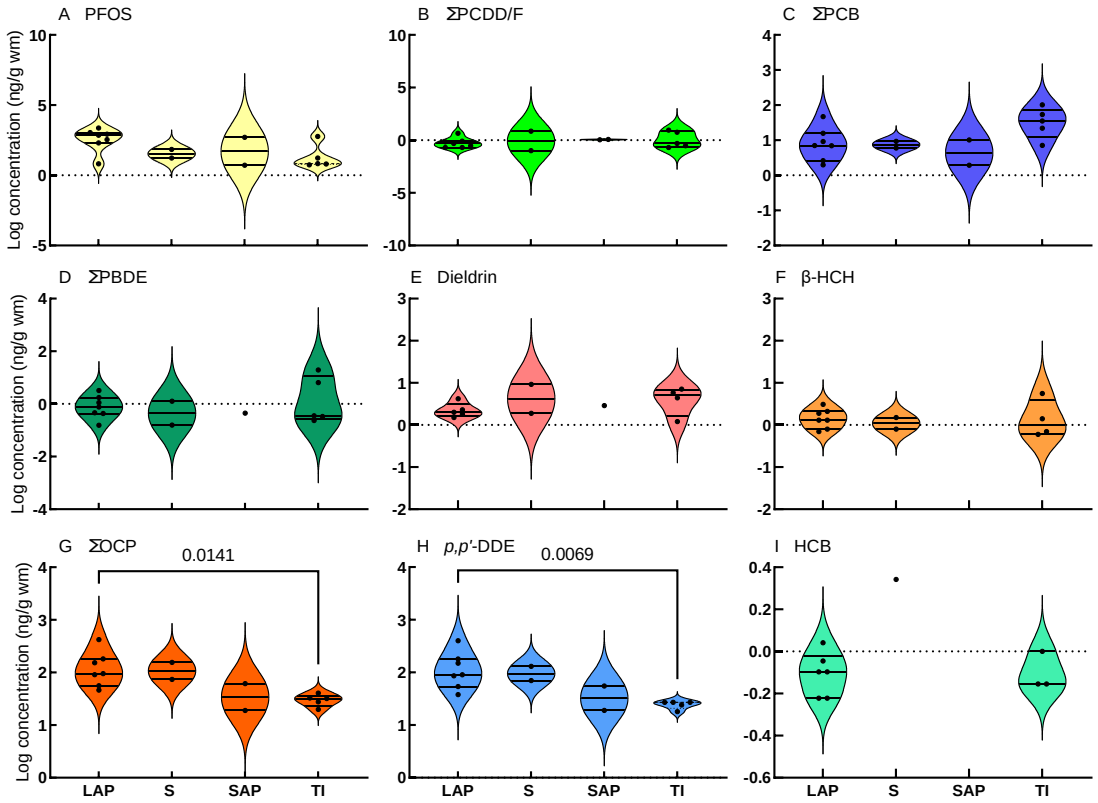


**Figure 5:** Violin plots (frequency distributions) of concentrations of selected compounds quantified in bird eggs according to feeding guilds. Horizontal lines are medians and 25 and 75% quartiles. Feeding guilds are expressed as LAP = large aquatic predators, S = scavengers, SAP = small aquatic predators, and TI = terrestrial insectivore. ANOVA p-values of guilds that were found to be statistically significant different are indicated with brackets. Two-way unpaired t-test was performed for HCB.

### 3.3 TEQ

Mean PCDD/F TEQ values of bird eggs were highest at Barbers Pan followed by Potchefstroom (Figure 6A), although the highest PCDD/F TEQ value were from eggs collected at Bloemhof Dam (1.6 ngTEQ/kg wm) (Table 2). PCB TEQ values in bird eggs were highest at Bloemhof Dam (12 ngTEQ/kg wm) followed by Potchefstroom (Figure 6B). PCDD/F and PCB TEQ values were highest in African Darter (12 ngTEQ/kg wm) and Black-headed Heron (7 ngTEQ/kg wm) eggs, followed by Reed Cormorant eggs (5 ngTEQ/kg wm) (Figure 6C and D). PCDD/F TEQ values were highest in terrestrial species (Figure 6E) while PCB TEQ values dominated in aquatic habitat guild eggs (Figure 6F). PCDD/F TEQ values were highest in terrestrial insectivores (Figure 6G) while PCB TEQ values were highest in large aquatic predators (Figure 6H). The PCB TEQs were higher in all species and at all sites compared to the PCDD/F TEQ values (Table 2).

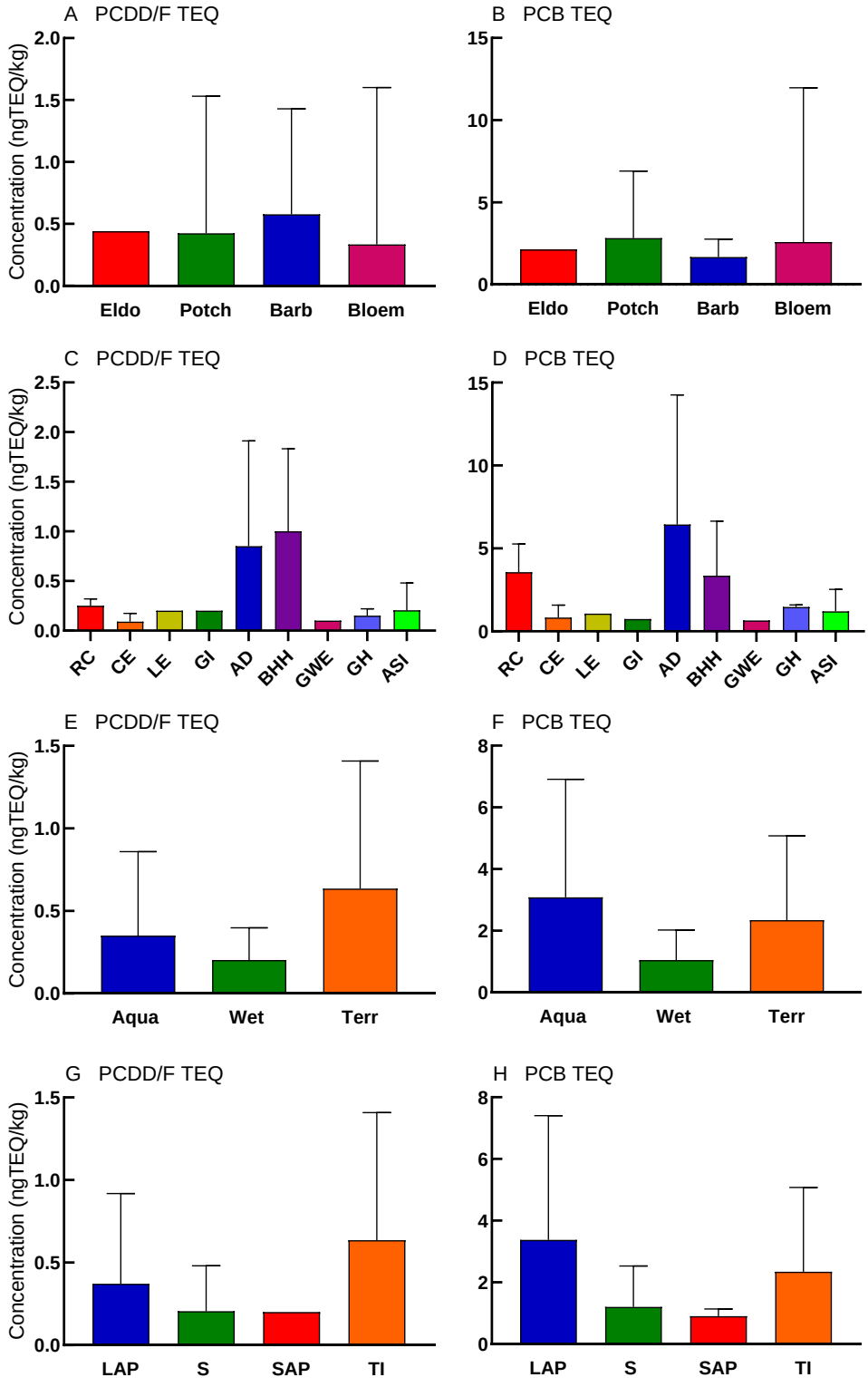


**Figure 6:** TEQ values in bird eggs. A) PCDD/F TEQ values according to sampling location. Data expressed as mean with range. B) PCB TEQ values according to sampling location. Data expressed as mean with range. Sampling locations are expressed as Barb = Barbers Pan, Bloem = Bloemhof Dam, Eldo = Eldorado Park and Potch = Potchefstroom. C) PCDD/F TEQ values according to species. D) PCB TEQ values according to species. Species are arranged according to increasing reported average egg mass. Species are expressed as RE = Reed

Cormorant, CE = Cattle Egret, LE = Little Egret, GI = Glossy Ibis, AD = African Darter, BHH = Black Headed Heron, GH = Grey Heron, GWE = Great White Egret, and ASI = African Sacred Ibis. E) PCDD/F TEQ values according to habitat guilds. F) PCB TEQ according to habitat guilds. Habitat guilds as expressed as Aqua = aquatic, Wet = wetland, and Terr = terrestrial. G) PCDD/F TEQ values according to feeding guilds. H) PCB TEQ according to feeding guilds. Feeding guilds are expressed as LAP = large aquatic predators, S = scavengers, SAP = small aquatic predators, and TI = terrestrial insectivore.

### 3.4 Influence of egg mass

We used linear regression to investigate the association of compound classes with egg mass (Figure 7). None of the slopes were significantly different from the x-axis. We also tested whether slopes and intercepts (vertical distances between the y-intercepts of each slope) were significantly different from each other. The slopes themselves were not significantly different ($p = 0.2773$). There was, however, a significant difference between y-intercepts ($p < 0.0001$).

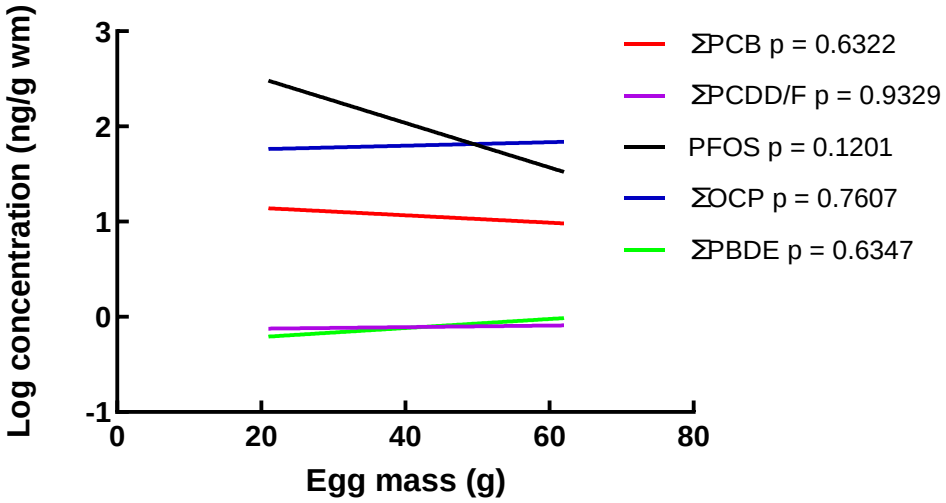


**Figure 7:** Simple linear regression. Concentrations of compound classes, regardless of species or sample location, regressed against egg mass.

## 4. Discussion

### 4.1 Bird egg concentrations

4.1.1 Feeding guilds

With eggs of nine species of birds collected from four locations and measured for 22 POPs, the current study is a large multi-species analyses investigating the pollution load of both aquatic and terrestrial birds. The concentrations of POPs differed greatly between species, sites, habitat guilds, and feeding guilds (Table 2 and Figures 2-5). This was as expected since the sites and species were collected over a large area where breeding colonies were available. The locations of the active breeding colonies at the time of sampling were found via aerial reconnaissance for this specific purpose. However, there were patterns based on guilds and localities close to sources that are apparent, with some exceptions.

Eggs of species occupying high trophic levels had higher PFOS and ΣOCP concentrations, while species that feed on insects had lower concentrations (Table 2 and 3 and Figure 5). This was reflected in habitat guilds where aquatic species had higher PFOS and ΣOCP concentrations (Figure 4). This pattern was also noted by Eriksson et al. (2016) that found higher PFAS concentrations in eggs from aquatic species compared with terrestrial species. The ΣOCP concentrations were dominated by *p,p'*-DDE, which is in agreement with others (Bouwman et al., 2008; Venugopal et al., 2020). Although we did not observe any pattern regarding PBDE concentrations in guilds. She et al. (2008) for instance, did find higher ΣPBDE concentrations in eggs of piscivorous birds compared with omnivorous species in the USA.

The ΣPCDD/F and ΣPCB concentrations suggest higher availability in terrestrial environments, which is counter to patterns found by Bouwman et al. (2021). Higher ΣPCB and ΣPCDD/F concentrations were found in soil rather than sediment (Quinn et al., 2009) from the same industrialised region sampled in this study. PCBs and PCDD/F tend to adhere to organic particles and concentrations may be greater in terrestrial environments as a result (Quinn et al., 2009). These patterns were not seen in other compound classes, perhaps due to the low concentrations in the environment and small sample sizes. Another possible explanation for the lack of patterns observed may be due to differences in foraging behaviour of species (Harris et al., 2003); some species spend prolonged time intervals around the nesting grounds while others roam over larger areas. In addition, differences in individual life histories among colony members can lead to differences in POP concentrations. It would have been insightful to compare the POP concentrations to those found in eggs of herbivorous, granivorous, and omnivorous species from the same sites (Bouwman et al., 2021).

We found few other studies with which to compare our findings. Lopez-Antia et al. (2017) reported no significant differences in PFOS concentrations between three species investigated. However, the PFOS concentration was greater in the more aquatic Mediterranean Gull (*Larus melanocephalus*). This pattern was also observed by Bouwman et al. (2021), reporting higher POP concentrations in species that inhabit aquatic habitats and species that are aquatic predators. Another multi-species analysis showed that eggs of omnivore species bio-accumulate higher ΣOCP concentration than species that only feed on one specific food source (Venugopal et al., 2020).

#### 4.1.2 Locations

Eggs of all species collected at Bloemhof Dam, except for African Sacred Ibis, had the highest PFOS concentrations in this survey (Table 2; Fig 3). This suggests high concentrations of environmentally available PFOS in this region. The African Sacred Ibis eggs from Eldorado Park, in contrast with the other POPs classes (Figure 3), had higher PFOS concentrations than those of the same species from Bloemhof Dam. Birds from industrialised areas are likely to be exposed to higher POP concentrations than rural birds (Elliott et al., 2015) which may explain the difference in PFOS concentrations between the Eldorado Park and Bloemhof Dam for the African Sacred Ibis (a scavenger, Table S1). Mean concentrations for all other compound classes, except PFOS and β-HCH, were highest at Eldorado Park, located in the highly industrialized Gauteng. Unfortunately, no eggs of other species aside from African Sacred Ibis were available at Eldorado Park at the time of sampling, complicating interpretation. A more detailed discussion on sources follows in Section 4.3.

#### 4.1.3 Egg mass

It could be argued that larger birds with larger eggs would eat larger prey from higher trophic levels. This would reflect in larger concentrations of POPs in their eggs. However, Bouwman et al. (2021) found no such effect, even when including eggs from a granivore trophic-level such as sparrows (small eggs at ca. 2 g) and high trophic-level African Darters and herons with large eggs (ca. 60 g). For POPs classes such as ΣDDTs, ΣPCBs, and ΣBDEs there were no associations (linear regressions) of any POP class concentrations with egg mass (Figure 7). Although the eggs of the current study were from birds from a generally high trophic level, we also found no association of POPs class concentrations such as DDTs and PCBs with egg mass, including for the first time PFOS and ΣPCDD/Fs (Figure 7). This phenomenon remains difficult to explain.

At higher concentrations of DDT and chordanes, eggs of Glaucous gulls (*Larus hyperboreus*) were smaller (Verboven et al., 2009). Verboven et al. (2009) include endocrine disruption, direct toxic effects, poor body condition, and food availability as contributing causes causing smaller eggs. DDT also cause thinner eggshells (Findholt, 1984; Peakall, 1993), suggesting that eggs with thinner shells weigh less per volume of egg. However, reverse causality should also be considered. During formation of eggs, those that are eventually lighter may have received proportionally more POPs deposited before the shell is formed. But, arguing this phenomenon across multiple species ranging in egg masses between 21 to 62 g would be difficult. Having observed this phenomenon twice (here, and in Bouwman et al., 2021) with POPs analyses done by two different laboratories invites further investigation.

### 4.2 Comparisons with international data

Many studies have reported POP concentrations in bird eggs. For the current study, we selected papers that used the same or similar species for comparisons (Table 3). The majority of published literature on POP concentrations in eggs primarily focused on PCBs and OCPs, especially DDT and its metabolites. ΣOCP concentrations in all species were generally lower compared with international studies. Little Egret eggs had ΣOCP concentrations two orders of magnitude lower than eggs from Spain (Huertas et al., 2016), and up to three orders of magnitude lower than eggs from France (Berny et al., 2002) and Romania (Aurigi et al., 2000).

∑PBDE concentrations in eggs of the present study were low compared with international data (Table 3). Concentrations quantified in Grey Heron eggs from Barbers Pan were two orders of magnitude lower than ΣPBDE concentrations in eggs from Spain, Canada, and the USA (Table 3; Custer et al., 2009; Eljarret et al., 2019; Miller et al., 2015). PFOS concentrations in eggs from the current study were generally lower, or of the same order of magnitude, than reported from other regions, except for eggs from Bloemhof Dam (Table 3). Night Heron (*Nycticorax nycticorax*) eggs from China had lower PFOS concentrations than Grey Heron eggs from Bloemhof Dam, but higher concentrations than eggs from Barbers Pan. Great Cormorant eggs from Sweden and Germany (Nordén et al., 2013; Rüdel et al., 2011) had lower PFOS concentrations than Reed Cormorants eggs from Bloemhof Dam, but higher concentration than those quantified in eggs from Potchefstroom. Only eggs of Blue Herons (*Ardea herodias*) collected in 1993 in the USA near a PFAS source (Custer et al., 2010), had similar PFOS concentrations than eggs from Bloemhof Dam. PFOS concentrations at Bloemhof Dam were therefore extraordinary high considering the absence of any local source.

ΣPCB concentrations were three orders of magnitude lower in eggs from the OSRB compared with internationally reported data (Table 3), especially when comparing similar species and guilds. Grey Heron eggs from Bloemhof Dam had ΣPCB concentrations two orders of magnitude lower than concentrations quantified in France (de Cruz et al., 1997), and one order of magnitude lower than eggs from Romania (Aurigi et al., 2000). A broad observation suggests a worldwide decline in PCB concentrations. Using the Grey Heron as example, the PCB concentrations from the 1970s to late 1990s were up to two orders of magnitude higher compared to post 2000 studies (Table 3). This decline was also observed in Double-crested Cormorants eggs in Canada, where there was an order of magnitude decline in PCDD/F concentrations from 1987 to 1997. Long-term monitoring of POPs in eggs can aid in assessing patterns and distribution profiles (Harris et al., 2003). We could not find a study that reported ΣPCB concentrations from similar species in African studies. However, free-range chicken eggs from Tanzania had even lower ΣPCB concentrations than in the current study (Polder et al., 2016).

ΣPCDD/F concentrations reported in eggs from other regions were generally lower or of the same order of magnitude than concentrations quantified in the current study (Table 3). In addition, a number of eggs had one order of magnitude higher ΣPCDD/F concentrations (Black-headed Heron: 9 ng/g wm) than eggs from other studies (Tables 2 and 3). Eggs of Double-crested Cormorants and Blue Herons from Canada (Elliott et al., 2001; Harris et al., 2003) measured the highest PCDD/F concentrations (1 ng/g wm) outside South Africa. We did not anticipate that bird eggs from South Africa would have the highest measured PCDD/F concentrations. Furthermore, all PCDD/F concentrations reported from international studies pre-date (1973–2000) the current data (2009). More recently reported PCDD/F concentrations in yellow-legged gull eggs (*Larus michahellis*) from in Spain (0.01 ng/g wm; Morales et al., 2016) and chicken eggs from Canada were also lower (Rawn et al., 2012). The current study is the first and only to report PCDD/F concentrations in wild bird eggs from South Africa, and to the best of knowledge, also Africa. TEQ values will be discussed in Section 4.4.

### 4.3 Hotspot identification

The ΣPCB and ΣOCP concentrations in all species were lower than those previously reported from nearby locations except for Cattle Egret eggs which were of the same order of magnitude (Bouwman et al., 2008 and 2021; Polder et al., 2008). However, African Sacred Ibis from Eldorado Park had lower ΣPCB, but higher ΣOCP concentration than those reported from Gauteng and northern Free State (Bouwman et al., 2021) (Table 3). In addition, the African Darter concentrations from those studies where an order of magnitude higher than the African Sacred Ibis concentrations. The Gauteng concentrations reported, had among others, eggs from a colony near Eldorado Park. This may at first suggest a decrease in ΣPCB, and increase in ΣOCP concentrations. However, eggs from some localities were pooled confounding interpretation. The ΣOCP concentrations were four orders of magnitude higher in Grey Heron eggs, and one order of magnitude higher in Cattle Egret eggs from areas of the country where DDT is still used (Bouwman et al., 2013) compared with the current study's locations where DDT has been banned since 1976 (Bouwman 2004).

∑PBDE concentrations in the current study were of the same order of magnitude or lower than those reported by other authors (Table 3). African Sacred Ibis eggs collected in Eldorado Park, had slightly lower ΣPBDE concentrations than those reported from nearby Soweto (Quinn et al., 2020) and Johannesburg (Bouwman et al., 2021). African Darter eggs from Gauteng

(Bouwman et al., 2021; Quinn et al., 2020) had an order of magnitude higher ΣPBDE concentrations than those reported from Bloemhof Dam and Barbers Pan of the current study.

Elevated PFOS concentrations were quantified at high concentrations in bird eggs, especially from Bloemhof Dam (Table 2). To the best of our knowledge, there is no PFAS production in South Africa, far less any near Bloemhof Dam which has no industries close by. This location appears to be a PFOS hotspot as PFOS was found to the dominant PFAS quantified in adult Odonata from there (median: 16 ng/g wm) (Lesch et al., 2017). In addition to PFOS, high concentrations of mercury (Hg) were also quantified in Great White Egrets eggs from Bloemhof Dam (van der Schyff et al., 2016). No other studies from South Africa reported on PFOS or PCDD/F concentrations in bird eggs of similar species. Compared with international reports, the high PCDD/F concentrations of the current study points towards a PCDD/F hotspot. Eggs from Barbers Pan had the highest PCDD/F concentration (9 ng/g wm; Table 2), this is concerning since this location is a Ramsar site. The four highest measured PCDD/F concentrations (BBH: 9 ng/g wm, ASI: 7 ng/g wm, BHH: 6 ng/g wm, and AD: 5 ng/g wm; Table 2) were in eggs from all four sites and from three different feeding and habitat guilds making it difficult interpret. It is concerning that concentrations quantified in eggs from all four sites were higher than internationally reported concentrations (Table 3), especially since Barbers Pan is a Ramsar site.

The data reported here represent the most current published insight into the pollution load of heron, ibis, egret, darter, and cormorants that breed in the OSRB. All other published reports were conducted during or prior to the current study. Chlordane and mirex were previously quantified in eggs of similar species (Bouwman et al., 2008; Polder et al., 2008). Studies conducted the same year as the current collection also found quantifiable concentrations of chlordane and mirex in eggs from Gauteng (Bouwman et al., 2021) and Limpopo (Bouwman et al., 2013). These compounds were also quantified in Little Egret and White-breasted Cormorant eggs collected in 2013 in KwaZulu-Natal that is not in the OSRB. The lack of quantifiable concentrations of these compounds may be due to concentrations below LOQ.

Therefore, POP hotspots identified in this study were Bloemhof Dam for PFOS, and Eldorado Park (Gauteng) for most other POPs. We could not identify hotspots for PCDD/F due to similar high concentrations detected at all four sites, nor could we explain these concentrations based on guilds. It would be reasonable to assume that the Vaal River catchment is a hotspot for PCDD/Fs, in general, and that more localised investigations need to be conducted. Bloemhof Dam has no associated industrial activities but is located approximately 450 km downstream of the most industrialised centre in the OSRB where African Sacred Ibis eggs were analysed. Eggs from Eldorado Park (in the industrialised centre) had factors to orders of magnitude higher concentrations of all compound classes at any other site, except for PFOS at Bloemhof Dam. The PFOS and PCDD/Fs results from Bloemhof Dam shows that single species studies cannot represent the picture of total avian exposure and risks as was also found by Bouwman et al. (2021). Also, assumptions about proximity to sources should not be assumed as the only factor when identifying hotspots.

### 4.4 Toxicity reference values for birds and risk characterization

The low TEQ values in eggs from Eldorado Park was unexpected, considering its proximity to industry. The high PCDD/F TEQ value from Barbers Pan was higher than expected, due to its isolation, remoteness from sources, and protection status as a nature sanctuary. However, the Black-headed Heron eggs from this location did have the highest quantifiable PCDD/F

concentration (Table 2). Bloemhof Dam on the other hand, had the highest PCB TEQ value, possibly a result of compounds accumulating at this point in the Vaal River. Bird embryos and foetuses are more sensitive to POPs than adults. In addition, exposure prior to organ development results in greater consequences when exposed after organ development (Caralson and Duby, 1973). However, the TEQ values reported in this study were low when compared with others (Harris et al., 2003; Hart et al., 1991). The TEQ values calculated for Double-crested Cormorant eggs close to a pulp mill were up to three orders of magnitude higher than any TEQ value of the current study (Harris et al., 2003). The Double-crested Cormorant hatchlings had elevated ethoxyresorufin-O-deethylase (EROD) activity and showed brain asymmetry. In addition, it is suggested that neurological activities in bird eggs are more effected by PCDD/F TEQs (Henshel, 1998), which were lower than PCB TEQs in the current study. In Blue Heron eggs from Canada a no-observed-adverse-effect-level (NOAEL) of 18 ngTEQ/kg wm was reported for developmental defects and reduced fledging (Hart et al., 1991), 10 ngTEQ/kg wm for intercerebral brain asymmetry (Henshel et al., 1995), and 100 ngTEQ/kg wm for gross abnormalities and oedema (Sanderson et al., 2009). A NOAEL of 200 ngTEQ/kg wm of coplanar PCBs were reported for Forster's tern eggs (*Sterna forsteri*) for reduced hatching success and fledging (Kubiak et al., 1989). These TEQ values were all well above all TEQ values from the current study and therefore we do not expect any adverse effects in eggs, hatchlings, or fledglings.

The number of PCB congeners measured affect the reported concentrations in bird eggs. While we investigated 18 congeners of which 12 are DL-PCBs and six NDL-PCBs, far higher ΣPCB residues were quantified in eggs that were investigated for fewer congeners. Field studies found mortality in Double-crested Cormorant eggs at $\Sigma PCB_?$ of 30 000 ng/kg wm (Tillitt et al., 1992) and developmental defects in Black-crowned Night herons at $\Sigma PCB_?$ concentrations of 800 ng/g wm (Hoffman et al., 1986). The highest ΣPCB concentrations quantified in any egg of the present study (African Darter: 102 ng/g wm) was far below the toxic reference value (TRV) that were derived by Hoffman et al. (1986) and Tilliet et al. (1992). We, therefore, do not expect adverse effects in birds as a result of PCB exposure for the regions sampled.

The probability for adverse effects on birds was investigated by comparing the concentrations quantified in the eggs to available POP TRVs. Unfortunately, TRVs are not available for all species. However, quantifiable concentrations can be compared to TVRs for other species, although it should be noted that these values are estimations and can differ greatly among species due to behavioural and biological differences.

The HCB concentrations measured in bird eggs were low compared with other studies (Table 2). This is reassuring since HCB is known to be very toxic to birds. The HCB concentrations measured in all eggs from the current study were far below the NOAEL of 1500 ng/g wm for herring gull (*Larus argentatus*) embryos (Boersma and Ellenton, 1986). DDT, specifically the metabolite *p,p'*-DDE, reduce eggshell thickness in eggs and may lead to reproductive failure and population decline (Dirksen et al., 1995; Peakall, 1993). In Snowy Egrets (*Egretta thula*) it was found that DDE residues of 5000 ng/g wm in eggs caused thinner eggshells and lower hatching success (Findholt, 1984). The *p,p'*-DDE residues in Little Egret eggs (19 ng/g wm) from Bloemhof Dam were well below this TRV. In addition, DDE residues of 8000 ng/g wm were found to increase eggshell breakage in Black-crowned Night Heron (*Nycticorax nycticorax*) populations (Henny et al., 1984). The *p,p'*-DDE concentrations of all species in the current study were well below that threshold. However, it should be noted that eggshell

thinning can occur at lower exposure concentrations. Eggshell thinning has been observed in Cattle Egret eggs with increased *p,p'*-DDE and *p,p'*-DDT concentrations of up to 290 ng/g wm (Bouwman et al., 2013). For insecticide POPs therefore, we do not expect adverse effects in birds for the region sampled.

Certain factors may influence the residue concentrations quantified in bird eggs such as the diet, habitat preference, age and health of the female bird, as well as the time and the number of eggs laid in the clutch (Dennis et al., 2021; Mineau, 1982). In addition, bioaccumulation of PFOS is 1.8 times greater in eggs when exposed through drinking water compared to food (Dennis et al., 2021). The concentrations of PFOS quantified in all eggs of all species from Bloemhof Dam and Eldorado Park (African Darter: 2330 ng/g wm) were two orders of magnitude above the TRV of PFOS estimated for Bobwhite quail (*Colinus virginianus*) whole egg (92.4 ng/g wm) (Dennis et al., 2021). The predicted no-effect concentration (PNEC) for PFOS in Bobwhite quail, regrading chick survival is 1700 ng/g wm (Newsted et al., 2005). African Darter eggs from Barbers Pan and Reed Cormorant eggs from Potchefstroom exceeded the TRV and PNEC for Bobwhite quail. PFOS therefore, poses a risk for adverse effects in birds for all regions sampled.

The ΣPBDE concentrations from African Sacred Ibis eggs from Eldorado Park was 20 ng/g wm, well below the NOEL of 1000 ng/g wm for the Osprey (*Pandion haliaetus*; Chen et al., 2010). The Osprey is a high-trophic level species when compared with the African Sacred Ibis. It may be that higher PBDE concentrations will bio-accumulate in higher trophic level species from Eldorado Park. However, PBDE does not pose a risk for adverse effects in bird species sampled.

## 5. Conclusions and recommendations

Concentrations of all compounds in eggs from the current study were generally lower or of the same magnitude than those reported from most local and international studies, except for PCDD/F and PFOS. OCP and PCB concentrations were lower than previously reported suggesting a decreases in the environment. Differences in POP concentrations in wild bird eggs were found between species, sites, habitat guilds, and feeding guilds. This was to be expected, since species from the same region have different life histories. In addition, POPs have multiple sources and as a result of their chemical and physical characteristics behave differently when exposed to the elements.

Large aquatic predators had greater OCP and PFOS concentrations compared to species that prey on insects, while PCBs and PCDD/Fs were more prominent in terrestrial species. No patterns were observed for the other compound groups. It is recommended that additional species occupying other feeding guilds, such as seedeaters and frugivorous, be included. It would also be instructive to determine trends and patterns over multiple years. DDT residues in bird eggs remain high in malaria endemic regions. However, Gauteng appears to be an OCP (*p,p'*-DDE) and PCB hotspot. It would be reasonable to assume that, given the lower *p,p'*-DDT concentrations and or lack of quantifiable concentrations in pooled eggs, that the ΣDDT quantified is of legacy use.

PFOS concentrations peak towards the west at Bloemhof Dam. The concentrations quantified are comparable to concentrations detected near a PFAS source. It appears that Bloemhof Dam act as a 'retainer' or 'trap' of some compounds coming from upstream or may reflect a local unknown PFOS source. These concentrations pose a risk for adverse effects and should

be monitored. It would be very informative to sample additional locations such as at Upington downstream of Bloemhof Dam, especially with regard to PFOS distribution. PCDD/F concentrations quantified were unexpected. Due to the widespread occurrence of high PCDD/F concentrations, it is difficult to pinpoint towards a specific hotspot. The high ΣPBDE concentrations at Barbers Pan is however, concerning given that the location is a Ramsar site. However, we recommend that more samples be collected and tested for PCDD/Fs. Overall, Bloemhof Dam in particular would be a good monitoring site of all POPs, given its remoteness from large sources and high breeding density.

The OCP concentrations detected in bird eggs were below TRVs. ΣPBDE concentrations in wild bird eggs were also low. However, the higher ΣPBDE concentrations from Eldorado Park are concerning and should be regarded as a PBDE hotspot. The combined POPs concentrations may lead to greater consequences than individual POPs. In addition, since 2010, seven POPs have been added to the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2016b), and others are in the process of being added. These new POPs need further investigation to determine possible threats and hotspots. PCB and PCDD/F concentrations and TEQ values in wild bird eggs were low and no adverse effects are expected. Therefore, we conclude that single-site and single-species studies would not effectively represent risks representative of the complexity of avian diversity as environmental, behavioural and physiological differences of species.

**Table 3:** Mean concentrations of compound groups (ng/g wm) in wild bird eggs reported by various authors. The country, location and year the samples were collected as well as the measuring unit the authors reported their data in are listed. All concentrations were converted to ng/g wm.

| Species | Country | Year sampled | Location | Reported in: | ΣPCB$_7$ | ΣPCB$^{\#}$ | ∑PCDD/F | PFOS | ∑BDE$_7$ | PBDE | ∑OCP | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Grey Heron (*Ardea cinerea*)** | | | | | | | | | | | | |
| | RSA | 2009 | Barbers Pan | ng/g wm | ΣPCB$_6$ | 9 | 0.5 | 7 | ΣBDE$_5$ | 2 | 46 | This study |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 10 | 0.2 | 725 | ΣBDE$_5$ | 0.4 | 56 | This study |
| | Turkey* | 2009 | SÖKE | ng/g wm | ΣPCB$_7$ | 70 | | | | | 1100 | Kocagöz et al., 2014 |
| | RSA* | 2009 | Nandoni Dam | ng/g lm | ΣPCB$_{20}$ | 38 | | | | | 14276 | Bouwman et al., 2013 |
| | Creece | 2004 | Lake Kerkini | ng/g wm | ΣPCB$_7$ | 64 | | | | | | Antoniadou et al., 2007 |
| | Spain | 2010-17 | Castrejón reservoir | ng/g lm | | | | | ΣBDE$_8$ | 119 | | Eljarrat et al., 2019 |
| | Romania* | 1997 | Danube Delta | ng/g dm | ΣPCB$_6$ | 620 | | | | | 2321 | Aurigi et al., 2000 |
| | Greece* | 2004 | Lake Kerkini | ng/g lm | | | | | | | 255 | Goutner et al., 2012 |
| | France | 1991 | Reserve Naturelle de Grandlieu | ug/kg wm | ΣPCB$_1$ | 1280 | | | | | 745 | de Cruz et al., 1997 |
| **Blue Heron (*Ardea herodias*)** | | | | | | | | | | | | |
| | Canada | 1979 | Quebec | ppm wm | ΣPCB$_7$ | 7880 | | | | | 5266 | Laporte 1982 |
| | Canada (2 and 3) | 1995 | Columbia River, Bachelor island | ng/g and pg/g wm | ΣPCB$_3$ | 615 | 0.04 | | | | 0.4 | Thomas and Anthony 1999 |
| | Canada (2 and 3) | 1994 | Columbia River, Ross island | ng/g and pg/g wm | ΣPCB$_3$ | 3454 | | | | | 2 | Thomas and Anthony 1999 |
| | USA | 1993 | Indiana Dunes | ng/g wm | | | | 245 | ΣBDE$_6$ | 347 | | Custer et al., 2009 |
| | Canada (3) | 2002 | Fraser River Estuary | ng/g wm | | | | | ΣBDE$_6$ | 457 | | Miller et al., 2015 |
| | Canada (2) | 1989 | University of Brithish Columbia | ng/kg wm | | | 0.4 | | | | | Elliott et al., 2001 |
| | Canada (2) | 1991 | Victoria | ng/kg wm | | | 0.1 | | | | | Elliott et al., 2001 |
| | Canada (2) | 1987 | Crofton | ng/kg wm | | | 1 | | | | | Elliott et al., 2001 |
| | USA (2) | 1993 | Mississippi river / Pig's Eye | ng/g wm | | | | 940 | ΣBDE$_7$ | 142 | | Custer et al., 2010 |
| **Black-headed Heron (*Ardea melanocephala*)** | | | | | | | | | | | | |
| | RSA | 2009 | Potchefstroom | ng/g wm | ΣPCB$_6$ | 2 | 0.2 | 6 | ΣBDE$_5$ | 1 | 33 | This study |
| | RSA | 2009 | Potchefstroom | ng/g wm | ΣPCB$_7$ | 47 | 6 | 17 | ΣBDE$_5$ | 3 | 32 | This study |
| | RSA | 2009 | Barbers Pan | ng/g wm | ΣPCB$_8$ | 16 | 9 | 6 | ΣBDE$_5$ | 0.5 | 41 | This study |
| **Night Heron (Genera: *Nycticora*, *Nyctanass*, *and Gorsachius)*** | | | | | | | | | | | | |
| | Romania* | 1997 | Danube Delta | ng/g dm | ΣPCB$_6$ | 127 | | | | | 1689 | Aurigi et al., 2000 |
| | Israel | 1975 | Coastal plain | ppm | ΣPCB$_7$ | 770 | | | | | 1620 | Perry et al., 1990 |
| | USA | 1996 | Alexander island | ng/g wm | ΣPCB$_{18}$ | 2100 | 0.2 | | | | 460 | Frank et al., 2001 |
| | Italy | 1994 | Riserva Naturale Garzaia di Villarasc | ppm | ΣPCB$_7$ | 40 | | | | | 200 | Fasola et al., 1998 |
| | Creece | 2004 | Lake Kerkini | ng/g wm | ΣPCB$_7$ | 26 | | | | | | Antoniadou et al., 2007 |
| | Hong Kong | 2000 | A Chau | pg/g lm | | | 0.07 | | | | | Wang et al., 2012 |
| | Hong Kong | 2000 | Mai Po Village | ng/g wm | ΣPCB$_7$ | 230 | | | | | 704 | Connell et al., 2003 |
| | China | 2004 | Xiamen | ng/g wm | | | | 123 | | | | Wang et al., 2008 |
| | Hong Kong | 2006 | A Chau | ng/g wm | | | | 115 | | | | Wang et al., 2008 |
| | Spain | 2010-17 | Castrejón reservoir | ng/g lm | | | | | ΣBDE$_8$ | 15 | | Eljarrat et al., 2019 |
| | Greece* | 2004 | Lake Kerkini | ng/g lm | | | | | | | 172 | Goutner et al., 2012 |
| | Hong Kong* | 2006 | A Chau | ng/g lm | ΣPCB$_7$ | 55 | | | | | 186 | Wang et al., 2011 |
| **Purple Heron (*Ardea purpurea*)** | | | | | | | | | | | | |
| | Spain | 2010-17 | Castrejón reservoir | ng/g lm | | | | | ΣBDE$_8$ | 43 | | Eljarrat et al., 2019 |
| **Reed Cormorant (*Microcarbo africanus*)** | | | | | | | | | | | | |
| | RSA | 2009 | Potchefstroom | ng/g wm | ΣPCB$_6$ | 54 | 1 | 201 | ΣBDE$_5$ | 6 | 154 | This study |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 22 | 1 | 1120 | ΣBDE$_5$ | 0.2 | 181 | This study |
| | RSA | 2004/5 | Vaal River | ng/g wm | ΣPCB$_{34}$ | 110 | | | | | 308 | Bouwman et al., 2008 |
| | RSA* | 2004/5 | Parys | ng/g lm | ΣPCB$_{34}$ | 165 | | | ΣBDE$_8$ | 1 | 449 | Polder et al., 2008 |
| **Great Cormorant (*Phalacrocorax carbo*)** | | | | | | | | | | | | |
| | Netherlands | 1988/9 | Rhine and Meuse rivers | ug/kg wm | ΣPCB$_6$ | 1583 | | | | | 5318 | Dirksen et al., 1995 |
| | Sweden (1) | 2007-9 | Lake Vänern | ng/g wm | | | | 552 | | | | Nordén et al., 2013 |
| | Germany | 2009 | Baltic sea / Heuwiese | ng/g wm | | | | 90 | | | | Rüsdel et al., 2011 |
| | Germany | 2009 | Elbe estuary/ Haseldorf | ng/g wm | | | | 540 | | | | Rüsdel et al., 2011 |
| | Greece* | 2004 | Lake Kerkini | ng/g lm | | | | | | | 355.136 | Goutner et al., 2012 |
| | Creece | 2004 | Lake Kerkini | ng/g wm | ΣPCB$_7$ | 60 | | | | | | Antoniadou et al., 2007 |

| Species | Country | Year sampled | Location | Reported in: | ΣPCB$_7$ | ΣPCB$^{\#}$ | ∑PCDD/F | PFOS | ∑BDE$_7$ | PBDE | ∑OCP | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Neotropic Cormorant (*Nannopterum brasilianum*)** | | | | | | | | | | | | |
| | USA | 1996 | Alexander island | ng/g wm | ΣPCB$_{18}$ | 5720 | 0.1 | | | | 1364 | Frank et al., 2001 |
| | USA | 1996 | San Bernard Wildlife refuge | ng/g wm | ΣPCB$_{18}$ | 404 | | | | | 493 | Frank et al., 2001 |
| | USA | 1996 | Smith Point | ng/g wm | ΣPCB$_{18}$ | 1640 | 0.01 | | | | 213 | Frank et al., 2001 |
| | USA | 1996 | Vingt-et-un | ng/g wm | ΣPCB$_{18}$ | 3140 | 0.01 | | | | 423 | Frank et al., 2001 |
| **White-breasted Cormorant (*Phalacrocorax lucidus*)** | | | | | | | | | | | | |
| | RSA | 2013 | KwaZulu-Natal | ng/g wm | | | | | | | 600 | Bouwman et al., 2019 |
| **Double-crested Cormorant (*Nannopterum auritum*)** | | | | | | | | | | | | |
| | Canada (5) | 1973 | Mandarte island | ng/kg wm | | | 0.1 | | | | | Harris et al., 2003 |
| | Canada (5) | 1998 | Mandarte island | ng/kg wm | | | 0.04 | | | | | Harris et al., 2003 |
| | Canada (5) | 1987 | Crofton | ng/kg wm | | | 1 | | | | | Harris et al., 2003 |
| | Canada (5) | 1997 | Crofton | ng/kg wm | | | 0.1 | | | | | Harris et al., 2003 |
| | Canada (3) | 1994 | Mandarte island | ng/g wm | | | | | ΣBDE$_9$ | 385 | | Miller et al., 2015 |
| **African Darter (*Anhinga rufa*)** | | | | | | | | | | | | |
| | RSA | 2009 | Barbers Pan | ng/g wm | ΣPCB$_6$ | 7 | 0.3 | 846 | ΣBDE$_5$ | 0.3 | 91 | This study |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 102 | 5 | 2330 | ΣBDE$_5$ | 0.4 | 95 | This study |
| | RSA | 2004/5 | Vaal River | ng/g wm | ΣPCB$_{34}$ | 300 | | | | | 370 | Bouwman et al., 2008 |
| | RSA* | 2004/5 | Parys | ng/g lm | ΣPCB$_{34}$ | 314 | | | ΣBDE$_8$ | 1 | 398 | Polder et al., 2008 |
| | RSA | 2008/9 | Gauteng/ Free state | ng/g wm | ΣPCB$_{34}$ | 310 | | | ΣBFR$_{11}$ | 8 | 590 | Bouwman et al., 2021 |
| | RSA* | 2008/9 | Kempton Park/ Parys | ng/g lm | | | | | ΣBDE$_9$ | 11 | | Quinn et al., 2020 |
| **Great White Egret (*Ardea alba*)** | | | | | | | | | | | | |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 6 | 0.2 | 352 | ΣBDE$_5$ | 0.2 | 423 | This study |
| | Romania* | 1997 | Danube Delta | ng/g dm | ΣPCB$_6$ | 740 | | | | | 4658 | Aurigi et al., 2000 |
| | Hong Kong* | 2006 | A Chau | ng/g lm | ΣPCB$_7$ | 126 | | | | | 1059 | Wang et al., 2011 |
| **Great Egret (*Ardea alba*)** | | | | | | | | | | | | |
| | USA | 1996 | Alexander island | ng/g and pg/g wm | ΣPCB$_{18}$ | 1510 | 0.1 | | | | 379 | Frank et al., 2001 |
| **Little Egret (*Egretta garzetta*)** | | | | | | | | | | | | |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 9 | 1 | 505 | ΣBDE$_5$ | 1 | 19 | This study |
| | Spain | 2006 | Aiguabarreig | ng/g wm | ΣPCB$_7$ | 230 | | | | | 277 | Huertas et al., 2016 |
| | France | 1996 | Rhône delta | ug/g | ΣPCB$_{12}$ | 3305 | | | | | 123 | Berny et al., 2002 |
| | Creece | 2004 | Lake Kerkini | ng/g wm | ΣPCB$_7$ | 18 | | | | | | Antoniadou et al., 2007 |
| | Hong Kong | 2000 | Mai Po Village | ng/g wm | ΣPCB$_7$ | 960 | | | | | 2440 | Connell et al., 2003 |
| | Hong Kong* | 2000 | Mai Po Village | ng/g lm | ΣPCB$_7$ | 288 | | | | | 417 | Wang et al., 2011 |
| | Italy | 1993/4 | Riserva Naturale Garzaia di Villarasc | ppm | ΣPCB$_7$ | 77 | | | | | 249 | Fasola et al., 1998 |
| | China | 2004 | Xiamen | ng/g wm | | | | 70 | | | | Wang et al., 2008 |
| | RSA | 2013 | KZN | ng/g wm | | | | | | | 500 | Bouwman et al., 2019 |
| | Romania* (4) | 1997 | Danube Delta | ng/g dm | ΣPCB$_6$ | 546 | | | | | 12448 | Aurigi et al., 2000 |
| | Israel | 1975 | Coastal plain | ppm | ΣPCB$_7$ | 540 | | | | | 1610 | Perry et al., 1990 |
| | Greece* | 2004 | Lake Kerkini | ng/g lm | | | | | | | 103 | Goutner et al., 2012 |
| **Cattle Egret (*Bubulcus ibis*)** | | | | | | | | | | | | |
| | RSA | 2009 | Potchefstroom | ng/g wm | ΣPCB$_6$ | 7 | 0.5 | 7 | ΣBDE$_5$ | 0.2 | 20 | This study |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 2 | 0.3 | 579 | ΣBDE$_5$ | 0 | 28 | This study |
| | RSA* | 2009 | Elim | ng/g lm | ΣPCB$_{20}$ | 9 | | | | | 26 | Bouwman et al., 2013 |
| | RSA* | 2009 | Tshakhuma Dam | ng/g lm | ΣPCB$_{20}$ | 5 | | | | | 104 | Bouwman et al., 2013 |
| | RSA* | 2009 | Xikundu dam | ng/g lm | ΣPCB$_{20}$ | 6 | | | | | 307 | Bouwman et al., 2013 |
| | RSA | 2004/5 | Baberspan | ng/g wm | ΣPCB$_{34}$ | 4 | | | | | 28 | Bouwman et al., 2008 |
| | RSA | 2004/5 | Vaal River | ng/g wm | ΣPCB$_{34}$ | 8 | | | | | 28 | Bouwman et al., 2008 |
| | RSA* | 2004/5 | Barberspan | ng/g lm | ΣPCB$_{34}$ | 3 | | | ΣBDE$_8$ | 0.1 | 23 | Polder et al., 2008 |
| | RSA* | 2004/5 | Parys | ng/g lm | ΣPCB$_{34}$ | 8 | | | ΣBDE$_8$ | 0.2 | 30 | Polder et al., 2008 |
| | RSA | 2008/9 | Gauteng/ Free state | ng/g wm | ΣPCB$_{34}$ | 16 | | | ΣBFR$_{11}$ | 4 | 21 | Bouwman et al., 2021 |
| | Spain | 2006 | Aiguabarreig | ng/g wm | ΣPCB$_7$ | 51 | | | | | 49 | Huertas et al., 2016 |
| | China* | 2000 | Tai Lake | ng/g dm | | | | | | | 56 | Dong et al., 2003 |
| | Israel | 1975 | Coastal plain | ppm | | | | | | | 620 | Perry et al., 1990 |
| | Hong Kong | 2000 | Mai Po Village | pg/g lm | | | 0.04 | | | | | Wang et al., 2012 |
| | RSA* | 2008/9 | Soweto/Parys/Sasolburg | ng/g lm | | | | | ΣBDE$_9$ | 5 | | Quinn et al., 2020 |
| **African Sacred Ibis (*Threskiornis aethiopicus*)** | | | | | | | | | | | | |
| | RSA | 2009 | Eldorado Park | ng/g wm | ΣPCB$_6$ | 35 | 7 | 69 | ΣBDE$_5$ | 20 | 156 | This study |
| | RSA | 2009 | Bloemhof Dam | ng/g wm | ΣPCB$_6$ | 3 | 0.1 | 17 | ΣBDE$_5$ | 0.4 | 74 | This study |
| | RSA | 2004/5 | Vaal River | ng/g wm | ΣPCB$_{34}$ | 59 | | | | | 94 | Bouwman et al., 2008 |
| | RSA* | 2004/5 | Parys | ng/g lm | ΣPCB$_{34}$ | 65 | | | ΣBDE$_8$ | 14 | 91 | Polder et al., 2008 |
| | RSA | 2008/9 | Gauteng/ Free state | ng/g wm | ΣPCB$_{34}$ | 59 | | | ΣBFR$_{11}$ | 53 | 56 | Bouwman et al., 2021 |
| | RSA* | 2008/9 | Soweto | ng/g lm | | | | | ΣBDE$_9$ | 54 | | Quinn et al., 2020 |
| **Glossy Ibis (*Plegadis falcinellus*)** | | | | | | | | | | | | |
| | RSA | 2009 | Potchefstroom | ng/g wm | ΣPCB$_6$ | 7 | 1 | 5 | ΣBDE$_5$ | 1 | 61 | This study |
| | Romania* | 1997 | Danube Delta | ng/g dm | ΣPCB$_6$ | 154 | | | | | 939 | Aurigi et al., 2000 |

All values reported from studies other than the current study, were reported in a concentration unit other than ng/kg and had to be converted.
* Values had to be converted from dry mass or lipid mass to wet mass
(1) Concentration was expressed as the median and not the mean
(2) Data from more than one location or site was reported, but only the location or site with the highest concentration was used in this table.
(3) Data from more than one yearly sample run was reported, but only the year with the highest concentration was used in this table.
(4) Concentration is of the egg yolk only
(5) Data from more than one year were reported, only selected data was used
# The sum concentration of DL-PCBs and NDL-PCBs as reported by authers

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This study was done under the auspices of ORASECOM in association with the UNDP and funded by the GEF via UNOPS. We thank Christof Mor for his kind assistance throughout the project. Mr Carlos Huisamen for the collection of bird eggs and the whole POPT group for assisting with sample preparation. Horst Rottler from Oekometrics is thanked for his inputs and efforts. We also thank Ian Smith, Claudine Roos, Laura Quinn, and Karin Blom.

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# Dioxins, PFOS, and 20 other persistent organic pollutants in nine species of wild bird eggs from the Vaal River, South Africa

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## Supplemental materials

Table S1: General distribution and description, habitat preference, breeding behaviour, diet and egg description of bird species investigated during this study.

| **Species name** | **Distribution** | **Habitat and breeding** | **General Description** | **Foraging and Diet** | **Egg description** | **Egg mass** | **Mean egg dimensions** | **Depiction of species** Images by Dr W. Tarboton |
|---|---|---|---|---|---|---|---|---|
| Grey Heron (GH) *Ardea cinerea* | Europe, Africa, Asia, East indies islands | Aquatic. Usually located near water. Colonial nester in tall tree patches. | Large heron having white and black accents, a white crown with black plumes, black belly, and white thighs. | Large aquatic predator. Hunts fish in solitary and feed at all times during the day. | Oval, slightly pointed at both ends. Pale blue eggs | 61 g | 57-61 x 41-43 |  |
| African Darter (AD) *Anhinga rufa* | Africa, Madagascar, and Iraq | Aquatic. Colonial, nests in trees near water | Dark coloured bird with a thin white lateral neck stripe, pointed bill | Large aquatic predator. Dives to actively catch fish. Will also consume frogs and molluscs. | Eggs are elongated, white in colour and normally smooth. | 37g | 53 x 35 mm |  |
| Black-headed heron (BBH) *Ardea melanocephala* | Africa | Terrestrial. Breeds in tall trees or reed beds in mixed species colonies. | Medium sized bird with a dark grey, black and white huge. | Small aquatic predator. Solitary feeders, primarily insectivore, but do occasionally feed on reptiles, fish and amphibians. | Oval, pale blue eggs | 60g | 60 x 43 mm |  |
| Great White Egret (GWE) *Ardea alba* | Located in the Americas , eastern Europe, Africa and northern Asia | Aquatic. Breeds near water between vegetation. | Large slender white bird with a long neck, dark legs and long black plumes when breeding | Large aquatic predator. Ambushes pray by impaling its long sharp bill into prey. | Smooth, pale greenish blue. | 61 g | 56-61 x 40-43 mm |  |
| Reed Cormorant (RC) *Microcarbo africanus* | Africa | Aquatic. Colonial, breeds near water in trees. Also on bare rock islands. | Small barred-backed black bird with short legs and a short dark crest above its bill. | Large aquatic predator. Hunts by propelling its feed in the water. | Eggs are a chalky blue colour | 21 g | 44 x 29 mm |  |
| African Sacred Ibis (ASI) *Threskiornis aethiopicus* | Africa | Wetland. Colonial, nests in trees near water. | All white body with a black bald head and neck, thick curved bill and legs. | Scavenger. Feeds on many organisms and will feed on other food sources not utilize by other species. | White with red/brown spots | 62 g | 66 x 44 mm |  |
| Little Egret (LE) *Egretta garzetta* | Europe, Africa, Madagascar, Asia, east indies, Australia, pacific ocean island, and west indies | Aquatic. Colonial breeder that nest in trees, bushes and reed beds | Small white heron with a slender black beak, long black legs and yellow feet. | Small aquatic predator. Hunts solitarily and feed on lower aquatic invertebrates. Will occasionally hunt fish. | Oval shaped blueish green white eggs | 28 g | 47-34 mm |  |
| Cattle Egret (CE) *Bubulcus ibis* | Worldwide distribution , found on all continents except Antarctica | Terrestrial. Colonial, breeds near water in trees and reed beds. | Medium sized bird with white feathers and a short yellow bill | Terrestrial insectivore. Feeds along large mammals or in groups usually during the day | Oval shaped blueish white eggs | 27 g | 45 x 34 mm |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Glossy Ibis (GI) *Plegadis falcinellus* | Americas, Europe, Asia, Africa and Australia | Wetland. Nests in trees and other vegetation's near water | Medium sized ibis with reddish-brown feathers and shiny bottle-green wing feathers. Brownish bill | Small aquatic predator. Primarily feeds on insects but also consume other small organisms. | Bright blue/green eggs | 34 g | 47-58 x 33-43 mm |  |

Table S2: Concentrations expressed as ng/kg wm of all individual congeners in pooled bird eggs samples.

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Polybrominated diphenyl ethers und biphenyls (PBDE/PBB)** | | | | | | | | | | | | | | | | |
| **Sample name** | **Pool 1** | **Pool 2** | **Pool 3** | **Pool 4** | **Pool 5** | **Pool 6** | **Pool 7** | **Pool 8** | **Pool 9** | **Pool 10** | **Pool 11** | **Pool 12** | **Pool 13** | **Pool 14** | **Pool 15** | **Pool 16** |
| **TetraBDE** | 547 | 158 | 132 | 914 | < 57 | < 50 | 1240 | < 50 | 181 | < 60 | < 57 | < 54 | < 54 | < 55 | < 55 | < 57 |
| **PentaBDE** | 260 | < 50 | < 65 | 4140 | 160 | 117 | 3340 | < 50 | 362 | < 80 | < 57 | < 54 | < 54 | < 83 | < 55 | < 57 |
| **HexaBDE** | 638 | < 50 | 315 | 6220 | 173 | 239 | 1790 | 234 | 357 | 197 | 776 | 427 | 1550 | 215 | 154 | < 57 |
| **HeptaBDE** | 303 | < 50 | < 76 | 8270 | < 57 | < 50 | 101 | < 50 | 220 | 234 | 498 | 341 | 1710 | 248 | < 62 | < 57 |
| **HexaBB** | < 100 | < 100 | < 100 | 529 | < 100 | < 100 | < 100 | < 100 | < 100 | < 100 | < 100 | 118 | < 100 | < 100 | < 100 | < 100 |
| **Indicator-polychlorinated biphenyls (PCB)** | | | | | | | | | | | | | | | | |
| **PCB 28** | 222 | 187 | 152 | 1790 | 197 | 1410 | 1140 | 436 | 169 | 117 | 228 | 72 | 410 | 413 | 149 | 152 |
| **PCB 52** | < 50 | 59 | < 50 | 64 | < 50 | 112 | 70 | < 50 | < 50 | < 50 | 99 | < 50 | < 50 | 71 | < 50 | < 50 |
| **PCB 101** | < 50 | 112 | < 50 | 223 | < 50 | 524 | 231 | 137 | 87 | < 50 | 191 | < 50 | 71 | 128 | 64 | < 50 |
| **PCB 138** | 1880 | 1430 | 2150 | 8510 | 1250 | 18100 | 12500 | 4640 | 1940 | 630 | 1970 | 334 | 5530 | 3010 | 1000 | 410 |
| **PCB 153** | 3830 | 2240 | 4430 | 12700 | 3060 | 44600 | 20500 | 9170 | 2460 | 913 | 3800 | 813 | 19900 | 6200 | 2990 | 719 |
| **PCB 180** | 2150 | 963 | 1960 | 9340 | 1570 | 22900 | 10500 | 3820 | 1390 | 641 | 1600 | 567 | 17000 | 4230 | 2020 | 359 |
| **Polychlorinated biphenyls (PCB) according to WHO** | | | | | | | | | | | | | | | | |
| **PCB 77** | < 3 | < 4 | < 4 | < 3 | < 4 | 65 | < 3 | < 3 | < 3 | < 3 | 6.2 | < 3 | < 3 | 4.7 | < 3 | < 3 |
| **PCB 81** | 1.4 | 0.9 | 1.2 | 4.3 | 1.4 | 16.5 | 7 | 3.7 | 1.2 | < 0.5 | 1.4 | < 0.5 | 3.5 | 2.7 | 1 | < 0.5 |
| **PCB 126** | 12 | 5.8 | 14.1 | 19.5 | 8.1 | 108 | 43.4 | 20.1 | 6.6 | 2.5 | 9.4 | 3.6 | 61.3 | 22.4 | 12.4 | 2.5 |
| **PCB 169** | 4.9 | 1.6 | 3.4 | 3.8 | 2.3 | 24.6 | 5.4 | 8.2 | 1.7 | < 0.5 | 2.8 | 1.5 | 21.4 | 15.9 | 3.3 | 1.1 |
| **PCB 105** | 124 | 169 | 184 | 423 | 168 | 1870 | 2060 | 717 | 237 | 52 | 238 | 31 | 271 | 262 | 97 | 42 |
| **PCB 114** | 7 | 14 | 14 | 36 | 16 | 162 | 90 | 35 | 16 | 6 | 10 | < 5 | 31 | 16 | 10 | < 5 |
| **PCB 118** | 504 | 566 | 913 | 1150 | 479 | 8060 | 4560 | 2100 | 361 | 161 | 847 | 85 | 1770 | 585 | 402 | 156 |
| **PCB 123** | < 5 | < 5 | < 5 | 6 | < 5 | 33 | 15 | 8 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 |
| **PCB 156** | 221 | 115 | 187 | 553 | 205 | 2010 | 1150 | 550 | 138 | 60 | 176 | 46 | 1070 | 413 | 161 | 27 |
| **PCB 157** | 22 | 22 | 25 | 89 | 29 | 384 | 247 | 95 | 33 | 8 | 34 | 7 | 128 | 62 | 36 | 7 |
| **PCB 167** | 133 | 65 | 131 | 243 | 82 | 1110 | 535 | 276 | 100 | 23 | 92 | 17 | 648 | 217 | 89 | 17 |
| **PCB 189** | 47 | 14 | 24 | 155 | 20 | 273 | 138 | 48 | 27 | 9 | 26 | 12 | 384 | 78 | 34 | 10 |
| **Polychlorierte Dibenzo-p-dioxine und Dibenzofurane (PCDD/F)** | | | | | | | | | | | | | | | | |
| **2,3,7,8-TCDD** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.2 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.15 | 0.11 | < 0.05 | < 0.05 |
| **1,2,3,7,8-PeCDD** | 0.11 | 0.08 | 0.06 | 0.07 | 0.07 | 0.87 | 0.14 | 0.23 | 0.07 | < 0.05 | 0.14 | < 0.05 | 0.53 | 0.73 | 0.08 | < 0.05 |
| **1,2,3,4,7,8-HxCDD** | < 0.05 | < 0.05 | < 0.05 | 0.09 | < 0.05 | 0.13 | 0.07 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.33 | 0.14 | < 0.05 | < 0.05 |
| **1,2,3,6,7,8-HxCDD** | 0.33 | 0.11 | 0.1 | 0.58 | 0.11 | 1.27 | 0.21 | 0.28 | 0.34 | 0.13 | 0.31 | 0.13 | 0.87 | 2.17 | 0.25 | 0.24 |
| **1,2,3,7,8,9-HxCDD** | < 0.05 | < 0.05 | < 0.05 | 0.12 | < 0.05 | 0.36 | < 0.05 | 0.08 | 0.24 | < 0.05 | 0.14 | < 0.05 | 0.11 | 0.38 | 0.07 | 0.09 |
| **1,2,3,4,6,7,8-HpCDD** | < 0.15 | < 0.15 | < 0.15 | 1.82 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | 0.33 | < 0.15 | 0.51 | < 0.15 | 0.39 | 1.36 | < 0.15 | < 0.15 |
| **OCDD** | < 0.5 | < 0.5 | < 0.5 | 2.83 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 2.4 | < 0.5 | < 0.5 |
| **2,3,7,8-TCDF** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.26 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **1,2,3,7,8-PeCDF** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.17 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.09 | < 0.05 | < 0.05 |
| **2,3,4,7,8-PeCDF** | 0.09 | < 0.05 | 0.07 | 0.36 | 0.1 | 0.95 | 0.2 | 0.12 | 0.23 | < 0.05 | < 0.05 | 0.1 | 1.94 | 0.72 | 0.13 | < 0.05 |
| **1,2,3,4,7,8-HxCDF** | < 0.05 | < 0.05 | < 0.05 | 0.27 | < 0.05 | 0.15 | 0.1 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.46 | 0.29 | < 0.05 | < 0.05 |
| **1,2,3,6,7,8-HxCDF** | < 0.05 | < 0.05 | < 0.05 | 0.22 | < 0.05 | 0.16 | 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.46 | 0.31 | < 0.05 | < 0.05 |
| **1,2,3,7,8,9-HxCDF** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **2,3,4,6,7,8-HxCDF** | < 0.05 | < 0.05 | < 0.05 | 0.48 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.42 | 0.27 | < 0.05 | < 0.05 |
| **1,2,3,4,6,7,8-HpCDF** | < 0.15 | < 0.15 | < 0.15 | 0.27 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 |
| **1,2,3,4,7,8,9-HpCDF** | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 | < 0.15 |
| **OCDF** | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 2 | < 0.5 |

## Chapter 4

# The first countrywide survey of dioxins (PCDDs), furans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) in South African sediment

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## Abstract

Dioxin-like polychlorinated biphenyls (DL-PCBs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) were quantified in sediment collected in 2002 from 22 sites across South Africa. Concentrations quantified in sediment ranged between 41–25000 ng/kg dry mass (dm) DL-PCB; 6–1100 ng/kg dm PCDD/F for industry, 22–37 ng/kg dm DL-PCB; 4–22 ng/kg dm PCDD/F, for agriculture, and 18–35 ng/kg dm DL-PCB; 3–8 ng/kg dm PCDD/F) for harbour or estuaries. The highest sum of toxic equivalency quotient (ΣTEQ) of 22 ngTEQ/kg dm was quantified from Riet Spruit channel, a location in the heart of the highly industrialised Gauteng province. Sediment collected from sites downstream or close to industrial activities had the highest DL-PCB and PCDD/F concentrations. Comparisons with subsequent data shows that POP concentrations at some locations have decreased while others have increased. This might be due to sampling sites being closely located but not identical, but more research in needed to assess temporal trends. The ∑TEQ concentrations from seven sites close to industrial activities exceeded international sediment quality guidelines, requiring close attention and possible intervention.

**Keywords**: Harbour, industry, soil, polychlorinated biphenyls, clay, silt, sand, Stockholm Convention

## 1. Introduction

Polychlorinated dibenzo-*p*-dioxin and dibenzofurans (PCDD/Fs) are unintended by-products of industrial processes and combustion of which incineration is the major source (Cheruiyot et al., 2016; Fiedler, 2007; Fiedler, 1996). Intentially produced dioxin-like polychlorinated biphenyls (DL-PCBs) gained notoriety as a ubiquitous contaminant mainly due to its use as transformer fluid and in capacitors and as hydraulic oil and in rubberised paints, glues, and plastics (Newman, 2015). The historic production of DL-PCBs primarily occurred in countries such as the United States of America, Europe, and Russia, where they were produced as mixtures of congeners (Newman, 2015). The use and production of DL-PCBs has drastically decreased. Remaining products mainly occur in old electrical equipment and in waste dumps. AlthoughDL-PCBs have not been produced in Africa, these compounds have been reported in air, water, sediment, and biota (Gioia et al., 2014; Ssebugere et al., 2019; White et al., 2020). African countries import large quantities of old electrical equipment that might contain such compounds (Gioia et al., 2014). Furthermore, it is not simply the importation of electronical goods that contribute to the DL-PCB concentrations, but also their fate. Illegal recycling and e-waste incineration, oil leaching from deconstructed transformers, and burning of electronic goods to recover metals are just some of the processes contributing to DL-PCB pollution in Africa (Gioia et al., 2011; Menad et al., 1998; Sepúlveda et al., 2010).

Dioxin-like PCBs and PCDD/Fs are environmentally persistent, can be found in most environmental matrixes, bioaccumulate, and can lead to severe health effects. Due to these reasons, DL-PCBs and PCDD/Fs form part of the 12 original persistent organic pollutant classes (POP), listed in Stockholm Convention of Persistent Organic Pollutants (SCPOPs) collectively referred to as the ‘dirty dozen’.

PCDD/Fs and DL-PCBs are multimedia pollutants—once released in the environment they distribute between environmental compartments. For instance, PCDD/Fs and DL-PCBs are primarily bound to particulate and organic matter in soil, sediment and air. They adsorb strongly to organic matter from whence they can either be ingested by fauna and move between trophic levels or settle and accumulate in sediments. In animals, POPs accumulate in tissues, mostly in proportion to the percentage of lipids (Newman, 2015). Because of their chemical characteristics and low solubility, these compounds accumulate in most soil types with little leaching and negligible degradation of the 2,3,7,8-substituted PCDD/F-congeners. The 2,3,7,8-substituted PCDD/Fs also strongly accumulate in the livers of mammals and birds (Pirard and De Pauw., 2006; Senthilkumar et al., 2002). Dioxin-like PCBs and PCDD/Fs are non-polar compounds that cannot be excreted or transformed to polar excretable compounds without the introduction of a polar functional group through metabolism. None of these compounds are metabolised to a significant degree by invertebrates, and therefore they bio-magnify in higher trophic species (Thomann, 1989; Gobas, 1993).

Elevated concentrations of POPs can lead to severe effects such as impaired reproduction and development, immunosuppression, and cancer (Jones and De Voogt., 1999; Johnson et al., 2013; Newman, 2015). All of these effects are considered ecologically significant because they have the potential of ultimately reducing populations of fish, birds, invertebrates and mammals exposed to POPs (Sonne et al., 2020; Vasseur and Cossu-Leguille, 2006). Wild bird egg monitoring studies near a pulp mill in Canada have shown a decline in PCDD/F and DL-PCB concentrations with time in bird eggs due to release interventions (Harris et al., 2003).

Accidental dioxin exposure incidents such as the Yusho and Yusheng rice bran oil indecent from Japan and Taiwan and the “Seveso incident” in Italy demonstrated the severe threat PCDD/Fs pose to human health (Fiedler, 1996; Ikeda, 1996; Yoshimura, 2003). Among organochlorine compounds tested, DL-PCBs and dioxins are the most potent immune-toxicants (active at low doses, causing a range of effects and multi-species toxicities) (Trizio et al., 1988). In addition, many of the identified POPs are known endocrine disruptors (Diamanti-Kandarakis et al., 2009). Hyperthyroidism in rodents has been observed after exposure to DL-PCBs (Goldey et al., 1995).

The multiple sources of DL-PCBs and PCDD/Fs and their potential to cause adverse effects at elevated concentrations emphasise the need to monitor these compounds in biota and the environment. Sediment acts as a sink of compounds released from nearby sources. This makes sediment a good abiotic matrix to use to monitor POP pollution. The literature available on DL-PCBs and PCDD/F concentrations in sediment from Africa is relatively few (Barakat et al., 2013; Gioia et al., 2014; Omwoma et al., 2015: Quinn et al., 2009) compared with other continents. Furthermore, PCDD/Fs have been neglected in research from Africa and should receive more interest (Olisah et al., 2022). The earliest published data for DL-PCB and PCDD/F concentrations in sediment from South Africa was by Nieuwoudt et al. (2009), but there has not been a country-wide survey.

Therefore, the aim of this study is to report DL-PCB and PCDD/F concentrations in sediment collected in 2002 from across South Africa. To the best of our knowledge, this is the only study designed to collect a country-wide dataset on DL-PCB s and PCDD/F in sediments from Africa. This dataset allows an assessment of temporal trends based on data published since 2002. We expect higher DL-PCB and PCDD/F concentrations in industrial regions and lower concentrations in remote agriculture regions. Due to industrial and population growth we expect the DL-PCB and PCDD/F concentrations to have increased since 2002.

## 2. Materials and methods

### 2.1 Study area, site selection and site classification

Twenty-two sites were selected to represent areas with potentially high and low DL-PCBs and PCDD/F concentrations in South Africa (Figure 1). Sites were selected based on proximity to or downstream of areas regarded as suspected sources of PCDD/Fs and DL-PCBs. The 22 sites selected for this study were categorised into harbour/ estuaries (3), industry (13), and agriculture (6).

The Vaal River catchment drains the large industrial region of South Africa before the confluence with the Orange-Senqu River (Figure 1). Sites 15, 16, and 17 were sampled to represent a large industrialized region of Gauteng that is adjacent to the Vaal River. Site 16 is a drainage channel running close to an iron and steel refinery before joining up with the Riet Spruit (Site 15) that feeds into Loch Vaal (Site 17). The Vaal Dam (Site 14) is approximately 70 km upstream of Loch Vaal (Site 17) and represents the Vaal River prior to the influence of large industries. The Vaal Dam receives runoff water from agricultural regions. Site 7 (Vaal River) near Douglas was selected to investigate the fate of expected pollution from Gauteng, approximately 450 km downstream of Gauteng. Site 1 (Orange-Senqu River estuary) was selected to investigate the long-range transport of pollutants and the influence of diamond mining and agriculture along the lower Orange-Senqu River but has no specific pollution source. The Orange-Senqu River was placed in the harbour/ estuary category. Modderfontein

Spruit (Site 22) was selected due to its proximity to industry, but is also surrounded by urban communities. Hartebeespoort Dam (Site 21) receives water from the rivers such as the Jukskei and Hennops rivers that drain urban and industrial regions.

Two sites along the Olifants River were selected. Site 20 (Loskop Dam) is mainly used for irrigation. Site 19 (Olifants River) drains a large area known for coal mining and electricity generation. The Crocodile (East) River (Site 18), in Mpumalanga was sampled in Mbombela. Possible pollution sources are pesticides use and a paper mill. This site was placed in the industry category rather than the agriculture category.

Umlazi (Site 10) and Umgeni (Site 11) estuaries were selected to represent Durban, a highly industrialised city in KwaZulu-Natal (KZN; Figure 1). The Umlazi River (Site 10) drains industrial and low-income urban areas and is located next to a paper mill. The Umgeni (Site 11) River drains industrial Durban areas. Even though the samples were collected in the estuaries, the sites were placed in the industry category due to immediate proximity. The Buffalo River (Site 8), and Mooi River (Site 9) in KZN are influenced by agriculture. The Swartkops estuary (Site 6) in the Eastern Cape drains industrial and low income housing regions similar to the Umgeni and Umlazi estuaries and were also subsequently placed in the industry category.

In Richard's Bay, a coastal city known for coal exports, chemical manufacturing, and other industrial processes were sampled. Both the harbour (Site 12) and a freshwater pan, Thulazihleka Pan (Site 13) next to a fertiliser plant, were sampled. Site 13 was placed into the industry category, while the harbour were categorised as such. Saldanha Bay harbour (Site 2) is known for steel manufacturing and the shipment thereof. However, this site although strictly speaking influenced by industry was placed in the harbour category. The Groot River estuary (Site 5) in the Western Cape was sampled. Possible pollution sources include plantation forests and wood preservation and process facilities and were thus rather included in the agriculture category. In addition, Berg River (Site 3) was also sampled due to possible pesticide pollution derived from agriculture. The Theewaterskloof Dam (Site 4) close to Cape Town is suspected of pesticide pollution and was added on the list because it is the main water source for the city. The GPS coordinates, sample date and site classifications of all 22 sites are listed in Table S1 in supplementary material.

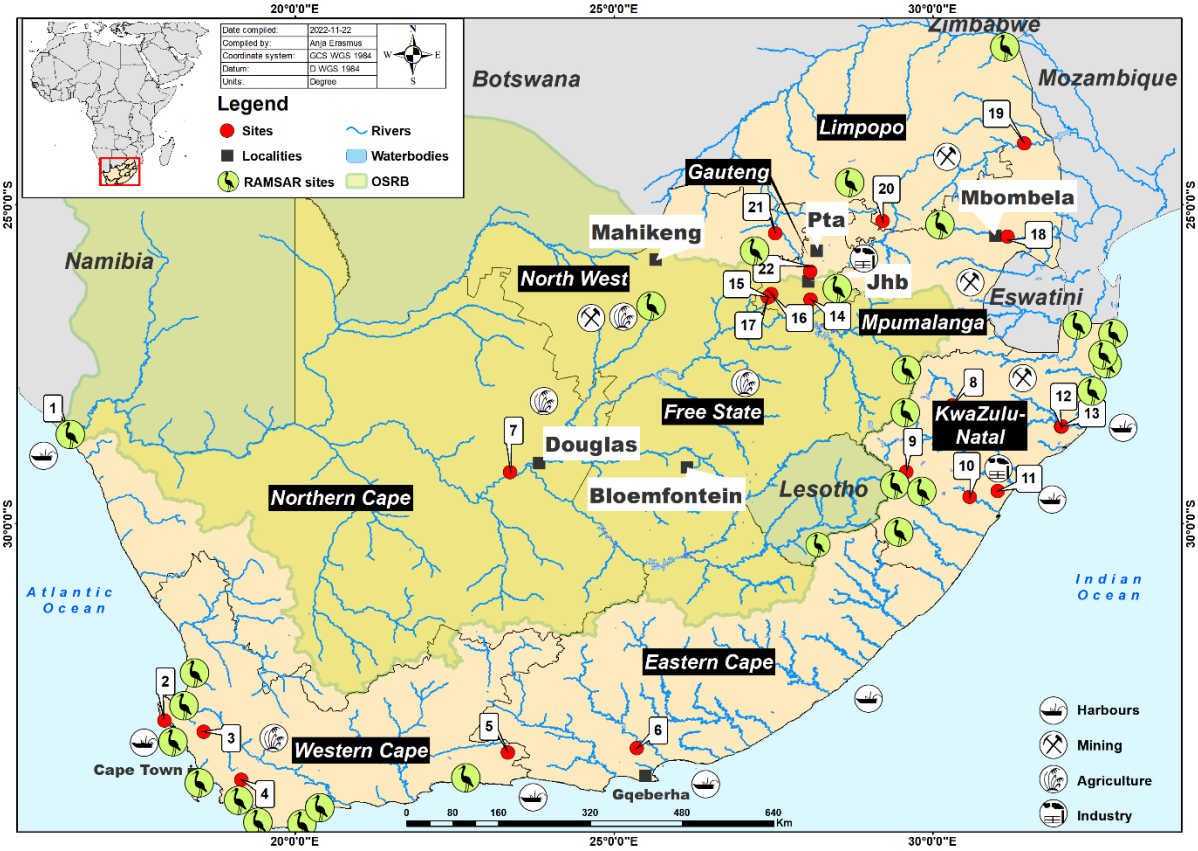


**Figure 1**: Map of south Africa with numbered sediment selection sites indicated.

### 2.2 Sediment collection and preparation

Sediment was collected in 2002 (Table S1) after the rainy season. All sampling equipment were cleaned before and after use to prevent potential cross-contamination. Samples were collected from the surface sediment (0-5 cm deep) of the river/dam/harbour with a brass grab sampler. At each site, five different locations were sampled, and 120 mL sediment of each was combined to provide a pooled sediment sample for each site. Samples were kept frozen to prevent bacterial degradation and protected from UV light by using dark coloured, pre-cleaned glass bottles, with a foil-lined cap until further processing.

The sediment samples were freeze-dried before small twigs and stones were manually removed and the sediment grounded to increase surface area for extraction. Thereafter, a 30 g sub-sample of each site was sent to an internationally accredited laboratory in Germany (Oëkometric GmbH- The Bayreuth Institute of Environmental Research in Bayreuth) for analyses.

### 2.3 Chemical and analytical analy ses

The analyses were done for 12 DL-PCBs and 17 PCDD/Fs (Table 1) listed in the Stockholm Convention (2016) at the time (2002). The analytical approaches and parameters are listed in Table 1. Concentrations are quantified based on dry mass (dm). Quality assurance and quality control protocols as per ISO/IEC 17025:2005 accreditation were included during the analyses of the DL-PCBs and PCDD/Fs. The oxidisable organic content (%OC) of each of the samples was determined using the Walkley-Black method (Schumacher, 2002). The %OC was then used to calculate the percentage total organic content (%TOC) (Sánchez-Monedero et al

1996) which was then used to calculate normalised toxic equivalency quotient (TEQ)-values (Table S2). The TEQ concentration were calculated by multiplying the concentration of each compound with the corresponding toxic equivalency factor (TEF) using the $WHO_{2005}$-TEFs for mammals (van den Berg et al., 2006). The TEQ concentrations are reported as limit of quantification inclusive.

Statistics were done using GraphPad Prism version 9.0.2 for summary and comparative statistics (ANOVA, 2-way ANOVA, and linear regressions) using log-transformed data where data were not normally distributed**.** Multivariate analyses (MjM Software PC-ORD version 6.07; www.pcord.com) was used to visualise the compositional relationships between DL-PCB and PCDD/F congeners, sediment characteristics and site classifications using non-metric multidimensionally scaled (NMS), ordinations of concentration data relativised to a sum of 1, per site. Gower-ignore 0 was the distance measure. The starting condition was a random number; six axes were initially allowed.

**Table 1:** List of compounds, analytical approaches and parameters.

| **Dioxin-like polychlorinated biphenyls** | **Polychlorinated dibenzo-*p*-dioxins and dibenzo-*p*-furans** | |
|---|---|---|
| PCB 77<br>PCB 81<br>PCB 126<br>PCB 169<br>PCB 105<br>PCB 114<br>PCB 118<br>PCB 123<br>PCB 156<br>PCB 157<br>PCB 167<br>PCB 189 | 2,3,7,8-TCDD<br>1,2,3,7,8-PeCDD<br>1,2,3,4,7,8-HxCDD<br>1,2,3,6,7,8-HxCDD<br>1,2,3,7,8,9-HxCDD<br>1,2,3,4,6,7,8-HpCDD<br>OCDD | 2,3,7,8-TCDF<br>1,2,3,7,8-PeCDF<br>2,3,4,7,8-PeCDF<br>1,2,3,4,7,8-HxCDF<br>1,2,3,6,7,8-HxCDF<br>1,2,3,7,8,9-HxCDF<br>2,3,4,6,7,8-HxCDF<br>1,2,3,4,6,7,8-HpCDF<br>1,2,3,4,7,8,9-HpCDF<br>OCDF |
| **Accredited analytical approaches** | | |
| DIN 38414-S20<br>EPA 1613 B<br>E-EN 1948-4 with High resolution Gas Chromatography–Mass Spectrometry (GC/MS) | DIN 38414-S24<br>EPA 1613 B<br>EN 1948-3 with High resolution GC/MS | |
| **Sensitivity parameters** | | |
| PCB 81, 126, 169 = 0,1 ng/kg dry mass (dm)<br>PCB 77, 105 = 3 ng/kg dm<br>PCB 114, 123, 156, 157, 167 = 1 ng/kg dm<br>PCB 118 = 10 ng/kg dm<br>PCB 189 = 50 ng/kg dm. | TCDD/F – HxCDD/F = 0,05 ng/kg dm<br>HpCDD/F = 0,15 ng/kg dm<br>OCDD/F = 0,5 ng/kg dm | |

## 3. Results

### 3.1 Concentrations

Dioxin-like PCBs and PCDD/Fs were quantifiable at all sites. The concentrations of individual PCDD/F and DL-PCB congeners, including TEQs, sediment particle size, and total organic carbon (%TOC) are presented in Table 2.

3.1.1 Industry

The total concentrations of the 13 industrial sites ranged between 41–25 000 ng/kg dm for ΣDL-PCB, 6–1100 ng/g dm for ΣPCDD/F, and 0.2–22 ngTEQ/kg dm for ΣTEQ (Table 2). The highest ΣDL-PCB (25 000 ng/kg dm), ΣPCDD/F (1100 ng/kg dm), ΣTEQ (10 ngTEQ/kg dm ΣDL-PCB and 12 ngTEQ/kg dm ΣPCDD/F = 22 ngTEQ/kg dm ΣTEQ), and normalized TEQ (1.9 ngTEQ/kg normalised to 1%TOC) concentrations were quantified in sediment from Riet Spruit channel that is close to an iron and steel refinery in Vanderbijlpark, Gauteng (Table 2). Another highly industrialised site in Gauteng, the Modderfontein Spruit (Site 22), had a ΣTEQ of 6 ng/kg dm, ΣPCDD/F concentration of 540 ng/kg dm, ΣDL-PCB concentration of 1500 ng/kg dm, and a normalised TEQ of 1.6 ngTEQ/kg dm (Table 2). Loch Vaal (Site 17) and Crocodile River (Site 18) had ΣTEQ concentrations of 3 ngTEQ/kg dm, ΣDL-PCB concentrations of 1400 and 1100 ng/kg dm and ΣPCDD/F concentrations of 220 and 230 ng/kg dm respectively. The lowest ΣTEQ (0.2 ngTEQ/kg dm) was quantified at Site 7 in the Vaal River near Douglas, while the Olifants River (Site 19) had the lowest quantified ΣDL-PCB (41 ng/kg dm) and ΣPCDD/F (6 ng/kg dm) concentrations from industrial sites (Table 2).

3.1.2 Harbours and estuaries

The highest normalised ΣTEQ concentration was quantified at Saldanha Bay (Site 2; 0.7 ngTEQ/kg dm; Table 2). The second highest ΣDL-PCB (28 ng/kg dm), ΣPCDD/F (4 ng/kg dm), and normalised ΣTEQ were quantified in sediment from the Orange-Senqu River estuary (Site 1: Table 2). The ΣTEQs were similar between all sites in this group.

3.1.3 Agriculture

The Mooi River (Site 9) had the highest ΣDL-PCB concentration (36 ng/kg dm), while Theewaterskloof Dam had the highest ΣPCDD/F concentration (22 ng/kg dm). The Vaal Dam (Site 14) had the lowest ΣDL-PCB (22 ng/kg dm) and ΣPCDD/F (4 ng/kg dm) concentrations (Table 2). However, the highest normalised ΣTEQ was at Vaal Dam (0.7 ngTEQ/kg dm). The Groot River (Site 5) had the second highest ΣDL-PCB (35 ng/kg dm), but a low ΣPCDD/F (8 ng/kg dm) and normalised ΣTEQ concentration (0.09 ngTEQ/kg dm; Table 2).

### 3.2 Sediment characteristics and congeners

The ΣTEQ normalised to 1% concentrations and percentage oxidised carbon (%OC) are presented in Table 2. The empty cells in the %C row are due to a TEQ-value for the site without quantifiable oxidised carbon. This means that even in the apparent absence of carbon, dioxin-like substances were present.

In Figure 2A, the TEQ contributions by the intentionally produced DL-PCB s are presented separately from the TEQ-concentrations of the unintentionally produced PCDD/Fs. Except for the Crocodile River (Site 18), the contribution of the PCDD/Fs were statistically significant higher than DL-PCB s at all the sites ($p = 0.0001$; Wilcoxon matched-pairs, signed rank test). In Figure 2B, the concentrations of DL-PCB are presented separately from the PCDD/F

concentrations ($p < 0.0001$; Wilcoxon matched-pairs, signed rank test). In Figure 2C the DL-PCB, PCDD, and PCDF concentrations are presented stacked within the bars. PCDD concentrations were higher than the furans. The log-transformed concentrations of DL-PCB, PCDD, and PCDF are illustrated in Figure 2D. A statistically significant difference was found between log concentrations (Two-way ANOVA, $p < 0.0001$).

The associations between TEQ and the %OC and the %TOC content are presented in Figures 3A-B. There were statistically significant linear regressions between %OC and ΣTEQ ($p = 0.002$) and between %TOC and ΣTEQ ($p = 0.0007$). DL-PCB 118 was the dominant mono-*ortho*-PCB, and DL-PCB 77 was the dominant non-*ortho*-PCB in all sediment that had quantifiable DL-PCB concentrations. DL-PCB s 118, 77, 105, and 156 were quantified at higher concentrations in Gauteng, while DL-PCB 105 was elevated in Durban (Sites 10 and 11; Table 1). Octa- and hepta PCDD/F congeners were dominant. OCDD was the dominant congener for PCDD at all sites followed by 1,2,3,4,6,7,8-HpCDD, while OCDF followed by 1,2,3,4,6,7,8-HpCDF were the dominant furans.

The NMS bi-plot needed only two axes for the entire ordination (Figure 4). Axis 1 represents 62.4 % of the variation, and axis 2, 27.6%. The final stress was 12.4 and final instability was < 0.00001. A final stress between 10 and 20 provides a general picture of the associations between sites and vectors. The site classifications are indicated by convex hulls. Care should be taken for interpretation as this ordination represents relative compositions of the compounds per site, not absolute concentrations. The finer sediment particles of organic content variables were associated with industry, as were all toxicity (TEQ), almost all DL-PCB vectors, and 2,3,7,8-TCDD. Most of the penta-, hexa- and hepta PCDD/Fs vectors had relatively higher proportional compositions with harbours and the estuary. A hepta PCDD and PCDF, together with the octa PCDD and PCDF, as well as 2,3,7,8-PCDF, had the highest proportional association with the agricultural sites. Interestingly, the 2,3,7,8-congeners vectors of PCDF and PCDD were diametrically opposed.

**Table 2**: Concentrations quantified in sediment from 22 sites. Concentrations are expressed in ng/kg dm.

| | Orange River estuary | Saldanha Bay Harbour | Berg River | Theewaterskloof Dam | Groot River estuary | Swartkops Estuary | Vaal River (Douglas) | Buffalo River | Mooi River | Umlazi River estuary | Umgeni River estuary | Richard's Bay Harbour | Thulazihleka Pan | Vaal Dam | Riet Spruit | RietSpruit channel | Lochi Vaal | Crocodile River | Olifants River | Loskop Dam | Harteebeespoort Dam | Modderfontein Spruit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site numbers** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** |
| **PCB congeners** | | | | | | | | | | | | | | | | | | | | | | |
| **PCB 77** | | | 6 | | | 45 | 6 | | | 33 | 53 | | 16 | | 80 | 2500 | 200 | 20 | | | 20 | 70 |
| **PCB 81** | | | 0.1 | | | 1 | 0.2 | | | 1 | 2 | | 1 | | 2 | 100 | 2 | 0.2 | | | 0.3 | 2 |
| **PCB 126** | | | | | | 5 | 0.2 | | 0.1 | 2 | 2 | | 0.2 | | 2 | 62 | 5 | 15 | | | 3 | 13 |
| **PCB 169** | | | 0.1 | | | 1 | | | 0.1 | 0.2 | 0.1 | | 0.2 | | | 7 | 0.3 | 2 | | | 0.4 | 1 |
| **PCB 105** | | | 10 | | | 170 | 9 | | | 140 | 130 | | 22 | | 120 | 6700 | 360 | 54 | 8 | | 150 | 300 |
| **PCB 114** | | | | | | 8 | | | | 10 | 10 | | 2 | | 12 | 580 | 33 | 4 | | | 7 | 18 |
| **PCB 118** | | | 24 | | | 420 | 20 | | | 340 | 270 | | 44 | | 230 | 10600 | 580 | 340 | 20 | | 420 | 690 |
| **PCB 123** | | | 1 | | | 16 | | | | 13 | 7 | | 2 | | 9 | 590 | 20 | 60 | | | 24 | 34 |
| **PCB 156** | | | 4 | 4 | | 70 | 3 | | 3 | 61 | 47 | | 7 | | 44 | 2500 | 100 | 340 | 3 | | 102 | 220 |
| **PCB 157** | | | 1 | 1 | | 14 | | | | 11 | 9 | | 1 | | 5 | 160 | 11 | 15 | | | 15 | 24 |
| **PCB 167** | | | 2 | 2 | | 34 | 2 | | 1 | 24 | 20 | | 3 | | 19 | 840 | 40 | 150 | 1 | | 56 | 98 |
| **PCB 189** | | | 1 | 1 | | 11 | | | | 5 | 6 | | | | 5 | 300 | 12 | 100 | | | 18 | 36 |
| **ΣPCB** | 28 | 18 | 51 | 31 | 35 | 790 | 45 | 22 | 37 | 640 | 550 | 23 | 98 | 22 | 530 | 25000 | 1400 | 1100 | 41 | 25 | 810 | 1500 |
| **TEQ** | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 1 | 0.03 | 0.01 | 0.02 | 0.30 | 0.3 | 0.01 | 0.04 | 0.01 | 0.3 | 10 | 1 | 2 | 0.02 | 0.01 | 0.5 | 2 |
| **PCDD/F congeners** | | | | | | | | | | | | | | | | | | | | | | |
| **2,3,7,8-TCDD** | | | | | | 0.1 | | | | | 0.1 | | 0.1 | | 0.1 | 1 | 0.1 | | | | | 0.1 |
| **1,2,3,7,8-PeCDD** | | | | | | 0.3 | | | | 0.1 | 0.1 | | 0.1 | | 0.1 | 1 | 0.2 | 0.1 | | | | 1 |
| **1,2,3,4,7,8-HxCDD** | | 0.1 | | 0.1 | | 0.5 | | | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 1 | 0.3 | 0.3 | | | 0.2 | 1 |
| **1,2,3,6,7,8-HxCDD** | 0.2 | 0.2 | 0.1 | 0.4 | 0.1 | 2 | 0.1 | 0.1 | 0.3 | 1 | 1 | 0.2 | 0.2 | 0.2 | 1 | 6 | 2 | 1 | 0.2 | 0.1 | 0.4 | 3 |
| **1,2,3,7,8,9-HxCDD** | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 1 | 0.2 | | 0.2 | 0.5 | 0.5 | 0.1 | 0.2 | 0.1 | 0.3 | 2 | 1 | 0.5 | 0.2 | 0.1 | 0.2 | 2 |
| **1,2,3,4,6,7,8-HpCDD** | 0.3 | | 2 | 2 | 1 | 31 | 1 | 1 | 2 | 16 | 19 | 0.3 | 2 | 0.3 | 8 | 100 | 26 | 16 | 1 | 0.2 | 7 | 40 |
| **OCDD** | 1 | 1 | 15 | 17 | 5 | 180 | 5 | 4 | 9 | 120 | 150 | 1 | 14 | 1 | 61 | 900 | 190 | 220 | 3 | 2 | 50 | 420 |
| **ΣPCDD** | 1 | 1 | 17 | 20 | 6 | 215 | 6 | 4 | 11 | 137 | 171 | 2 | 17 | 2 | 71 | 1012 | 220 | 237 | 4 | 3 | 57 | 467 |
| **2,3,7,8-TCDF** | | | 0.1 | 0.1 | 0.1 | 1 | 0.1 | | 0.1 | 0.3 | 0.4 | | 1 | 0.1 | 1 | 14 | 2 | 0.2 | 0.1 | | 0.3 | 3 |
| **1,2,3,7,8-PeCDF** | | 0.1 | 0.1 | 0.1 | | 0.5 | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 7 | 1 | 0.1 | 0.1 | | 0.2 | 2 |
| **2,3,4,7,8-PeCDF** | | 0.1 | 0.1 | 0.1 | 0.1 | 0.5 | 0.1 | 0.1 | 0.1 | 0.4 | 1 | 0.1 | 0.2 | 0.1 | 0.4 | 8 | 1 | 0.2 | 0.1 | | 0.3 | 2 |
| **1,2,3,4,7,8-HxCDF** | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 1 | 0.1 | 0.1 | 0.2 | 0.4 | 1 | 0.1 | 0.2 | 0.1 | 0.4 | 7 | 1 | 0.3 | 0.1 | 0.1 | 0.3 | 3 |
| **1,2,3,6,7,8-HxCDF** | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 1 | 0.1 | 0.1 | 0.2 | 1 | 1 | 0.2 | 0.3 | 0.1 | 0.5 | 7 | 1 | 0.4 | 0.1 | 0.1 | 0.3 | 3 |
| **1,2,3,7,8,9-HxCDF** | | | | 0.1 | | | 0.1 | | 0.1 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.2 | 1 | 1 | 0.2 | 0.1 | 0.1 | 0.1 | 1 |
| **2,3,4,6,7,8-HxCDF** | 0.1 | 0.1 | 0.1 | 0.1 | | 0.5 | | | 0.1 | 0.4 | 1 | 0.1 | 0.1 | 0.1 | 0.4 | 5 | 1 | 0.2 | 0.1 | | 0.2 | 2 |
| **1,2,3,4,6,7,8-HpCDF** | 1 | 0.3 | 1 | 1 | 0.4 | 5 | 0.3 | 0.4 | 1 | 4 | 5 | 0.3 | 1 | 0.4 | 2 | 26 | 7 | 7 | 0.3 | 0.2 | 2 | 18 |
| **1,2,3,4,7,8,9-HpCDF** | | | 0.2 | 0.2 | 0.2 | 0.2 | | | | | 0.2 | | | 0.2 | 0.2 | 2 | 1 | | | | | 1 |
| **OCDF** | 1 | | 1 | 1 | 1 | 10 | | 1 | 1 | 9 | 12 | | 1 | 1 | 4 | 38 | 11 | 7 | | | 3 | 33 |
| **ΣPCDF** | 2 | 1 | 3 | 2 | 2 | 19 | 1 | 1 | 2 | 15 | 20 | 1 | 4 | 2 | 9 | 120 | 27 | 15 | 1 | 0.4 | 6 | 68 |
| **ΣPCDD/F** | 4 | 3 | 20 | 22 | 8 | 240 | 8 | 6 | 13 | 160 | 190 | 4 | 20 | 4 | 80 | 1100 | 250 | 250 | 6 | 4 | 61 | 540 |
| **TEQ** | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 2 | 0.2 | 0.2 | 0.3 | 1 | 1 | 0.2 | 0.5 | 0.2 | 1 | 12 | 2 | 1 | 0.2 | 0.2 | 1 | 4 |
| **ΣTEQ** | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 2 | 0.2 | 0.2 | 0.3 | 1 | 2 | 0.3 | 1 | 0.2 | 1 | 22 | 3 | 3 | 0.3 | 0.2 | 1 | 6 |
| **ΣTEQ normalised** | 53 | 73 | 75 | 22 | 9 | 120 | 12 | 40 | 11 | 70 | 110 | 40 | 7 | 65 | 40 | 190 | 85 | 130 | 15 | 35 | 95 | 160 |
| **ΣTEQ normalised to 1% TOC** | 0.5 | 0.7 | 0.8 | 0.2 | 0.1 | 1.2 | 0.1 | 0.4 | 0.1 | 0.7 | 1.1 | 0.4 | 0.1 | 0.7 | 0.4 | 1.9 | 0.9 | 1.3 | 0.2 | 0.4 | 1.0 | 1.6 |
| **%TOC** | 0.4 | 0.35* | 0.35* | 1 | 2 | 2 | 2 | 1 | 3 | 2 | 1 | 1 | 6 | 0.35* | 3 | 9 | 3 | 2 | 1 | 1 | 1 | 3 |
| **%C** | 0.1 | | | 1 | 2 | 1 | 1 | 0.2 | 2 | 1 | 1 | 0.2 | 5 | | 2 | 7 | 2 | 1 | 1 | 0.2 | 0.5 | 2 |
| **% > 2mm** | 0.2 | 0.1 | | 12 | 1 | 4 | 4 | 2 | 4 | 0.2 | 2 | 2 | 1 | 8 | 0.3 | 0.3 | | 3 | 0.2 | 2 | 4 | 5 |
| **Sand** | 50 | 90 | 95 | 63 | 90 | 76 | 49 | 75 | 67 | 85 | 79 | 87 | 25 | 90 | 62 | 22 | 5 | 60 | 44 | 83 | 69 | 65 |
| **% < 2mm Silt** | 40 | 3 | 1 | 22 | 1 | 14 | 23 | 12 | 17 | 5 | 9 | 5 | 66 | 5 | 14 | 69 | 32 | 17 | 22 | 8 | 18 | 18 |
| **Clay** | 8 | 7 | 5 | 15 | 9 | 10 | 28 | 13 | 17 | 10 | 13 | 9 | 9 | 5 | 25 | 10 | 63 | 23 | 35 | 9 | 14 | 18 |

Concentrations not presented were below limit of quantification

*Walkley-Black results: 0% oxidisable organic carbon

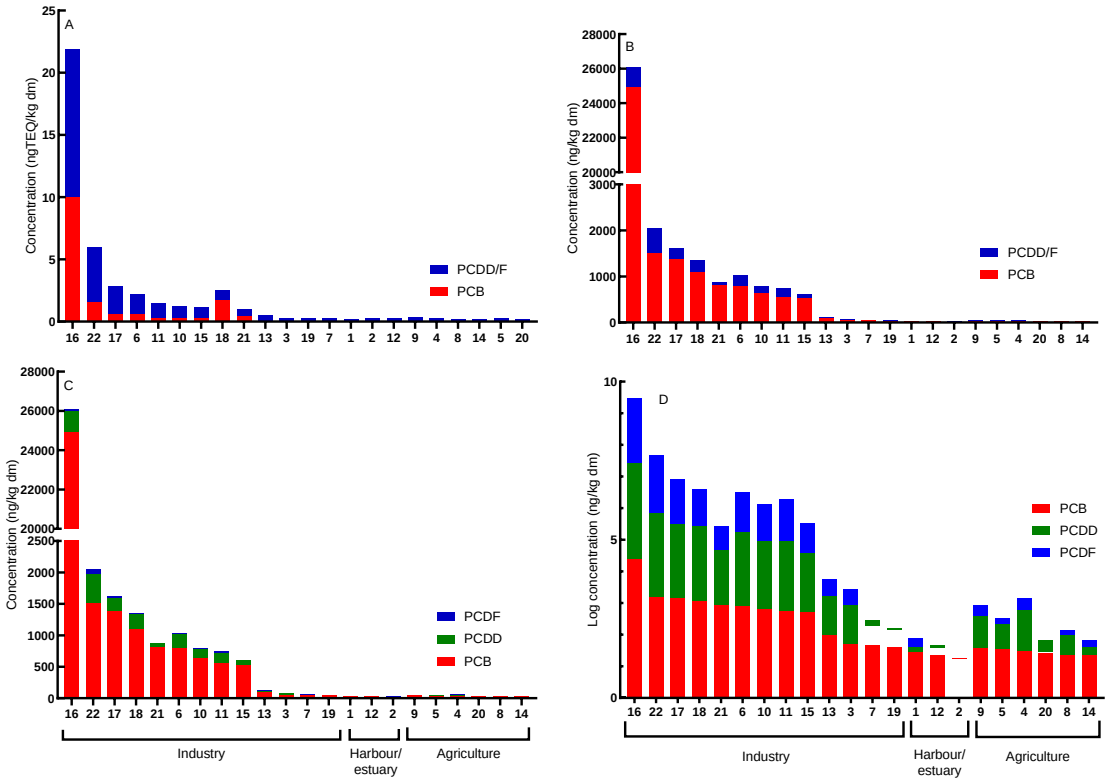


**Figure 2:** The PCBs (DL-PCB) and PCDD/F TEQ concentrations quantified in sediment at each site, expressed as ngTEQ/kg dm (A). Dioxin-like PCB and PCDD/F concentrations expressed as ng/kg dm (B). The contributions of DL-PCB, PCDD, and PCDF concentrations at each sampling site, expressed as ng/kg dm (C). Log DL-PCB, PCDD, and PCDF concentrations (D).

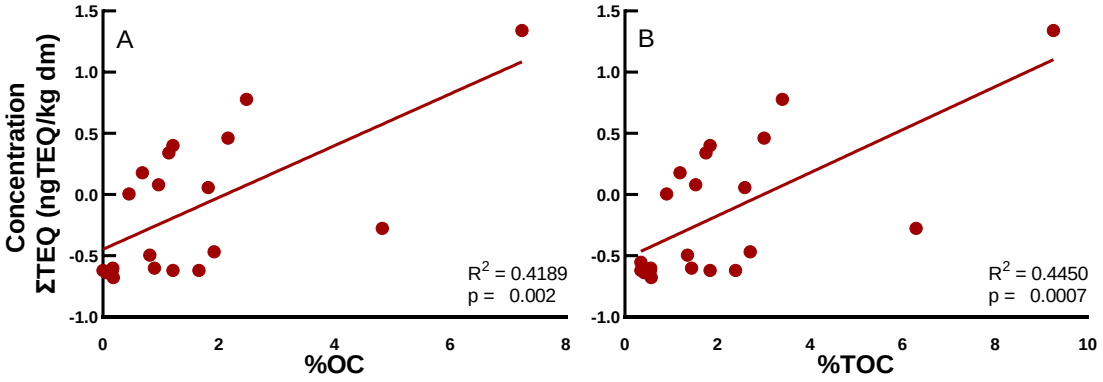


**Figure 3:** Linear regressions between the TEQ and the oxidised carbon (%OC) (A) and total organic content (%TOC) (B) of the sediment.

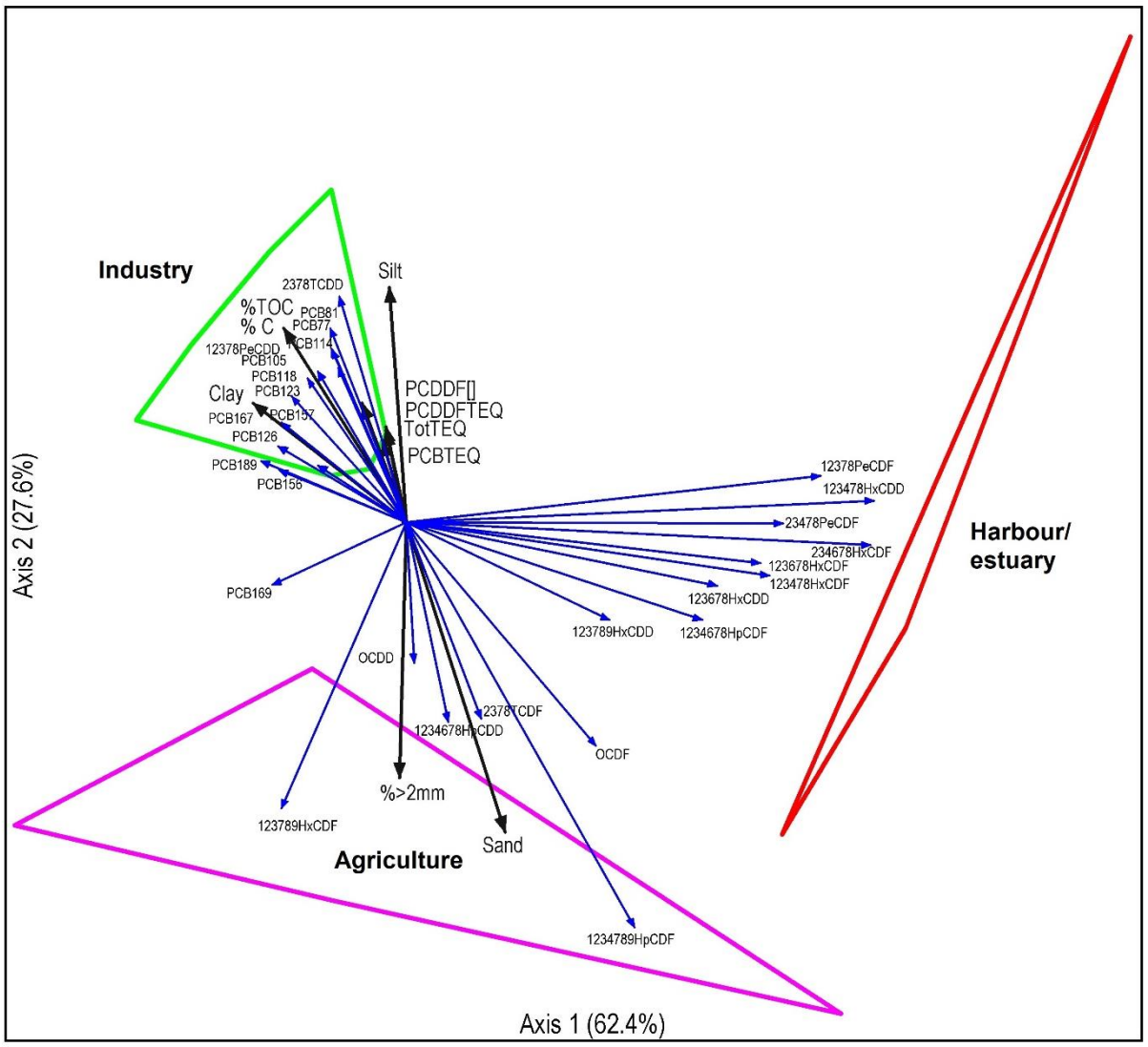


**Figure 4:** Non-metric multidimensionally scaled ordination of concentrations relativised per sample, using Gower-ignore-0, as distance measure. Sediment characteristics are represented as a bi-plot (black vectors). Final instability was <0.00001, and final stress was 12.4.

## 4. Discussion

The current study is the earliest reported data set on dioxin and DL-PCBsin sediment covering South Africa. The patterns and trends seen in the current study should be used as point of reference for future studies. The current study predates all other South African studies used for comparison, while international studies that predate the current data will be mentioned as such.

### 4.1 Concentrations

4.1.1 Industry

Located in a highly industrialised region of Gauteng, the sediment from the Riet Spruit channel had the highest ΣDL-PCB, ΣPCDD/F, and ΣTEQ concentrations, as well as the second highest normalised TEQ concentrations (Table 2). Riet Spruit channel connects a steel refinery with Riet Spruit that had two orders of magnitude lower ΣDL-PCB and one order of magnitude

lower ΣPCDD/F and ΣTEQ concentrations than the Riet Spruit channel, probably due to the dilution or chromatographic effect (retention) when moving down stream. Sediment collected in 2006 from Riet Spruit had similar $\Sigma$DL-PCB$_{12}$ and $\Sigma$PCDD/F$_{17}$ concentrations (Nieuwoudt et al., 2009). Loch Vaal in the Vaal River, however, had an order of magnitude higher ΣDL-PCB, ΣPCDD/F and ΣTEQ concentrations than Riet Spruit that feeds into the Vaal River. The dilution effect was also observed in the Klip River which drains the highly industrialised regions south of Johannesburg (Rimayi et al., 2017). Higher $\Sigma$PCB$_{31}$ concentrations were quantified in Alberton (61 000 ng/kg dm) compared with downstream Henley (27 000 ng/kg dm: Rimayi et al., 2017). Just as with Loch Vaal, the sediment at the confluence of the Klip River and Vaal River had higher $\Sigma$PCB$_{31}$ concentrations than quantified upstream (Rimayi et al., 2017: Table 3). Similar concentrations to Loch Vaal were quantified in sediment slightly upstream in the Vaal River near Vanderbijlpark (1300 ng/kg dm; Nieuwoudt et al., 2009). The Klip River confluences with the Vaal River upstream of Riet Spruit. Nieuwoudt et al. (2009) quantified an order of magnitude higher $\Sigma$DL-PCB $_{12}$ concentrations in sediment from the Klip River than in Riet Spruit. The influence of pollution from Gauteng into the Vaal River is evident as ΣDL-PCB and ΣPCDD/F concentrations were two orders of magnitude, and ΣTEQs one order of magnitude higher at Loch Vaal, then concentrations in sediment upstream in the Vaal Dam (Table 2).

ΣDL-PCB and ΣPCDD/F concentrations in the Vaal River downstream of Gauteng (site 7), before the confluence with the Orange-Senqu River, were two orders of magnitude lower, while ΣTEQs were one order of magnitude lower (Table 2). This indicates dilution, retention of the compounds upstream, or accumulation in biota as higher ΣPCB, ΣDL-PCBs and ΣPCDD/F concentrations occur in biota from this region (Bouwman et al., 2021; Lesch et al., Chapter 3). The concentrations quantified in sediment at the estuary of the Orange-Senqu River were even lower (Table 2). The Modderfontein Spruit had the second highest ΣDL-PCB, ΣPCDD/F, and ΣTEQ concentrations and the third highest normalized TEQ concentration (Table 2). The Modderfontein Spruit confluences with the Jukskei River. Approximately 90% of the yearly inflow of water into the Hartebeespoort Dam comes from the Crocodile River in Gauteng (Toerien and Walmsley, 1978) that receive water from the Jukskei and Hennops rivers. The Hartebeespoort Dam and Riet Spruit had similar concentrations (Table 2). The Jukskei River drains industries and receives runoff from illegal waste dumps, sewage spills, and agricultural practices (DWAFD, 2004). Sediment from the Jukskei River had $\Sigma$PCB$_{31}$ concentrations an order of magnitude higher (1900 ng/kg dm; Rimayi et al., 2017; Table 3) than concentrations in the Hartebeespoort Dam of this study.

The Crocodile River in Mpumalanga had concentrations similar to Loch Vaal, even though it is located in a less populated and industrialized region. The influence of industry, specifically Ngodwana paper mill may have contributed to the concentrations quantified in sediment. Paper and pulp mills are known dioxin sources (Newman, 2015). Elevated ΣDL-PCB and ΣPCDD/F concentrations have been linked to embryo mortality in eggs of wild birds nesting near paper and pulp mills in Canada (Harris et al., 2003). The two sites along the Olifants River (Sites 29 and 20) had similar concentrations but were one to two orders of magnitude lower than quantified in sediment from Gauteng, confirming large industrial regions and cities as dioxin hotspots. The slightly higher ΣDL-PCB concentrations at Site 19 compared with Site 20 can be attributed to nearby coal mining and coal-powered electricity generation. Up to two orders of magnitude higher $\Sigma$PCB$_{33}$ concentrations were quantified in sediment from the Olifants River closer to the Kruger National Park almost 10 years after the current samples

were collected (Verhaert et al., 2017: Table 3), indicating that the pollution in the Olifants River is ongoing and may be increasing.

The Umlazi and Umgeni rivers, draining low-income urban areas and industry in Durban, had concentrations similar to those of Riet Spruit in Gauteng (Table 1). Three orders of magnitude higher $\Sigma PCB_8$ concentrations were quantified in sediment from the Umgeni River in 2013 (Gakuba et al., 2015; Table 3). The Msunduzi River that later confluences with the Umgeni River had six orders of magnitude higher $\Sigma PCB_8$ concentrations than the Umgeni River in the current study (Adeyinka et al., 2018). The Swartkops River had ΣPCB and ΣPCDD/F concentrations of the same order magnitude than sediments from the Umlazi and Umgeni rivers (Table 1). $\Sigma PCB_{17}$ concentrations in sediment collected in 2017 from the Swartkops and Sunday rivers had four orders of magnitude higher concentrations than quantified in the current study (Olisah et al., 2020). These trends show that increased industrialization and urbanization are producing higher quantities of pollution. The Thulazihleka Pan had lower concentrations than expected, given its proximity to industry. The ΣDL-PCB and ΣPCDD/F concentrations quantified in sediment from Thulazihleka Pan were up to two orders of magnitude lower compared with other industrial sites. However, the concentrations were higher than quantified in the Richard's Bay Harbour nearby (Table 2).

#### 4.1.2 Harbours and estuaries

The Orange-Senqu River had lower ΣDL-PCB, ΣPCDD/F, and ΣTEQ concentrations than quantified in sediment from inland locations. The ΣTEQ normalised concentrations were an order of magnitude higher in the Orange-Senqu River estuary (53 ngTEQ/kg dm) than upstream near Douglas (9 ngTEQ/kg dm; Table 2). The countryside through which the river flows between Douglas and the Orange-Senqu River estuary is mostly arid and used for agriculture activities and diamond mining that may have contributed to pollution. However, solvent depletion (reduction in the organic content of the sediment as the river flows downstream while PCBs and PCDD/Fs break down slower) may also explain the high normalised ΣTEQ concentrations.

Higher concentrations were expected in sediment from Richard's Bay Harbour due to its proximity to a paper mill, steel and automobile construction, and its status as a large exporting port and coal terminal. The Durban Harbour had three orders of magnitude higher $\Sigma PCB_{34}$ concentrations than Richard's Bay Harbour (Vogt et al., 2018), while Port Elizabeth Harbour had two orders of magnitude higher $\Sigma PCB_6$ concentrations (Kampire et al., 2015; Table 3). The ΣDL-PCB and ΣPCDD/F concentrations in the Saldana Bay Harbour were similar to the concentrations quantified in Richard's Bay Harbour. The higher concentrations quantified in Durban and Port Elizabeth harbours can either be as a result of increased pollution in recent years or as a consequence of the size of the harbours and associated large industries. Even though the concentrations quantified in the sediment from Richard's Bay and Saldana Bay Harbours were lower than reported elsewhere, tidal flushing may have played a role. The tidal-flushing effect was observed in Lake Shihwa in Korea, where PCB and PCDD/F concentrations decreased with increasing distance between the freshwater streams and marine sampling sites offshore (Moon et al., 2012).

#### 4.1.3 Agriculture

The agricultural sites classified according to their proximity to agriculture and possible pesticide pollution had the lowest quantifiable ΣDL-PCB and ΣPCDD/F concentrations (Table

2). All ΣDL-PCB concentrations quantified from agricultural sites were of the same order of magnitude, while Mooi River and Theewaterskloof Dam had one order of magnitude higher ΣPCDD/F concentrations than the other sites within this classification (Table 2). The ΣDL-PCB concentrations quantified in sediment from Mooi River were higher than concentrations of other sites classified in the agriculture group followed by Groot River. The agriculture influenced site with the highest ΣPCDD/F concentration quantified in sediment were Theewaterskloof Dam, which is one of the main water storage impoundments in the Western Cape.

The Mooi River, far removed from industry, had ΣDL-PCB and ΣPCDD/F concentrations comparable to concentrations quantified at Richard's Bay Harbour and Thulazihleka Pan. The Vaal Dam had the lowest ΣDL-PCB and ΣPCDD/F concentrations of all sites in the agriculture category followed closely by Buffalo River.

Higher ΣDL-PCB and ΣPCDD/F concentrations were quantified in sediment collected near industrial activities. The association between high POP concentrations and industrial regions are well documented in many matrixes including plants (Odabasi et al., 2015), soil, sediment (Nieuwoudt et al., 2009), bird eggs (Polder et al., 2008; Harris et al., 2003), air (Cetin et al., 2007), and fish (Polder et al., 2014). In addition, the ΣTEQ concentrations were also generally higher near large cities such as Johannesburg and Durban. This is in accordance with patterns observed by Rabodonirina et al. (2015) in sediment from the Scheldt River in Europe. The two harbour sites of the present study had lower than expected ΣTEQ concentrations compared with sites with high industrial activity. This could be due to tidal flushing and low organic carbon at these sites. The industrialised Umgeni and Umlazi rivers, however, had ΣTEQ concentrations comparable with large inland cities. This is in accordance to patterns found by Kanzari et al. (2014) in the industrialized urban river of Huveaune in France.

### 4.2 Concentration comparisons with continental Africa

Sediment samples collected from Alexandra Harbour in Egypt (Barakat et al., 2002; Table 3) had $\Sigma PCB_{99}$ concentrations four orders of magnitude higher than $\Sigma PCB_{12}$ concentrations from Richard's Bay and Saldanha Bay harbours, possibly due to the many congeners measured. Barakat et al (2002) reported that the NDL-PCBs made up the bulk of the ΣPCB and that the DL-PCBs were lower. The Nile River though (El-Kady et al., 2007), had $\Sigma PCB_{18}$ concentrations two orders of magnitude higher, and $PCDD/F_{12}$ concentrations one order of magnitude higher than the harbour sites. ΣPCB concentrations from an estuary (Falia) in Senegal were three orders of magnitude higher than estuaries in the current study (Table 3). The dams sampled in the current study (Sites 4, 14, 20, and 21), although not as large as Lake Victoria, had up to two orders of magnitude lower ΣPCB and ΣPCDD/F concentrations than sediment from Lake Victoria (Omwoma et al., 2015).

### 4.3 Sediment characteristics and congeners

DL-PCB concentrations at each site was higher than ΣPCDD/F concentrations (Figure 2B). However, PCDD/Fs contributed most to the total toxic TEQ (Figure 2A). This agrees with trends from elsewhere (Castro-Jiménez et al., 2008; Okay et al., 2009; Zou et al., 2018). In addition, concentrations in PCDFs were lower compared with PCDDs (Figure 2C). The sites with the highest concentrations of furans such as Riet Spruit channel and Modderfontein Spruit were near industrial activities (Table 2)

The dominance of mono-ortho-PCB (DL-PCB 118) and non-ortho-PCB (DL-PCB 77) congeners is a pattern also reported by Eljarrat et al. (2005). DL-PCB 118 is usually found in greater concentrations compaird with other DL-PCBs (Barakat et al., 2002; Hu et al., 2010; Okay et al., 2009)

OCDD was the major contributor to ΣPCDD/F concentrations (Table 2). The higher OCDD concentrations in environmental samples is a pattern often found (Mari et al., 2009; Moon et al., 2012: Castro-Jiménez et al., 2008). OCDD is environmentally stable, more so than the lesser chlorinated congeners due to its high molecular mass and high vapour pressure. Higher concentrations and occurrence of octa chlorinated PCDD/Fs such as OCDD and OCDF were quantified at all sites. This is in accordance to studies conducted in Spain (Eljarrat et al., 2005), and France (Castro-Jiménez et al., 2008). Some of the sources associated with this pattern include wood preservatives, pesticides, and most certainly combustion processes (Baran et al., 2020; Lee et al., 2006; Li et al., 2013). Lower chlorinated PCDD/Fs might be more concentrated in air since they are mainly found in their vapour phase and not bound to organic matter (Fiedler, 2003).

The physical properties of sediment such as grain size are important in understanding the sedimentation and the fate of compounds within the water column. Smaller sediment particles have a larger surface area and are likely to contain higher quantities of organic content for compounds to bind with. This is likely why higher POP concentrations are quantified in sediment consisting of finer particles such as clay and silt (Lee et al., 2006). In contrast, larger sediment particles such as sand, rocks, corals and shells have less organic content, lower surface area to bind with, resulting in lower POP content. The relative composition of each site classification showed a distinct distribution between DL-PCB s, and higher and lower chlorinated PCDD/Fs (Figure 4).

Sand and particles larger than 2 mm were strongly associated with agriculture, while clay, slit and higher organic content ordinated strongly with industry (Figure 4). The Vaal River along the industrial region of Gauteng contained mostly clay and small particles (Wepener et al., 2011) that may have aided in the concentration and retention of ΣDL-PCB and ΣPCDD/F, specifically from Riet Spruit channel that had the highest percentage silt (69%). Lower chlorinated compounds are more rapidly biodegraded and predominantly found in the vapour phase (Fiedler, 2003) and may explain why higher chlorinated PCDD/F ordinated away from the industry convex hulls. The higher chlorinated compounds such as OCDD are more persistent and had a relative higher composition towards agriculture and harbours. The relative composition suggests that industrial activities are the main contributor to DL-PCB and PCDD/F pollution and that these compounds bind to organic matter within the sediment. However, as the sediment moves downriver, the lower chlorinated compounds are degraded faster leaving behind only the higher chlorinated compounds such as OCDD and OCDF, that are environmentally stable. This may explain why higher chlorinated compounds and larger sediment particles were associated with agriculture. The strong association between PCDD/F compounds and the category “harbours and estuaries” is more difficult to interpret. On possible explanation is that PCDD/F compounds make their way to estuaries and harbours where the higher chlorinated PCDD/F remain stable but that tidal flushing and low organic carbon at these marine sites cause the lower chlorinated compounds to biodegrade faster or are more easily flushed. However, the reason behind this pattern remain unclear.

### 4.4 Sediment quality guidelines

Sediment quality guidelines (SQGs) are useful tools to assess the risk posed to organisms by POP concentrations quantified within the sediment. Countries aim to develop SQGs according to their environment, climate, and landscape attributes. However, due to a lack of data many countries have not yet developed their own. Comparing concentrations in sediment from an arid, water scarce region to SQG developed for other conditions may be misleading. However, safety factors are applied to incorporate uncertainty. South Africa does not have its own SQG, which is why we use the guidelines from Canada and Australia. The Canadian SQG and Australian DGVs are based on 1% TOC. Dioxin-like PCB and PCDD/F concentrations quantified in sediment in the current study were converted to 1% TOC for comparison to guidelines (Australian Government Initiative, 2019; Environmental Law Alliance Worldwide, 2015)

Sediments of seven sites of the current study exceeded the Canadian interim sediment quality guideline (ISQG) or threshold effect level (TEL) of 0.85 ng/TEQ/kg dm PCDD/F and DL-PCB s namely; Riet Spruit channel (Site 16), Modderfontein Spruit (Site 22), Swartkops estuary (Site 5), Loch Vaal (Site 17), Crocodile River, (Site 18) Hartebeespoort Dam (Site 21) and Umgeni estuary (Site11; Table 2). None of the sites however, exceeded the Canadian ISQG of 34 100 ng/kg dm for DL-PCB s. Australian DVG concentrations for DL-PCB s (34 000 ng/kg dm) is of the same order of magnitude as that of the Canadian ISQGs. No Australian DVG concentrations for PCDD/F and dioxin like DL-PCB s are available. All seven sites exceeding the Canadian ISQG are close to or downriver from industry and should be further investigated.

**Table 3:** Summary table of reported ΣPCB and ΣPCDD/F concentrations quantified in sediment from across South Africa and Africa by various authors (ng/kg dm).

| Country | Provance | Location | Year Sampled | $PCB_?$ | ∑PCB | $PCDD/F_?$ | ∑PCDD/F | Reference |
|---|---|---|---|---|---|---|---|---|
| South Africa | KZN | Umgeni River | | $\sum PCB_8$ | 190000 | | | Gakuba et al., 2015 |
| | KZN | Msunduzi River (winter) | 2013 | $\sum PCB_8$ | 2200000 | | | Adeyinka et al., 2018 |
| | KZN | Msunduzi River (spring) | 2013 | $\sum PCB_8$ | 1200000 | | | Adeyinka et al., 2018 |
| | Eastern Province | Sundays estuary (winter)* | 2017/18 | $\sum PCB_{17}$ | 1700000 | | | Olisah et al., 2020 |
| | Eastern Province | Swartkop estuary (winter)* | 2017/18 | $\sum PCB_{17}$ | 3600000 | | | |
| | Gauteng, JHB | Marie Louise landfill | 2017 | $\sum PCB_7$ | 6900 | | | Sibiya et al., 2019 |
| | Gauteng, PTA | Harherly | 2017 | $\sum PCB_7$ | 2300 | | | |
| | Eastern Province | Port Elizabeth Harbour | 2012/2013 | $\sum PCB_6$ | 17000 | | | Kampire et al., 2015 |
| | KZN | Umgeni River (Mouth) | 2012 | $\sum PCB_{34}$ | 2000 | | | Vogt et al., 2018 |
| | KZN | Durban Bay Harbour | 2012 | $\sum PCB_{34}$ | 40000 | | | |
| | Gauteng | Suikerbosrand River 1 Upstream | 2006 | $\Sigma PCB_{12}$ | 220 | $\Sigma PCDD_{17}$ | 1 | Nieuwoudt et al., 2009 |
| | Gauteng | Vaal River | 2006 | $\Sigma PCB_{12}$ | 1300 | $\Sigma PCDD_{17}$ | 7 | |
| | Gauteng | Klip River | 2006 | $\Sigma PCB_{12}$ | 1800 | $\Sigma PCDD_{17}$ | 180 | |
| | Free State | Taaibos Spruit | 2006 | $\Sigma PCB_{12}$ | 130 | $\Sigma PCDD_{17}$ | 4 | |
| | Gauteng | Riet Spruit | 2006 | $\Sigma PCB_{12}$ | 610 | $\Sigma PCDD_{17}$ | 90 | |
| | Gauteng | Suikerbosrand River 2 Upstream | 2006 | $\Sigma PCB_{12}$ | 120 | $\Sigma PCDD_{17}$ | 4 | |
| | Gauteng | Suikerbosrand River 3 Downstream | 2006 | $\Sigma PCB_{12}$ | 1000 | $\Sigma PCDD_{17}$ | 11 | |
| | Gauteng | Klip River (Alberton) | 2013 | $\Sigma PCB_{31}$ | 61000 | | | Rimayi et al., 2017 |
| | Gauteng | Klip River (Henley) | 2013 | $\Sigma PCB_{31}$ | 27000 | | | |
| | Gauteng | Klip River (Confluence) | 2013 | $\Sigma PCB_{31}$ | 3000 | | | |
| | Gauteng | Jukskei River | 2013 | $\Sigma PCB_{31}$ | 1900 | | | |
| | Limpopo | Flag Boshielo Dam | 2012 | $\Sigma PCB_{33}$ | 390 | | | Verhaert et al., 2017 |
| | Limpopo | Phalaborwa Bar-rage | 2012 | $\Sigma PCB_{33}$ | 160 | | | |
| | Limpopo | Mamba Weir | 2012 | $\Sigma PCB_{33}$ | 2000 | | | |
| | Limpopo | Olifants Gorge | 2012 | $\Sigma PCB_{33}$ | 280 | | | |
| Senegal | | Falia | 2017 | $\Sigma PCB_7$ | 160000 | | | Bodin et al., 2011 |
| Egypt | | Alexandria Harbour | 1998 | $\Sigma PCB_{99}$ | 260000 | | | Barakat et al., 2002 |
| Kenya | | Lake Victoria | 2010 | $\Sigma PCB_{18}$ | 1800 | $\Sigma PCDD/F_{17}$ | 361 | Omwoma et al., 2015 |
| Egypt | | River Nile | 2003 | $\Sigma PCB_{18}$ | 1900 | $\Sigma PCDD/F_{12}$ | 482 | El- Kady et al., 2007 |

* highest reported concentration

## 5. Conclusion

This is the first report on DL-PCB and PCDD/F concentrations in sediment from sites across South Africa investigating possible sources that include industry, agriculture and harbours/estuaries. The majority of published literature from South Africa focused on these compounds in sediment only reported DL-PCB concentrations, with few also analysing for the unintentionally produced, but highly toxic PCDD/Fs. That in itself shows the significance of the current study. The current study has the earliest available DL-PCB and PCDD/F data to compare with subsequent studies.

DL-PCB s and PCDD/F concentrations were quantified in all sediment samples from all 22 sites. Higher ΣDL-PCB and ΣPCDD/F concentrations were quantified in sediment from industrial regions compared with agriculture and harbours/estuaries. This shows that regions with high industrialisation and urbanisation are the major contributors to DL-PCB and PCDD/F concentrations quantified in the environment.

Several of the sampling sites from the current study were also sampled by others. The ΣPCB and ΣPCDD/F concentrations from Gauteng did not vary between the current study and other reports, suggesting that PCB and PCDD/F pollution from this region has not improved. However, it is recommended that this region be monitored regularly since the highest ΣDL-PCB and PCDD/F concentrations were quantifiable in sediment from Gauteng. PCB concentrations in sediment from the Olifants River, Umgeni River and Swartkops River increased since 2002.

Sediment from industrial areas had distinctly different relative compositions of congeners compared with agricultural sites and harbours and estuaries, that were also distinctly different between them. The probable reason for this is that sources, uses, and the influence of environmental factors interacted with the physical and chemical characteristics of the congeners, causing distinct congener compositions. This illustrates that sediment quality guidelines need to take local conditions, sources, and use patterns into account. Biota at the different sites would also come into contact with different congener compositions that should be taken into account when interpreting concentrations in biotic matrixes such as wild bird eggs and fish.

The expectation at the outset was that DL-PCB and PCDD/F concentrations will have increased with an increase in industry and human population. Based on the current data, PCBs and PCDD/F concentrations in Gauteng have essentially not changed between 2002 and 2006, but concentrations in Durban have increased by up to two orders of magnitude. The respective sampling sites are close together but do not correspond. This might affect the interpretation of temporal trends. It would be advisable to sample sediment repeatedly from the same sites. Seven sites also exceeded the international sediment quality guideline for PCDD/Fs and DL-PCB s, all of whom were in industrial and urban regions. The current study highlights the importance of regular monitoring and reporting of PCBs and PCDD/F concentrations in environmental samples. The current data can and should be used as a point of

reference for future studies to assess the progress or lack thereof in limiting PCB and PCDD/F pollution.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors would like to thank the following the Water Research Commission for the funding of this project and Claudine Roos (neé Nieuwoudt), Laura Quinn, Adrian Hudson, and Werner Conradie for assistance with sample collection.

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# The first countrywide survey of dioxins (PCDDs), furans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) in South African sediment

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**Supplementary material**

Table S1: Site sampling location with GPS coordinates, closest town and sample date.

| # | Site classification | Name | Closest town/farm/resort | Date of collection | Coordinates | |
|---|---|---|---|---|---|---|
| | | | | | S | E |
| 1 | Harbour/Estuary | Orange-Senqu River estuary | Alexander Bay | 31/03/2002 | 28°31.900’ | 16°36.338’ |
| 2 | Harbour/Estuary | Saldanha Bay Harbour | Saldanha Bay | 1/4/2002 | 33°00.010’ | 17°59.805’ |
| 3 | Industry | Berg River | Hermon, Wellington, Paarl | 2/4/2002 | 33°26.011’ | 18°57.374’ |
| 4 | Agriculture | Theewaterskloof Dam | Villiersdorp | 2/4/2002 | 34°01.616’ | 19°15.795’ |
| 5 | Agriculture | Groot Estuary | Nature’s Valley | 4/4/2002 | 33°58.799’ | 23°33.913’ |
| 6 | Industry | Zwartkops Estuary | Port Elizabeth/ Gqeberha | 6/4/2002 | 33°52.030’ | 25°36.400’ |
| 7 | Industry | Vaal River | Douglas | 7/4/2002 | 29°03.132’ | 23°45.594’ |
| 8 | Agriculture | Buffalo River | Dundee | 21/04/2002 | 28°14.777’ | 30°30.599’ |
| 9 | Agriculture | Mooi River | Rosetta | 22/04/2002 | 29°18.315’ | 29°58.561’ |
| 10 | Industry | Umlazi Estuary | Durban | 23/04/2002 | 29°57.800’ | 30°58.210’ |
| 11 | Industry | Umgeni Estuary | Durban | 24/04/2002 | 29°48.480’ | 31°01.969’ |
| 12 | Harbour/Estuary | Richard’s Bay Harbour | Richard’s Bay | 25/04/2002 | 28°47.496’ | 32°01.705’ |
| 13 | Industry | Thulazihleka Pan | Richard’s Bay | 25/04/2002 | 28°46.889’ | 32°02.430’ |
| 14 | Agriculture | Vaal Dam | Leboya Bay | 19/06/2002 | 26°48.566 | 28°08.607’ |
| 15 | Industry | Riet Spruit | Vanderbijl Park | 19/06/2002 | 26°44.205’ | 27°42.673’ |
| 16 | Industry | Riet Spruit channel | Louisrus | 19/06/2002 | 26°39.825’ | 27°47.185' |
| 17 | Industry | Loch Vaal | Vanderbijl Park | 3/7/2002 | 26°45.039’ | 27°42.026’ |
| 18 | Industry | Crocodile River | Nelspruit/ Mbombela | 8/7/2002 | 25°29.238’ | 31°09.535’ |
| 19 | Industry | Olifants River | Phalaborwa | 9/7/2002 | 24°03.085’ | 31°43.801’ |
| 20 | Agriculture | Loskop Dam | Groblersdal | 10/7/2002 | 25°25.088’ | 29°21.689’ |
| 21 | Industry | Hartbeespoort Dam | Oberon | 10/7/2002 | 25°44.250’ | 27°52.931’ |
| 22 | Industry | Modderfontein Spruit | Modderfontein | 11/7/2002 | 26°04.468’ | 28°08.167’ |

## Chapter 5

# Dioxins and 21 other persistent organic pollutants in 61 sediment samples from the Orange-Senqu River Basin in Southern Africa.

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## Abstract

The Orange-Senqu River Basin stretches over four southern Africa countries that are also Parties to the Stockholm Convention on persistent organic pollutants (POPs). The main tributary, the Vaal River on the eastern side of the basin drains a large industrial region before it confluences with the Orange-Senqu River, flowing past mining, rural, and agricultural activities, discharging into the Atlantic Ocean. Under the auspices of the Orange-Senqu River Commission (ORASECOM), 61 sediment samples were collected in 2009 across the basin and analysed for all POPs listed in the Stockholm Convention on Persistent Organic Pollutants (SCPOP) in 2010. This is the largest multi-pollutant survey in Africa. Nine sites around the industrial region had quantifiable concentrations of dichlorodiphenyltrichloroethanes (DDTs) (max: 9 ng/g dry mass (dm)). Lindane was quantified at three sites in the upper Orange-Senqu River; no other pesticides were quantified. Higher polybrominated diphenyl ether (PBDE) concentrations (max: 15 ng/g dm) were quantified near the confluence than in the industrial region. Polychlorinated biphenyl (PCB) concentrations were high in industrial regions and even higher near mining activities (1053 ng/kg dm). The majority of sediment samples had quantifiable concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (max: 83 ng/kg dm), all sites associated with industry. This pattern was reflected in toxic equivalency quotient concentrations. Three sites had lindane concentrations above sediment quality guidelines, while ΣDDT from four locations and dichlorodiphenyldichloroethylene (*p,p'*-DDE) from one location were above international sediment quality guidelines. Concentrations quantified were comparable with many other studies. Industry is likely the main contributor to POPs pollution in the basin. Future studies should therefore concentrate on PCBs and DDTs at selected sites on the eastern side of the basin, while the POPs added to the SCPOP since 2010 would need a wider survey. Studies should prioritise biota rather than sediments to enable better assessments.

**Key words:** PCB, PCDD/F, POPs, Vaal River, pesticide, PBDE, DDT, ORASECOM

## 1. Introduction

The Orange-Senqu River Basin (OSRB) is the third largest basin in southern Africa, after the Zambezi and the Congo, covering approximately 1 000 000 $km^2$. The Orange-Senqu River rises in the Lesotho Highlands (where the river is named Senqu), from where it flows westwards to discharge into the Atlantic Ocean between the settlements of Alexander Bay and Oranjemund on the west coast (Figure 1). The Vaal River is the largest tributary of the Orange-Senqu River. Four countries–Botswana, Lesotho, Namibia, and South Africa—share the basin and are all parties to the Stockholm Convention on Persistent Organic Pollutants (SCPOPs). Lesotho lies entirely within the basin (Figure 1). While it makes up less than 5% of the total basin area, it contributes over 40% of the stream flow, but uses little of this water. South Africa, with more than 60% of the OSRB area, is by far the largest user of the basin's water supporting the economic heartland of the country. The Botswana part of the basin, making up 7.9% of its area, is entirely covered by the Kalahari Desert so there is little surface runoff, and water demand is largely met by groundwater abstraction. Namibia contributes 24.5% of the basin's area but less than 5% of the runoff, and is heavily dependent on the basin's water to support agricultural activities along the lower Orange River (Kistin and Ashton, 2008; Lange et al., 2007; Reig, 2013).

Indeed, irrigation demand from both Namibia and South Africa largely determines the water requirements in the lower reaches of the river, but there is also a requirement to maintain sufficient flow to safeguard the environment of the estuary. The Orange-Senqu River estuary (Figure 1) on the border of South Africa and Namibia, is an important wetland and a Ramsar site, but it was placed on the Montreux Record following recognition of its environmental degradation (Ramsar Sites Information Service, 1991a). The middle and lower reaches of the river are subject to periodic and often devastating floods, while agricultural, urban, mining, and industrial activities throughout the basin impact water quality (Cambray et al., 1986). There are seven Ramsar Sites in the OSRB. Lets'eng-la-Letsie in Lesotho is 200 km southeast of the capital city Maseru and the only site in the upper Orange-Senqu River. Barbers Pan, Blesbok Spruit, Kgaswane Mountain Reserve, Seekoeivlei Nature Reserve and Ingula Nature Reserve are all within the Vaal River catchment in South Africa (Ramsar Sites Information Service, 1991b).

Bird eggs from the upper Vaal River near the Vaal Triangle, suspected of being persistent organic pollutants (POP) hotspots, had quantifiable concentrations of dichlorodiphenyltrichloroethane (DDT) and its degradation products, as well as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Bouwman et al., 2008; Lesch et al., Chapter 3; Polder et al., 2008). The Vaal Triangle is a collective name given to an industrialised region between Vanderbijlpark and Vereeniging in Gauteng and Sasolburg in the Free State province that drain into the Vaal River. A risk assessment of this region has shown the devastating impact of industry, inadequate waste management and urbanisation in the Vaal River (Wepener et al., 2011). Per- and polyfluorinated Substances (PFAS) has been quantified in bird

eggs, fish, and invertebrates (Groffen et al., 2018; Lesch et al., 2017; Lesch et al., Chapter 3) along the Vaal River catchment. DDT and its metabolites were also reported in water, sediment, fish, amphibians, chicken eggs, and wild bird eggs in areas in the Limpopo Province still sprayed for malaria control (Barnhoorn et al., 2009; Bouwman et al., 2019; Bouwman et al., 2015; Viljoen et al., 2016). Concentrations of polycyclic aromatic hydrocarbons (PAHs), PCBs, dioxin-like chemicals, and organochlorine pesticides in soils and sediments were reported for the central part of South Africa (Nieuwoudt et al., 2009; Quinn et al., 2009). It is important to note that DDT use is banned throughout the OSRB (Bouwman et al., 2004).

Sediment acts as depot for POPs and is a primary source of exposure for microorganisms and small invertebrates. Numerous papers report concentrations of organochlorine pesticides (OCPs) in South African sediments (Barnhoorn et al., 2010; Gerber et al., 2016; Grobler, 1994; Humphries, 2013; Kampire et al., 2017; Quinn et al., 2009; Verhaert et al., 2017; Vogt et al., 2018). However, no survey has been conducted to assess the POP concentration in sediment from the largest freshwater basin in South Africa.

Under the auspices of the Orange-Senqu River Commission's (ORASECOM) 2010 Joint Basin Survey on POPs, 61 sediment samples were collected in the OSRB as part of the transboundary diagnostic analysis of the OSRB. The aim of the study was to quantify and assess all POPs listed in the Stockholm Convention on Persistent Organic Pollution at that time (2010); compare the results with other reports, identify hotspots, trends, and patterns, and compare with international sediment quality guidelines. For a survey on this large scale, we would expect higher concentrations of POPs associated with industrial areas and lower concentrations in arid areas with little anthropogenic influences. To the best of our knowledge, this is the second largest geographical survey of 2010 POPs in sediment, after the Joint Danube Survey conducted in 2007 (JDS2, 2007).

## 2. Materials and methods

### 2.1 Sediment sample collection

Sediments were sampled from 61 locations in the Orange-Senqu River catchment in September 2010 (Figure 1). Thirty-three were in the Vaal River catchment and the remaining 28 were in the Orange-Senqu River or its other tributaries, with one site in Namibia and five in Lesotho (Figure 1 and Table 2). Note that Site 38 is not included in this paper due to the site not falling in the target basin. However, all concentrations for this location can be viewed in Table S3 in the supplementary material.

### 2.2 Sediment sampling

All sampling equipment was stainless steel or glass. Sample contamination or cross-contamination was prevented by rinsing sampling utensils before and after sampling with acetone and hexane. Sediment samples were prepared at each site by collecting the top 5 cm of five collection points within a 10 m radius and stirring the pooled

mixture thoroughly. Sub-samples were stored in high-density polyethylene bottles at -20°C and protected from sunlight. The sediment samples were air-dried, ground, and sieved (mesh size 0.5 mm), before being sent to an accredited laboratory in Germany (Oëkometric GmbH- The Bayreuth Institute of Environmental Research in Bayreuth) for analyses.

### 2.3 Chemical and analytical analyses

Analyses were done for the 21 organochlorine pesticides, six non dixion-like PCBs (NDL-PCBs), 12 dioxin-like PCBs (DL-PCBs), 17 PCDD/F congeners, five brominated flame retardants (PBDE), and one per-fluorinated chemical, perfluorooctanesulfonic acid (PFOS), listed in 2010 under the Stockholm Convention (2016) (Table 1). Concentrations are quantified based on dry mass (dm). The analytical technique used for each compound group is listed in Table 1.

Quality assurance and quality control protocols as per ISO/IEC 17025:2005 accreditation were applied during the analyses of POPs. The TEQs were calculated by multiplying dry mass (dm) concentration of each dioxin and dioxin-like compound with their corresponding Toxic Equivalency Factor (TEF) value according to WHO:2005 (van den Berg et al., 2006). Upper bound TEQ concentrations were used. Descriptive and comparative statistics were done using GraphPad Prism version 9.0.2.

### 2.4 Characterisation of land use associated with the sampling sites

The 61 sampling sites were categorised according to their land use and the nearest possible source of pollution (although at some sites there may be more than one; Table 2). Four sites were upstream of agricultural activity, 15 sites downstream of agricultural activities and 24 sites within agricultural areas. Four sites were urban and two were in informal settlements. Four sites were located near mining activities, and four sites nestled in remote mountain regions (all in Lesotho).

## 3. Results

The ΣOCP, ΣPBDE, ΣPCDD/F, and all PCB concentrations along with the DL-PCB and PCDD/F TEQ values are presented in Table 2. None of the 61 sample locations had quantifiable concentrations of PFOS, α- and β-HCH, HCB, heptachlor, aldrin, dieldrin, endrin, heptachloroepoxide, Σchlordane, *o,p'*-DDE, *p,p'*-DDD, mirex, pentachlorobenzene, chlordecone, toxaphene, and hexaBB. The normalized TOC concentrations of ΣPCB and ΣOCP are in Table S2 in supplementary material.

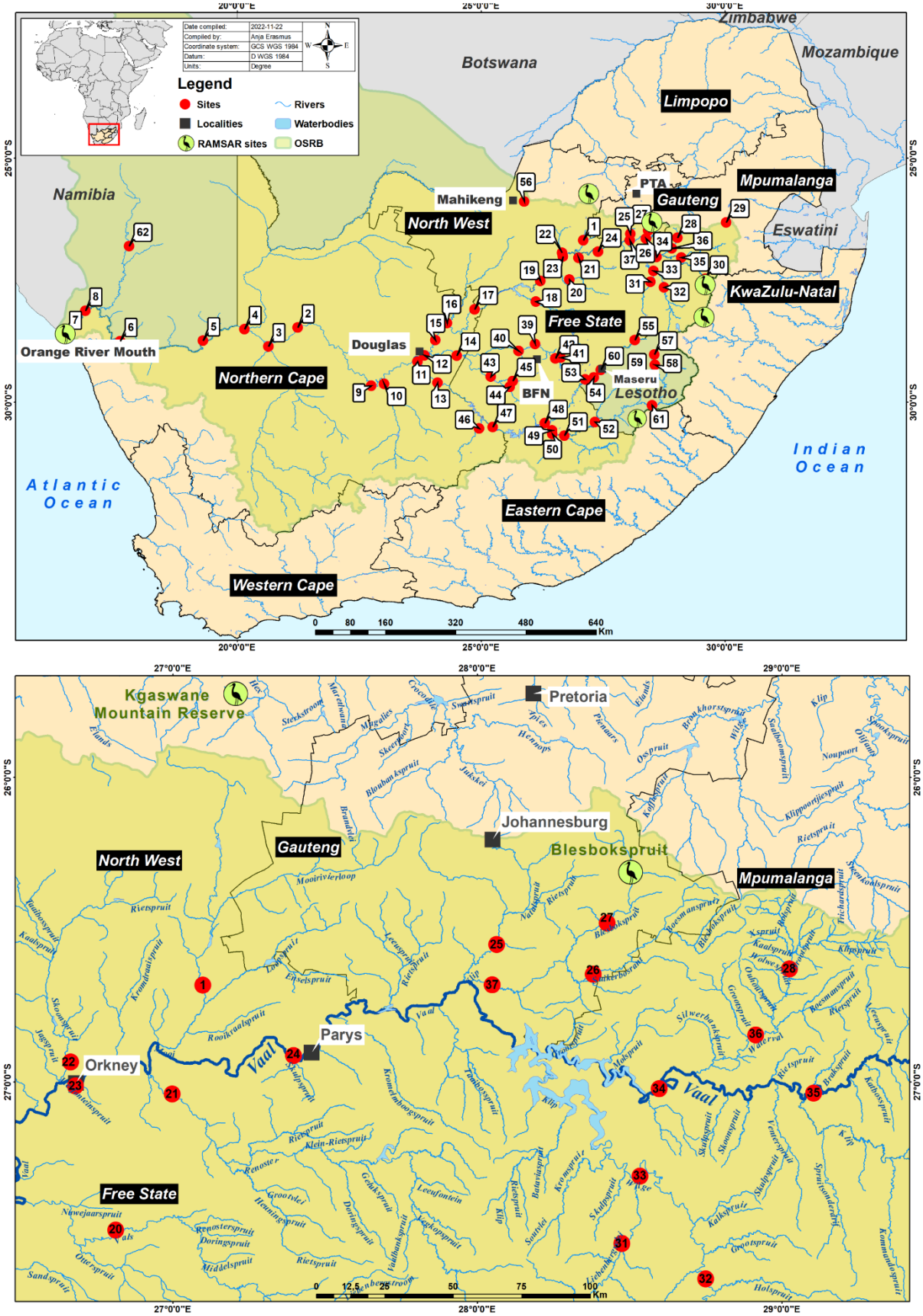


**Figure 1:** Site numbers of the 61 sediment sampling sites in the Orange-Senqu River Basin. Coordinates are provided in Table S1.

**Table 1:** The POPs analysed in the sediment samples collected and the analytical methods used.

| **Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)** | **Polychlorinated biphenyls (PCBs)** | **Polybrominated diphenyl ether (PBDE)** | **Per- and polyfluorinated substances** | **Pesticides** |
|---|---|---|---|---|
| 2,3,7,8-TCDD | PCB 77 | TetraBDE | PFOS | α-Hexachlorocyclohexane (α-HCH) |
| 1,2,3,7,8-PeCDD | PCB 81 | PentaBDE | | β-Hexachlorocyclohexane (β-HCH) |
| 1,2,3,4,7,8-HxCDD | PCB 126 | HexaBDE | | γ-Hexachlorocyclohexane (Lindane) |
| 1,2,3,6,7,8-HxCDD | PCB 169 | HeptaBDE | | Hexachlorobenzene (HCB) |
| 1,2,3,7,8,9-HxCDD | PCB 105 | HexaBB | | Heptachlor |
| 1,2,3,4,6,7,8-HpCDD | PCB 114 | | | Aldrin |
| OCDD | PCB 118 | | | Dieldrin |
| 2,3,7,8-TCDF | PCB 123 | | | Endrin |
| 1,2,3,7,8-PeCDF | PCB 156 | | | Heptachloroepoxide |
| 2,3,4,7,8-PeCDF | PCB 157 | | | Chlordane (trans-) |
| 1,2,3,4,7,8-HxCDF | PCB 167 | | | Chlordane (cis-) |
| 1,2,3,6,7,8-HxCDF | PCB 189 | | | *o,p'*-DDE |
| 1,2,3,7,8,9-HxCDF | *PCB 28 | | | *p,p'*-DDE |
| 2,3,4,6,7,8-HxCDF | *PCB 52 | | | *o,p'*-DDD |
| 1,2,3,4,6,7,8-HpCDF | *PCB 101 | | | *p,p'*-DDD |
| 1,2,3,4,7,8,9-HpCDF | *PCB 138 | | | *o,p'*-DDT |
| OCDF | *PCB 153 | | | *p,p'*-DDT |
| | *PCB 180 | | | Mirex |
| | | | | Pentachlorobenzene |
| | | | | Chlordecone |
| | | | | Toxaphene |
| **Accredited analytical approaches** | | | | |
| DIN 38414-S24<br>EPA 1613 B<br>EN 1948-3 with High resolution Gas Chromatography–Mass Spectrometry (GC/MS) | DIN 38414-S20<br>EPA 1613 B<br>E-EN 1948-4 with High resolution GC/MS | DIN EN ISO 22032<br>EPA 1614 with GC/MS<br>(in case of confirmation by high resolution GC/MS) | Internal Method with Liquid Chromatography Tandem mass Spectrometry | DIN 38407-2<br>Internal Method (based on S19 multimethod)<br>DIN EN ISO 6468 with GC/MS<br>(in case of confirmation by high resolution GC/MS) |
| **Sensitivity parameters** | | | | |
| TCDD/F – HxCDD/F = 0.05 ng/kg dry mass (dm)<br>HpCDD/F = 0.15 ng/kg dm<br>OCDD/F = 0.5 ng/kg dm | PCB 81, 126, 169 = 0,1 ng/kg dm<br>PCB 77, 105 = 3 ng/kg dm<br>PCB 114, 123, 156, 157, 167, 169 = 1 ng/kg dm<br>PCB 118 = 10 ng/kg dm<br>PCB 28, 52, 101, 138, 180 = 50 ng/kg dm | 50 - 100 ng/kg dm | PFOS = 1 ng/g dm | 0.1 - 2 ng/g dm |

Perfluorooctanesulfonic acid (PFOS), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyltrichloroethane (DDT), Environmental Protection Agency (EPA) .

* Non dioxin-like PCBs

### 3.1 Organochlorine pesticides

Nine out of the 61 sample locations had quantifiable concentrations of OCPs. Blesbok Spruit (Site 27), Mooi River (1) Suikerbosrand River (37), and Skoon Spruit (22) had the highest ΣOCP (Table 2). The highest ∑OCP concentration of 9 ng/g dm was found at Blesbok Spruit (Site 27) a location surrounded by agricultural activity but also near an oil refinery. Furthermore, a town and informal settlement are situated upstream of this location. The second highest ∑OCP concentration (8 ng/g dm) was found at Mooi River (Site 1; Table 2) that flows through both mining and agricultural landscapes and which happens to be located close to the boarder of Gauteng. Sites 39, 49, 59, and 60, more towards the southeast of the OSRB, had lower concentrations than those

found in and around Gauteng (Figure 2a). Although Sites 39, 49, and 59 had quantifiable concentrations of lindane; there were no quantifiable DDTs, while Site 60 had low concentrations of *p,p'*-DDT. Site 27 had quantifiable concentrations of DDT but no lindane. In addition, the highest *p,p'*-DDT (4 ng/g dm) and *o,p'*-DDT (3 ng/g dm) concentrations were at this location. The highest *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) (4 ng/g dm) and *o,p'*-dichlorodiphenyldichloroethane (*o,p'*-DDD) (2 ng/g dm) concentrations were at Site 1 (Table 2). Site 22 was the only location to have quantifiable concentrations of both lindane (1 ng/g dm) and DDT metabolites (*p,p'*-DDE 1 ng/g dm; *p,p'*-DDT 1 ng/g dm).

### 3.2 Polybrominated diphenyl ethers

Five sites had quantifiable concentrations of PBDEs (Table 2). The highest ΣPBDE concentrations were quantified at Sites 12 (15 ng/g dm) and 11 (4 ng/g dm) in the Vaal River near Douglas. The three other sites are all located on the eastern side of the OSRB with two sites, Skoon Spruit (Site 22) and Klip River (Site 25) within the Vaal River catchment and Mohokare River (Site 60) falling under the Orange-Senqu catchment in Lesotho (Figure 2b). Site 22 is next to a gold mine, while Site 25 is in an urban region. Site 60 is in the capital city of Lesotho, Masaru.

### 3.3 Polychlorinated biphenyls and polychlorinated dibenzo(p)dioxins and dibenzofurans

The highest ΣPCDD/F concentration in sediment was at Leeu River (Site 53; 83 ng/kg dm), followed by Blesbok Spruit (Site 27; 66 ng/kg dm; Table 2; Figure 2c). The highest ΣPCB concentrations (DL-PCB and NDL-PCB) in sediment was from the Vaal River (Site 23; 1053 ng/kg dm; Figure 2d). The highest PCDD/F TEQ value of 1 ng TEQ/kg dm was found at Suikerbosrand River (Site 37) while the sediment had a relatively low TOC (0.81%) (Table 2). In addition, the Suikerbosrand River is surrounded by agricultural activities and flows past a major drinking water treatment plant. Three sites had PCDD/F TEQ concentrations of 0.5 ngTEQ/kg namely Modder River (Site 39), Skoon Spruit (Site 22) and Mohokare River (Site 60) (Table 2; Figure 2e).

The Skoon Spruit also has the highest DL-PCB TEQ of 1 ngTEQ/kg dm. Skoon Spruit flows through several agricultural sections before it flows through the city of Klerksdorp, a large informal settlement, and then past the largest gold mining operation (Minerals Council of South Africa, 2022) in South Africa (Figure 2f). In contrast, the Mohokare River (Site 60) in Lesotho had similar PCDD/F TEQ concentrations as Skoon Spruit, but lower DL-PCB TEQ concentrations. That said, the Mohokare River had twice as much total organic carbon (1.7 %) as the sediment from the Skoon Spruit. The Malibamatso River (Site 57) had the highest TOC of 4.94% but had no quantifiable ΣPCB and relatively low ΣPCDD/F concentrations of 2.75 ng/kg dm in addition to no quantifiable TEQ concentrations (Table 2). The Kromellenboog Spruit (Site 44) had the second highest TOC (4.53%) but had no quantifiable PCB or PCDD/F concentrations or TEQ values. The concentrations of individual congeners for all 61 sites can be viewed in Table S3 in the supplementary material.

In order to determine if there were any statistically significant differences between the 61 sites characterised according to land use, we performed a one-way ANOVA along with a Tukey's multiple comparison post-test. We found no statistically significant differences between characterised sites for ΣOCP, ΣPBDE, and ΣPCDD/F including TEQ values. However, a statistically significant difference was found for PCB concentrations and TEQ values for mining vs all other characterised site concentrations (ANOVA p-value = 0.0029; Brown-Forsythe test = $p<0.0001$).

### 3.4 Vaal River vs Orange-Senqu River

Six out of 33 sites along the Vaal River had quantifiable concentrations of ΣOCP compared with the three out of 28 sites along Orange-Senqu River. In addition, the Vaal River sites had higher ΣOCP concentrations than the Orange-Senqu River. Three sites along the Vaal River had quantifiable ΣPBDE concentrations, including the highest ΣPBDE concentration (Site 12; 15 ng/g dm) quantified in this survey (Table 2). Only two (Sites 11 and 60) sites along the Orange-Senqu River had quantifiable ΣPBDE concentrations. Three sites (Site 53, 59, and 60) along the Orange-Senqu River had PCDD/F TEQ values. Interestingly, the Senquenyane and Mohokare rivers not only had quantifiable concentrations of PCDD/F and organochlorine pesticides but also PCBs. Two other sites also had quantifiable concentrations of PCBs namely the Matsuko River (0.01 ngTEQ/kg dm), and Molopo Eye (0.34 ngTEQ/kg dm). The majority of the sites along the Vaal River had quantifiable PCDD/Fs with seven sites having quantifiable concentrations of PCBs. Both the highest concentration PCDD/F TEQ (Site 37; 0.6 ngTEQ/kg dm) and PCB TEQ (Site 22; 0.5 ngTEQ/kg dm) were found along the Vaal River.

**Table 2:** Concentrations of all compounds in quantifiable sediment samples. Concentrations of all PCBs and PCDD/Fs, as well as PCDD/F and DL-PCB TEQ concentrations are expressed as ng/kg dm and ngTEQ/kg dm. OCPs and PBDEs are expressed as ng/g dm. The total organic carbon (TOC%) and land use characterisation are indicated. Sites are arranged according to the latter.

| | | | ng/kg dm | | | | ng/g dm | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PCDD/F | | PCB | | | | | | | | | | | | | | |
| Site number | Site name | TOC (%) | ΣConcentration | WHO-TE 2005: | ΣConcentration | WHO-TE 2005: | TetraBDE | PentaBDE | HexaBDE | HeptaBDE | ΣPBDE | Lindane | *p,p'*-DDE | *o,p'*-DDD | *o,p'*-DDT | *p,p'*-DDT | ΣDDT | ΣOCP | Land use characterisation |
| 3 | Hartbees River | 1 | | | | | | | | | | | | | | | | | |
| 10 | Brak River | 1 | 2 | | | | | | | | | | | | | | | | |
| 43 | Riet River | 2 | 2 | | | | | | | | | | | | | | | | Upstream |
| 50 | Stormberg Spruit | 2 | 1 | | | | | | | | | | | | | | | | Agriculture |
| 7 | Orange River (Mouth) | 1 | | | | | | | | | | | | | | | | | |
| 9 | Orange River | 2 | | | | | | | | | | | | | | | | | |
| 12 | Vaal River | 2 | 3 | 0.1 | | | 1 | 2 | 6 | 6 | 15 | | | | | | | | |
| 14 | Riet River | 1 | 5 | 0.1 | | | | | | | | | | | | | | | |
| 16 | Harts River | 2 | 1 | | | | | | | | | | | | | | | | |
| 17 | Vaal River | 2 | 8 | 0.1 | | | | | | | | | | | | | | | |
| 27 | Blesbok Spruit | 3 | 66 | 0.2 | 100 | 0.2 | | | | | | | 1 | 1 | 3 | 4 | 9 | 9 | |
| 39 | Modder River | 1 | 53 | 0.5 | 160 | 0.1 | | | | | | 1 | | | | | | 1 | |
| 40 | Kaal River | 2 | 2 | | | | | | | | | | | | | | | | |
| 41 | Karonna Spruit | 1 | 3 | | | | | | | | | | | | | | | | |
| 44 | Kromellenboog Spruit | 5 | | | | | | | | | | | | | | | | | |
| 45 | Riet River | 2 | 2 | | | | | | | | | | | | | | | | |
| 47 | Orange River | 1 | 2 | | | | | | | | | | | | | | | | |
| 48 | Caledon river | 3 | 1 | | | | | | | | | | | | | | | | Downstream |
| 49 | Orange River | 1 | 2 | | | | | | | | | 1 | | | | | | 1 | Agriculture |
| 2 | Orange River | 1 | 1 | | | | | | | | | | | | | | | | |
| 4 | Orange River | 1 | | | | | | | | | | | | | | | | | |
| 5 | Orange River | 1 | 0.1 | | | | | | | | | | | | | | | | |
| 6 | Orange River | 1 | | | | | | | | | | | | | | | | | |
| 11 | Orange River | 3 | 2 | | | | 0.1 | 1 | 1 | 1 | 4 | | | | | | | | |
| 13 | Orange River | 2 | 4 | | | | | | | | | | | | | | | | |
| 18 | Vet River | 1 | 1 | | | | | | | | | | | | | | | | |
| 19 | Vaal River | 1 | 18 | 0.2 | 69 | | | | | | | | | | | | | | |
| 20 | Vals River | 1 | | | | | | | | | | | | | | | | | |
| 21 | Renoster River | 1 | 1 | 0.1 | | | | | | | | | | | | | | | |
| 24 | Vaal River | 2 | 6 | | 38 | | | | | | | | | | | | | | |
| 26 | Suikerbosrand River | 2 | 3 | 0.1 | | | | | | | | | | | | | | | |
| 28 | Waterval River | 2 | 9 | 0.1 | | | | | | | | | | | | | | | |
| 30 | Klip River | 1 | | | | | | | | | | | | | | | | | |
| 31 | Liebenbergvallei River | 1 | 2 | 0.1 | | | | | | | | | | | | | | | |
| 32 | Wilge River | 3 | | | | | | | | | | | | | | | | | |
| 33 | Wilge River | 2 | 7 | 0.3 | | | | | | | | | | | | | | | |
| 35 | Vaal River | 2 | 1 | | | | | | | | | | | | | | | | |
| 36 | Waterval River | 1 | 2 | 0.1 | | | | | | | | | | | | | | | |
| 37 | Suikerbosrand River | 1 | 30 | 1 | 130 | 0.1 | | | | | | | 1 | | 1 | 1 | 3 | 3 | |
| 46 | Seekoei River | 1 | 1 | | | | | | | | | | | | | | | | |
| 53 | Leeu River | 1 | 83 | 0.2 | | | | | | | | | | | | | | | |
| 54 | Caledon River | 1 | 6 | | | | | | | | | | | | | | | | Within |
| 55 | Caledon River | 1 | 2 | | | | | | | | | | | | | | | | Agriculture |
| 1 | Mooi River | 1 | 4 | | | | | | | | | | 4 | 2 | 2 | | 8 | 8 | |
| 25 | Klip River | 1 | 62 | 0.3 | 340 | 0.2 | 0.1 | 0.1 | | 0.2 | 1 | | 1 | | | | | 1 | |
| 34 | Vaal River | 2 | 8 | 0.1 | | | | | | | | | | | | | | | |
| 51 | Orange River | 2 | 0 | | | | | | | | | | | | | | | | Urban |
| 29 | Vaal River (origin) | 1 | 6 | 0.1 | | | | | | | | | | | | | | | |
| 42 | Modder River | 2 | 2 | | | | | | | | | | | | | | | | |
| 56 | Molopo Eye | 1 | 11 | | 160 | 0.3 | | | | | | | | | | | | | |
| 62 | Fish River | 1 | 3 | | | | | | | | | | | | | | | | Arid Region |
| 8 | Orange River | 1 | | | | | | | | | | | | | | | | | |
| 15 | Vaal River | 1 | 5 | 0.1 | | | | | | | | | | | | | | | |
| 22 | Skoon Spruit | 1 | 57 | 0.5 | 340 | 1 | 0.1 | 0.2 | | 0.2 | 1 | 1 | 1 | | | 1 | 2 | 2 | |
| 23 | Vaal River | 2 | 12 | 0.1 | 1050 | 0.1 | | | | | | | | | | | | | Mining |
| 57 | Malibamatso River | 5 | 3 | | | | | | | | | | | | | | | | |
| 58 | Matsuko River | 3 | 3 | | 0.4 | | | | | | | | | | | | | | |
| 59 | Senquenyane River | 2 | 5 | 0.1 | 0.3 | | | | | | | 1 | | | | | | 1 | |
| 61 | Senqu River | 2 | 4 | | | | | | | | | | | | | | | | Mountains |
| 52 | Orange River | 2 | 1 | | | | | | | | | | | | | | | | Informal |
| 60 | Mohokare River | 2 | 66 | 1 | 31 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 1 | 1 | | | | | | 1 | settlement |

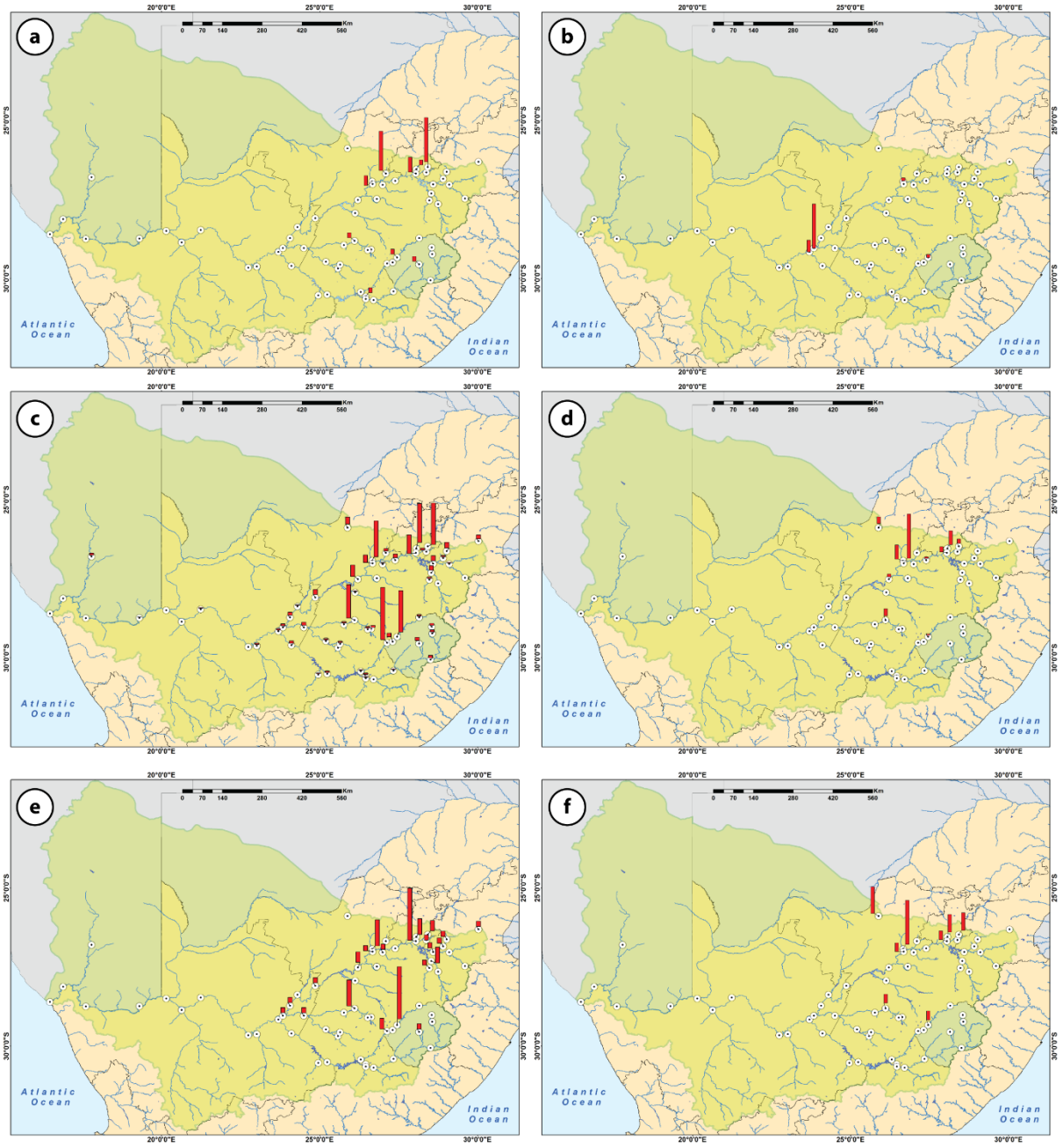


**Figure 2**: Visual representation of POPs quanified in OSRB. a) ΣOCP, b) ΣPBDE, c) ΣPCDD/F d) PCB, e) PCDD/F TEQ, f) PCB TEQ.

**Table 3:** Mean concentrations (ng/g dm) of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) found in sediment from locations in freshwater systems in South Africa.

| | | Compounds | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **River name** | **Year sampled** | **ΣDDT** | **ΣHCH** | **ΣDrins** | **ΣChlors** | **ΣEndos** | **HCB** | **Heptachlor** | **Mirex** | **Aldrin** | **Dieldrin** | **Endrin** | **ΣOCP** | **BDE** | **ΣPBDE** | **Reference** |
| Vaal River | 2006 | 2 | 0.5 | | | 0.5 | 0.1 | | | | | | 3.1 | | | Quinn et al., 2009 |
| uMngeni River, South Africa | 2013 | 100 | 41 | | | | 13 | 30 | 42 | 22 | 27 | 56 | 330 | | | Gakuba et al., 2018 |
| Lake St Lucia, South Africa | 2015/16 | 87 | 83 | 94 | 35 | 73 | | | | | | | 370 | | | Buah-Kwofie and Humphries., 2017 |
| Mkhuze, South Africa | 2015/16 | 140 | 190 | 120 | 76 | 140 | | | | | | | 670 | | | |
| Late Sibaya, South Africa | 2015/16 | 160 | 92 | 160 | 34 | 100 | | | | | | | 550 | | | |
| Kosi Bay, South Africa | 2015/16 | 200 | 84 | 180 | 32 | 98 | | | | | | | 590 | | | |
| Selati River | 1994 | 0.03 | | | | | | | | | | | 0.03 | | | Grobler., 1994 |
| Jukskei river | 2005 | | | | | | | | | | | | 1200 | | | Sibali et al., 2008 |
| Buffalo river | 2002 | 88 | | | | | 17 | 53 | | 18 | 13 | 24 | 212 | | | Awofolu and Fatoki., 2003 |
| Keiskammahoek river | 2002 | 16 | | | | | 5 | 2 | | 3 | 3 | 3 | 31 | | | |
| Tyume river | 2002 | 17 | | | | | 4 | 3 | | 3 | | 6 | 33 | | | |
| Swartkops river | 2002 | 4 | | | | | 8 | 2 | | 0.4 | 7 | | 21 | | | |
| Mfolozi catchment | 2019 | 54 | 53 | | | | | 8 | | 9 | 36 | 15 | 260 | | | Buah-Kwofie and Humphries., 2021 |
| Mkhuze catchment | 2018 | 40 | 67 | | | | | 8 | | 9 | 21 | 16 | 240 | | | |
| Vaal Triangle | 2006 | 2 | 1 | | | 0.1 | 0.1 | | | | | | 2 | | | Quinn et al., 2009 |
| Olifants River | 2010 | | | | 0.2 | | | 0.2 | | | | | 0.4 | | | Gerber et al., 2016 |
| Letaba River | 2010 | 1 | 0.2 | | | | | | | | | | 1 | | | |
| Luvuvhu River | 2010 | 3 | 2 | | | | | | | | | | 4 | | | |
| Loskop dam | 2015-17 | | | | | | | | | | | | 1 | | | Seopela et al., 2021 |
| Lake Sibaya | 2012 | 43 | | | | | | | | | | | 43 | | | Humphries., 2013 |
| Pongolo floodplain | 1995 | 740 | | | | | | | | | | | 740 | | | Naude et al., 1998 |
| Letaba River | 1994 | 86 | | | | | | | | | | | 86 | | | |
| Mthambalala river | 2000 | 0.1 | | | | | | | | | | | 0.1 | | | Sereda and Meinhardt, 2003 |
| Emhlangeni river | 2000 | 1 | | | | | | | | | | | 1 | | | |
| Cezwana river | 2000 | 0.2 | | | | | | | | | | | 0.2 | | | |
| Mthala river | 2000 | 0.4 | | | | | | | | | | | 0.4 | | | |
| Ingwavuma river | 2000 | 0.2 | | | | | | | | | | | 0.2 | | | |
| Namanini pan | 2000 | 0.2 | | | | | | | | | | | 0.2 | | | |
| Namanini pan | 2001 | 0.3 | | | | | | | | | | | 0.3 | | | |
| Msunduzi pan | 2001 | 1 | | | | | | | | | | | 1 | | | |
| Emhlangeni river | 2001 | 0.4 | | | | | | | | | | | 0 | | | |
| Balemhlanga pan | 2001 | 1 | | | | | | | | | | | 1 | | | |
| Mozi Swamp | 2001 | 1 | | | | | | | | | | | 1 | | | |
| Emhlangeni pan | 2001 | 10 | | | | | | | | | | | 10 | | | |
| Cezwana river | 2001 | 1.3 | | | | | | | | | | | 1 | | | |
| Lourens river | 2001/3 | 14 | | | | 7 | | | | | | | 20 | | | Bollmohr et al., 2007 |
| Ga-Selati River | 2014 | 27 | 0.01 | | | | 0.01 | | | | | | 27 | | | Govaerts et al., 2018 |
| Olifants River, FBD | 2012 | 3.4 | 0.1 | | 0.1 | | 0.03 | | | | | | 4 | | | Verhaert et al., 2017 |
| Olifants River, PB | 2012 | 0.6 | | | 0.03 | | 0.1 | | | | | | 1 | | | |
| Olifants River, MW | 2012 | 0.2 | 0.1 | | 0.04 | | 0.02 | | | | | | 0.3 | | | |
| Olifants River, OG | 2012 | 0.1 | | | | | | | | | | | 0.1 | | | |
| Soshanguve | 2014 | | | | | | | | | | | | | $BDE_9$ | 4 | Sibiya et al., 2017 |
| Hatherly (PTA) | 2014 | | | | | | | | | | | | | $BDE_9$ | 3 | |
| Garankuwa | 2014 | | | | | | | | | | | | | $BDE_9$ | 3 | |
| Onderstepoort | 2014 | | | | | | | | | | | | | $BDE_9$ | 3 | |
| Goudkoppies | 2014 | | | | | | | | | | | | | $BDE_9$ | 3 | |
| Marie Louis (JHB) | 2014 | | | | | | | | | | | | | $BDE_9$ | 4 | |
| Robinson Deep | 2014 | | | | | | | | | | | | | $BDE_9$ | 3 | |
| eThekwini metropolitan municipality | 2011 | | | | | | | | | | | | | $BDE_{11}$ | 3800 | Guardia et al., 2013 |
| Jukskei river | 2010 | | | | | | | | | | | | | $BDE_{16}$ | 24 | Olukunle et al., 2012 |
| Marie Louis (JHB) | 2017 | | | | | | | | | | | | 14 | $BDE_5$ | 1 | Sibiya et al., 2019 |
| Hatherly (PTA) | 2017 | | | | | | | | | | | | 21 | $BDE_5$ | 1 | |
| Diep River (Cape Town) | 2010 | | | | | | | | | | | | | $BDE_8$ | 3 | Daso et al., 2016 |
| Kuils River (Cape Town) | 2010 | | | | | | | | | | | | | $BDE_8$ | 11 | |
| Vaal River | 2017 | | | | | | | | | | | | | $BDE_7$ | 48 | Chokwe et al., 2019 |
| Vaal River @ Barrage Outlet | 2013 | | | | | | | | | | | | | $BDE_6$ | 10 | Chokwe et al., 2015 |
| Meyerton | 2013 | | | | | | | | | | | | | $BDE_6$ | 11 | |
| Rietspruit | 2013 | | | | | | | | | | | | | $BDE_6$ | 25 | |

**Table 4:** Mean concentrations (ng/kg dm) ΣPCB and ΣPCDD/F found in sediment from locations in freshwater systems in South Africa.

| | | Compounds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | Year sampled | PCB | ΣPCB | PCB TEQ | ΣPCDD | PCDD TEQ | ΣPCDF | PCDF TEQ | ΣPCDD/F TEQ | Reference |
| Umgeni River | 2013 | $\Sigma PCB_8$ | 200000 | | | | | | | Gakuba et al., 2015 |
| Msunduzi River | 2013 | $\Sigma PCB_8$ | 1500000 | | | | | | | Adeyinka et al., 2018 |
| uMngeni River | 2012 | $\Sigma PCB_{34}$ | 1400 | | | | | | | Vogt et al., 2018 |
| Amanzimnyama River | 2012 | $\Sigma PCB_{34}$ | 5000 | | | | | | | |
| Umbilo River | 2012 | $\Sigma PCB_{34}$ | 0.1 | | | | | | | |
| Amanzimnyama River | 2012 | $\Sigma PCB_{34}$ | 27000 | | | | | | | |
| Sasolburg low-income residential | 2006 | $\Sigma PCB_{12}$ | 600 | 0.2 | 170 | 0.5 | 22 | 0.8 | 1 | Nieuwoudt et al., 2009 |
| Vanderbijlpark, low-income residential | 2006 | $\Sigma PCB_{12}$ | 2700 | 0.5 | 5 | 0.1 | 3 | 0.2 | 0.3 | |
| Suikerbosrand River | 2006 | $\Sigma PCB_{12}$ | 220 | 0.04 | 1.0 | 0.1 | 0.4 | 0.02 | 0.1 | |
| Vaal River | 2006 | $\Sigma PCB_{12}$ | 1300 | 0.2 | 7 | 0.1 | 1 | 0.02 | 0.1 | |
| Klip River | 2006 | $\Sigma PCB_{12}$ | 1800 | 1 | 170 | 0.4 | 13 | 0.4 | 0.8 | |
| Taaibos Spruit | 2006 | $\Sigma PCB_{12}$ | 130 | 0.1 | 3 | 0.1 | 1 | 0.1 | 0.1 | |
| Riet Spruit | 2006 | $\Sigma PCB_{12}$ | 610 | 0.2 | 85 | 0.3 | 4 | 0.2 | 0.4 | |
| Suikerbosrand River | 2006 | $\Sigma PCB_{12}$ | 120 | 0.04 | 3 | 0.1 | 1 | 0.03 | 0.1 | |
| Suikerbosrand River | 2006 | $\Sigma PCB_{12}$ | 1000 | 0.2 | 9 | 0.1 | 2 | 0.11 | 0.2 | |
| Sasolburg bindustrial | 2006 | $\Sigma PCB_{12}$ | 300 | 0.7 | 870 | 4 | 160 | 2 | 6 | |
| Vanderbijlpark, industrial | 2006 | $\Sigma PCB_{12}$ | 4700 | 4 | 100 | 2 | 160 | 10 | 11 | |
| Ga-Selati River | 2014 | $\Sigma PCB_{21}$ | 210 | | | | | | | Govaerts et al., 2018 |
| Olifants River, FBD | 2012 | $\Sigma PCB_{33}$ | 360 | | | | | | | Verhaert et al., 2017 |
| Olifants River, PB | 2012 | $\Sigma PCB_{33}$ | 160 | | | | | | | |
| Olifants River, MW | 2012 | $\Sigma PCB_{33}$ | 1030 | | | | | | | |
| Olifants River, OG | 2012 | $\Sigma PCB_{33}$ | 180 | | | | | | | |
| Vaal River | 2006 | $\Sigma PCB_7$ | 3000 | | 30 | | | | | Quinn et al., 2009 |

Nieuw oudt et al., 2009 reported $PCDD_{17}$ and $PCDF_{10}$

## 4. Discussion

### 4.1 Compounds < LOQ

None of the sediment samples had quantifiable PFOS. This was comparable with results obtained by Groffen et al. (2018) that found no PFASs concentrations in sediment above the limit of quantification (LOQ) along the Vaal River, with the exception of Thabela Thabeng (2.36 ng/g dm PFOS). This would suggest relatively low levels of PFOS in sediment in the Vaal River. In contrast, water, invertebrates, wild bird eggs, and fish had quantifiable concentrations of PFOS along the Vaal River (Groffen et al., 2018; Lesch et al., 2017; Lesch et al., Chapter 3). This pattern was also observed in freshwater systems in Tanzania (Groffen et al., 2021). Furthermore, sediment from the Diep and Plankenburg river systems (Fagbayigbo et al., 2022) in the Western Cape, as well as sediment from Roodeplaat and Hartbeespoort dams in Pretoria (Batay et al., 2020) had quantifiable PFOS concentrations. PFOS concentrations in wild bird eggs (Lesch et al., Chapter 3) and adult Odonata (Lesch et al., 2017) from Bloemhof Dam were exceptionally high. The PFOS concentrations poses a risk for adverse effects in birds. Thus, the lack of quantifiable PFOS in sediment from Site 19, was unexpected (Table 1 and S1). This suggests that sediment might not be an effective matrix to use to assess PFOS concentrations in the environment.

HCH and HCB concentrations were quantifiable in sediment from the Vaal Triangle (Quinn et al., 2009; Table 3). This region is known for high industrial and municipality pollution (Iloms et al., 2020). However, we found no quantifiable concentrations of HCB and HCH in sediment from Sites 37 (Vereeniging; Suikerbosrand River), or 25 (Meyerton; Klip River) (Table 2 and S1). Compounds may settle into sediment closer to sources and may significantly differ in concentrations from sites close to each other. ΣHCH were found in sediment from iSimangaliso Wetland Park and uMngeni River in KwaZulu-Natal (KZN) (Buah-Kwofie and Humphries., 2017; Gakuba et al., 2018; Table 3) but this area does not fall withing the OSRB. Due to POPs' lipophilic nature, their concentrations are usually higher in biota than in sediment (Groffen et al., 2021). ΣHCH concentrations in muscle tissue of fish (*Clarias gariepinus*) from Gauteng ranged from ND–11 ng/g wet mass (wm), and Σchlordane ranged from 29–44 ng/g wm (Pheiffer et al., 2018) while muscle tissue of *Labeo capensis* had quantifiable ΣHCH concentrations between 10–156 ng/g dm and HCB concentrations between 9–588 ng/g dm (Wepener et al., 2011). Nile crocodile (*Crocodylus niloticus*) eggs from isolated areas of the Kruger National Park had quantifiable concentrations of OCPs not quantified in the current study (Bouwman et al., 2014). This shows that sediment alone, may not be sufficient in reflecting the pollution load of such compounds.

**4.2 Organochlorine pesticides**

All sites with quantifiable concentrations of ΣOCP were on the eastern side of the OSRB (Figure 2a) with the highest concentrations in and around Gauteng, a highly industrialised region and the densest human populated provinces of South Africa (South African Government, 2022). The highest ΣOCP concentration (9 ng/g dm) was quantified at Blesbok Spruit (Site 27), a Ramsar Site (Table 2; Figure 1). The lower, but quantifiable ΣOCP concentrations in the Orange-Senqu River, may be due to isolated instances of lindane used in agriculture, as sites up and downstream of this location had no quantifiable ΣOCP concentrations (Table 2: Figure 2a). DDT and its metabolites made the largest contribution (91%) to ΣOCP concentrations. This is comparable with patterns found in sediment (Phele et al., 2015), fish (Pheiffer et al., 2018), and bird eggs (Bouwman et al., 2021) along the Vaal River.

The use of DDT was banned in 1976 (Bouwman et al., 2004) in all regions of the OSRB. However, due to DDTs' persistent nature we still detected it in regions that no longer use or produce the toxic compounds. The legal use of DDT for malaria control is limited to KwaZulu-Natal (KZN) and the north-eastern side of South Africa where higher DDT concentrations have been quantified in wildlife (Barnhoorn et al., 2009; Bouwman et al., 2013; Bouwman et al., 2014; Bouwman et al., 2019; McHugh et al., 2011). Organochlorine pesticides have been quantified in both aquatic and terrestrial bird eggs along the Vaal River with mean ΣDDT concentrations ranging between 18–420 ng/g wm (Bouwman et al., 2008: Lesch et al. Chapter 3). Furthermore, two species of fish had up to two orders of magnitude higher ΣDDT concentrations than quantified in sediment from the same region (Pheiffer et al., 2018: Wepener et al., 2011). The DDT concentrations quantified in wildlife far exceed the concentrations

quantified in sediment, as is to be expected due to their chemical and physical properties.

Higher ΣOCP concentrations were quantified in sediment from Sites 25, 26, 27, and 37 in the Vaal Triangle in Gauteng (Figure 2a) than reported by (Quinn et al., 2009: Table 3). DDE, a degradation product of DDT is usually found in greater quantities than other metabolites (Bouwman et al., 2008; Giuliani et al., 2019, Lesch et al., Chapter 3; Polder et al., 2014). However, the higher *p,p'*-DDT concentrations quantified at Site 27 and to some extent Site 37 were unexpected. Both these sites are near each other, implying a possible mutual source. However, *p,p'*-DDT can persist in the environment for decades under the appropriate conditions (Chattopadhyay and Chattopadhyay, 2015). Therefore, attributing the DDT residues found in Gauteng to recent DDT use would be rash. Instead, the concentrations may be due to past DDT use with unfavourable sediment conditions for aerobic and anaerobic breakdown, or illegal application thereof, the former being more unlikely. The *p,p'*-DDT/*p,p'*-DDE ratios are often used as indicator of recency of *p,p'*-DDT use has also been questioned by Bouwman et al. (2021). The high ΣOCP concentrations (8 ng/g dm) from Site 1 may be attributed to historical DDT use in agriculture.

Lindane was the only other OCP, aside from ΣDDT, quantified in sediment samples. Four sites had quantifiable lindane concentrations (Table 2), three (Sites 39, 49, and 59) of which had quantifiable lindane only, no ΣDDTs. There was no clear pattern found other than their proximity to possible agricultural practices. Prior to this study, lindane was legally still available, however, it has since been banned (South Africa, 2009). Gauteng is therefore a DDT hotspot while isolated occurrences of lindane were quantified in the lower Orange-Senqu River (Figure 2a).

### 4.3 Polybrominated diphenyl ethers

The higher ΣPBDE concentrations quantified in sediment downstream of Gauteng (Sites 11 and 12) were unexpected. It was expected that sites in and around Gauteng would have had higher quantifiable ΣPBDE concentrations. However, Site 12 is in the Vaal River just before it merges with the Orange-Senqu River, and Site 11 is in the Orange-Senqu River before it confluences with the Vaal River (Figure 2b). The ΣPBDE concentrations in sediment from Site 12 were comparable to concentrations quantified in sediment from the Jukskei River and Riet Spruit in Gauteng, both of which are impacted by waste-water treatment works and industry (Chokwe et al., 2015; Olukunle et al., 2012; Table 3). The ΣPBDE concentrations quantified at Site 11 were of the same magnitude as concentrations in sediment from landfill sites in Gauteng (Sibiya et al., 2017: Table 3). The ΣPBDE concentrations at Site 22 may be attributed to pollution from the nearby industries and informal settlement, while Site 25 is located in the Vaal Triangle (Figure 2b). ΣPBDE concentrations in fish from the Vaal River near Gauteng were an order of magnitude higher than quantified in sediment (Wepener et al., 2011). Gauteng can therefore be considered a PBDE source region. Quantities of these compounds make their way downstream and eventually settle out

(or break down) before the Vaal River and Orange-Senqu River confluences as there were no quantifiable ΣPBDE concentrations further downstream (Figure 2b).

### 4.4 Polychlorinated biphenyls and polychlorinated dibenzo(p)dioxin and furan

The current study is the first to report concentrations of PCDD/Fs, DL-PCBs, and NDL-PCBs in sediment across any large catchment in Africa. The addition of PCDD/F data is especially important, as this compound is under represented in literature (Olisah et al., 2022). PCDD/F concentrations in sediment from the current study ranged between 0-83 ng/kg dm (Table 2). The highest PCDD/F concentrations were quantified in sediment on the eastern side of the OSRB (Figure 2c), with the highest concentration at Leeu River (Site 53; 83 ng/kg dm) on the border of South Africa and Lesotho. In addition, similar high PCDD/F concentrations were found in sediment from the Mohokare River in Lesotho (Site 60; 66 ng/kg dm) that also had quantifiable concentrations of other compound classes. Sites closer to the industrialised Gauteng (Sites 22, 25, and 27) had similar PCDD/F concentrations than those from the Lesotho border (Figure 2e).

Sediment from Sasolburg and Klip River in the Vaal Triangle had quantifiable concentrations of PCDD/F an order of magnitude higher than the present study (Nieuwoudt et al., 2009: Table 4). Similar PCDD/F concentrations were quantified in sediment from Riet Spruit, Vanderbijlpark's industrial region, and pooled sediment samples from the Vaal Triangle (Lesch et al., Chapter 4; Nieuwoudt et al., 2009; Quinn et al., 2009; Table 4). The PCDD/F concentrations quantified in sediment from Suikerbosrand River (Site 37) from the current study were two order levels higher than concentrations quantified by Nieuwoudt et al. (2009) from the same location. In addition, this site also had the highest TEQ value (0.6 ngTEQ/kg dm) in the current study. The capital city of Lesotho, Maseru and Gauteng province of South Africa release PCDD/Fs from a variety of sources, more than any other region in the OSRB. These sites also had quantifiable concentrations of other compound classes, further strengthening the argument that they are pollution hotspots.

The highest ΣPCB concentration (1050 ng/kg dm) were quantified in sediment from the Vaal River (Site 23) near Orkney (Figure 2d). This site is located downstream of the largest gold mining complex in South Africa and an informal settlement. In addition, the concentrations quantified in sediment from this site had more than double the PCB concentrations of any other site (Table 2; Figure 2d). The second highest PCB concentration (340 ng/kg dm) was in sediment from Skoon Spruit (Site 22) that eventually drains into the Vaal River near Site 23. This suggests the informal settlement and the gold mine complex as a common source. Similar PCB concentrations were quantified in sediment from Gauteng (Sites 25, 27, and 37). The PCB concentrations quantified in sediment from Gauteng in the current study had one to two orders of magnitude lower concentrations than those reported by others (Nieuwoudt et al., 2009; Quinn et al., 2009: Table 4: Lesch et al., Chapter 4). While two studies reported on DL-PCBs from Gauteng (Lesch et al., Chapter 4 and Nieuwoudt et al., 2009). Sediment collected from the Olifants River in Limpopo and

uMngeni River in KZN followed similar patterns (Govaerts et al., 2018; Verhaert et al., 2017; Vogt et al., 2018), while the Msunduzi and Umgeni rivers in KZN had more than three orders of magnitude higher concentrations than those quantified in the current study (Adeyinka et al., 2018; Gakuba et al., 2015). The highest PCB TEQ concentration (0.5 ngTEQ/kg dm) was in sediment from Skoon Spruit (Site 22), followed by the Molopo Eye (0.3 ngTEQ/kg dm; Site 56; Figure 2f). The statistically significant difference between PCBs from sites in mining regions compared with other regions suggest that mining activity as the largest source of PCBs in the OSRB.

### 4.5 Vaal River vs Orange-Senqu River

The differences between the Vaal River and the Orange-Senqu River, not only in concentrations but also the number of sites with quantifiable POPs differences were expected. The Vaal River and its tributaries flow through the highly industrialised Gauteng province and past several mines, waste-water treatment plants, urban regions, rural and agricultural communities. Skoon Spruit (Site 22) near the goldmine and Klip River (Site 25) in the Vaal Triangle both had quantifiable concentrations of all POP classes along the Vaal River catchment whereas the Orange-Senqu River flows through mountains and semi secluded agricultural communities, except for the city of Maseru, before confluence with the Vaal River. The Mohokare River (Site 60) in the City of Maseru, in Lesotho is the only site of the Orange-Senqu River with quantifiable concentrations of all POP classes. The higher POP concentrations in the vicinity of urban/industrial areas are comparable to patterns found in the Scheldt catchment between France and Belgium et al. (2015) and in the Huveaune River in France (Kanzari et al. (2014).

### 4.6 Other large-scale multi-pollutant surveys

The International cooperation GVC-UCODEP project was launched to assess the pollution load of the Cầu River in Bắc Giang Province, Vietnam (Giuliani et al., 2019). Similar to the OSRB, the Cầu River is of economic importance as it provides water for drinking, irrigation and industry to the Bắc Giang Province. The survey collected sediment from 30 sites in 2011 and 2012, that were analysed for POPs. Mean ΣPBDE concentrations in the Cầu River were similar to those of the OSRB. However, the maximum concentration quantified in the Cầu River (60 ng/g dm) was four times greater than that of the OSRB (15 ng/g dm). Only *p,p'*-DDE were detected in low concentrations (1.44.4 ng/g dm) in the Cầu River. This is in accordance to patterns observed in sediment and biota in South Africa (Bouwman et al., 2008; Giuliani et al., 2019, Lesch et al., Chapter 3; Polder et al., 2014) where *p,p'*-DDE is usually quantified in greater concentrations than other metabolites. ΣPCDD/F (160–370 ng/kg dm) and ΣPCB (2900–31 000 ng/kg dm) concentrations quantified in sediment from the Cầu River exceeded concentrations quantified in the OSRB.

The Joint Danube Survey 2 (JD2), launched in 2007, was the largest comparable survey in the world with over 100 sampling locations. The Danube is the second-longest river in Europe. Originating in Germany, it flows through nine other countries,

forming the boundaries of eight of these countries and, receives water from 19 nations before draining into the Black sea (Sommerwerk et al., 2022). The Danube River therefore may be influenced by industrial, urban, municipal, and agricultural waste. The ΣPBDE concentrations (0.25–5 ng/g dm) quantified in sediment from the Danube River were of the same range as in the current study, except for Site 12 (15 ng/g dm) that had three times the maximum ΣPBDE concentrations quantified in the Danube River. In addition, the PBDE concentrations were more representative of distinct areas of contamination, in contrast to patterns observed in the current study.

In contrast to the patterns observed in the OSRB, more sediment in the lower part of the Danube River had quantifiable concentrations of DDTs, while other OCPs such as aldrin and dieldrin (among others) were also quantified in low concentrations in a small number of samples. Most of the OCP quantified were attributed to historical use. The mean ΣPCB concentrations quantified in the Danube River were 4300 ng/kg dm with the highest quantifiable concentration of 46 000 ng/kg dm. The mean ΣPCB concentration is four times the maximum concentration (1053 ng/kg dm) detected in the current study. In addition, a mean DL-PCB TEQ concentration of 0.6 ng/kg dm and a maximum DL-PCB TEQ concentration of 2.6 ng/kg were quantified in the Danube River survey. Only Site 22 in the current study had quantifiable DL-PCB TEQ concentrations (1 ng/kg dm) on par with the mean PCB TEQ concentrations reported in the Danube River, while all other quantifiable DL-PCB TEQ concentrations were below the mean Danube sediment concentration. The patterns seen with regards to ΣPCB dispersal in the Danube is similar to the OSRB, with higher ΣPCB concentrations quantified in areas presumed polluted by industry. The mean PCDD/F TEQ quantified in sediment from the Danube River was 2.8 ngTEQ/kg dm, almost three times the maximum quantified PCDD/F TEQ (1 ngTEQ/kg dm), and an order of magnitude higher than most other PCDD/F TEQs concentrations (0.1–0.5 ngTEQ/kg dm) in the current study.

The POP concentrations in the current study were lower than reported in the GVC-UCODEP project and JD2 survey. However, PBDE concentrations at Site 12 were higher than reported in the JD2 survey. This was to be expected since the Danube River is influenced by a larger number of possible pollution sources and flows past more urban regions than the OSRB. In contrast, the Cầu River in Vietnam had less influence from large cities. Instead, mining, agriculture, and rapid population growth were identified as the main contributors of POPs.

The POPs profile of the OSRB therefore, has more in common with the Cầu River in Vietnam than the Danube. The developing nature of South Africa (also having a rapid population growth) and Vietnam, therefore, seems to translate to comparable POPs patterns in sediment. The significance of the present study is shown when the data can be brought into relation with the other two large-scale studies.

### 4.7 International standards and sediment quality guidelines.

The hydrophobic nature of POPs causes the compounds to associate with organic matter in both suspended and settled sediment. Benthic organisms are exposed to compounds by either direct contact or ingestion of contaminated sediments. Biomagnification of compounds in increasingly higher trophic organisms leads to amplified POP concentrations (Verhaert et al., 2017). Increasingly concentrated POPs in biota may lead to adverse health effects and population declines (Vasseur and Cossu-Leguille, 2006). Countries around the world have different approaches to sediment quality guidelines and many countries are still developing their own. In addition, there seems to be much deliberation to which degree some variables, such as sediment grain size and TOC, affect the probable bioavailability. Moreover, although many countries aim to develop their own sediment quality guidelines, they have to deal with a lack of data for many compounds.

In Canada, the guidelines for the protection of aquatic life has a two-fold approach. Both the threshold effect level (TEL) and the probable effect level (PEL) are used as assessments tools to determine the interim sediment quality guidelines (ISQG) (Cly., 2001). In addition, safety factors are applied to control variables and address concerns as sediment composition varies over terrain and with climate. Canada in the northern hemisphere differs drastically in economy, climate, and environment compared with South Africa. Australia with a climate similar to South Africa may have more applicable standards. However, the Australian default guidelines were adopted from Canada with minor adaptations. Two values per compound are reported, the default guideline values (DGV) that represents concentrations below the risk of detrimental effects, and the GV-high, that represents concentrations that may already have caused detrimental toxicity-related effects. All concentrations were normalised to 1% TOC for comparison (Australian Government Initiative, 2019; Environmental Law Alliance Worldwide, 2015)

There were three sites (Sites 39, 49, and 22) with lindane values between the Australian DGV (0.9 ng/g dm) and GV-High (1.4 ng/g dm) and the Canadian ISQG (0.9 ng/g dm) and PEL (1.38 ng/g dm). Lindane concentrations at these levels/concentrations are concerning. Although, lindane has since been banned for use in South Africa it would be recommended to include these sites for future analyses in order to determine if the ban was successfully implemented.

The Mooi River (Site 1) had *p,p'*-DDE values (4 ng/g dm) between the Australian DGV and GV-high concentrations (1.4–7 ng/g dm) and Canadian ISQG (1.46 ng/g dm) and PEL (6.75 ng/g dm). Four sites (22. 25, 27, and 37) had ΣDDT concentrations between the Australian DGV (1.2 ng/g dm) and GV-High (5 ng/g dm) values, including Site 1 that exceeded the GV-high value. There was no explicit ΣDDT value given for the Canadian ISQG. However, the sum of the DDD, DDT, and DDE ISQG (6.15 ng/g dm) is below the ΣDDT concentrations quantified at Site 1, indicating elevated ΣDDT concentrations in sediment from the Mooi River especially. DDT has been for banned for over two decades from the region where the Mooi River flows. To attribute the

concentrations to historical use would be premature, instead future investigation on DDT concentrations from the Mooi River is advised for future studies.

None of the sites had ΣPCB concentrations exceeding the Australian DGV (34 000 ng/g dm) values or Canadian ISQG (34 100 ng/g dm), while PCDD/F values were not available for Australian DGV. Only Skoon Spruit (Site 22), had ΣTEQ concentrations quantified and normalized to 1% TOC that exceeded the Canadian ISQG (0.85 ngTEQ/kg dm).

Based on the comparison of measured concentrations with international sediment quality guidelines, ΣPCBs concentrations, and ΣPCDD/F TEQs concentrations measured in the current study were below standards of concern. However, lindane and DDT remain an issue at selective sites and require investigation. The samples from the current survey was taken in 2009—follow-up sampling and analyses is advisable due to rapid population growth and industrialisation.

## 5. Conclusions and recommendations

Surveys conducted on the scale of the current study are rare. They are time consuming, costly, and are usually conducted under a joint agreement between multiple states. Large scale studies aid in assessing the pollution along important freshwater systems that significantly contribute to economic, environmental, and human wellbeing.

Concentrations of selected POPs in sediment have been reported by others for isolated rivers, sites, or regions in South Africa. The current study is the largest and only published survey reporting POP concentrations in sediment of the entire OSRB. In addition, the current survey is the second largest freshwater survey investigating POP concentrations in sediment in the world after the Danube survey.

The majority of sites sampled in the current study had no quantifiable concentrations of OCPs, PCBs, and PBDEs in sediments and these sites can be excluded from future surveys for these compounds. Higher levels of POPs were quantified in sediment towards the east, decreasing downstream towards the west, except for PBDEs at the confluence of the Orange-Senqu and Vaal rivers. ΣOCP concentrations were dominated by *p,p'*-DDE. It would not be recommended in future surveys to analyse sediment for OCPs other than DDTs, specifically *p,p'*-DDE, lindane, and OCPs added to the Stockholm Convention since 2010 when the samples for the present study were collected. The trend of lindane in the upper region of the Orange-Senqu River after the implementation of the ban on its use and production will be insightful, while the sources of DDT should be investigated.

None of the sediment samples had quantifiable concentrations of PFOS, although this compound has been detected in elevated concentrations in biota. Thus, exclusively using sediment concentrations to determine the toxic threat of PFOS is misleading. It would be insightful for future studies to sample sediment and biota from the same localities, especially considering that there are seven Ramsar sites within the basin.

The PCB and PCDD/F concentrations quantified in sediments were low compared with international sediment quality guidelines, while lindane in the upper region of the Orange-Senqu River and ΣDDT, especially *p,p'*-DDE from Site 1 exceeded international sediment quality guidelines. The ΣTEQ concentrations from Skoon Spruit was the only site to exceed the international sediment quality guidelines.

The POP hotspots in Gauteng, Maseru, Skoon Spruit and Mooi River are associated with industry, urban activities, and mining that greatly influence the freshwater systems. Due to the low quantifiable concentrations of POPs towards the west of the basin, future studies could focus on localities in and around Gauteng, other larger cities, and near mining operations in the OSRB. The fate and sources of PBDE downriver of Gauteng must be investigated. In addition, the influence of Maseru on the Mohokare River should be investigated due to the importance of the river for agriculture. Therefore, we conclude that sediment is a good indicator of point source pollution but biota and other abiotic mediums need to be sampled as well to fully comprehend the pollution within the area under investigation, especially near urban and industrial activities.

This study, the largest of its kind in Africa, provides a valuable baseline for assessments and planning future studies. It concentrated on the Stockholm Convention POPs and has given insights into its source areas and geographical patterns that would otherwise not be illustrated. The OSRB ranks as one of the few large river basins with a rich POPs dataset and assessment, enriching the information contributions of the four countries towards its responsibility towards their inhabitants, environment, and international commitments.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This study was done under the auspices of ORASECOM in association with the UNDP and funded by the GEF via UNOPS. We thank Christof Mor for his kind assistance throughout the project. We also thank Ian Smith, Claudine Roos, Laura Quinn, Karin Blom, and Carlos Huisman for collection and assistance.

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# Dioxins and 21 other persistent organic pollutants in 61 sediment samples from the Orange-Senqu River Basin in Southern Africa.

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**Supplementary material**

**Table S1**: The 61 sampling locations, site numbers, names, river system, latitude and longitude coordinates, the country the site is located in, site description and the nearest town.

| Site no | River | Nearest town/ settelment | Latitude | Longitude | Country | River system | Site description |
|---|---|---|---|---|---|---|---|
| 1 | Mooi River | Potchefstroom | -26.68283 | 27.09856 | RSA | Vaal | Urban |
| 2 | Orange River | Upington | -28.46746 | 21.24162 | RSA | Orange-Senqu | Surrounded Agri |
| 3 | Hartbees River | Kakamas | -28.85786 | 20.6419 | RSA | Orange-Senqu | Upstream Agri |
| 4 | Orange River | Augrabies | -28.49907 | 20.15425 | RSA | Orange-Senqu | Surrounded Agri |
| 5 | Orange River | Onseepkans | -28.73718 | 19.30323 | RSA | Orange-Senqu | Surrounded Agri |
| 6 | Orange River | Vioolsdrif | -28.74892 | 17.60789 | RSA | Orange-Senqu | Surrounded Agri |
| 7 | Orange River (Mouth) | Alexander Bay | -28.60166 | 16.47148 | RSA | Orange-Senqu | Downstream Agri |
| 8 | Orange River | Sendelingsdrift | -28.12284 | 16.89034 | RSA | Orange-Senqu | Mining |
| 9 | Orange River | Prieska | -29.65938 | 22.74801 | RSA | Orange-Senqu | Downstream Agri |
| 10 | Brak River | Prieska | -29.62295 | 23.01586 | RSA | Orange-Senqu | Upstream Agri |
| 11 | Orange River | Douglas | -29.16249 | 23.69918 | RSA | Orange-Senqu | Surrounded Agri |
| 12 | Vaal River | Douglas | -29.03875 | 23.84134 | RSA | Vaal | Downstream Agri |
| 13 | Orange River | Hopetown | -29.60061 | 24.1106 | RSA | Orange-Senqu | Surrounded Agri |
| 14 | Riet River | Riet River | -29.0418 | 24.50267 | RSA | Vaal | Downstream Agri |
| 15 | Vaal River | Schmidtsdrif | -28.72198 | 24.06684 | RSA | Vaal | Mining |
| 16 | Harts River | Delportshoop | -28.37735 | 24.30343 | RSA | Vaal | Downstream Agri |
| 17 | Vaal River | Warrenton | -28.0946 | 24.86716 | RSA | Vaal | Downstream Agri |
| 18 | Vet river | Hoopstad | -27.93642 | 26.12745 | RSA | Vaal | Surrounded Agri |
| 19 | Bloemhof Dam | Bloemhof | -27.51618 | 26.21607 | RSA | Vaal | Surrounded Agri |
| 20 | Vals River | Bothaville | -27.48678 | 26.81273 | RSA | Vaal | Surrounded Agri |
| 21 | Renoster River | Viljoenskroon | -27.04003 | 26.99795 | RSA | Vaal | Surrounded Agri |
| 22 | Skoon Spruit | Klerksdorp | -26.93476 | 26.66436 | RSA | Vaal | Mining |
| 23 | Vaal River | Orkney | -27.01357 | 26.6798 | RSA | Vaal | Mining |
| 24 | Vaal River | Parys | -26.91408 | 27.39709 | RSA | Vaal | Surrounded Agri |
| 25 | Klip River | Meyerton | -26.54934 | 28.06397 | RSA | Vaal | Urban |
| 26 | Suikerbosrand | Heidelberg | -26.64659 | 28.38109 | RSA | Vaal | Surrounded Agri |
| 27 | Blesbok Spruit | Nigel | -26.47889 | 28.42627 | RSA | Vaal | Downstream Agri |
| 28 | Waterval River | Secunda | -26.6291 | 29.02522 | RSA | Vaal | Surrounded Agri |
| 29 | Vaal River (origin) | Breyten | -26.31296 | 30.02597 | RSA | Vaal | Arid Region |
| 30 | Klip River | Volksrust | -27.47026 | 29.60028 | RSA | Vaal | Surrounded Agri |
| 31 | Liebenbergvallei River | Tweeling | -27.53055 | 28.47546 | RSA | Vaal | Surrounded Agri |
| 32 | Wilge River | Reitz | -27.6469 | 28.75031 | RSA | Vaal | Surrounded Agri |
| 33 | Wilge River | Frankfort | -27.30974 | 28.53447 | RSA | Vaal | Surrounded Agri |
| 34 | Vaal River | Villiers | -27.02274 | 28.59754 | RSA | Vaal | Urban |
| 35 | Vaal River | Standerton | -27.03672 | 29.10451 | RSA | Vaal | Surrounded Agri |
| 36 | Waterval River | Greylingstad | -26.84641 | 28.91446 | RSA | Vaal | Surrounded Agri |
| 37 | Suikerbosrand River | Vereeniging | -26.68187 | 28.0495 | RSA | Vaal | Surrounded Agri |
| 39 | Modder river | Bloemfontein | -28.80712 | 26.10722 | RSA | Vaal | Downstream Agri |
| 40 | Kaal river | Bloemfontein | -28.94511 | 25.77264 | RSA | Vaal | Downstream Agri |
| 41 | Karonna Spruit | Bloemfontein | -29.08591 | 26.6364 | RSA | Vaal | Downstream Agri |
| 42 | Modder river | Bloemfontein | -29.10065 | 26.52457 | RSA | Vaal | Arid Region |
| 43 | Riet River | Koffiefontein | -29.47411 | 25.20197 | RSA | Vaal | Upstream Agri |
| 44 | Kromellenboog Spruit | Bloemfontein | -29.68127 | 25.58665 | RSA | Vaal | Downstream Agri |
| 45 | Riet River | Koffiefontein | -29.56313 | 25.64912 | RSA | Vaal | Downstream Agri |
| 46 | Seekoei River | Colesberg | -30.53463 | 24.96297 | RSA | Orange-Senqu | Surrounded Agri |
| 47 | Orange River | Colesberg | -30.50689 | 25.2421 | RSA | Orange-Senqu | Downstream Agri |
| 48 | Caledon river | Bethulie | -30.42789 | 26.30583 | RSA | Orange-Senqu | Downstream Agri |
| 49 | Orange River | Aliwal north | -30.57493 | 26.45936 | RSA | Orange-Senqu | Downstream Agri |
| 50 | Stormberg Spruit | Aliwal North | -30.65019 | 26.46547 | RSA | Orange-Senqu | Upstream Agri |
| 51 | Orange River | Aliwal North | -30.68384 | 26.71092 | RSA | Orange-Senqu | Urban |
| 52 | Orange River | Sterkspruit | -30.40496 | 27.33713 | RSA | Orange-Senqu | Informal settlement |
| 53 | Leeu River | Hobhouse | -29.52812 | 27.13656 | RSA | Orange-Senqu | Surrounded Agri |
| 54 | Caledon River | Hobhouse | -29.48965 | 27.31554 | RSA | Orange-Senqu | Surrounded Agri |
| 55 | Caledon River | Fouriesburg | -28.72445 | 28.15129 | RSA | Orange-Senqu | Surrounded Agri |
| 56 | Molopo Eye | Mahikeng | -25.88732 | 25.88732 | RSA | Orange-Senqu | Arid Region |
| 57 | Malibamatso River | Lejone | -29.02042 | 28.55085 | Lesotho | Orange-Senqu | Mountains |
| 58 | Matsuko River | Lejone | -29.23028 | 28.56345 | Lesotho | Orange-Senqu | Mountains |
| 59 | Senquenyane River | Marakabeis | -29.5531 | 28.14809 | Lesotho | Orange-Senqu | Mountains |
| 60 | Mohokare River | Maseru | -29.33583 | 27.45385 | Lesotho | Orange-Senqu | Informal settlement |
| 61 | Senqu River | Seapa | -30.06218 | 28.5099 | Lesotho | Orange-Senqu | Mountains |
| 62 | Fish River | Seeheim | -26.8 | 17.79 | Namibia | Orange-Senqu | Arid Region |

**Table S2**: All concentrations represented in Table 2 and ΣOCP and ΣPCB normalized to 1% TOC.

| | | | ng/kg dw | | | | ng/g dw | | Normalised to 1% TOC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PCDD/F | | PCB | | | | | | | |
| Site number | Site name | TOC (%) | ΣConcentration | WHO-TE 2005: | ΣConcentration | WHO-TE 2005: | ΣPBDE | ΣOCP | ΣPCB | ΣOCP | River system | Site description |
| 3 | Hartbees River | 1 | | | | | | | | | Orange-Senqu | |
| 10 | Brak River | 1 | 2 | | | | | | | | Orange-Senqu | |
| 43 | Riet River | 2 | 2 | | | | | | | | Vaal | Upstream |
| 50 | Stormberg Spruit | 2 | 1 | | | | | | | | Orange-Senqu | Agriculture |
| 7 | Orange River (Mouth) | 1 | | | | | | | | | Orange-Senqu | |
| 9 | Orange River | 2 | | | | | | | | | Orange-Senqu | |
| 12 | Vaal River | 2 | 3 | 0.1 | | | 15 | | | | Vaal | |
| 14 | Riet River | 1 | 5 | 0.1 | | | | | | | Vaal | |
| 16 | Harts River | 2 | 1 | | | | | | | | Vaal | |
| 17 | Vaal River | 2 | 8 | 0.1 | | | | | | | Vaal | |
| 27 | Blesbok Spruit | 3 | 66 | 0.2 | 104 | 0.2 | | 9 | 34 | 3 | Vaal | |
| 39 | Modder River | 1 | 53 | 0.5 | 164 | 0.1 | | 1 | 191 | 1 | Vaal | |
| 40 | Kaal River | 2 | 2 | | | | | | | | Vaal | |
| 41 | Karonna Spruit | 1 | 3 | | | | | | | | Vaal | |
| 44 | Kromellenboog Spruit | 5 | | | | | | | | | Vaal | |
| 45 | Riet River | 2 | 2 | | | | | | | | Vaal | |
| 47 | Orange River | 1 | 2 | | | | | | | | Orange-Senqu | |
| 48 | Caledon river | 3 | 1 | | | | | | | | Orange-Senqu | Downstream |
| 49 | Orange River | 1 | 2 | | | | | 1 | | 0.5 | Orange-Senqu | Agriculture |
| 2 | Orange River | 1 | 1 | | | | | | | | Orange-Senqu | |
| 4 | Orange River | 1 | | | | | | | | | Orange-Senqu | |
| 5 | Orange River | 1 | 0.1 | | | | | | | | Orange-Senqu | |
| 6 | Orange River | 1 | | | | | | | | | Orange-Senqu | |
| 11 | Orange River | 3 | 2 | | | | 4 | | | | Orange-Senqu | |
| 13 | Orange River | 2 | 4 | | | | | | | | Orange-Senqu | |
| 18 | Vet River | 1 | 1 | | | | | | | | Vaal | |
| 19 | Vaal River | 1 | 18 | 0.2 | 69 | | | | 65 | | Vaal | |
| 20 | Vals River | 1 | | | | | | | | | Vaal | |
| 21 | Renoster River | 1 | 1 | 0.1 | | | | | | | Vaal | |
| 24 | Vaal River | 2 | 6 | | 38 | | | | 18 | | Vaal | |
| 26 | Suikerbosrand River | 2 | 3 | 0.1 | | | | | | | Vaal | |
| 28 | Waterval River | 2 | 9 | 0.1 | | | | | | | Vaal | |
| 30 | Klip River | 1 | | | | | | | | | Vaal | |
| 31 | Liebenbergvallei River | 1 | 2 | 0.1 | | | | | | | Vaal | |
| 32 | Wilge River | 3 | | | | | | | | | Vaal | |
| 33 | Wilge River | 2 | 7 | 0.3 | | | | | | | Vaal | |
| 35 | Vaal River | 2 | 1 | | | | | | | | Vaal | |
| 36 | Waterval River | 1 | 2 | 0.1 | | | | | | | Vaal | |
| 37 | Suikerbosrand River | 1 | 30 | 1 | 126 | 0.1 | | 3 | 155 | 3 | Vaal | |
| 46 | Seekoei River | 1 | 1 | | | | | | | | Orange-Senqu | |
| 53 | Leeu River | 1 | 83 | 0.2 | | | | | | | Orange-Senqu | |
| 54 | Caledon River | 1 | 6 | | | | | | | | Orange-Senqu | |
| 55 | Caledon River | 1 | 2 | | | | | | | | Orange-Senqu | Within Agriculture |
| 1 | Mooi River | 1 | 4 | | | | | 8 | | 8 | Vaal | |
| 25 | Klip River | 1 | 62 | 0.3 | 335 | 0.2 | 1 | 1 | 361 | 1 | Vaal | |
| 34 | Vaal River | 2 | 8 | 0.1 | | | | | | | Vaal | |
| 51 | Orange River | 2 | 0 | | | | | | | | Orange-Senqu | Urban |
| 29 | Vaal River (origin) | 1 | 6 | 0.1 | | | | | | | Vaal | |
| 42 | Modder River | 2 | 2 | | | | | | | | Vaal | |
| 56 | Molopo Eye | 1 | 11 | | 159 | 0.3 | | | 118 | | Orange-Senqu | |
| 62 | Fish River | 1 | 3 | | | | | | | | Orange-Senqu | Arid Region |
| 8 | Orange River | 1 | | | | | | | | | Orange-Senqu | |
| 15 | Vaal River | 1 | 5 | 0.1 | | | | | | | Vaal | |
| 22 | Skoon Spruit | 1 | 57 | 0.5 | 338 | 1 | 1 | 2 | 367 | 2 | Vaal | |
| 23 | Vaal River | 2 | 12 | 0.1 | 1053 | 0.1 | | | 688 | | Vaal | Mining |
| 57 | Malibamatso River | 5 | 3 | | | | | | | | Orange-Senqu | |
| 58 | Matsuko River | 3 | 3 | | 0.4 | | | | 0.1 | | Orange-Senqu | |
| 59 | Senquenyane River | 2 | 5 | 0.1 | 0.3 | | | 1 | 0.1 | 0.2 | Orange-Senqu | |
| 61 | Senqu River | 2 | 4 | | | | | | | | Orange-Senqu | Mountains |
| 52 | Orange River | 2 | 1 | | | | | | | | Orange-Senqu | |
| 60 | Mohokare River | 2 | 66 | 1 | 31 | 0.1 | 1 | 1 | 18 | 0.4 | Orange-Senqu | Informal settlement |

## **Table S3**: PCB and PCDD/F concentrations of individual congeners

| Site no | 1 | 2 | 4 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCDD/F | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2,3,7,8-TCDD | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1,2,3,7,8-PeCDD | | | | | | | | | | | | | | | | | | | | | | 0.1 | | | | | | | | | | | 0.1 | | | | 0.1 | 0.1 | 0.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.1 | | | |
| 1,2,3,4,7,8-HxCDD | | | | | | | | | | | | | | | | | | | | | | 0.1 | | | 0.1 | | 0.1 | | | | | | | | | | 0.1 | 0.2 | 0.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.1 | | | |
| 1,2,3,6,7,8-HxCDD | | | | | | | | | | | | 0.1 | 0.1 | 0.1 | 0.1 | | 0.1 | | 0.1 | | | 0.3 | | | 0.3 | | 0.2 | | 0.1 | | 0.1 | | 0.1 | 0.1 | | | 0.3 | 0.7 | 0.4 | | | 0.1 | 0.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.2 | | | |
| 1,2,3,7,8,9-HxCDD | | | | | | | | | | | | 0.2 | | 0.2 | 0.3 | 0.1 | 0.2 | | 0.2 | | 0.1 | 0.2 | | | 0.3 | 0.1 | 0.2 | | 0.2 | | 0.1 | | 0.3 | 0.1 | 0.1 | 0.1 | 0.3 | 0.4 | 0.4 | | 0.1 | 0.1 | 0.2 | | | | 0.1 | | | | | | | | | | | | | | | | 0.2 | | 0.1 | | | 0.1 | 0.1 | 0.2 | | |
| 1,2,3,4,6,7,8-HpCDD | 1 | | | | | | | | | 0.3 | 0.2 | 0.5 | 1 | 1 | 0.4 | | 1 | | 2 | | 0.2 | 7 | 1 | 1 | 6 | 0.3 | 4 | 1 | 1 | | 0.2 | | 1 | 1 | | 0.3 | 3 | 19 | 6 | 0.4 | 0.2 | 0.2 | 0.3 | | 1 | | 0.3 | 0.2 | | | | | | 8 | 0.2 | 0.2 | 2 | 0.2 | 0.3 | 0.2 | 6 | 0.3 | | |
| OCDD | 4 | 1 | | | | | | | | 2 | 2 | 2 | 3 | 4 | 3 | 1.3 | 6 | 1 | 15 | | 1 | 43 | 5 | 5 | 51 | 1.7 | 57 | 3 | 3 | | 1 | | 4 | 4 | 1 | 1 | 16 | 164 | 39 | 1 | 1 | 1 | 2 | | 2 | | 1 | 1 | 2 | | | | 68 | 2 | 1 | 8 | 1 | 2 | 2 | 52 | 2 | 1 |
| 2,3,7,8-TCDF | 0.1 | | | | | | | | | | | 0.1 | | 0.1 | 0.1 | | 0.1 | | 0.2 | | | 0.4 | 0.1 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 | | | | 0.4 | 0.3 | 0.1 | 0.1 | 0.4 | 0.4 | 1 | | | | | | | 0.1 | | 0.1 | 0.2 | | | 0.1 | 0.1 | | | 0.1 | 1 | 0.1 | | |
| 1,2,3,7,8-PeCDF | | | | | | | | | | | | 0.1 | | 0.1 | 0.1 | | 0.1 | | 0.1 | | | 0.2 | 0.1 | | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | | 0.1 | | 0.2 | 0.2 | | | 0.4 | 0.2 | 0.2 | | | | | | | | | | | | | | | | | | | | | | | | 0.1 | 0.4 | | |
| 2,3,4,7,8-PeCDF | | | | | | | | | | | | | | | | | 0.1 | | 0.1 | | | 0.2 | 0.1 | | 0.2 | | 0.1 | 0.1 | | | | 0.1 | | | | 0.4 | 0.3 | 0.3 | | | | | | | | | | | | | | | | | | | | | | | | 0.3 | | | |
| 1,2,3,4,7,8-HxCDF | | | | | | | | | | | | | | | 0.1 | | | | 0.1 | | | 0.3 | 0.2 | | 0.2 | 0.1 | 0.2 | 0.2 | 0.1 | | | 0.1 | 0.1 | | | 1 | 0.4 | 0.2 | | | | | | | 0.1 | | | | | | | | 0.1 | 0.1 | 0.1 | | 0.1 | 0.1 | 0.2 | 1 | 0.1 | 0.1 |
| 1,2,3,6,7,8-HxCDF | | | | | | | | | | | | | | | 0.1 | | | | 0.1 | | | 0.2 | 0.1 | | 0.2 | | 0.2 | 0.2 | 0.1 | | | 0.1 | 0.1 | | | 1 | 0.3 | 0.2 | | | | | | | | | | | | | | | 0.1 | 0.1 | 0.1 | | 0.1 | 0.1 | 0.2 | 0.4 | 0.1 | 0.1 |
| 1,2,3,7,8,9-HxCDF | | | | | | | | | | | | 0.1 | | | 0.2 | 0.1 | 0.1 | | 0.2 | | | 0.1 | | | | 0.4 | 0.1 | | 0.4 | | 0.3 | 0.5 | 1 | | 0.4 | 0.4 | 0.2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2,3,4,6,7,8-HxCDF | | | | | | | | | | | | | | | | | 0.1 | | 0.1 | | | 0.4 | 0.1 | | 0.3 | | 0.2 | 0.1 | | | | 0.1 | 0.1 | | | 1 | 0.4 | 0.2 | | | | | | | | | | | | | | | 0.1 | | | | | | 0.3 | | | |
| 1,2,3,4,6,7,8-HpCDF | | | | | | | | | | | | | | | 0.2 | | 0.3 | | 1 | | | 2.2 | 1 | | 2 | 0.3 | 1 | 1 | 0.5 | | | 1 | 1 | | 0.2 | 3 | 14 | 1 | | 0.4 | | | 0.2 | 0.3 | 0.2 | 0.2 | | 0.3 | | 0.2 | 2 | 1 | 0.3 | 1 | 0.4 | 0.3 | 1 | 1 | 0.4 | 0.3 |
| 1,2,3,4,7,8,9-HpCDF | | | | | | | | | | | | | | | | | | | | | | 0.3 | 0.2 | | | | | 0.2 | | | | | | | | 0.4 | 0.4 | | | | | | | | | | | | | | | | | | | | | | | | 0.2 | | | |
| OCDF | | | | | | | | | | | | | | | | | | | | | | 3 | 5 | | 2 | | 2 | 3 | 2 | | | | 1 | | | 3 | 14 | 4 | | 2 | | | | 1 | | | 1 | | | 1 | 5 | 3 | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 1 |
| PCB 77 | | | | | | | | | | | | | | | | | | | 6 | | | 40 | 3 | 5 | 49 | | 17 | | | | | | | | | 13 | 11 | 9 | | | | | | | | | | | | | | | | | | | | | | | | 13 | | | 4 | | |
| PCB 81 | | | | | | | | | | | | | | | | | | | 0.3 | | | 2 | | 0 | 2 | | 0 | | | | | | | | | 1 | 0.4 | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | |
| PCB 126 | | | | | | | | | | | | | | | | | | | 0.4 | | | 5 | 1 | | 1 | | 2 | | | | | | | | | 1 | 2 | 1 | | | | | | | | | | | | | | | | | | | | | | | | 3 | | | 1 | | |
| PCB 169 | | | | | | | | | | | | | | | | | | | | | | 1 | | | 0.3 | | 1 | | | | | | | | | 0.4 | 0.3 | | | | | | | | | | | | | | | | | | | | | | | | 0.2 | | 0.4 | 0.3 | 0.3 | | |
| PCB 105 | | | | | | | | | | | | | | | | | | | 17 | | | 69 | 229 | 7 | 59 | | 6 | | | | | | | | | 24 | 26 | 36 | | | | | | | | | | | | | | | | | | | | | | | | 31 | | | 7 | | |
| PCB 114 | | | | | | | | | | | | | | | | | | | | | | 4 | 13 | | 6 | | | | | | | | | | | 2 | 2 | 3 | | | | | | | | | | | | | | | | | | | | | | | | 2 | | | | | |
| PCB 118 | | | | | | | | | | | | | | | | | | | 30 | | | 151 | 628 | 23 | 170 | | 24 | | | | | | | | | 47 | 56 | 88 | | | | | | | | | | | | | | | | | | | | | | | | 53 | | | 15 | | |
| PCB 123 | | | | | | | | | | | | | | | | | | | | | | 1 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PCB 156 | | | | | | | | | | | | | | | | | | | 7 | | | 29 | 118 | 3 | 27 | | 27 | 3 | | | | | | | | 11 | 23 | 16 | | | | | | | | | | | | | | | | | | | | | | | | 32 | | | 4 | | |
| PCB 157 | | | | | | | | | | | | | | | | | | | 1 | | | 7 | 21 | | 6 | | 8 | | | | | | | | | 4 | 4 | 3 | | | | | | | | | | | | | | | | | | | | | | | | 5 | | | | | |
| PCB 167 | | | | | | | | | | | | | | | | | | | 7 | | | 22 | 32 | | 10 | | 13 | | | | | | | | | 7 | 19 | 6 | | | | | | | | | | | | | | | | | | | | | | | | 14 | | | | | |
| PCB 189 | | | | | | | | | | | | | | | | | | | | | | 8 | 6 | | 5 | | 6 | | | | | | | | | 3 | 4 | 2 | | | | | | | | | | | | | | | | | | | | | | | | 4 | | | | | |
| PCB 28 | | | | | | | | | | | | | | | | | | | | | | 215 | | | 335 | | 93 | | | | | | | | | | 82 | 117 | | | | | | | | | | | | | | | | | | | | | | | | | | | 611 | | |
| PCB 52 | | | | | | | | | | | | | | | | | | | | | | 126 | 115 | | 252 | | 71 | | | | | | | | | 56 | | 97 | | | | | | | | | | | | | | | | | | | | | | | | 92 | | | 463 | | |
| PCB 101 | | | | | | | | | | | | | | | | | | | 59 | | | 226 | 1000 | | 233 | | 216 | | | | | | | | | 73 | 117 | 164 | | | | | | | | | | | | | | | | | | | | | | | | 453 | | | | | |
| PCB 138 | | | | | | | | | | | | | | | | | | | 89 | | | 435 | 906 | | 363 | | 360 | | | | | | | | | 165 | 249 | 220 | | | | | | | | | | | | | | | | | | | | | | | | 623 | | | 61 | | |
| PCB 153 | | | | | | | | | | | | | | | | | | | 96 | | | 479 | 695 | | 398 | | 376 | | | | | | | | | 149 | 277 | 174 | | | | | | | | | | | | | | | | | | | | | | | | 674 | | | 69 | | |
| PCB 180 | | | | | | | | | | | | | | | | | | | | | | 221 | 112 | | 226 | | 282 | | | | | | | | | 88 | 166 | 181 | | | | | | | | | | | | | | | | | | | | | | | | 316 | | | | | |

# Chapter 6

## 1. Discussion and conclusions

### 1.1 Overview

The aim of this thesis was to assess persistent organic pollutants (POPs) in biotic and abiotic components of the Orange-Senqu River Basin (OSRB; Figure 1). I identified the Cattle Egret *Bubulcus ibis* as a near-global indicator of terrestrial pollution. I also report on POP concentrations in eggs of eight bird species, including Cattle Egrets, occupying different trophic levels collected from four sites in the Vaal River catchment. I report polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins and dibenzo-*p*-furans (PCDD/F) concentrations in sediment collected in 2002 and 2009 which were analysed for 22 POPs. The evaluation concerns the presence, concentrations, and threats POPs may pose in the Southern African environment making use of two often-used matrices; sediment and wild bird eggs.

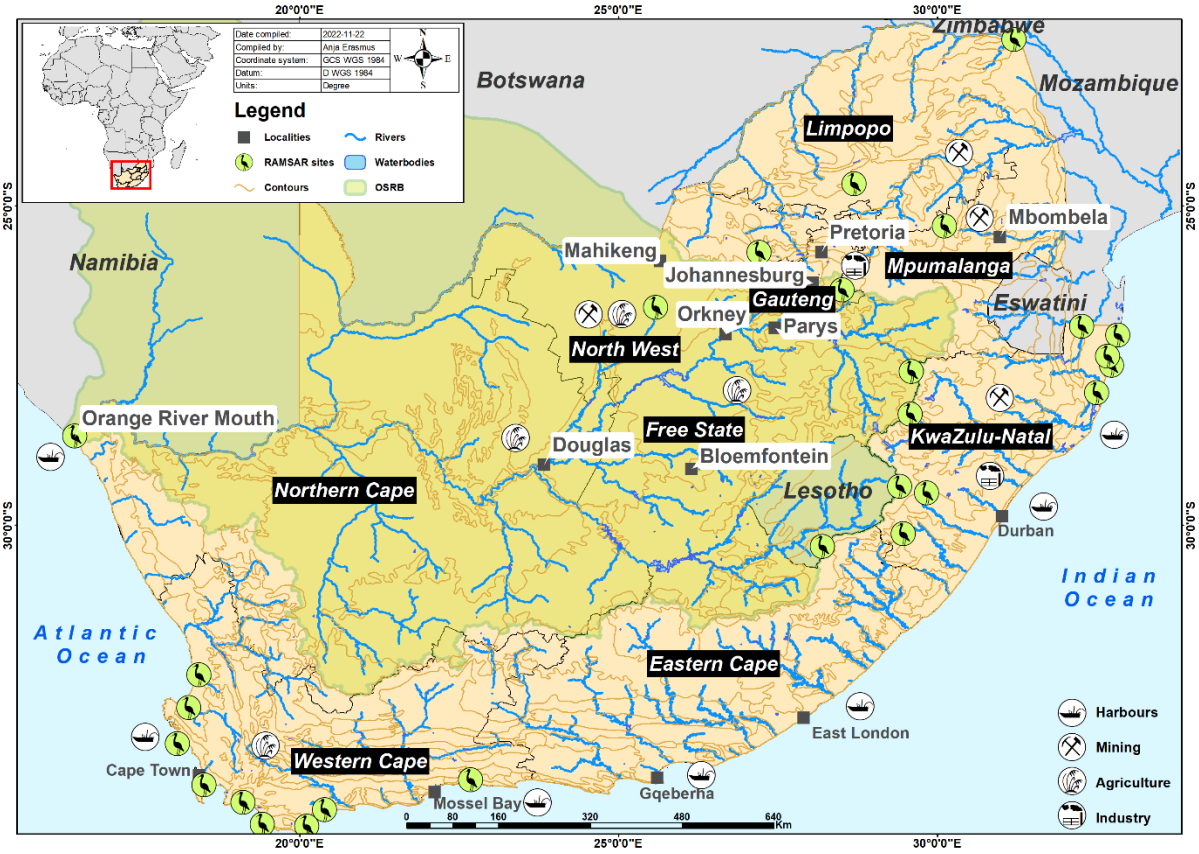


**Figure 1:** Map of Southern Africa showing the major rivers, the Orange-Senqu River Basin boundaries RAMSAR sites, towns and cities mentioned in text, major industrial and mining sites, and harbours.

### 1.2 Discussion according to matrices

1.2.1 Wild bird eggs

Wild bird eggs are frequently used as monitoring tools to assess pollution since they reflect contamination in the environment where the female bird fed prior to and during egg formation (Aurigi et al., 2000). Elevated concentrations of POPs in bird eggs may lead to eggshell

thinning (Bitman et al., 1970; Holm et al., 2006; Lundholm, 1997), endocrine disruption (Jensen and Leffers., 2008), deformities (Fernie et al., 2006 and 2008), and mortality of the developing embryo that may lead to population declines (Dirksen et al., 1995; Peakall, 1993).

In Chapter 2, I reviewed and assessed the Cattle Egret as a suitable near-global terrestrial indicator of pollution. Several studies show that Cattle Egret eggs bioaccumulate POPs (Bouwman etal., 2021; Elliott et al, 2001; Harris et al., 2003). Using eggs of the same species across multiple countries, catchments, and continents will provide highly comparable data to assess terrestrial pollution.

In Chapter 3, I investigated POP concentrations in wild bird eggs occupying four trophic levels, including the terrestrial Cattle Egret, in the Vaal River. The results showed that aquatic predatory birds had higher perfluorooctane sulfonic acid (PFOS), organochlorine pesticides (OCPs) and PCBs concentrations, while PCDD/Fs dominated in eggs of terrestrial species. The study showed the importance of multi-species studies from multiple locations to better comprehend the risks that POPs pose to avian populations.

Thus, it would be recommended that Cattle Egrets eggs should be included as a near-global terrestrial indicator of pollution in order to assess pollution on an equal level across continents, while eggs of aquatic Ardeids and eggs from birds from other trophic levels be sampled alongside that of the Cattle Egret in order to accurately identify hotspots and trends.

#### 1.2.2 Sediment

Sediment is a popular matrix to assess pollution in aquatic environments. It is cost effective and relatively easy to obtain compared with other matrixes and can be collected close to suspected pollution sources, whereas bird breeding colonies might not be so conveniently co-located with sources. Sediment act as pollutant sinks as many compounds bind to organic content, concentrating in the sediment. In Chapter 4, I report and assess the earliest dioxin-like PCB (DL-PCB) and PCDD/F concentration dataset in sediment from 22 sites across South Africa collected in 2002. Higher DL-PCB and PCDD/F concentrations occurred in sediment collected from highly industrialised regions, of which seven sites exceeded PCDD/F toxic equivalency quotients according to international quality guidelines.

In Chapter 5, I report the largest multi-pollutant freshwater survey on 22 POPs in sediment collected in 2009 in Southern Africa covering the Orange-Senqu River Basin. Similar patterns were seen during this survey compared with the survey in Chapter 4. Higher concentrations of POPs were quantified in sediment near industrial activities, with the highest concentrations found at sites in Gauteng. PCDD/F concentrations were quantified in more samples compared with other POPs. Dichlorodiphenyltrichloroethanes (DDTs) were mainly quantified in samples near Gauteng, while polybrominated biphenyl ether (PBDE) concentrations peaked near the confluence of the Orange-Senqu and Vaal rivers.

### 1.3 Historical trends

Chapters 3 and 5 were parts of a large, multi-pollutant, transboundary survey under the auspices of the Orange-Senqu River Commission's (ORASECOM). Chapter 4 was funded by the Water Research Commission. Several of the 2002 sediment sampled locations were also sampled in 2009. Here, I will briefly discuss the temporal trends based on ΣPCB of all PCBs quantified and PCDD/F concentrations and patterns found. Site 1 in Chapter 4 and Site 7 in Chapter 5 are the Orange-Senqu River mouth near Alexander Bay. No ΣPCB and ΣPCDD/F

concentrations were quantified in the 2009 survey, while low concentrations were quantified in 2002 (Figure 2). The Vaal River near Douglas also showed a decline in ΣPCB and ΣPCDD/F concentrations from 2002 to 2009 (Figure 2). Sites from the industrial region in Gauteng adjacent to the Vaal River were grouped in both surveys. Figure 2 shows a decline in ΣPCB and ΣPCDD/F concentrations from 2002 to 2009.

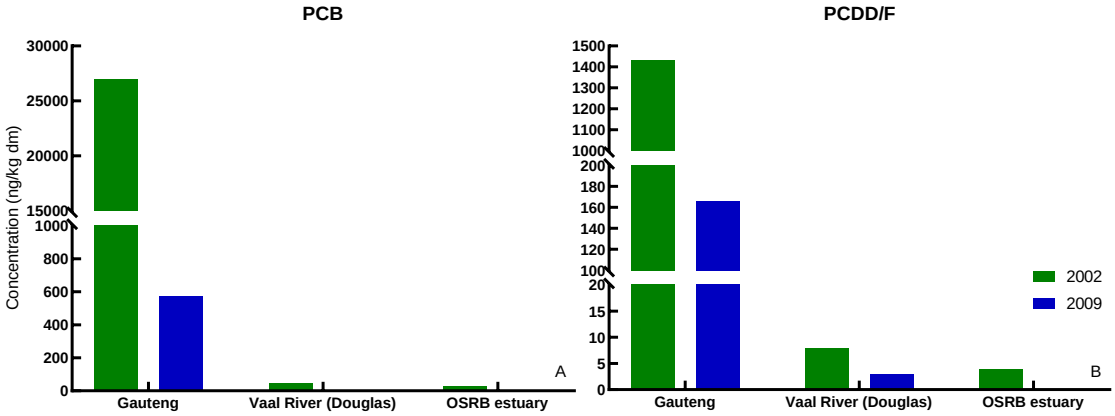


**Figure 2:** ΣPCB (A) and ΣPCDD/F (B) concentrations in sediment samples collected in 2002 and 2009 near the same locations. Sites in Gauteng's lower industrial region were grouped to give a mean concentration for Gauteng. The Vaal River near Douglas and the Orange-Senqu River estuary were sampled in both surveys.

The wild bird eggs from Chapter 3 and the sediment from Chapter 5 were collected the same year (2009). Three out of the four bird egg sampling locations (Chapter 3) were also used for sampling of sediment at approximately the same locality. Although the comparison between concentrations in sediment expressed as ng/kg dm and the wild bird eggs expressed as ng/kg wm for PCBs and PCDD/F and ng/g wm for OCPs and PFOS is impossible, the relative patterns can be discussed.

Sediment from Chapter 5, Site 1 (Mooi River), exceeded the international sediment quality guidelines for ΣDDT. However, no adverse effects are expected in wild birds from Potchefstroom due to DDT. The PFOS concentrations quantified in wild bird eggs from all four sampling localities is a risk for adverse effects, but none of the corresponding sediments had quantifiable concentrations of PFOS. From Chapter 3 it is recommend that bird eggs be sampled from Upington in order to assess if the high PFOS in bird eggs from Bloemhof Dam is also seen at Upington, even though Upington sediment (Site 2 in Chapter 5) had no quantifiable PFOS concentrations.

The highest ΣPCB concentrations in bird eggs and sediment were from Gauteng, highlighting this region as a PCB hotspot. However, not all sediment sites in Gauteng had quantifiable concentrations of PCBs. Mooi River in Potchefstroom had no quantifiable PCBs in sediment but was quantifiable in bird eggs from there. PCDD/F concentrations were quantifiable in more sediment samples than other POPs. Moreover, all bird eggs had quantifiable concentrations of PCDD/Fs. The higher PBDE concentrations in sediment from Douglas, approximately 450 km downstream of Gauteng, contrasted with the highest PBDE concentrations found in wild bird eggs from Gauteng.

The general message of this thesis is that using only one matrix to assess environmental POPs pollution ishas its limitations.

### 1.5 Stockholm Convention on Persistent Organic pollutants

The SCPOPs obliges Parties to support and report studies on POPs and the effectiveness of the implementation of the SCPOPs. Monitoring the presence of chemicals listed in Annexes A, B, and C makes provision for the evaluation of the effectiveness of the SCPOPs in Article 16 of the Convention. In this thesis, I present studies in support of such assessments in achieving the objective of the SCPOPs, "…to protect human health and the environment from persistent organic pollutants".

### 1.6 Future studies and recommendations

Based on the conclusions I recommend the following:

- It is recommended to use the near-global terrestrial Cattle Egret eggs in analyses for POPs on an equal level across continents.
- Bird eggs of species occupying multiple trophic levels should be sampled to comprehend the risks representative of the complexity of avian diversity as environmental, behavioural and physiological differences of species.
- Sites that had no quantifiable concentrations of POPs in sediment can be excluded in future surveys.
- It is recommended that additional localities in identified hotspot regions be sampled.
- It would be advisable to use biota in future studies focused around PFAS, as sediment are not good indicators of PFOS pollution within South Africa.
- Sediment quality guidelines appropriate to Southern Africa should be developed.
- Wild bird eggs are more suitable and sensitive sample matrixes than sediment to assess pollution and should be preferred over abiotic matrixes.
- Biotic and abiotic samples should be collected and analysed in tandem in identified POP hotspot regions.

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